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Canadian Intellectual Property Office

CA 2965414 C 2024/01/09

(11)(21) 2 965 414

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2015/10/23
(87) Date publication PCT/PCT Publication Date: 2016/05/06
(45) Date de délivrance/Issue Date: 2024/01/09
(85) Entrée phase nationale/National Entry: 2017/04/21
(86) N° demande PCT/PCT Application No.: AU 2015/050654
(87) N° publication PCT/PCT Publication No.: 2016/065409
(30) Priorité/Priority: 2014/10/29 (AU2014904326)

(51) Cl.Int./Int.Cl. *A61K 38/21* (2006.01),
A61K 39/395 (2006.01), *A61P 39/00* (2006.01),
C07K 14/56 (2006.01), *C07K 16/28* (2006.01),
C12N 15/21 (2006.01)

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(54) Titre : VARIANTES DE L'INTERFERON α2B
(54) Title: INTERFERON α2B VARIANTS

(57) Abrégé/Abstract:
The present invention provides a fusion polypeptide comprising a first domain and a second domain, wherein the first domain comprises a polypeptide ligand which binds to a cell surface-associated antigen and the second domain comprises aglycosylated interferon α 2b (IFNα2b) having a sequence of SEQ ID NO: 1 or SEQ ID NO: 2. The aglycosylated IFNα2b further comprises one or more amino acid substitutions or deletions which attenuate the activity of the aglycosylated IFNα2b.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 May 2016 (06.05.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/065409 A1

(51) International Patent Classification:
A61K 38/21 (2006.01) *C07K 16/28* (2006.01)
A61K 39/395 (2006.01) *C12N 15/21* (2006.01)
C07K 14/56 (2006.01) *A61P 39/00* (2006.01)

(21) International Application Number:
PCT/AU2015/050654

(22) International Filing Date:
23 October 2015 (23.10.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2014904326 29 October 2014 (29.10.2014) AU

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2016/065409 A1

(54) Title: INTERFERON α2B VARIANTS

(57) Abstract: The present invention provides a fusion polypeptide comprising a first domain and a second domain, wherein the first domain comprises a polypeptide ligand which binds to a cell surface-associated antigen and the second domain comprises aglycosylated interferon α 2b (IFNα2b) having a sequence of SEQ ID NO: 1 or SEQ ID NO: 2. The aglycosylated IFNα2b further comprises one or more amino acid substitutions or deletions which attenuate the activity of the aglycosylated IFNα2b.

INTERFERON α2b VARIANTS

FIELD OF INVENTION

[0001] The present invention relates to polypeptides comprising ligands targeted against cell surface antigens and aglycosylated interferon α 2b (IFNα2b) and the use of these polypeptides in the treatment of cancer.

BACKGROUND OF INVENTION

[0002] Numerous peptide and polypeptide molecules 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 molecules include peptide and polypeptide hormones, cytokines, chemokines, growth factors, apoptosis-inducing factors and the like. These molecules can be either soluble or can be attached to the surface of another cell.

[0003] Due to the biological activity of such molecules, some have potential use as therapeutics. Several peptide or polypeptide molecules have been approved by regulatory agencies as therapeutic products, including, for example, human growth hormone, insulin, interferon IFNα2b, IFNα2a, IFNβ, erythropoietin, G-CSF and GM-CSF. Many of these and other peptides have demonstrated potential in therapeutic applications, but have also exhibited toxicity when administered to human patients. One reason for toxicity is that most of these molecules trigger receptors on a variety of cells, including cells other than those that mediate the therapeutic effect. For example, when IFNα2b 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 reduced proliferation and hence limits disease progression. Unfortunately, however, this IFN also binds to numerous other, normal cells within the body, triggering a variety of other cellular responses, some of which are harmful (e.g. flu-like symptoms, neutropenia, depression). A consequence of such "off target" activity of peptides is that many peptides are not suitable as drug candidates. In this context, "off target activity" refers to activity on the peptide's natural

receptor, but on the surface of cells other than those that mediate therapeutically beneficial effects.

[0004] Even though some peptides, such as IFNα2b, are approved for the treatment of medical conditions, they are poorly tolerated due to their "off target" biological activity. The off-target activity and associated poor tolerability also mean that some of these peptide based drugs cannot be administered at sufficiently high dosages to produce optimal therapeutic effects on the target cells which mediate the therapeutic effect.

[0005] Similarly, it has been known since the mid-1980's that interferons, in particular IFNα, are able to increase apoptosis and decrease proliferation of certain cancer cells. 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. IFNα 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 IFNα on the tumour cells is mediated by the IFNα binding directly to the type I IFN receptor on those cells and stimulating apoptosis, terminal differentiation or reduced proliferation. One "indirect" effect of IFNα 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 tumour.

[0006] Unfortunately, the type I interferon receptor is also present on most non-cancerous cells. Activation of this receptor on such cells by IFNα causes the expression of numerous pro-inflammatory cytokines and chemokines, leading to toxicity. Such toxicity prevents the dosing of IFNα to a subject at levels that exert the maximum anti-proliferative and pro-apoptotic activity on the cancer cells.

[0007] Ozzello *et al.* (Breast Cancer Research and Treatment 25:265-76, 1993) described covalently attaching human IFNα to a tumour-targeting antibody, thereby localizing the direct inhibitory activity of IFNα to the tumour as a way of reducing tumour growth rates, and demonstrated that such conjugates have anti-tumour activity in a xenograft model of a human cancer. The mechanism of the observed anti-cancer activity

was attributed to a direct effect of IFNα on the cancer cells, since the human IFNα used in the experiments did not interact appreciably with the murine type I IFN receptor, which could have lead to an indirect anti-cancer effect. Because of this lack of binding of the human IFNα to the murine cells, however, the authors could not evaluate the toxicity of the antibody-IFNα conjugate relative to free INFα. These authors used a chemical method to attach the IFNα to the antibody.

[0008] Alkan *et al.*, (Journal of Interferon Research, volume 4, number 3, p. 355-63, 1984) demonstrated that attaching human IFNα to an antibody that binds to the Epstein-Barr virus (EBV) membrane antigen (MA) increased its antiproliferative activities towards cells that express the EBV-MA antigen. This increased potency was dependent on both antigen expression by the target cells and the binding specificity of the antibody. The cell line tested was the cancer cell line QIMR-WIL, a myeloblastic leukemia. The authors suggested that the attachment of IFNα to an antibody could be used as a treatment for cancer since it would reduce tumour growth. Alkan *et al* did not address the potential toxicity of these antibody-IFNα conjugates arising from their interactions with normal, antigen-negative cells.

[0009] It is also known that the linkage between an antibody and IFNα may be accomplished by making a fusion protein construct. For example, IDEC (WO01/97844) disclose a direct fusion of human IFNα to the C terminus of the heavy chain of an IgG targeting the tumour antigen CD20. Other groups have disclosed the use of various linkers between the C-terminus of an IgG heavy chain and the IFNα. For example, US 7,456,257 discloses that the C-terminus of an antibody heavy chain constant region may be connected to IFNα via an intervening serine-glycine rich (S/G) linker of the sequence $(GGGGS)_n$, where n may be 1, 2 or 3, and that there are no significant differences in the IFNα activity of the fusion protein construct regardless of linker length.

[0010] Morrison *et al.* (US2011/0104112 A1; and Xuan C, Steward KK, Timmerman JM, Morrison SL. Targeted delivery of interferon-α via fusion to anti-CD20 results in potent antitumor activity against B-cell lymphoma. Blood 2010;115:2864–71) also disclose IFNα linked to the C-terminus of the heavy chain of a cancer-targeting IgG

antibody, with an intervening S/G linker, and observed that the fusion of the IgG and linker to the IFNα reduced the activity of IFNα on cells that did not express the corresponding antigen on the cell surface. The decreased IFN activity of these fusion protein constructs was modest when compared to human non-fusion protein IFNα (free IFNα) acting on human cells, but appeared to be more significant for murine IFNα on murine cells. The decrease in the activity of human IFNα that results from fusing it to the C-terminus of an antibody, as observed by Morrison *et al*, and in US 7,456,257 is modest and is generally considered to be a disadvantage since it reduces potency of the IFN. This disadvantage was pointed out, for example, by Rossi *et al* (Blood vol. 114, No. 18, pp3864-71), who used an alternative strategy of attaching the IFNα to a tumor targeting antibody in such a way that no loss in IFNα activity was observed.

[0011] In general the prior art teaches to use a potent IFN and to target this IFN to cancer cells. While this approach results in an increase in activity of the IFN against cancer cells, it does not address the issue of activity of the IFN on normal "off-target" cells. In prior art examples referred to above, the human IFNα portion of the antibody-IFNα fusion protein maintained a high proportion of native IFNα activity when exposed to human cells that do not express the corresponding antigen on their cell surfaces. This activity may lead to toxicity arising from the activation of non-cancerous, normal ("off target") cells by the IFNα portion of the fusion protein. Accordingly, there exists a need to decrease the "off-target" activity of IFN-based drugs, while retaining the "on-target", therapeutic effect of such drugs. The maintenance of target-specific activity and at the same time a reduction in non-target toxicity of these types of therapeutic agents would create a greater therapeutic concentration window for therapeutically useful peptides. It would for example be desirable to use human IFNα in a form such that its activity can be directed to the cancer cells while minimizing its effects on normal human cells. Ideally the type I interferon receptor on the cancer cells would be maximally stimulated, while the same receptor on non-cancerous cells would experience minimal stimulation. There is a need to target human IFNα to the cancer cells in such a way that it has dramatically more activity on the cancer cells, which display the antigen, than on the normal cells, which do not display the antigen. The same logic applies to other potentially therapeutic molecules,

e.g. other cytokines, peptide and polypeptide hormones, chemokines, growth factors, apoptosis-inducing factors and the like.

[0012] The logic of this approach has been demonstrated in WO 2013/059885, and WO 2014/178820.

SUMMARY OF INVENTION

[0013] In a first aspect the present invention provides a fusion polypeptide comprising a first domain and a second domain, wherein the first domain comprises a polypeptide ligand which binds to a cell surface-associated antigen and the second domain comprises human aglycosylated interferon α 2b (IFNα2b) having a sequence of SEQ ID NO: 1 or SEQ ID NO: 2 and wherein the aglycosylated IFNα2b further comprises one or more amino acid substitutions or deletions which attenuate the activity of the aglycosylated IFNα2b.

[0014] In another aspect the present invention provides a fusion polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs: 31, 61 to 77, 83 and 87, and a sequence selected from the group consisting of SEQ ID NOs: 81, 82 and 84.

[0015] In another aspect the present invention provides a fusion polypeptide comprising SEQ ID NO: 87 and SEQ ID NO: 81.

[0016] In another aspect the present invention provides a fusion polypeptide comprising SEQ ID NO: 79 and SEQ ID NO: 85.

[0017] In another aspect the present invention provides a fusion polypeptide comprising SEQ ID NO: 80 and SEQ ID NO: 86.

[0018] In another aspect the present invention provides a fusion polypeptide comprising SEQ ID NO: 78.

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[0019] In another aspect the present invention provides a composition comprising the fusion polypeptide of the present invention and a pharmaceutically acceptable carrier or diluent.

[0020] In another aspect the present invention provides a method of treating a tumour in a subject comprising administering to the subject the fusion polypeptide of the present invention or the composition of the present invention wherein the first domain of the fusion polypeptide binds to cells of the tumour.

[0021] In another aspect the present invention provides the use of the fusion polypeptide of the present invention in the treatment of a tumour wherein first domain of the fusion polypeptide binds to the tumour.

[0022] In another aspect the present invention provides an isolated polynucleotide(s) encoding the fusion polypeptide(s) of the present invention.

[0023] In another aspect the present invention provides a vector comprising the one or more polynucleotides of the present invention.

[0024] In another aspect the present invention provides a transformed cell comprising the vector of the present invention.

[0025] In another aspect the present invention provides a method of generating a polypeptide ligand-attenuated IFNα2b fusion polypeptide in mammalian cells, wherein the polypeptide ligand-attenuated IFNα2b fusion polypeptide has reduced heterogeneity and/or enhanced FcRn binding and/or improved target selectivity, the method comprising culturing a recombinant mammalian cell comprising a polynucleotide encoding the polypeptide ligand-attenuated IFNα2b fusion polypeptide wherein T106 of the IFNα2b sequence is replaced with another amino acid or is deleted such that on expression in mammalian cells the IFNα2b component of the fusion protein is aglycosylated.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Figure 1: Anti-proliferative activity upon treatment of (A) ARP1 and (B) NCI-H929 cells with anti-CD38-attenuated IFNα2b fusion proteins in IgG1 or IgG4 format with and without O-linked glycosylation of the IFNα2b.

[0027] Figure 2 A, B, C, D and E. Anti-proliferative activity of anti-CD38-attenuated IFNα2b fusion proteins with different amino acid substitutions removing the O-linked glycosylation site from the attenuated IFNα2b.

[0028] Figure 3: On-target activities of (A) A10.21 and (B) A10.43 anti-CD38-attenuated IFNα2b fusion proteins with (T106T) and without (T106A) O-linked glycosylation of the IFNα2b.

[0029] Figure 4: Off-target activities of (A) A10.21 and (B) A10.43 anti-CD38-attenuated IFNα2b fusion proteins with (T106T) and without (T106A) O-linked glycosylation of the IFNα2b.

[0030] Figure 5: Off-target activity by anti-CD38-attenuated IFNα2b fusion proteins with (T106T) and without (T106A or ΔT106) O-linked glycosylation of the IFNα2b.

[0031] Figure 6: A, B, C, D, E and F: Off-target activity of anti-CD38-attenuated IFNα2b fusion proteins with different amino acid substitutions removing the O-linked glycosylation site from the attenuated IFNα2b.

[0032] Figure 7: Off-target activity of A10.43 anti-CD38 –attenuated IFNα2b fusion proteins with (T106T) or without (T106A) O-linked glycosylation of the IFNα2b.

[0033] Figure 8: Potency of sub-optimal dosages of anti-CD38-attenuated IFNα2b fusion proteins with (T106T) or without (T106A) O-linked glycosylation in treating tumours in a murine model of multiple myeloma.

[0034] Figure 9: Number of charged species of A10.21 anti-CD38-attenuated IFNα2b fusion proteins with (T106T) or without (T106A, ΔT106, T106S, T106V, T106G, T106E) O-linked glycosylation of the IFNα2b, as assessed by the number of bands on IEF gel.

[0035] Figure 10: Number of charged species of A10.21 anti-CD38-attenuated IFNα2b fusion proteins with (T106T) or without (T106A) O-linked glycosylation of the IFNα2b and with varying Fc isotypes, as assessed by the number of bands on IEF gel.

[0036] Figure 11: Number of charged species of A10.21 (IgG4 with S228P) anti-CD38-attenuated IFNα2b fusion proteins with (T106T) or without (T106A) O-linked glycosylation of the IFNα2b in the presence of YTE substitutions in the antibody constant region, as assessed by the number of bands on IEF gel.

[0037] Figure 12: Number of charged species of A10.21 (IgG4 with S228P) anti-CD38-attenuated IFNα2b fusion proteins with (T106T) or without (T106A) O-linked glycosylation of the IFNα2b in the presence of a variety of IFN attenuating substitutions, as assessed by the number of bands on IEF gel.

[0038] Figure 13: Number of charged species of antibodies with differing target specificities; anti-CD138 antibody, anti-HLA antibody and anti- CD38 antibody (A02.12) (all IgG4 with S228P) fused to attenuated IFNα2b with (T106T) or without (T106A) O-linked glycosylation of the IFNα2b, as assessed by the number of bands on IEF gel.

[0039] Figure 14: "On-target" activities of anti-CD38-attenuated IFNα2b fusion proteins (A10.21 IgG4 (S228P) IFN (A145D)) with (T106T) and without (T106A, ΔT106, T106S, T106V, T106G, T106E) O-linked glycosylation of the IFNα2b.

[0040] Figure 15: "On-target" activities of two different anti-CD38 antibody-attenuated IFNα2b fusion proteins (A02.12 and A10.21, both IgG4 with S228P) which bind different epitopes on CD38 with (T106T) or without (T106A) O-linked glycosylation of the IFNα2b.

[0041] Figure 16: "On-target" activities of A10.21 anti-CD38-attenuated IFNα2b fusion proteins (A10.21 IgG4 (S228P) IFN) with (T106T) and without (T106A) O-linked glycosylation of the IFNα2b, with a variety of IFN attenuating substitutions (R33A, R144I, R145Q, A145K or A145G).

[0042] Figure 17: "On-target" activities of antibodies with differing target specificities; anti-CD138 antibody and anti-HLA antibody (both IgG4 with S228P) fused to attenuated IFNα2b with (T106T) or without (T106A) O-linked glycosylation of the IFNα2b.

[0043] Figure 18: "On-target" activities of A10.21 anti-CD38-attenuated IFNα2b fusion proteins (A10.21 IgG4 (S228P) IFN (A145D)) with (T106T) and without (T106A) O-linked glycosylation of the IFNα2b, in the presence of YTE substitution in the antibody heavy chain.

[0044] Figure 19: "On-target" activities of A10.21 anti-CD38-attenuated IFNα2b (A145D) fusion proteins with (T106T) and without (T106A) O-linked glycosylation of the IFNα2b, with with a variety of immunoglobulin Fc isotypes.

[0045] Figure 20: Selectivity index of A10.21 anti-CD38-attenuated IFNα2b fusion proteins with and without O-linked glycosylation of the IFNα2b in the presence of a variety of amino acid substitutions to remove glycosylation of the IFN, YTE substitutions in the imunoglobulin constant region for extended half-life, IFN attenuation and Fc isotypes.

DETAILED DESCRIPTION OF THE INVENTION

[0046] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

[0047] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[0048] It must be noted that, as used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "an agent" includes a single agent, as well as two or more agents; reference to "a molecule" includes a single molecule, as well as two or more molecules; and so forth.

[0049] The constructs of the present invention are polypeptide ligand-attenuated aglycosylated IFNα2b fusion constructs, which show an elevated antigen-selectivity index with respect to activating signaling pathways due to the action of both the ligand targeting to a cell surface receptor on a cell of interest and the attenuated IFNα2b having reduced affinity to a cell surface IFN receptor. These constructs are based on the discovery outlined in WO 2013/059885 that, in the context of an antibody-IFN fusion construct, the IFN portion can be mutated in such a way that the IFN activity on antigen-negative cells is dramatically attenuated, while the IFN 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 IFN.

[0050] In one embodiment, the antibody-attenuated IFN construct retains 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) IFN. In addition, in one embodiment the antibody-attenuated IFN construct retains 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) IFN; in this context, "maximal activity" should be understood as meaning the amount of signaling activity (or downstream effect thereof) at the high, plateau portion of a dose-response curve, where further increases in the agent does not further increase the amount of response).

[0051] The present inventors have now found that an unexpected advantage is obtained by using constructs comprising aglycosylated IFNα2b as compared to the constructs comprising O-glycosylated IFNα2b. In some embodiments these advantages include one or more of an increase in ON-target activity, increased target selectivity and

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enhanced affinity to FcRn, whilst providing a less heterogeneous product than the O-glycosylated IFNα2b when produced in a mammalian cell expression system. Enhanced FcRn binding is desirable to improve the pK of a biological therapeutic agent which comprises an Fc region. Increased target selectivity is desirable as it potentially reduces OFF-target toxicity whilst substantially maintaining ON-target activity. A reduction in heterogeneity allows increases in yields of purified product from a mammalian cell culture system.

[0052] Accordingly, in a first aspect, the present invention provides a fusion polypeptide comprising a first and a second domain, wherein the first domain comprises a polypeptide ligand which binds to a cell surface-associated antigen and the second domain comprises aglycosylated interferon α 2b (IFNα2b) having a sequence of SEQ ID NO: 1 or SEQ ID NO: 2, and wherein the aglycosylated IFNα2b further comprises one or more amino acid substitutions or deletions which attenuate the activity of the aglycosylated IFNα2b.

[0053] In an embodiment of the present invention the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W or Y. This represents a substitution of the T106 normally present in human IFNα2b with a naturally occurring amino acid which does not permit O-linked glycosylation at this position when produced in mammalian cell culture. In another embodiment of the present invention the sequence of the aglycosylated IFNα2b is SEQ ID NO: 2. This represents a deletion of residue T106 found in normal human IFNα2b, which also removes the O-linked glycosylation site found in this molecule. As demonstrated herein, each of the substitutions or deletion removes the O-glycosylation site from human attenuated IFNα2b and reduces the heterogeneity of the molecule as measured in IEF gels, when expressed by CHO cells, and whilst at least substantially maintaining the activity of the attenuated IFNα2b to bind cell surface IFN receptors and to initiate downstream signaling.

[0054] In additional embodiments the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by an attenuating mutation selected from the group consisting of L15A,

R22A, R23A, S25A, L26A, F27A, L30A, L30V, K31A, D32A, R33A, R33K, R33Q, H34A, Q40A, D114R, L117A, R120A, R120E, R125A, R125E, K131A, E132A, K133A, K134A, M148A, R149A, S152A, L153A, N156A, (L30A, H57Y, E58N and Q61S), (M148A, H57Y, E58N and Q61S), (L153A, H57Y, E58N and Q61S), (R144A, H57Y, E58N and Q61S), (N65A, L80A, Y85A and Y89A,) (N65A, L80A, Y85A, Y89A and D114A), (N65A, L80A, Y85A, Y89A and L117A), (N65A, L80A, Y85A, Y89A and R120A), (Y85A, Y89A and D114A), (D114A and R120A), (L117A and R120A), (L117A, R120A and K121A), (R120A and K121A), (R120E and K121E), replacement of R at position 144 with A, D, E, G, H, I, K, L, N, Q, S, T, V or Y, replacement of A at position 145 with D, E, G, H, I, K, L, M, N, Q, S, T, V or Y, and deletion of residues L161 to E165.

[0055] In additional embodiments the sequence of the aglycosylated IFNα2b is SEQ ID NO: 2 modified by an attenuating mutation selected from the group consisting of L15A, R22A, R23A, S25A, L26A, F27A, L30A, L30V, K31A, D32A, R33A, R33K, R33Q, H34A, Q40A, D113R, L116A, R119A, R119E, R124A, R124E, K130A, E131A, K132A, K133A, M147A, R148A, S149A, L152A, N155A, (L30A, H57Y, E58N and Q61S), (M147A, H57Y, E58N and Q61S), (L152A, H57Y, E58N and Q61S), (R143A, H57Y, E58N and Q61S), (N65A, L80A, Y85A and Y89A,) (N65A, L80A, Y85A, Y89A and D113A), (N65A, L80A, Y85A, Y89A and L116A), (N65A, L80A, Y85A, Y89A and R1190A), (Y85A, Y89A and D113A), (D113A and R119A), (L116A and R119A), (L116A, R119A and K120A), (R119A and K120A), (R119E and K120E), replacement of R at position 143 with A, D, E, G, H, I, K, L, N, Q, S, T, V or Y, replacement of A at position 144 with D, E, G, H, I, K, L, M, N, Q, S, T, V or Y, and deletion of residues L160 to E164.

[0056] In another embodiment the sequence of the aglycosylated IFNα2b modified by an attenuating mutation is selected from the group consisting of SEQ ID NOs: 3 to 30 and SEQ ID NOs: 32 to 47.

[0057] In another embodiment the cell surface-associated antigen is selected from the group consisting of CD38, CD138, RANK-Ligand, HM1.24, CD56, CS1, CD20, CD74, IL-6R, Blys (BAFF), BCMA, HLA-SR, HLA-DR, Kininogen, beta2 microglobulin, FGFR3, ICAM-1, matriptase, CD52, EGFR, GM2, alpha4-integrin, IFG-1R, KIR, CD3,

CD4, CD8, CD24, CD44, CD69, CD71, CD79, CD83, CD86, CD96, HLA, PD-1, ICOS, CD33, CD115, CD11c, CD19, CD52, CD14, FSP1, FAP, PDGFR alpha, PDGFR beta, ASGR1, ASGR2, FSP1, RTI140/Ti-alpha, HTI56, VEGF receptor, CD241 the product of the RCHE gene, CD117 (c-kit), CD71 (transferrin receptor), CD36 (thrombospondin receptor), CD34, CD45RO, CD45RA, CD115, CD168, CD235, CD236, CD237, CD238, CD239 and CD240.

[0058] In certain embodiments the polypeptide ligand is an antibody or antigen binding portion thereof.

[0059] In another embodiment the polypeptide ligand is an antibody which binds CD38. It is preferred that the V_H sequence of the antibody is selected from the group consisting of SEQ ID Nos: 48 to 56 and 58 and that the V_L sequence of the antibody is selected from the group consisting of SEQ ID Nos: 81, 82 and 84.

[0060] In another embodiment the polypeptide ligand is an antibody which binds CD138. It is preferred that the V_H sequence of the antibody is SEQ ID NO: 59 and that the V_L sequence of the antibody is SEQ ID NO: 85.

[0061] In another embodiment the polypeptide ligand binds RANK-Ligand. It is preferred that the sequence of the polypeptide ligand is SEQ ID NO: 57.

[0062] In another embodiment the first domain is linked to the second domain via a peptide bond. The first domain may be linked to the second domain directly by a peptide bond (a "zero-length linker") or via a peptide linker of from 1 to 20 amino acids in length. The linker may be $(SGGGGS)_n$ where n is 1 to 3. Examples of linkers include SGGGGS and SGGGGSGGGGSGGGGS.

[0063] In another embodiment the C-terminus of the first domain is linked to N-terminus of the second domain.

[0064] In another embodiment the amino acid sequence of the first domain is glycosylated.

[0065] In another aspect the present invention provides a fusion polypeptide comprising a sequence selected from the group consisting of SEQ ID NOs: 31, 61 to 77, 83 and 87, and a sequence selected from the group consisting of SEQ ID NOs: 81, 82 and 84.

[0066] In another aspect the present invention provides a fusion polypeptide comprising SEQ ID NO: 87 and SEQ ID NO: 81.

[0067] In another aspect the present invention provides a fusion polypeptide comprising SEQ ID NO: 79 and SEQ ID NO: 85.

[0068] In another aspect the present invention provides a fusion polypeptide comprising SEQ ID NO: 80 and SEQ ID NO: 86.

[0069] In another aspect the present invention provides a fusion polypeptide comprising SEQ ID NO: 78.

[0070] As will be understood from the discussion above particular forms of the fusion polypeptide of the current invention are as follows:

a. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is A.

b. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is C.

c. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is D.

d. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is E.

e. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is F.

f. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is G.

g. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is H.

h. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is I.

i. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is K.

j. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is L.

k. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is M.

l. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is N.

m. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is P.

n. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is Q.

o. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is R.

p. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is V.

q. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is W.

r. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is Y.

s. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation L15A.

t. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation A19W.

u. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation R22A.

v. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation R23A.

w. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation S25A.

x. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation L26A.

y. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation F27A.

z. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation L30A or L30V.

aa. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation K31A.

bb. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation D32A.

cc. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation R33A, R33K or R33Q.

dd. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation H34A.

ee. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutation Q40A.

ff. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation D114R or SEQ ID NO: 2 modified by the attenuating mutation D113R.

gg. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation L117A or SEQ ID NO: 2 modified by the attenuating mutation L116A.

hh. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation R120A or R120E or SEQ ID NO: 2 modified by the attenuating mutation R119A or R119E.

ii. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation R125A or R125E or SEQ ID NO: 2 modified by the attenuating mutation R124A or R124E.

jj. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation K131A or SEQ ID NO: 2 modified by the attenuating mutation K130A.

kk. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation E132A or SEQ ID NO: 2 modified by the attenuating mutation E131A.

ll. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation K133A or SEQ ID NO: 2 modified by the attenuating mutation K132A.

mm. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation K134A or SEQ ID NO: 2 modified by the attenuating mutation K133A.

nn. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation M148A or SEQ ID NO: 2 modified by the attenuating mutation M147A.

oo. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation R149A or SEQ ID NO: 2 modified by the attenuating mutation R148A.

pp. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation S152A or SEQ ID NO: 2 modified by the attenuating mutation S151A.

qq. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation L153A or SEQ ID NO: 2 modified by the attenuating mutation L152A.

rr. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutation N156A or SEQ ID NO: 2 modified by the attenuating mutation N155A.

ss. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutations L30A, H57Y, E58N and Q61S.

tt. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutations M148A, H57Y, E58N and Q61S or SEQ ID NO: 2 modified by the attenuating mutations M147A, H57Y, E58N and Q61S.

uu. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutations L153A, H57Y, E58N and Q61S or SEQ ID NO: 2 modified by the attenuating mutations L152A, H57Y, E58N and Q61S.

vv. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutations R144A, H57Y, E58N and Q61S or SEQ ID NO: 2 modified by the attenuating mutations R143A, H57Y, E58N and Q61S.

ww. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 or SEQ ID NO: 2 modified by the attenuating mutations N65A, L80A, Y85A and Y89A.

xx. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutations N65A, L80A, Y85A, Y89A and D114A or SEQ ID NO: 2 modified by the attenuating mutations N65A, L80A, Y85A, Y89A and D113A.

yy. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutations N65A, L80A, Y85A, Y89A and L117A or SEQ ID NO: 2 modified by the attenuating mutations N65A, L80A, Y85A, Y89A and L116A.

zz. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutations N65A, L80A, Y85A, Y89A and R120A or SEQ ID NO: 2 modified by the attenuating mutations N65A, L80A, Y85A, Y89A and R119A.

aaa. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutations Y85A, Y89A and D114A or SEQ ID NO: 2 modified by the attenuating mutations Y85A, Y89A and D113A.

bbb. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutations D114A and R120A or SEQ ID NO: 2 modified by the attenuating mutations D113A and R119A.

ccc. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating mutations L117A and R120A or SEQ ID NO: 2 modified by the attenuating mutations L116A and R119A.

ddd. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the mutations L117A, R120A and K121A or SEQ ID NO: 2 modified by the mutations L116A, R119A and K120A.

eee. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the mutations R120A and K121A or SEQ ID NO: 2 modified by the mutations R119A and K120A.

fff. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the mutations R120E and K121E or SEQ ID NO: 2 modified by the mutations R119E and K120E.

ggg. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b SEQ ID NO: 1 is modified by replacement of R at position 144 with A, D, E, G, H, I, K, L, N, Q, S, T, V or Y or SEQ ID NO: 2 is modified by replacement of R at position 143 with A, D, E, G, H, I, K, L, N, Q, S, T, V or Y.

hhh. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by replacement of A at position 145 with D, E, G, H, I, K, L, M, N, Q, S, T, V or Y or SEQ ID NO: 2 modified by replacement of A at position 144 with D, E, G, H, I, K, L, M, N, Q, S, T, V or Y.

iii. The fusion polypeptide in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by deletion of residues L161 to E165 or SEQ ID NO: 2 modified by deletion of residues L161 to E165.

jjj. The fusion polypeptide as claimed in claim 1 in which the sequence of the aglycosylated IFNα2b is selected from the group consisting of SEQ ID NOs: 3 to 30 and SEQ ID NOs: 32 to 47.

[0071] The term "antibody", as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art, non-limiting embodiments of which are discussed below.

[0072] In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or V_H) 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 V_L) and a light chain constant region. The light chain constant region is comprised of one domain, CL, which in humans may be of either the κ or λ class. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FRl, CDRl, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass.

[0073] The term "antigen binding domain" or “antigen binding portion” of an antibody, as used herein, refers to one or more fragments of an antibody or protein that retain the ability to specifically bind to an antigen (e.g., CD38). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats, specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH1

domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments in addition to a portion of the hinge region, linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the V_H and CH1 domains; (iv) an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody , (v) a domain antibody (dAb) (Ward *et al.* 1989 Nature 341 544-6, Winter *et al.*, PCT publication WO 90/05144 Al), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird *et al.* 1988 Science 242 423-6; Huston *et al.* 1988 Proc Natl Acad Sci U S A 85 5879-83). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies, are also encompassed. Diabodies are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., *et al.*, 1994, Structure 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering 2001 Springer-Verlag. New York. 790 pp., ISBN 3-540-41354-5). In an embodiment the antibody binding portion is a Fab fragment.

[0074] The antibody described herein may be a humanized antibody. The term "humanized antibody" shall be understood to refer to a protein comprising a human-like variable region, which includes CDRs from an antibody from a non-human species (e.g., mouse or rat or non-human primate) grafted onto or inserted into FRs from a human antibody (this type of antibody is also referred to a "CDR-grafted antibody"). Humanized antibodies also include proteins in which one or more residues of the human protein are modified by one or more amino acid substitutions and/or one or more FR 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-

Date Reçue/Date Received 2022-01-26

human antibody. Any additional regions of the protein (e.g., Fc region) are generally human. Humanization can be performed using a method known in the art, e.g., US5,225,539, US6,054,297, US7,566,771 or US5,585,089. The term "humanized antibody" also encompasses a super-humanized antibody, e.g., as described in US7,732,578.

[0075] The antibody described herein may be human. The term "human antibody" as used herein refers to proteins having variable and, optionally, constant antibody regions found in humans, e.g. in the human germline or somatic cells or from libraries produced using such regions. The "human" antibodies can include amino acid residues not encoded by human sequences, e.g. mutations introduced by random or site directed mutations in vitro (in particular mutations which involve conservative substitutions or mutations in a small number of residues of the protein, e.g. in 1, 2, 3, 4 or 5 of the residues of the protein). These "human antibodies" do not necessarily need to be generated as a result of an immune response of a human, rather, they can be generated using recombinant means (e.g., screening a phage display library) and/or by a transgenic animal (e.g., a mouse) comprising nucleic acid encoding human antibody constant and/or variable regions and/or using guided selection (e.g., as described in or US5,565,332). This term also encompasses affinity matured forms of such antibodies. For the purposes of the present disclosure, a human protein will also be considered to include a protein comprising FRs from a human antibody or FRs comprising sequences from a consensus sequence of human FRs and in which one or more of the CDRs are random or semi-random, e.g., as described in US6,300,064 and/or US6,248,516.

[0076] The antibody portions of polypeptides of the present invention may be full length antibodies of any class, preferably 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, be of human origin or be humanized. Antibody fragments may also be used in place of the full length antibodies.

[0077] The term "antibody" also includes engineered antibodies. As will be appreciated there are many variations of engineered antibodies (e.g. mouse monoclonal, chimeric, humanized and human monoclonal antibodies, single chain variable antibody

fragments (scFv's), minibodies, aptamers, as well as bispecific antibodies and diabodies as described above).

[0078] Single variable region domains (termed dAbs) are, for example, disclosed in (Ward et al., 1989, Nature 341: 544-546; Hamers-Casterman et al., 1993, Nature 363: 446-448; Davies & Riechmann, 1994, FEBS Lett. 339: 285-290).

[0079] Minibodies are small versions of whole antibodies, which encode in a single chain the essential elements of a whole antibody. Suitably, the minibody is comprised of the VH and VL domains of a native antibody fused to the hinge region and CH3 domain of the immunoglobulin molecule as, for example, disclosed in U.S. Patent No 5,837,821.

[0080] In an alternate embodiment, the engineered antibody may comprise non-immunoglobulin derived, protein frameworks. For example, reference may be made to (Ku & Schutz, 1995, Proc. Natl. Acad. Sci. USA 92: 6552-6556) which discloses a four-helix bundle protein cytochrome b562 having two loops randomized to create CDRs, which have been selected for antigen binding.

[0081] There is a plethora of non-antibody recognition protein or protein domain scaffolds that may be utilised as the antigen binding domains in the constructs of this invention. These include scaffolds based on cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (Evibody; US7,166,697); human transferrin (Trans-body); a three-helix bundle from the Z-domain of Protein A (Affibody); a monomeric or trimeric human C-type lectin domain (Tetranectin); the tenth human fibronectin type III domain (AdNectin); the Kunitz-type domain of human or bovine trypsin inhibitor; insect Defensin A (IICA29), APPI (Kuntiz domains); lipocalins, FABP, Bilin-binding protein, Apoloproptein D (Anticalins); human α-crystallin or ubiquitin molecule (Affilin); trypsin inhibitor II (Microbody); α2p8 or Ankyrin repeat (repeat-motif proteins), Charybdotoxin (Scorpion toxins), Min-23, Cellulose binding domain (Knottins); Neocarzinostatin, CBM4-2 and Tendamistat.

[0082] Further, in addition to scaffolds provided for by antibody-derived domains or non-antibody folds as described above, there are naturally occurring ligand binding proteins or protein domains that may be utilised as the ligand binding domains in this invention. For example, protein domains that possess ligand binding properties include

extracellular domains of receptors, PDZ modules of signaling proteins, such as Ras-binding protein AF-6, adhesion molecules, and enzymes.

[0083] Using methods well known in the art to increase binding, by for example, affinity maturation, or to decrease immunogenicity by removing predicted MHC class II-binding motifs. The therapeutic utility of the antibodies described herein can 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.

[0084] An example of glyco-engineering used the Potelligent® method as described in Shinkawa T. et al., 2003 (J Biol Chem 278: 3466-73).

[0085] Numerous methods for affinity maturation of antibodies are known in the art. Many of these are based on the general strategy of generating panels or libraries of variant proteins by mutagenesis followed by selection and/or screening for improved affinity. Mutagenesis is often performed at the DNA level, for example by error prone PCR (Thie, Voedisch et al. 2009, Methods Mol Biol 525: 309-322), by gene shuffling (Kolkman and Stemmer 2001, Nat Biotechnol. May; 19(5):423-8), by use of mutagenic chemicals or irradiation, by use of 'mutator' strains with error prone replication machinery (Greener 1996, In Vitro Mutagenesis Protocols. Humana press, NJ) or by somatic hypermutation approaches that harness natural affinity maturation machinery (Peled, Kuang et al. 2008, Annu Rev Immunol. 26:481-511). Mutagenesis can also be performed at the RNA level, for example by use of Qβ replicase (Kopsidas, Roberts et al. 2006, Immunol Lett. 2006 Nov 15; 107(2):163-8). Library-based methods allowing screening for improved variant proteins can be based on various display technologies such as phage, yeast, ribosome, bacterial or mammalian cells, and are well known in the art (Benhar 2007, Expert Opin Biol Ther. May; 7(5): 763-79). Affinity maturation can be achieved by more directed/predictive methods for example by site-directed mutagenesis or gene synthesis

guided by findings from 3D protein modeling (see for example Queen, Schneider et al. 1989, PNAS, 86(24): 10029-33 or US patent 6,180,370 or US patent 5,225,539).

[0086] Methods of increasing ADCC have been described by Ferrara, Brunker et al. 2006, Biotechnol Bioeng; 93:851-61; Li, Sethuraman et al. 2006, Nat Biotechnol; 24:210-5; Stavenhagen, Gorlatov et al. 2007, Cancer Res; 67:8882-90; Shields, Namenuk et al. 2001, J Biol Chem; 276:6591-604; Shinkawa, Nakamura et al. 2003, J Biol Chem; 278:3466-73; and WO 2008/006554.

[0087] Methods of increasing CDC have been described by Idusogie, Wong et al. 2001, J Immunol; 176:346-56; Dall'Acqua, Cook et al. 2006, J Biol Chem; 281:23514-24; Michaelsen, Aase et al. 1990, Scand J Immunol; 32:517-28; Brekke, Bremnes et al. 1993, Mol Immunol; 30:1419-25; Tan, Shopes et al. 1990, PNAS; 87:162-6; and Norderhaug, Brekke et al. 1991, Eur J Immunol; 21:2379-84.

[0088] References describing methods of increasing ADCC and CDC include Natsume, In et al. 2008, Cancer Res; 68:3863-72. The disclosure of each of these references is included herein by cross reference. In certain embodiments it may be advantageous to reduce or eliminate ADCC and CDC activities of the antibody component of the polypeptide of the invention, so that the IFNα2b activity is the principal activity of the polypeptide which modulates target cell survival.

[0089] A number of methods for modulating antibody serum half-life and biodistribution are based on modifying the interaction between antibody and the neonatal Fc receptor (FcRn), a receptor with a key role in protecting IgG from catabolism, and maintaining high serum antibody concentration. Dall'Acqua et al describe substitutions in the Fc region of IgG1 that enhance binding affinity to FcRn, thereby increasing serum half-life (Dall'Acqua, Woods et al. 2002, J Immunol; 169:5171-80) and further demonstrate enhanced bioavailability and modulation of ADCC activity with triple substitution of M252Y/S254T/T256E (with residue numbering according to the EU Index) or M265Y/S267T/T269 (with residue numbering according to the Kabat numbering system) (Dall'Acqua, Kiener et al. 2006, J Biol Chem; 279:6213-6). See also U.S Pat. Nos 6,277,375; 6,821,505; and 7,083,784. Hinton et al have described constant domain amino

acid substitutions at positions 250 and 428 that confer increased *in vivo* half-life (Hinton, Johlfs et al. 2004, J Biol Chem; 279:6213-6; Hinton, Xiong et al. 2006, J Immunol; 176:346-56). See also U.S Pat. No 7,217,797. Petkova et al have described constant domain amino acid substitutions at positions 307, 380 and 434 that confer increased in vivo half-life (Petkova, Akilesh et al. 2006, Int Immunol; 18:1759-69). See also Shields et al 2001, J Biol Chem; 276:6591-604 and WO 2000/42072. Other 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 Pat. Application Nos 20090142340; 20090068175 and 20090092599. The substitution referred to herein as "S228P" which is numbered according to the EU index as in Kabat has also been referred to as "S241P" according to Kabat et al. (1987 Sequences of proteins of immunological interest. United States Department of Health and Human Services, Washington DC.). This substitution stabilizes the hinge region of IgG4 molecules, having the effect of making the sequence of the core of the hinge region the same as that of an IgGl or IgG2 isotype antibody. This results in a reduction in the spontaneous dissociation and reassociation of the heavy chains which often leads to the production of heterodimeric IgG4 antibodies.

[0090] 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 (Kaneko, Nimmerjahn et al. 2006, Science; 313:670-3; Jones, Papac et al. 2007, Glcobiology; 17:529-40; and Kanda, Yamada et al. 2007, Glycobiology; 17:104-18). Hence, certain glycoforms that modulate desired antibody activities can confer therapeutic advantage. Methods for generating engineered glycoforms are known in the art and include but are not limited to those described in U.S. Pat. Nos US6,602,684; US7,326,681; US7,388,081 and in WO 2008/006554.

[0091] Extension of half-life by addition of polyethylene glycol (PEG) has been widely used to extend the serum half-life of proteins, as reviewed, for example, by Fishburn 2008, J Pharm Sci; 97:4167-83.

[0092] As will be recognised it is possible to make conservative amino acid substitutions within the sequences of the current invention. By "conservative substitution"

is meant amino acids having similar properties. As used in this specification the following groups of amino acids are to be seen as conservative substitutions: H, R and K; D, E, N and Q; V, I and L; C and M; S, T, P, A and G; and F, Y and W. It is not intended, however, that substitutions other than those specifically recited are made at the sites of attenuation and/or glycosylation.

[0093] The term "cell surface-associated antigen", as used herein, broadly refers to any antigen expressed on surfaces of cells, including without limitation malignant cells or infectious or foreign cells.

[0094] In certain aspects of the present invention, the fusion polypeptide constructs or compositions of the present invention may be used to treat patients with cancer. Cancers contemplated herein include: a group of diseases and disorders that are characterized by uncontrolled cellular growth (e.g. formation of tumor) without any differentiation of those cells into specialized and different cells. Such diseases and disorders include ABL1 protooncogene, AIDS related cancers, acoustic neuroma, acute lymphocytic leukaemia, acute myeloid leukaemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, 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, gynaecological cancers, hematological malignancies, hairy cell leukaemia, 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, oesophageal 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.

[0095] As contemplated for the treatment of cancer, the antibody portions of the fusion constructs of the present invention may bind to tumour-associated antigens, *i.e.*, cell surface antigens that are selectively expressed by cancer cells or over-expressed in cancer cells relative to most normal cells. There are many tumour-associated antigens (TAAs) known in the art. Non-limiting examples of TAAs include enzyme tyrosinase; melanoma antigen GM2; alphafetoprotein (AFP); carcinoembryonic antigen (CEA); Mucin 1 (MUC1); Human epidermal growth factor receptor (Her2/Neu); T-cell leukemia/lymphoma 1 (TCL1) oncoprotein. Exemplary TAAs associated with a number of different cancers are telomerase (hTERT); prostate–specific membrane antigen (PSMA); urokinase plasminogen activator and its receptor (uPA/uPAR); vascular

endothelial growth factor and its receptor (VEGF/VEGFR); extracellular matrix metalloproteinase inducer (EMMPRIN/CD147); epidermal growth factor (EGFR); platelet-derived growth factor and its receptor (PDGF/PDGFR) and c-kit (CD117).

[0096] A list of other TAAs is provided in US 2010/0297076, the disclosure of which is included herein by reference. Of particular interest are cell surface antigens associated with multiple myeloma leukemia or lymphoma cells, including but not limited to CD38, CD138, CD79, CS1, and HM1.24. In one embodiment an antigen for ligand-attenuated IFN constructs, for example, an antibody-attenuated interferon construct, is CD38.

[0097] 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 (Bergsagel, P., Blood; 85:436, 1995 and Liu, Q., Structure, 13:1331, 2005). 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 (Malavasi, F., Hum. Immunol. 9:9, 1984). In lymphocytes, however, the expression appears to be dependent on the differentiation and activation state of the cell. Resting T and B cells are negative while immature and activated lymphocytes are predominantly positive for CD38 expression (Funaro, A., J. Immunol. 145:2390, 1990). Additional studies indicate mRNA expression in non-hemopoeitic organs such as pancreas, brain, spleen and liver (Koguma, T., Biochim. Biophys. Acta 1223:160, 1994.)

[0098] 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 (reviewed in Deaglio, S., Trends in Mol. Med. 14:210, 2008.)

[0099] CD38 is expressed at high levels on multiple myeloma cells, in most cases of T- and B-lineage acute lymphoblastic leukemias, some acute myelocytic leukemias, follicular center cell lymphomas and T lymphoblastic lymphomas. (Malavasi, F., J. Clin Lab Res. 22:73, 1992). More recently, CD38 expression has become a reliable prognostic marker in B-lineage chronic lymphoblastic leukemia (B-CLL) (Ibrahim, S., Blood. 98:181, 2001 and Durig, J., Leuk. Res. 25:927, 2002). Independent groups have demonstrated that B-CLL patients presenting with a $CD38^+$ clone are characterized by an unfavorable clinical course with a more advance stage of disease, poor responsiveness to chemotherapy and shorter survival time (Morabito, F., Haematologica. 87:217,2002). The consistent and enhanced expression of CD38 on lymphoid tumors makes this an attractive target for therapeutic antibody technologies.

[0100] Preferred antigens for the development of antibody-attenuated aglycosylated IFNα2b fusion protein constructs which target cancer are antigens which show selective or greater expression on the cancer cells than on most other, non-transformed cells within the body. Non-protein examples of such antigens include, sphingolipids, ganglioside GD2 (Saleh *et al.*, 1993, J. Immunol., 151, 3390-3398), ganglioside GD3 (Shitara *et al.*, 1993, Cancer Immunol. Immunother. 36:373-380), ganglioside GM2 (Livingston *et al.*, 1994, J. Clin. Oncol. 12:1036-1044), ganglioside GM3 (Hoon *et al.*, 1993, Cancer Res. 53:5244-5250) and $Lewis^x$, $lewis^y$ and $lewis^{xy}$ carbohydrate antigens that can be displayed on proteins or glycolipids. Examples of protein antigens are HER-2/neu, human papillomavirus-E6 or -E7, MUC-1; KS 1/4 pan-carcinoma antigen (Perez and Walker, 1990, J. Immunol. 142:3662-3667; Bumal, 1988, Hybridoma 7(4):407-415); ovarian carcinoma antigen CA125 (Yu *et al.*, 1991, Cancer Res. 51(2):468-475); prostatic acid phosphate (Tailor *et al.*, 1990, Nucl. Acids Res. 18(16):4928); prostate specific antigen (Henttu and Vihko, 1989, Biochem. Biophys. Res. Comm. 160(2):903-910; Israeli *et al.*, 1993, Cancer Res. 53:227-230); melanoma-associated antigen p97 (Estin *et al.*, 1989, J. Natl. Cancer Instit. 81(6):445-446); melanoma antigen gp75 (Vijayasardahl *et al.*, 1990, J. Exp. Med. 171(4):1375-1380); prostate specific membrane antigen; carcinoembryonic antigen (CEA) (Foon *et al.*, 1994, Proc. Am. Soc. Clin. Oncol. 13:294), MUC16 (antibodies include MJ-170, MJ-171, MJ-172 and MJ-173 [US 7,202,346],3A5 [US 7,723,485]).NMB (US 8,039,593), malignant human lymphocyte antigen-APO-1

(Bernhard *et al.*, 1989, Science 245:301-304); high molecular weight melanoma antigen (HMW-MAA) (Natali *et al.*, 1987, Cancer 59:55-63; Mittelman *et al.*, 1990, J. Clin. Invest. 86:2136-2144); Burkitt's lymphoma antigen-38.13; CD19 (Ghetie *et al.*, 1994, Blood 83:1329-1336); human B-lymphoma antigen-CD20 (Reff *et al.*, 1994, Blood 83:435-445); GICA 19-9 (Herlyn *et al.*, 1982, J. Clin. Immunol. 2:135), CTA-1 and LEA; CD33 (Sgouros *et al.*, 1993, J. Nucl. Med. 34:422-430); oncofetal antigens such as alpha-fetoprotein for liver cancer or bladder tumor oncofetal antigen (Hellstrom *et al.*, 1985, Cancer. Res. 45:2210-2188); differentiation antigens such as human lung carcinoma antigen L6 or L20 (Hellstrom *et al.*, 1986, Cancer Res. 46:3917-3923); antigens of fibrosarcoma; human leukemia T cell antigen-Gp37 (Bhattacharya-Chatterjee *et al.*, 1988, J. Immunol. 141:1398-1403); tumor-specific transplantation type of cell-surface antigen (TSTA) such as virally-induced tumor antigens including T-antigen, DNA tumor virus and envelope antigens of RNA tumor viruses; neoglycoproteins, breast cancer antigens such as EGFR (Epidermal growth factor receptor), polymorphic epithelial mucin (PEM) (Hilkens *et al.*, 1992, Trends in Bio. Chem. Sci. 17:359); polymorphic epithelial mucin antigen; human milk fat globule antigen; colorectal tumor-associated antigens such as TAG-72 (Yokata *et al.*, 1992, Cancer Res. 52:3402-3408), CO 17-1A (Ragnhammar *et al.*, 1993, Int. J. Cancer 53:751-758); differentiation antigens (Feizi, 1985, Nature 314:53-57) such as I(Ma) found in gastric adenocarcinomas, SSEA-1 found in myeloid cells, VEP8, VEP9, Myl, VIM-D5, M18 and M39 found in breast epithelial cancers, D_{156-22} found in colorectal cancer, TRA-1-85 (blood group H), C14 found in colonic adenocarcinoma, F3 found in lung adenocarcinoma, AH6 found in gastric cancer, Y hapten found in embryonal carcinoma cells, TL5 (blood group A), E1 series (blood group B) antigens found in pancreatic cancer, FC10.2 found in embryonal carcinoma cells, gastric adenocarcinoma antigen, CO-514 (blood group Le^a) found in adenocarcinoma, NS-10 found in adenocarcinomas, CO-43 (blood group Le^b), G49 found in A431 cells, 19.9 found in colon cancer; gastric cancer mucins; R_{24} found in melanoma, MH2 (blood group ALe^b/Le^y) found in colonic adenocarcinoma, 4.2, D1.1, OFA-1, G_{M2}, OFA-2 and M1:22:25:8 found in embryonal carcinoma cells and SSEA-3 and SSEA-4. HMW-MAA (SEQ ID NO:433), also known as melanoma chondroitin sulfate proteoglycan, is a membrane-bound protein of 2322 residues which is overexpressed on over 90% of the surgically removed benign

nevi and melanoma lesions (Camploi, et. al, Crit Rev Immunol.;24:267,2004). Accordingly it may be a potential target cell surface associated antigen.

[0101] Other example cancer antigens for targeting with fusion protein constructs of the present invention include (exemplary cancers are shown in parentheses): CD5 (T-cell leukemia/lymphoma), CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), CA 242 (colorectal), placental alkaline phosphatase (carcinomas), prostatic acid phosphatase (prostate), MAGE-1 (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE -4 (carcinomas), transferrin receptor (carcinomas), p97 (melanoma), MUC1 (breast cancer), MART1 (melanoma), CD20 (non Hodgkin's lymphoma), CD52 (leukemia), CD33 (leukemia), human chorionic gonadotropin (carcinoma), CD38 (multiple myeloma), CD21 (B-cell lymphoma), CD22 (lymphoma), CD25 (B-cell Lymphoma), CD37 (B-cell lymphoma), CD45 (acute myeloblastic leukemia), HLA-DR (B-cell lymphoma), IL-2 receptor (T-cell leukemia and lymphomas), CD40 (lymphoma), CD79 (B cell leukemia or lymphoma, Hodgkin lymphoma), various mucins (carcinomas), P21 (carcinomas), MPG (melanoma), Ep-CAM (Epithelial Tumors), Folate-receptor alpha (Ovarian), A33 (Colorectal), G250 (renal), Ferritin (Hodgkin lymphoma), de2-7 EGFR (glioblastoma, breast, and lung), Fibroblast activation protein (epithelial) and tenascin metalloproteinases (glioblastoma). Some specific, useful antibodies include, but are not limited to, BR64 (Trail *et al.*, 1997, Cancer Research 57:100 105), BR96 mAb (Trail *et al.*, 1993, Science 261:212-215), mAbs against the CD40 antigen, such as S2C6 mAb (Francisco *et al.*, 2000, Cancer Res. 60:3225-3231) or other anti-CD40 antibodies, such as those disclosed in U.S Patent Publication Nos. 2003-0211100 and 2002-0142358; mAbs against the CD30 antigen, such as AC10 (Bowen *et al.*, 1993, J. Immunol. 151:5896-5906; Wahl *et al.*, 2002 Cancer Res. 62(13):3736-42) or MDX-0060 (U.S. Patent Publication No. 2004-0006215) and mAbs against the CD70 antigen, such as 1F6 mAb and 2F2 mAb (see, e.g., U.S. Patent Publication No. 2006-0083736) or antibodies 2H5, 10B4, 8B5, 18E7, 69A7 (US 8,124,738). Other antibodies have been reviewed elsewhere (Franke *et al.*, 2000, Cancer Biother. Radiopharm. 15:459 76; Murray, 2000, Semin. Oncol. 27:64 70; Breitling, F., and Dubel, S., Recombinant Antibodies, John Wiley, and Sons, New York, 1998).

[0102] In certain embodiments, useful antibodies can bind to a receptor or a complex of receptors expressed on a target cell. The receptor or receptor complex can comprise an

immunoglobulin gene superfamily member, a major histocompatibility protein, a cytokine receptor, a TNF receptor superfamily member, a chemokine receptor, an integrin, a lectin, a complement control protein, a growth factor receptor, a hormone receptor or a neurotransmitter receptor. Non-limiting examples of appropriate immunoglobulin superfamily members are CD2, CD3, CD4, CD8, CD19, CD22, CD79, CD90, CD152/CTLA-4, PD-1, B7-H4, B7-H3, and ICOS. Non-limiting examples of suitable TNF receptor superfamily members are TACI, BCMA, CD27, CD40, CD95/Fas, CD134/0X40, CD137/4-1BB, TNFR1, TNFR2, RANK, osteoprotegerin, APO 3, Apo2/TRAIL R1, TRAIL R2, TRAIL R3, and TRAIL R4. Non-limiting examples of suitable integrins are CD11a, CD11b, CD11c, CD18, CD29, CD41, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD103 and CD104. Non-limiting examples of suitable lectins are S type, C type, and I type lectin. Examples of antibodies to CEA are shown in Table 1.

Table 1

CEA Antibodies			
Ab Clones	**Patent**	**Assignee**	**Comments**
COL-1	US 6,417,337	The Dow Chemical Company	Humanized
806.077	US 6,903,203	AstraZeneca UK Ltd.	Humanized
T84.66	US 7,776,330	City of Hope	Humanized

[0103] Antibodies that bind the CD22 antigen expressed on human B cells include, for example, HD6, RFB4, UV22-2, To15, 4KB128 and a humanized anti-CD22 antibody (hLL2) (see, e.g., Li *et al.* (1989) Cell. Immunol. 111: 85-99; Mason *et al.* (1987) Blood

69: 836-40; Behr *et al.* (1999) Clin. Cancer Res. 5: 3304s-3314s; Bonardi *et al.* (1993) Cancer Res. 53: 3015-3021).

[0104] Antibodies to CD33 include, for example, HuM195 (see, e.g., Kossman *et al.* (1999) Clin. Cancer Res. 5: 2748-2755; US5693761) and CMA-676 (see, e.g., Sievers *et al.*, (1999) Blood 93: 3678-3684).

[0105] Illustrative anti-MUC-1 antibodies include, but are not limited to Mc5 (see, e.g., Peterson *et al.* (1997) Cancer Res. 57: 1103-1108; Ozzello *et al.* (1993) Breast Cancer Res. Treat. 25: 265-276), and hCTMO1 (see, e.g., Van Hof *et al.* (1996) Cancer Res. 56: 5179-5185).

[0106] Illustrative anti-TAG-72 antibodies include, but are not limited to CC49 (see, e.g., Pavlinkova *et al.* (1999) Clin. Cancer Res. 5: 2613-2619), B72.3 (see, e.g., Divgi *et al.* (1994) Nucl. Med. Biol. 21: 9-15), and those disclosed in U.S. Pat. No. 5,976,531.

[0107] Illustrative anti-HM1.24 antibodies include, but are not limited to a mouse monoclonal anti-HM1.24 and a humanized anti-HM1.24 IgG1kappa antibody (see, e.g., Ono *et al.* (1999) Mol. Immuno. 36: 387-395).

[0108] In certain embodiments the targeting moiety comprises an anti-Her2 antibody. The erBB 2 gene, more commonly known as (Her-2/neu), is an oncogene encoding a transmembrane receptor. Several antibodies have been developed against Her-2/neu, and some of these are in clinical use. These include trastuzumab (e.g., HERCEPTIN™; Fornir *et al.* (1999) Oncology (Huntingt) 13: 647-58), TAB-250 (Rosenblum *et al.* (1999) Clin. Cancer Res. 5: 865-874), BACH-250 (Id.), TA1 (Maier *et al.* (1991) Cancer Res. 51: 5361-5369), and the mAbs described in U.S. Pat. Nos. 5,772,997; 5,770,195 (mAb 4D5; ATCC CRL 10463); and U.S. Pat. No. 5,677,171.

[0109] Other fully human anti-Her2/neu antibodies are well known to those of skill in the art. Such antibodies include, but are not limited to the C6 antibodies such as C6.5, DPL5, G98A, C6MH3-B1, B1D2, C6VLB, C6VLD, C6VLE, C6VLF, C6MH3-D7, C6MH3-D6, C6MH3-D5, C6MH3-D3, C6MH3-D2, C6MH3-D1, C6MH3-C4, C6MH3-C3, C6MH3-B9, C6MH3-B5, C6MH3-B48, C6MH3-B47, C6MH3-B46, C6MH3-B43,

C6MH3-B41, C6MH3-B39, C6MH3-B34, C6MH3-B33, C6MH3-B31, C6MH3-B27, C6MH3-B25, C6MH3-B21, C6MH3-B20, C6MH3-B2, C6MH3-B16, C6MH3-B15, C6MH3-B11, C6MH3-B1, C6MH3-A3, C6MH3-A2, and C6ML3-9. These and other anti-HER2/neu antibodies are described in U.S. Pat. Nos. 6,512,097 and 5,977,322, in PCT Publication WO 97/00271, in Schier *et al.* (1996) J Mol Biol 255: 28-43, Schier *et al.* (1996) J Mol Biol 263: 551-567, and the like.

[0110] More generally, antibodies directed to various members of the epidermal growth factor receptor family are well suited for use as targeting antibodies or antigen binding portions thereof in the constructs of the present invention. Such antibodies include, but are not limited to anti-EGFR antibodies as described in U.S. Pat. Nos. 5,844,093 and 5,558,864, and in European Patent No. 706,799A. Other illustrative anti-EGFR family antibodies include, but are not limited to antibodies such as C6.5, C6ML3-9, C6MH3-B1, C6-B1D2, F5, HER3.A5, HER3.F4, HER3.H1, HER3.H3, HER3.E12, HER3.B12, EGFR.E12, EGFR.C10, EGFR.B11, EGFR.E8, HER4.B4, HER4.G4, HER4.F4, HER4.A8, HER4.B6, HER4.D4, HER4.D7, HER4.D11, HER4.D12, HER4.E3, HER4.E7, HER4.F8 and HER4.C7 and the like (see, e.g., U.S. Patent publications US 2006/0099205 A1 and US 2004/0071696 A1).

[0111] CD38 is of particular interest as an antibody target for fusion protein constructs of the present invention. Antibodies to CD38 include for example, AT13/5 (see, e.g., Ellis *et al.* (1995) J. Immunol. 155: 925-937), HB7, and the like.

[0112] The present invention also provides compositions comprising the fusion polypeptides of the present invention. These compositions can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabiliser, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but not limited to, Gennaro, Ed., Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Co. (Easton, Pa.) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the antibody composition as well known in the art or as described herein.

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[0113] Pharmaceutical excipients and additives useful in the present composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatised sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin, such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. 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, aspartame, and the like. One preferred amino acid is histidine. A second preferred amino acid is arginine.

[0114] Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides, such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

[0115] 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, phosphate buffers or amino acid buffers. Preferred buffers for use in the present compositions are organic acid salts, such as citrate or amino acids.

[0116] Additionally, the compositions of the invention can include polymeric excipients/additives, such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, 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).

[0117] These and additional known pharmaceutical excipients and/or additives suitable for use in the antibody compositions according to the invention are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy", 19 th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52 nd ed., Medical Economics, Montvale, N.J. (1998). Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

[0118] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

[0119] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any materials and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred materials and methods are now described.

EXAMPLES

General Methods

Production of antibody-fusion constructs in HEK-293E cells.

[0120] The DNA sequences of a number of the domains in the fusion polypeptides of the present are provided in the attached Sequence listing incorporated herein. DNA plasmids encoding protein constructs (antibody-attenuated IFNα2b fusion 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

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supplemented with 0.45% (w/v) D-(+)-Glucose (Sigma, Castle Hill, NSW), 25 µg/mL Geneticin (Invitrogen, Carlsbad, CA), and 1 x 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 antibody-fusion constructs in EXPI293 cells.

[0121] DNA plasmids encoding protein constructs (antibody- IFNα2b related constructs) were prepared using HiSpeed Plasmid Maxi Kit (Qiagen, Valencia, CA) and then transfected into EXPI293 cells (Life Technologies, Carlsbad, CA), grown in EXPI Expression medium (Life Technologies, Carlsbad, CA) using transfection reagent provided in the EXPI293 transfection kit and OptiMEM medium (Invitrogen, Carlsbad, CA). After allowing for expression for 3 days in an incubator supplied with 5% CO_2 and 125 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, 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 antibody-fusion constructs in CHO cells.

[0122] DNA plasmids encoding protein constructs (antibody- IFNα2b 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 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.

Method for measuring antigen-targeted activity of antibody-IFNα2b fusion protein constructs

[0123] "On target (Daudi) assay": This assay was used to quantify the anti-proliferative activity of IFNα2b and antibody-IFNα2b fusion protein constructs on cells that display both IFN receptor and the antigen targeted by the antibody to which the IFNα2b is fused. Daudi cells express both CD20 and CD38 as cell surface associated antigens, as well as cell surface IFN receptors. The viability of the Daudi 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:

[0124] 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 x 10^5 and 0.8 x 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 400g 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 x 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 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.

[0125] A mixture of the manufacturer-supplied assay buffer and assay substrate (hereafter, "CellTiterGlo 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. 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 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)".

[0126] 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). For each test article, potency relative to free IFNα2b (or some other form of IFN with a known potency relative to IFNα2b) was calculated as a ratio of EC50s.

[0127] One of ordinary skill in the art will appreciate that there are many other commonly used assays for measuring cell viability that could also be used.

[0128] "On target (ARP) assay" (also sometimes referred to herein as a "targeted assay"): 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. (Interleukin-6 prevents dexamethasone-induced myeloma cell death. Blood; 84:3063, 1994). ARP-1 cells ($CD38^+$) were used to test CD38 targeting antibody-IFN fusion protein constructs. Culture and assay conditions were the same as for Daudi-based assay outlined above, with the following exceptions:

ARP-1 was cultured to a density of 4.0 x 10^5 to 6.0 x 10^5 cells/ml. ARP-1 concentration was adjusted to 1.0 x 10^4 cells/ml prior to assay.

EXAMPLE 1

ISOELECTRIC POINTS OF ANTI-CD38 ANTIBODY ATTENUATED IFNα2b FUSION PROTEINS

[0129] Various transiently transfected cells expressing an anti-CD38 antibody-attenuated IFNα2b fusion constructs (Table 2) were harvested and purified using a Mab Select Sure Protein A column. Samples were desalted into 200mM Arginine, 25mM Histidine pH 6.5 using a HiLoad Superdex 200 column.

Table 2: Table of Constructs

Antibody Constructs	**SEQ ID No.**			
	Heavy Chain	**Light Chain**	**IFN**	**VH + IFN**
A10.21 IgG4 (S228P) IFN (A145D, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145D, T106A)	48	81	25	87, 31
A10.21 IgG4 (S228P) IFN (A145D, ΔT106)	48	81	110	61
A10.21 IgG1 IFN (A145D, T106T)	50	81	107	
A10.21 IgG1 IFN (A145D, T106A)	50	81	25	68
A10.21 IgG1 IFN (A145D, ΔT106)	50	81		83
A10.43 IgG4 (S228P) IFN (A145D, T106T)	55	81	107	
A10.43 IgG4 (S228P) IFN (A145D, T106A)	55	81	25	74

Antibody Constructs	SEQ ID No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (A145D, T106S)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106V)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106G)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106E)	48	81	25	31
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106T)	49	81	107	
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106A)	49	81	25	62
A10.21 IgG4 (S228P) IFN (R144I T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144I, T106A)	48	81	24	63
A10.21 IgG4 (S228P) IFN (A145K, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145K, T106A)	48	81	25	64
A10.21 IgG4 (S228P) IFN (A145G, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145G, T106A)	48	81	25	64
A10.21 IgG4 (S228P) IFN (R33A, T106T)	48	81	109	
A10.21 IgG4 (S228P) IFN (R33A, T106A)	48	81	13	65

Antibody Constructs	SEQ ID No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (A145Q, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145Q, T106A)	48	81	25	66
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106T)	51	81	107	
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106A)	51	81	25	69
A10.21 IgG2 IFN (A145D, T106T)	53	81	107	
A10.21 IgG2 IFN (A145D, T106A)	53	81	25	72
A10.21 IgG2 (A330S, P331S) IFN (A145D, T106T)		81	107	
A10.21 IgG2 (A330S, P331S) IFN (A145D, T106A)		81	25	
A02.12 IgG4 (S228P) IFN (A145D, T106T)	58	84	107	
A02.12 IgG4 (S228P) IFN (A145D, T106A)	58	84	25	77
Anti-CD138 IgG4 (S228P) IFN (A145D, T106T)	59	85	107	
Anti-CD138 IgG4 (S228P) IFN (A145D, T106A)	59	85	25	
Anti-HLA IgG4 (S228P) IFN (A145D, T106T)	60	86	107	
Anti-HLA IgG4 (S228P) IFN (A145D, T106A)	60	86	25	

[0130] Isoelectric focusing gels were used to determine the isoelectric point (pI) of the fusion polypeptide and to detect minor changes in the protein due to post-translational modifications such as phosphorylation and glycosylation.

[0131] Pre-cast IEF gel was setup in gel tanks ensuring a tight seal between gel and buffer. Then 200mL of 1x Cathode buffer was poured into inner chamber ensuring no buffer enters the outer chamber. 500mL of 1x Anode buffer was then poured into the outer chamber and filled ¾ of the tank. After the samples and ladder were loaded onto the gel, it was then run for 1hour at 100 volt, 1 hour at 200 volt and ½ hour at 500 volt. As soon as gel run was finished, the gel was taken out and fixed in TCA solution in a glass container for 30 min. The gel was then immediately washed with deionised water 3 times. The gel was stained in SimplyBlue SafeStain (Invitrogen Life Technologies) for a full hour, and left overnight in water to destain. The final image scanned using a scanner.

[0132] The O-linked glycosylation site of the IFNα2b portion of the antibody-attenuated interferon fusion constructs was removed by either substituting the interferon's threonine 106 (T106) to alanine (shown as T106A), serine (T106S), valine (T106V), glycine (T106G) or glutamic acid (T106E) or by deleting T106 (shown as ΔT106). The effect of these changes on pI and the heterogeneity of the fusion constructs was investigated by comparing constructs with and without O-linked glycosylation by separation on IEF gels.

[0133] In each case, deleting T106 or substituting T106 with alanine, serine, valine, glycine or glutamic acid decreased the number of observed charged species on an IEF gel, as evidenced by a reduced number of bands when directly compared to unmodified T106, and hence the heterogeneity of the fusion construct was reduced (Figure 9). The reduced number of charged species on the IEF gel and therefore the reduced heterogeneity of the molecule incorporating T106S is consistent with removal of the O-linked glycosylation at residue 106 of IFNα2b.

[0134] Removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody fusion constructs resulted in an increased pI relative to O-link glycosylated proteins. Using the same antibody front-end of A10.21, this trend was consistent regardless

of whether the isotype of the antibody was IgG4, IgG1, IgG1 AA (IgG1 L235A, G237A, an effector function reduced form of IgG1), IgG2 or IgG2 SS (IgG2 (A330S, P331S)) in the constructs (Figure 10).

[0135] YTE substitutions (M252Y, S254T, T256E) have been shown to confer increased affinity to FcRn, presumably increasing the half-life of antibodies. Further experiments examined whether substitutions in other parts of the antibody IFN fused constructs will affect the reduced heterogeneity which resulted from a T106 deletion or substitution. The heterogeneity of glycosylated A10.21 anti-CD38-attenuated IFNα2b fusion construct (YTE, T106T) and the non-glycosylated IFN fusion construct with the YTE substitutions (YTE, T106A) was assessed on IEF gels. Removal of glycosylation of the IFNα2b component of fusion constructs bearing the YTE mutations decreased heterogeneity (Figure 11).

[0136] Attenuation of IFNα2b is attained by substitutions of key amino acid residues which are responsible for binding to IFN receptors. The number of charged species of A10.21 IgG4 (S228P) IFN constructs with various attenuating amino acid substitutions in the IFNα2b, together with (T106T) or without O-linked glycosylation (T106A) of the IFNα2b component was evaluated. Individual amino acid residues Arginine-33, Arginine-144 and Alanine-145 of IFNα2b were substituted one at a time with Alanine for residue 33 (R33A), Isoleucine for residue 144 (R144I) or Lysine, Glycine or Glutamine for residue 145 (A145K, A145G, A145Q). When directly compared the aglycosylated IFN fusion constructs were consistently less heterogeneous than their glycosylated counterparts (Figure 12).

[0137] The reduction in heterogeneity exhibited was independent of the antibody portion of the construct. Removal of the O-linked glycosylation site in the attenuated IFNα2b portion of the antibody (IgG4 (S228P)) –attenuated IFN fusion constructs with specificity against HLA, CD138 and CD38 (a different epitope on CD38 to antibody A10.21 - antibody A02.12) also resulted in a decrease in heterogeneity (Figure 13) as detected by IEF.

EXAMPLE 2

ANTI-PROLIFERATIVE ACTIVITY OF ANTIBODY ATTENUATED INTERFERONα2b FUSION PROTEINS

[0138] The anti-proliferative effects of IFNα2b consist of direct and indirect activities. Direct activity occurs through cancer cell growth inhibition by cell cycle arrest (Matsui et al. 2003), apoptosis by death receptor–dependent (Crowder et al. 2005) and -independent (Otsuki et al. 1998) pathways, or differentiation (Matsui et al. 2003). Target-specific direct cytotoxicity of antibody attenuated interferon fusion proteins were measured against target positive cell lines using a luminescent cell viability assay.

Anti CD38-IFN Lead Sequence Samples

[0139] The constructs (Table 3) were either stably cloned or transiently transfected and were harvested and purified using a Mab Select Sure Protein A column. Samples were desalted into 200mM Arginine, 25mM Histidine pH 6.5 using a HiLoad Superdex 200 column.

Table 3: Table of Constructs

Antibody Constructs		**Seq Id No.**		
	Heavy Chain	**Light Chain**	**IFN**	**VH + IFN**
A10.21 IgG4 (S228P) IFN (A145D, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145D, T106A)	48	81	25	87, 31
A10.21 IgG4 (S228P) IFN (A145D, ΔT106)	48	81	110	61
A10.21 IgG4 (S228P) IFN (A145D, T106S)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106V)	48	81	25	31

Antibody Constructs		Seq Id No.		
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (A145D, T106G)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106E)	48	81	25	31
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106T)	49	81	107	
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106A)	49	81	25	62
A10.21 IgG4 (S228P) IFN (R144I T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144I, T106A)	48	81	24	63
A10.21 IgG4 (S228P) IFN (A145K, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145K, T106A)	48	81	25	64
A10.21 IgG4 (S228P) IFN (A145G, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145G, T106A)	48	81	25	64
A10.21 IgG4 (S228P) IFN (R33A, T106T)	48	81	109	
A10.21 IgG4 (S228P) IFN (R33A, T106A)	48	81	13	65
A10.21 IgG4 (S228P) IFN (A145Q, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145Q, T106A)	48	81	25	66

Antibody Constructs		Seq Id No.		
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (A145N, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145N, T106A)	48	81	25	66
A10.21 IgG4 (S228P) IFN (R144N, T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144N, T106A)	48	81	24	67
A10.21 IgG4 (S228P) IFN (R144H, T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144H, T106A)	48	81	24	67
A10.21 IgG1 IFN (A145D, T106T)	50	81	107	
A10.21 IgG1 IFN (A145D, T106A)	50	81	25	68
A10.21 IgG1 IFN (A145D, ΔT106)	50	81	110	83
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106T)	51	81	107	
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106A)	51	81	25	69
A10.21 IgG1 (L235A, G237A, M252Y, S254T, T256E) IFN (A145D, T106T)	52	81	107	
A10.21 IgG1 (L235A, G237A, M252Y, S254T, T256E) IFN (A145D, T106A)	52	81	25	
A10.21 IgG1 (M252Y, S254T, T256E) IFN (A145D, T106T)	52	81	107	
A10.21 IgG1 (M252Y, S254T, T256E) IFN (A145D, T106A)		81	25	70
A10.21 IgG2 IFN (A145D, T106T)	53	81	107	

Antibody Constructs		Seq Id No.		
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG2 IFN (A145D, T106A)	53	81	25	72
A10.21 IgG2 (A330S, P331S) IFN (A145D, T106T)		81	107	
A10.21 IgG2 (A330S, P331S) IFN (A145D, T106A)		81	25	
A10.21 IgG2 (M252Y, S254T, T256E) IFN (A145D, T106T)	54	81	107	
A10.21 IgG2 (M252Y, S254T, T256E) IFN (A145D, T106A)	54	81	25	73
A10.43 IgG4 (S228P) IFN (A145D, T106T)	55	81	107	
A10.43 IgG4 (S228P) IFN (A145D, T106A)	55	81	25	74
R10A2 IgG4 (S228P) IFN (A145D, T106T)	75	82	107	
R10A2 IgG4 (S228P) IFN (A145D, T106A)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106R)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106N)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106D)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106C)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106E)	75	82	25	

Antibody Constructs		Seq Id No.		
	Heavy Chain	Light Chain	IFN	VH + IFN
R10A2 IgG4 (S228P) IFN (A145D, T106Q)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106G)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106H)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106I)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106L)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106K)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106M)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106F)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106P)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106S)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106W)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106Y)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106V)	75	82	25	
A02.12 IgG4 (S228P) IFN (A145D, T106T)	58	84	107	

Antibody Constructs		Seq Id No.		
	Heavy Chain	Light Chain	IFN	VH + IFN
A02.12 IgG4 (S228P) IFN (A145D, T106A)	58	84	25	77
OPG-Fc (IgG2) IFN (A145D, T106T)	57		107	
OPG-Fc (IgG2) IFN (A145D, T106A)	57		25	78
Anti-CD138 IgG4 (S228P) IFN (A145D, T106T)	59	85	107	
Anti-CD138 IgG4 (S228P) IFN (A145D, T106A)	59	85	25	
Anti-HLA IgG4 (S228P) IFN (A145D, T106T)	60	86	107	
Anti-HLA IgG4 (S228P) IFN (A145D, T106A)	60	86	25	

Commercial IFNα2b

[0140] INTRON® A, a commercial bacterially produced IFNα2b from Schering-Plough, was used as a positive control.

Anti-Proliferative activity measurement

[0141] Anti-proliferative activity was measured using the methods described above "Daudi cell proliferation assay" and "ARP-1 Cell proliferation assay". The ARP-1 cell proliferation assay method was used with an additional cell line NCI-H929 for the measurement of the anti-proliferative activity of antibody-attenuated IFNα2b fusion protein. In some experiments the plates were read on a dedicated GloMax® 96 Microplate Luminometer and CellTiter-Glo® 2.0 reagents were used instead of the original CellTiter-Glo, neither of which impacted the results. The ARP-1/ NCI-H929 or Daudi cell proliferation assay was used to quantify the anti-proliferative activity of IFNs and antibody-attentuated IFNα2b fusion protein constructs on cells that display CD38. Daudi,

ARP1 and NCI-H929 cells express CD38 as a cell surface associated antigen. The details of the assays are as follows.

[0142] In the ARP-1/ NCI-H929 cell proliferation assay viability of cells was measured using the reagent CellTiter-Glo® 2.0, Cat # G9242, 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. Cells (NCI-H929 from ATCC, Manassas, VA and ARP-1, a gift from Bart Barlogie MD, PhD, Director of the Myeloma Institute at the University of Arkansas Medical Center ; Little Rock, AK) were cultured in a T75 flask (TPP, Trasadingen, Switzerland, cat# 90076) to a preferred density of between 0.5 x 10^5 and 0.8 x 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 x 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 x 10^5 cells/mL in RPMI 1640 + 10% FBS. 50µL of cell suspension was seeded into each well of a 96 well round bottom tissue culture plate (hereafter, "experimental plate") (TPP, cat# 92067). Cells were incubated at 4°C for 1 hour prior to the addition of test compounds. 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. 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 "CellTiter-Glo® reagent 2.0", was added to the experimental plate at 100 µL/well. The plate was shaken for two minutes. 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 GloMax® 96 Microplate Luminometer. Results are presented as "relative luminescence units" (RLU).

[0143] Data was analyzed using Prism 5 (Graphpad, San Diego, CA) using non-linear regression and four parameter curve fit to determine the IC50.

[0144] The O-linked glycosylation site of anti-CD38 antibody attenuated interferon fusion constructs were removed by either substituting threonine 106 (T106) to alanine (T106A), serine (T106S), valine (T106V), glycine (T106G) or glutamic acid (T106E) or deleting T106 (shown as ΔT106). The effect on cell proliferation was investigated by comparing A10.21 anti-CD38 antibody fused to attenuated IFNα2b with and without O-linked glycosylation. Removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody fusion constructs resulted in increased anti-proliferative activity as shown by lower IC50 (nM) relative to the corresponding O-linked glycosylated fusion proteins i.e. A10.21 IgG4 (S228P) IFN (A145D, T106T) and A10.21 IgG1 IFN (A145D, T106T) in both ARP1 (Figure 1A) and NCI-H929 cells (Figure 1B). This trend was consistent regardless of whether the antibody isotype was IgG4 and IgG1.

[0145] Substitution of threonine 106 (T106) to alanine (T106A), serine (T106S), valine (T106V), glycine (T106G) or glutamic acid (T106E) resulted in increased anti-proliferative activity as shown by lower IC50 (nM) relative to O-linked glycosylated fusion proteins. All non-glycosylated constructs showed higher "on-target" potency relative to their glycosylated counterpart in NCI-H929 cells (Figure 14).

[0146] The impact on anti-proliferative activity of the removal of the O-linked glycosylation site from the attenuated IFNα2b component was examined in a range of anti-CD38 antibody-attenuated IFNα2b fusion proteins. Variants of the anti-CD38 antibody-attenuated IFNα2b fusion protein R10A2 IgG4 (S228P) IFN (A145D) possessing different amino acid substitutions at T106 to remove the O-linked glycosylation site on the IFN component (T106A, T106G, T106N, T106F, T106R, T106D, T106E, T106Q, T106H, T106I, T106L, T106K, T106M, T106F, T106P, T106S, T106V, T106Y and T106W, (Table 4) were examined and the results shown in Figure 2.

[0147] While the level of potency varied between different substitutions at the T106 site, all substitutions that are predicted to result in the removal of O-linked glycosylation increased potency relative to the corresponding O-linked glycosylated protein (Figure 2).

Table 4: R10A2 Anti-CD38 antibody- attenuated IFN fusion construct variants with various amino acid substitutions at T106 for removal of O-linked glycosylation.

Construct Name	Seq Id No.		
	Heavy Chain	Light Chain	IFN
R10A2 IgG4 (S228P) IFN (A145D, T106T)	75	82	107
R10A2 IgG4 (S228P) IFN (A145D, T106A)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106R)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106N)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106D)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106C)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106E)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106Q)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106G)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106H)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106I)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106L)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106K)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106M)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106F)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106P)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106S)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106W)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106Y)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106V)	75	82	25

[0148] The relative changes in the potency between two different anti-CD38 antibody interferon fusion proteins (A02.12 and A10.21) which bind different epitopes on CD38, with or without the O-linked glycosylation (A02.12 IgG4 (S228P) IFN (A145D, T106T), A02.12 IgG4 (S228P) IFN (A145D, T106A), A10.21 IgG4 (S228P) IFN (A145D, T106T) and A10.21 IgG4 (S228P) IFN (A145D, T106A) was assessed in a similar manner.

Removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody (IgG4 (S228P)) fusion constructs with differing specificity against CD38 resulted in an increase in anti-proliferative activity regardless of the target of the antibody portion of the construct (Figure 15).

[0149] Attenuation of IFNα2b is achieved by various attenuating substitutions. The anti-proliferative activity of A10.21 IgG4 (S228P) IFN constructs with various attenuating substitutions in the IFNα2b with (T106T) and without O-linked glycosylation (T106A) (Table 5) was also evaluated. The removal of the O-linked glycosylation site in the IFNα2b component of constructs with various attenuating substitutions all had higher potency than their glycosylated counterpart (Figure 16).

Table 5: A10.21 IgG4 (S228P) IFN constructs with various attenuating substitutions in the IFNα2b with (T106T) and without O-linked glycosylation (T106A).

Construct Name	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (R144I T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144I, T106A)	48	81	24	63
A10.21 IgG4 (S228P) IFN (A145K, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145K, T106A)	48	81	25	64
A10.21 IgG4 (S228P) IFN (A145G, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145G, T106A)	48	81	25	64
A10.21 IgG4 (S228P) IFN (R33A, T106T)	48	81	109	
A10.21 IgG4 (S228P) IFN (R33A, T106A)	48	81	13	65
A10.21 IgG4 (S228P) IFN (A145Q, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145Q, T106A)	48	81	25	66
A10.21 IgG4 (S228P) IFN (A145N, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145N, T106A)	48	81	25	66
A10.21 IgG4 (S228P) IFN (R144N, T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144N, T106A)	48	81	24	67

Construct Name	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (R144H, T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144H, T106A)	48	81	24	67

[0150] To investigate whether other attenuated IFNα2b fused antibodies/proteins which have different binding targets have modulated anti-proliferative activity as a result of removal of O-linked glycosylation, 2 constructs were created (an anti-CD138 antibody and an anti-HLA antibody and fused to IFN (A145D) with or without O-linked glycosylation (Table 6) and were tested for anti-proliferative activity.

[0151] Removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody (IgG4 (S228P)) fusion constructs with specificity against HLA and CD138 resulted in an increase in anti-proliferative activity regardless of the target of the antibody portion of the construct. This was demonstrated in antibody fusion constructs against HLA and CD138 (Figure 17).

Table 6: Constructs of various IFN fusion proteins -anti-CD138 antibody and anti-HLA antibody fused to IFN (A145D) with or without O-linked glycosylation

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
Anti-CD138 IgG4 (S228P) IFN (A145D, T106T)	59	85	107	59
Anti-CD138 IgG4 (S228P) IFN (A145D, T106A)	59	85	25	59
Anti-HLA IgG4 (S228P) IFN (A145D, T106T)	60	86	107	
Anti-HLA IgG4 (S228P) IFN (A145D, T106A)	60	86	25	

Effect on anti-proliferative activity of the removal of O-linked glycosylation from fusion proteins comprising substitutions in the Fc regions for half-life extension or reduced effector function

[0152] YTE substitutions have been shown to confer increased affinity to FcRn, presumably increasing the half-life of antibodies. IFN fused antibodies containing the YTE substitutions were tested for anti-proliferative activity in the presence and absence of O-linked glycosylation. The following variants were made of the A10.21 anti-CD38 antibody attenuated IFN fusion protein (Table 7).

[0153] The introduction of the YTE substitution to A10.21 IgG4 (S228P) (A145D, T106A) did not impact the increase in potency caused by removal of the O-linked glycosylation from the attenuated IFN (Figure 18).

[0154] L235A and G237A substitutions in the Fc portion of IgG1 variants and A330S and P331S substitutions in the Fc portion of IgG2 variants results in reduced effector function. Attenuated IFN fused IgG1 antibodies containing L235A and G237A substitutions and attenuated IFN fused IgG2 antibodies containing A330S and P331S substitutions were tested for anti-proliferative activity in the presence and absence of O-linked glycosylation. These variants were made of the A10.21 anti-CD38 antibody attenuated IFN fusion protein (Table 7). Removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody fusion constructs resulted in an increase in potency regardless of whether the isotype of the antibody in the antibody constructs was IgG1 or IgG1 AA (IgG1 (L235A, G237A)), IgG2 or IgG2 SS (IgG2 (A330S, P331S)) (Figure 19).

Table 7: A10.21 fusion proteins composed of substitutions in the Fc regions for half-life extension or reduced effector function

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106T)	49	81	107	
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106A)	49	81	25	62
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106T)	51	81	107	
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106A)	51	81	25	69
A10.21 IgG1 (L235A, G237A, M252Y, S254T, T256E) IFN (A145D, T106T)	52	81	107	
A10.21 IgG1 (L235A, G237A, M252Y, S254T, T256E) IFN (A145D, T106A)	52	81	25	
A10.21 IgG1 (M252Y, S254T, T256E) IFN (A145D, T106T)	52	81	107	
A10.21 IgG1 (M252Y, S254T, T256E) IFN (A145D, T106A)	111	81	25	70
A10.21 IgG2 IFN (A145D, T106T)	53	81	107	
A10.21 IgG2 IFN (A145D, T106A)	53	81	25	72
A10.21 IgG2 (A330S, P331S) IFN (A145D, T106T)	112	81	107	
A10.21 IgG2 (A330S, P331S) IFN (A145D, T106A)	112	81	25	
A10.21 IgG2 (M252Y, S254T, T256E) IFN (A145D, T106T)	54	81	107	
A10.21 IgG2 (M252Y, S254T, T256E) IFN (A145D, T106A)	54	81	25	73

[0155] Removal of the O-linked glycosylation site from the attenuated interferon portion of the anti-CD38 antibody interferon fusion proteins by amino acid substitution or deletion of T106 resulted in an increase in anti-proliferative activity on target CD38, CD138 or HLA positive cells (1.3-12 fold).

EXAMPLE 3

ON/OFF TARGET ACTIVITY OF ANTIBODY-ATTENUATED IFNα2b FUSION PROTEINS

[0156] The iLite™ reporter gene assay was performed for the quantitative determination of Human Interferon Alpha (IFNα2b) bioactivity (IU/ml) using luciferase generated-bioluminescence. The cells used in this assay express CD38 and were used to measure the 'ON-target' activity of anti-CD38-attenuated IFNα2b fusion proteins. These cells can also be used to measure 'OFF-target' activity when CD38 is blocked with an anti-CD38 antibody recognizing the same epitope. These assays can be used to determine the Selectivity Index (SI) which is a measure of how selectively active anti-CD38 IFN fusion proteins are against CD38+ target cells and non-active on cells where CD38 is blocked (mimicking $CD38^-$ cells). The larger the SI the more selective the agents are against the target, while a number close to 1 indicates that there is no selectivity against the target or non-target. Intron A was used as a positive control as it is active against cells that express interferon alpha receptors, IFNAR1/2 but not selective against other cell surface expressed antigens (i.e. CD38) having an SI of approximately 1.

Antibody-attenuated IFN constructs

[0157] The sequences for the constructs used are set out in Sequence Listing and are listed in the following Table 8.

Table 8

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (A145D, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145D, T106A)	48	81	25	87, 31
A10.21 IgG4 (S228P) IFN (A145D, ΔT106)	48	81	110	61
A10.21 IgG4 (S228P) IFN (A145D, T106S)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106V)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106G)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106E)	48	81	25	31
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106T)	49	81	107	
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106A)	49	81	25	62
A10.21 IgG4 (S228P) IFN (R144I T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144I, T106A)	48	81	24	63
A10.21 IgG4 (S228P) IFN (A145K, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145K, T106A)	48	81	25	64
A10.21 IgG4 (S228P) IFN (A145G, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145G, T106A)	48	81	25	64
A10.21 IgG4 (S228P) IFN (R33A, T106T)	48	81	109	
A10.21 IgG4 (S228P) IFN (R33A, T106A)	48	81	13	65
A10.21 IgG4 (S228P) IFN (A145Q, T106T)	48	81	107	

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (A145Q, T106A)	48	81	25	66
A10.21 IgG4 (S228P) IFN (A145N, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145N, T106A)	48	81	25	66
A10.21 IgG4 (S228P) IFN (R144N, T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144N, T106A)	48	81	24	67
A10.21 IgG4 (S228P) IFN (R144H, T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144H, T106A)	48	81	24	67
A10.21 IgG1 IFN (A145D, T106T)	50	81	107	
A10.21 IgG1 IFN (A145D, T106A)	50	81	25	68
A10.21 IgG1 IFN (A145D, ΔT106)	50	81	110	83
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106T)	51	81	107	
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106A)	51	81	25	69
A10.21 IgG1 (L235A, G237A, M252Y, S254T, T256E) IFN (A145D, T106T)	52	81	107	
A10.21 IgG1 (L235A, G237A, M252Y, S254T, T256E) IFN (A145D, T106A)	52	81	25	
A10.21 IgG1 (M252Y, S254T, T256E) IFN (A145D, T106T)	52	81	107	
A10.21 IgG1 (M252Y, S254T, T256E) IFN (A145D, T106A)	111	81	25	70
A10.21 IgG2 IFN (A145D, T106T)	53	81	107	
A10.21 IgG2 IFN (A145D, T106A)	53	81	25	72
A10.21 IgG2 (M252Y, S254T, T256E) IFN (A145D, T106T)	54	81	107	

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG2 (M252Y, S254T, T256E) IFN (A145D, T106A)	54	81	25	73
A10.43 IgG4 (S228P) IFN (A145D, T106T)	55	81	107	
A10.43 IgG4 (S228P) IFN (A145D, T106A)	55	81	25	74
R10A2 IgG4 (S228P) IFN (A145D, T106T)	75	82	107	
R10A2 IgG4 (S228P) IFN (A145D, T106A)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106R)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106N)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106D)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106C)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106E)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106Q)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106G)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106H)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106I)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106L)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106K)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106M)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106F)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106P)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106S)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106W)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106Y)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106V)	75	82	25	
A02.12 IgG4 (S228P) IFN (A145D, T106T)	58	84	107	

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A02.12 IgG4 (S228P) IFN (A145D, T106A)	58	84	25	77
OPG-Fc (IgG2) IFN (A145D, T106T)	57		107	
OPG-Fc (IgG2) IFN (A145D, T106A)	57		25	78
Anti-CD138 IgG4 (S228P) IFN (A145D, T106T)	59	85	107	
Anti-CD138 IgG4 (S228P) IFN (A145D, T106A)	59	85	25	
Anti-HLA IgG4 (S228P) IFN (A145D, T106T)	60	86	107	
Anti-HLA IgG4 (S228P) IFN (A145D, T106A)	60	86	25	

[0158] INTRON® A was used as a positive control.

ON/OFF-target activity measurement

[0159] The ON/OFF-target activity was measured using the same method described above as "iLite gene reporter assay"

[0160] iLite reporter gene 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 expresses CD38 and 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.

[0161] The manufacturer supplied iLite plate (hereafter, assay plate) and diluent were removed from the -80° C freezer and allowed to equilibrate to room temperature. 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. 25 µL aliquots of cells were dispensed into each well of the assay plate. Next, 12.5 µL of 8 mg/mL human IgG diluted in RPMI 1640 + 10% FBS (Sigma Chemicals, St. Louis, MO; cat# I4506) 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 read on a Victor 3V Multilabel Counter on a luminometry channel and the luminescence measured and presented as RLU.

[0162] To test the off-target activity of anti-CD38 antibody-IFN fusion protein constructs in the iLite assay, manufacturer-supplied diluent was supplemented with 0.25 mg/mL anti-CD38 antibody (an antibody recognizing the same epitope on CD38 as the antibody-IFN fusion protein construct being tested, to block any binding of the anti-CD38 antibody-IFN fusion protein constructs to the CD38 expressed on the iLite cells). This blocking stage was followed by treatment with anti-CD38 antibody-IFN fusion protein or IFNα2b.

[0163] 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). Selectivity Index (SI) was calculated by EC50 (OFF-target activity)/ EC50 (ON-target activity). Selectivity Index (SI) is a measure of how selectively active anti-CD38 IFN constructs are against CD38-expressing cells and non-active in cells with no CD38. The

larger the number the more selective it is against the target, while a number close to 1 indicates that there is no selectivity against the target. Intron A was used as a positive control, has an SI of approximately 1.

[0164] The O-linked glycosylation site of anti-CD38 antibody attenuated interferon fusion constructs were removed by substituting threonine 106 (T106) to alanine (shown as T106A). The activity was investigated by comparing A10.21 and A10.43 anti-CD38 antibodies fused to IFNα2b with and without O-linked glycosylation.

[0165] Removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody fusion constructs (shown as T106A) resulted in slight increase in ON-target activity (Figure 3 and Table 9) as well as slight to no increase in the OFF-target activity relative to O-linked glycosylated proteins. Both O-linked glycosylated and non-glycosylated proteins showed high selectivity towards CD38+ cells, while Intron A did not show selectivity.

Table 9: EC50 and SI of anti-CD38 antibody attenuated IFNα2b fusion proteins A10.21 and A10.43 with and without O-linked glycosylation

	EC50 (nM)		
Test Article	**ON Target**	**OFF Target**	**SI**
Intron A	0.00017	0.00016	0.90
A10.21 IgG4 (S228P) IFN(A145D, T106T)	0.00240	2.33	972
A10.21 IgG4 (S228P) IFN (A145D, T106A)	0.00182	2.23	1223
A10.43 IgG4 (S228P) IFN (A145D, T106T)	0.00560	2.31	412
A10.43 IgG4 (S228P) IFN (A145D, T106A)	0.00124	2.25	1822

[0166] The impact on ON/OFF target activity of the removal of the O-linked glycosylation site from the attenuated IFN of fusion proteins may also be examined in a similar manner.

[0167] The iLiteTM reporter gene ON-OFF target activity assays were conducted to demonstrate the selective activity of anti-CD38 attenuated IFN fusion proteins on target CD38+ cells and the limited activity on cells when CD38 is blocked with an anti-CD38 antibody (recognizing the same epitope) mimicking the activity anticipated on target negative cells.

[0168] Removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody fusion constructs (by T106A substitution) resulted in a slight increase in ON-target activity as well as slight to no increase in the OFF-target activity relative to O-linked glycosylated proteins. Both O-linked glycosylated and non-glycosylated anti-CD38 antibody attenuated interferon fusion proteins showed high selectivity towards CD38+ cells, while Intron A did not show selectivity.

[0169] To further examine the modulation in selectivity index as a result of the removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody fusion constructs, ON-OFF target activity was examined in a range of constructs. The changes examined were based on the A10.21 IgG4 (S228P) IFN (A145D, T106T) construct. These included deletion of threonine 106 (ΔT106), substitution of T106 to serine (T106S), YTE substitution (YTE T106T and YTE T106A), IFN attenuation by substitution from alanine 145 to glutamine (A145Q) and varying antibody isotype to IgG1 (IgG1 T106T and IgG1 T106A).

[0170] The majority of constructs demonstrated a selectivity index >1, the magnitude of selectivity towards targeted CD38+ cells varied depending on the anti-CD38 IFN fused construct (Figure 20). The highest selectivity was observed in A10.21 IgG4 (S228P) IFN (A145D, T106A).

EXAMPLE 4

OFF-TARGET ACTIVITY OF ANTI-CD38 ANTIBODY-ATTENUATED IFNα2b FUSION PROTEINS

[0171] HEK-Blue™ IFN-α/β cells allow the detection of bioactive human type I IFNs by monitoring the activation of the ISGF3 pathway. Stimulation of HEK-Blue™ IFN-α/β

cells with human IFN-α activates the JAK/STAT/ISGF3 pathway and subsequently induces the production of SEAP (a reporter gene under the control of the IFN-α/β inducible ISG54 promoter). Levels of SEAP in the supernatant can be easily determined with QUANTI-Blue™. The effects of removal of O-linked glycosylation from the attenuated interferon portion of the antibody interferon fusion proteins were evaluated using this reporter gene assay.

Antibody/Fc fusion protein construct production

[0172] Various transiently transfected cells expressing interferon fusion proteins were harvested and purified using a Mab Select Sure Protein A column. Samples were desalted into 200mM Arginine, 25mM Histidine pH 6.5 using a HiLoad Superdex 200 column.

Table 10

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (A145D, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145D, T106A)	48	81	25	87, 31
A10.21 IgG4 (S228P) IFN (A145D, ΔT106)	48	81	110	61
A10.21 IgG4 (S228P) IFN (A145D, T106S)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106V)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106G)	48	81	25	31
A10.21 IgG4 (S228P) IFN (A145D, T106E)	48	81	25	31
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106T)	49	81	107	
A10.21 IgG4 (S228P, M252Y, S254T, T256E) IFN (A145D, T106A)	49	81	25	62
A10.21 IgG4 (S228P) IFN (R144I T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144I, T106A)	48	81	24	63
A10.21 IgG4 (S228P) IFN (A145K, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145K, T106A)	48	81	25	64

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG4 (S228P) IFN (A145G, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145G, T106A)	48	81	25	64
A10.21 IgG4 (S228P) IFN (R33A, T106T)	48	81	109	
A10.21 IgG4 (S228P) IFN (R33A, T106A)	48	81	13	65
A10.21 IgG4 (S228P) IFN (A145Q, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145Q, T106A)	48	81	25	66
A10.21 IgG4 (S228P) IFN (A145N, T106T)	48	81	107	
A10.21 IgG4 (S228P) IFN (A145N, T106A)	48	81	25	66
A10.21 IgG4 (S228P) IFN (R144N, T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144N, T106A)	48	81	24	67
A10.21 IgG4 (S228P) IFN (R144H, T106T)	48	81	108	
A10.21 IgG4 (S228P) IFN (R144H, T106A)	48	81	24	67
A10.21 IgG1 IFN (A145D, T106T)	50	81	107	
A10.21 IgG1 IFN (A145D, T106A)	50	81	25	68
A10.21 IgG1 IFN (A145D, ΔT106)	50	81	110	83
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106T)	51	81	107	
A10.21 IgG1 (L235A, G237A) IFN (A145D, T106A)	51	81	25	69
A10.21 IgG1 (L235A, G237A, M252Y, S254T, T256E) IFN (A145D, T106T)	52	81	107	
A10.21 IgG1 (L235A, G237A, M252Y, S254T, T256E) IFN (A145D, T106A)	52	81	25	
A10.21 IgG1 (M252Y, S254T, T256E) IFN (A145D, T106T)	52	81	107	
A10.21 IgG1 (M252Y, S254T, T256E) IFN (A145D, T106A)		81	25	70
A10.21 IgG2 IFN (A145D, T106T)	53	81	107	

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A10.21 IgG2 IFN (A145D, T106A)	53	81	25	72
A10.21 IgG2 (M252Y, S254T, T256E) IFN (A145D, T106T)	54	81	107	
A10.21 IgG2 (M252Y, S254T, T256E) IFN (A145D, T106A)	54	81	25	73
A10.43 IgG4 (S228P) IFN (A145D, T106T)	55	81	107	
A10.43 IgG4 (S228P) IFN (A145D, T106A)	55	81	25	74
R10A2 IgG4 (S228P) IFN (A145D, T106T)	75	82	107	
R10A2 IgG4 (S228P) IFN (A145D, T106A)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106R)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106N)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106D)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106C)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106E)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106Q)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106G)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106H)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106I)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106L)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106K)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106M)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106F)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106P)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106S)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106W)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106Y)	75	82	25	
R10A2 IgG4 (S228P) IFN (A145D, T106V)	75	82	25	
A02.12 IgG4 (S228P) IFN (A145D, T106T)	58	84	107	

Antibody Constructs	Seq Id No.			
	Heavy Chain	Light Chain	IFN	VH + IFN
A02.12 IgG4 (S228P) IFN (A145D, T106A)	58	84	25	77
OPG-Fc (IgG2) IFN (A145D, T106T)	57		107	
OPG-Fc (IgG2) IFN (A145D, T106A)	57		25	78
Anti-CD138 IgG4 (S228P) IFN (A145D, T106T)	59	85	107	
Anti-CD138 IgG4 (S228P) IFN (A145D, T106A)	59	85	25	
Anti-HLA IgG4 (S228P) IFN (A145D, T106T)	60	86	107	
Anti-HLA IgG4 (S228P) IFN (A145D, T106A)	60	86	25	

[0173] INTRON® A was used as a positive control.

Off-target activity measurement by HEK-Blue assay

[0174] Off-target activity of antibody-IFN fusion was measured using the same method as described in "HEK-Blue Off-target assay" except for the volumes of cells seeded, diluted antibodies and supernatant used. The details of the assay are as follows.

[0175] HEK-Blue Off-target 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 signaling 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™.

[0176] 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-Cl). Cells were washed twice in DMEM + HI FBS and counted. Cells were adjusted to 2.77 x 10^5 viable cells/mL in DMEM + HI FBS and was seeded at180 µL per well into a flat bottom 96 well tissue culture plate (hereafter, the "experimental plate"). Then, 20µL 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 (180 µL) was seeded into each well of a flat bottom plate (hereafter, the "assay plate"). Then, 20 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 620 nm was read on a model SpectraMaxPlus 384 Microplate Reader from Molecular Devices.The data was analyzed using Graph Pad Prism.

[0177] The impact the presence or the absence of O-linked glycosylation in IFNα2b has on the off-target activity of the attenuated IFN fused anti-CD38 antibodies was evaluated.

[0178] The O-linked glycosylation site of an anti-CD38 antibody with attenuated interferon fusion constructs was removed by either substituting threonine 106 (T106) to alanine (shown as T106A) or deleting T106 (shown as ΔT106). The effect on off-target activity was investigated by comparing X10.21 anti-CD38 antibody fused to attenuated IFNα2b with and without O-linked glycosylation.

[0179] Removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody fusion constructs resulted in a slight increase in off-target activity relative to O-linked glycosylated proteins i.e. A10.21 IgG4 IFN (145D, T106T) and A10.21 IgG1 IFN

(145D, T106T) (Figure 5). This trend was consistent regardless of isotype (Figure 5). However IFNα2b (A145D) is so attenuated that the level of induction of SEAP in this assay is limited even at the highest doses used. Therefore the EC50 is an approximation only.

[0180] The off-target activity of antibodies fused with attenuated IFN (R10A2 IgG4 (S228P) IFN (A145D)) possessing different amino acid substitutions for the removal of O-linked glycosylation was examined. All possible amino acid substitutions were tested and the construct are listed in Table 11.

Table 11

Construct Name	Seq Id No.		
	Heavy Chain	Light Chain	IFN
R10A2 IgG4 (S228P) IFN (A145D, T106T)	75	82	107
R10A2 IgG4 (S228P) IFN (A145D, T106A)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106R)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106N)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106D)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106C)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106E)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106Q)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106G)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106H)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106I)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106L)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106K)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106M)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106F)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106P)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106S)	75	82	25

Construct Name	Seq Id No.		
	Heavy Chain	Light Chain	IFN
R10A2 IgG4 (S228P) IFN (A145D, T106W)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106Y)	75	82	25
R10A2 IgG4 (S228P) IFN (A145D, T106V)	75	82	25

[0181] It was shown that each of these substitutions demonstrated similar or slightly less off-target activity (increased EC_{50}) relative to the O-glycosylated IFN fused antibody R10A2 IgG4 (S228P) IFN (A145D, T106T). However, IFNα2b (A145D) is so attenuated that the level of induction of SEAP in this assay is limited even at the highest doses used. Therefore the EC_{50} is an approximation only.

[0182] The off-target activity of another anti-CD38 antibody attenuated IFNα2b construct and the impact of removal of O-linked glycosylation by the T106A substitution was investigated. A10.43 has 7 amino acid changes in the heavy chain compared to A10.21 while the two constructs share the same light chain sequence (see Sequence Listing). The results demonstrated that removal of the O-linked glycosylation site by T106A substitution slightly increased the off-target activity of A10.43. However IFNα2b (A145D) is so attenuated that the level of induction of SEAP in this assay is limited even at the highest doses used. Therefore the EC_{50} is an approximation only.

[0183] Removal of the O-linked glycosylation site in the attenuated IFN portion of the antibody interferon fusion constructs resulted in slight to no increase in the OFF-target activity as well as a slight increase in ON-target activity relative to O-linked glycosylated proteins. Both O-linked glycosylated and non-glycosylated anti-CD38 antibody attenuated interferon fusion proteins showed high selectivity towards CD38+ cells, while Intron A did not show selectivity.

EXAMPLE 5

EVALUATION OF HUMAN NEONATAL FC RECEPTOR (FCRN) BINDING TO ANTI-CD38-ATTENUATED IFN

[0184] Poly-his-tagged FcRn was immobilized onto the designated active flow cells of a CM5 sensor chip in a Biacore T200 enhanced instrument using an amine coupling protocol, while a blank immobilization was performed on the reference flow cells. Pulses of poly-his-tagged FcRn were injected over the active surfaces to ensure that the solution would pre-concentrate onto the flow cell. The surfaces were then washed with 50 mM NaOH. Both the reference and active surfaces were activated for seven minutes with a mix of 50:50 EDC/NHS. This was followed by a series of pulses of poly-his-tagged FcRn at 2 µg/mL in 10 mM Sodium Acetate pH 5.0 over the active surfaces only. Once the target of 150 RU was reached, both surfaces were deactivated with 1 M ethanolamine pH 8.5 for seven minutes. This protocol resulted in the immobilization of approximately 150 RU of poly-his-tagged FcRn onto the active surfaces. Each active surface was used for one run.

[0185] The test antibodies were desalted in PBS-P running buffer (DPBS, pH 7.4 and 0.005% Tween-20 adjusted to pH 6.0 with HCl at 25°C) using Zeba Spin Desalting Column, 7K MWCO, 0.5 mL (Pierce Product # 89882). Following desalting, the concentration of each sample was adjusted to 1 mg/mL.

[0186] On the day of the assay the 1 mg/mL solutions were further diluted in running buffer to prepare the highest concentration, and then diluted 1:2 to create the concentration series. The test samples were passed over the surface using a flow rate of 50 µL/minute. The association phase was 100 seconds while the dissociation phase was 300 seconds for all concentrations tested. The active and reference surfaces were regenerated using a 60 second injection of 100 mM Tris, 50 mM NaCl at pH 8.0 to remove the test antibody. Binding constants were determined at 25°C.

[0187] The antibody poly-his-FcRn binding interaction was evaluated using the two state reaction model where the R_{max} was set to local and RI (refractive index) parameter was set to local. All data was double reference subtracted: first, the signal in the reference

cell (blank immobilization) resulting from antibody binding to the dextran matrix was subtracted, and second, the signal of 0 nM antibody on the active surface was subtracted.

[0188] Binding constants were determined on at least two separate runs for each antibody. Prolia Lot 1035726 was tested alongside the anti-CD38/IFN samples as a positive control for each run. Average values for k_{a1}. k_{d1}, k_{a2}, k_{d2} and K_D were determined.

[0189] The average kinetic and affinity values for FcRn binding for the 4 anti-CD38/IFN (A10.21 IgG4 (S228P) IFN (A145D, T106A), A10.21 IgG4 (S228P) IFN (A145D, T106T) A10.43 IgG4 (S228P) IFN (A145D, T106A) A10.43 IgG4 (S228P) IFN (A145D, T106T)) were measured. In this sample set the clone A10.21 IgG4 (S228P) IFN (A145D, T106A) displayed the highest affinity for FcRn.

EXAMPLE 6

EFFICACY OF ANTI-CD38 ANTIBODIES FUSED TO ATTENUATED INTERFERON ALPHA 2B WITH AND WITHOUT O-LINKED GLYCOSYLATION IN THE MOUSE NCI-H929 MULTIPLE MYELOMA MODEL

Drugs and Treatment:

Table 12

Gr.	N	Regimen 1			
		Agent	µg/animal	Route	Schedule
1#	8	vehicle	20	ip	biwk x 5
2	8	A10.21 IgG4 (S228P) IFN (A145D, T106T)	20	ip	biwk x 5
3	8	A10.21 IgG4 (S228P) IFN (A145D, T106A)	20	ip	biwk x 5
4	8	A10.43 IgG4 (S228P) IFN (A145D, T106T)	20	ip	biwk x 5
5	8	A10.43 IgG4 (S228P) IFN (A145D, T106A)	20	ip	biwk x 5

- Control Group

Procedures:

- Set up CR female CB.17 SCID mice with $1x10^7$ H929 tumor cells in 50% Matrigel sc in flank.
- Cell Injection Volume is 0.2 mL/mouse.
- Age at Start Date: 8 to 12 weeks.
- Perform a pair match when tumors reach an average size of 170 - 350 mm^3, and begin treatment.
- Dosing volume = 0.2 mL/mouse. Do not adjust for body weight.
- Body Weight: qd x 5 then biwk to end
- Caliper Measurement: biwk to end
- Endpoint TGD. Animals are to be monitored individually. The endpoint of the experiment is a tumor volume of 2000 mm^3 or 60 days, whichever comes first. Responders can be followed longer. When the endpoint is reached, the animals are to be euthanized per SOP #687.

[0190] The results are shown in Figure 8. Roughly equivalent efficacy was seen with and without O-linked glycosylation, with the aglycosylated form slightly more potent in impeding tumour growth.

EXAMPLE 7

[0191] In the evaluation of human neonatal Fc receptor (FcRn) binding to anti-CD38 antibodies fused to attenuated IFN with and without O-linked glycosylation the proteins without O-linked glycosylation displayed the highest affinity for FcRn. The effect of this may be evaluated in cynomolgus monkeys and humanised FcRn mice.

Cynomolgus Monkey Study

Monkey study design- to compare +/- O-glyc

- Single 3mg/kg intravenous infusion (1 hour) through an indwelling catheter
  - A10.21 IgG4 (S228P) IFN (145D, T106A) (n=4)
  - A10.21 IgG4 (S228P) IFN (145D, T106T) (n=4)
  - A10.21 IgG4 (S228P) IFN (145D, T106A) (n=4)
  - A10.21 IgG4 (S228P) IFN (145D, T106T) (n=4)
- Compare PK and PD (biological effect i.e. serum neopterin levels)
- PK: all monkeys, ≤1 ml, 11 timepoints (Pre·dose, 0 minutes (immediately post end of infusion). 2, 6, 12, 24, 48, 96, 120, 168 and at 240 hours post infusion. Samples (80) are analysed by ELISA.
- TK modelling: WinNonlin Table Assembly {non-compartmental analysis)
- Clinical pathology: Hematology and blood chemistry- all monkeys, 3 occasions (pre-treatment, 24 hours post dose and Day 8)
- Serum neopterin· all monkeys, ~0.5ml 3 occasions at 5 timepoints (Predose, 12, 24, 96, 168 hours post dose)

Pharmacokinetic study in humanised FcRN mice

1. Thirty two (32) B6.Cg-*Fcgr*^{tm1Dcr}Tg(CAG-FCGRT)276Dcr/DcrJ (JAX stock# 004919) female mice are dosed on day 1 by IP injection of 1mg/kg of;

  - A10.21 IgG4 (S228P) IFN (145D, T106A) (n=8)
  - A10.21 IgG4 (S228P) IFN (145D, T106T) (n=8)
  - A10.43 IgG4 (S228P) IFN (145D, T106A) (n=8)

- A10.43 IgG4 (S228P) IFN (145D, T106T) (n=8)

2. Body weight is measured 3 days prior to dosing, the day of dosing and then weekly.

3. Cage side observations are made daily and clinical observations made weekly.

4. Pharmacokinetic blood collection: Mice are bled (25 μl) at pre-dose 3 days and postdose 1, 12, 24, 48 and 72 hours and 5, 7, 10, and 14 days. Mice are bled in 2 cohorts (4 mice/group/cohort).

5. All mice are sacrificed on day 14. Terminal cardiocentesis is performed to collect blood.

6. Blood is collected in lithium heparin tubes and centrifuged at 10,000 rpm for 2 min at 4°C.

7. Plasma samples are diluted 1:10 in PBS and are frozen prior to analysis by binding ELISA for A10.21 IgG4 IFN(145D) A106, A10.21 IgG4 IFN(145D) T106, A10.43 IgG4 IFN(145D) A106 or A10.43 IgG4 IFN(145D) T106.

The embodiments of the present invention for which an exclusive property or privilege is claimed are defined as follows:

1. A fusion polypeptide comprising a first and a second domain, wherein the first domain comprises an antibody, or antigen-binding fragment thereof, which binds to a cell surface-associated antigen and the second domain comprises aglycosylated interferon α 2b (IFNα2b) having a sequence of SEQ ID NO: 1, where T106 is deleted or substituted with A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W or Y, and wherein the aglycosylated IFNα2b further comprises one or more amino acid substitutions or deletions which attenuate the activity of the aglycosylated IFNα2b.

2. The fusion polypeptide as claimed in claim 1 in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 in which the residue at position 106 is A.

3. The fusion polypeptide as claimed in claim 1 or 2 in which the sequence of the aglycosylated IFNα2b is SEQ ID NO: 1 modified by the attenuating substitution(s) or deletions selected from the group consisting of L15A, R22A, R23A, S25A, L26A, F27A, L30A, L30A, L30V, K31A, D32A, R33A, R33K, R33Q, H34A, Q40A, D114R, L117A, R120A, R120E, R125A, R125E, K131A, E132A, K133A, K134A, M148A, R149A, S152A, L153A, N156A, (L30A, H57Y, E58N and Q61S), (M148A, H57Y, E58N and Q61S), (L153A, H57Y, E58N and Q61S), (R144A, H57Y, E58N and Q61S), (N65A, L80A, Y85A and Y89A), (N65A, L80A, Y85A, Y89A and D114A), (N65A, L80A, Y85A, Y89A and L117A), (N65A, L80A, Y85A, Y89A and R120A), (Y85A, Y89A and D114A), (D114A and R120A), (L117A and R120A), (L117A, R120A and K121A), (R120A and K121A), (R120E and K121E), replacement of R at position 144 with A, D, E, G, H, I, K, L, N, Q, S, T, V or Y, replacement of A at position 145 with D, E, G, H, I, K, L, M, N, Q, S, T, V or Y, and deletion of residues L161 to E165.

4. The fusion polypeptide as claimed in any one of claims 1 to 3 in which the sequence of the aglycosylated IFNα2b is selected from the group consisting of SEQ ID NOs: 3 to 30 and SEQ ID NOs: 32 to 47.

5. The fusion polypeptide as claimed in any one of claims 1 to 4 in which the cell surface-associated antigen is selected from the group consisting of CD38, CD138, RANK-Ligand, HM1.24, CD56, CS1, CD20, CD74, IL-6R, Blys (BAFF), BCMA, HLA-SR, HLA-DR,

Kininogen, beta2 microglobulin, FGFR3, ICAM-1, matriptase, CD52, EGFR, GM2, alpha4-integrin, IFG-1R, KIR, CD3, CD4, CD8, CD24, CD44, CD69, CD71, CD79, CD83, CD86, CD96, HLA, PD-1, ICOS, CD33, CD115, CD11c, CD19, CD52, CD14, FSP1, FAP, PDGFR alpha, PDGFR beta, ASGR1, ASGR2, FSP1, RTI140/Ti-alpha, HTI56, VEGF receptor, CD241 the product of the RCHE gene, CD117 (c-kit), CD71 (transferrin receptor), CD36 (thrombospondin receptor), CD34, CD45RO, CD45RA, CD115, CD168, CD235, CD236, CD237, CD238, CD239 and CD240.

6. The fusion polypeptide as claimed in any one of claims 1 to 5 in which the first domain is an antibody which binds CD38.

7. The fusion polypeptide as claimed in claim 6 in which the V_H sequence of the antibody is selected from the group consisting of SEQ ID Nos: 48 to 56 and 58.

8. The fusion polypeptide as claimed in claim 6 or claim 7 in which the V_L sequence of the antibody is selected from the group consisting of SEQ ID Nos: 81, 82 and 84.

9. The fusion polypeptide as claimed in any one of claims 1 to 8 in which the first domain is linked to the second domain via a peptide bond.

10. The fusion polypeptide as claimed in any one of claims 1 to 8 in which the first domain is linked to the second domain directly via a peptide bond.

11. The fusion polypeptide as claimed in any one of claims 1 to 8 in which the C-terminus of the first domain is linked to N-terminus of the second domain.

12. A fusion polypeptide comprising a sequence selected from the group consisting of SEQ ID Nos: 31, 61 to 77, 83 and 87, and a sequence selected from the group consisting of SEQ ID Nos: 81, 82 and 84.

13. A fusion polypeptide comprising SEQ ID NO: 87 and SEQ ID NO: 81.

14. A composition comprising the fusion polypeptide as claimed in any one of claims 1 to 13 and a pharmaceutically acceptable carrier or diluent.

15. Use of the fusion polypeptide as claimed in any one of claims 1 to 13 in the treatment of a tumour wherein the first domain of the fusion polypeptide binds to cells of the tumour.

16. The use of the fusion polypeptide as claimed in claim 15 in the treatment of cancer in which the cancer is multiple myeloma or non-Hodgkin's lymphoma.

17. An isolated polynucleotide(s) encoding the fusion polypeptide(s) as claimed in any one of claims 1 to 13.

18. A vector comprising one or more of the polynucleotide(s) of claim 17.

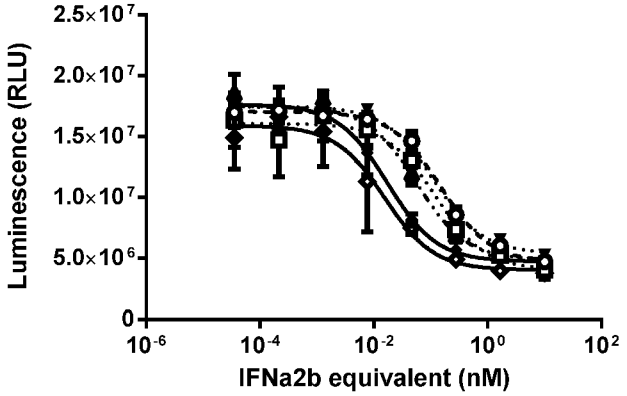
19. A transformed cell comprising the vector of claim 18.

20. A method of generating a polypeptide ligand-attenuated IFNα2b fusion polypeptide having the sequence of SEQ ID NO:1 in mammalian cells, wherein the polypeptide ligand-attenuated IFNα2b fusion polypeptide has reduced heterogeneity and/or enhanced FcRn binding and/or enhanced target selectivity, the method comprising culturing a recombinant mammalian cell comprising one or more polynucleotides encoding the polypeptide ligand-attenuated IFNα2b fusion polypeptide wherein T106 of the IFNα2b sequence is deleted or substituted with A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W or Y such that on expression the IFNα2b component of the fusion protein is aglycosylated; and wherein the polypeptide ligand is an antibody, or an antigen-binding fragment thereof.

Date Reçue/Date Received 2023-01-11

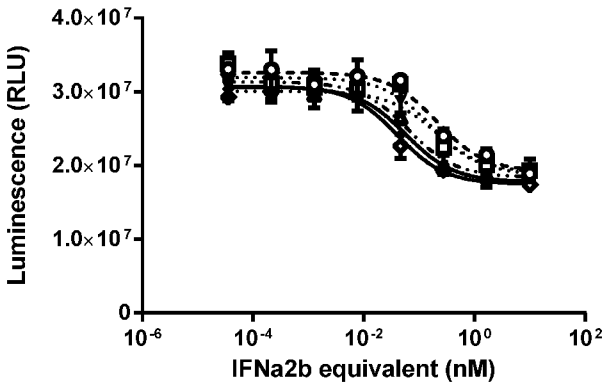
Figure 1

A.



	IC_{50} (nM)
A10.21 IgG4 (S228P) IFN (A145D, T106T)	0.15
A10.21 IgG4 (S228P) IFN (A145D, T106A)	0.12
A10.21 IgG4 (S228P) IFN (A145D, ΔT106)	0.06
A10.21 IgG1 IFN (A145D, T106T)	0.11
A10.21 IgG1 IFN (A145D, T106A)	0.02
A10.21 IgG1 IFN (A145D, ΔT106)	0.02

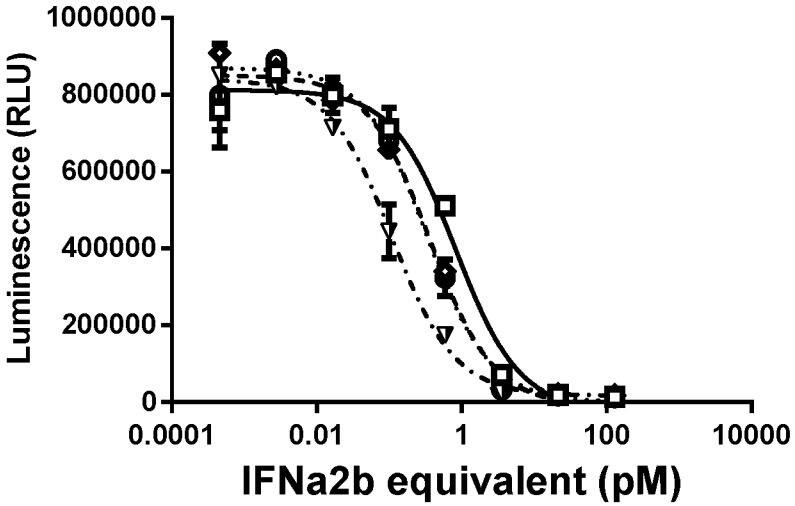
B.



	IC_{50} (nM)
A10.21 IgG4 (S228P) IFN (A145D, T106T)	0.21
A10.21 IgG4 (S228P) IFN (A145D, T106A)	0.17
A10.21 IgG4 (S228P) IFN (A145D, ΔT106)	0.06
A10.21 IgG1 IFN (A145D, T106T)	0.19
A10.21 IgG1 IFN (A145D, T106A)	0.06
A10.21 IgG1 IFN (A145D, ΔT106)	0.04

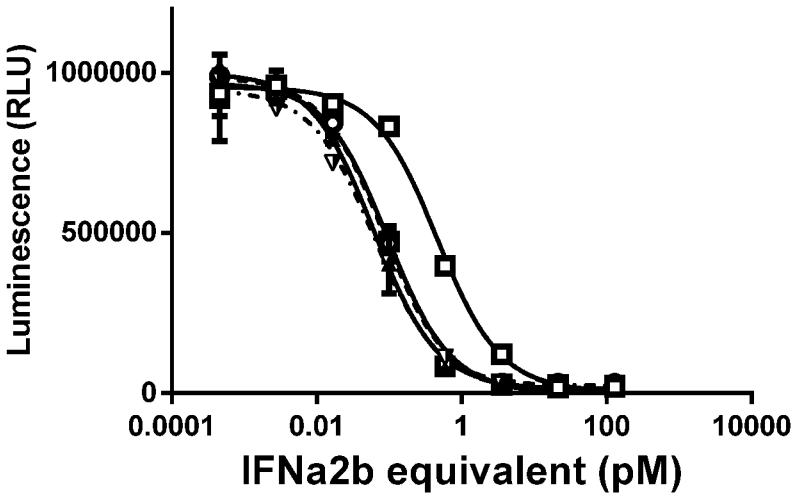
Figure 2.

Figure 2A



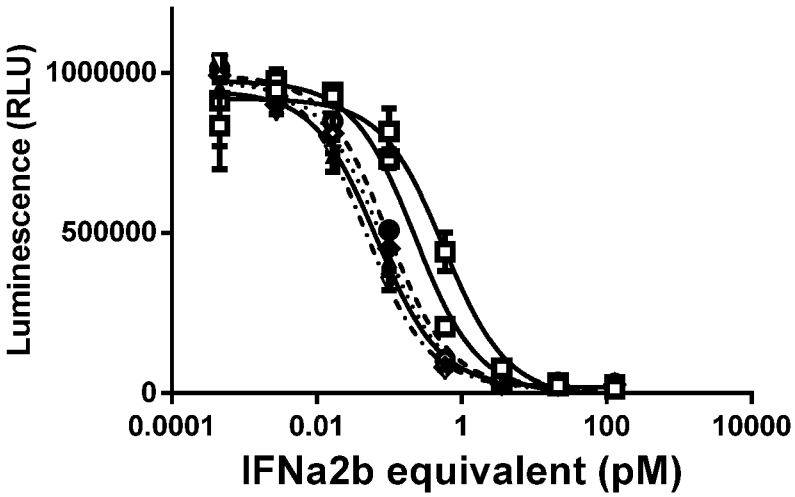
	IC_{50} (pM)
R10A2 IgG4 (S228P) IFN (A145D, T106T)	0.84
R10A2 IgG4 (S228P) IFN (A145D, T106A)	0.11
R10A2 IgG4 (S228P) IFN (A145D, T106D)	0.33
R10A2 IgG4 (S228P) IFN (A145D, T106E)	0.37

Figure 2B.



	IC_{50} (pM)
R10A2 IgG4 (S228P) IFN (A145D, T106T)	0.46
R10A2 IgG4 (S228P) IFN (A145D, T106F)	0.06
R10A2 IgG4 (S228P) IFN (A145D, T106G)	0.06
R10A2 IgG4 (S228P) IFN (A145D, T106H)	0.08
R10A2 IgG4 (S228P) IFN (A145D, T106I)	0.09

Figure 2C.



	IC_{50} (pM)
R10A2 IgG4 (S228P) IFN (A145D, T106T)	0.57
R10A2 IgG4 (S228P) IFN (A145D, T106K)	0.07
R10A2 IgG4 (S228P) IFN (A145D, T106L)	0.05
R10A2 IgG4 (S228P) IFN (A145D, T106M)	0.08
R10A2 IgG4 (S228P) IFN (A145D, T106N)	0.09
Intron A	0.22

Figure 2D.

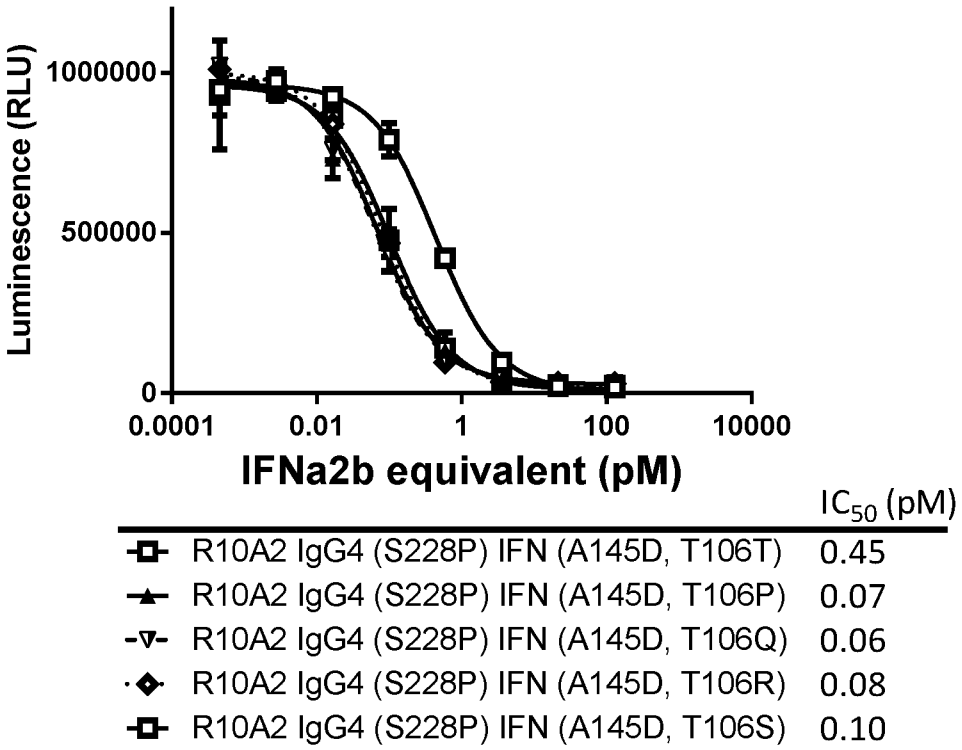
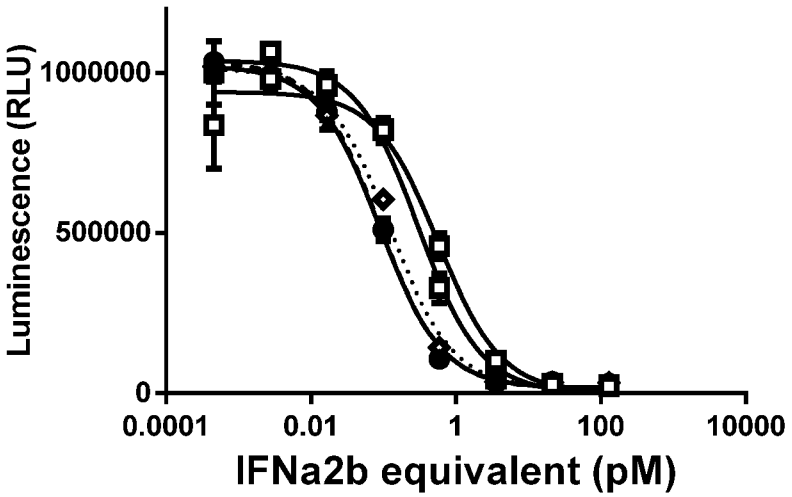

Luminescence (RLU)
1000000
500000
0
0.0001
0.01
1
100
10000
IFNa2b equivalent (pM)
IC50 (pM)
R10A2 IgG4 (S228P) IFN (A145D, T106T) 0.45
R10A2 IgG4 (S228P) IFN (A145D, T106P) 0.07
R10A2 IgG4 (S228P) IFN (A145D, T106Q) 0.06
R10A2 IgG4 (S228P) IFN (A145D, T106R) 0.08
R10A2 IgG4 (S228P) IFN (A145D, T106S) 0.10

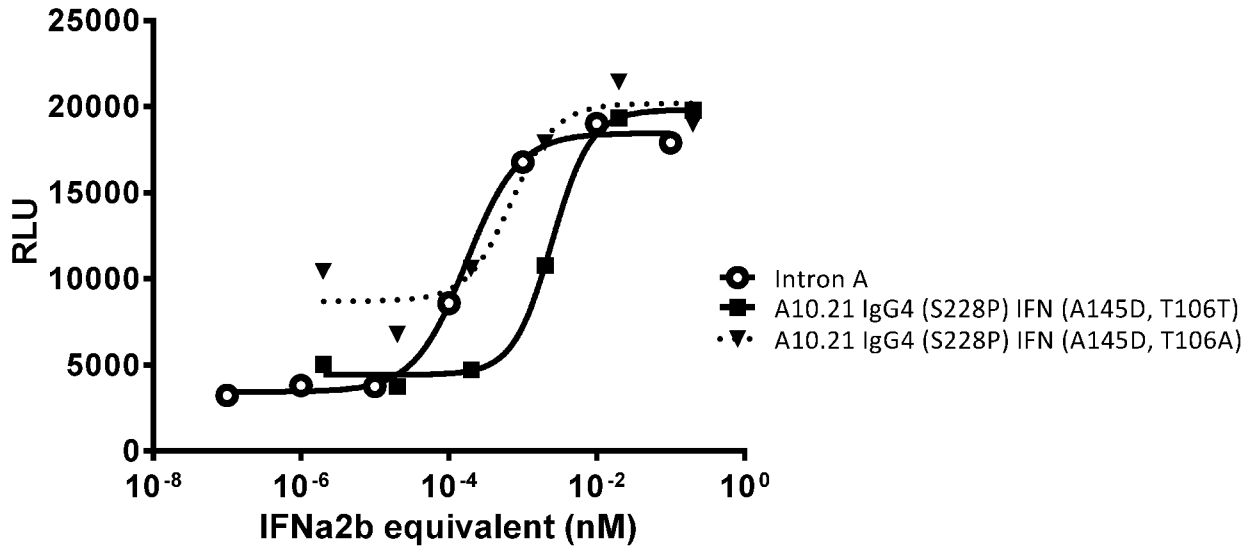
Figure 2E.



	IC_{50} (pM)
R10A2 IgG4 (S228P) IFN (A145D, T106T)	0.57
R10A2 IgG4 (S228P) IFN (A145D, T106V)	0.09
R10A2 IgG4 (S228P) IFN (A145D, T106W)	0.12
R10A2 IgG4 (S228P) IFN (A145D, T106Y)	0.09
Intron A	0.30

Figure 3

A.


25000
20000
15000
10000
5000
0
RLU
10-8
10-6
10-4
10-2
100
IFNa2b equivalent (nM)
Intron A
A10.21 IgG4 (S228P) IFN (A145D, T106T)
A10.21 IgG4 (S228P) IFN (A145D, T106A)

B.

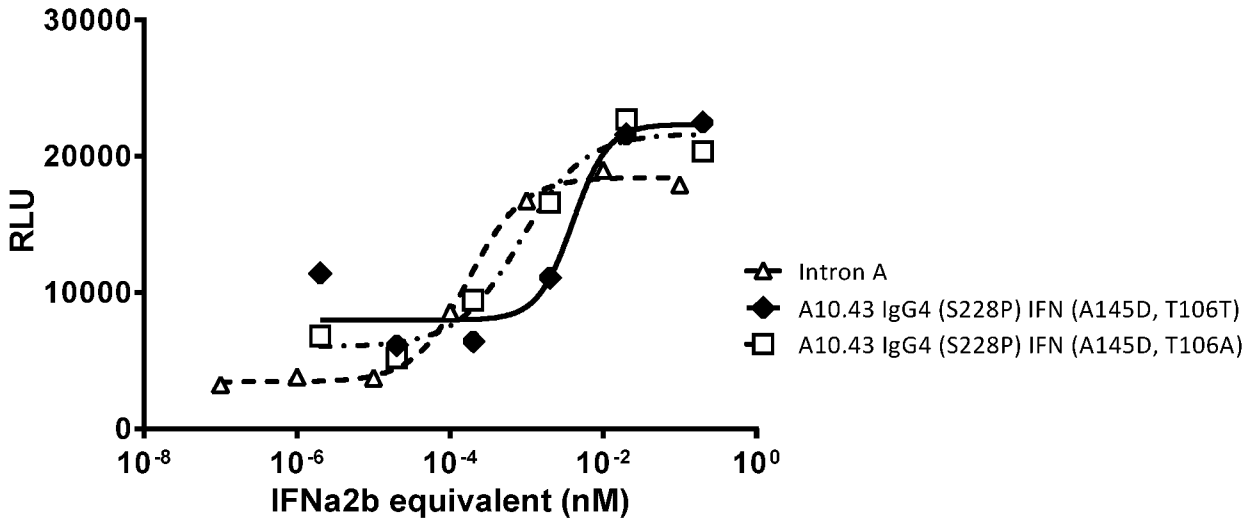
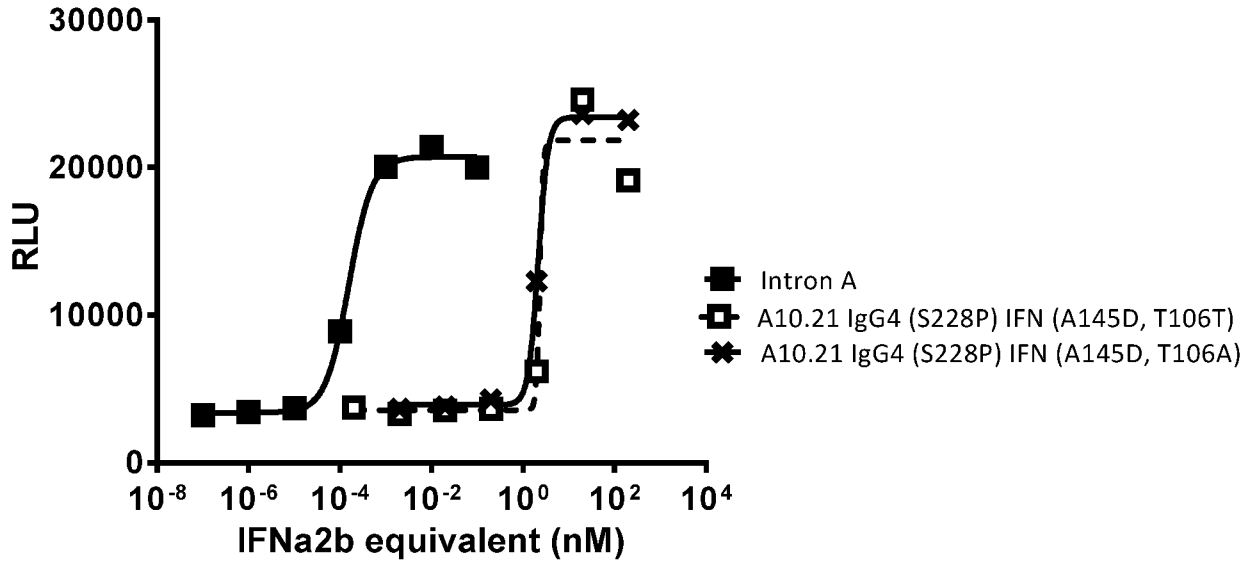

30000
20000
10000
0
RLU
10-8
10-6
10-4
10-2
100
IFNa2b equivalent (nM)
Intron A
A10.43 IgG4 (S228P) IFN (A145D, T106T)
A10.43 IgG4 (S228P) IFN (A145D, T106A)

Figure 4

A.


30000
20000
10000
0
RLU
10-8 10-6 10-4 10-2 100 102 104
IFNa2b equivalent (nM)
Intron A
A10.21 IgG4 (S228P) IFN (A145D, T106T)
A10.21 IgG4 (S228P) IFN (A145D, T106A)

B.

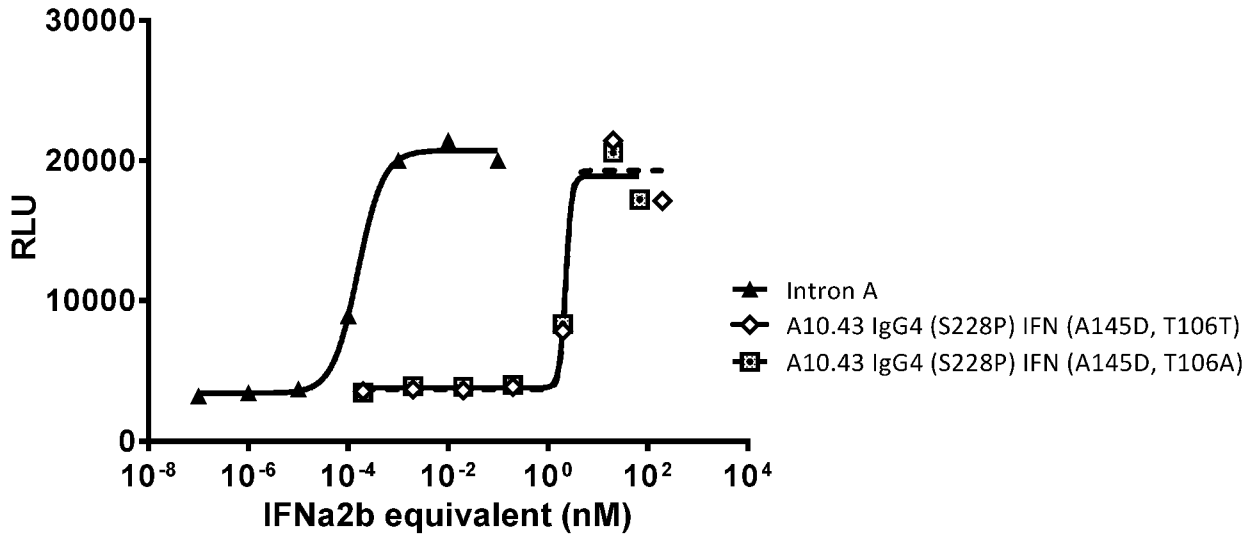
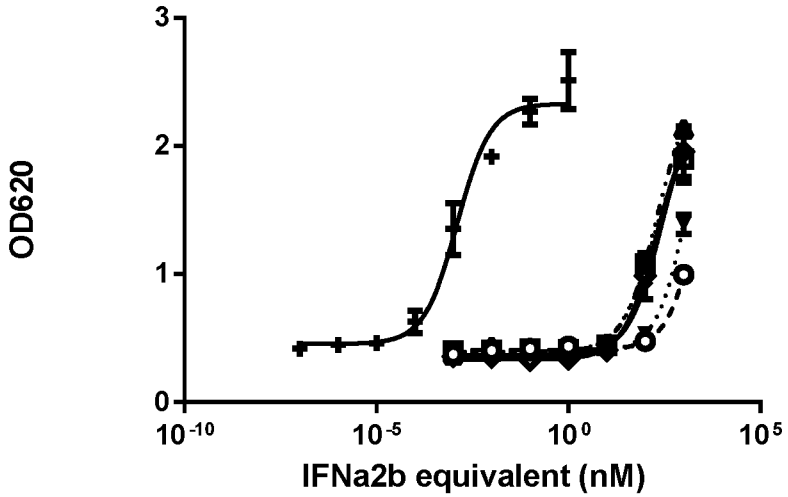

30000
20000
10000
0
RLU
10-8 10-6 10-4 10-2 100 102 104
IFNa2b equivalent (nM)
Intron A
A10.43 IgG4 (S228P) IFN (A145D, T106T)
A10.43 IgG4 (S228P) IFN (A145D, T106A)

Figure 5



	EC_{50} (nM)
A10.21 IgG4 (S228P) IFN (A145D, T106T)	>1000
A10.21 IgG4 (S228P) IFN (A145D, T106A)	160.6
A10.21 IgG4 (S228P) IFN (A145D, ΔT106)	202.8
A10.21 IgG1 IFN (A145D, T106T)	>1000
A10.21 IgG1 IFN (A145D, T106A)	274.1
A10.21 IgG1 IFN (A145D, ΔT106)	200.2
Intron A	$1.3e^{-3}$

Figure 6.

Figure 6A

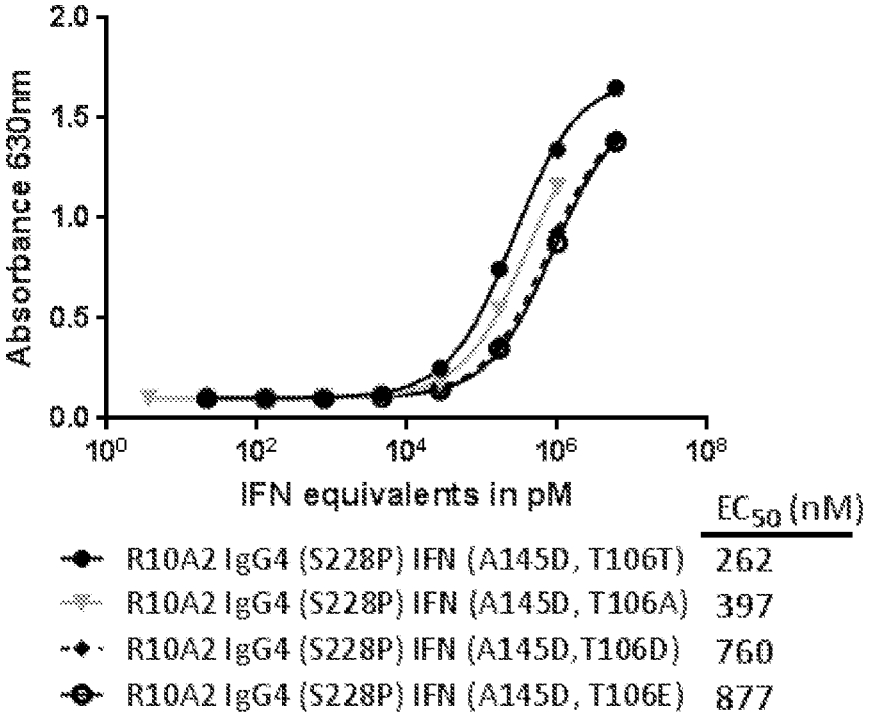


Figure 6B

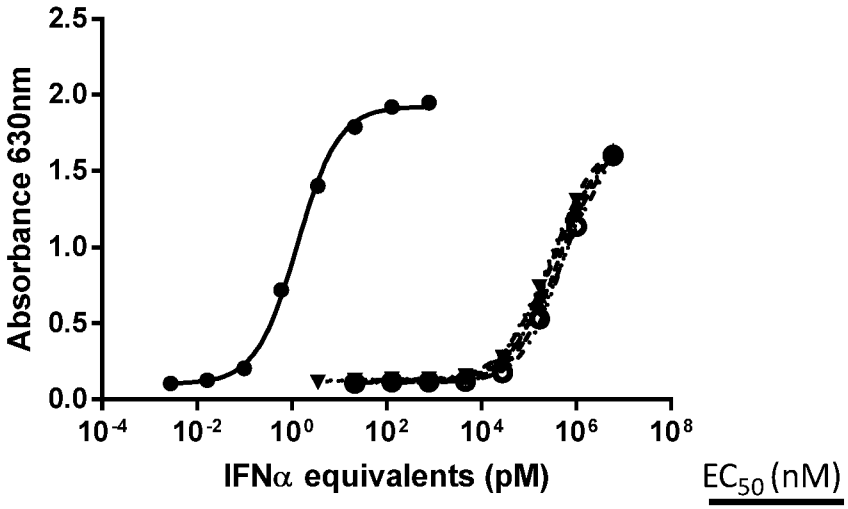

Absorbance 630nm
2.5
2.0
1.5
1.0
0.5
0.0
10^{-4} 10^{-2} 10^{0} 10^{2} 10^{4} 10^{6} 10^{8}
IFNα equivalents (pM)
EC_{50} (nM)
Intron A $1.3e^{-3}$
R10A2 IgG4 (S228P) IFN (A145D, T106S) 312
R10A2 IgG4 (S228P) IFN (A145D, T106G) 227
R10A2 IgG4 (S228P) IFN (A145D, T106V) 414
R10A2 IgG4 (S228P) IFN (A145D, T106A) 526

Figure 6C

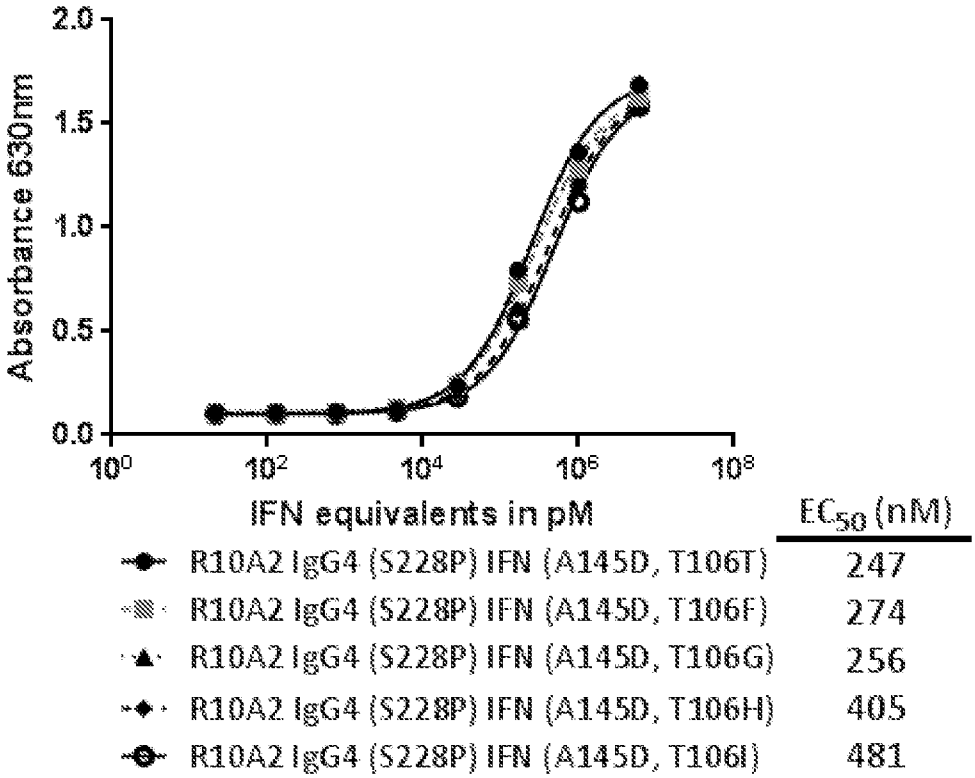
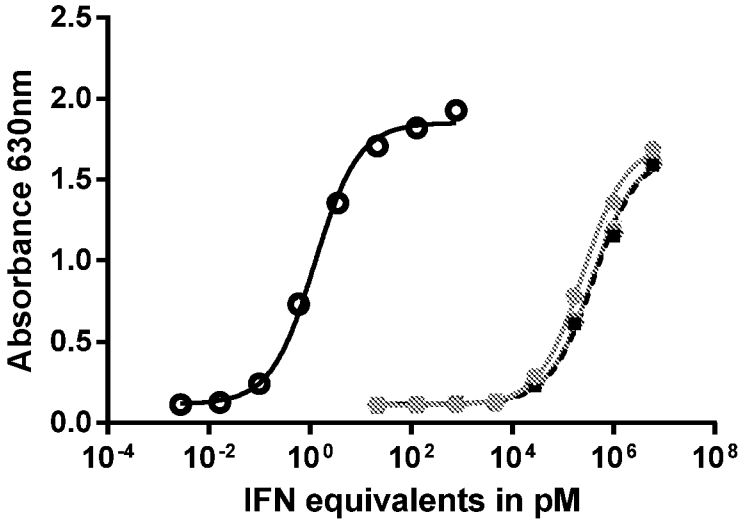
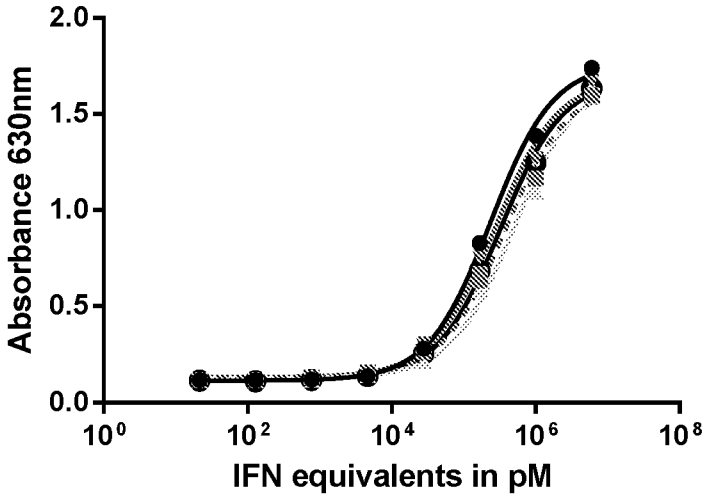

2.0
1.5
1.0
0.5
0.0
Absorbance 630nm
10^0
10^2
10^4
10^6
10^8
IFN equivalents in pM
EC_{50} (nM)
R10A2 IgG4 (S228P) IFN (A145D, T106T) 247
R10A2 IgG4 (S228P) IFN (A145D, T106F) 274
R10A2 IgG4 (S228P) IFN (A145D, T106G) 256
R10A2 IgG4 (S228P) IFN (A145D, T106H) 405
R10A2 IgG4 (S228P) IFN (A145D, T106I) 481

Figure 6D



	EC_{50} (nM)
R10A2 IgG4 (S228P) IFN (A145D, T106T)	247
R10A2 IgG4 (S228P) IFN (A145D, T106K)	409
R10A2 IgG4 (S228P) IFN (A145D, T106L)	388
R10A2 IgG4 (S228P) IFN (A145D, T106M)	365
Intron A	$1.2e^{-3}$

Figure 6E



	EC_{50} (nM)
R10A2 IgG4 (S228P) IFN (A145D, T106T)	239
R10A2 IgG4 (S228P) IFN (A145D, T106P)	372
R10A2 IgG4 (S228P) IFN (A145D, T106Q)	495
R10A2 IgG4 (S228P) IFN (A145D, T106R)	277
R10A2 IgG4 (S228P) IFN (A145D, T106S)	324

Figure 6F

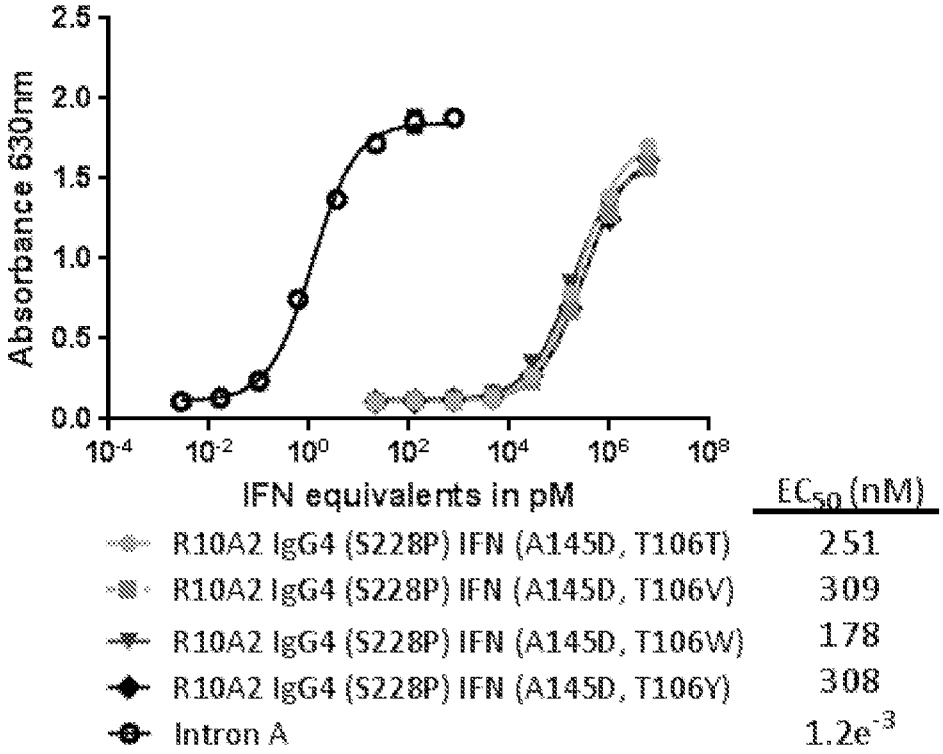
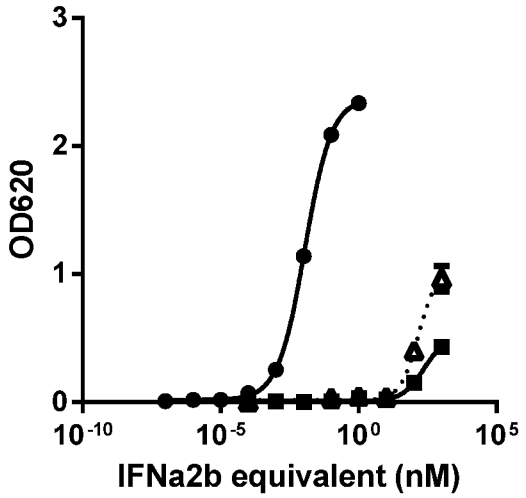

Absorbance 630nm
2.5
2.0
1.5
1.0
0.5
0.0
10-4
10-2
100
102
104
106
108
IFN equivalents in pM
EC50 (nM)
R10A2 IgG4 (S228P) IFN (A145D, T106T) 251
R10A2 IgG4 (S228P) IFN (A145D, T106V) 309
R10A2 IgG4 (S228P) IFN (A145D, T106W) 178
R10A2 IgG4 (S228P) IFN (A145D, T106Y) 308
Intron A 1.2e-3

Figure 7.



	EC_{50} (nM)
Intron A	0.01
A10.43 IgG4 (S228P) IFN (A145D, T106T)	230.7
A10.43 IgG4 (S228P) IFN (A145D, T106A)	145.2

Figure 8.

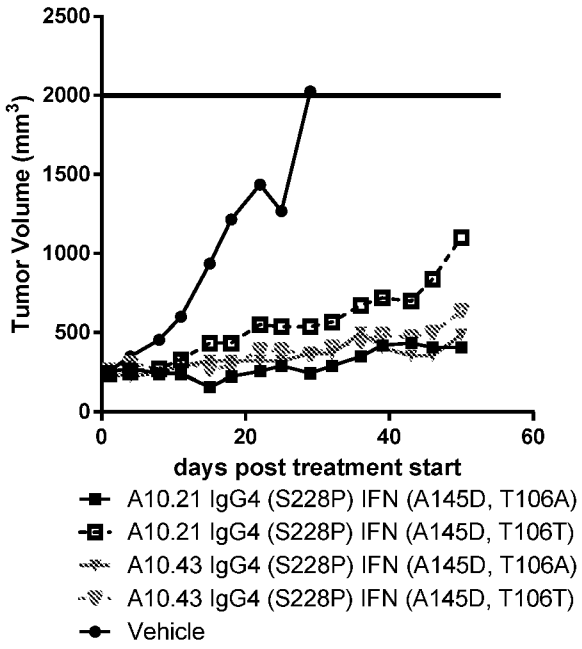

2500
2000
1500
1000
500
0
Tumor Volume (mm3)
0
20
40
60
days post treatment start
A10.21 IgG4 (S228P) IFN (A145D, T106A)
A10.21 IgG4 (S228P) IFN (A145D, T106T)
A10.43 IgG4 (S228P) IFN (A145D, T106A)
A10.43 IgG4 (S228P) IFN (A145D, T106T)
Vehicle

Figure 9.

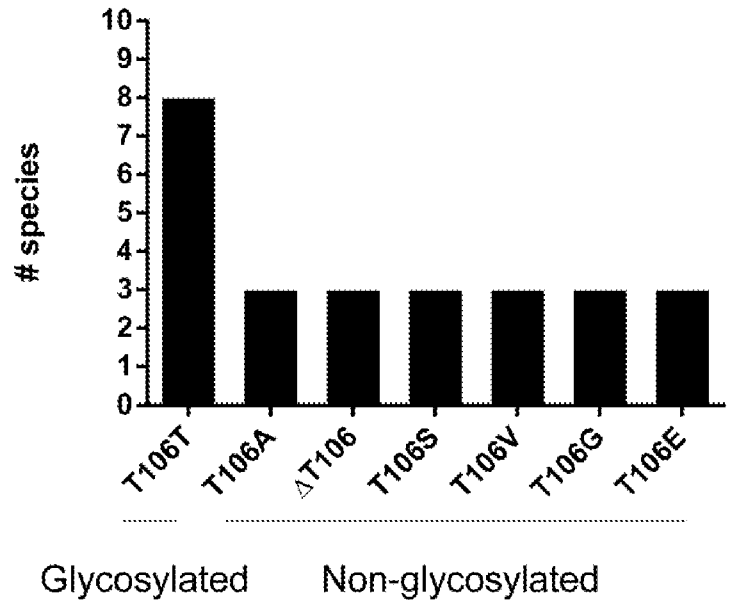

species
10
9
8
7
6
5
4
3
2
1
0
T106T
T106A
ΔT106
T106S
T106V
T106G
T106E
Glycosylated
Non-glycosylated

Figure 10.

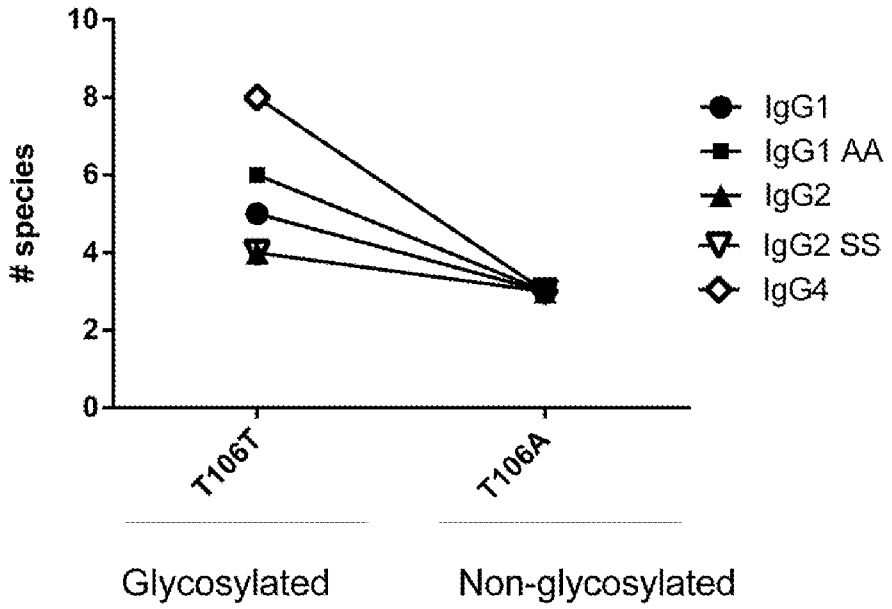

10
8
6
4
2
0
species
T106T
T106A
Glycosylated
Non-glycosylated
IgG1
IgG1 AA
IgG2
IgG2 SS
IgG4

Figure 11.

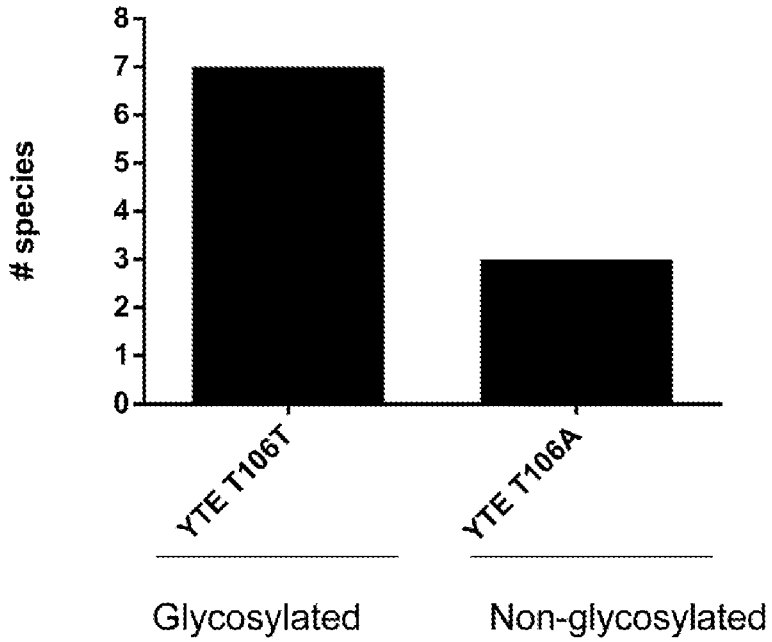

species
8
7
6
5
4
3
2
1
0
YTE T106T
YTE T106A
Glycosylated
Non-glycosylated

Figure 12.

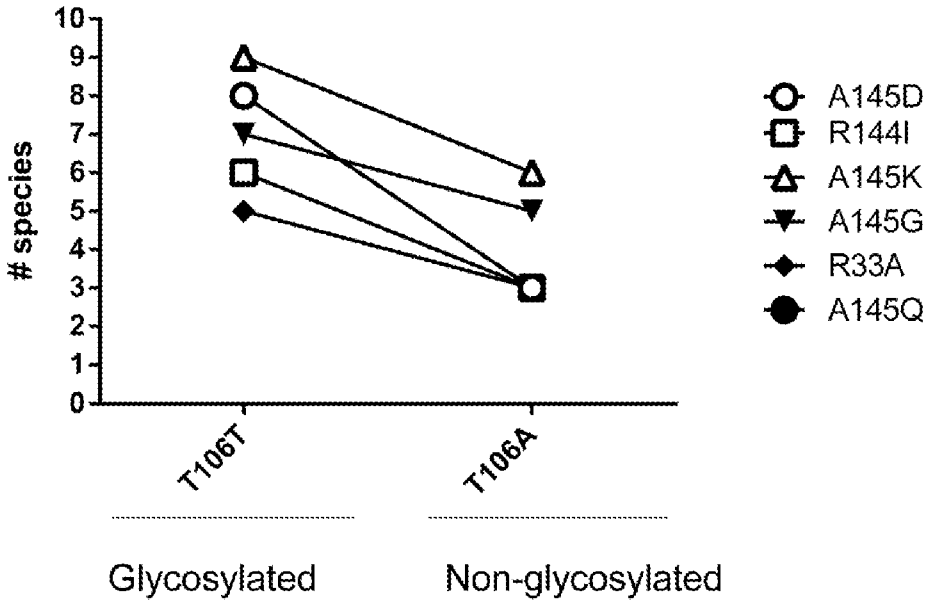

10
9
8
7
6
5
4
3
2
1
0
species
T106T
T106A
Glycosylated
Non-glycosylated
A145D
R144I
A145K
A145G
R33A
A145Q

Figure 13.

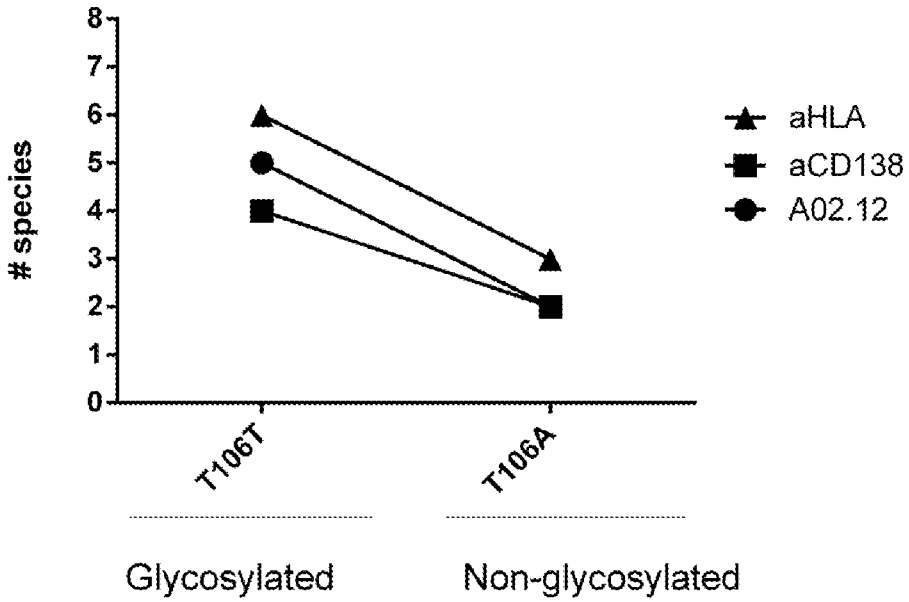

species
8
7
6
5
4
3
2
1
0
T106T
T106A
aHLA
aCD138
A02.12
Glycosylated
Non-glycosylated

Figure 14.

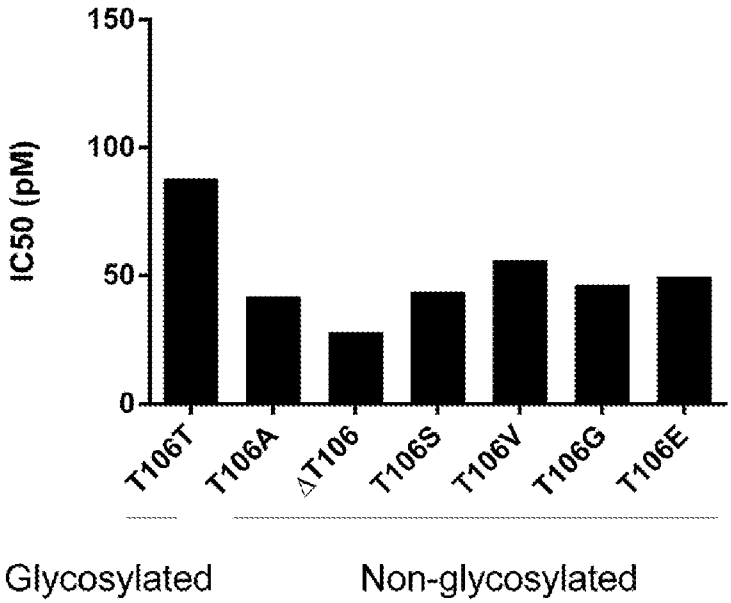

150
100
50
0
IC50 (pM)
T106T
T106A
ΔT106
T106S
T106V
T106G
T106E
Glycosylated
Non-glycosylated

Figure 15.

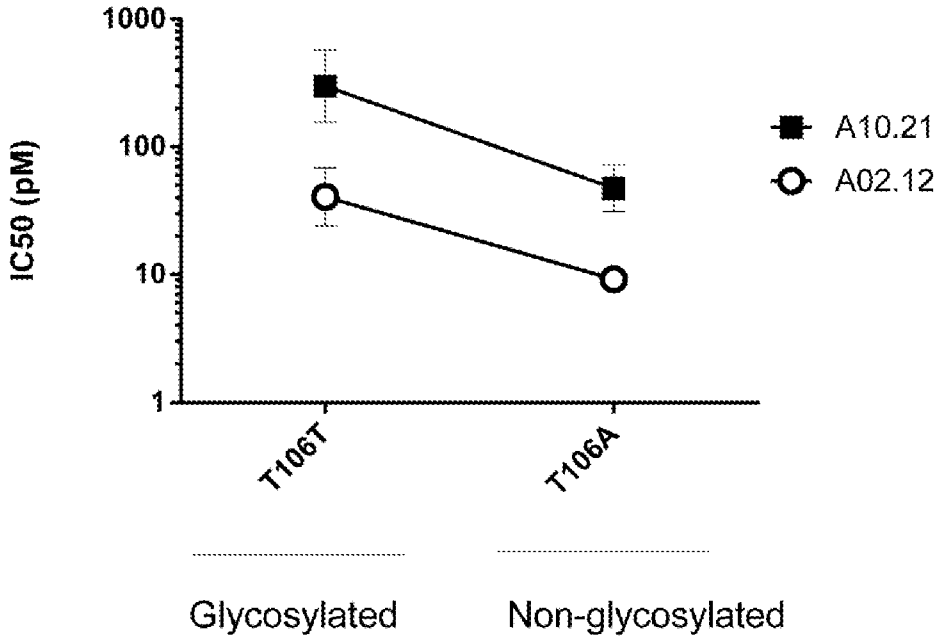

1000
100
10
1
IC50 (pM)
A10.21
A02.12
T106T
T106A
Glycosylated
Non-glycosylated

Figure 16.

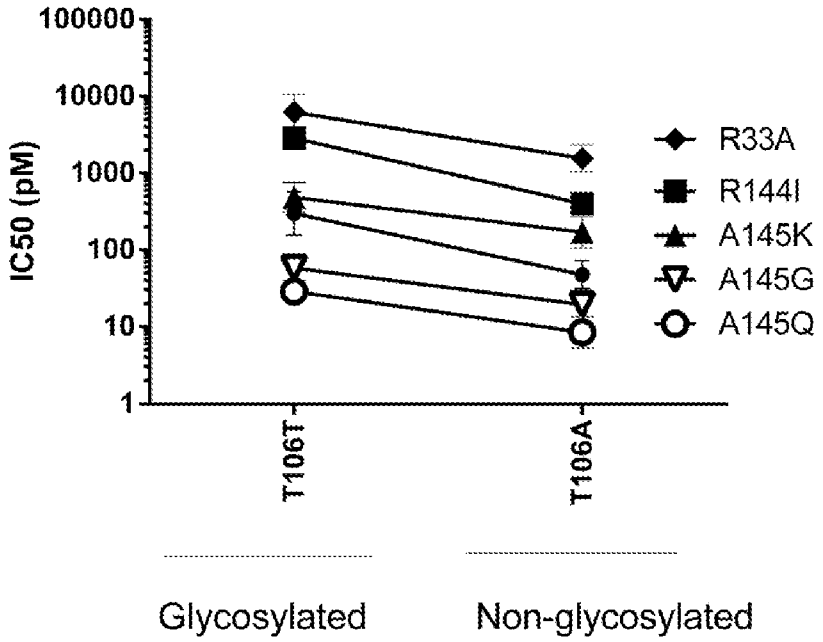

100000
10000
1000
100
10
1
IC50 (pM)
T106T
T106A
R33A
R144I
A145K
A145G
A145Q
Glycosylated
Non-glycosylated

Figure 17.

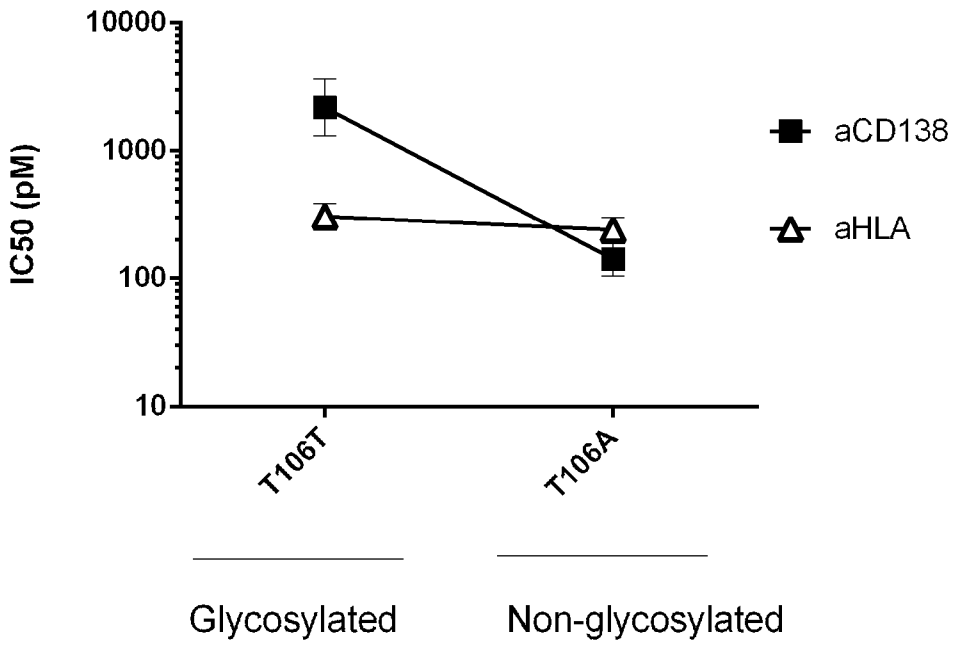

10000
1000
100
10
IC50 (pM)
aCD138
aHLA
T106T
T106A
Glycosylated
Non-glycosylated

Figure 18.

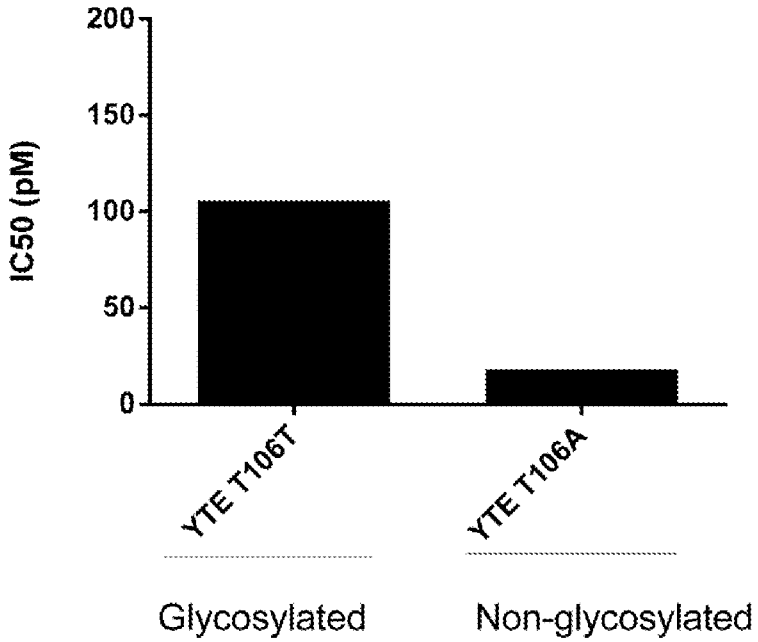

200
150
100
50
0
IC50 (pM)
YTE T106T
YTE T106A
Glycosylated
Non-glycosylated

Figure 19.

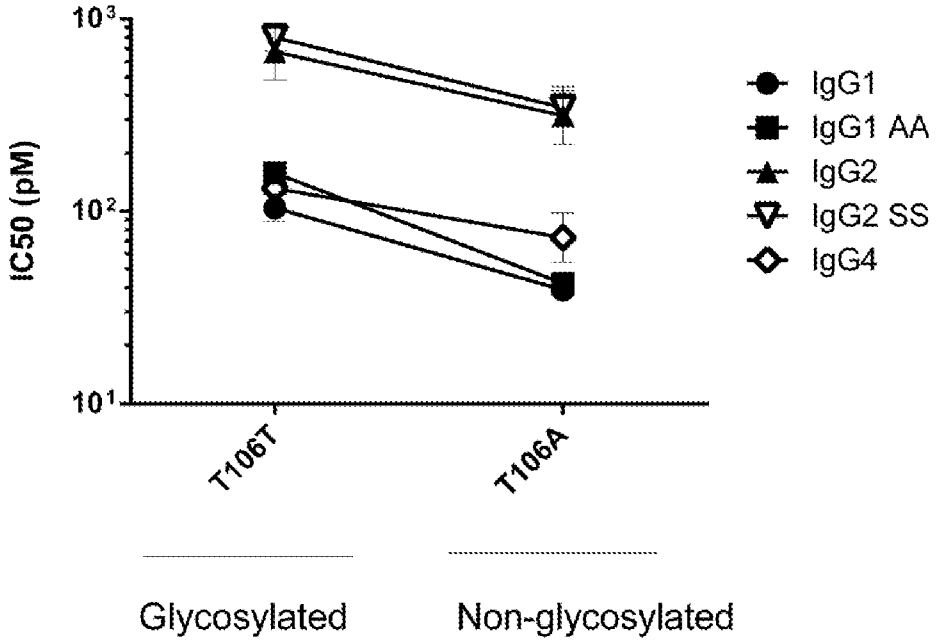
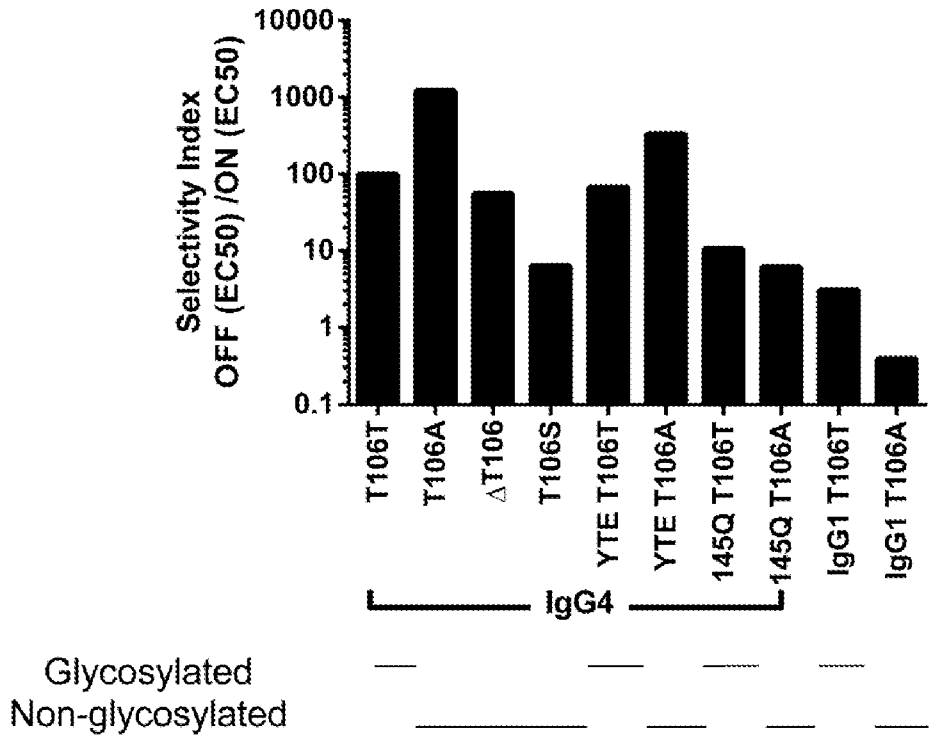

IC50 (pM)
10³
10²
10¹
T106T
T106A
IgG1
IgG1 AA
IgG2
IgG2 SS
IgG4
Glycosylated
Non-glycosylated

Figure 20.


Selectivity Index
OFF (EC50) /ON (EC50)
10000
1000
100
10
1
0.1
T106T
T106A
ΔT106
T106S
YTE T106T
YTE T106A
145Q T106T
145Q T106A
IgG1 T106T
IgG1 T106A
IgG4
Glycosylated
Non-glycosylated