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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 March 2020 (26.03.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/056522 A1

(51) International Patent Classification:
C07K 16/40 (2006.01) *C12N 15/63* (2006.01)
C07K 19/00 (2006.01) *C12N 5/10* (2006.01)
C12N 15/13 (2006.01) *C12P 21/00* (2006.01)
C12N 15/62 (2006.01) *C12P 21/08* (2006.01)

(21) International Application Number:
PCT/CA2019/051345

(22) International Filing Date:
20 September 2019 (20.09.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/734,426 21 September 2018 (21.09.2018) US

(71) Applicant: **NATIONAL RESEARCH COUNCIL OF CANADA** [CA/CA]; M-55, Room 29, 1200 Montreal Rd, Ottawa, Québec K1A 0R6 (CA).

(72) Inventors: **DUROCHER, Yves**; 5335 Place d'Argenteuil, Montreal, Québec H4J 1M1 (CA). **JOUBERT, Simon**; 280 Croissant de Sabrevois, Laval, Québec H7G 4M1 (CA).

(74) Agent: **BENOIT & COTE INC.**; 560 Cremazie Blvd. East, Suite 300, Montreal, Québec H2P 1E8 (CA).

(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, 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))*

(54) Title: INTRABODIES FOR REDUCING FUT8 ACTIVITY

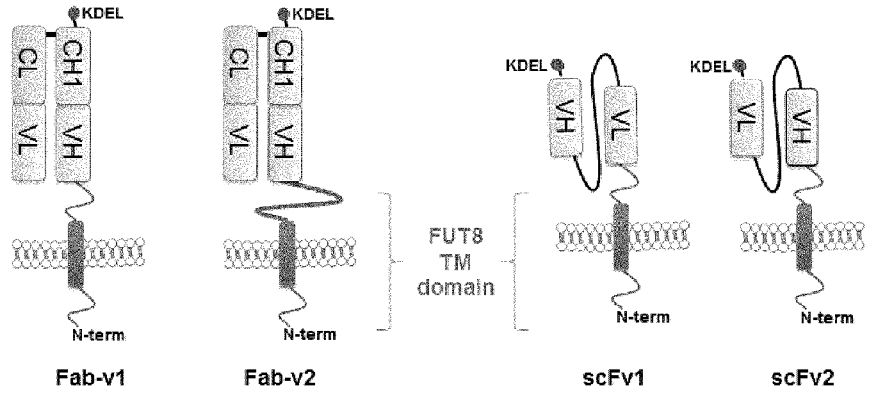


FIG. 8

(57) Abstract: The present document describes an alpha-(1,6)-fucosyltransferase (FUT8) antibody, antigen binding domain thereof, or a fusion protein thereof, operable to inhibit FUT8 activity in a cell, and methods of producing recombinant proteins, in particular antibodies, having reduced fucosylation. The present document also describes methods of inhibiting expression and/or activity of a protein in a cell by expressing an antibody and/or a fusion protein operable to inhibit expression and/or activity of the protein. The antibody, antigen binding domain thereof, or fusion protein thereof may comprise a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

WO 2020/056522 A1

Title: INTRABODIES FOR REDUCING FUT8 ACTIVITY

BACKGROUND

(a) Field

[0001] The subject matter disclosed generally relates to antibodies, antigen binding fragment thereof, or fusion proteins operable to inhibit activity and/or expression of a protein in a cell. The subject matter disclosed more specifically relates to an alpha-(1,6)-fucosyltransferase (FUT8) antibody, an antigen binding fragment thereof, or a fusion protein thereof, operable to inhibit FUT8 activity in a cell, and methods of producing recombinant proteins, in particular antibodies, having reduced fucosylation. The subject matter disclosed also specifically relates to methods of inhibiting expression and/or activity of a protein in a cell by expressing an antibody and/or a fusion protein operable to inhibit expression and/or activity of the protein.

(b) Related Prior Art

[0002] Reducing fucosylation of recombinant proteins, especially therapeutic monoclonal antibodies (mAbs), is highly desirable for increasing effector functions [e.g. in antibody-dependent cell-mediated cytotoxicity (ADCC) and cell dependent cytotoxicity (CDC)] and to enhance their therapeutic efficacy.

[0003] FUT8 is the only fucosyltransferase that catalyzes the transfer of fucose from GDP-fucose to GlcNAc via α-1,6 linkage (medial Golgi). Therefore, the inhibition of FUT8 is a pertinent approach for reduction of fucosylation *in vivo*. Several approaches have been developed to reduce recombinant protein fucosylation, particularly of mAbs fucosylation. For example, FUT8 knockout (KO) CHO cells lines have been generated using zinc finger nucleases, meganucleases, siRNA, or the CRISPR / CAS9 system by companies such as Biowa, Genentech and Lonza. Also, the Co-expression of glycosylation enzyme beta1-4-N-acetylglucosaminyltransferase III (GnTIII) to generate altered glycoforms (Glycart), and the use of small FUT8 inhibitor molecules (Amgen), or

of fucose diversion pathways [Probiogen: overexpression of the bacterial oxidoreductase GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD); Kyowa: GDP-d-mannose-4,6-dehydratase (GMD)] were used. However, the use of these systems is often expensive and represents a significant barrier to commercial and non-commercial production of recombinant proteins and mAbs.

[0004] Therefore, there is a need for alternative affordable technology for the production of CHO afucosylated proteins and/or antibodies.

SUMMARY

[0005] According to an embodiment, there is provided an alpha-(1,6)-fucosyltransferase (FUT8) antibody, an antigen binding fragment thereof, or a fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, operable to inhibit FUT8 activity in a cell. The provided antibody or fusion protein thereof may comprise a heavy (V_H) and a light (V_L) chain or may be any antigen binding antibody or fragment capable of inhibiting FUT8 activity in a cell.

[0006] The fusion protein may comprise a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

[0007] According to another embodiment, there is provided a cell expressing the FUT8 antibody, antigen binding fragment thereof or fusion protein thereof, of the present invention.

[0008] According to another embodiment, there is provided a method for producing a recombinant antibody having reduced fucosylation comprising :

a) culturing a host cell expressing a FUT8 antibody, an antigen binding fragment thereof, or a fusion protein thereof, of the present invention,

wherein the host cell expresses the recombinant antibody under conditions which permit the production of the recombinant antibody; and

b) isolating the recombinant antibody.

[0009] According to another embodiment, there is provided a method for producing a recombinant protein having reduced fucosylation comprising :

a) culturing a host cell expressing a FUT8 antibody, an antigen binding fragment thereof, or a fusion protein thereof, of the present invention,

wherein the host cell expresses the recombinant protein under conditions which permit the production of the recombinant protein; and

b) isolating the recombinant protein.

[0010] According to another embodiment, there is provided a nucleic acid vector comprising a nucleotide sequence encoding an alpha-(1,6)-fucosyltransferase (FUT8) antibody, an antigen binding fragment thereof, or a fusion protein thereof according to the present invention.

[0011] According to another embodiment, there is provided a method for inhibiting expression and/or activity of a secreted protein in a cell comprising culturing a cell expressing an antibody, an antigen binding fragment thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, operable to inhibit expression and/or activity of the protein in the cell, the antibody, antigen binding fragment thereof, or the fusion protein thereof comprising a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

[0012] According to another embodiment, there is provided a FUT8 antibody, antigen binding fragment thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, wherein the variable region heavy (V_H) chain comprises an amino acid sequence comprising SEQ ID NO:1, and the variable region light (V_L) chain comprises an amino acid sequence comprising SEQ ID NO:2.

[0013] According to another embodiment, there is provided a FUT8 antibody, antigen binding fragment thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof,

wherein the variable region heavy (V_H) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5, respectively;

and wherein the variable region light (V_L) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, respectively.

[0014] The FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof may be antibody 1D2.

[0015] According to another embodiment, there is provided a FUT8 antibody, an antigen binding fragment thereof, or a fusion protein comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, wherein the variable region heavy (V_H) chain comprises an amino acid sequence comprising SEQ ID NO:9, and the variable region light (V_L) chain comprises an amino acid sequence comprising SEQ ID NO:10.

[0016] According to another embodiment, there is provided a FUT8 antibody, antigen binding fragment thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof,

wherein the variable region heavy (V_H) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13, respectively;

and wherein the variable region light (V_L) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16, respectively.

[0017] The FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof may be antibody 5C9.

[0018] According to another embodiment, there is provided a FUT8 antibody, antigen binding fragment thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, wherein the variable region heavy (V_H) chain comprises an amino acid sequence comprising SEQ ID NO:17, and the variable region light (V_L) chain comprises an amino acid sequence comprising SEQ ID NO:18.

[0019] According to another embodiment, there is provided a FUT8 antibody, an antigen binding fragment thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof,

wherein the variable region heavy (V_H) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, respectively;

and wherein the variable region light (V_L) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively.

[0020] The FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof may be antibody 1H9.

[0021] The FUT8 antibody, antigen binding fragment thereof or fusion protein thereof may further comprise a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

[0022] The FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof, of the present invention, or the cell of the present invention, or the method of the present invention, or the nucleic acid vector of the present

invention, wherein the FUT8 antibody and/or the fusion protein further comprises an endoplasmic reticulum retention signal.

[0023] The FUT8 antibody, antigen binding fragment thereof or fusion protein thereof, of the present invention, the cell of the present invention, the method of the present invention, or the nucleic acid vector the present invention, wherein the FUT8 antibody may be an IgA, an IgD, an IgE, and IgG, an IgM, n Fab or combinations thereof.

[0024] The FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention, the cell of the present invention, the method of the present invention, or the nucleic acid vector of the present invention, wherein the antibody, antigen binding fragment or fusion protein may comprise a scFab, a scFv, a sdAb, or combinations thereof.

[0025] In the method of the present invention, the fusion protein may be a scFv.

[0026] The cell of the present invention, the method of the present invention, or the nucleic acid vector of the present invention, wherein the FUT8 antibody, antigen binding fragment thereof, or the fusion protein thereof may be the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof according to the present invention.

[0027] The FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention, the cell of the present invention, the method of the present invention, or the nucleic acid vector of the present invention, wherein the transmembrane domain may be chosen from a transmembrane domain of FUT8, a transmembrane domain of beta-1,4-galactosyltransferase 1 (B4GT1), and a transmembrane domain of human calnexin (hCNX).

[0028] The FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof, cell or method according to the present invention, wherein the

transmembrane domain of FUT8 may comprise an amino acid sequence comprising SEQ ID NO:25, the transmembrane domain of B4GT1 may comprise an amino acid sequence comprising SEQ ID NO:26, and the transmembrane domain of hCNX may comprise an amino acid sequence comprising SEQ ID NO:27.

[0029] The FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof, cell or method according to the present invention, wherein the endoplasmic reticulum retention signal may comprise an amino acid sequence comprising SEQ ID NO:28.

[0030] The following terms are defined below.

[0031] The terms “intrabody” or “intrabodies” (from intracellular and antibody) refers to an antibody that works within the cell to bind to an intracellular protein. Introducing an antibody within the cell typically requires the expression of the antibody within the target cell. As a result, intrabodies are defined as antibodies that have been modified for intracellular localization. The terms are also used even when antibodies are produced in prokaryotes or other non-target cells.

[0032] The term “antibody”, which is also referred to in the art as “immunoglobulin” (Ig), as used herein refers to a protein constructed from paired heavy and light polypeptide chains; various Ig isotypes exist, including IgA, IgD, IgE, IgG, and IgM. When an antibody is correctly folded, each chain folds into a number of distinct globular domains joined by more linear polypeptide sequences. For example, the immunoglobulin light chain folds into a variable (V_L) and a constant (C_L) domain, while the heavy chain folds into a variable (V_H) and three constant (C_H, C_{H2}, C_{H3}) domains. Interaction of the heavy and light chain variable domains (V_H and V_L) results in the formation of an antigen binding region (Fv). Each domain has a well-established structure familiar to those of skill in the art.

[0033] The light and heavy chain variable regions are responsible for binding the target antigen and can therefore show significant sequence diversity between antibodies. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. The variable region of an antibody contains the antigen-binding determinants of the molecule, and thus determines the specificity of an antibody for its target antigen. The majority of sequence variability occurs in six hypervariable regions, three each per variable heavy (V_H) and light (V_L) chain; the hypervariable regions combine to form the antigen-binding site, and contribute to binding and recognition of an antigenic determinant. The specificity and affinity of an antibody for its antigen is determined by the structure of the hypervariable regions, as well as their size, shape, and chemistry of the surface they present to the antigen. Various schemes exist for identification of the regions of hypervariability, the two most common being those of Kabat and of Chothia and Lesk [Kabat et al, 1991, J Immunol (1991) 147(5):1709-1719; Chothia and Lesk 1987, J Mol Biol (1987) 196(4):901-917], define the "complementarity-determining regions" (CDR) based on sequence variability at the antigen-binding regions of the V_H and V_L domains. Chothia and Lesk 1987, J Mol Biol (1987) 196(4):901-917 define the "hypervariable loops" (H or L) based on the location of the structural loop regions in the V_H and V_L domains. These individual schemes define CDR and hypervariable loop regions that are adjacent or overlapping, those of skill in the antibody art often utilize the terms "CDR" and "hypervariable loop" interchangeably, and they may be so used herein. The CDR/loops are identified herein according to the Kabat scheme (i.e. CDR1, 2 and 3, for each variable region).

[0034] An "antibody fragment" or an "antigen binding domain", or an "antigen binding fragment" as referred to herein may include any suitable antigen-binding antibody fragment known in the art. The antibody fragment may be a naturally-occurring antibody fragment, or may be obtained by manipulation

of a naturally-occurring antibody or by using recombinant methods. For example, an antibody fragment may include, but is not limited to a Fv, single-chain Fv (scFv; a molecule consisting of V_L and V_H connected with a peptide linker), Fab, $F(ab')_2$, single-domain antibody (sdAb; a fragment composed of a single V_L or V_H), and multivalent presentations of any of these. Antibody fragments such as those just described may require linker sequences, disulfide bonds, or other type of covalent bond to link different portions of the fragments; those of skill in the art will be familiar with the requirements of the different types of fragments and various approaches and various approaches for their construction.

[0035] In a non-limiting example, the antibody fragment may be an sdAb derived from naturally-occurring sources. Heavy chain antibodies of camelid origin (Hamers-Casterman et al, 1993, Nature *363*: 446–448) lack light chains and thus their antigen binding sites consist of one domain, termed V_HH. sdAb have also been observed in shark and are termed V_{NAR} (Nuttall et al, 2003, Eur. J. Biochem. *270*: 3543–3554). Other sdAb may be engineered based on human Ig heavy and light chain sequences (Jespers et al, 2004, Nat. Biotechnol. *22*: 1161–1165; To et al, 2005, J. Biol. Chem. *280*: 41395–41403). As used herein, the term "sdAb" includes those sdAb directly isolated from V_H, V_HH, V_L, or V_{NAR} reservoir of any origin through phage display or other technologies, sdAb derived from the aforementioned sdAb, recombinantly produced sdAb, as well as those sdAb generated through further modification of such sdAb by humanization, affinity maturation, stabilization, solubilization, camelization, or other methods of antibody engineering. Also encompassed by the present invention are homologues, derivatives, or fragments that retain the antigen-binding function and specificity of the sdAb.

[0036] SdAb possess desirable properties for antibody molecules, such as high thermostability, high detergent resistance, relatively high resistance to proteases (Dumoulin et al, 2002, Protein Sci. *11*: 500–15) and high production yield (Arbabi-Ghahroudi et al, 1997); they can also be engineered to have very

high affinity by isolation from an immune library (Li et al, 2009, Mol. Immunol. *46*: 1718–1726) or by *in vitro* affinity maturation (Davies & Riechmann, 1996, Immunotechnology *2*: 169–79). Further modifications to increase stability, such as the introduction of non-canonical disulfide bonds (Hussack et al, 2011a,b; Kim et al, 2012, J. Biol. Chem. *286*: 8961–8976), may also be brought to the sdAb.

[0037] A person of skill in the art would be well-acquainted with the structure of a single-domain antibody (see, for example, 3DWT, 2P42 in Protein Data Bank). An sdAb comprises a single immunoglobulin domain that retains the immunoglobulin fold; most notably, only three CDR/hypervariable loops form the antigen-binding site. However, and as would be understood by those of skill in the art, not all CDR may be required for binding the antigen. For example, and without wishing to be limiting, one, two, or three of the CDR may contribute to binding and recognition of the antigen by the sdAb of the present invention. The CDR of the sdAb or variable domain are referred to herein as CDR1, CDR2, and CDR3.

[0038] The term "scFv" is intended to refer to single-chain variable fragment, although an scFv is not actually a fragment of an antibody, but instead is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa. This scFv protein retains the specificity of the original immunoglobulin, despite removal of the constant Fc regions and the introduction of the linker. ScFv molecules were created to facilitate phage display, where it is highly convenient to express the antigen-binding domain as a single peptide. As an alternative, scFv can be created directly from subcloned heavy and light chains derived from a hybridoma.

[0039] Divalent (or bivalent) scFvs (di-scFvs, bi-scFvs) can be engineered by linking two scFvs. This can be done by producing a single peptide chain with

two V_H and two V_L regions, yielding tandem scFvs. Another possibility is the creation of scFvs with linker peptides that are too short for the two variable regions to fold together (about five amino acids), forcing scFvs to dimerize. This type is known as diabodies. Diabodies have been shown to have dissociation constants up to 40-fold lower than corresponding scFvs, meaning that they have a much higher affinity to their target. For example, a diabody drugs could be dosed much lower than other therapeutic antibodies and are capable of highly specific targeting of tumors *in vivo*. Still shorter linkers (one or two amino acids) lead to the formation of trimers, so-called triabodies or tribodies. Tetrabodies have also been produced. They exhibit an even higher affinity to their targets than diabodies.

[0040] All of these formats can be composed from variable fragments with specificity for two different antigens, in which case they are types of bispecific antibodies. The furthest developed of these are bispecific tandem di-scFvs, known as bi-specific T-cell engagers (BiTE antibody constructs).

[0041] The present invention further encompasses an antibody or fragment that is "humanized" using any suitable method known in the art, for example, but not limited to CDR grafting and veneering. Humanization of an antibody or antibody fragment comprises replacing an amino acid in the sequence with its human counterpart, as found in the human consensus sequence, without loss of antigen-binding ability or specificity; this approach reduces immunogenicity of the antibody or fragment thereof when introduced into human subjects. In the process of CDR grafting, one or more than one of the CDR defined herein may be fused or grafted to a human variable region (V_H, or V_L), to other human antibody (IgA, IgD, IgE, IgG, and IgM), to other human antibody fragment framework regions (Fv, scFv, Fab) or to other proteins of similar size and nature onto which CDR can be grafted (Nicaise et al, 2004). In such a case, the conformation of said one or more than one hypervariable loop is likely preserved, and the affinity and specificity of the sdAb for its target (i.e.,

IGF1R) is likely minimally affected. CDR grafting is known in the art and is described in at least the following: US Patent No. 6180370, US Patent No. 5693761, US Patent No. 6054297, US Patent No. 5859205, and European Patent No. 626390. Veneering, also referred to in the art as "variable region resurfacing", involves humanizing solvent-exposed positions of the antibody or fragment; thus, buried nonhumanized residues, which may be important for CDR conformation, are preserved while the potential for immunological reaction against solvent-exposed regions is minimized. Veneering is known in the art and is described in at least the following: US Patent No. 5869619, US Patent No. 5766886, US Patent No. 5821123, and European Patent No. 519596. Persons of skill in the art would also be amply familiar with methods of preparing such humanized antibody fragments and humanizing amino acid positions.

[0042] The antibody, antigen binding fragment thereof, or fusion protein thereof, of the present invention may also comprise additional sequences to aid in expression, detection, localization or purification. Any such sequences or tags known to those of skill in the art may be used. For example, and without wishing to be limiting, the antibody or fragment thereof may comprise a targeting or signal sequence {for example, but not limited to ompA, a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof [e.g. a transmembrane domain of FUT8, a transmembrane domain of beta-1,4-galactosyltransferase 1 (B4GT1), and a transmembrane domain of human calnexin (hCNX)], an endoplasmic reticulum retention signal (KDEL)}, a detection/purification tag (for example, but not limited to c-Myc, His5, or His6), or a combination thereof. In another example, the additional sequence may be a biotin recognition site such as that described by Cronan et al in WO 95/04069 or Voges et al in WO/2004/076670. As is also known to those of skill in the art, linker sequences may be used in conjunction with the additional sequences or tags, or may serve as a detection/purification tag.

[0043] The antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention may also be in a multivalent display format, also referred to herein as multivalent presentation. Multimerization may be achieved by any suitable method of known in the art. For example, and without wishing to be limiting in any manner, multimerization may be achieved using self-assembly molecules such as those described in Zhang et al (2004a, Mol. Biol. *341*: 161–169; and 2004b, J. Mol. Biol. *335*: 49–56) and W02003/046560, where pentabodies are produced by expressing a fusion protein comprising the antibody or fragment thereof of the present invention and the pentamerization domain of the B-subunit of an AB5 toxin family (Merritt & Hol, 1995, Curr. Opin. Struct. Biol. *5*: 165–171). A multimer may also be formed using the multimerization domains described by Zhu et al. (2010, Immunol. Cell Biol. *88*: 667–675); this form, referred to herein as a "combody" form, is a fusion of the antibody or fragment of the present invention with a coiled-coil peptide resulting in a multimeric molecule (Zhu et al., 2010, Immunol. Cell Biol. *88*: 667–675). Other forms of multivalent display are also encompassed by the present invention. For example, and without wishing to be limiting, the antibody or fragment thereof may be presented as a dimer, a trimer, or any other suitable oligomer. This may be achieved by methods known in the art, for example direct linking connection (Nielson et al, 2000, Cancer Res. *60*: 6434–6440), c-jun/Fos interaction (de Kruif & Logtenberg, 1996, J. Biol. Chem. *271*: 7630–7634), "Knob into holes" interaction (Ridgway et al, 1996, Protein Eng. *9*: 617–621).

[0044] Another method known in the art for multimerization is to dimerize the antibody or fragment thereof using an Fc domain, for example, but not limited to human Fc domains. The Fc domains may be selected from various classes including, but not limited to, IgG, IgM, or various subclasses including, but not limited to IgG1, IgG2, etc. In this approach, the Fc gene in inserted into a vector along with the sdAb gene to generate a sdAb-Fc fusion protein (Bell et al, 2010, Cancer Lett. *289*: 81–90; Iqbal et al, 2010, Br. J. Pharmacol. *160*: 1016–28); the

fusion protein is recombinantly expressed then purified. For example, and without wishing to be limiting in any manner, multivalent display formats may encompass chimeric or humanized formats of antibodies V_HH linked to an Fc domain, or bi or tri-specific antibody fusions with two or three antibodies V_HH recognizing unique epitopes. Such antibodies are easy to engineer and to produce, can greatly extend the serum half-life of sdAb, and may be excellent tumor imaging reagents (Bell et al., 2010, Cancer Lett. *289*: 81–90).

[0045] The Fc domain in the multimeric complex as just described may be any suitable Fc fragment known in the art. The Fc fragment may be from any suitable source; for example, the Fc may be of mouse or human origin. In a specific, non-limiting example, the Fc may be the mouse Fc2b fragment or human Fc1 fragment (Bell et al, 2010, Cancer Lett. *289*: 81–90; Iqbal et al, 2010, Br. J. Pharmacol. *160*: 1016–28). The Fc fragment may be fused to the N-terminal or C-terminal end of the VHH or humanized versions of the present invention.

[0046] Each subunit of the multimers described above may comprise the same or different antibodies or fragments thereof of the present invention, which may have the same or different specificity. Additionally, the multimerization domains may be linked to the antibody or antibody fragment using a linker, as required; such a linker should be of sufficient length and appropriate composition to provide flexible attachment of the two molecules, but should not hamper the antigen-binding properties of the antibody.

[0047] Features and advantages of the subject matter hereof will become more apparent in light of the following detailed description of selected embodiments, as illustrated in the accompanying figures. As will be realized, the subject matter disclosed and claimed is capable of modifications in various respects, all without departing from the scope of the claims. Accordingly, the drawings and the description are to be regarded as illustrative in nature, and not as restrictive and the full scope of the subject matter is set forth in the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] Further features and advantages of the present disclosure will become apparent from the following detailed description, taken in combination with the appended drawings, in which:

[0049] Fig. 1 illustrates the inhibition of FUT8 activity by 50 mAbs produced from tested hybridomas. The 1H9, 1D2 and 5C9 were selected as potent inhibitors.

[0050] Fig. 2 illustrates the sequence of the mAb sequence of the selected hybridomas.

[0051] Fig. 3 illustrates the expression of recombinant mAbs produced in CHO-3E7 cells from the VH and VL of the 3 mAbs 1H9, 1D2 and 5C9 cloned into PTT5-CH123 (hIgG1) and PTT5-kappa vectors. (S: supernatant), (FT: flow-through) and (E: elution).

[0052] Fig. 4 illustrates that mock transfected cells are fully fucosylated, while cells transfected with RMD show decreased fucosylation.

[0053] Fig. 5 illustrates that mAbs 1H9, 1D2 and 5C9 fused with a retention signal (KDEL) are able to inhibit fucosylation with various efficacies when transiently expressed in CHO cells.

[0054] Fig. 6 illustrates recombinant FUT8 antibodies 1H9, 1D2 and 5C9 with a C-terminal KDEL sequence of the heavy chain coexpressed with a one-arm monoclonal antibody (OAA) in CHO cells. The results show that the C-terminal KDEL sequence is not sufficient to fully retain the anti-FUT8 mAbs within the cells as we can clearly detect them in the supernatants.

[0055] Fig. 7 illustrates the binding of FUT8 to indirectly capture anti-FUT8 antibody 1H9, 1D2 or 5C9 as determined by surface plasmon resonance using a BioRad® Proteon® (Mississauga, ON).

[0056] Fig. 8 illustrates a graphical representation of Fab and scFv versions of the mAbs of the present invention, which were prepared.

[0057] Fig. 9 illustrates the measured cell surface fucosylation levels as evidenced by labeling with biotinylated *Aleuria Aurantia* Lectin (AAL) and detection with fluorescein isothiocyanate (FITC)-streptavidin, of the of Fab and scFv versions of the mAbs illustrated in Fig. 8.

[0058] Fig. 10 illustrates schematic representations of additional constructs according to the present invention that were prepared.

[0059] Fig. 11 illustrates that cells expressing the identified constructs of Fig. 10 were able to reduce fucosylation to various levels, especially the scFv5 construct bearing a signal peptide (SP), the calnexin transmembrane and cytoplasmic domains, which was able to reduce fucosylation by 8-fold.

[0060] Fig. 12 illustrates schematic representations of constructs that were prepared with and without the KDEL sequence according to embodiments of the present invention.

[0061] Fig. 13 illustrates that when variable regions of the heavy (V_H) and light (V_L) chains of the identified hybridoma were introduced into the scFv5 format, cells expressing the identified constructs were able to reduce fucosylation of a co-transfected IgG1 model antibody to various levels compared to the control indicated by the dashed line (upper panel: antibody 1 – 2G4, an anti-ebola glycoprotein antibody, lower panel: antibody 2- rituximab).

[0062] It will be noted that throughout the appended drawings, like features are identified by like reference numerals.

DETAILED DESCRIPTION

[0063] The present invention is directed to a technology for inhibiting the activity and/or expression of a protein *in vivo*. In embodiments, the technology uses intrabodies directed to the protein of interest to inhibit the activity and/or

expression thereof. More specifically, in embodiments, the intrabodies are directed to FUT8, to inhibit the fucosyltransferase activity of this enzyme, and therefore reduce overall fucosylation of the proteins produced therein.

[0064] In a first embodiment there is disclosed an alpha-(1,6)-fucosyltransferase (FUT8) antibody, antigen binding fragment thereof, or a fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, operable to inhibit FUT8 activity in a cell. The provided antibody or fusion protein thereof may comprise a heavy (V_H) and a light (V_L) chain or may be any antigen binding antibody or fragment capable of inhibiting FUT8 activity in a cell.

[0065] The FUT8 antibody, antigen binding fragment thereof,or the fusion protein thereof may comprise a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

[0066] According to other embodiments of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention, the transmembrane domain is chosen from a transmembrane domain of FUT8, a transmembrane domain of beta-1,4-galactosyltransferase 1 (B4GT1), and a transmembrane domain of human calnexin (hCNX). For example, the transmembrane domain of FUT8 may comprise an amino acid sequence comprising SEQ ID NO:25, the transmembrane domain of B4GT1 may comprise an amino acid sequence comprising SEQ ID NO:26, and the transmembrane domain of hCNX may comprise an amino acid sequence comprising SEQ ID NO:27.

[0067] According to an embodiment of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, the variable region heavy (V_H) chain may comprise an amino acid sequence comprising SEQ ID NO:1, and the

variable region light (V_L) chain may comprise an amino acid sequence comprising SEQ ID NO:2.

[0068] According to another embodiment of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, the variable region heavy (V_H) chain may comprise CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5, respectively; and the variable region light (V_L) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, respectively.

[0069] According to an embodiment, the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof may be antibody 1D2.

[0070] According to another embodiment of the FUT8 antibody, antigen binding fragment thereof, or a fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, the variable region heavy (V_H) chain may comprise an amino acid sequence comprising SEQ ID NO:9, and the variable region light (V_L) chain may comprise an amino acid sequence comprising SEQ ID NO:10.

[0071] According to another embodiment of the FUT8 antibody, antigen binding fragment thereof, or a fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, the variable region heavy (V_H) chain may comprise CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13, respectively; and the variable region light (V_L) chain may comprise CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16, respectively.

[0072] According to an embodiment, the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof may be antibody 5C9.

[0073] According to another embodiment of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, the variable region heavy (V_H) chain may comprise an amino acid sequence comprising SEQ ID NO:17, and the variable region light (V_L) chain may comprise an amino acid sequence comprising SEQ ID NO:18.

[0074] According to another embodiment of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, the variable region heavy (V_H) chain may comprise CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, respectively; and the variable region light (V_L) chain may comprise CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively.

[0075] According to another embodiment, the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof may be antibody 1H9.

[0076] According to other embodiments of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof, of the present invention, the FUT8 antibody and/or the fusion protein may further comprises an endoplasmic reticulum retention signal. For example, the endoplasmic reticulum retention signal is comprising an amino acid sequence comprising SEQ ID NO:28 - KDEL.

[0077] According to other embodiments of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention, the FUT8 antibody may be an IgA, an IgD, an IgE, and IgG, an IgM, an Fab or combinations thereof.

[0078] According to other embodiments of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention, the fusion protein may be a scFab, a scFv, a sbAb, or combinations thereof.

[0079] According to another embodiment, there is disclosed a cell expressing the FUT8 antibody, antigen binding fragment thereof, or a fusion protein thereof of the present invention.

[0080] According to another embodiment, there is disclosed a method for producing a recombinant antibody having reduced fucosylation comprising :

a) culturing a host cell expressing the FUT8 antibody, antigen binding fragment thereof or fusion protein thereof, of the present invention, wherein the host cell expresses the recombinant antibody under conditions which permit the production of the recombinant antibody; and

b) isolating the recombinant antibody.

[0081] According to another embodiment, there is disclosed a method for producing a recombinant protein having reduced fucosylation comprising :

a) culturing a host cell expressing a FUT8 antibody, antigen binding fragment thereof, or a fusion protein thereof, of the present invention, wherein the host cell expresses the recombinant protein under conditions which permit the production of the recombinant protein; and

b) isolating the recombinant protein.

[0082] According to an embodiment, the fusion protein is a scFv.

[0083] According to an embodiment, the FUT8 antibody, antigen binding fragment thereof, or said fusion protein thereof, is the FUT8 antibody or fusion protein according to the present invention.

[0084] According to an embodiment, the said transmembrane domain is chosen from a transmembrane domain of FUT8, a transmembrane domain of beta-1,4-galactosyltransferase 1 (B4GT1), and a transmembrane domain of human calnexin (hCNX). The transmembrane domain of FUT8 may comprise an amino acid sequence comprising SEQ ID NO:25, the transmembrane domain of B4GT1 may comprise an amino acid sequence comprising SEQ ID NO:26, and

the transmembrane domain of hCNX may comprise an amino acid sequence comprising SEQ ID NO:27.

[0085] According to other embodiments of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention, the FUT8 antibody and/or the fusion protein may further comprises an endoplasmic reticulum retention signal. For example, the endoplasmic reticulum retention signal is comprising an amino acid sequence comprising SEQ ID NO:28 - KDEL.

[0086] According to another embodiment, there is disclosed a nucleic acid vector comprising a nucleotide sequence encoding an alpha-(1,6)-fucosyltransferase (FUT8) antibody, antigen binding fragment thereof, or a fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, operable to inhibit FUT8 activity in a cell, the FUT8 antibody and/or the fusion protein comprising a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

[0087] According to an embodiment, the FUT8 antibody, antigen binding fragment thereof, and/or said fusion protein thereof is the FUT8 antibody or fusion protein according to the present invention.

[0088] According to an embodiment, the said transmembrane domain is chosen from a transmembrane domain of FUT8, a transmembrane domain of beta-1,4-galactosyltransferase 1 (B4GT1), and a transmembrane domain of human calnexin (hCNX). The transmembrane domain of FUT8 may comprise an amino acid sequence comprising SEQ ID NO:25, the transmembrane domain of B4GT1 may comprise an amino acid sequence comprising SEQ ID NO:26, and the transmembrane domain of hCNX may comprise an amino acid sequence comprising SEQ ID NO:27.

[0089] According to other embodiments of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention, the

FUT8 antibody, antigen binding fragment thereof, or the fusion protein thereof may further comprises an endoplasmic reticulum retention signal. For example, the endoplasmic reticulum retention signal is comprising an amino acid sequence comprising SEQ ID NO:28 - KDEL.

[0090] According to another embodiment, there is disclosed a cell expressing an antibody and/or a fusion protein comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, operable to inhibit expression and/or activity of a protein in the cell, the antibody and/or the fusion protein comprising a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

[0091] According to an embodiment, the FUT8 antibody, antigen binding fragment thereof, or said fusion protein thereof is the FUT8 antibody or fusion protein according to the present invention.

[0092] According to an embodiment, the said transmembrane domain is chosen from a transmembrane domain of FUT8, a transmembrane domain of beta-1,4-galactosyltransferase 1 (B4GT1), and a transmembrane domain of human calnexin (hCNX). The transmembrane domain of FUT8 may comprise an amino acid sequence comprising SEQ ID NO:25, the transmembrane domain of B4GT1 may comprise an amino acid sequence comprising SEQ ID NO:26, and the transmembrane domain of hCNX may comprise an amino acid sequence comprising SEQ ID NO:27.

[0093] According to other embodiments of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention, the FUT8 antibody, antigen binding fragment thereof, or the fusion protein thereof may further comprises an endoplasmic reticulum retention signal. For example, the endoplasmic reticulum retention signal is comprising an amino acid sequence comprising SEQ ID NO:28 - KDEL.

[0094] According to another embodiment, there is disclosed a method for inhibiting expression and/or activity of a secreted protein in a cell comprising culturing a cell expressing an antibody and/or a fusion protein comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, operable to inhibit expression and/or activity of the protein in the cell, the antibody and/or the fusion protein comprising a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

[0095] According to an embodiment, the FUT8 antibody, antigen binding fragment thereof, or said fusion protein thereof is the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof according to the present invention.

[0096] According to an embodiment, the transmembrane domain is chosen from a transmembrane domain of FUT8, a transmembrane domain of beta-1,4-galactosyltransferase 1 (B4GT1), and a transmembrane domain of human calnexin (hCNX). The transmembrane domain of FUT8 may comprise an amino acid sequence comprising SEQ ID NO:25, the transmembrane domain of B4GT1 may comprise an amino acid sequence comprising SEQ ID NO:26, and the transmembrane domain of hCNX may comprise an amino acid sequence comprising SEQ ID NO:27.

[0097] According to other embodiments of the FUT8 antibody, antigen binding fragment thereof, or fusion protein thereof of the present invention, the FUT8 antibody, antigen binding fragment thereof, or the fusion protein thereof may further comprises an endoplasmic reticulum retention signal. For example, the endoplasmic reticulum retention signal may comprise an amino acid sequence comprising SEQ ID NO:28 - KDEL.

[0098] The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE 1

Soluble FUT8 expression and purification

[0099] A pTT5 expression vector (Shi 2005, Biochemistry, 44(48), 15705-15714) encoding a codon-optimized soluble and secreted FUT8 (sFUT8) enzyme (aa 87-575; UniProtKB #Q9BYC5) with a N-terminal signal peptide (SEQ ID NO: 29 - MRLPAQLLGLLMLWVSGSSGDV) and a C-terminal polyhistidine tag (SEQ ID NO: 30 - GGGHHHHHHHHHHG) was transiently transfected in 293-6E cells as described previously (Raymond 2011, Methods (San Diego, Calif.), 55(1), 44-51). Culture medium was harvested 5 days post-transfection and purified by immobilized metal affinity chromatography as described in Dorion-Thibaudeau *et al.* 2014, J Immunol Methods, 408, 24-34.

EXAMPLE 2

Anti-sFUT8 monoclonal antibody generation

[00100] Animal immunization. Four six-week old female A/J mice (The Jackson Laboratory, Bar Harbor, ME) were bled (pre-immune serum) and injected intraperitoneally and subcutaneously with 100 µg of sFUT8 antigen emulsified in Titermax adjuvant (Cedarlane Labs, Burlington, ON) at day 0 and at day 21. Blood was collected in microvette CB 300Z (Sarstedt, Montreal, QC) at day 31 or 38, and serum was stored at -20°C until further use.

[00101] *ELISA (serum titer determination).* Pre- and post-immune sera titers of animals were assessed by ELISA. Unless otherwise stated, all incubations were performed at room temperature. Briefly, half-area 96-well plates (Costar® #3690) were coated with 25 µl per well of immunogen at 20 µg/ml in PBS and incubated overnight at 4°C. Microplates were washed three times in PBS and

blocked for 30 min with PBS containing 1% bovine serum albumin (BSA, Sigma® Cat#A7030). Blocking buffer was removed and 25 µl of serial dilutions of sera samples were added. After a 2-h incubation, microplates were washed 4 times with PBS-Tween 20 0,05% and 25 µl of a 1/5,000 dilution of alkaline phosphatase conjugated goat anti-mouse IgG (H+L) (#115-056-062, Jackson Immunoresearch®, Cedarlane, Burlington, ON) in blocking buffer was added. After a 1-h incubation, microplates were washed 4 times and 25 µl of p-nitrophenyl phosphate (pNPP) substrate (Sigma-Aldrich Canada Co.®, Oakville, ON) at 1 mg/ml in carbonate buffer at pH 9.6 was added and further incubated for 30 min. Absorbance was read at 405 nm using a SpectraMax® plate reader (Molecular Devices®, Sunnyvale, CA). All pre-immune bleeds were negative and all post-immune bleeds were very strong (above 1/12800) on recombinant protein. A final intraperitoneal booster injection using 100 µg of recombinant protein in PBS was done 3 days prior to fusion experiment.

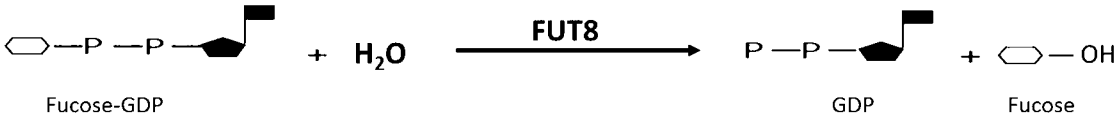
[00102] *Fusion of the harvested spleen cells*. All manipulations were done under sterile conditions. Spleen cells were harvested in Iscove's Modified Dulbecco's medium (IMDM, Gibco® Cat. #31980-030) and fused to NS0 myeloma cell line using polyethylene glycol. Spleen cells and myeloma cells were washed in IMDM, counted in RBC lysing buffer (Sigma, Cat#7757-100ML) and mixed together at a 5:1 ratio. Pelleted cells were fused together by adding 1 ml of a 50% solution of PEG 4000 (EMD-Millipore® Cat#9727-2) in PBS preheated at 37°C drop-wise over one minute, and incubated at 37°C for an additional 90 sec. The reaction was stopped by addition of 30 ml of IMDM at 22°C over 2 min. After a 10 min incubation, freshly fused cells were spun at 233xg for 10 min. Cells were washed once in IMDM supplemented with 10% heat inactivated FBS (Sigma Cat #F1051) and suspended at a concentration of $2x10^5$ input myeloma cells per ml in HAT selection medium (IMDM containing 20% heat inactivated FBS, penicillin-streptomycin (Sigma® Cat#P7539), 1 ng/ml mouse IL-6 (Biolegend Cat#575706), HAT media supplement (Sigma® Cat#H0262) and L-

glutamine (Hy-Clone® Cat#SH30034.01) and incubated at 37°C, 5% CO_2. The next day, hybridoma cells were washed and suspended at a concentration of 2-3x10^5 input myeloma cells per ml in semi-solid medium D (StemCell Technologies® Cat.#03804) supplemented with 5% heat inactivated FBS, 1 ng/ml mouse IL-6 and 10 µg/ml FITC- F(ab')2 Goat anti-mouse IgG (Jackson® # 115-056-062). The cell mixture was plated in Omnitray® dish (Nunc® cat#242811) and further incubated for 6-7 days at 37°C, 5% CO_2. Fluorescent secretor clones were then transferred using a mammalian cell clone picker (ClonepixFL®, Molecular Devices®) into sterile 96-w plates (Costar® #3595) containing 200 µl of IMDM supplemented with 20% heat inactivated FBS, penicillin-streptomycin, 1 ng/ml mouse IL-6, HT media supplement (Sigma® Cat# H0137) and L-glutamine and incubated for 2-3 days at 37°C, 5% CO2.

[00103] *Screening*. Hybridoma supernatant were screened by ELISA to detect specific binders. To this end, 96-wells half-area plates (Costar® #3690) were coated with 25 µl of sFUT8 at 20 µg/ml or an irrelevant control protein at 5 µg/ml in PBS and incubated overnight at 4°C. Microplates were washed 3 times with PBS, blocked with PBS-BSA 1%, and 25 µl of hybridoma supernatant were added and incubated at 37°C, 5% CO_2 for 2 hours. Plates were washed 4 times with PBS-Tween 20 0,05% and incubated for one hour at 37°C, 5% CO_2 with 25 µl of secondary antibody alkaline phosphatase conjugated F(ab')2 goat anti-mouse IgG (Jackson Immunoresearch # 115-056-062) diluted 1/5000 in blocking buffer. After 4 washes with PBS-Tween 20 0,05%, 25 µl of a 1 mg/ml pNPP substrate solution was added and further incubated for one hour at 37°C. OD405nm measurements were done using a microplate reader (Spectramax® 340 PC, Molecular Devices®). Hits were confirmed using alkaline phosphatase conjugated F(ab')2 goat anti-mouse IgG Fc gamma specific (Jackson Immunoresearch® # 115-056-071) and 50 mAbs were selected for further characterization.

[00104] *Recloning of hybridomas*. Selected hybridoma were recloned by limiting dilution to ensure their monoclonality.

[00105] *FUT8 enzyme inhibition assay*. The assay is shown below:



[00106] The assay reaction was done using 2 mM FUT8 enzyme, 60 mM Fucose-GDP, purified mAb (1-5 µM) in DPBS and 30 mM MES buffer pH7.0 for 2 hours at room temperature. The % inhibition was determined based on the changes in signals for the GDP-fucose and GDP measured by LC-MS relative to control experiments (i.e. substrate alone, and substrate + enzyme in absence of mAb). The results of this assay are shown in Fig. 1 and the mAbs 1H9, 1D2 and 5C9 were selected as potent FUT8 inhibitors. The sequence of these mAbs are shown in Fig. 2.

EXAMPLE 3

Making of recombinant anti-FUT8

[00107] Now referring to Fig. 3, the V_H and V_L regions of the candidate mAbs 1H9, 1D2 and 5C9 were sequenced, synthesized and cloned into the pTT5 vector in-frame with a constant domain of a human IgG1 heavy chain (comprising CH1, CH2 and CH3 regions) or in-frame with a constant domain of a human kappa light chain, and recombinant mAbs were produced in CHO-3E7 cells by transient transfection according to Delafosse et al 2016, J Biotechnol, 227, 103-111.

EXAMPLE 4

Cell surface fucosylation measurement by flow cytometry

[00108] Approximately $0{,}75 \times 10^6$ CHO cells were centrifuged (280g for 3 min) and resuspended in 1 ml PBS containing 0,5% (w/v) BSA. Ten µl of

biotinylated *Aleuria Aurantia* Lectin, (bAAL; Vector Laboratories® Inc. cat#B-1395) was added to the cell suspension followed by a 30 min incubation on ice. Cells were centrifuged and resuspended into 1 ml PBS containing 0,5% (w/v) BSA. To the cell suspension, 10 µl of Streptavidin-FITC was added followed by 30 min incubation on ice. Cells were washed once with 50 µl PBS/0,5% (w/v) BSA, resuspended in 500 µl of PBS/0,5% (w/v) BSA and filtered through a 30 µm mesh Nytex® filter prior to flow cytometry analysis. Now referring to Fig. 4, the results show that mock transfected cells are fully fucosylated, while cells transfected with RMD show decreased fucosylation. Now referring to Fig. 5, the results show that mAbs 1H9, 1D2 and 5C9 fused with a retention signal (KDEL) are able to inhibit fucosylation with various efficacies when transiently expressed in CHO cells.

EXAMPLE 5

Western blot analysis using lectin

[00109] Following protein separation by SDS-PAGE and transfer to a nitrocellulose membrane, protein fucosylation was revealed by incubating the membrane in the presence of biotinylated LCA (10 µg/mL) or AAL (10 µg/mL) lectins diluted in blocking solution for 1h at RT. Following extensive washing, the membranes were incubated with Streptavidin-HRP for 1h at RT. Membranes were then revealed using Biorad®'s ECL detection kit (Clarity™ Western ECL Substrate).

EXAMPLE 6

Inhibition of FUT8 activity with intrabodies

[00110] Now referring to Fig. 6, the recombinant FUT8 antibodies (1H9, 1D2 & 5C9) with a C-terminal KDEL sequence of the heavy chain were coexpressed with a one-arm monoclonal antibody (OAA) in CHO cells constructed using the Azymetric Het-Fc scaffold (Von Kreudenstein 2013, MAbs 5(5):646-654). The OAA was selected because of its smaller size (100 kDa)

compared to the anti-FUT8 mabs (~150 kDa; arrow), allowing for their discrimination on the gel. As a positive control for reduced fucosylation, the OAA antibody was co-expressed with RMD encoding plasmid (Von Horsten 2010, Glycobiology 20(12):1607-1618). Five days later, supernatants were harvested and analyzed by SDS-PAGE and lectin-blot detection using biotinylated Lens Culinaris agglutinin (bLCA).

[00111] The Coomassie-blue stained SDS-PAGE shows that the OAA was present in the supernatant for all co-transfection experiment. However, the gel also indicate that the C-terminal KDEL sequence is not sufficient to fully retain the anti-FUT8 mabs within the cells as we can clearly detect them in the supernatants (as shown by the "intrabodies" arrow at ~150 kDa). The corresponding bLCA lectin-blot shows that fucosylation of the OAA is easily detected in the control lane (transfection of OAA only) while it is strongly reduced by RMD co-expression. For the anti-FUT8 intrabodies, reduction of fucosylation was most apparent when OAA was co-transfected with 1H9.

EXAMPLE 7

Surface Plasmon Resonance (SPR)

[00112] Now referring to Fig. 7, the binding of FUT8 to indirectly capture anti-FUT8 antibody was determined by surface plasmon resonance using a BioRad® Proteon® (Mississauga, ON). Each of the recombinant FUT8 antibodies 1H9, 1D2 & 5C9 was tested. PBS containing 0.05% Tween 20 (Teknova® Inc., Hollister, CA) and 3.4 mM EDTA was used as a running buffer at 25°C. An anti-human Fc capture surface was made with goat anti-human-Fc polyclonal antibody (Jackson ImmunoResearch®, West Grove, PA) immobilized to approximately 5000 RUs on a GLC sensorchip using standard amine coupling of a 25 µg/mL solution in 10 mM NaOAc pH 4.5. For the binding assay, approximately 1000 RUs of each anti-FUT8 antibody to be tested was captured onto the anti-human Fc surface by injecting 5 µg/mL anti-FUT8 solution for 240

seconds at flow rate 25 µL/min. This was followed by injection of a FUT8 dilution series (100 / 33.3 / 11.1 / 3.7 / 1.23 nM) and PBST running buffer for referencing. 120 second injections of each FUT8 concentration were used at a flow rate of 50 µL/min and with 900 second dissociation. Surfaces were regenerated with two 18 secs pulses of 0.85% phosphoric acid at a flow rate 100 µL/min. Sensorgrams were double referenced using mock-activated reference spots, and data were analyzed within BioRad Proteon evaluation software v3.1.

EXAMPLE 8

Cell surface fucosylation measurement by flow cytometry

[00113] Now referring to Figs. 8 and 9. Fig. 8 shows graphical representations of Fab and scFv versions of the mAbs of the present invention, which were prepared. Fig. 9 shows the measured cell surface fucosylation levels as evidenced by labeling with biotinylated AAL and detection with FITC-streptavidin. Total cell surface fluorescence level inhibited by membrane anchored scFv's and Fab variants of the mAbs 1H9 and 5C9, as was measured by flow cytometry. The %-positive cells is multiplied by the mean fluorescence intensity to get the total cell-surface fucosylation level as measured by lectin. The results show that the 1H9 is the most efficient to decrease fucosylation. However, Fab cannot be used to produce afucosylated mAb because of light chain scrambling.

EXAMPLE 9

Test of additional scFv constructs

[00114] Fig. 10 illustrates schematic representations of additional constructs according to the present invention that were prepared. Fig. 11 shows that cells expressing the identified constructs were able to reduce fucosylation to various levels, especially the scFv5 construct bearing a signal peptide (SP) and the calnexin transmembrane and cytoplasmic domain was able to reduce fucosylation by 8-fold.

[00115] While preferred embodiments have been described above and illustrated in the accompanying drawings, it will be evident to those skilled in the art that modifications may be made without departing from this disclosure. Such modifications are considered as possible variants comprised in the scope of the disclosure.

EXAMPLE 10

Test of additional scFv sequences

[00116] Fig. 12 illustrates schematic representations of additional constructs that were prepared with and without the KDEL sequence according to embodiments of the present invention.

[00117] Table 1 shows the comparison between FUT8 reactivity measured by ELISA versus the inhibitory activity of FUT8 activity, for specific anti-FUT8 mAbs obtained from hybridoma sequences which were selected based on corresponding in vitro FUT8 activity inhibition percentage.

Hybridoma name	Bonding to FUT8 by ELISA	FUT8 enzymatic activity (% inhibition)
1H9	Yes	60%
5C9	Yes	87%
1D2	Yes	82%
4G1	Yes	15%
4A10	Yes	15%
4F1	Yes	0%
1C4	Yes	0%

Table 1

[00118] Fig. 13 shows that when the variable regions of the heavy (V_H) and light (V_L) chains of the selected hybridomas were introduced into the scFv5 format, cells expressing the identified constructs were able to reduce fucosylation of a co-transfected antibody to various levels compared to the control indicated

by the dashed line (upper panel: antibody 1– 2G4, an anti-ebola glycoprotein antibody, lower panel: antibody 2 - rituximab). Especially, the heavy (V_H) and light (V_L) chain variable regions sequences from the 5C9, 1D2, and 1H9 hybridomas were able to significantly reduce fucosylation (about 4-, 3.5-, and 7.5-8.7-fold, respectively). Variable region sequences which were identified in the in vitro FUT8 activity assay as moderately inhibitory or non-inhibitory (4G1, 4A10, 4F1, 1C4) were also non-significantly inhibitory in this cell based assay. In the context of the scFv2 format, adding a KDEL sequence slightly reduced antibody fucosylation.

SEQUENCE TABLE

SEQ ID NO: 1 - 1D2 VH	QVQLKES**GPGLVAPSQSLS**ITCTVSGFSLTGYGVN W**VRQPPGKGLE**WLGMIWGDGSTDYNSALKSRLSIS KDNSKSQVFLKMNSL**QTDDTASYYCARD**FYDGYLY AMDYWGQGTSVTVSSAS
SEQ ID NO: 2 - 1D2 VL	DIVMTQ**SQKFMSTSLGDR**VSVTCKASQNVGSYVAW **YQQKPGQSPK**ALIYSASYRYSGVPDRFAGSGSGTD FTLTISNVQSEDLA**EYFCQQYYTYPYT**FGGGTWSTR LASWAFRSL
SEQ ID NO: 3 - 1D2 VH CDR1	GPGLVAPSQSLS
SEQ ID NO: 4 - 1D2 VH CDR2	VRQPPGKGLE
SEQ ID NO: 5 - 1D2 VH CDR3	QTDDTASYYCARD
SEQ ID NO: 6 - 1D2 VL CDR1	SQKFMSTSLGDR
SEQ ID NO: 7 - 1D2 VL CDR2	YQQKPGQSPK
SEQ ID NO: 8 - 1D2 VL CDR3	EYFCQQYYTYPYT
SEQ ID NO:9 - 5C9 VH	QVQLKES**GPGLVAPSQSLS**ITCTVSGFSLTGYGVN W**VRQPPGKGLE**WLGTIWGDGSTDYNSALKSRLSIS KDNSKTQVFLKMHSL**QTDDTAIYYCARG**GYDDYFG YAMDYWGQGTSVTVSS
SEQ ID NO:10 - 5C9 VL	DIVMTQ**SQKFMSTVVGDR**VSVTCKASQNVGTNVAW **YQQKPGQSPK**ALIYSASYRYSGVPDRLTGSGSGTD FTLTFSYVQSEDLA**EYFCQQYYTYPYT**FGGGTKLEIK

SEQ ID NO:11 - 5C9 VH CDR1	GPGLVAPSQSLS
SEQ ID NO:12 - 5C9 VH CDR2	VRQPPGKGLE
SEQ ID NO:13 - 5C9 VH CDR3	QTDDTAIYYCARG
SEQ ID NO:14 - 5C9 VL CDR1	SQKFMSTVVGDR
SEQ ID NO:15 - 5C9 VL CDR2	YQQKPGQSPK
SEQ ID NO:16 - 5C9 VL CDR3	EYFCQQYYTYPYT
SEQ ID NO:17 - 1H9 VH	EVQLQQS**GPELVKPGASVK**MSCKASGYIFTDYVMH W**VKQSNGKSLE**WIGYINPYNDYSNYNQKFKGKATL TVDKSSNTAYMQLNS**LTSEDSAVYFCAR**SGDVWLA YWGQGTLVTISAAS
SEQ ID NO:18 - 1H9 VL	DIVLTQ**SPASLAISLGQR**ATISCRASKSVSTSGYS**YM HWYQQKP**GQPPRLLIYLASNLESGVPARFSGSGSG TDFTLNIHPVEE**EDGATYYCQHSRE**LPWTFGGGT
SEQ ID NO:19 - 1H9 VH CDR1	GPGLVAPSQSLS
SEQ ID NO:20 - 1H9 VH CDR2	VRQPPGKGLE
SEQ ID NO:21 - 1H9 VH CDR3	QTDDTAIYYCARG
SEQ ID NO:22 - 1H9 VL CDR1	SQKFMSTVVGDR

SEQ ID NO:23 - 1H9 VL CDR2	YQQKPGQSPK
SEQ ID NO:24 - 1H9 VL CDR3	EYFCQQYYTYPYT
SEQ ID NO :25 – Human fucosyltransferase-8 transmembrane domain	WIMLILFAWGTLLFYIGGHL
SEQ ID NO :26 – Human β4-galactosyltransferase-1 transmembrane domain	LLVAVCALHLGVTLVYYLAG
SEQ ID NO :27 – Human Calnexin transmembrane domain	WLWVVYILTVALPVFLVILFC
SEQ ID NO:28 - endoplasmic reticulum retention signal	KDEL
SEQ ID NO:29 – N-Terminal signal peptide	MRLPAQLLGLLMLWVSGSSGDV
SEQ ID NO:30 - C-terminal polyhistidine tag	GGGHHHHHHHHHHG

CLAIMS :

1. An alpha-(1,6)-fucosyltransferase (FUT8) antibody, an antigen binding domain thereof, or a fusion protein thereof, comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, the antibody or antigen binding domain thereof, or fusion protein thereof being operable to inhibit FUT8 activity in a cell.

2. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof of claim 1, wherein said fusion protein comprises a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

3. A cell expressing the FUT8 antibody, antigen binding domain thereof or fusion protein thereof of any one of claims 1 - 2.

4. A method for producing a recombinant antibody having reduced fucosylation comprising :

a) culturing a host cell expressing a FUT8 antibody, antigen binding domain thereof or fusion protein thereof of any one of claims 1 - 2,
wherein said host cell expresses said recombinant antibody under conditions which permit the production of said recombinant antibody; and
b) isolating said recombinant antibody.

5. A method for producing a recombinant protein having reduced fucosylation comprising :

a) culturing a host cell expressing a FUT8 antibody, antigen binding domain thereof, or fusion protein thereof of any one of claims 1 - 2,
wherein said host cell expresses said recombinant protein under conditions which permit the production of said recombinant protein; and

b) isolating said recombinant protein.

6. A nucleic acid vector comprising a nucleotide sequence encoding an alpha-(1,6)-fucosyltransferase (FUT8) antibody, antigen binding domain thereof or fusion protein thereof according to any one of claims 1 – 2..

7. A method for inhibiting expression and/or activity of a secreted protein in a cell comprising culturing a cell expressing an antibody, antigen binding domain thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, operable to inhibit expression and/or activity of said protein in said cell, said antibody, antigen binding domain thereof, or said fusion protein thereof comprising a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

8. A FUT8 antibody, an antigen binding domain thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, wherein said variable region heavy (V_H) chain comprises an amino acid sequence comprising SEQ ID NO:1, and said variable region light (V_L) chain comprises an amino acid sequence comprising SEQ ID NO:2.

9. A FUT8 antibody, an antigen binding domain thereof or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof,
wherein said variable region heavy (V_H) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5, respectively;

and wherein said variable region light (V_L) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8, respectively.

10. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof of any one of claims 8-9, wherein said antibody is antibody 1D2.

11. A FUT8 antibody, an antigen binding domain thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, wherein said variable region heavy (V_H) chain comprises an amino acid sequence comprising SEQ ID NO:9, and said variable region light (V_L) chain comprises an amino acid sequence comprising SEQ ID NO:10.

12. A FUT8 antibody, an antigen binding domain thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof,
wherein said variable region heavy (V_H) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13, respectively;
and wherein said variable region light (V_L) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16, respectively.

13. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof of any one of claims 11-12, wherein said antibody is antibody 5C9.

14. A FUT8 antibody, an antigen binding domain thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof, wherein said variable region heavy (V_H) chain comprises an amino acid

sequence comprising SEQ ID NO:17, and said variable region light (V_L) chain comprises an amino acid sequence comprising SEQ ID NO:18.

15. A FUT8 antibody, an antigen binding domain thereof, or a fusion protein thereof comprising a variable region of a heavy (V_H) and a light (V_L) chain thereof,
wherein said variable region heavy (V_H) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, respectively;
and wherein said variable region light (V_L) chain comprises CDR 1, CDR2 and CDR3 comprising an amino acid sequence comprising SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively.

16. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof of any one of claims 14-15, wherein said antibody is antibody 1H9.

17. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof of any one of claims 8 - 16, further comprising a transmembrane domain of a protein resident in an endoplasmic reticulum (ER), a cis Golgi apparatus, a trans Golgi apparatus, or a combination thereof.

18. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof of any one of claims 1-2, 8-17, the cell of claim 3, the method of any one of claims 4-5 and 7, or the nucleic acid vector of claim 6, wherein said FUT8 antibody or a fusion protein thereof further comprises an endoplasmic reticulum retention signal.

19. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof of any one of claims 1-2, 8-17, the cell of claim 3, the method of any one

of claims 4-5 and 7, or the nucleic acid vector of claim 6, wherein said FUT8 antibody, antigen binding domain, or fusion protein is an IgA, IgD, IgE, IgM, Fab, scFv, scFab, sdAb, any antigen binding protein, or any combination thereof.

20. The method of claim 4, wherein said fusion protein is a scFv.

21. The cell of claim 3, the method of any one of claims 4-5 and 7, or the nucleic acid vector of claim 6, wherein said FUT8 antibody and/or said fusion protein is the FUT8 antibody, antigen binding domain thereof, or fusion protein thereof according to any one of claims 1-2, 8-17.

22. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof of any one of claims 1-2, 8-17, the cell of claim 3, the method of any one of claims 4-5 and 7, or the nucleic acid vector of claim 6, wherein said transmembrane domain is chosen from a transmembrane domain of FUT8, a transmembrane domain of beta-1,4-galactosyltransferase 1 (B4GT1), and a transmembrane domain of human calnexin (hCNX).

23. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof, cell or method according to claim 22, wherein said transmembrane domain of FUT8 is comprising an amino acid sequence comprising SEQ ID NO:25, said transmembrane domain of B4GT1 is comprising an amino acid sequence comprising SEQ ID NO:26, and said transmembrane domain of hCNX is comprising an amino acid sequence comprising SEQ ID NO:27.

24. The FUT8 antibody, antigen binding domain thereof, or fusion protein thereof, cell or method according to claim 18, wherein said endoplasmic reticulum retention signal is comprising an amino acid sequence comprising SEQ ID NO:28.

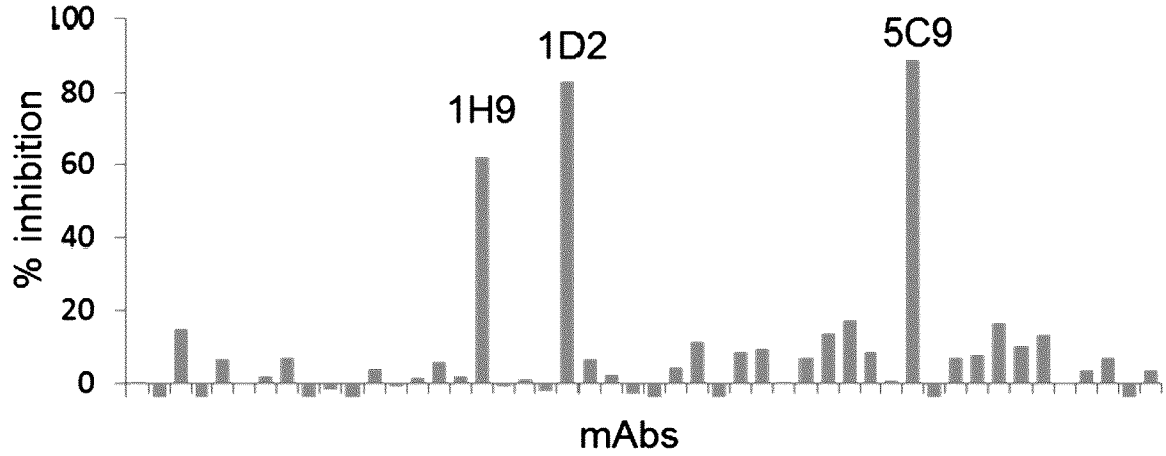


FIG. 1

```
1H9VH     EVQLQQSGPELVKPGASVKMSCKASGYIFTDYVMHWVKQSNGKSLEWIGY
1D2VH     Q...KE...G..A.SQ.LSIT.TV..FSL.G.GVN..R.PP..G...L.M
5C9VH     Q...KE...G..A.SQ.LSIT.TV..FSL.G.GVN..R.PP..G...L.T

1H9VH     INPYNDYS-NYNQKFKGKATLTVDKSSNTAYMQLNSLTSEDSAVYFCARS
1D2VH     .--WG.G.TD..SAL.SRLSISK.N.KSQVFLKM...QTD.T.S.Y...D
5C9VH     .--WG.G.TD..SAL.SRLSISK.N.KTQVFLKMH..QTD.T.I.Y...G

1H9VH     ---G--DVW---LAYWGQGTLVTISA
1D2VH     FYD.--YLYA--MD......S..V.S
5C9VH     ---.YD.YFGYAMD......S..V.S


1H9VL     EFATMETDTLLLWVLLLWVPGSTGDIVLTQSPASLAISLGQRATISCRAS
1D2VL     ......SQ.QVFVYM...LS.VD....M...QKFMST...D.VSVT.K..
5C9VL     ......SQ.QVFVYM...LS.VD....M...QKFMSTVV.D.VSVT.K..

1H9VL     KSVSTSGYSYMHWYQQKPGQPPRLLIYLASNLESGVPARFSGSGSGTDFT
1D2VL     QN.G-----..VA........S.KA...S..YRY....D..A.........
5C9VL     QN.G.N----VA........S.KA...S..YRY....D.LT.........

1H9VL     LNIHPVEEEDGATYYCQHSRELPWTFGGGT
1D2VL     .T.SN.QS..L.E.F..QYYTY.Y......
5C9VL     .TFSY.QS..L.E.F..QYYTY.Y......
```

FIG. 2

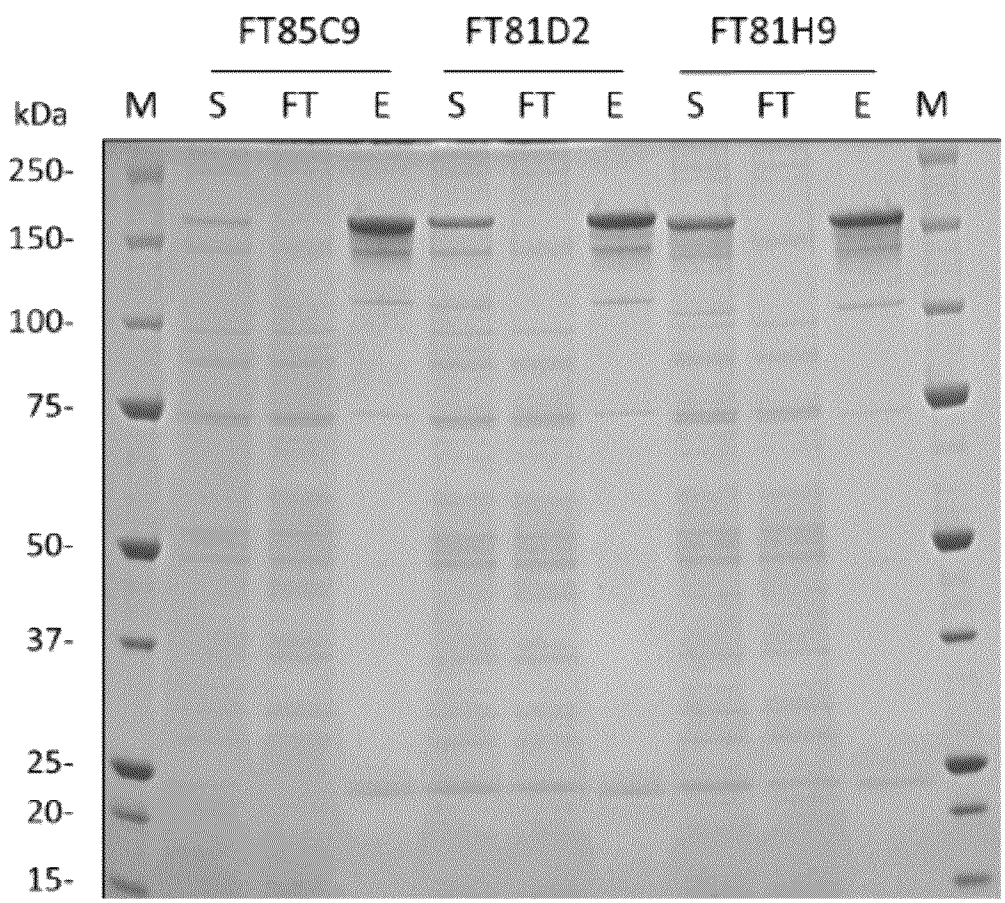

FT85C9
FT81D2
FT81H9
kDa
M
S
FT
E
S
FT
E
S
FT
E
M
250-
150-
100-
75-
50-
37-
25-
20-
15-

FIG. 3

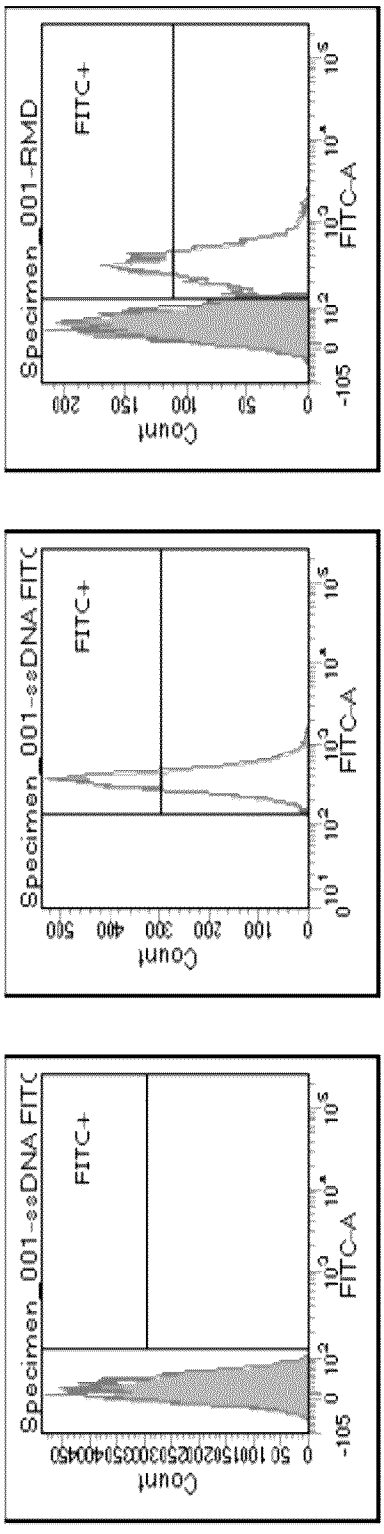

Specimen_001-ssDNA FITC
FITC+
Count
FITC-A
StrepFITC only
(neg control)
Specimen_001-ssDNA FITC
FITC+
Count
FITC-A
B-AAL+StrepFITC
Mock-transfected cells
= 100% fucosylated
Specimen_001-RMD
FITC+
Count
FITC-A
B-AAL+StrepFITC
RMD-transfected cells
= decreased fucosylation
(Probiogen's technology)

FIG. 4

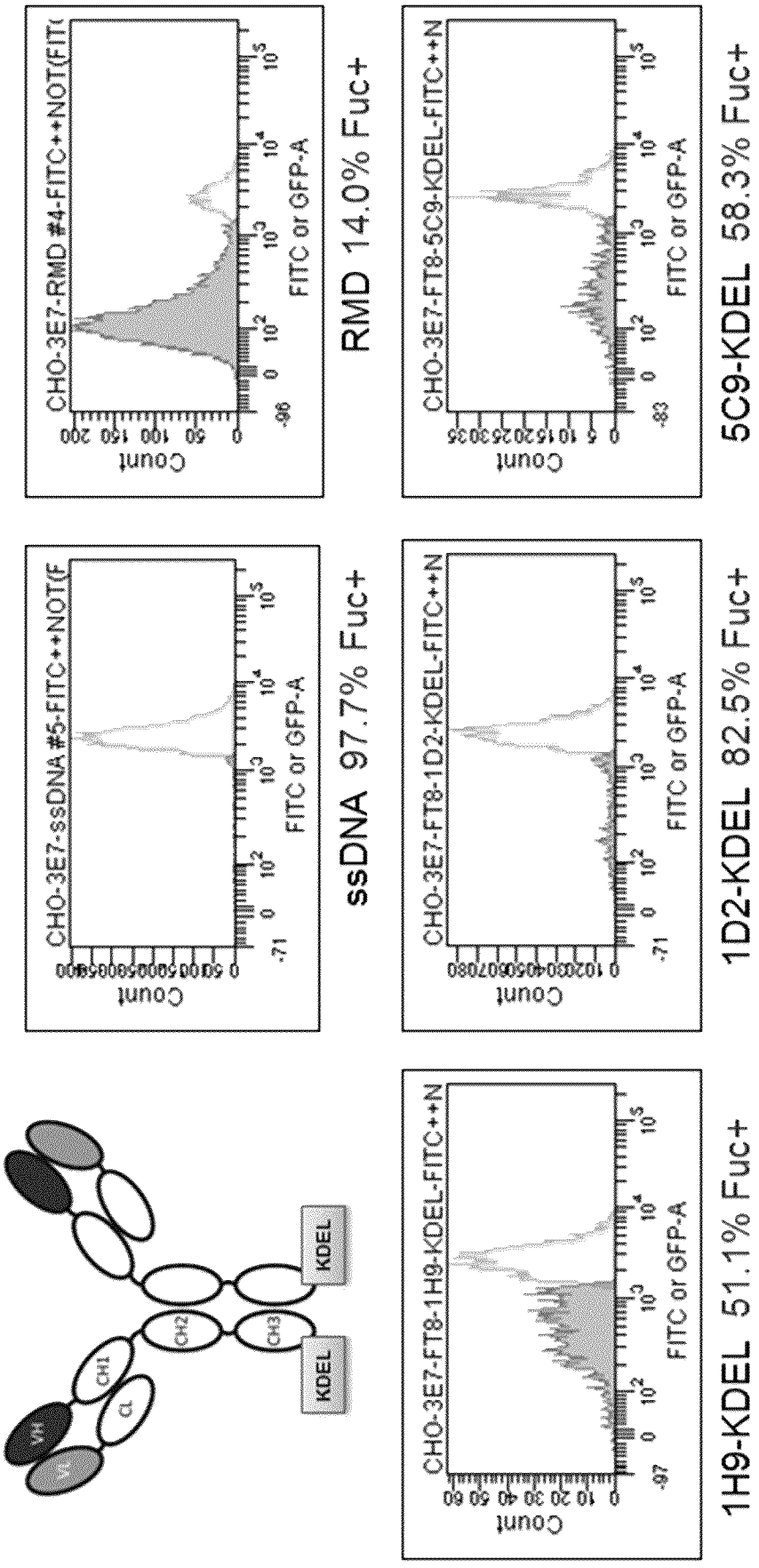

CHO-3E7-RMD #4-FITC++NOT(FIT(
Count
FITC or GFP-A
RMD 14.0% Fuc+
CHO-3E7-FT8-5C9-KDEL-FITC++N
5C9-KDEL 58.3% Fuc+
CHO-3E7-ssDNA #5-FITC++NOT(F
ssDNA 97.7% Fuc+
CHO-3E7-FT8-1D2-KDEL-FITC++N
1D2-KDEL 82.5% Fuc+
CHO-3E7-FT8-1H9-KDEL-FITC++N
1H9-KDEL 51.1% Fuc+
VH
VL
CH1
CL
CH2
CH3
KDEL
KDEL

FIG. 5

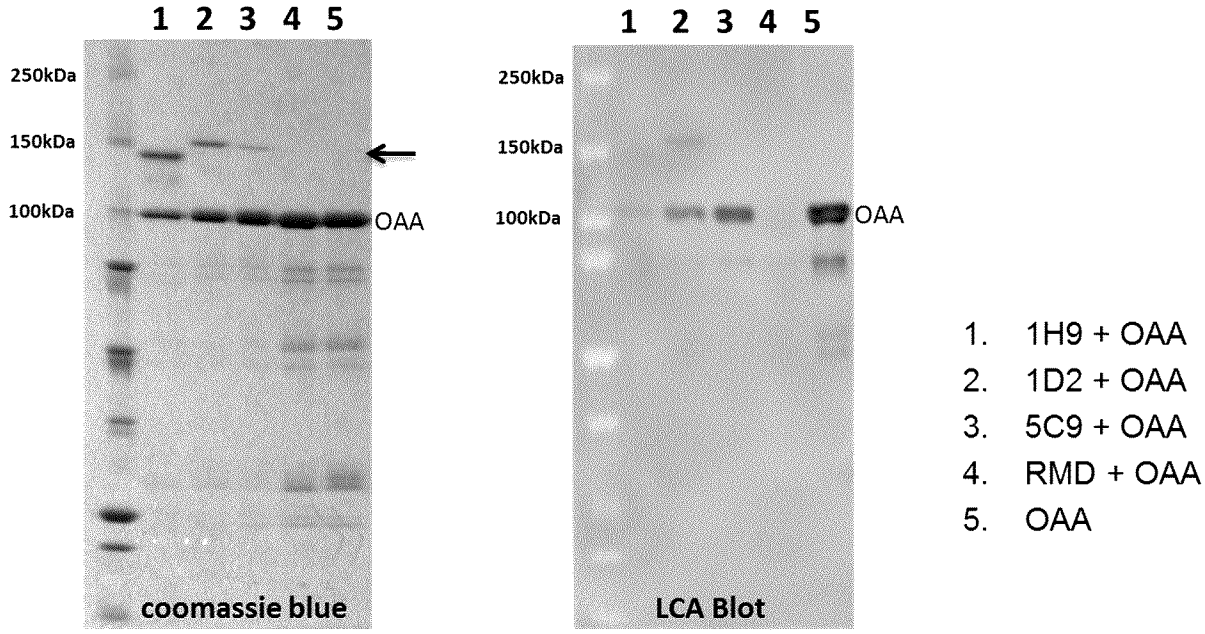
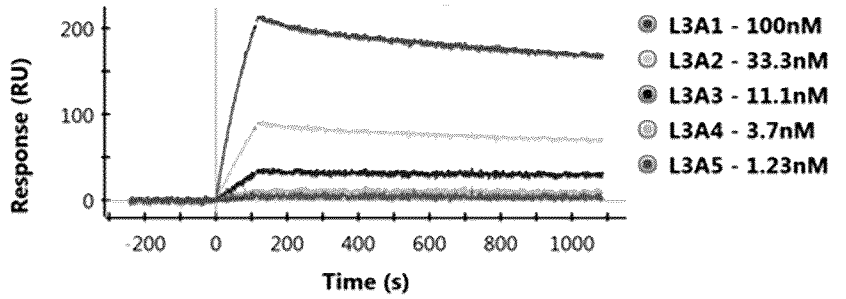
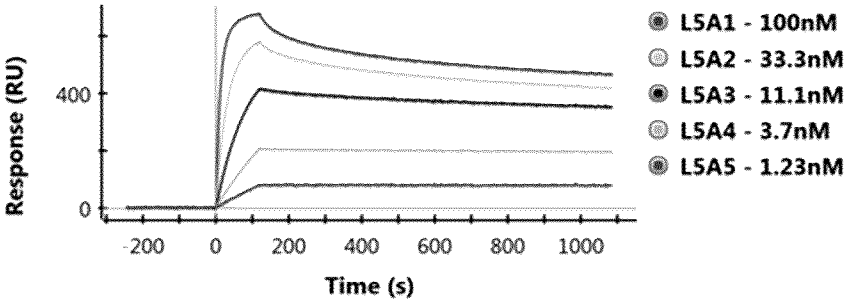

1 2 3 4 5
250kDa
150kDa
100kDa
OAA
coomassie blue
1 2 3 4 5
250kDa
150kDa
100kDa
OAA
LCA Blot
1. 1H9 + OAA
2. 1D2 + OAA
3. 5C9 + OAA
4. RMD + OAA
5. OAA

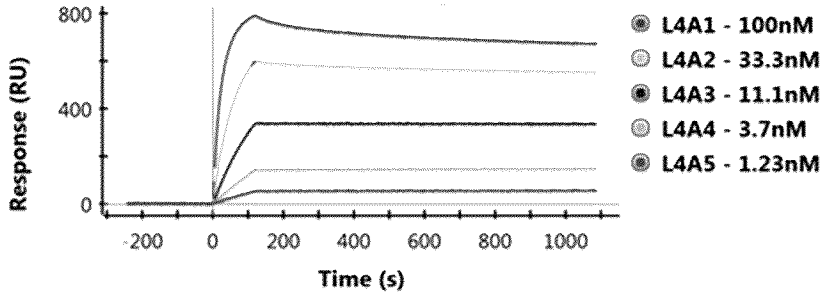
FIG. 6



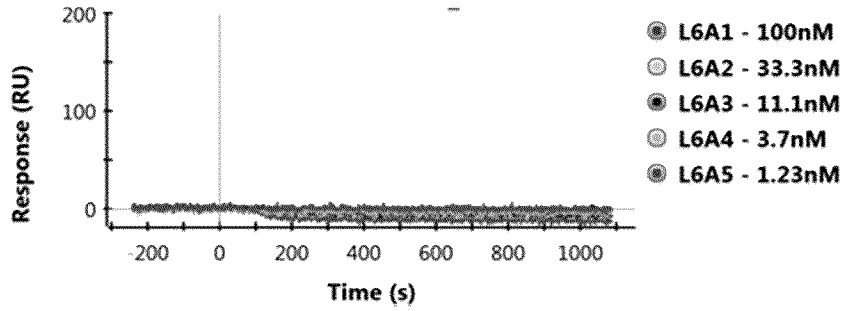
1H9



1D2



5C9



Buffer

FIG. 7

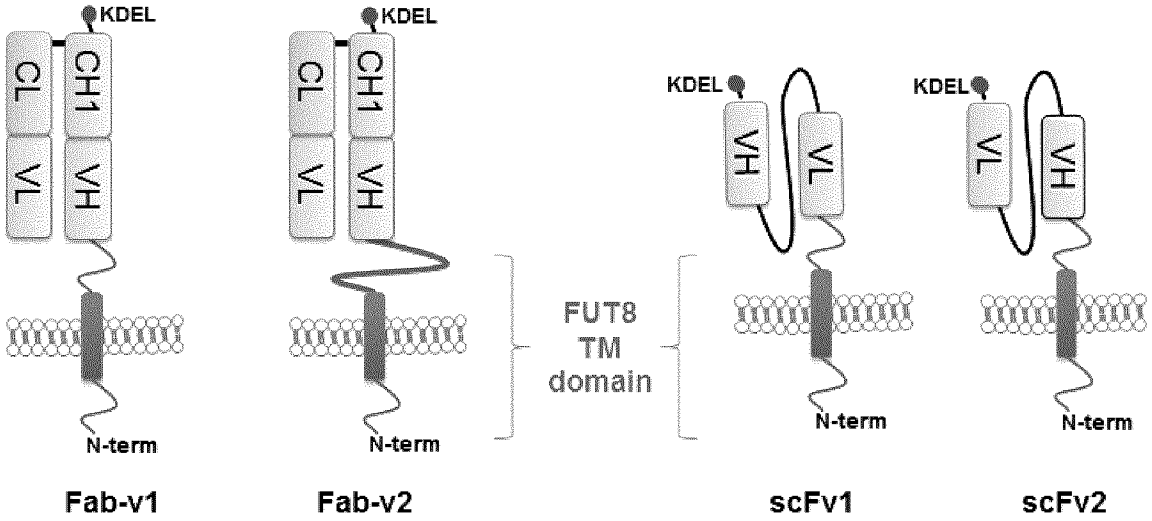

KDEL
CL
CH1
VL
VH
N-term
Fab-v1
KDEL
CL
CH1
VL
VH
N-term
Fab-v2
FUT8
TM
domain
KDEL
VH
VL
N-term
scFv1
KDEL
VL
VH
N-term
scFv2

FIG. 8

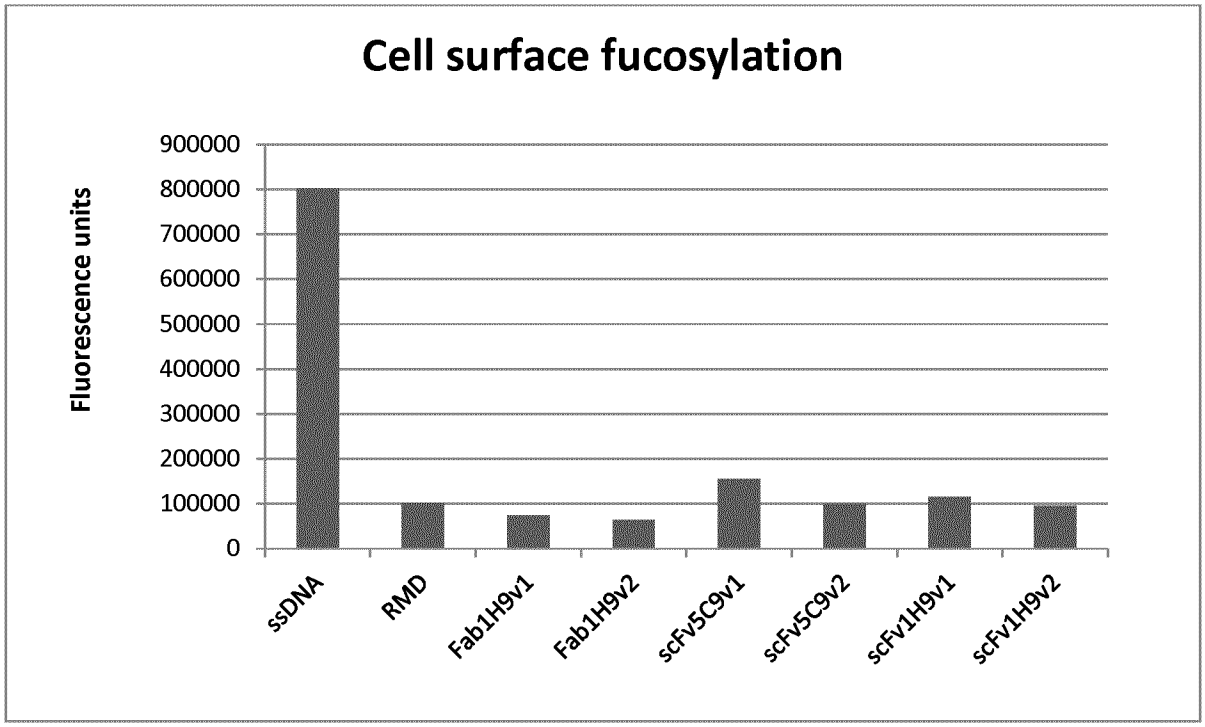

Cell surface fucosylation
Fluorescence units
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
ssDNA
RMD
Fab1H9v1
Fab1H9v2
scFv5C9v1
scFv5C9v2
scFv1H9v1
scFv1H9v2

FIG. 9

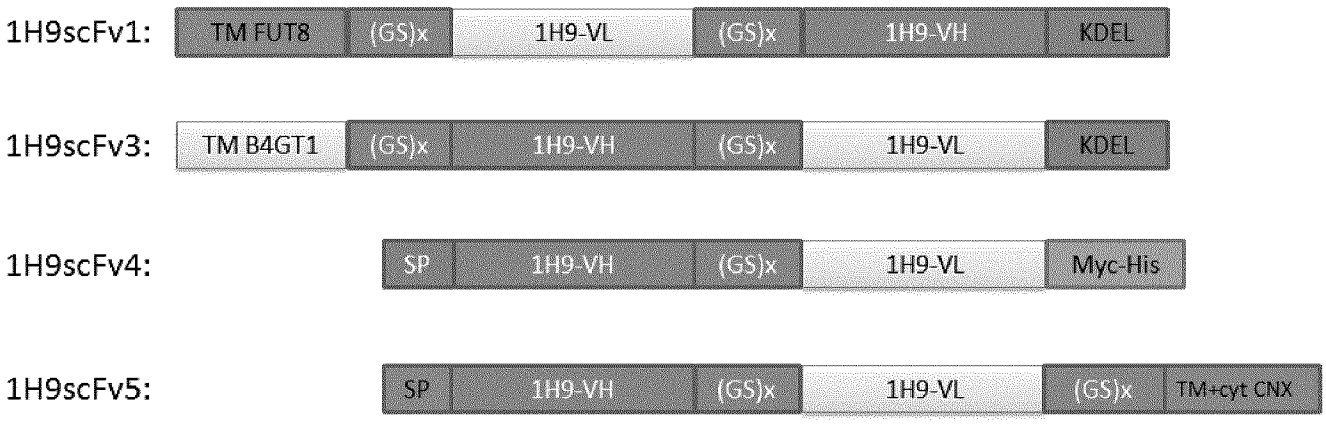
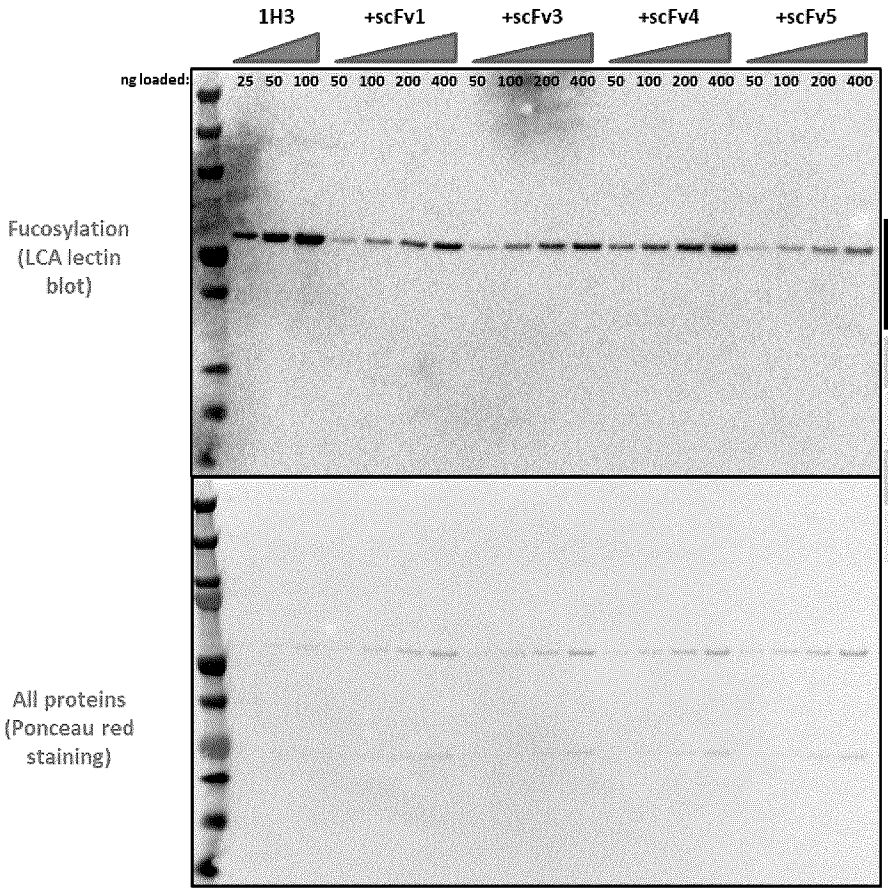


FIG. 10



Construct	Fold reduction in fucosylation vs control
scFv1	6
scFv3	6
scFv4	3
scFv5	8

FIG. 11

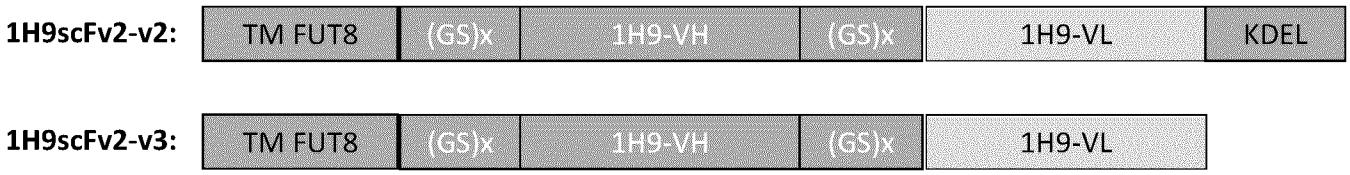
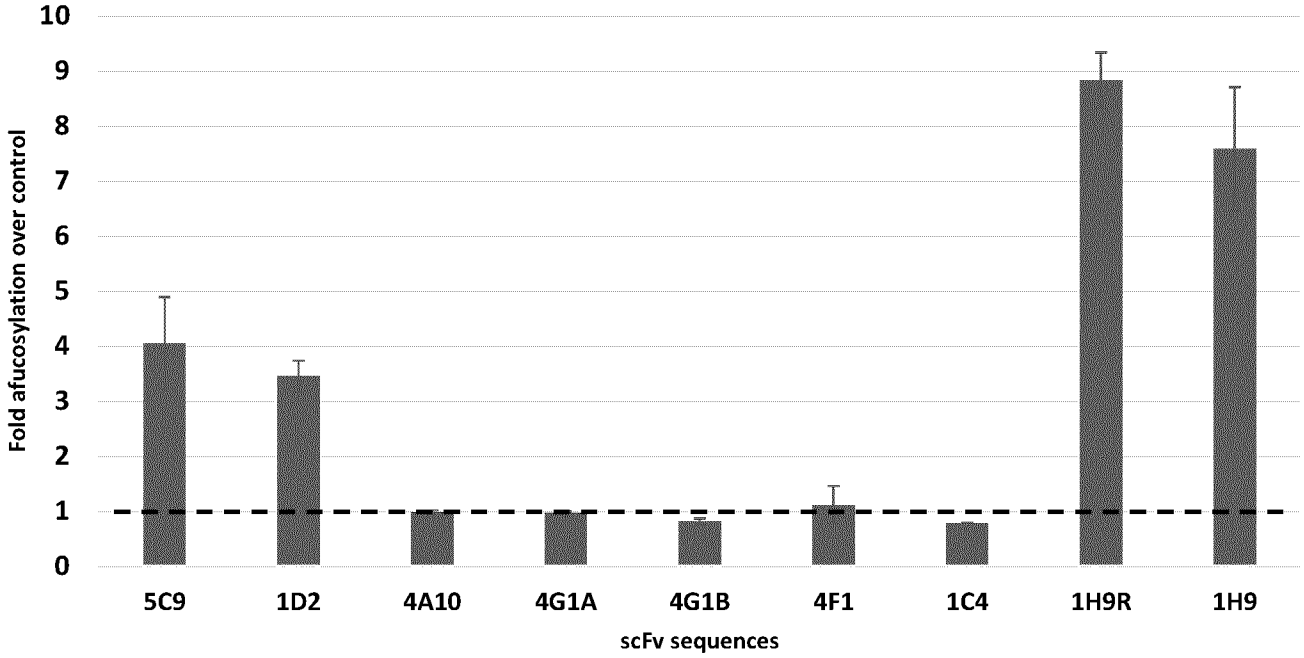

1H9scFv2-v2:
TM FUT8
(GS)x
1H9-VH
(GS)x
1H9-VL
KDEL
1H9scFv2-v3:
TM FUT8
(GS)x
1H9-VH
(GS)x
1H9-VL

FIG. 12


Fold afucosylation over control
10
9
8
7
6
5
4
3
2
1
0
5C9
1D2
4A10
4G1A
4G1B
4F1
1C4
1H9R
1H9
scFv sequences

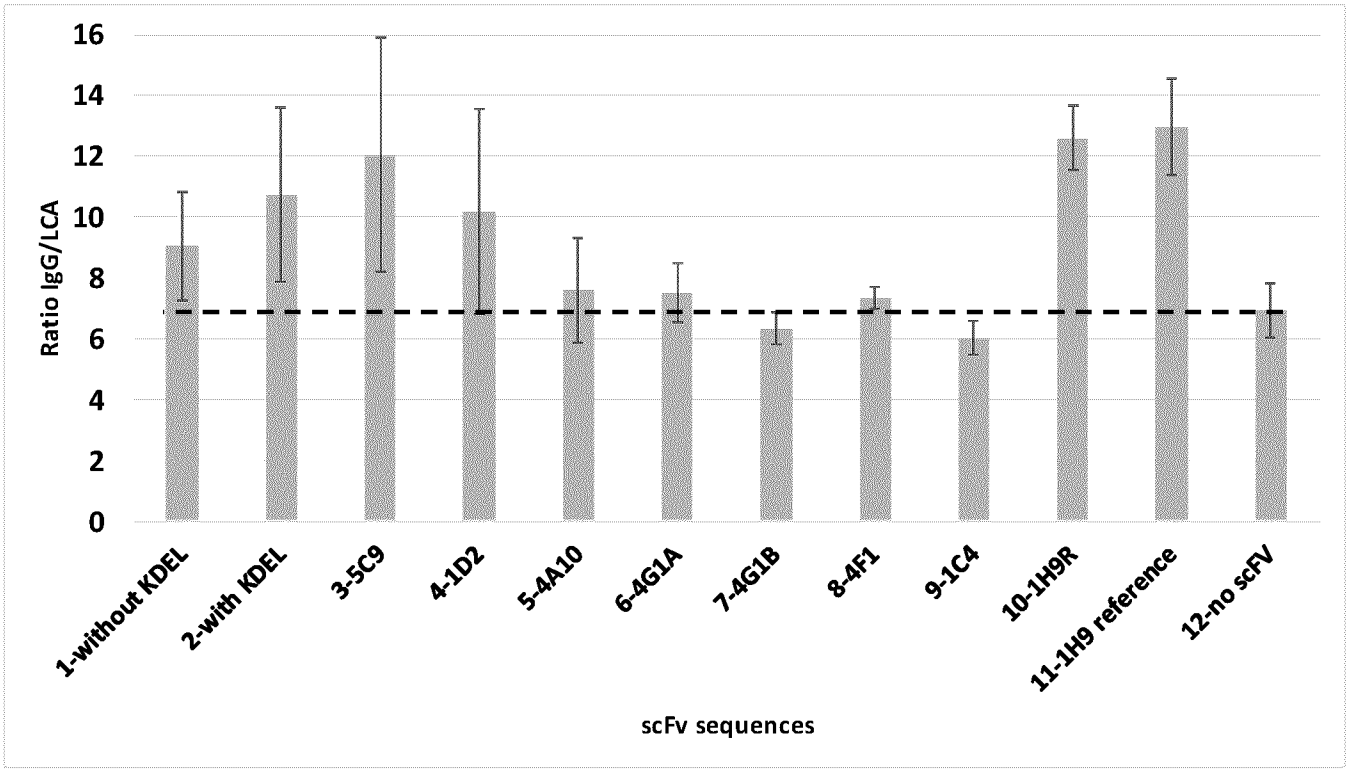

Ratio IgG/LCA
16
14
12
10
8
6
4
2
0
1-without KDEL
2-with KDEL
3-5C9
4-1D2
5-4A10
6-4G1A
7-4G1B
8-4F1
9-1C4
10-1H9R
11-1H9 reference
12-no scFV
scFv sequences

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2019/051345

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 16/40*** (2006.01) , ***C07K 19/00*** (2006.01) , ***C12N 15/13*** (2006.01) , ***C12N 15/62*** (2006.01) , ***C12N 15/63*** (2006.01) , ***C12N 5/10*** (2006.01) , ***C12P 21/00*** (2006.01) , ***C12P 21/08*** (2006.01)
(more IPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: all

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Genomequest, Google Scholar, Scopus, Questel Orbit

Keywords: FUT8, antibody, anti-FUT8, bind*, antibod*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/009086 (COLLINGWOOD, T et al) 15 January 2009 (15-01-2009)	1-6 and 8-24
A	WO 2006/133148 (JOLY, J. C. et al) 14 December 2006 (14-12-2006)	1-6 and 8-24
A	AGRAWAL, P et al (17 June 2017) A Systems Biology Approach Identifies FUT8 as a Driver of Melanoma Metastasis. CANCER CELL 31: 804-819, ISSN: 1535-6108.	1-6 and 8-24
A	NODA, M et al (5 July 2018) Prognostic role of FUT8 expression in relation to p53 status in stage II and III colorectal cancer. PLOS ONE 13(17): e0200315, ISSN: 1932-6203.	1-6 and 8-24
A	ITO, Y. et al (2003) Expression of α1,6-fucosyltransferase (FUT8) in papillary carcinoma of the thyroid: its linkage to biological aggressiveness and anaplastic transformation. CANCER LETTERS 200:167-172, ISSN: 1872-7980.	1-6 and 8-24

☐ Further documents are listed in the continuation of Box C.

☑ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"D"	document cited by the applicant in the international application
"E"	earlier application or patent but published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 13 November 2019 (13-11-2019)	Date of mailing of the international search report 25 November 2019 (25-11-2019)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Kerry Ferguson (819) 639-7804

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2019/051345

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group A - Claims 1-6, 8-17, and 20 (all completely) and 18, 19, and 21-24 (all partially) are directed to a FUT8 antibody or antigen domain thereof, associated vectors, cells, and methods of making a recombinant protein having reduced fucosylation; and

Group B - Claim 7 (completely) and claims 18, 19, and 21-24 (all partially) are directed to a method for inhibiting expression and/or activity of a secreted protein in a cell comprising culturing an antibody, antigen binding domain or fusion protein thereof comprising a transmembrane domain of a protein resident in an endoplasmic reticulum, a cis or trans Golgi apparatus, or combination thereof.

The claims must be limited to one inventive concept as set out in PCT Rule 13.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☑ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
1-6 and 8-24 with respect to Group A

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2019/051345

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2009009086A2	15 January 2009 (15-01-2009)	WO2009009086A3	27 August 2009 (27-08-2009)
		AU2008275649A1	15 January 2009 (15-01-2009)
		AU2008275649B2	05 September 2013 (05-09-2013)
		CA2692453A1	15 January 2009 (15-01-2009)
		CA2692453C	09 January 2018 (09-01-2018)
		CN101883850A	10 November 2010 (10-11-2010)
		CN101883850B	12 November 2014 (12-11-2014)
		EP2171052A2	07 April 2010 (07-04-2010)
		EP2171052B1	27 August 2014 (27-08-2014)
		EP2527435A2	28 November 2012 (28-11-2012)
		EP2527435A3	13 February 2013 (13-02-2013)
		EP2527435A9	04 September 2013 (04-09-2013)
		EP2527435B1	17 December 2014 (17-12-2014)
		JP2010532995A	21 October 2010 (21-10-2010)
		JP5770471B2	26 August 2015 (26-08-2015)
		JP2015172081A	01 October 2015 (01-10-2015)
		KR20100052474A	19 May 2010 (19-05-2010)
		KR101510778B1	10 April 2015 (10-04-2015)
		US2009042250A1	12 February 2009 (12-02-2009)
		US7919313B2	05 April 2011 (05-04-2011)
		US2011159541A1	30 June 2011 (30-06-2011)
		US8772025B2	08 July 2014 (08-07-2014)
		US2014273097A1	18 September 2014 (18-09-2014)
		US9322036B2	26 April 2016 (26-04-2016)
		US2016194665A1	07 July 2016 (07-07-2016)
		US9890395B2	13 February 2018 (13-02-2018)
WO2006133148A2	14 December 2006 (14-12-2006)	WO2006133148A3	10 May 2007 (10-05-2007)
		AU2006255085A1	14 December 2006 (14-12-2006)
		CA2608818A1	14 December 2006 (14-12-2006)
		CN101238148A	06 August 2008 (06-08-2008)
		EP1888638A2	20 February 2008 (20-02-2008)
		IL187308D0	13 April 2008 (13-04-2008)
		JP2008541770A	27 November 2008 (27-11-2008)
		KR20080032065A	14 April 2008 (14-04-2008)
		MX2007015107A	15 February 2008 (15-02-2008)
		US2009208500A1	20 August 2009 (20-08-2009)