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(71) Applicant *(for all designated States except US)*: **ALNYLAM PHARMACEUTICALS, INC.** [US/US]; 300 Third Street, Cambridge, Massachusetts 02141 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROSSOMANDO, Anthony** [US/US]; 7 Pratt Street, South Grafton, Massachusetts 01560 (US). **MARAGANORE, John M.** [US/US]; c/o Alnylam Pharmaceuticals, Inc., 300 Third Street, Cambridge, Massachusetts 02142 (US). **THILL, Gregory P.** [US/US]; 48 Windsor Road, Milton, Massachusetts 02186 (US). **BETTENCOURT, Brian** [US/US]; c/o Alnylam Pharmaceuticals, Inc., 300 Third Street, Cambridge, Massachusetts 02142 (US).

(74) Agents: **UNDERWOOD, Robert H.** et al.; McDermott Will & Emery LLP, 28 State Street, Boston, Massachusetts 02109 (US).

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[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR PRODUCING MODIFIED GLYCOPROTEINS

(57) Abstract: The invention generally relates to compositions and methods for producing glycoproteins that have alter glycan structure and improved properties. The glycoproteins are produced by modifying the glycosylation pathways in a host cell using an RNA effector molecule, such as an siRNA. Glycan-modified proteins produced using the methods described herein have improved properties, such as improved effector activity, improved pharmacokinetic properties, reduced immunogenicity in humans and the like.

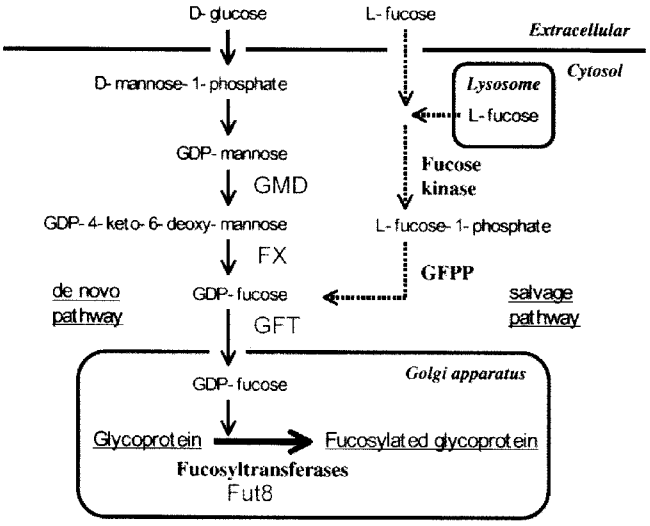


Figure 3

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COMPOSITIONS AND METHODS FOR PRODUCING MODIFIED GLYCOPROTEINS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/510,437, filed July 21, 2011, and U.S. Provisional Application No. 61/617,322, filed March 29, 2012, each of the foregoing applications is incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Glycosylation is the enzymatic process that attaches glycans to proteins, lipids, or other organic molecules. Glycosylation is a form of co-translational or post-translational modification. Glycans serve a variety of structural and functional roles in membrane and secreted

[0003] N-glycans are covalently attached to protein at asparagine residues (Asn; particularly an Asp which occurs in the sequence Asn-Xaa-Ser/Thr/Cys, where Xaa represents any amino acid) by an N-glycosidic bond. Following attachment of the saccharide moiety, further modifications may occur *in vivo*. Typically these modifications occur *via* an ordered sequence of enzymatic reactions, known as a cascade.

[0004] All N-glycans share a common core sugar sequence, Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ1-[Asn-X-Ser/Thr/Cys], and are classified into three types: (1) oligomannose, in which only mannose residues are attached to the core; (2) complex, in which "antennae" initiated by N-acetylglucosaminyltransferases (GlcNAcTs) are attached to the core; and (3) hybrid, in which only mannose residues are attached to the Manα1-6 arm of the core and one or two antennae are on the Manα1-3 arm.

[0005] Complex N-glycans often also comprise galactose, fucose, and terminal sialic acid residues. In humans, nucleotide sugar precursors (*e.g.*, UDP-N-acetylglucosamine, UDP-N-acetylgalactosamine, CMP-N-acetylneuraminic acid, UDP-galactose, GDP-fucose, *etc.*) are generally synthesized in the cytosol and transported into the Golgi, where they are attached to the core oligosaccharide by glycosyltransferases. *See, e.g.,* Sommers and Hirschberg, 1981 J. Cell Biol. 91(2): A406-A406; Sommers and Hirschberg 1982 J. Biol. Chem.257(18): 811-817; Perez and Hirschberg 1987 Methods in Enzymology 138: 709-715. Glycosyl transfer reactions

typically yield a side product which is a nucleoside diphosphate or monophosphate. While monophosphates can be directly exported in exchange for nucleoside triphosphate sugars by an antiport mechanism, diphosphonucleosides (*e.g.*, GDP) have to be cleaved by phosphatases (*e.g.*, GDPase) to yield nucleoside monophosphates and inorganic phosphate prior to being exported.

[0006] Glycosyltransferases and mannosidases are located in the inner (luminal) surface of the ER and Golgi apparatus, which provides a catalytic surface for sequential processing of glycoproteins as they proceed through the ER and Golgi network. As a glycoprotein proceeds from synthesis in the ER to full maturation in the late Golgi or trans Golgi Network (TGN), it is sequentially exposed to different glycosidases, mannosidases and glycosyltransferases such that a specific N-glycan structure may be synthesized.

[0007] The modification of serine or threonine residues on proteins by addition of a GalNAc residue results in an O-linked oligosaccharide or O-glycan. O-glycan biosynthesis is initiated by the addition of the monosaccharide GalNAc (from UDP-GalNAc) to serine and threonine residues catalyzed by a polypeptide GalNAc transferase (GalNAcT). Many O-glycans are extended into long chains with variable termini that may be similar to the termini of N-glycans. However, O-glycans are less branched than most N-glycans and are commonly biantennary structures.

[0008] Many therapeutic proteins are glycosylated, including, for example, interferons (IFNs), erythropoietin (EPO), tissue plasminogen activator (tPA), antithrombin, granulocyte-macrophage colony stimulating factor (GM-CSF), and therapeutic monoclonal antibodies. The structures of N-glycans can affect therapeutic efficacies of glycoproteins in many ways, such as pharmacokinetics (*e.g.*, half life), physical stability, protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, or immunogenicity. *See, e.g.*, Jenkins et al. (1996) Nature Biotechnololy, 14:975-981; Ghaderi et al. (2010) Nature Biotechnololy, 28:863-867.

[0009] For many antibodies, the N-glycosylation status of the Fc region of the antibody heavy chain (H-chain) plays an important role in antibody dependent dell mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). The structure and extent

of heterogeneity of these N-glycans are among the considerations in selecting a protein expression platform for a therapeutic antibody.

[0010] Accordingly, there is a need for improved methods for making glycoproteins, and for therapeutic glycoproteins that contain glycans that provide improved properties.

SUMMARY OF THE INVENTION

[0011] The invention generally relates to compositions and methods for producing glycoproteins that have alter glycan structure and improved properties. The glycoproteins are produced by modifying the glycosylation pathways in a host cell using an RNA effector molecule, such as an siRNA. Glycan-modified proteins produced using the methods described herein have improved properties, such as improved effector activity, improved pharmacokinetic properties, reduced immunogenicity in humans and the like.

BRIEF DESCRIPTION OF THE FIGURES

[0012] Figure 1 shows examples of G0, G1, and G2 N-glycans. In this figure, the glycans are fucosylated.

[0013] Figure 2 provides a schematic illustration of the glycosylation pathway in CHO cells.

[0014] Figure 3 provides a schematic illustration of fucosylation pathway in CHO cells.

[0015] Figure 4 shows the growth curve and cell viability of an exemplary 3L bioreactor. siRNA dosing days are indicated by the blue arrows.

[0016] Figure 5 shows the qPCR results that confirmed both Fut8 and GMD silencing over the time course of the bioreactor. Arrows indicate siRNA dosing days. RNAi-treated sample mRNA levels were normalized to the mRNA levels in the untreated control bioreactor.

[0017] Figure 6 shows the detection of the conversion of fucosylated glycans to their a-fucosylated forms by CE-LIF. RNAi-treated antibody samples are indicated in light blue and

the untreated control antibody samples in dark blue. The structures of the indicated glycoforms are represented below the graph. Each antibody sample was analyzed four times by CE-LIF.

[0018] Figure 7 shows the detection of the conversion of fucosylated glycans to their a-fucosylated forms by LC-MS. RNAi-treated antibody samples are indicated in light blue and the untreated control antibody samples in dark blue. The structures of the indicated glycoforms are represented below the graph. Each antibody sample was analyzed three times by LC-MS.

[0019] Figure 8 shows that RNAi-treated CD20 antibody has improved FcγRIIIa binding affinity and capacity. An approximate two-fold improvement in both affinity and binding capacity was observed with the a-fucosylated CD20 antibody (square) compared to the non-RNAi treated control (triangle) and to rituximab (inverted triangle; Mabthera). The CD20 control antibody and rituximab (a commercially available anti-CD20 antibody) had similar FcγRIIIa characteristics. Figure to the right of the curve is a schematic representation of the ELISA with expected results. Labels are indicated in figure.

[0020] Figure 9 shows that RNAi-treated CD20 antibody has improved ADCC activity. An approximate three-fold improvement in specific cell lysis was observed with the a-fucosylated CD20 antibody (square) compared to the non-RNAi treated control (triangle) and approximately a two-fold improvement over rituximab (inverted triangle; Mabthera). The CD20 control antibody and rituximab (a commercially available anti-CD20 antibody) had similar ADCC activity. Figure to the right of the curve is a schematic representation of the ADCC. Labels are indicated in figure.

DETAILED DESCRIPTION OF THE INVENTION

1. OVERVIEW

[0021] As described herein, glycoproteins that have altered glycan structures can be produced on a commercial scale by transiently reducing the expression of target genes that encode enzymes or transporters that are involved in glycosylation pathways. Transient reduction of target genes in commercial scale bioreactors can be accomplished using RNA effector molecules, such as an siRNA. Glycoproteins produced in this way have improved properties. For example, as described and exemplified herein, siRNAs were used to transiently reduce the expression of enzymes and/or transporters that are involved in the fucosylation

pathway in CHO cells. Significantly higher amount of afucosylated monoclonal antibodies were produced upon addition of siRNAs to the cell culture, and the afucosylated antibodies showed improved ADCC activity as compared to the corresponding fucosylated antibodies.

[0022] RNA effectors, such as siRNAs, can also be used to reduce the expression levels of enzymes or transporters that are involved in the production of immunogenic epitopes on glycans. Examples of epitopes on glycans that are immunogenic in humans include, e.g., N-glycans that comprise a N-glycolylneuraminic acid (Neu5Gc) residue, and the αGal epitope (galactose-alpha(1,3)-galactose-beta(1,4)N-acetylglucosamine-R; or Gal-α(1,3)-Gal-β(1,4)-GlcNAc-R; where R is the glycan-protein structure). Epitopes on glycans that are immunogenic in humans are often present on glycoproteins that are produced in non-human host cells, such as CHO cells. Glycoproteins that lack Neu5Gc or αGal epitope can reduce the likelihood of adverse immune responses (such as anaphylactic responses) when a glycoprotein is administered to a human patient, and facilitate the clinical application of many therapeutic glycoproteins. Reducing immunogenicity can also preserve efficacy and potency of the glycoprotein, especially when the glycoprotein is intended for repeated (e.g., chronic) use.

[0023] The serum half life of glycoproteins is also influenced by the composition and structure of its N-glycans, and RNA effectors, such as siRNAs, can also be used to improve pharmacokinetic properties of glycoproteins. In general, maximal serum half life of a glycoprotein requires that its N-glycans terminate with a sialic acid cap (e.g., N-acetylneuraminic acid (Neu5Ac)). RNA effectors can be used to transiently reduce the expression of sialidases, which remove the terminal sialic acid (e.g., Neu5Ac) cap from glycoprotein-glycans, thereby stabilizing the caped glycan and promoting a longer serum half life. By reducing the hydrolysis of terminal sialic acid from N-linked N-glycans, therapeutic efficacies of glycoproteins can be improved.

[0024] Accordingly, in one aspect, the invention provides glycan-modified antibodies, or fusion proteins that comprise an Fc domain of an antibody, that have improved effector activities (such as ADCC).

[0025] In another aspect, the invention provides glycan-modified glycoproteins that have reduced immunogenicity in humans.

[0026] In another aspect, the invention provides glycan-modified glycoproteins that have an increased serum half life.

[0027] A single species of RNA effector molecule can be used to reduce the expression of a single gene that encodes a protein involved in a desired glycosylation reaction. Alternatively, two or more different species of RNA effector molecules may be used, to reduce expression of two or more genes that encode a proteins involved in a desired glycosylation reaction(s).

[0028] The glycan-modified glycoproteins described herein can be formulated into a pharmaceutical formulation that is suitable for *in vivo* administration. The invention also relates to the use of the glycoproteins described herein, or pharmaceutical compositions comprising the glycoproteins, in therapy, and to the use of the glycoproteins, or pharmaceutical compositions comprising the glycoproteins, for the manufacture of a medicament for use in therapy.

[0029] Also provided are methods for producing glycan-modified glycoproteins, by treating a large scale host cell culture with RNA effector molecules.

2. DEFINITIONS

[0030] The term "about", as used here, refers to +/- 10% of a value.

[0031] The term "antibody" covers fully assembled immunoglobulins that comprise two heavy chains and two light chains (e.g., human, humanized, chimeric antibodies), as well as an antigen-binding fragment of an antibody (e.g., Fab, Fab', F(ab')2, Fv, scFv, single domain antibody) that may contain natural or introduced glycosylation site(s).

[0032] The terms "complementary," "fully complementary" and "substantially complementary" are used herein to describe the base matching between the sense strand and the antisense strand of a double-stranded RNA (dsRNA), or between the antisense strand of a RNA effector molecule and a target sequence. A nucleotide sequence is "fully complementary" to another nucleotide sequence when there are no mismatched base pairs across the length of the shorter sequence. A nucleotide sequence is "substantially complementary" to another nucleotide sequence when there are no more than 20% of the mismatched base pairs across the length of the shorter sequence (e.g., no more than 5, 4, 3, 2, or 1 mismatched base pair(s) upon hybridization for a duplex up to 30 base pairs). Where two oligonucleotides are designed to form, upon

hybridization, one or more single-stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as "fully complementary."

[0033] The term "Fc domain" refers to the Fc region of an antibody, i.e., the antibody fragment that comprises the heavy-chain constant region 2 (C_H2) and the heavy-chain constant region (C_H3) of an IgA, IgD, and IgG, and heavy-chain constant regions 2, 3, and 4 (C_H2-C_H3-C_H4) of IgE and IgM. Typically, the Fc region of an antibody comprises a dimer of two C_H2-C_H3 (or C_H2-C_H3-C_H4) chains. The Fc region does not include the variable regions of the heavy and light chains, the heavy-chain constant region 1 (C_H1), and the light-chain constant region 1 (C_L1) of the immunoglobulin. An Fc domain may further include the hinge region at the heavy-chain constant region. The term "Fc domain" also compasses fragments of the Fc region of an antibody, such as the CH_2 domain, or the C_H3 domain.

[0034] The term "fusion polypeptide" refers to a single polypeptide in which the amino acid sequence is derived from at least two different naturally occurring proteins or polypeptide chains.

[0035] The term "glycoform" of a protein refers to a protein comprising a particular glycan structure or structures. It is recognized that a glycoprotein having more than one glycosylation site can have the same glycan species attached to each glycosylation site, or can have different glycan species attached to different glycosylation sites. In this manner, different patterns of glycan attachment yield different glycoforms of a glycoprotein.

[0036] A "G0 glycoform" refers to a glycoform in which the N-glycan has the core structure of $GlcNAc_2Man_3GlcNAc_2$, wherein no terminal galactose (Gal) residue is attached to either the two mannose arms. A "G1 glycoform" or "G1' glycoform" refers to a glycoform in which the N-glycan has the core structure of $GlcNAc_2Man_3GlcNAc_2$, wherein one terminal galactose (Gal) residue is attached to one of the mannose arms. A "G2 glycoform" refers to a glycoform in which the N-glycan has the core structure of $GlcNAc_2Man_3GlcNAc_2$, wherein one

terminal galactose (Gal) residue is attached to each of two mannose arms. Examples of G0, G1 (G1'), and G2 N-glycans are shown in Figure 1.

[0037] The "immunogenicity" of a molecule refers to the ability of the molecule to induce a response by the immune system. The immune response may be a cell or antibody-mediated response. Immunogenicity may be determined by use of any suitable method known in the art, e.g. in vivo or in vitro immunogenicity test that quantifying the presence of antigen-specific antibodies or T-cells. The term "reduced immunogenicity", when referring to composition, means that the composition gives rise to a measurably lower immune response than a reference composition, as determined under comparable conditions. Reduced immunogenicity can be demonstrated by showing, e.g., decreased amount of an immunogenic epitope (e.g., an α-Gal epitope) that is present in the composition; decreased binding affinity for an antibody; or lower antibody titers induced by the composition; as compared to a reference composition, determined under comparable conditions. The reference composition may be a commercially available product (such as Rituximab, Cetuximab, Trastuzumab, Abatacept etc.).

[0038] A "large scale culture" refers to a culture that is at least about a 10 liter in size, (e.g., a volume of at least about 10L, least about 20L, least about 30L, least about 40L, at least about 50L, least about 60L, least about 70L, least about 80L, least about 90L, at least about 100L, least about 150L, least about 200L, at least about 250L, least about 300L, least about 400L, at least about 500L, least about 600L, least about 700L, least about 800L, least about 900L, at least about 1000 L, at least about 2000 L, at least about 3000 L, at least about 4000 L, at least about 5000 L, at least about 6000 L, at least about 10,000 L, at least about 15,000 L, at least about 20,000 L, at least about 25,000 L, at least about 30,000 L, at least about 35,000 L, at least about 40,000 L, at least about 45,000 L, at least about 50,000 L, at least about 55,000 L, at least about 60,000 L, at least about 65,000 L, at least about 70,000 L, at least about 75,000 L, at least about 80,000 L, at least about 85,000 L, at least about 90,000 L, at least about 95,000 L, at least about 100,000 L, etc.).

[0039] The expression of the target gene in a host cell is "transiently" reduced by an RNA effector molecule when the RNA effector molecule reduces the expression level of the target gene for a defined period of time (e.g., at least about 24 hours, at least about 48 hours, at least about 72 hours, at least about 96 hours, etc), but the reduction in the expression level is not

permanent. In other words, the RNA effector, or a nucleic acid construct encoding the RNA effector, does not integrate into the genome of the host cell.

3. GLYCAN-MODIFIED PROTEINS

[0040] In one aspect, the invention provides glycan-modified proteins that have improved effector activity, improved pharmacokinetic properties, or reduced immunogenicity in humans. "Glycan-modified" or "glycan modification" refer to a change in the glycan structure of a glycoprotein produced by a host cell in the presence of an RNA effector molecule that transiently reduces the expression of a target gene that encodes an enzyme or a transporter protein that is involved in a glycosylation pathway, as compared the glycan structure of the glycoprotein produced by the host cell under substantially the same conditions but in the absence of the RNA effector.

A. Glycan-Modified Proteins That Have Improved Effector Activity

[0041] In one aspect, the invention provides glycan-modified antibodies, or fusion proteins that comprise the Fc domain of an antibody (Fc-fusion proteins), that have improved effector activity. For example, the invention provides a composition comprising an antibody or a fusion protein that comprises the Fc domain of an antibody, wherein the composition is characterized by: (a) at least about 70% of the antibody molecules, or the fusion protein molecules, comprise a complex N-glycan; and (b) about 20% to about 100% of the N-glycans are afucosyl glycans. Preferably, about 25% to about 100%, about 30% to about 100% , about 35% to about 100% , about 40% to about 100%, about 45% to about 100%, or about 50% to about 100% of the N-glycans are afucosyl glycans.

[0042] In certain embodiment, the composition comprises an antibody. The antibodies described herein are preferably monoclonal antibodies. The antibodies can be monospecific, or polyspecific (e.g., bispecific). The antibodies can be from any species, but preferably are humanized, human, or chimeric, all of which are well-known in the art.

[0043] In other embodiments, composition comprises a fusion protein that comprises the Fc domain of an antibody. For example, the Fc domain may be fused with an enzyme, a toxin, a ligand (e.g., a growth factor), a cytokine (e.g., a chemokine), etc. The Fc fusion proteins may optionally comprise a linker.

[0044] Preferably, the N-glycan is linked to the Fc domain of the antibody or the Fc-fusion protein. Naturally-occurring antibody molecules have conserved N-linked glycosylation at the Fc region of each of the two heavy chains. For example, an IgG molecule contains an N-linked glycan covalently attached at the conserved Asn297 (Kabat numbering) in each of the CH2 domains in the Fc region. The glycans found in the Fc region of serum IgGs are mostly biantennary complex glycans. Variations of IgG glycosylation patterns include attachment of terminal sialic acid, a third GlcNAc arm (bisecting GlcNAc), terminal galactosylation, and α-1,6-linked core fucosylation. The N-glycans can contain zero (G0), one (G1 and G1'), or two (G2) galactoses (see Figure 1 for examples of G0, G1, and G2 glycans). The exact pattern of glycosylation depends on the structural properties of IgG subcomponents, in particular, C_H2 and C_H3 domains (see, e.g., Lund et al. (2000) Eur. J. Biochem., 267:7246-7257).

[0045] In addition, about 15%-20% polyclonal IgG molecules bear N-linked glycans in the variable regions of the light and/or heavy chains. There N-linked glycans are attached at the Asn residue of the consensus motif Asn-Xaa-Ser/Thr/Cys, where Xaa represents any amino acid). The glycans in the Fab region of serum IgGs are mostly biantennary oligosaccharides that are extensively galactosylated, fucosylated, and substantially sialylated. *See, e.g.*, Gary Walsh (ed.), Post-translational Modification of Protein Biopharmaceuticals, Wiley-VCH, 1st edition (2009), Chapter 4, Antibodies. For example, Cetuximab (Erbitux®) contains an N-glycan at Asn 99 of the VH region. N-glycosylation at the Fab region can influence antigen binding affinity of the antibody.

[0046] The compositions described herein may comprise a mixture of glycosylated and aglycosylated antibodies, or Fc-fusion proteins. Preferably, at least about 50%, and preferably, at least about 70% of antibody molecules, or Fc-fusion proteins, comprise a complex N-glycan. For example, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, or at least about 80% of the antibody molecules, or Fc-fusion proteins, comprise a complex N-glycan. Preferably, the N-glycan is linked to the Fc domain of the antibody or the Fc-fusion protein.

[0047] The Fc domain of the antibodies or Fc-fusion proteins described herein can be a naturally occurring Fc region of an antibody, which may optionally include the hinge region. Alternatively, the Fc domain can a truncated form of a naturally occurring Fc region (e.g., C_H2 domain only); a fusion form that comprising a naturally occurring Fc region fused with a

heterologous sequence; a mutated form of a naturally occurring Fc domain (e.g., a Fc region having amino acid substitution(s), addition(s), or deletion(s)); or a combination thereof. For example, Fc variants T256A, K290A, S298A, E333A, K334A, A339T have been described as having enhanced ADCC activity as compared to naturally occurring Fc sequences (*see, e.g.*, Shields et al. (2001) J. Biol. Chem., 276:6591-6604). Additional mutation variants of Fc regions are disclosed in U.S. Patent Application No. 2004/0228856.

[0048] The Fc domain can be derived from the Fc region of IgG, IgA, IgD, IgE and IgM (including various isotypes, such as, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 etc). Preferably, the Fc domain is derived from IgG1.

[0049] As described herein, reducing or inhibiting N-glycan fucosylation of antibodies, or Fc-fusion proteins, can enhance the antibody dependent cell-mediated cytotoxicity (ADCC). ADCC typically involves the activation of natural killer (NK) cells and is dependent on the recognition of antibody-coated cells by Fc receptors, most commonly CD16 (FcγRIII)) on the surface of the NK cell. Binding of the Fc domain to Fc receptors on the NK cells is affected by the glycosylation state of the Fc domain. In addition, the type of the N-glycan at the Fc domain also affects ADCC activity. Therefore, for an antibody composition, or a Fc-fusion protein composition, increasing the relative amount of afucosyl N-glycans can increase the binding affinity for an FcγRIII, or ADCC activity, of the composition.

[0050] The N-glycosylated antibodies, or Fc-fusion proteins, as described herein, may comprise a mixture of fucosylated and afucosylated molecules. For example, from about 20% to about 100%, preferably, from about 40% to about 80% of the glycans are afucosyl glycans. For example, from about 20% to about 100%, from about 25% to about 100%, from about 30% to about 100%, from about 35% to about 100%, from about 40% to about 100%, from about 45% to about 100%, or from about 20% to about 100% of the N-glycans are afucosyl glycans. It is not required that all, or substantially all, of the N-glycosylated antibodies, or Fc-fusion proteins are afucosylated. In fact, as shown in Example 1, significant enhancement in ADCC activity of a monoclonal antibody that binds to CD20 was achieve when about 48% of the N-glycans were afucosylated.

[0051] The antibody composition, or Fc-fusion protein composition described herein may comprise a mixture of G0, G1, G1'and G2 glycoforms (e.g., at the Fc-linked N-glycan).

For example, from about 0% to about 70%, from about 0% to about 65%, from about 0% to about 60%, from about 0% to about 55%, from about 0% to about 50%, from about 0% to about 45%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, of the N-glycans may be G0 glycans. Alternatively or in addition, from about 0% to about 70%, from about 0% to about 65%, from about 0% to about 60%, from about 0% to about 55%, from about 0% to about 50%, from about 0% to about 45%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, of the N-glycans may be G1 and G1' glycans. Alternatively or in addition, from about 0% to about 70%, from about 0% to about 65%, from about 0% to about 60%, from about 0% to about 55%, from about 0% to about 50%, from about 0% to about 45%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, of the N-glycans may be G2 glycans. The N-glycans of the antibody composition, or Fc-fusion protein composition may also be any combination of the G0, G1, G1', G2 profiles described above.

[0052] Preferably, from about 20% to about 100%, such as from about 25% to about 100%, from about 30% to about 100%, from about 40% to about 100%, from about 20% to about 95%, from about 20% to about 90%, from about 20% to about 85%, from about 20% to about 80%, from about 25% to about 95%, from about 30% to about 90%, from about 35% to about 85%, or from about 40% to about 80%, of the G0 N-glycans are afucosyl G0 glycans.

[0053] Alternatively or in addition, from about 0% to about 100%, such as from about 1% to about 90%, from about 1% to about 80%, from about 1% to about 70%, from about 1% to about 60%, from about 1% to about 55%, from about 1% to about 50%, from about 1% to about 45%, from about 1% to about 40%, from about 35%, of the G1 and G1' N-glycans are afucosyl G1 and G1' glycans.

[0054] Alternatively or in addition, from about 0% to about 100%, such as from about 1% to about 90%, from about 1% to about 80%, from about 1% to about 70%, from about 1% to about 60%, from about 1% to about 55%, from about 1% to about 50%, from about 1% to about 45%, from about 1% to about 40%, from about 35%, of the G2 N-glycans are afucosyl G2 glycans.

[0055] The afucosyl N-glycans of the antibody composition, or Fc-fusion protein composition may also be any combination of the G0, G1, G1', G2 profiles described above.

[0056] The glycan structure of the antibodies or Fc-fusion proteins described herein can be determined using art-known methods for analyzing glycan structures of glycoproteins, as described below.

[0057] Antibodies or Fc-fusion proteins having improved ADCC activity are useful for treating or preventing various diseases including cancers, inflammatory diseases, immune diseases such as autoimmune diseases, allergies and the like, circulatory organ diseases (e.g., arteriosclerosis) and viral or bacterial infections. Preferably, the antibody or Fc-fusion protein binds to a cell-surface molecule, or a cell-surface associated molecule, such as a transmembrane receptor. If desired, the antibody or Fc-fusion protein may bind to a soluble molecule that can associate with the cell surface, for example, with a cell-surface molecule, such as a ligand that binds to a transmembrane receptor.

[0058] Examples of the antibody which recognizes a tumor-related antigen include anti-GD2 antibody (Ohta et al., Anticancer Res., 13, 331-336, 1993), anti-GD3 antibody (Ohta et al., Cancer Immunol. Immunother., 36, 260-266, 1993), anti-GM2 antibody (Nakamura et al., Cancer Res., 54, 1511-1516, 1994), anti-HER2 antibody (Carter et al., Proc. Natl. Acad. Sci. USA, 89, 4285-4289, 1992), anti-CD52 antibody (Carter et al., Proc. Natl. Acad. Sci. USA, 89, 4285-4289, 1992), anti-MAGE antibody (Jungbluth et al., British J. Cancer, 83, 493-497, 2000), anti-HM124 antibody (Ono et al., Molecular Immunol, 36, 387-395, 1999), anti-parathyroid hormone-related protein (PTHrP) antibody (Ogata et al., Cancer, 88, 2909-2911, 2000), anti-basic fibroblast growth factor antibody and anti-FGF8 antibody (Matsuzaki et al., Proc. Natl. Acad. Sci. USA, 86, 9911-9915, 1989), anti-basic fibroblast growth factor receptor antibody and anti-FGF8 receptor antibody (Kuo et al., J. Biol. Chem., 265, 16455-16463, 1990), anti-insulin-like growth factor antibody (Yao et al., J. Neurosci. Res., 40, 647-659, 1995), anti-insulin-like growth factor receptor antibody (Yao et al., J. Neurosci. Res., 40, 647-659, 1995), anti-PMSA antibody (Murphy et al., J. Urology, 160, 2396-2401, 1998), anti-vascular endothelial cell growth factor antibody (Presta et al., Cancer Res., 57, 4593-4599, 1997), anti-vascular endothelial cell growth factor receptor antibody (Kanno et al., Oncogene, 19, 2138-2146, 2000) and the like.

[0059] Examples of the antibody which recognizes an allergy- or inflammation-related antigen include anti-interleukin 6 antibody (Abrams et al., Immunol. Rev., 121, 5-24, 1992), anti-interleukin 6 receptor antibody (Sato et al., Molecular Immunol., 31, 371-381, 1994),

anti-interleukin 5 antibody (Abrams et al., Immunol. Rev., 127, 5-24, 1992), anti-interleukin 5 receptor antibody and anti-interleukin 4 antibody (Biord et al., Cytokine, 3, 562-567, 1991), anti-tumor necrosis factor antibody (Tempest et al., Hybridoma, 13, 183-190, 1994), anti-tumor necrosis factor receptor antibody (Amrani et al., Molecular Pharmacol., 58, 237-245, 2000), anti-CCR4 antibody (Campbell et al., Nature, 400, 776-780, 1999), anti-chemokine antibody (Peri et al., J. Immuno. Meth., 174, 249-257, 1994), anti-chemokine receptor antibody (Wu et al., J. Exp. Med., 186, 1373-1381, 1997) and the like. Examples of the antibody which recognizes a circulatory organ disease-related antigen include anti-GpIIb/IIIa antibody (Co et al., J. Immunol., 152, 2968-2976, 1994), anti-platelet-derived growth factor antibody (Ferns et al., Science, 253, 1129-1132, 1991), anti-platelet-derived growth factor receptor antibody (Shulman et al., J. Biol. Chem., 272, 17400-17404, 1997) and anti-blood coagulation factor antibody (Peter et al., Circulation, 101, 1158-1164, 2000) and the like.

[0060] Examples of the antibody which recognizes a viral or bacterial infection-related antigen include anti-gp120 antibody (Tugarinov et al., Structure, 8, 385-395, 2000), anti-CD4 antibody (Schulze-Koops et al., J. Rheumatology, 25, 2065-2076, 1998), anti-CCR4 antibody and anti-Vero toxin antibody (Karnali et al., J. Clin. Microbiol., 3, 396-399, 1999) and the like.

[0061] Several therapeutic antibodies and Fc fusion proteins are commercially available, such as antibodies that bind to VEGF (e.g., Bevacizumab (Avastin)), EGFR (e.g., Cetuximab (Erbitux®)), HER2 (e.g., Trastuzumab (Herceptin®)), and CD20 (e.g., Rituximab (Rituxan®)), and Fc-fusion proteins that bind to TNFα (e.g., Etanecept (Enbrel), which comprises the receptor-binding domain of a TNF receptor (p75)), CD2 (e.g., Alefacept (Amevive), which contains the CD2-binding domain of LFA-3), or B7 (Abatacept (Orencia®), which comprises the B7-binding domain of CTLA4).

[0062] All of the antibodies above can be modified according to the invention to improve ADCC activities.

(1). Glycan-modified anti-CD20 antibodies

[0063] In one aspect, the invention provides a composition comprising an antibody that binds to CD20, wherein the composition is characterized by: (a) at least about 70% of the

antibody molecules comprise a complex N-glycan (e.g., linked to the Fc domain of the antibody); and (b) about 40% to about 100% of the N-glycans are afucosylated.

[0064] CD20 is a transmembrane protein with a molecular weight of approximately 35 kD located on pre-B and mature B lymphocytes (Valentine et al. (1989) J. Biol. Chem. 264(19):11282-11287; and Einfield et al. (1988) EMBO J. 7(3):311-717). CD20 is found on the surface of greater than 90% of B cells from peripheral blood or lymphoid organs and is expressed during early pre-B cell development and remains until plasma cell differentiation. Although CD20 is expressed on normal B cells, this surface antigen is usually expressed at very high levels on neoplastic B cells. More than 90% of B-cell lymphomas and chronic lymphocytic leukemias, and about 50% of pre-B-cell acute lymphoblastic leukemias express this surface antigen. CD20 is not found on hematopoietic stem cells, pro-B cells, normal plasma cells, or other normal tissue (Tedder et al. (1985) J. Immunol. 135(2):973-979).

[0065] CD20 is expressed by malignant cells of B-cell origin, including B-cell lymphoma and chronic lymphocytic leukemia (CLL). CD20 is not expressed by malignancies of pre-B-cells, such as acute lymphoblastic leukemia. CD20 is therefore a good target for therapy of B-cell lymphoma, CLL, and other diseases in which B-cells are involved in the disease etiology. Other B-cell disorders include autoimmune diseases in which auto-antibodies are produced during the differentiation of B-cells into plasma cells.

[0066] In addition, CD20 has also been targeted by radioimmunotherapeutic agents to treat B-cell related diseases. One treatment consists of anti-CD20 antibodies prepared in the form of radionuclides for treating B-cell lymphoma (e.g., ^{131}I-labeled anti-CD20 antibody), as well as a ^{89}Sr-labeled form for the palliation of bone pain caused by prostate and breast cancer metastases (Endo, *Gan To Kagaku Ryoho* 1999, 26: 744-748).

[0067] Rituximab (Rituxan®) is a commercially available recombinant mouse/human IgG1 chimeric monoclonal antibody (mAb) in which variable domains of the heavy and light chains of a murine anti-CD20 mAb are fused to the human constant regions of IgG1.

[0068] In certain embodiments, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Rituximab. Alternatively

or in addition, the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Rituximab.

[0069] Preferably, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Rituximab; and the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Rituximab.

[0070] The anti-CD20 antibody composition may comprise a mixture of G0, G1, G1', and G2 glycoforms (e.g., at the Fc-linked N-glycan), as described above.

[0071] As described above, an antibody comprising an afucosylated N-glycan at the Fc region has an increased binding affinity for FcγRIII, as compared to a corresponding antibody (antibody having the same amino acid sequence) comprising a fucosylated N-glycan. Therefore, the antibody composition as described herein has an increased antibody-dependent cellular cytotoxicity (ADCC) activity, as compared to a composition comprising the same amount of the glycosylated antibody, but less than 40% of the N-glycans are afucosylated. Preferably, the anti-CD20 composition described herein has an increased antibody-dependent cellular cytotoxicity (ADCC) activity as compared to Rituximab under substantially the same conditions.

[0072] The anti-CD20 antibodies described herein may be used for treating Non-Hodgkins lymphoma.

(2). Glycan-modified anti-EGFR antibodies

[0073] In one aspect, the invention provides a composition comprising an antibody that binds to EGFR, wherein the composition is characterized by: (a) at least about 70% of the antibody molecules comprise a complex N-glycan (e.g, linked to the Fc domain of the antibody); and (b) about 40% to about 100% of the N-glycans are afucosylated.

[0074] EGFR (also known as ErbB-1 and HER1 in humans) is the cell-surface receptor for members of the epidermal growth factor family (EGF-family) of extracellular protein ligands (Herbst, 2004, Int. J. Radiat. Oncol. Biol. Phys. 59 (2 Suppl):21-6). Mutations

affecting EGFR expression or activity can result in cancer. EGF receptors are over expressed in most epithelial malignancies including those of the colon and the rectum. The EGFR is constitutively expressed in many normal epithelial tissues, including the skin and hair follicle.

[0075] Cetuximab (Erbitux®) is a commercially available recombinant mouse/human IgG1 chimeric monoclonal antibody (mAb) in which variable domains of the heavy and light chains of a murine anti-EGFR mAb (known as 225) are fused to the human constant regions of IgG1. Cetuximab has been approved for use in combination with radiation therapy for treating squamous cell carcinoma of the head and neck (SCCHN) or as a single agent in patients who have had prior platinum-based therapy. Cetuximab is also indicated for treatment of metastatic colon cancer in combination with irinotecan (Camptosar) a DNA topoisomerase blocker. Cetuximab is believed to function mainly by blocking the EGF binding to EGFR, thereby starving the tumor of needed growth factor.

[0076] In certain embodiments, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Cetuximab. Alternatively or in addition, the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Cetuximab.

[0077] Preferably, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Cetuximab; and the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Cetuximab.

[0078] The anti- EGFR antibody composition may comprise a mixture of G0, G1, G1', and G2 glycoforms (e.g, at the Fc-linked N-glycan), as described above.

[0079] As described herein, an antibody comprising an afucosylated N-glycan at the Fc region has an increased binding affinity for FcγRIII, as compared to a corresponding antibody (antibody having the same amino acid sequence) comprising a fucosylated N-glycan. Therefore, the antibody composition as described herein has an increased antibody-dependent cellular

cytotoxicity (ADCC) activity, as compared to a composition comprising the same amount of the glycosylated antibody, but less than 40% of the N-glycans are afucosylated. Preferably, the anti-EGFR composition described herein has an increased antibody-dependent cellular cytotoxicity (ADCC) activity as compared to Cetuximab under substantially the same conditions.

[0080] The anti-EGFR antibodies described herein may be used for treating colorectal cancer or head and neck squamous cell carcinoma.

(3). Glycan-modified anti-HER2 antibodies

[0081] In one aspect, the invention provides a composition comprising an antibody that binds to HER2, wherein the composition is characterized by: (a) at least about 70% of the antibody molecules comprise a complex N-glycan (e.g, linked to the Fc domain of the antibody); and (b) about 40% to about 100% of the N-glycans are afucosylated.

[0082] HER2 (also known as ErbB-2) is a 185 kD transmembrane glycoprotein receptor that is encoded by the erbB2 gene. HER2 is related to the epidermal growth factor receptor (EGFR, or HER1 in human), and is over expressed in about 25% to 30% of human breast cancer (Slamon et al., Science 235:177-182 , (1987); Slamon et al., Science 244:707-712 (1989)). HER2 overexpression is commonly regarded as a predictor of a poor prognosis, especially in patients with primary disease that involves axillary lymph nodes (Slamon et al., (1987) and (1989), supra; Ravdin and Chamness, Gene 159:19-27 (1995); and Hynes and Stern, Biochim Biophys Acta 1198:165-184 (1994)), and has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluoruracil) and anthracyclines (Baselga et al., Oncology 11(3 Suppl 1):43-48 (1997)).

[0083] Trastuzumab (Herceptin®) is a humanized anti-ErbB2 monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2). Trastuzumab has been clinically active in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior anti-cancer therapy (Baselga et al., J. Clin. Oncol. 14:737-744 (1996)).

[0084] In certain embodiments, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least

97%, at least 98%, at least 99%, or 100% identical to the light chain of Trastuzumab. Alternatively or in addition, the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Trastuzumab.

[0085] Preferably, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Trastuzumab; and the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Trastuzumab.

[0086] The anti-HER2 antibody composition may comprise a mixture of G0, G1, G1', and G2 glycoforms (e.g, at the Fc-linked N-glycan), as described above.

[0087] As described above, an antibody comprising an afucosylated N-glycan at the Fc region has an increased binding affinity for FcγRIII, as compared to a corresponding antibody (antibody having the same amino acid sequence) comprising a fucosylated N-glycan. Therefore, the antibody composition as described herein has an increased antibody-dependent cellular cytotoxicity (ADCC) activity, as compared to a composition comprising the same amount of the glycosylated antibody, but less than 40% of the N-glycans are afucosylated. Preferably, the anti-HER2 composition described herein has an increased antibody-dependent cellular cytotoxicity (ADCC) activity as compared to Trastuzumab under substantially the same conditions.

[0088] The anti-HER2 antibodies described herein may be used for treating breast cancer.

(4). Glycan-modified B7-Binding Molecule

[0089] In one aspect, the invention provides a composition comprising a Fc-fusion protein that binds to B7, wherein the composition is characterized by: (a) (a) at least about 70% of the antibody molecules comprise a complex N-glycan (e.g., linked to the Fc domain of the fusion protein), and (b) about 40% to about 100% of the N-glycans are afucosylated.

[0090] B7 is a peripheral membrane protein found on activated antigen presenting cells (APC) that, when paired with either a CD28 or CD152 (CTLA-4) surface protein on a T

cell, can produce a costimulatory signal to enhance or decrease the activity of a MHC-TCR signal between the APC and the T cell, respectively (see, e.g., Coico, et al. (2003) *Immunology: A Short Course*; Wiley-Liss. p. 131). Besides being present on activated APCs, B7 is also found on T-cells themselves (see, e.g., Taylor et al., *J Immunol.* 172 (1): 34-39). Binding of the B7 on T-cells to CTLA-4 causes inhibition of the activity of T-cells. There are two major types of B7 proteins, B7.1 and B7.2 or CD80 and CD86 respectively.

[0091] In certain embodiments, the Fc-fusion protein comprises the extracellular domain of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4).

[0092] CTLA4 (Cytotoxic T-Lymphocyte Antigen 4) also known as CD152 (Cluster of differentiation 152) is a protein that plays an important regulatory role in the immune system. In humans, the CTLA4 protein is encoded by the CTLA4 gene (Dariavach P, et al., *Eur. J. Immunol.* **18** (12): 1901-5). CTLA4 is a member of the immunoglobulin superfamily, which is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells. CTLA4 is similar to the T-cell costimulatory protein CD28, and both molecules bind to CD80 and CD86 (B7) on antigen-presenting cells. CTLA4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA4 is also found in regulatory T cells and may be important to their function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules (CD80 and CD86).

[0093] In certain embodiments, the fusion protein comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Abatacept.

[0094] Orencia® (Abatacept) is a soluble fusion protein that consisting of the extracellular domain of human cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) linked to the modified Fc (hinge, CH2, and CH3 domains) portion of human immunoglobulin G1 (IgG1). Abatacept can bind to B7 and is a selective costimulation modulator as it inhibits the costimulation of T cells.

[0095] The Fc-fusion protein composition may comprise a mixture of G0, G1, G1', and G2 glycoforms (e.g, at the Fc-linked N-glycan), as described above.

[0096] As described above, an Fc-fusion protein comprising an afucosylated N-glycan at the Fc region has an increased binding affinity for FcγRIII, as compared to a corresponding fusion protein (fusion protein having the same amino acid sequence) comprising a fucosylated N-glycan. Therefore, the Fc-fusion protein composition as described herein has an increased antibody-dependent cellular cytotoxicity (ADCC) activity, as compared to a composition comprising the same amount of the glycosylated Fc-fusion protein, but less than 40% of the N-glycans are afucosylated. Preferably, the Fc-fusion protein composition described herein has an increased antibody-dependent cellular cytotoxicity (ADCC) activity as compared to Abatacept under substantially the same conditions.

B. Glycan-Modified Proteins That Have Reduced Immunogenicity in Human

[0097] As described herein, RNA effectors, such as siRNAs, can also be used to reduce the expression levels of enzymes or transporters that are involved in the production of immunogenic epitopes. Examples of immunogenic epitopes in humans include, e.g., glycans, such as N-glycans, that comprise a N-glycolylneuraminic acid (Neu5Gc) residue, and the αGal epitope (galactose-alpha(1,3)-galactose-beta(1,4)N-acetylglucosamine-R; or Gal-α(1,3)-Gal-β(1,4)-GlcNAc-R; where R is the glycan-protein structure).

[0098] The compositions described herein may comprise a mixture of glycosylated and aglycosylated proteins. Preferably, at least about 50%, and preferably, at least about 70% of the proteins comprise a glycan. For example, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, or at least about 80% of the protein molecules comprise a glycan. Preferably, the glycan is a complex N-glycan.

(1). αGal Epitope

[0099] In another aspect, the invention provides a composition comprising a protein, wherein at least about 70% of the protein molecules comprise a glycan (e.g., a complex N-glycan); and wherein (a). the protein is produced by a cell that is not a human, ape, or Old World monkey cell; and at least about 40% of the glycosylated molecules do not comprise the α-Gal epitope.

[00100] Mammalian cells, such as Chinese hamster ovary (CHO) cells, and rodent myeloma derived NS/0 and SP2/0 cells, are widely used for producing therapeutic

glycoproteins. However, glycoproteins produced by these cells often contain oligosaccharides terminated with α-galactose residues. The α-galactose residues are linked to the penultimate galactose residues at a hydroxyl of the third sugar carbon position, α(1-3) linkage. Human, apes, and old-world monkey cells, however, do not express functional α-galactosyltransferase (α1-3GT), which participates in the glycosylation of cell membrane glycoconjugates in nonprimate mammals, prosimians, and New World monkeys. The absence of α1-3Gal epitopes from human cells is due to silencing of the gene for the enzyme α1,3 galactosyltransferase (GGTA1 gene). As a result, humans have up to 1% of circulating antibodies (such as anti-αGal IgA, IgG, and IgM) directed against the enzymatic product of α1,3-galactosyltransferase (Gal α1-3Galβ1-4GlcNAc), also called Galili antigen (Galili, U., Clark, M. R., Shohet, S. B., Buehler, J., and Macher, B. A. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 1369-1373), or αGal epitope. As such, there is a potential risk for clinical application of glycoprotein that are produced by non-human, non-ape, and non-Old World monkey cells.

[00101] Accordingly, the invention provides compositions of glycoproteins in which at least about 20% of the glycosylated molecules do not comprise the α-Gal epitope. Preferably, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, of the glycosylated molecules do not comprise the α-Gal epitope.

[00102] Preferably, the compositions described herein comprises glycoproteins in which at least about 40% of the glycans do not comprise the α-Gal epitope, and the composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated protein in which more than 40% of the glycans comprise the α-Gal epitope.

(2). N-glycolylneuraminic acid

[00103] In another aspect, the invention provides a composition comprising a protein, wherein at least about 70% of the protein molecules comprise a glycan (e.g., a complex N-glycan); and wherein (a) the protein is produced by a non-human cell; and; (b) the glycans (e.g., complex N-glycans) of said protein molecules are characterized by a total sialic acid content that contain no more than about 20% of N-glycolylneuraminic acid (Neu5Gc).

[00104] Sialic acids are 9-carbon backbone acidic sugars terminating the glycan chains of various glycoproteins and glycolipids at vertebrate cell surfaces. N-acetylneuraminic acid (Neu5Ac) and its hydroxylated form Neu5Gc are the two major Sialic acids in mammals, with the activated form CMP-Neu5Ac serving as the precursor for synthesis of CMP-Neu5Gc, catalyzed by the enzyme CMP-Neu5Ac hydroxylase (CMAH), encoded by the CMAH gene. This enzyme is specifically inactivated in humans, in contrast to other mammals studied to date, including old world primates and pigs (Varki A., Proc. Natl. Acad. Sci. USA 2010; 107(Suppl. 2): 8939-8946). In addition, humans lack an alternative pathway for Neu5Gc synthesis. Accordingly, human immune system recognizes Neu5Gc as foreign, resulting in a humoral response involving a polyclonal highly diverse antibody profile in all humans.

[00105] Accordingly, the invention provides compositions of glycoproteins in which the total sialic acid content of the glycans (e.g., complex N-glycans) contain no more than about 40% of Neu5Gc. Preferably, the total sialic acid content of the glycans (e.g., complex N-glycans) contain no more than about 35%, no more than about 30%, no more than about 25%, no more than about 20%, no more than about 15%, or no more than about 10% of Neu5Gc.

[00106] Preferably, the compositions described herein comprises glycoproteins in which the total sialic acid content of the glycans (e.g., complex N-glycans) contain no more than about 20% of Neu5Gc, and the composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated protein, but the total sialic acid content of the glycans (e.g., complex N-glycans) contain more than about 20% of Neu5Gc.

(3). Glycan-modified antibodies and Fc-fusion proteins that have reduced immunogenicity in humans

[00107] Any suitable glycoprotein may be modified according to the teachings of the invention to reduce the amount of immunogenic epitopes, such as the αGal and Neu5Gc epitopes described above. Preferred classes of glycoproteins include, e.g., antibodies or Fc-fusion proteins. Therefore, in certain embodiments, the composition described herein comprises a protein that is an antibody or a fusion protein that comprises the Fc domain of an antibody; and wherein said N-glycan is linked to the Fc domain of the antibody molecules or the fusion protein.

[00108] As described above, the antibody composition, or Fc-fusion protein composition described herein may comprise a mixture of G0, G1, G1', and G2 glycoforms (e.g., at the Fc-linked N-glycan). For example, from about 0% to about 70%, from about 0% to about 65%, from about 0% to about 60%, from about 0% to about 55%, from about 0% to about 50%, from about 0% to about 45%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, of the N-glycans may be G0 glycans. Alternatively or in addition, from about 0% to about 70%, from about 0% to about 65%, from about 0% to about 60%, from about 0% to about 55%, from about 0% to about 50%, from about 0% to about 45%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, of the N-glycans may be G1 and G1' glycans. Alternatively or in addition, from about 0% to about 70%, from about 0% to about 65%, from about 0% to about 60%, from about 0% to about 55%, from about 0% to about 50%, from about 0% to about 45%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, of the N-glycans may be G2 glycans. The N-glycans of the antibody composition, or Fc-fusion protein composition may also be any combination of the G0, G1, G1', G2 profiles described above.

[00109] In addition, an antibody or Fc-fusion protein may be modified such that it has both improved effector activity (e.g., ADCC activity), and reduced immunogenicity in humans. For example, two or more different species of RNA effector molecules may be added to a host cell culture. At least one RNA effector targets the fucosylation pathway to increase the amount of afucosylated antibody or Fc-fusion protein that is produced by the host cells, and at least another RNA effector targets α-galactosyltransferase or CMAH to reduce the amount of immunogenic epitopes produced by the host cells.

[00110] The glycan structure of the antibodies or Fc-fusion proteins described herein can be determined using art-known methods for analyzing glycan structures of glycoproteins, as described below.

[00111] In certain embodiments, the composition described herein comprises a protein that is an anti-EGFR antibody. Preferably, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Cetuximab; and/or the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%

identical to the heavy chain of Cetuximab. Preferably, the anti-EGFR antibody composition described herein has reduced immunogenicity as compared to Cetuximab under substantially the same conditions (for example, as a result of reduced amount of αGal epitope, Neu5Gc epitope, or both).

[00112] One study reported that among 76 Cetuximab -treated subjects, 25 had a hypersensitivity reaction to the drug. In most subjects who had a hypersensitivity reaction, IgE antibodies that are specific for the αGal epitope were detected. Therefore, preferably, the anti-EGFR antibody composition described herein has reduced immunogenicity in human, as compared Cetuximab to under similar conditions. In addition, it has been reported that Neu5Gc is present in Cetuximab (see, e.g. Ghaderi et al., Nature Biotechnology Vol. 28, 863-867 (2010)).

[00113] In certain embodiments, the composition described herein comprises a protein that is an anti-CD20 antibody. Preferably, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Rituximab; and/or the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Rituximab. Preferably, the anti-CD20 antibody composition described herein has reduced immunogenicity as compared to Rituximab under substantially the same conditions (for example, as a result of reduced amount of αGal epitope, Neu5Gc epitope, or both).

[00114] In certain embodiments, the composition described herein comprises a protein that is an anti-HER2 antibody. Preferably, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Trastuzumab; and/or the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Trastuzumab. Preferably, the anti-HER2 antibody composition described herein has reduced immunogenicity as compared to Trastuzumab under substantially the same conditions (for example, as a result of reduced amount of αGal epitope, Neu5Gc epitope, or both).

[00115] In certain embodiments, the composition described herein comprises a Fc-fusion protein that binds to B7.

[00116] In certain embodiments, the Fc-fusion protein comprises the extracellular domain of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4).

[00117] In certain embodiments, the fusion protein comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Abatacept.

[00118] Preferably, the Fc-fusion protein composition described herein has reduced immunogenicity as compared to Abatacept under substantially the same conditions (for example, as a result of reduced amount of αGal epitope, Neu5Gc epitope, or both). The Fc-fusion proteins described herein may be used to for treating rheumatoid arthritis.

C. Glycan-Modified Proteins That Have Increased Serum Half Life

[00119] As described herein, RNA effectors, such as siRNAs, can also be used to improve pharmacokinetic properties of glycoproteins. In another aspect, the invention provides a composition comprising a protein, wherein one or more glycans comprise a sialic acid cap (e.g., N-acetylneuraminic acid (Neu5Ac)).

[00120] The serum half life of glycoproteins is dependent on the composition and structure of its N-glycans. In general, maximal serum half life of a glycoprotein requires that its N-glycans terminate with a sialic acid cap (e.g., N-acetylneuraminic acid (Neu5Ac)). RNA effectors can be used to transiently reduce the expression of sialidases that remove the terminal sialic acid (e.g., Neu5Ac) cap from a glycoprotein. By reducing the hydrolysis of terminal sialic acid from N-linked N-glycans, therapeutic efficacies of glycoproteins can be improved.

[00121] Preferably, the composition described herein has an increased serum half-life in human, as compared to a composition comprising the same amount of the glycosylated protein in which the N-glycans does not comprise Neu5Ac.

[00122] In addition, a glycoprotein may be modified such that it has both reduced immunogenicity in humans, and increased serum half-life. For example, two or more different species of RNA effector molecules may be added to a host cell culture. At least one RNA

effector targets α-galactosyltransferase or CMAH to reduce the amount of immunogenic epitopes produced by the host cells, and at least another RNA effector targets a sialidase to increase the amount of sialic acid-capped N-glycans that are produced by the host cell.

[00123] Any suitable glycoprotein may be modified accordingly to reduce the amount of immunogenic epitopes, such as the αGal and Neu5Gc epitopes described above. Preferred classes of glycoproteins include, e.g., antibodies or Fc-fusion proteins. Therefore, in certain embodiments, the composition described herein comprises a protein that is an antibody or a fusion protein that comprises the Fc domain of an antibody; and wherein said antibody or fusion protein comprises a glycan. Preferably, the glycan is an N-glycan. Preferably, the N-glycan is linked to the antibody or Fc-fusion protein at the Fc domain.

[00124] As described above, the antibody composition, or Fc-fusion protein composition described herein may comprise a mixture of G0, G1, G1', and G2 glycoforms (e.g., at the Fc-linked N-glycan). For example, from about 0% to about 70%, from about 0% to about 65%, from about 0% to about 60%, from about 0% to about 55%, from about 0% to about 50%, from about 0% to about 45%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, of the N-glycans may be G0 glycans. Alternatively or in addition, from about 0% to about 70%, from about 0% to about 65%, from about 0% to about 60%, from about 0% to about 55%, from about 0% to about 50%, from about 0% to about 45%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, of the N-glycans may be G1 and G1' glycans. Alternatively or in addition, from about 0% to about 70%, from about 0% to about 65%, from about 0% to about 60%, from about 0% to about 55%, from about 0% to about 50%, from about 0% to about 45%, from about 0% to about 40%, from about 0% to about 35%, from about 0% to about 30%, of the N-glycans may be G2 glycans. The N-glycans of the antibody composition, or Fc-fusion protein composition may also be any combination of the G0, G1, G1', G2 profiles described above.

[00125] The glycan structure of the antibodies or Fc-fusion proteins described herein can be determined using art-known methods for analyzing glycan structures of glycoproteins, as described below.

[00126] In addition, an antibody or Fc-fusion protein may be modified such that it has any combination of improved effector activity (e.g., ADCC activity), reduced immunogenicity

in humans, or increased serum half life. For example, two or more different species of RNA effector molecules may be added to a host cell culture. At least one RNA effector targets the fucosylation pathway to increase the amount of afucosylated antibody or Fc-fusion protein that is produced by the host cells, and at least another RNA effector targets a sialidase to increase the amount of sialic acid-capped N-glycans that are produced by the host cell, or targets α-galactosyltransferase or CMAH to reduce the amount of immunogenic epitopes produced by the host cells. If desired, three or more different species of RNA effector molecules may be added to a host cell culture, at least one targets the fucosylation pathway, at least one targets a sialidase, and at least one targets α-galactosyltransferase or CMAH.

[00127] In certain embodiments, the composition described herein comprises a protein that is an anti-EGFR antibody. Preferably, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Cetuximab; and/or the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Cetuximab. Preferably, the anti-EGFR antibody composition described herein has an increased serum half life as compared to Cetuximab under substantially the same conditions (for example, as a result of reduced hydrolysis of the sialic acid cap).

[00128] In certain embodiments, the composition described herein comprises a protein that is an anti-CD20 antibody. Preferably, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Rituximab; and/or the heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Rituximab. Preferably, the anti-CD20 antibody composition described herein has an increased serum half life as compared to Rituximab under substantially the same conditions (for example, as a result of reduced hydrolysis of the sialic acid cap).

[00129] In certain embodiments, the composition described herein comprises a protein that is an anti-HER2 antibody. Preferably, the light chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the light chain of Trastuzumab; and/or the

heavy chain of the antibody comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the heavy chain of Trastuzumab. Preferably, the anti-HER2 antibody composition described herein has an increased serum half life as compared to Trastuzumab under substantially the same conditions (for example, as a result of reduced hydrolysis of the sialic acid cap).

[00130] In certain embodiments, the composition described herein comprises an Fc-fusion protein that binds to B7. In certain embodiments, the Fc-fusion protein comprises the extracellular domain of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4). Preferably, the fusion protein comprises a sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to Abatacept. Preferably, the Fc-fusion protein composition described herein has an increased serum half life as compared to Abatacept under substantially the same conditions (for example, as a result of reduced hydrolysis of the sialic acid cap).

4. METHODS FOR MODIFYING PROTEIN GLYCOSYLATION

[00131] In one aspect, the invention provides methods for producing glycan-modified glycoproteins, in particular on a large or commercial scale. The method comprises culturing a host cell in a large scale cell culture in the presence of an RNA effector that targets a gene that encodes an enzyme or a transporter protein that is involved in a glycosylation pathway. The RNA effector transiently reduces the expression level of the target gene, thereby altering the glycosylation profile of a glycoprotein.

[00132] Examples of commercially available glycoproteins that can be modified using the methods described herein are listed in Table 1.

Table 1

Product	Class	Mode of action	Indication	Cell line	Company
Aranesp (Darbepoetin alfa)	Erythropoiesis stimulating protein	Regulates red blood cell production	Anemia	CHO	Amgen
Arcalyst (Rilonacept)	IL-1 Trap	Binds IL-1β to prevent the interaction to cell surface receptors	Cryoporin-associated periodic syndromes	CHO	Regeneron
Avastin	rMab	Binds to the vascular	Colorectal cancer	CHO	Genentech

Product	Class	Mode of action	Indication	Cell line	Company
(Bevacizumab)		endothelial growth factor (VEGF) to inhibit angiogenesis			
Avonex (Interferon β−1a)	Interferon	Binds to type I interferon receptors to activate two Jak tyrosine kinases	Multiple sclerosis (MS)	CHO	Biogen Idec
Cerezyme (Imiglucerase)	Enzyme	Engineered to have mannose-terminated oligosaccharide chains that are recognized by endocytic carbohydrate receptors on macrophages. Catalyzes hydrolysis of glycolipid glucocerebroside to glucose and ceramide in those macrophages which accumulate lipids in Gaucher disease	Gaucher Disease	CHO	Genzyme
Elaprase (Iduronate sulfatase)	Enzyme	Hydrolyzes the 2-sulfate esters of terminal iduronate sulfate residues from the glycosaminoglycans dermatan sulfate and heparan sulfate in the lysosomes of various cell types	Hunter syndrome	HT-1080	Shire
Enbrel (Etanercept)	Fusion protein	Mimics inhibitory effects of naturally occurring soluble TNF receptors to reduce inflammatory response	Rheumatoid arthritis	CHO	Amgen
Epogen (Epoetin alfa)	Erythropoiesis stimulating protein	Recombinant human erythropoietin interacts with erythropoietin (EPO) receptors to stimulate production of red blood cells from bone marrow stem cells	Anemia	CHO	Amgen, Kirin
Erbitux (Cetuximab)	rMab	Binds to the extracellular domain of the epidermal growth factor (EGFR) preventing activation of EGFR to impair cell growth and proliferation	Colorectal cancer	SP2/0	Imclone/BMS
Herceptin (Trastuzumab)	rMab	Binds to HER2+ tumor cells, blocks downstream HER2	Breast cancer	CHO	Genentech

Product	Class	Mode of action	Indication	Cell line	Company
		signaling to inhibit proliferation of cells			
Wellferon (Interferon α)	Cytokine	Binds to type I interferon receptors (IFNAR1 and IFNAR2c) which, upon dimerization, activate two Jak (Janus kinase) tyrosine kinases (Jak1 and Tyk2). Upregulates expression of MHC I proteins	Chronic hepatitis C	Human lympho-blastoid	Wellcome
Mircera (Methoxy polyethylene glycol-epoetin beta)	Erythropoiesis stimulating protein	Recombinant human erythropoietin interacts with erythropoietin (EPO) receptors to stimulate production of red blood cells from bone marrow stem cells	Anemia	CHO	Hoffmann-La Roche
Myozyme (Alglucosidase alfa)	Enzyme	Recombinant acid a-glucosidase (GAA) to replace GAA deficiencies	Pompe disease	CHO	Genzyme
Naglazyme (Galsulfase)	Enzyme	Recombinant form of polymorphic human enzyme *N*-acetylgalactosamine 4-sulfatase which catabolizes glycosaminoglycans (GAG)	Mucopolysaccharidosis VI (MPS VI)	CHO	Biomarin
NeoRecormon (Epoetin beta)	Erythropoiesis stimulating protein	Recombinant human erythropoietin interacts with erythropoietin (EPO) receptors to stimulate production of red blood cells from bone marrow stem cells	Anemia	CHO	Roche
Orencia (Abatacept)	Fusion Protein	CTLA4-Ig acts as a selective modulator of the costimulatory signal required for full T-cell activation	Rheumatoid arthritis	CHO	Bristol-Myers Squibb
Procrit/Eprex (Epoetin alfa)	Erythropoiesis stimulating protein	Recombinant human erythropoietin interacts with erythropoietin (EPO) receptors to stimulate production of red blood cells from bone marrow stem cells	Anemia	CHO	Johnson & Johnson, Schering-Plough
Rebif (Interferon β-	Cytokine	Immunomodulation through the induction	Multiple sclerosis (MS)	CHO	Merck Serono

Product	Class	Mode of action	Indication	Cell line	Company
1a)		of cell membrane components of the major histocompatibility complex			
Remicade (Infliximab)	rMab	Binds to TNF and inhibits TNF action	Crohn's disease & Rheumatoid arthritis	SP2/0	Centocor/Johnson & Johnson
Rituxan (Rituximab)	rMab	Binds to the cluster of differentiation 20 (CD20) which is expressed on B-cells. Fc portion mediates ADCC and CDC	Non-Hodgkins lymphoma	CHO	Genentech (IDEC)
Soliris (Eculizumab)	rMab	Binds to the complement protein C5, inhibiting terminal complement mediated intravascular hemolysis	Paroxysmal nocturnal hemoglobinuria	CHO	Alexion
Synagis (Palivizumab)	rMab	Targets an epitope in the A antigenic site of the F protein of respiratory syncytial virus (RSV)	Respiratory syncytial virus	NS0	MedImmune
TNKase (Tenecteplase) (tPA)	Enzyme	Recombinant fibrin-specific plasminogen activator	Acute myocardial infarction	CHO	Genetech
Tysabri (Natalizumab)	rMab	Binds to α4 integrin of adhesion molecule VLA-4 and sterically inhibits binding of VLA-4 to VCAM-1	Multiple sclerosis (MS)	CHO	Biogen Idec
Vectibix (Panitumumab)	rMab	Binds to EGFR	Colorectal carcinoma	CHO	Amgen
Zenapax (Daclizumab)	rMab	Binds to the α subunit (p55 α, CD25, or Tac subunit) of human IL-2 receptor expressed on the surface of activated lymphocytes	Acute organ rejection	SP2/0	Hoffmann-La Roche

A. Target Proteins for Modifying Glycosylation

[00133] The biosynthesis of all eukaryotic N-glycans begins on the cytoplasmic face of the ER membrane with the transfer of GlcNAc-P from UDP-GlcNAc to the lipid-like precursor dolichol phosphate (Dol-P) to generate dolichol pyrophosphate N-acetylglucosamine (Dol-P-P-GlcNAc). Fourteen sugars are sequentially added to Dol-P before en bloc transfer of the entire glycan to an Asn-X-Ser/Thr/Cys motif in a protein that is being synthesized and translocated through the ER membrane. The protein-bound N-glycan is subsequently remodeled

in the ER and Golgi by a complex series of reactions catalyzed by membrane-bound glycosidases and glycosyltransferases. Many of these enzymes are exquisitely sensitive to the physiological and biochemical state of the cell in which the glycoprotein is expressed. Thus, the populations of sugars attached to each glycosylated asparagine in a mature glycoprotein will depend on the cell type in which the glycoprotein is expressed and on the physiological status of the cell, a status that may be regulated during development and differentiation and altered in disease.

[00134] Glycosylation is a nonlinear non-template driven process. Regulation of a particular glycan may be due to a number of orthogonal inputs such as precursor levels, donor levels, transferase levels etc. Figure 2 provides a schematic illustration of the glycosylation pathway in CHO cell line. Each one of the enzymes may be targeted by RNA effectors.

[00135] In one aspect, the invention provides a method for producing a composition comprising a glycoprotein, the method comprises: (a) culturing a host cell in a large scale cell culture, wherein (i) said host cell expresses the glycoprotein; and (ii) said host cell comprises a target gene that encodes a protein that is selected from the group consisting of: GDP-fucose transporter (GFT), solute carrier-35C1 (SLC35C1), solute carrier-35C2 (SLC35C2), MPDU1, and Ggta1; and (b) adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to a target gene of (a), and reduces or prevents the expression of said target gene.

[00136] If desired, two or more genes may be targeted. For example, the host cell may further comprises a second target gene that encodes a protein that is selected from the group consisting of: dolichyl-diphosphooligosaccharide-protein glycosyltransferase, UDP glycosyltransferase, UDP-Gal:βGlcNAcβ1,4-galactosyltransferase, UDP-galactose-ceramide galactosyltransferase, fucosyltransferase, protein O-fucosyltransferase, N-acetylgalactosaminyltransferase, O-GlcNAc transferase, oligosaccharyl transferase, O-linked N-acetylgrucosamine transferase, α-galactosidase, β-galactosidase, sialyltransferase, GMD dehydratase, FX epimerase, α-1,3-galactosyltransferase, mannosyl (α-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT1), MGAT4B, SLC35D1, ST6GALNAC6, and glucosamine (UDP-N-acetyl)-2-epimerase; and step (b) may further comprise adding an effective amount of a second RNA effector molecule to the large scale cell culture, wherein the RNA effector is substantially complementary to a target gene that encodes a protein that is

selected from the group consisting of: dolichyl-diphosphooligosaccharide-protein glycosyltransferase, UDP glycosyltransferase, UDP-Gal:βGlcNAcβ1,4-galactosyltransferase, UDP-galactose-ceramide galactosyltransferase, fucosyltransferase, protein O-fucosyltransferase, N-acetylgalactosaminyltransferase, O-GlcNAc transferase, oligosaccharyl transferase, O-linked N-acetylgrucosamine transferase, α-galactosidase, β-galactosidase, sialyltransferase, GMD dehydratase, FX epimerase, α-1,3-galactosyltransferase, mannosyl (α-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT1), MGAT4B, SLC35D1, ST6GALNAC6, and glucosamine (UDP-N-acetyl)-2-epimerase, and wherein said RNA effector reduces or prevents the expression of said second target gene.

[00137] In one aspect, the invention provides a method for producing a composition comprising a glycoprotein, the method comprises: (a) culturing a host cell in a large scale cell culture, wherein (i) said host cell expresses the glycoprotein; and (ii) said host cell comprises at least two target genes, the target genes independently encode a protein selected from the group consisting of: dolichyl-diphosphooligosaccharide-protein glycosyltransferase, UDP glycosyltransferase, UDP-Gal:βGlcNAcβ1,4-galactosyltransferase, UDP-galactose-ceramide galactosyltransferase, fucosyltransferase, protein O-fucosyltransferase, N-acetylgalactosaminyltransferase, O-GlcNAc transferase, oligosaccharyl transferase, O-linked N-acetylgrucosamine transferase, α-galactosidase, β-galactosidase, sialyltransferase, GMD dehydratase, FX epimerase, α-1,3-galactosyltransferase, mannosyl (α-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT1), MGAT4B, SLC35D1, ST6GALNAC6, and glucosamine (UDP-N-acetyl)-2-epimerase; and (b) adding an effective amount of two or more RNA effector molecules to said large scale cell culture, wherein each of said RNA effector is substantially complementary to a target gene of (a), and reduces or prevents the expression of its target gene. In certain embodiments, the host cell is a rodent cell, such as a CHO cell.

[00138] Table 2 provides exemplary target genes that encode an enzyme or a transporter protein of the glycosylation pathway.

Table 2

Protein Name	Gene Name
sialidase	NEU2
α-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3-N-acetylgalactosaminide α-2,6-sialyltransferase 6	ST6GALNAC6
CMP-N-acetylneuraminic acid hydroxylase	CMAH

α1,3 galactosyltransferase	ABO
GDP-mannose 4,6-dehydratase	GMD
GDP-4-keto-6-deoxy-D-mannose epimerase-reductase	FX
GDP-fucose transporter	GFT
Fucosyltransferase	FUT (e.g., FUT1 to FUT12)
solute carrier-35C1	SLC35C1
solute carrier-35C2	SLC35C2
dolichyl-diphosphooligosaccharide-protein glycosyltransferase	DAD1, RPN2, RPN1, STT3A, STT3B, OST4, DDOST
UDP glycosyltransferase	UGT (e.g., UGT1a1, UGT1a2, UGT1a5, etc.)
UDP-Gal:βGlcNAcβ1,4-galactosyltransferase	B4GALT1, B4GALT2, B4GALT3, B4GALT4, B4GALT5, B4GALT6
UDP-galactose-ceramide galactosyltransferase	UGT8A
protein O-fucosyltransferase	POFUT1, POFUT2
N-acetylgalactosaminyltransferase	MGAT1, MGAT4B
O-GlcNAc transferase	OGT
oligosaccharyl transferase	STT3A, STT3B, DAD1, DDOST
O-linked N-acetylglucosamine transferase	OGT
α-galactosidase	GLA
β-galactosidase	GLB1
sialyltransferase	CMAH
GDP-fucose transporter	GFT
GMD dehydratase	GMDS
FX epimerase	FX
α-1,3-galactosyltransferase	A3GALT2
α-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	MGAT2
α-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	MGAT1
α-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase B	MGAT4B
Slc35d1 solute carrier family 35	SLC35D1
α-N-acetylgalactosaminide alpha-2,6-sialyltransferase 6	ST6GALNAC6
mannose-P-dolichol utilization defect 1	MPDU1
N-acetyllactosaminide α-1,3-galactosyltransferase	Ggta1

[00139] Preferred genes that may be targeted for modifying the glycosylation include FUT8, GMDS, TSTA3, ABO, CMAH, MGAT1, MGAT4B, SLC35D1, TSTA3, SLC35C1, SLC35C2, NEU2, ST6GALNAC6, and UGGT1.

[00140] In certain embodiments, the RNA effector transiently reduces the expression of its target gene. The nucleotide sequences of exemplary target genes from Chinese hamster

and siRNA molecules that modulate the expression of these genes, are provided in the Appendices.

[00141] In certain embodiments, the method further comprising harvesting said glycoprotein from said large scale culture.

(1). Modification of Fucosylation pathways

[00142] In one aspect, the invention provides a method for producing a composition comprising an afucosylated glycoprotein, comprising: (a) culturing a host cell in a large scale cell culture, wherein (i) said host cell expresses the glycoprotein; and (ii) said host cell comprises a target gene that encodes a protein that is selected from the group consisting of: GDP-fucose transporter (GFT), solute carrier-35C1 (SLC35C1), and solute carrier-35C2 (SLC35C2); and (b) adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said target gene, and reduces or prevents the expression of said target gene.

[00143] If desired, two or more genes may be targeted. For example, the host cell may further comprises a second target gene that encodes a protein that is selected from the group consisting of: GDP-mannose 4,6-dehydratase (GMD), GDP-4-keto-6-deoxy-D-mannose epimerase-reductase (FX), and Fucosyltransferase (Fut); and wherein step (b) further comprises adding an effective amount of a second RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to a target gene that encodes a protein that is selected from the group consisting of: GDP-mannose 4,6-dehydratase (GMD), GDP-4-keto-6-deoxy-D-mannose epimerase-reductase (FX), and Fucosyltransferase (Fut), and wherein said RNA effector reduces or prevents the expression of said second target gene.

[00144] In one aspect, the invention provides a method for producing a composition comprising an afucosylated glycoprotein, the method comprises: (a) culturing a host cell in a large scale cell culture, wherein (i) said host cell expresses the glycoprotein; and (ii) said host cell comprises at least two target genes that independently encode a protein that is selected from the group consisting of: GDP-mannose 4,6-dehydratase (GMD), GDP-4-keto-6-deoxy-D-mannose epimerase-reductase (FX), and Fucosyltransferase (Fut); and (b) adding an effective amount of two or more RNA effector molecules to said large scale cell culture, wherein each of

said RNA effector is substantially complementary to a target gene of (a), and reduces or prevents the expression of its target gene.

[00145] Preferably, at least one of the target genes encodes a fucosyltransferase.

[00146] In certain embodiment, the target gene is selected from the group consisting of: Fut8, GMD, and TSTA3.

[00147] Figure 3 provides a schematic illustration of fucosylation pathway in CHO cells. Each one of the enzymes may be targeted by RNA effectors. Preferably, two or more of the enzymes or transporter proteins are targeted. When two or more enzymes transporter proteins are targeted, it may be desirable that at least two enzymes or transporter proteins act sequentially along a metabolic pathway. For example, GMD, FX, GTF, and Fut8 (Figure 3) are considered act sequentially along a metabolic pathway. Similarly, Fucose kinase, GFPP, GTF, and Fut8 (Figure 3) are also considered act sequentially along a metabolic pathway. As such, any two of these four proteins may be targeted. By "sequentially," the two enzymes or transporter proteins do not have to act immediately one after another, as long as they align a metabolic pathway, such that a molecule is sequentially processed by the two enzymes or protein transporters (e.g., GMD and Fut8 are two enzymes act sequentially). By targeting two enzymes or transporter proteins act sequentially along a metabolic pathway, potential synergistic effect may be achieved.

[00148] Alternatively, it may be desirable that to target two or more enzymes or transporter proteins, each is involved in an alternative metabolic pathway that leads to the same product (see e.g., Figure 3, GMD in the de novo pathway, and fucose kinase in the salvage pathway). By targeting two enzymes or transporter proteins are involved in alternative metabolic pathways that lead to the same product, potential synergistic effect may be achieved.

[00149] In certain embodiments, the glycoprotein is an antibody or a fusion protein that comprises the Fc domain of an antibody.

[00150] Genes that may be targeted for modifying the fucosylation of N-glycans include, e.g., Fut8 (encodes Fucosyltransferase), GMD, TSTA3, SLC35C1, SLC35C2, etc.

(2). Reduction of Immunogenic Epitopes

[00151] In one aspect, the invention provides a method for producing a composition comprising a glycoprotein, the method comprises: (a) culturing a host cell in a large scale cell culture, wherein (i) said host cell expresses the glycoprotein; and (ii) said host cell comprises a target gene that encodes ABO α-1,3 galactosyltransferase; and (b) adding an effective amount an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said target gene, and reduces or prevents the expression of the target gene. Preferably, said glycoprotein has reduced immunogenicity in a human when compared to the same glycoprotein produced in the absence of said RNA effector molecule.

[00152] In one aspect, the invention provides a method for producing a composition comprising a glycoprotein, the method comprises: (a) culturing a host cell in a large scale cell culture, with the proviso that said host cell is not a human, ape, or Old World monkey cell; wherein (i) said host cell expresses the glycoprotein; and (ii) said host cell comprises a target gene that encodes a protein selected from the group consisting of ABO α-1,3 galactosyltransferase and Ggta1; and (b) adding an effective amount an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said target gene, and reduces or prevents the expression of the target gene.

[00153] In one aspect, the invention provides a method for producing a composition comprising a glycoprotein, wherein said glycoprotein comprises a N-glycan, the method comprises: (a) culturing a host cell in a large scale cell culture, with the proviso that said host cell is not a human cell; wherein: (i) said host cell expresses the glycoprotein; and (ii) said host cell comprises a target gene that encodes CMP-N-acetylneuraminic acid hydroxylase (CMAH); and (b) adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said gene, and reduces or prevents the expression of said target gene.

(3). Increasing Serum Half-Life

[00154] In one aspect, the invention provides a method for producing a composition comprising a glycoprotein, wherein said glycoprotein comprises a N-glycan, the method comprises: (a) culturing a host cell in a large scale cell culture; wherein (i) said host cell expresses the glycoprotein; and (ii) said host cell comprises a target gene that encodes a sialidase; and (b) adding an RNA effector molecule to said large scale cell culture, wherein said

RNA effector is substantially complementary to said gene, and reduces or prevents the expression of said target gene.

[00155] Preferably, the sialidase is NEU2 sialidase or α-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6 (ST6GALNAC6).

[00156] Two or more RNA effectors may be used, such as the glycan modified glycoproteins show a combination of improved effector activities (such as ADCC), reduced immunogenicity in humans, or increased serum half life. For example, one RNA effector may target fucosyltransferase to reduce the level of fucosylation, and is used in combination with another RNA effector that targets a sialidase to reduce the hydrolysis of terminal sialic acid. Alternatively, one RNA effector may target ABO α1,3 galactosyltransferase or CMAH to reduce the level of immunogenic epitope, and is used in combination with another RNA effector that targets a sialidase to reduce the hydrolysis of terminal sialic acid.

[00157] In another aspect, the invention provides a method for producing a glycoprotein, comprising: (a) culturing a host cell that expresses said glycoprotein in a large scale cell culture, wherein said host cell expresses target genes that are necessary for the glycosylation of said glycoprotein with two or more of N-glycolylneuraminic acid, fucose and galactose-α-1,3-galactose; (b) adding two or more RNA effector molecules to said large scale cell culture, wherein each of said RNA effectors are substantially complementary to said target genes, and cultivating the large scale culture for a sufficient period of time such that the expression of the target genes are reduced or inhibited in said host cell, thereby producing a glycoprotein with reductions in two or more of N-glycolylneuraminic acid, fucose and galactose-α-1,3-galactose as compared to a glycoprotein expressed under the same conditions but in the absence of said two or more RNA effector molecules.

B. RNA effector molecules

[00158] RNA effector molecules that are suitable for modifying glycosylation process of a host cell has been disclosed in detail in WO 2011/005786, and is described brief below.

[00159] RNA effector molecules are ribonucleotide agents that are capable of reducing or preventing the expression of a target gene within a host cell, or ribonucleotide agents capable of forming a molecule that can reduce the expression level of a target gene within a host

cell. A portion of a RNA effector molecule, wherein the portion is at least 10, at least 12, at least 15, at least 17, at least 18, at least 19, or at least 20 nucleotide long, is substantially complementary to the target gene. The complementary region may be the coding region, the promoter region, the 3' untranslated region (3'-UTR), and/or the 5'-UTR of the target gene. Preferably, at least 16 contiguous nucleotides of the RNA effector molecule are complementary to the target sequence (e.g., at least 17, at least 18, at least 19, or more contiguous nucleotides of the RNA effector molecule are complementary to the target sequence). The RNA effector molecules interact with RNA transcripts of target genes and mediate their selective degradation or otherwise prevent their translation.

[00160] RNA effector molecules can comprise a single RNA strand or more than one RNA strand. Examples of RNA effector molecules include, e.g., double stranded RNA (dsRNA), microRNA (miRNA), antisense RNA, promoter-directed RNA (pdRNA), Piwi-interacting RNA (piRNA), expressed interfering RNA (eiRNA), short hairpin RNA (shRNA), antagomirs, decoy RNA, DNA, plasmids and aptamers. The RNA effector molecule can be single-stranded or double-stranded. A single-stranded RNA effector molecule can have double-stranded regions and a double-stranded RNA effector can have single-stranded regions. Preferably, the RNA effector molecules are double-stranded RNA, wherein the antisense strand comprises a sequence that is substantially complementary to the target gene.

[00161] Complementary sequences within a RNA effector molecule, e.g., within a dsRNA (a double-stranded ribonucleic acid) may be fully complementary or substantially complementary. Generally, for a duplex up to 30 base pairs, the dsRNA comprises no more than 5, 4, 3 or 2 mismatched base pairs upon hybridization, while retaining the ability to regulate the expression of its target gene.

[00162] In some embodiments, the RNA effector molecule comprises a single-stranded oligonucleotide that interacts with and directs the cleavage of RNA transcripts of a target gene. For example, single stranded RNA effector molecules comprise a 5' modification including one or more phosphate groups or analogs thereof to protect the effector molecule from nuclease degradation. The RNA effector molecule can be a single-stranded antisense nucleic acid having a nucleotide sequence that is complementary to a "sense" nucleic acid of a target gene, e.g., the coding strand of a double-stranded cDNA molecule or a RNA sequence, e.g., a

pre-mRNA, mRNA, miRNA, or pre-miRNA. Accordingly, an antisense nucleic acid can form hydrogen bonds with a sense nucleic acid target.

[00163] Given a coding strand sequence (e.g., the sequence of a sense strand of a cDNA molecule), antisense nucleic acids can be designed according to the rules of Watson-Crick base pairing. The antisense nucleic acid can be complementary to the coding or noncoding region of a RNA, e.g., the region surrounding the translation start site of a pre-mRNA or mRNA, e.g., the 5' UTR. An antisense oligonucleotide can be, for example, about 10 to 25 nucleotides in length (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 nucleotides in length). In some embodiments, the antisense oligonucleotide comprises one or more modified nucleotides, e.g., phosphorothioate derivatives and/or acridine substituted nucleotides, designed to increase its biological stability of the molecule and/or the physical stability of the duplexes formed between the antisense and target nucleic acids. Antisense oligonucleotides can comprise ribonucleotides only, deoxyribonucleotides only (e.g., oligodeoxynucleotides), or both deoxyribonucleotides and ribonucleotides. For example, an antisense agent consisting only of ribonucleotides can hybridize to a complementary RNA and prevent access of the translation machinery to the target RNA transcript, thereby preventing protein synthesis. An antisense molecule including only deoxyribonucleotides, or deoxyribonucleotides and ribonucleotides, can hybridize to a complementary RNA and the RNA target can be subsequently cleaved by an enzyme, e.g., RNAse H, to prevent translation. The flanking RNA sequences can include 2'-O-methylated nucleotides, and phosphorothioate linkages, and the internal DNA sequence can include phosphorothioate internucleotide linkages. The internal DNA sequence is preferably at least five nucleotides in length when targeting by RNAseH activity is desired.

[00164] In certain embodiments, the RNA effector comprises a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA (a) comprises a sense strand and an antisense strand that are substantially complementary to each other; and (b) wherein said antisense strand comprises a region of complementarity that is substantially complementary to one of the target genes, and wherein said region of complementarity is from 10 to 30 nucleotides in length.

[00165] In some embodiments, RNA effector molecule is a double-stranded oligonucleotide . Typically, the duplex region formed by the two strands is small, about 30 nucleotides or less in length. Such dsRNA is also referred to as siRNA. For example, the siRNA may be from 15 to 30 nucleotides in length, from 10 to 26 nucleotides in length, from 17

to 28 nucleotides in length, from 18 to 25 nucleotides in length, or from 19 to 24 nucleotides in length, etc.

[00166] The duplex region can be of any length that permits specific degradation of a desired target RNA through a RISC pathway, but will typically range from 9 to 36 base pairs in length, e.g., 15 to 30 base pairs in length. For example, the duplex region may be 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, or any sub-range there between, including, e.g., 15 to 30 base pairs, 15 to 26 base pairs, 15 to 23 base pairs, 15 to 22 base pairs, 15 to 21 base pairs, 15 to 20 base pairs, 15 to 19 base pairs, 15 to 18 base pairs, 15 to 17 base pairs, 18 to 30 base pairs, 18 to 26 base pairs, 18 to 23 base pairs, 18 to 22 base pairs, 18 to 21 base pairs, 18 to 20 base pairs, 19 to 30 base pairs, 19 to 26 base pairs, 19 to 23 base pairs, 19 to 22 base pairs, 19 to 21 base pairs, 19 to 20 base pairs, 20 to 30 base pairs, 20 to 26 base pairs, 20 to 25 base pairs, 20 to 24 base pairs, 20 to 23 base pairs, 20 to 22 base pairs, 20 to 21 base pairs, 21 to 30 base pairs, 21 to 26 base pairs, 21 to 25 base pairs, 21 to 24 base pairs, 21 to 23 base pairs, or 21 to 22 base pairs.

[00167] The two strands forming the duplex structure of a dsRNA can be from a single RNA molecule having at least one self-complementary region, or can be formed from two or more separate RNA molecules. Where the duplex region is formed from two strands of a single molecule, the molecule can have a duplex region separated by a single stranded chain of nucleotides (a "hairpin loop") between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure. The hairpin loop can comprise at least one unpaired nucleotide; in some embodiments the hairpin loop can comprise at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides. Where the two substantially complementary strands of a dsRNA are formed by separate RNA strands, the two strands can be optionally covalently linked. Where the two strands are connected covalently by means other than a hairpin loop, the connecting structure is referred to as a "linker."

[00168] It is known that dsRNAs having a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference. Elbashir et al., 20 EMBO 6877-88 (2001).

[00169] A double-stranded oligonucleotide can include one or more single-stranded nucleotide overhangs, which are one or more unpaired nucleotide that protrudes from the terminus of a duplex structure of a double-stranded oligonucleotide, e.g., a dsRNA. A double-stranded oligonucleotide can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5' end, 3' end, or both ends of either an antisense or sense strand of a dsRNA.

[00170] In one embodiment, at least one end of a dsRNA has a single-stranded nucleotide overhang of 1 to 4, generally 1 or 2 nucleotides.

[00171] The overhang can comprise a deoxyribonucleoside or a nucleoside analog. Further, one or more of the internucloside linkages in the overhang can be replaced with a phosphorothioate. In some embodiments, the overhang comprises one or more deoxyribonucleoside or the overhang comprises one or more dT, e.g.,the sequence 5'-dTdT-3' or 5'-dTdTdT-3'. In some embodiments, overhang comprises the sequence 5'-dT*dT-3, wherein * is a phosphorothioate internucleoside linkage.

[00172] An RNA effector molecule as described herein can contain one or more mismatches to the target sequence. Preferably, a RNA effector molecule as described herein contains no more than three mismatches. If the antisense strand of the RNA effector molecule contains one or more mismatches to a target sequence, it is preferable that the mismatch(s) is (are) not located in the center of the region of complementarity, but are restricted to be within the last 5 nucleotides from either the 5' or 3' end of the region of complementarity. For example, for a 23-nucleotide RNA effector molecule agent RNA, the antisense strand generally does not contain any mismatch within the central 13 nucleotides.

[00173] The dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch Technologies (Novato, CA).

[00174] In some embodiments, the RNA effector molecule is a promoter-directed RNA (pdRNA) which is substantially complementary to a noncoding region of an mRNA transcript of a target gene. In one embodiment, the pdRNA is substantially complementary to

the promoter region of a target gene mRNA at a site located upstream from the transcription start site, e.g., more than 100, more than 200, or more than 1,000 bases upstream from the transcription start site. In another embodiment, the pdRNA is substantially complementary to the 3'-UTR of a target gene mRNA transcript. In one embodiment, the pdRNA comprises dsRNA of 18-28 bases optionally having 3' di- or tri-nucleotide overhangs on each strand. In another embodiment, the pdRNA comprises a gapmer consisting of a single stranded polynucleotide comprising a DNA sequence which is substantially complementary to the promoter or the 3'-UTR of a target gene mRNA transcript, and flanking the polynucleotide sequences (e.g., comprising the 5 terminal bases at each of the 5' and 3' ends of the gapmer) comprises one or more modified nucleotides, such as 2' MOE, 2'OMe, or Locked Nucleic Acid bases (LNA), which protect the gapmer from cellular nucleases.

[00175] pdRNA can be used to selectively increase, decrease, or otherwise modulate expression of a target gene. Without being limited to theory, it is believed that pdRNAs modulate expression of target genes by binding to endogenous antisense RNA transcripts which overlap with noncoding regions of a target gene mRNA transcript, and recruiting Argonaute proteins (in the case of dsRNA) or host cell nucleases (e.g., RNase H) (in the case of gapmers) to selectively degrade the endogenous antisense RNAs. In some embodiments, the endogenous antisense RNA negatively regulates expression of the target gene and the pdRNA effector molecule activates expression of the target gene. Thus, in some embodiments, pdRNAs can be used to selectively activate the expression of a target gene by inhibiting the negative regulation of target gene expression by endogenous antisense RNA. Methods for identifying antisense transcripts encoded by promoter sequences of target genes and for making and using promoter-directed RNAs are known, *see, e.g.*, WO 2009/046397.

[00176] In some embodiments, the RNA effector molecule comprises an aptamer which binds to a non-nucleic acid ligand, such as a small organic molecule or protein, e.g., a transcription or translation factor, and subsequently modifies (e.g., inhibits) activity. An aptamer can fold into a specific structure that directs the recognition of a targeted binding site on the non-nucleic acid ligand. Aptamers can contain any of the modifications described herein.

[00177] In some embodiments, the RNA effector molecule comprises an antagomir. Antagomirs are single stranded, double stranded, partially double stranded or hairpin structures that target a microRNA. An antagomir consists essentially of or comprises at least 10 or more

contiguous nucleotides substantially complementary to an endogenous miRNA and more particularly a target sequence of an miRNA or pre-miRNA nucleotide sequence. Antagomirs preferably have a nucleotide sequence sufficiently complementary to a miRNA target sequence of about 12 to 25 nucleotides, such as about 15 to 23 nucleotides, to allow the antagomir to hybridize to the target sequence. More preferably, the target sequence differs by no more than 1, 2, or 3 nucleotides from the sequence of the antagomir. In some embodiments, the antagomir includes a non-nucleotide moiety, e.g., a cholesterol moiety, which can be attached, e.g., to the 3' or 5' end of the oligonucleotide agent.

[00178] In some embodiments, antagomirs are stabilized against nucleolytic degradation by the incorporation of a modification, e.g., a nucleotide modification. For example, in some embodiments, antagomirs contain a phosphorothioate comprising at least the first, second, and/or third internucleotide linkages at the 5' or 3' end of the nucleotide sequence. In further embodiments, antagomirs include a 2'-modified nucleotide, e.g., a 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA). In some embodiments, antagomirs include at least one 2'-O-methyl-modified nucleotide.

[00179] In some embodiments, the RNA effector molecule is a promoter-directed RNA (pdRNA) which is substantially complementary to a noncoding region of an mRNA transcript of a target gene. The pdRNA can be substantially complementary to the promoter region of a target gene mRNA at a site located upstream from the transcription start site, e.g., more than 100, more than 200, or more than 1,000 bases upstream from the transcription start site. Also, the pdRNA can substantially complementary to the 3'-UTR of a target gene mRNA transcript. For example, the pdRNA comprises dsRNA of 18 to 28 bases optionally having 3' di- or tri-nucleotide overhangs on each strand. The dsRNA is substantially complementary to the promoter region or the 3'-UTR region of a target gene mRNA transcript. In another embodiment, the pdRNA comprises a gapmer consisting of a single stranded polynucleotide comprising a DNA sequence which is substantially complementary to the promoter or the 3'-UTR of a target gene mRNA transcript, and flanking the polynucleotide sequences (e.g., comprising the five terminal bases at each of the 5' and 3' ends of the gapmer) comprising one or more modified nucleotides, such as 2'MOE, 2'OMe, or Locked Nucleic Acid bases (LNA), which protect the gapmer from cellular nucleases.

[00180] Expressed interfering RNA (eiRNA) can be used to selectively increase, decrease, or otherwise modulate expression of a target gene. Typically, eiRNA, the dsRNA is expressed in the first transfected cell from an expression vector. In such a vector, the sense strand and the antisense strand of the dsRNA can be transcribed from the same nucleic acid sequence using e.g., two convergent promoters at either end of the nucleic acid sequence or separate promoters transcribing either a sense or antisense sequence. Alternatively, two plasmids can be cotransfected, with one of the plasmids designed to transcribe one strand of the dsRNA while the other is designed to transcribe the other strand. Methods for making and using eiRNA effector molecules are known in the art. *See, e.g.,* WO 2006/033756; U.S. Patent Pubs. No. 2005/0239728 and No. 2006/0035344.

[00181] In some embodiments, the RNA effector molecule comprises a small single-stranded Piwi-interacting RNA (piRNA effector molecule) which is substantially complementary to a target gene, and which selectively binds to proteins of the Piwi or Aubergine subclasses of Argonaute proteins. A piRNA effector molecule can be about 10 to 50 nucleotides in length, about 25 to 39 nucleotides in length, or about 26 to 31 nucleotides in length. *See, e.g.,* U.S. Patent Application Pub. No. 2009/0062228.

[00182] MicroRNAs are a highly conserved class of small RNA molecules that are transcribed from DNA in the genomes of plants and animals, but are not translated into protein. Pre-microRNAs are processed into miRNAs. Processed microRNAs are single stranded ~17 to 25 nucleotide (nt) RNA molecules that become incorporated into the RNA-induced silencing complex (RISC) and have been identified as key regulators of development, cell proliferation, apoptosis and differentiation. They are believed to play a role in regulation of gene expression by binding to the 3'-untranslated region of specific mRNAs. MicroRNAs cause post-transcriptional silencing of specific target genes, e.g., by inhibiting translation or initiating degradation of the targeted mRNA. In some embodiments, the miRNA is completely complementary with the target nucleic acid. In other embodiments, the miRNA has a region of noncomplementarity with the target nucleic acid, resulting in a "bulge" at the region of non-complementarity. In some embodiments, the region of noncomplementarity (the bulge) is flanked by regions of sufficient complementarity, e.g., complete complementarity, to allow duplex formation. For example, the regions of complementarity are at least 8 to 10 nucleotides long (e.g., 8, 9, or 10 nucleotides long).

[00183] miRNA can inhibit gene expression by, e.g., repressing translation, such as when the miRNA is not completely complementary to the target nucleic acid, or by causing target RNA degradation, when the miRNA binds its target with perfect or a high degree of complementarity.In further embodiments, the RNA effector molecule can include an oligonucleotide agent which targets an endogenous miRNA or pre-miRNA. For example, the RNA effector can target an endogenous miRNA which negatively regulates expression of a target gene, such that the RNA effector alleviates miRNA-based inhibition of the target gene.

[00184] The miRNA can comprise naturally occurring nucleobases, sugars, and covalent internucleotide (backbone) linkages, or comprise one or more non-naturally-occurring features that confer desirable properties, such as enhanced cellular uptake, enhanced affinity for the endogenous miRNA target, and/or increased stability in the presence of nucleases. In some embodiments, an miRNA designed to bind to a specific endogenous miRNA has substantial complementarity, e.g., at least 70%, 80%, 90%, or 100% complementary, with at least 10, 20, or 25 or more bases of the target miRNA. Exemplary oligonucleiotde agents that target miRNAs and pre-miRNAs are described, for example, in U.S. Patent Pubs. No. 20090317907, No. 20090298174, No. 20090291907, No. 20090291906, No. 20090286969, No. 20090236225, No. 20090221685, No. 20090203893, No. 20070049547, No. 20050261218, No. 20090275729, No. 20090043082, No. 20070287179, No. 20060212950, No. 20060166910, No. 20050227934, No. 20050222067, No. 20050221490, No. 20050221293, No. 20050182005, and No. 20050059005.

[00185] A miRNA or pre-miRNA can be 10 to 200 nucleotides in length, for example from 16 to 80 nucleotides in length. Mature miRNAs can have a length of 16 to 30 nucleotides, such as 21 to 25 nucleotides, particularly 21, 22, 23, 24, or 25 nucleotides in length. miRNA precursors can have a length of 70 to 100 nucleotides and can have a hairpin conformation. In some embodiments, miRNAs are generated *in vivo* from pre-miRNAs by the enzymes cDicer and Drosha. miRNAs or pre-miRNAs can be synthesized *in vivo* by a cell-based system or can be chemically synthesized. miRNAs can comprise modifications which impart one or more desired properties, such as superior stability, hybridization thermodynamics with a target nucleic acid, targeting to a particular tissue or cell-type, and/or cell permeability, e.g., by an endocytosis-dependent or -independent mechanism. Modifications can also increase sequence specificity, and consequently decrease off-site targeting.

[00186] Upon contact with a cell expressing the target gene, the RNA effector molecule inhibits the expression of the target gene by at least 10%, as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by western blot. Expression of a target gene in cell culture can be assayed by measuring target gene mRNA levels, e.g., by bDNA or TAQMAN® assay, or by measuring protein levels, e.g., by immunofluorescence analysis or quantitative immunoblot.

RNA Effector Modification

[00187] Optionally, an RNA effector may bechemically modified to enhance stability or other beneficial characteristics.

[00188] Oligonucleotides can be modified to prevent rapid degradation of the oligonucleotides by endo- and exo-nucleases and avoid undesirable off-target effects. The nucleic acids featured in the invention can be synthesized and/or modified by methods well established in the art, such as those described in CURRENT PROTOCOLS IN NUCLEIC ACID CHEMISTRY (Beaucage et al., eds., John Wiley & Sons, Inc., NY). Modifications include, for example, (a) end modifications, e.g., 5' end modifications (phosphorylation, conjugation, inverted linkages, etc.), or 3' end modifications (conjugation, DNA nucleotides, inverted linkages, etc.); (b) base modifications, e.g., replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; (c) sugar modifications (e.g., at the 2' position or 4' position) or replacement of the sugar; as well as (d) internucleoside linkage modifications, including modification or replacement of the phosphodiester linkages. Specific examples of oligonucleotide compounds useful in this invention include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. Specific examples of oligonucleotide compounds useful in this invention include, but are not limited to oligonucleotides containing modified or non-natural internucleoside linkages. Oligonucleotides having modified internucloside linkages include, among others, those that do not have a phosphorus atom in the internucleoside linkage.

[00189] Modified internucleoside linkages include (e.g., RNA backbones) include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters,

aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those) having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[00190] Representative patents that teach the preparation of the above phosphorus-containing linkages include, e.g., U.S. Patents No. 3,687,808; No. 4,469,863; No. 4,476,301; No. 5,023,243; No. 5,177,195; No. 5,188,897; No. 5,264,423; No. 5,276,019; No. 5,278,302; No. 5,286,717; No. 5,321,131; No. 5,399,676; No. 5,405,939; No. 5,453,496; No. 5,455,233; No. 5,466,677; No. 5,476,925; No. 5,519,126; No. 5,536,821; No. 5,541,316; No. 5,550,111; No. 5,563,253; No. 5,571,799; No. 5,587,361; No. 5,625,050; No. 6,028,188; No. 6,124,445; No. 6,160,109; No. 6,169,170; No. 6,172,209; No. 6, 239,265; No. 6,277,603; No. 6,326,199; No. 6,346,614; No. 6,444,423; No. 6,531,590; No. 6,534,639; No. 6,608,035; No. 6,683,167; No. 6,858,715; No. 6,867,294; No. 6,878,805; No. 7,015,315; No. 7,041,816; No. 7,273,933; No. 7,321,029; and No. RE39464.

[00191] Additionally, both the sugar and the internucleoside linkage may be modified, i.e., the backbone, of the nucleotide units are replaced with novel groups. One such oligomeric compound, a RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). See, e.g., U.S. Patents No. 5,539,082; No. 5,714,331; and No. 5,719,262. Further teaching of PNA compounds can be found, for example, in Nielsen et al., 254 Science 1497-1500 (1991).

[00192] Modified oligonucleotides can also contain one or more substituted sugar moieties. The RNA effector molecules, e.g., dsRNAs, can include one of the following at the 2' position: H (deoxyribose); OH (ribose); F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to 10, inclusive. In some embodiments, oligonucleotides include one of the following at the 2' position: C_1 to C_{10} lower alkyl,

substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, a RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide (e.g., a RNA effector molecule), or a group for improving the pharmacodynamic properties of an oligonucleotide (e.g., a RNA effector molecule), and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., 78 Helv. Chim. Acta 486-504 (1995)), i.e., an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O-CH_2-O-CH_2-$N(CH_2)_2$.

[00193] Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotide and the 5' position of 5' terminal nucleotide. Oligonucleotides can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Patents No. 4,981,957; No. 5,118,800; No. 5,319,080; No. 5,359,044; No. 5,393,878; No. 5,446,137; No. 5,466,786; No. 5,514,785; No. 5,519,134; No. 5,567,811; No. 5,576,427; No. 5,591,722; No. 5,597,909; No. 5,610,300; No. 5,627,053; No. 5,639,873; No. 5,646,265; No. 5,658,873; No. 5,670,633; and No. 5,700,920, certain of which are commonly owned with the instant application.

[00194] An oligonucleotide (e.g., a RNA effector molecule) can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as inosine, xanthine, hypoxanthine, nubularine, isoguanisine, tubercidine, 2-(halo)adenine, 2-(alkyl)adenine, 2-(propyl)adenine, 2 (amino)adenine, 2-(aminoalkyll)adenine, 2 (aminopropyl)adenine, 2 (methylthio) N6 (isopentenyl)adenine, 6 (alkyl)adenine, 6 (methyl)adenine, 7 (deaza)adenine, 8 (alkenyl)adenine, 8-(alkyl)adenine, 8 (alkynyl)adenine, 8 (amino)adenine, 8-(halo)adenine, 8-(hydroxyl)adenine,

8 (thioalkyl)adenine, 8-(thiol)adenine, N6-(isopentyl)adenine, N6 (methyl)adenine, N6, N6 (dimethyl)adenine, 2-(alkyl)guanine,2 (propyl)guanine, 6-(alkyl)guanine, 6 (methyl)guanine, 7 (alkyl)guanine, 7 (methyl)guanine, 7 (deaza)guanine, 8 (alkyl)guanine, 8-(alkenyl)guanine, 8 (alkynyl)guanine, 8-(amino)guanine, 8 (halo)guanine, 8-(hydroxyl)guanine, 8 (thioalkyl)guanine, 8-(thiol)guanine, N (methyl)guanine, 2-(thio)cytosine, 3 (deaza) 5 (aza)cytosine, 3-(alkyl)cytosine, 3 (methyl)cytosine, 5-(alkyl)cytosine, 5-(alkynyl)cytosine, 5 (halo)cytosine, 5 (methyl)cytosine, 5 (propynyl)cytosine, 5 (propynyl)cytosine, 5 (trifluoromethyl)cytosine, 6-(azo)cytosine, N4 (acetyl)cytosine, 3 (3 amino-3 carboxypropyl)uracil, 2-(thio)uracil, 5 (methyl) 2 (thio)uracil, 5 (methylaminomethyl)-2 (thio)uracil, 4-(thio)uracil, 5 (methyl) 4 (thio)uracil, 5 (methylaminomethyl)-4 (thio)uracil, 5 (methyl) 2,4 (dithio)uracil, 5 (methylaminomethyl)-2,4 (dithio)uracil, 5 (2-aminopropyl)uracil, 5-(alkyl)uracil, 5-(alkynyl)uracil, 5-(allylamino)uracil, 5 (aminoallyl)uracil, 5 (aminoalkyl)uracil, 5 (guanidiniumalkyl)uracil, 5 (1,3-diazole-1-alkyl)uracil, 5-(cyanoalkyl)uracil, 5-(dialkylaminoalkyl)uracil, 5 (dimethylaminoalkyl)uracil, 5-(halo)uracil, 5-(methoxy)uracil, uracil-5 oxyacetic acid, 5 (methoxycarbonylmethyl)-2-(thio)uracil, 5 (methoxycarbonyl-methyl)uracil, 5 (propynyl)uracil, 5 (propynyl)uracil, 5 (trifluoromethyl)uracil, 6 (azo)uracil, dihydrouracil, N3 (methyl)uracil, 5-uracil (i.e., pseudouracil), 2 (thio)pseudouracil,4 (thio)pseudouracil,2,4-(dithio)psuedouracil,5-(alkyl)pseudouracil, 5-(methyl)pseudouracil, 5-(alkyl)-2-(thio)pseudouracil, 5-(methyl)-2-(thio)pseudouracil, 5-(alkyl)-4 (thio)pseudouracil, 5-(methyl)-4 (thio)pseudouracil, 5-(alkyl)-2,4 (dithio)pseudouracil, 5-(methyl)-2,4 (dithio)pseudouracil, 1 substituted pseudouracil, 1 substituted 2(thio)-pseudouracil, 1 substituted 4 (thio)pseudouracil, 1 substituted 2,4-(dithio)pseudouracil, 1 (aminocarbonylethylenyl)-pseudouracil, 1 (aminocarbonylethylenyl)-2(thio)-pseudouracil, 1 (aminocarbonylethylenyl)-4 (thio)pseudouracil, 1 (aminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1 (aminoalkylaminocarbonylethylenyl)-pseudouracil, 1 (aminoalkylamino-carbonylethylenyl)-2(thio)-pseudouracil, 1 (aminoalkylaminocarbonylethylenyl)-4 (thio)pseudouracil, 1 (aminoalkylaminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-substituted 1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-substituted 1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-(aminoalkylhydroxy)-1-(aza)-2-

(thio)-3-(aza)-phenoxazin-1-yl, 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-(guanidiniumalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-(guanidiniumalkyl-hydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 1,3,5-(triaza)-2,6-(dioxa)-naphthalene, inosine, xanthine, hypoxanthine, nubularine, tubercidine, isoguanisine, inosinyl, 2-aza-inosinyl, 7-deaza-inosinyl, nitroimidazolyl, nitropyrazolyl, nitrobenzimidazolyl, nitroindazolyl, aminoindolyl, pyrrolopyrimidinyl, 3-(methyl)isocarbostyrilyl, 5-(methyl)isocarbostyrilyl, 3-(methyl)-7-(propynyl)isocarbostyrilyl, 7-(aza)indolyl, 6-(methyl)-7-(aza)indolyl, imidizopyridinyl, 9-(methyl)-imidizopyridinyl, pyrrolopyrizinyl, isocarbostyrilyl, 7-(propynyl)isocarbostyrilyl, propynyl-7-(aza)indolyl, 2,4,5-(trimethyl)phenyl, 4-(methyl)indolyl, 4,6-(dimethyl)indolyl, phenyl, napthalenyl, anthracenyl, phenanthracenyl, pyrenyl, stilbenyl, tetracenyl, pentacenyl, difluorotolyl, 4-(fluoro)-6-(methyl)benzimidazole, 4-(methyl)benzimidazole, 6-(azo)thymine, 2-pyridinone, 5 nitroindole, 3 nitropyrrole, 6-(aza)pyrimidine, 2 (amino)purine, 2,6-(diamino)purine, 5 substituted pyrimidines, N2-substituted purines, N6-substituted purines, O6-substituted purines, substituted 1,2,4-triazoles, pyrrolo-pyrimidin-2-on-3-yl, 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, para-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, bis-ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, para-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, ortho-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, bis-ortho-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, pyridopyrimidin-3-yl, 2-oxo-7-amino-pyridopyrimidin-3-yl, 2-oxo-pyridopyrimidine-3-yl, or any O-alkylated or N-alkylated derivatives thereof. Modified nucleobases also include natural bases that comprise conjugated moieties, e.g., a ligand.

[00195] The oligonucleotides can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to oligonucleotide molecules has been shown to increase oligonucleotide molecule stability in serum, and to reduce off-target effects. Elmen et al., 33 Nucl. Acids Res. 439-47 (2005); Mook et al., 6 Mol. Cancer Ther. 833-43 (2007); Grunweller et al., 31 Nucl.

Acids Res. 3185-93 (2003); U.S. Patents No. 6,268,490; No. 6,670,461; No. 6,794,499; No. 6,998,484; No. 7,053,207; No. 7,084,125; and No. 7,399,845.

[00196] In certain instances, the oligonucleotides of a RNA effector molecule can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution or cellular uptake of the oligonucleotides, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo et al., 365 Biochem. Biophys. Res. Comm. 54-61 (2007)); Letsinger et al., 86 PNAS 6553 (1989)); cholic acid (Manoharan et al., 1994); a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., 1992; Manoharan et al., 1993); a thiocholesterol (Oberhauser et al., 1992); an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., 1991; Kabanov et al., 259 FEBS Lett. 327 (1990); Svinarchuk et al., 75 Biochimie 75 (1993)); a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., 1995); Shea et al., 18 Nucl. Acids Res. 3777 (1990)); a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995); or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995); a palmityl moiety (Mishra et al., 1995); or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., 1996). Representative United States patents that teach the preparation of such RNA conjugates have been listed herein. Typical conjugation protocols involve the synthesis of an oligonucleotide bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

Delivery Methods of RNA Effector Molecules

[00197] The delivery of RNA effector molecules to cells can be achieved in a number of different ways. Several suitable delivery methods are well known in the art. For example, the skilled person is directed to WO 2011/005786, which discloses exemplary delivery methods can be used in this invention at pages 187-219, the teachings of which are incorporated herein by reference. For example, delivery can be performed directly by administering a composition comprising a RNA effector molecule, e.g., an siRNA, into cell culture. Alternatively, delivery

can be performed indirectly by administering into the cell one or more vectors that encode and direct the expression of the RNA effector molecule.

[00198] A reagent that facilitates RNA effector molecule uptake may be used. For example, an emulsion, a cationic lipid, a non-cationic lipid, a charged lipid, a liposome, an anionic lipid, a penetration enhancer, a transfection reagent or a modification to the RNA effector molecule for attachment, e.g., a ligand, a targeting moiety, a peptide, a lipophillic group, etc.

[00199] For example, RNA effector molecules can be delivered using a drug delivery system such as a nanoparticle, a dendrimer, a polymer, a liposome, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of a RNA effector molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient cellular uptake. Cationic lipids, dendrimers, or polymers can either be bound to RNA effector molecules, or induced to form a vesicle, liposome, or micelle that encases the RNA effector molecule. *See, e.g.*, Kim et al., 129 J. Contr. Release 107-16 (2008). Methods for making and using cationic-RNA effector molecule complexes are well within the abilities of those skilled in the art. *See e.g.*, Sorensen et al 327 J. Mol. Biol. 761-66 (2003); Verma et al., 9 Clin. Cancer Res. 1291-1300 (2003); Arnold et al., 25 J. Hypertens. 197-205 (2007).

[00200] In one embodiment, the reagent that facilitates RNA effector molecule uptake used herein comprises a charged lipid as described in U.S. Application Ser. No. 61/267,419, filed 7 December 2009.

[00201] The RNA effector molecules described herein can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, the RNA effector molecules can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C1-20 alkyl ester (e.g., isopropylmyristate IPM), monoglyceride, diglyceride, or acceptable salts thereof.

[00202] In one embodiment, the RNA effector molecules are fully encapsulated in the lipid formulation (e.g., to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle).

The term "SNALP" refers to a stable nucleic acid-lipid particle: a vesicle of lipids coating a reduced aqueous interior comprising a nucleic acid such as a RNA effector molecule or a plasmid from which a RNA effector molecule is transcribed. SNALPs are described, e.g., in U.S. Patent Pubs. No. 2006/0240093, No. 2007/0135372; No. 2009/0291131; U.S. Patent Applications Ser. No. 12/343,342; No.12/424,367. The term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SNALPs and SPLPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in WO 00/03683. The particles in this embodiment typically have a mean diameter of about 50 nm to about 150 nm, or about 60 nm to about 130 nm, or about 70 nm to about 110 nm, or typically about 70 nm to about 90 nm, inclusive, and are substantially nontoxic. I n addition, the nucleic acids when present in the nucleic acid- lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are reported in, e.g., U.S. Patents No. 5,976,567; No. 5,981,501; No. 6,534,484; No. 6,586,410; No. 6,815,432; and WO 96/40964.

[00203] The lipid to RNA ratio (mass/mass ratio) (e.g., lipid to dsRNA ratio) can be in ranges of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1, inclusive.

[00204] A cationic lipid of the formulation can comprise at least one protonatable group having a pKa of from 4 to 15. The cationic lipid can be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I-(2,3- dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I-(2,3- dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1 ,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl),

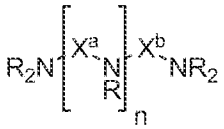
1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane, or a mixture thereof. The cationic lipid can comprise from about 20 mol% to about 70 mol%, inclusive, or about 40 mol% to about 60 mol%, inclusive, of the total lipid present in the particle. In one embodiment, cationic lipid can be further conjugated to a ligand.

[00205] A non-cationic lipid can be an anionic lipid or a neutral lipid, such as distearoyl-phosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoyl-phosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoyl-phosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoyl-phosphatidylcholine (POPC), palmitoyloleoyl- phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l- carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1 -stearoyl-2-oleoyl- phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid can be from about 5 mol% to about 90 mol%, inclusive, of about 10 mol%, to about 58 mol%, inclusive, if cholesterol is included, of the total lipid present in the particle.

[00206] The lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA can be, for example, a PEG-dilauryloxypropyl (C12), a PEG-dimyristyloxypropyl (C14), a PEG-dipalmityloxypropyl (C16), or a PEG- distearyloxypropyl (C18). The lipid that prevents aggregation of particles can be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle. In one embodiment, PEG lipid can be further conjugated to a ligand.

[00207] In some embodiments, the nucleic acid-lipid particle further includes a steroid such as, cholesterol at, e.g., about 10 mol% to about 60 mol%, inclusive, or about 48 mol% of the total lipid present in the particle.

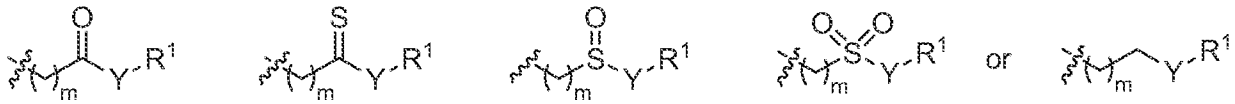
[00208] In one embodiment, the lipid particle comprises a steroid, a PEG lipid and a cationic lipid of formula (I):



formula (I)

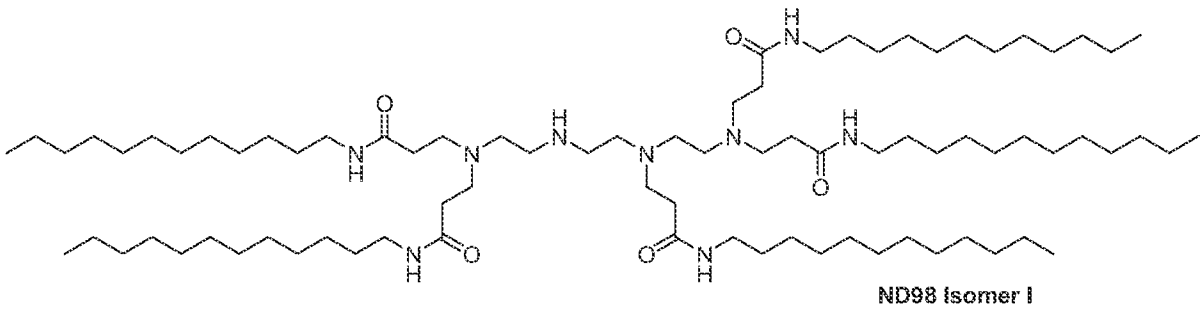
wherein each Xa and Xb, for each occurrence, is independently C1-6 alkylene;

n is 0, 1, 2, 3, 4, or 5; each R is independently H,



m is 0, 1, 2, 3 or 4; Y is absent, O, NR^2, or S; R^1 is alkyl alkenyl or alkynyl; each of which is optionally substituted with one or more substituents; and R^2 is H, alkyl alkenyl or alkynyl; each of which is optionally substituted each of which is optionally substituted with one or more substituents.

[00209] In one example, the lipidoid ND98·4HCl (MW 1487) (Formula 2), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid RNA effector molecule nanoparticles (e.g., LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/mL; Cholesterol, 25 mg/mL, PEG-Ceramide C16, 100 mg/mL. The ND98, Cholesterol, and PEG-Ceramide C16 stock solutions can then be combined in, e.g., a 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous RNA effector molecule (e.g., in sodium acetate pH 5) such that the final ethanol concentration is about 35% to 45% and the final sodium acetate concentration is about 100 mM to 300 mM, inclusive. Lipid RNA effector molecule nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (e.g., 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, e.g., about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.

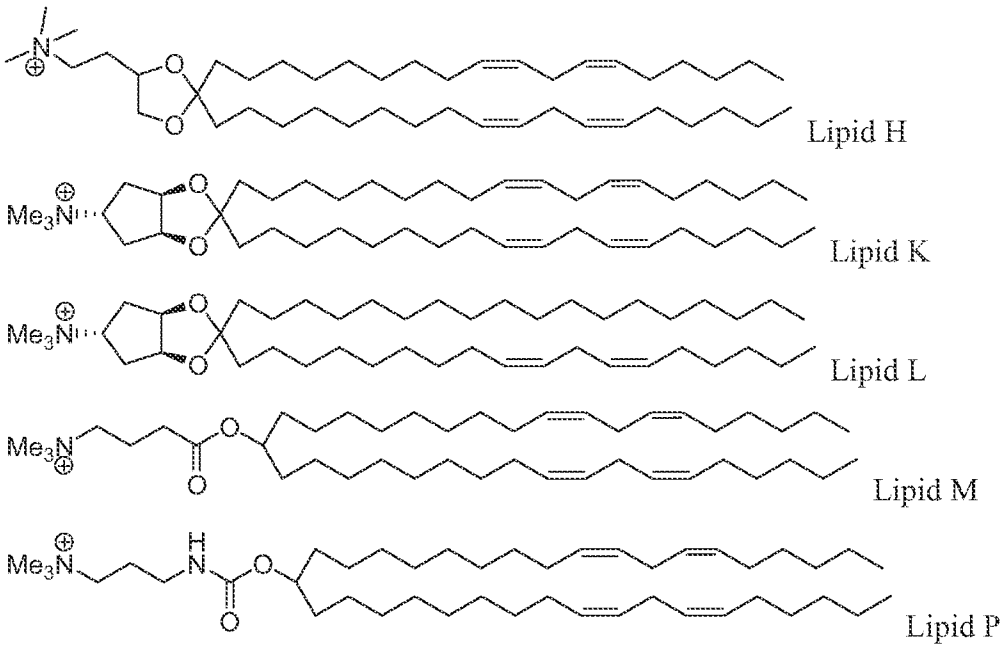


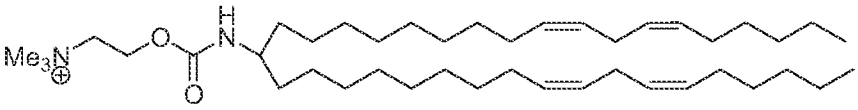
Formula 2

[00210] In some embodiments, the nucleic acid-lipid particle further includes a steroid such as, cholesterol at, e.g., about 10 mol% to about 60 mol%, inclusive, or about 48 mol% of the total lipid present in the particle.

[00211] LNP01 formulations are described elsewhere, e.g., WO 2008/042973.

[00212] In one embodiment, the reagent that facilitates RNA effector molecule uptake used herein comprises a cationic lipid as described in e.g., U.S. Application Ser. No. 61/267,419, filed 7 December 2009, and U.S. Application Ser. No. 61/334,398, filed 13 May 2010. In various embodiments, the RNA effector molecule composition described herein comprisescomprises a cationic lipid selected from the group consisting of: "Lipid H", "Lipid K"; "Lipid L", "Lipid M"; "Lipid P"; or "Lipid R", whose formulas are indicated as follows:





Lipid R

[00213] Also contemplated herein are various formulations of the lipids described above, such as, e.g., K8, P8 and L8 which refer to formulations comprising Lipid K, P, and L, respectively. Some exemplary lipid formulations for use with the methods and compositions described herein are found in e.g., Table 3:

Table 3. Example lipid formulations

Formulation Number	Cationic Lipid Number	Cationic Lipid Mol %	DOPE %	Cholesterol %
1	200 (Lipid H)	48.08	51.92	-
2	200 (Lipid H)	47.94	47.06	5
3	201 (Lipid K)	45.56	54.44	-
4 (K8)	201 (Lipid K)	47.94	47.06	5
5 (L8)	202 (Lipid L)	47.94	47.06	5
6	203 (Lipid M)	53.01	44.49	2.5
7	203 (Lipid M)	47.94	47.06	5
8 (P8)	204 (Lipid P)	47.94	47.06	5
9	205 (Lipid R)	47.94	47.06	5

[00214] In another embodiment, the RNA effector molecule composition described herein further comprises a lipid formulation comprising a lipid selected from the group consisting of Lipid H, Lipid K, Lipid L, Lipid M, Lipid P, and Lipid R, and further comprises a neutral lipid and a sterol. In particular embodiments, the lipid formulation comprises between approximately 25 mol % - 100 mol% of the lipid. In another embodiment, the lipid formulation comprises between 0 mol% - 50 mol% cholesterol. In still another embodiment, the lipid formulation comprises between 30 mol% - 65 mol% of a neutral lipid. In particular embodiments, the lipid formulation comprises the relative mol% of the components as listed in Table 4 as follows:

Table 4. Example lipid formulae

Series	Lipid (Mol%)	DOPE	Chol
1	45.56	54.44	0
2	48.08	51.92	0
3	50.60	49.40	0
4	53.10	46.90	0
5	52.73	37.27	10
6	52.92	42.08	5
7	53.01	44.49	2.5

8	47.94	47.06	5

[00215] Additional exemplary lipid-siRNA formulations are as shown below in Table 5.

TABLE 5: lipid-siRNA formulations

	Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio**	**Process**
SNALP	1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1	
SNALP-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1	
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1	Extrusion
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1	Extrusion
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1	In-line mixing
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1	In-line mixing
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1	In-line mixing

[00216] LNP09 formulations and XTC comprising formulations are described, *e.g.*, in U.S. Provisional Serial No. 61/239,686, filed September 3, 2009, which is hereby incorporated by reference.

[00217] LNP11 formulations and MC3 comprising formulations are described, *e.g.*, in U.S. Provisional Serial No. 61/244,834, filed September 22, 2009, which is hereby incorporated by reference.

[00218] LNP12 formulations and TechG1 comprising formulations are described, *e.g.*, in U.S. Provisional Serial No. 61/175,770, filed May 5, 2009, which is hereby incorporated by reference.

[00219] Formulations prepared by either the standard or extrusion-free method can be characterized in similar manners. For example, formulations are typically characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles can be measured by light scattering using, for example, a Malvern Zetasizer Nano ZS (Malvern, PA). Particles should be about 20-300 nm, such as 40-100 nm in size. The particle size distribution should be unimodal. The total dsRNA effector molecule concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated RNA effector molecule can be incubated with a RNA-binding dye, such as Ribogreen (Molecular Probes) in the presence or absence of a formulation disrupting surfactant, e.g., 0.5% Triton-X100. The total RNA effector molecule in the formulation can be determined by the signal from the sample containing the surfactant, relative to a standard curve. The entrapped fraction is determined by subtracting the "free" RNA effector molecule content (as measured by the signal in the absence of surfactant) from the total RNA effector molecule content. Percent entrapped RNA effector molecule is typically >85%. For lipid nanoparticle formulation, the particle size is at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm, at least 100 nm, at least 110 nm, or at least 120 nm. The suitable range is typically about at least 50 nm to about at least 110 nm, about at least 60 nm to about at least 100 nm, or about at least 80 nm to about at least 90 nm, inclusive.

[00220] Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the

composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages *in vivo*. In order to cross intact cell membranes, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

[00221] Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; and liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation. *See, e.g.,* Wang et al., DRUG DELIV. PRINCIPLES & APPL. (John Wiley & Sons, Hoboken, NJ, 2005); Rosoff, 1988. Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

[00222] Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes and as the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act. Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

[00223] Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged polynucleotide molecules to form a stable complex. The positively charged polynucleotide/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm. Wang et al., 147 Biochem. Biophys. Res. Commun., 980-85 (1987).

[00224] Liposomes which are pH-sensitive or negatively-charged, entrap polynucleotide rather than complex with it. Because both the polynucleotide and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some polynucleotide is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells. Zhou et al., 19 J. Controlled Rel. 269-74 (1992).

[00225] One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

[00226] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside GM1, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES). Allen et al., 223 FEBS Lett. 42 (1987); Wu et al., 53 Cancer Res. 3765 (1993).

[00227] Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (507 Ann. NY Acad. Sci. 64 (1987)), reported the ability of monosialoganglioside GM1, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (85

PNAS 6949 (1988)). U.S. Patent No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside GM1 or a galactocerebroside sulfate ester. U.S. Patent No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al.).

[00228] Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto et al. (53 Bull. Chem. Soc. Jpn. 2778 (1980)) described liposomes comprising a nonionic detergent, 2C1215G, that contains a PEG moiety. Illum et al. (167 FEBS Lett. 79 (1984)), noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (e.g., PEG) are described by Sears (U.S. Patent No. 4,426,330 and No. 4,534,899). In addition, antibodies can be conjugated to a polyakylene derivatized liposome (see e.g., PCT Application US 2008/0014255). Klibanov et al. (268 FEBS Lett. 235 (1990)), described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (1029 Biochim. Biophys. Acta 1029, (1990)), extended such observations to other PEG-derivatized phospholipids, e.g., DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. 0445131 B1 and WO 90/04384 to Fisher.

[00229] Liposome compositions containing 1-20 mol% of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Patents No. 5,013,556; No. 5,356,633) and Martin et al. (U.S. Patent No. 5,213,804; European Patent No. 0 496813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Patent No. 5,225,212 and in WO 94/20073. Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391. U.S. Patents No. 5,540,935 and No. 5,556,948 describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces. Methods and compositions relating to liposomes comprising PEG can be found in, e.g., U.S. Patents No. 6,049,094; No. 6,224,903; No. 6,270,806; No. 6,471,326; No. 6,958,241.

[00230] As noted above, liposomes can optionally be prepared to contain surface groups, such as antibodies or antibody fragments, small effector molecules for interacting with cell-surface receptors, antigens, and other like compounds, and these groups can facilitate delivery of liposomes and their contents to specific cell populations. Such ligands can be included in the liposomes by including in the liposomal lipids a lipid derivatized with the targeting molecule, or a lipid having a polar-head chemical group that can be derivatized with the targeting molecule in preformed liposomes. Alternatively, a targeting moiety can be inserted into preformed liposomes by incubating the preformed liposomes with a ligand-polymer-lipid conjugate.

[00231] Lipids can be derivatized using a variety of targeting moieties, such as ligands, cell surface receptors, glycoproteins, vitamins (e.g., riboflavin) and monoclonal antibodies by covalently attaching the ligand to the free distal end of a hydrophilic polymer chain, which is attached at its proximal end to a vesicle-forming lipid. There are a wide variety of techniques for attaching a selected hydrophilic polymer to a selected lipid and activating the free, unattached end of the polymer for reaction with a selected ligand, and as noted above, the hydrophilic polymer polyethyleneglycol (PEG) has been studied widely. Allen et al., 1237 Biochem. Biophys. Acta 99-108 (1995); Zalipsky, 4 Bioconj. Chem. 296-99 (1993); Zalipsky et al., 353 FEBS Lett. 1-74 (1994); Zalipsky et al., Bioconj. Chem. 705-08 (1995); Zalipsky, *in* STEALTH LIPOSOMES (Lasic & Martin, eds. CRC Press, Boca Raton, FL, 1995).

[00232] A number of liposomes comprising nucleic acids are known in the art, such as methods for encapsulating high molecular weight nucleic acids in liposomes. WO 96/40062. U.S. Patent No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes can include a dsRNA. U.S. Patent No. 5,665,710 to Rahman et al. describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love et al. discloses liposomes comprising dsRNAs targeted to the *raf* gene. In addition, methods for preparing a liposome composition comprising a nucleic acid can be found in, e.g., U.S. Patents No. 6,011,020; No. 6,074,667; No. 6,110,490; No. 6,147,204; No. 6,271,206; No. 6,312,956; No. 6,465,188; No. 6,506,564; No. 6,750,016; No. 7,112,337.

[00233] Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate

through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g., they are self-optimizing, self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition.

[00234] Encapsulated nanoparticles can also be used for delivery of RNA effector molecules. Examples of such encapsulated nanoparticles include those created using yeast cell wall particles (YCWP). For example, glucan-encapsulated siRNA particles (GeRPs) are payload delivery systems made up of a yeast cell wall particle (YCWP) exterior and a multilayered nanoparticle interior, wherein the multilayered nanoparticle interior has a core comprising a payload complexed with a trapping agent. Glucan-encapsulated delivery systems, such as those described in U.S. Patent Applications Ser. No. 12/260,998, filed October 29, 2008, can be used to deliver siRNA duplexes to achieve silencing *in vitro* and *in vivo*..

C. Cell Cultures

[00235] Methods described herein use host cells to produce glycoproteins having modified glycans. A host cell can be derived from a yeast, insect, amphibian, fish, reptile, bird, mammal or human, or can be a hybridoma cell. Host cells can be unmodified cells or cell lines, or cell lines which have been genetically modified (e.g., to facilitate production of a biological product). In some embodiments, the host cell is a cell line that has been modified to allow for growth under desired conditions, such as in serum-free media, in cell suspension culture, or in adherent cell culture.

[00236] A mammalian host cell can be advantageous where the glycoprotein is a mammalian glycoprotein, particularly if the glycoprotein is a biotherapeutic agent or is otherwise intended for administration to or consumption by humans. In some embodiments, the host cell is a CHO cell, which is a cell line used for the expression of many recombinant proteins. Additional mammalian cell lines used commonly for the expression of recombinant proteins include 293HEK cells, HeLa cells, COS cells, NIH/3T3 cells, Jurkat Cells, NSO cells. and HUVEC cells.

[00237] In some embodiments, the host cell is a CHO cell derivative that has been modified genetically to facilitate production of recombinant proteins. For example, various CHO cell strains have been developed which permit stable insertion of recombinant DNA into a

specific gene or expression region of the cells, amplification of the inserted DNA, and selection of cells exhibiting high level expression of the recombinant protein. Examples of CHO cell derivatives useful in methods provided herein include, but are not limited to, CHO-K1 cells, CHO-DUKX, CHO-DUKX B1, CHO-DG44 cells, CHO-ICAM-1 cells, and CHO-hIFNγ cells. Methods for expressing recombinant proteins in CHO cells are known in the art and are described, e.g., in U.S. Patents No. 4,816,567 and No. 5,981,214.

[00238] Examples of human cell lines useful in methods provided herein include the cell lines 293T (embryonic kidney), 786-0 (renal), A498 (renal), A549 (alveolar basal epithelial), ACHN (renal), BT-549 (breast), BxPC-3 (pancreatic), CAKI-1 (renal), Capan-1 (pancreatic), CCRF-CEM (leukemia), COLO 205 (colon), DLD-1 (colon), DMS 114 (small cell lung), DU145 (prostate), EKVX (non-small cell lung), HCC-2998 (colon), HCT-15 (colon), HCT-116 (colon), HT29 (colon), HT-1080 (fibrosarcoma), HEK 293 (embryonic kidney), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), HL-60(TB) (leukemia), HOP-62 (non-small cell lung), HOP-92 (non-small cell lung), HS 578T (breast), HT-29 (colon adenocarcinoma), IGR-OV1 (ovarian), IMR32 (neuroblastoma), Jurkat (T lymphocyte), K-562 (leukemia), KM12 (colon), KM20L2 (colon), LAN5 (neuroblastoma), LNCap.FGC (Caucasian prostate adenocarcinoma), LOX IMVI (melanoma), LXFL 529 (non-small cell lung), M14 (melanoma), M19-MEL (melanoma), MALME-3M (melanoma), MCF10A (mammary epithelial), MCF7 (mammary), MDA-MB-453 (mammary epithelial), MDA-MB-468 (breast), MDA-MB-231 (breast), MDA-N (breast), MOLT-4 (leukemia), NCI/ADR-RES (ovarian), NCI-H226 (non-small cell lung), NCI-H23 (non-small cell lung), NCI-H322M (non-small cell lung), NCI-H460 (non-small cell lung), NCI-H522 (non-small cell lung), OVCAR-3 (ovarian), OVCAR-4 (ovarian), OVCAR-5 (ovarian), OVCAR-8 (ovarian), P388 (leukemia), P388/ADR (leukemia), PC-3 (prostate), PERC6® (E1-transformed embryonal retina), RPMI-7951 (melanoma), RPMI-8226 (leukemia), RXF 393 (renal), RXF-631 (renal), Saos-2 (bone), SF-268 (CNS), SF-295 (CNS), SF-539 (CNS), SHP-77 (small cell lung), SH-SY5Y (neuroblastoma), SK-BR3 (breast), SK-MEL-2 (melanoma), SK-MEL-5 (melanoma), SK-MEL-28 (melanoma), SK-OV-3 (ovarian), SN12K1 (renal), SN12C (renal), SNB-19 (CNS), SNB-75 (CNS) SNB-78 (CNS), SR (leukemia), SW-620 (colon), T-47D (breast), THP-1 (monocyte-derived macrophages), TK-10 (renal), U87 (glioblastoma), U293 (kidney), U251 (CNS), UACC-257 (melanoma), UACC-62 (melanoma), UO-31 (renal), W138 (lung), and XF 498 (CNS).

[00239] Examples of non-human primate cell lines useful in methods provided herein include the cell lines monkey kidney (CVI-76), African green monkey kidney (VERO-76), green monkey fibroblast (COS-1), and monkey kidney (CVI) cells transformed by SV40 (COS-7). Additional mammalian cell lines are known to those of ordinary skill in the art and are catalogued at the American Type Culture Collection catalog (Manassas, VA).

[00240] Examples of rodent cell lines useful in methods provided herein include the cell lines baby hamster kidney (BHK) (e.g., BHK21, BHK TK), mouse Sertoli (TM4), buffalo rat liver (BRL 3A), mouse mammary tumor (MMT), rat hepatoma (HTC), mouse myeloma (NS0), murine hybridoma (Sp2/0), mouse thymoma (EL4), Chinese Hamster Ovary (CHO) and CHO cell derivatives, murine embryonic (NIH/3T3, 3T3 LI), rat myocardial (H9c2), mouse myoblast (C2C12), and mouse kidney (miMCD-3).

[00241] In some embodiments, the host cell is a multipotent stem cell or progenitor cell. Examples of multipotent cells useful in methods provided herein include murine embryonic stem (ES-D3) cells, human umbilical vein endothelial (HuVEC) cells, human umbilical artery smooth muscle (HuASMC) cells, human differentiated stem (HKB-II) cells, human mesenchymal stem (hMSC) cells, and induced pluripotent stem (iPS) cells.

[00242] In some embodiments, the host cell is an insect cell, such as Sf9 cell line (derived from pupal ovarian tissue of *Spodoptera frugiperda*); Hi-5 (derived from *Trichoplusia ni* egg cell homogenates); or S2 cells (from *Drosophila melanogaster*).

[00243] In some embodiments, the host cells are suitable for growth in suspension cultures. Suspension-competent host cells are generally monodisperse or grow in loose aggregates without substantial aggregation. Suspension-competent host cells include cells that are suitable for suspension culture without adaptation or manipulation (e.g., hematopoietic cells, lymphoid cells) and cells that have been made suspension-competent by modification or adaptation of attachment-dependent cells (e.g., epithelial cells, fibroblasts).

[00244] In some embodiments, the host cell is an attachment dependent cell which is grown and maintained in adherent culture. Examples of human adherent cell lines useful in methods provided herein include the cell lines human neuroblastoma (SH-SY5Y, IMR32, and LAN5), human cervical carcinoma (HeLa), human breast epithelial (MCFlOA), human embryonic kidney (293T), and human breast carcinoma (SK-BR3).

[00245] In some embodiments, the host cell is a cell line that has been modified to allow for growth under desired conditions, such as in serum-free media, in cell suspension culture, or in adherent cell culture. The host cell can be, for example, a human Namalwa Burkitt lymphoma cell (BLcl-kar-Namalwa), baby hamster kidney fibroblast (BHK), CHO cell, Murine myeloma cell (NS0, SP2/0), hybridoma cell, human embryonic kidney cell (293 HEK), human retina-derived cell (PER.C6® cells, U.S. Patent No. 7,550,284), insect cell line (Sf9, derived from pupal ovarian tissue of *Spodoptera frugiperda*; or Hi-5, derived from *Trichoplusia ni* egg cell homogenates; *see also* U.S. Patent No. 7,041,500), Madin-Darby canine kidney cell (MDCK), primary mouse brain cells or tissue, primary calf lymph cells or tissue, primary monkey kidney cells, embryonated chicken egg, primary chicken embryo fibroblast (CEF), Rhesus fetal lung cell (FRhL-2), Human fetal lung cell (WI-38, MRC-5), African green monkey kidney epithelial cell (Vero, CV-1), Rhesus monkey kidney cell (LLC-MK2), or yeast cell. Additional mammalian cell lines commonly used for the expression of recombinant proteins include, but are not limited to, HeLa cells, COS cells, NIH/3T3 cells, Jurkat Cells, and human umbilical vein endothelial cells (HUVEC) cells.

[00246] Host cells can be unmodified or genetically modified (e.g., a cell from a transgenic animal). For example, CEFs from transgenic chicken eggs can have one or more genes essential for the IFN pathway, e.g.,interferon receptor, STAT1, etc., has been disrupted, i.e., is a "knockout." *See, e.g.*, Sang, 12 Trends Biotech. 415 (1994); Perry et al., 2 Transgenic Res. 125 (1993); Stern, 212 Curr Top Micro. Immunol. 195-206 (1996); Shuman, 47 Experientia 897 (1991). Also, the cell can be modified to allow for growth under desired conditions, e.g., incubation at 30°C.

[00247] The host cells may express the glycoprotein of interest endogenously, or alternatively, the host cell may be engineered to express an exogenous glycoprotein. For example, a host cell may be transfected with one or more expression vectors that encode the glycoprotein. The nucleic acid molecule encoding the glycoprotein may be transiently introduced into the host cell, or stably integrated into the genome of the host cell. For example, in case of an antibody, one or more recombinant expression vectors encoding the light and/or heavy chains of the antibody (or an antigen-binding fragment of the antibody) may be transfected, such that the light and/or heavy chains are expressed in the host cell. If desired, the glycoprotein may be secreted into the medium in which the host cell is cultured, from which medium the glycoprotein can be recovered.

[00248] Standard recombinant DNA methodologies may be used to obtain a nucleic acid that encodes a glycoprotein, incorporate the nucleic acid into an expression vector and introduce the vector into a host cell, such as those described in Sambrook, et al. (eds), Molecular Cloning; A Laboratory Manual, Third Edition, Cold Spring Harbor, (2001); Ausubel, F. M. et al. (eds.) Current Protocols in Molecular Biology, John Wiley & Sons (1995). A nucleic acid encoding the glycoprotein may be inserted into an expression vector or vectors such that the nucleic acids are operably linked to transcriptional and translational control sequences. The expression vector and expression control sequences are generally chosen to be compatible with the expression host cell used.

[00249] For example, to express an antibody, or an antigen-binding fragment thereof, nucleic acids encoding the light and heavy chain variable regions may be first obtained. These nucleic acids can be obtained by amplification and modification of human germline light and heavy chain variable region genes using PCR. Germline DNA sequences for human heavy and light chain variable region genes are known in the art.

[00250] In addition to the nucleic acid that encodes the glycoprotein, the expression vector may additionally carry regulatory sequences that control the expression of the glycoprotein in a host cell, such as promoters, enhancers or other expression control elements (e. g. , polyadenylation signals) that control the transcription or translation of the nucleic acid(s). Such regulatory sequences are known in the art (see, e.g., Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press (1990)). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Exemplary regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer),adenovirus, (e. g. , the adenovirus major late promoter (AdMLP)) and polyoma virus.

[00251] In addition to sequences encoding the glycoprotein and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as

sequences that regulate replication of the vector in host cells (e. g. , origins of replication) and selectable marker genes.

[00252] The expression vector(s) encoding the glycoprotein may be transfected into a host cell by standard techniques, such as electroporation, calcium-phosphate precipitation, or DEAE-dextran transfection. If desired, viral vectors, such as retro-viral vectors, may also be used to generate stable cell lines (as a source of a continuous supply of the glycoprotein).

[00253] The methods described herein can be applied to any size of cell culture flask and/or bioreactor. For example, the methods can be applied in bioreactors or cell cultures of 10 L, 30 L, 50 L, 100 L, 150 L, 200 L, 300 L, 500 L, 1000 L, 2000 L, 3000 L, 4000 L, 5000 L, 10,000 L or larger. In some embodiments, the cell culture size can range from 10 L to 5000 L, from 10 L to 10,000 L, from 10 L to 20,000 L, from 10 L to 50,000 L, from 40 L to 50,000 L, from 100 L to 50,000 L, from 500 L to 50,000 L, from 1000 L to 50,000 L, from 2000 L to 50,000 L, from 3000 L to 50,000 L, from 4000 L to 50,000 L, from 4500 L to 50,000 L, from 1000 L to 10,000 L, from 1000 L to 20,000 L, from 1000 L to 25,000 L, from 1000 L to 30,000 L, from 15 L to 2000 L, from 40 L to 1000 L, from 100 L to 500 L, from 200 L to 400 L, or any integer there between.

[00254] Media components include, e.g., buffer, amino acid content, vitamin content, salt content, mineral content, serum content, carbon source content, lipid content, nucleic acid content, hormone content, trace element content, ammonia content, co-factor content, indicator content, small molecule content, hydrolysate content and enzyme modulator content. Preferably, the growth medium is a chemically defined media such as Biowhittaker® POWERCHO® (Lonza, Basel, Switzerland), HYCLONE PF CHO™ (Thermo Scientific, Fisher Scientific), GIBCO® CD DG44 (Invitrogen, Carlsbad, CA), Medium M199 (Sigma-Aldrich), OPTIPRO™ SFM (Gibco), etc.).

D. Regulation of Host Cell Gene Expression.

[00255] One or more RNA effector molecules are added to the cell culture to regulate the expression level(s) of target gene(s). If more than two or more RNA effector molecules are used, they may be provided at the same concentration, or different concentrations. The RNA effectors may be added simultaneously into the cell culture, or added at different times into the cell culture.

[00256] An effective amount of an RNA effector is added to the cell culture to allow sufficient reduction of the expression of a target gene. For example, an effective amount of an RNA effector is added to the cell culture such that the expression level of its target gene is reduced by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85%.

[00257] In general, a suitable dose of RNA effector molecule will be in the range of 0.001 to 200.0 milligrams per unit volume per day. For example, the RNA effector molecule may be provided in the range of 0.001 nM to 200 mM per day, generally in the range of 0.1 nM to 500 nM. For example, a dsRNA can be administered at 0.01 nM, 0.05 nM, 0.1 nM, 0.5 nM, 0.75 nM, 1 nM, 1.5 nM, 2 nM, 3 nM, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 100 nM, 200 nM, 400 nM, or 500 nM per single dose. In one embodiment, the RNA effector molecule is administered a cell culture at a concentration less than about 50nM.

[00258] The composition can be added to the cell culture once daily, or the RNA effector molecule can be added as two, three, or more sub-doses at appropriate intervals throughout the day or delivery through a controlled release formulation. In that case, the RNA effector molecule contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, e.g., using a conventional sustained release formulation, which provides sustained release of the RNA effector molecule over a several-day-period.

[00259] The effect of a single dose on target gene transcript levels can be long-lasting, such that subsequent doses are administered at not more than 3-, 4-, or 5-day intervals, or at not more than 1-, 2-, 3-, or 4-week intervals.

[00260] The administration of the RNA effector molecule may be ceased at least 6 hr, at least 12 hr, at least 18 hr, at least 36 hr, at least 48 hr, at least 60 hr, at least 72 hr, at least 96 hr, or at least 120 hr, or at least 1 week, before isolation of the biological product. Thus in one embodiment, contacting a host cell (e.g., in a large scale host cell culture) with a RNA effector molecule is complete at least 6 hr, at least 12 hr, at least 18 hr, at least 36 hr, at least 48 hr, at least 60 hr, at least 72 hr, at least 96 hr, or at least 120 hr, or at least 1 week, before isolation of the biological product.

[00261] Sometimes, it may be beneficial to provide a RNA effector molecule to the host cell cultures in a way that a constant number (or at least a minimum number) of RNA effector molecules per each cell is maintained. Maintaining the levels of the RNA effector molecule as such can ensure that modulation of target gene expression is maintained even at high cell densities.

[00262] The amount of a RNA effector molecule can also be administered according to the cell density. In such embodiments, the RNA effector molecule(s) is added at a concentration of at least 0.01 fmol/10^6 cells, at least 0.1 fmol/10^6 cells, at least 0.5 fmol/10^6 cells, at least 0.75 fmol/10^6 cells, at least 1 fmol/10^6 cells, at least 2 fmol/10^6 cells, at least 5 fmol/10^6 cells, at least 10 fmol/10^6 cells, at least 20 fmol/10^6 cells, at least 30 fmol/10^6 cells, at least 40 fmol/10^6 cells, at least 50 fmol/10^6 cells, at least 60 fmol/10^6 cells, at least 100 fmol/10^6 cells, at least 200 fmol/10^6 cells, at least 300 fmol/10^6 cells, at least 400 fmol/10^6 cells, at least 500 fmol/10^6 cells, at least 700 fmol/10^6 cells, at least 800 fmol/10^6 cells, at least 900 fmol/10^6 cells, or at least 1 pmol/10^6 cells, or more.

[00263] For example, the RNA effector molecule may be administered at a dose of at least 10 molecules per cell, at least 20 molecules per cell (molecules/cell), at least 30 molecules/cell, at least 40 molecules/cell, at least 50 molecules/cell, at least 60 molecules/cell, at least 70 molecules/cell, at least 80 molecules/cell, at least 90 molecules/cell at least 100 molecules/cell, at least 200 molecules/cell, at least 300 molecules/cell, at least 400 molecules/cell, at least 500 molecules/cell, at least 600 molecules/cell, at least 700 molecules/cell, at least 800 molecules/cell, at least 900 molecules/cell, at least 1000 molecules/cell, at least 2000 molecules/cell, at least 5000 molecules/cell or more, inclusive.

[00264] In some embodiments, the RNA effector molecule is administered at a dose within the range of 10-100 molecules/cell, 10-90 molecules/cell, 10-80 molecules/cell, 10-70 molecules/cell, 10-60 molecules/cell, 10-50 molecules/cell, 10-40 molecules/cell, 10-30 molecules/cell, 10-20 molecules/cell, 90-100 molecules/cell, 80-100 molecules/cell, 70-100 molecules/cell, 60-100 molecules/cell, 50-100 molecules/cell, 40-100 molecules/cell, 30-100 molecules/cell, 20-100 molecules/cell, 30-60 molecules/cell, 30-50 molecules/cell, 40-50 molecules/cell, 40-60 molecules/cell, or any range there between.

[00265] In one embodiment, the RNA effector molecule is administered as a sterile aqueous solution. In one embodiment, the RNA effector molecule is formulated in a non-lipid formulation. In another embodiment, the RNA effector molecule is formulated in a cationic or non-cationic lipid formulation. In still another embodiment, the RNA effector molecule is formulated in a cell medium suitable for culturing a host cell (e.g., a serum-free medium).

E. Purification of Glycoproteins

[00266] The glycoproteins produced in accordance with the methods described herein can be harvested from host cells, and purified using any suitable methods. For example, methods for purifying polypeptides by immune-affinity chromatography are known in the art. *Ruiz-Arguello et al., J. Gen. Virol., 85*:3677-3687 (2004). Suitable methods for purifying desired glycoprotein including precipitation and various types of chromatography, such as hydrophobic interaction, ion exchange, affinity, chelating and size exclusion are well-known in the art. Suitable purification schemes can be created using two or more of these or other suitable methods. If desired, the glycoprotein can include a "tag" that facilitates purification, such as an epitope tag or a HIS tag. Such tagged polypeptides can conveniently be purified, for example from conditioned media, by chelating chromatography or affinity chromatography. Optionally, the tag sequence may be cleaved post-purification.

[00267] For example, normal phase liquid chromatography can be used to separate glycans and/or glycoproteins based on polarity. Reverse-phase chromatography can be used, e.g., with derivatized sugars. Anion-exchange columns can be used to purify sialylated, phosphorylated, and sulfated sugars. Other methods include high pH anion exchange chromatography and size exclusion chromatography can be used and is based on size separation.

[00268] Affinity based methods can be selected that preferentially bind certain chemical units and glycan structures. Matrices such as m-aminophenylboronic acid, immobilized lectins and antibodies can bind particular glycan structures. M-aminophenylboronic acid matrices can form a temporary covalent bond with any molecule (such as a carbohydrate) that contains a 1,2-cis-diol group. The covalent bond can be subsequently disrupted to elute the protein of interest. Lectins are a family of carbohydrate-recognizing proteins that exhibit affinities for various monosaccharides. Lectins bind carbohydrates specifically and reversibly. Primary monosaccharides recognized by lectins include

mannose/glucose, galactose/N-acetylgalactosamine, N-acetylglucosamine, fucose, and sialic acid (QProteome Glycoarray Handbook, Qiagen, September 2005, available at: http://wolfson.huji.ac.il/urification/PDF/Lectins/QIAGEN_GlycoArrayHa-ndbook.pdf) or similar references. Lectin matrices (e.g., columns or arrays) can consist of a number of lectins with varying and/or overlapping specificities to bind glycoproteins with specific glycan compositions. Some lectins commonly used to purify glycoproteins include concavalin A (often coupled to Sepharose or agarose) and Wheat Germ. Anti-glycan antibodies can also be generated by methods known in the art and used in affinity columns to hind and purify glycoproteins.

[00269] The interaction of a lectin or antibody with a ligand, such as a glycoprotein, allows for the formation of cross-linked complexes, which are often insoluble and can be identified as precipitates (Varki et al., ed., "Protein-Glycan Interactions" in Essentials of Glycobiology available at world wide web at http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=glyco.section.269) or similar references. In this technique, a fixed amount of lectin or antibody (receptor) is titrated with a glycoprotein or a glycan, and at a precise ratio of ligand to receptor, a precipitate is formed (Varki et al.). Such precipitation is highly specific to the affinity constant of the ligand to the receptor (Varki et al.). Another precipitation approach takes advantage of the fact that a complex between a lectin and a glycan can be "salted" out or precipitated by ammonium sulfate (Varki et al.).

F. Analysis of Glycoproteins

(1). Analysis of the structure and composition of N-linked glycans

[00270] The glycan structure of the glycoproteins (such as antibodies or Fc-fusion proteins) described herein can be determined using art-known methods for analyzing glycan structures of glycoproteins, such as chromatography, mass spectrometry (MS), chromatography followed by MS, electrophoresis, electrophoresis followed by MS, nuclear magnetic resonance (NMR), and any combinations thereof. A preferred technique is Liquid chromatography–mass spectrometry (LC-MS, or alternatively HPLC-MS).

[00271] For example, an enzyme, such as an N-glycanase (e.g, N-glycanase F, N-glycanase-A), can be used to cleave the N-glycan moiety from a glycoprotein. Further, exoglycosidases (e.g., sialidase, galactosidase, hexosaminidase, fucosidase, mannosidase etc.) can be used cleave terminal glycosidic bonds from the non-reducing end of glycans.

Alternatively, acid hydrolysis (e.g., trifluoroacetic acid) can be used to release neutral saccharides (e.g., galactose, mannose, fucose) or amino saccharides (e.g., N-acetylglucosamine) from a glycan. The cleaved or hydrolyzed saccharides can be analyzed using chromatography spectrometry, or electrophoresis methods described above.

[00272] For example, glycan structure and composition can be analyzed by chromatography, including, e.g., liquid chromatography (LC), high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), thin layer chromatography (TLC), amide column chromatography, or combinations thereof.

[00273] Another method to analyze glycan structure and composition is mass spectrometry (MS), including, e.g., tandem MS, LC-MS, LC-MS/MS, matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS), Fourier transform mass spectrometry (FTMS), ion mobility separation with mass spectrometry (IMS-MS), electron transfer dissociation (ETD-MS), or combinations thereof.

[00274] Another method to analyze glycan structure and composition is electrophoresis, including, e.g., capillary electrophoresis (CE), CE-MS, gel electrophoresis, agarose gel electrophoresis, acrylamide gel electrophoresis, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting using antibodies that recognize specific glycan structures, or combinations thereof. For example, the structure of an N-glycan can be determined by two dimensional sugar chain mapping (see, e.g., Anal. Biochem., 171, 73 (1988); Biochemical Experimentation Methods 23-Methods for Studying Glycoprotein Sugar Chains (Japan Scientific Societies Press) edited by Reiko Takahashi (1989)). Two dimensional sugar chain mapping is a method for deducing the structure of a saccharide chain by plotting the retention time or elution position of the saccharide chain by reverse phase chromatography as the X axis, and the retention time or elution position of the saccharide chain by normal phase chromatography as the Y axis, respectively, and comparing them with such results of known sugar chains. The structure deduced by two dimensional sugar chain mapping can be confirmed by mass spectrometry.

[00275] Another method to analyze glycan structure and composition is nuclear magnetic resonance (NMR), including, e.g., one-dimensional NMR (1D-NMR), two-dimensional NMR (2D-NMR), correlation spectroscopy magnetic-angle spinning NMR (COSY-

NMR), total correlated spectroscopy NMR (TOCSY-NMR), heteronuclear single-quantum coherence NMR (HSQC-NMR), heteronuclear multiple quantum coherence (HMQC-NMR), rotational nuclear overhauser effect spectroscopy NMR (ROESY-NMR), nuclear overhauser effect spectroscopy (NOESY-NMR), or combinations thereof.

[00276] Saccharide composition of a glycan can also be analyzed by fluorescence labeling. For example, acid-hydrolyzed glycans can be labeled with 2-aminopyridine and then analyzed by HPLC.

[00277] Immunological methods (e.g., antibody staining, lectin staining) may also be used to determine the structures of N-glycan. For example, lectin molecules can bind to the carbohydrate moieties of glycoproteins. Therefore, a lectin that binds to a specific N-glycan can be used to identify the presence and quantity of such glycoforms in a composition (e.g., by determining the amount of glycan-bound lectin using a secondary antibody). Examples of lectins that can be used for identifying the glycan structure of an antibody, or a Fc-fusion protein, include, e.g., WGA (wheat-germ agglutinin derived from T. vulgaris), ConA (cocanavalin A derived from C. ensiformis), RIC (a toxin derived from R. communis), L-PHA (leucoagglutinin derived from P. vulgaris), LCA (lentil agglutinin derived from L. culinaris), PSA (pea lectin derived from P. sativum), AAL (Aleuria aurantia lectin), ACL (Amaranthus caudatus lectin), BPL (Bauhinia purpurea lectin), DSL (Datura stramonium lectin), DBA (Dolichos biflorus agglutinin), EBL (elderberry balk lectin), ECL (Erythrina cristagalli lectin), EEL (Euonymus eoropaeus lecin), GNL (Galanthus nivalis lectin), GSL (Griffonia simplicifolia lectin), HPA (Helix pomatia agglutinin), HHL (Hippeastrum hybrid lectin), Jacalin, LTL (Lotus tetragonolobus lectin), LEL (Lycopersicon esculentum lectin), MAL (Maackia amurensis lectin), MPL (Maclura pomifera lectin), NPL (Narcissus pseudonarcissus lectin), PNA (peanut agglutinin), E-PHA (Phaseolus vulgaris erythroagglutinin), PTL (Psophocarpus tetragonolobus lectin), RCA (Ricinus communis agglutinin), STL (Solanum tuberosum lectin), SJA (Sophora japonica agglutinin), SBA (soybean agglutinin), UEA (Ulex europaeus agglutinin), VVL (Vicia villosa lectin) and WFA (Wisteria floribunda agglutinin).

[00278] For example, a lectin that specifically recognizes a complex N-glycan in which a fucose residue is linked to the N-acetylglucosamine in the reducing end of the N-glycan may be used. Exemplary lectins include, e.g., Lens culinaris lectin LCA (lentil agglutinin derived from Lens culinaris), pea lectin PSA (pea lectin derived from Pisum sativum), broad

bean lectin VFA (agglutinin derived from Vicia faba) and Aleuria aurantia lectin AAL (lectin derived from Aleuria aurantia).

[00279] Another method to analyze glycan structure and composition is by capillary electrophoresis (CE), which is described e.g., in Szabo et al., Electrophoresis, 2010 April; 31(8): 1389–1395. doi:10.1002/elps.201000037.

[00280] Techniques described herein may be combined with one or more other technologies for the detection, analysis, and or isolation of glycans or glycoproteins. For example, any combination of NMR, mass spectrometry, liquid chromatography, 2-dimensional chromatography, SDS-PAGE, antibody staining, lectin staining, monosaccharide quantitation, capillary electrophoresis, fluorophore-assisted carbohydrate electrophoresis (FACE), micellar electrokinetic chromatography (MEKC), exoglycosidase or endoglycosidase treatments may be used. See, e.g., Anumula, Anal. Biochem. 350(1): 1, 2006; Klein et al., Anal. Biochem., 179:162, 1989; Townsend, R.R. Carbohydrate Analysis, High Performance Liquid Chromatography and Capillary Electrophoresis, Ed. Z. El Rassi, pp 181-209, 1995. For example, Qian et al. (Analytical Biochemistry 364 (2007) 8-18) discloses a method for determining the structures of glycans using orthogonal matrix-assisted laser desorption/ionization hybrid quadrupole-quadrupole time-of-fight mass spectrometry (oMALDI Qq–TOF MS) and tandem mass spectrometry (MS/MS) in combination with exoglycosidase digestion. The N-linked glycans are released by treatment with N-glycanase F, reductively aminated with anthranilic acid, and fractionated by normal phase high-performance liquid chromatography (NP-HPLC). The Xuorescent-labeled oligosaccharide pool and fractions are then analyzed by oMALDI Qq-TOF MS and MS/MS in negative ion mode. Each fraction is further digested with an array of exoglycosidase mixtures, and subsequent MALDI TOF MS analysis of the resulting products yields information about structural features of the glycan.

[00281] One exemplary saccharide composition analyzer is BioLC, manufactured by Dionex, which analyzes saccharide composition by HPAEC-PAD (high performance anion-exchange chromatography-pulsed amperometric detection).

(2). Analysis of the activity of glycoproteins

[00282] The biological activity of the glycoprotein compositions described herein may be assessed using any art known method. Such biological activities include, e.g., binding

affinity or specificity of a glycoprotein (e.g., antibody, or a ligand for a receptor), bioavailability, pharmacokinetics, pharmacodynamics, etc. Additionally, therapeutic activity of a glycoprotein may be assessed (e.g., ADCC activity of an antibody, efficacy of a glycoprotein in decreasing severity or symptom of a disease or condition, or in delaying appearance of a symptom of a disease or condition).

[00283] Methods of analyzing bioavailability, pharmacokinetics, and pharmacodynamics of glycoprotein therapeutics have been described. See, e.g., Weiner et al., /. Pharm. Biomed. Anal. 15(5):571-9, 1997; Srinivas et al., /. Pharm. ScL 85(l):l-4, 1996; and Srinivas et al., Pharm. Res. 14(7):911-6, 1997. Assays for measuring ADCC activity mediated by therapeutic antibodies are also known. See, e.g., Schnueriger A. et al., Mol. Immunol. (2011) 48(12-13):1512-7; Cancer Immunology Immunotherapy, 36, 373 (1993); Cancer Research, 5A, 1511 (1994) . As would be understood to one of skill in the art, the particular biological activity or therapeutic activity that can be tested will vary depending on the particular glycoprotein or glycan structure.

[00284] The potential adverse activity or toxicity (e.g., propensity to cause hypertension, immunogenicity/allergic reactions, thrombotic events, seizures, or other adverse events) of glycoprotein preparations can be analyzed by any available method. For example, immunogenicity of a glycoprotein composition can be assessed, e.g., by determining in vitro by immunoassay (e.g., using an antibody that binds to a recognized immunogenic epitope, such as the αGal epitope, or Neu5Gc epitope), or by in vivo administration to determine whether the composition elicits an antibody response in a subject.

5. PHARMACEUTICAL COMPOSITIONS, METHODS OF ADMINISTRATION, AND KITS

A. Pharmaceutical Compositions

[00285] In one aspect, the invention relates to pharmaceutical compositions comprising the glycoproteins (such as antibodies or Fc-fusion proteins) described herein.

[00286] The pharmaceutical compositions usually one or more pharmaceutical carrier(s) and/or excipient(s). A thorough discussion of such components is available in Gennaro (2000) Remington: The Science and Practice of Pharmacy (20th edition). Examples of such carriers or additives include water, a pharmaceutical acceptable organic solvent, collagen,

polyvinyl alcohol, polyvinylpyrrolidone, a carboxyvinyl polymer, carboxymethylcellulose sodium, polyacrylic sodium, sodium alginate, water-soluble dextran, carboxymethyl starch sodium, pectin, methyl cellulose, ethyl cellulose, xanthan gum, gum Arabic, casein, gelatin, agar, diglycerin, glycerin, propylene glycol, polyethylene glycol, Vaseline, paraffin, stearyl alcohol, stearic acid, human serum albumin (HSA), mannitol, sorbitol, lactose, a pharmaceutically acceptable surfactant and the like. Formulation of the pharmaceutical composition will vary according to the route of administration selected.

[00287] Optionally, the glycoprotein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of activity loss and that use levels may have to be adjusted to compensate.

[00288] A variety of aqueous carriers can be used to formulate suitable pharmaceutical compositions for administration, such as plain water (*e.g.* w.f.i.) or a buffer *e.g.* a phosphate buffer, a Tris buffer, a borate buffer, a succinate buffer, a histidine buffer, or a citrate buffer. Buffer salts will typically be included in the 5-20mM range.

[00289] The pharmaceutical compositions are preferably sterile, and may be sterilized by conventional sterilization techniques.

[00290] The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, and tonicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. Preferably, the pharmaceutical compositions of the invention may have a pH between 5.0 and 9.5, *e.g.* between 6.0 and 8.0.

[00291] Pharmaceutical compositions of the invention may include sodium salts (*e.g.* sodium chloride) to give tonicity. A concentration of 10 ± 2 mg/ml NaCl is typical *e.g.* about 9 mg/ml.

[00292] Pharmaceutical compositions of the invention may have an osmolarity of between 200 mOsm/kg and 400 mOsm/kg, *e.g.* between 240-360 mOsm/kg, or between 290-310 mOsm/kg.

B. Methods of Administration

[00293] In another aspect, the invention provide a method for treating Non-Hodgkins lymphoma, comprising administering to a subject in need thereof a therapeutically effectively amount of an anti-CD20 antibody as described herein. The subject is preferably human.

[00294] In another aspect, the invention provide a method for treating cancer or head and neck squamous cell carcinoma, comprising administering to a subject in need thereof a therapeutically effectively amount of an anti-EGFR antibody as described herein. The subject is preferably human.

[00295] In another aspect, the invention provide a method for treating breast cancer, comprising administering to a subject in need thereof a therapeutically effectively amount of an anti-HER2 antibody as described herein. The subject is preferably human.

[00296] In another aspect, the invention provide a method for treating breast cancer, comprising administering to a subject in need thereof a therapeutically effectively amount of an B7-binding Fc-fusion protein as described herein. The subject is preferably human.

[00297] The compositions described herein may be administered to a subject orally, topically, transdermally, parenterally, by inhalation spray, vaginally, rectally, or by intracranial injection. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or infusion techniques. Administration by intravenous, intradermal, intramusclar, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary injection and or surgical implantation at a particular site is contemplated as well. In certain embodiments, injection, especially intravenous, is preferred.

[00298] The amounts of a glycoprotein in a given dosage will vary according to the size of the individual to whom the therapy is being administered as well as the characteristics of the disorder being treated. In exemplary treatments, it may be necessary to administer about 1 mg/day, about 5 mg/day, about 10 mg/day, about 20 mg/day, about 50 mg/day, about 75 mg/day, about 100 mg/day, about 150 mg/day, about 200 mg/day, about 250 mg/day, about 400

mg/day, about 500 mg/day, about 800 mg/day, about 1000 mg/day, about 1600 mg/day or about 2000 mg/day. The doses may also be administered based on weight of the patient, at a dose of 0.01 to 50 mg/kg. The glycoprotein may be administered in a dose range of 0.015 to 30 mg/kg, such as in a dose of about 0.015, about 0.05, about 0.15, about 0.5, about 1.5, about 5, about 15 or about 30 mg/kg.

[00299] Dosage can be by a single dose schedule or a multiple dose schedule. Multiple doses will typically be administered at least 1 week apart (*e.g.*, about 2 weeks, about 3 weeks, about 4 weeks, about 6 weeks, about 8 weeks, about 10 weeks, about 12 weeks, about 16 weeks, etc.).

[00300] Standard dose-response studies, first in animal models and then in clinical testing, can reveal optimal dosages for particular diseases and patient populations.

[00301] The glycoprotein compositions described herein may be administered in combination with a second therapeutic agent. For example, for cancer treatment, a chemotherapeutic agent may be used as the second agent. For treatment of autoimmune diseases, non-steroidal anti-inflammatory drugs (NSAIDs), analgesiscs, glucocorticoids, disease-modifying antirheumatic drugs (DMARDs), may be used as the second agent. Examples of such therapeutic agents can be found, e.g., in WO 2008/156713.

C. Kits

[00302] In another aspect, the invention provides kits that comprise the glycoprotein compositions described herein packaged in a manner that facilitates their use for therapy. For example, such a kit includes a glycoprotein (e.g., an antibody or an Fc-fusion protein) as described herein, packaged in a container such as a sealed bottle or vessel, with a label affixed to the container or included in the package that describes use of the composition in practicing the method. The kit can further comprise another container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution, or dextrose solution. Preferably, the composition is packaged in a unit dosage form. The kit may further include a device suitable for administering the composition according to a specific route of administration or for practicing a screening assay. Preferably, the kit contains a label that describes use of the composition.

[00303] In another aspect, the invention provides kits for testing the effect of a RNA effector molecule or a series of RNA effector molecules on the production of a glycoprotein by the host cell, where the kits comprise a substrate having one or more assay surfaces suitable for culturing cells under conditions that allow production of the glycoprotein. In some embodiments, the exterior of the substrate comprises wells, indentations, demarcations, or the like at positions corresponding to the assay surfaces. In some embodiments, the wells, indentations, demarcations, or the like retain fluid, such as cell culture media, over the assay surfaces.

[00304] In some embodiments, the assay surfaces on the substrate are sterile and are suitable for culturing host cells under conditions representative of the culture conditions during large-scale (e.g., industrial scale) production of the glycoprotein. Advantageously, kits provided herein offer a rapid, cost-effective means for testing a wide-range of agents and/or conditions on the production of the glycoprotein, allowing the cell culture conditions to be established prior to full-scale production of the glycoprotein.

[00305] In some embodiments, one or more assay surfaces of the substrate comprise a concentrated test agent, such as a RNA effector molecule, such that the addition of suitable media to the assay surfaces results in a desired concentration of the RNA effector molecule surrounding the assay surface. In some embodiments, the RNA effector molecules may be printed or ingrained onto the assay surface, or provided in a lyophilized form, e.g., within wells, such that the effector molecules can be reconstituted upon addition of an appropriate amount of media. In some embodiments, the RNA effector molecules are reconstituted by plating cells onto assay surfaces of the substrate.

[00306] In some embodiments, kits provided herein further comprise cell culture media suitable for culturing a cell under conditions allowing for the production of the glycoprotein of interest. The media can be in a ready to use form or can be concentrated (e.g., as a stock solution), lyophilized, or provided in another reconstitutable form.

[00307] In further embodiments, kits provided herein further comprise one or more reagents suitable for detecting production of the glycoprotein by the cell, cell culture, or tissue culture. In further embodiments, the reagent(s) are suitable for detecting a property of the cell, such as maximum cell density, cell viability, or the like, which is indicative of production of the

desired glycoprotein. In some embodiments, the reagent(s) are suitable for detecting the glycoprotein or a property thereof, such as the *in vitro* or *in vivo* biological activity, homogeneity, or structure of the glycoprotein.

[00308] In some embodiments, one or more assay surfaces of the substrate further comprise a carrier for which facilitates uptake of RNA effector molecules by cells. Carriers for RNA effector molecules are known in the art and are described herein. For example, in some embodiments, the carrier is a lipid formulation such as Lipofectamine™ transfection reagent (Invitrogen; Carlsbad, CA) or a related formulation. Examples of such carrier formulations are described herein. In some embodiments, the reagent that facilitates RNA effector molecule uptake comprises a charged lipid, an emulsion, a liposome, a cationic or non-cationic lipid, an anionic lipid, a transfection reagent or a penetration enhancer as described throughout the application herein. In particular embodiments, the reagent that facilitates RNA effector molecule uptake comprises a charged lipid as described in U.S. Application Ser. No. 61/267,419, filed on December 7, 2009.

[00309] In some embodiments, one or more assay surfaces of the substrate comprise a RNA effector molecule or series of RNA effector molecules and a carrier, each in concentrated form, such that plating test cells onto the assay surface(s) results in a concentration the RNA effector molecule(s) and the carrier effective for facilitating uptake of the RNA effector molecule(s) by the cells and modulation of the expression of one or more genes targeted by the RNA effector molecules.

[00310] In some embodiments, the substrate further comprises a matrix which facilitates 3-dimensional cell growth and/or production of the glycoprotein by the cells. In further embodiments, the matrix facilitates anchorage-dependent growth of cells. Non-limiting examples of matrix materials suitable for use with various kits described herein include agar, agarose, methylcellulose, alginate hydrogel (e.g., 5% alginate + 5% collagen type I), chitosan, hydroactive hydrocolloid polymer gels, polyvinyl alcohol-hydrogel (PVA-H), polylactide-co-glycolide (PLGA), collagen vitrigel, PHEMA (poly(2-hydroxylmethacrylate)) hydrogels, PVP/PEO hydrogels, BD PuraMatrix™ hydrogels, and copolymers of 2-methacryloyloxyethyl phophorylcholine (MPC).

[00311] In some embodiments, the substrate comprises a microarray plate, a biochip, or the like which allows for the high-throughput, automated testing of a range of test agents, conditions, and/or combinations thereof on the production of a glycoprotein by cultured cells. For example, the substrate may comprise a 2-dimensional microarray plate or biochip having m columns and n rows of assay surfaces (e.g., residing within wells) which allow for the testing of m x n combinations of test agents and/or conditions (e.g., on a 24-, 96- or 384-well microarray plate). The microarray substrates are preferably designed such that all necessary positive and negative controls can be carried out in parallel with testing of the agents and/or conditions.

[00312] In some embodiments, kits provided herein allow for the selection or optimization of at least one factor for enhancing production of the biological product. For example, the kits may allow for the selection of a RNA effector molecule from among a series of candidate RNA effector molecules, or for the selection of a concentration or concentration range from a wider range of concentrations of a given RNA effector molecule. In some embodiments, the kits allow for selection of one or more RNA effector molecules from a series of candidate RNA effector molecules directed against a common target gene. In further embodiments, the kits allow for selection of one or more RNA effector molecules from a series of candidate RNA effector molecules directed against two or more functionally related target genes or two or more target genes of a common host cell glycosylation pathway.

[00313] In another aspect, the invention provides kits that comprise one or more container that independently contain one or more RNA effector molecules and one or more suitable host cells.

EXEMPLIFICATION

[00314] The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

EXAMPLE 1. GLYCAN-MODIFIED ANTI-CD20 ANTIBODY

[00315] In this Example, glycan-modified anti-CD20 antibody, based on Rituximab, was produced in a large scale culture using RNAi technology. The glycan-modified antibody

showed enhanced ADCC activity as compared to Rituxamb under substantially the same conditions.

[00316] Rituximab (Rituxan®) is a chimeric anti-CD20 monoclonal antibody for treating non-Hodgkin's B-cell lymphoma (NHL). Rituximab is recombinantly produced in CHO cells, and has a heterogeneous mixture of glycoforms.

[00317] The response rate for Rituximab in NHL patients is about 50-60% and is significantly correlated with a FcγRIIIa receptor polymorphism (Cartron et al. (2002) Blood 99:754-758). About 90% of patients homozygous for valine at position 158 respond to Rituximab treatment, whereas patients hetero- or homozygous for phenylalanine at position 158 have a considerably lower response rate. It is believed that a lower affinity for FcγRIIIa F158 than for FcγRIIIa V158 leads to lower ADCC activity. It has been shown that afucosylated IgG1 has a higher affinity for FcγRIIIa F158 (consequently higher ADCC activity) than the corresponding fucosylated IgG1 (Shields et al. (2002) J. Biol. Chem. 277: 26733-26740). An afucosylated Rituximab therefore would be a more potent therapeutic product regardless of the FcγRIIIa genotype.

[00318] In addition to ADCC, Rituximab also mediates tumor cell killing through CDC (Cragg and Glennie (2004) Blood 103: 2738-2743). A correlation between the galactose content of Rituximab N-glycans and CDC activity has been documented. As the number of galactose residues increases from 0 to 2 moles/mole of heavy chain, the level of CDC activity increases from 80% to 150% of the maximum CDC activity of the antibody having 1 mole galactose/mole of heavy chain (FDA, 1997; see, IDEC BLA 97-0260 at www.fda.gov/Cder/biologics/review/ritugen112697).

1. Bioreactor operation and dosing for modifying a-fucosylation

Bioreactor preparation for cell seeding & growth

[00319] Inoculate bioreactor with exponentially growing Anti-CD20 CHO cells to an initial seeding density of approximately 0.6 x 10^6 cells/mL.

[00320] Sample bioreactor cultures daily for the following: 1) Growth (Viable Cell Density, Viability (**Figure 4**), and Total Cell Density) - 1mL, 2) Metabolism (Glucose,

glutamine, glutamate, pH, and lactate)- 1mL, 3) mRNA analysis (*fut8*, *GMD*)- 0.5mL (**Figure 5**), and 4) Titer, product quality analysis (fucosylation)- 30-60 mL after Day 6.

[00321] On day 3 and 6 feed with 5% each of CHO CD Efficient Feed media A and B, (Invitrogen, Catalog#'s A10234-01 and A10240-01, respectively). Starting daily on Day 7, add 1% RPMI amino acid solution (Sigma, Catalog# R7131). Add 1M glucose and glutamax (Invitrogen, Catalog# 35050-061) as necessary to maintain ~2-6g/L glucose and >0.5 mmol/L glutamine.

2. Culture harvest & protein purification

[00322] Harvest culture at < 70% viability. Clarified culture supernatant is passed through a 50 mL Protein A Sepharose (GE Biosciences) column. The resin washed with 20 mM phosphate buffer (pH 7.) and the protein eluted with 0.1 M citrate (pH 3.0). Eluted protein is dialyzed against 20 mM phosphate (pH 6.5) and 100 mM NaCl. Protein is sufficiently pure at this point for glycan analysis. When further polishing of the protein product is desired, the Protein A eluate is dialyzed against 50 mM NaAcetate (pH 5.0) and applied to a 50 mL SP Sepharose column (GE Biosciences) that was pre-equilibrated using the same buffer. The column is washed with 10-15 column volumes using 50 mM NaAcetate (pH 5.0) and the protein eluted with 250 mM NaCl in the same buffer. Eluted protein is dialyzed against 20 mM phosphate (pH 6.5) and 100 mM NaCl. The protein concentration is determined at UV280 using a scanning spectrophotometer ranging from OD240 to OD320. Protein samples are aliquoted and stored at -20 degree Celsius until needed.

3. Glycan characterization of CD20 monoclonal antibody

[00323] Purified CD20 monoclonal antibody from siRNA-treated or untreated cells was subjected to both LC/MS and Capillary Electrophoresis – Laser-induced Fluorescence (CE-LIF) to determine the extent of fucose knockdown. Both detection methods yielded similar results with an approximate 70% increase in a-fucosylated glycans. Using CE-LIF detection, the G0 glycoform had the largest increase with approximately 60% conversion from G0 with fucose to without followed by G1 and G2 (**Figure 6**).

[00324] Similar results were obtained using the LC-MS method to quantitate the glycoforms (**Figure 7**). A 70% increase in the a-fucosylated glycans was observed. Again, a-

fucosylated G0 had the largest increase with approximately 60% followed by G1 at 10%. A-fucosylated G2 was not detected by this method.

4. CD20 monoclonal antibody bioassays

RNAi improves FcγRIIIa binding to CD20 monoclonal antibody

[00325] A soluble HIS-tagged FcγRIIIa was utilized in a CD20 MAb binding ELISA to demonstrate RNAi targeting both fucosyltransferase and GMD-Dehydratase significantly decreases fucosylated CD20 MAb (**Figure 6 & 7**) and improves both maximum receptor binding and receptor affinity (**Figure 8**) by approximately two-fold.

RNAi improves CD20 antibody Antibody-Dependent Cellular Cytotoxicity (ADCC) activity

[00326] Antibody-Dependent Cellular Cytotoxicity (ADCC) is a cell-based immunity mechanism that promotes the specific lyses of target cells bound with antibody (CD20 MAb) by effector cells (NK) through binding interactions with FcγRIIIa on the surface of effector cells. The target Cell line used in this study was Jeko-1 (Mantle cell lymphoma). Rituximab was used as a positive control. Freshly-isolated human PBMCs were used as effector cells at an Effector to Target (E/T) cell ratio of 25:1. The antibodies were tested in triplicate at 8 concentrations. The RNAi-treated CD20 antibody gave approximately 70% specific cell lysis compared to 20-30% lysis for both the control CD20 antibody and rituximab (**Figure 9**).

5. Conclusion

[00327] RNAi is an effective means to modulate the glycan profile of therapeutic biologics to improve activity and decrease potential immunogenicity. Targeting key enzymes required for de novo GDP-fucose synthesis, GDP-fucose transport, and/or fucosylation with siRNA duplexes added directly to bioreactors gives the bioprocessing engineer highly specific control over the amount of fucose present on therapeutic monoclonal antibodies improving product quality that is currently unavailable today.

EXAMPLE 2. SIRNAS THAT TARGET FUT8, GMDS AND NEU2

[00328] In this Example, activities of siRNAs targeting Fut8, GMDS, and Neu2 were determined. Table 6 shows the results.

Table 6

Target	Fold Change	Antisense Seq	Dose	SEQ ID NO
Fut8	0.710826	AAAUGACCACCUAUAUAAA	10	1554
Fut8	0.340191	AUCUGUCAUGAUAGACCUU	10	1555
Fut8	0.273006	AUGAGUGUUCGCUGGGUGC	10	1556
Fut8	0.256985	UUACAGGUCUAAACACAGU	10	1557
Fut8	0.226638	AAUUGGAGUACUUUGUCUU	10	1558
Fut8	0.22144	AUAAUUGGAGUACUUUGUC	10	1559
Fut8	0.197256	UUAUUCUCCGCUGGACCAG	10	1560
Fut8	0.182816	UAAUUGGAGUACUUUGUCU	10	1561
Fut8	0.182563	AAGUGUAUAUCCAGGAUCA	10	1562
Fut8	0.161942	AACCAAAAUGACCACCUAUA	10	1563
Fut8	0.156643	AACUGGAUGUUUGAAGCCA	10	1564
Fut8	0.134463	UUGGAGUACUUUGUCUUUG	10	1565
Fut8	0.121371	UUAUCUCGAACCAAAUGAC	10	1566
Fut8	0.0800275	UUGCAAGAAUCUUGGAGAG	10	1567
Fut8	0.0698774	AAAAGAAUGAGCAUAAUCC	10	1568
Fut8	0.0676092	AACCAGAGCUCUUUAGCUC	10	1569
Fut8	0.0668146	AAAACACGGACUCUUCCUG	10	1570
Fut8	0.0566435	UAUCUCGAACCAAAUGACC	10	1571
Fut8	0.0518515	AUGCCCGCAUUUUCAGAGU	10	1572
Fut8	0.0335188	UAAUCCAACGCCAGGAACC	10	1573
Gmds	0.369709	UUGGCAUGGCCCCAGUCUC	10	1574
Gmds	0.226638	UGUCCAAGGUAAAUCUUAG	10	1575
Gmds	0.211225	UUCAUGUUUCCUUCAAUAU	10	1576
Gmds	0.191691	UUCCCCAGUAGCUAUGACA	10	1577
Gmds	0.191102	AAAGGCAACGCGGGGCUUC	10	1578
Gmds	0.186461	AUGUUCAAUUCGACCUGUA	10	1579
Gmds	0.166711	UUGUCCAAGGUAAAUCUUA	10	1580
Gmds	0.149004	UUGCCGGUCUCUUUACAUC	10	1581
Gmds	0.128617	UUGUCUAGUGACAUAAUUU	10	1582
Gmds	0.121371	AUAGUCCUUGGCAUGGCCC	10	1583
Gmds	0.102414	AGUGCAACUUCAUGUUUCC	10	1584
Gmds	0.100891	UUCCUUCCCACACAAUGGU	10	1585
Gmds	0.0939335	UCCAAGGUAAAUCUUAGCU	10	1586
Gmds	0.0917034	UCUCUUUACAUCUGCCCAC	10	1587
Gmds	0.087858	AAAAACAAUCUCAAGACUC	10	1588
Gmds	0.087352	UAUAAAUGUUCAAUUCGAC	10	1589
Gmds	0.0731123	UAGUGACAUAAUUUCAAGU	10	1590
Gmds	0.0730004	AAGUAAAAUGAGUAUGUGA	10	1591
Gmds	0.0685559	UUCGACCUGUAUUAAAUGA	10	1592
Gmds	0.0612937	AAUUCGACCUGUAUUAAAU	10	1593

Neu2	0.388030164	AGGACUUUGUGCCUGGACC	2	1594
Neu2	0.218618546	AGAUAGACCACCCUCUCAG	2	1595
Neu2	0.307993119	AACCGAAGCACUUUGGGCC	2	1596
Neu2	0.476607506	CUGAUUGAGGUACACACCC	2	1597
Neu2	0.08951285	UUUAUUAAGACUCAUCCAA	2	1598
Neu2	0.119298165	UCUCCUGUCUGGAAUAGCG	2	1599
Neu2	0.236577331	UCUGCUUGUCAUACAAAGG	2	1600
Neu2	0.231697907	AAGUUUACUCACUACCUGG	2	1601
Neu2	0.104061837	UUUACUCACUACCUGGUUG	2	1602
Neu2	0.487300265	UGAUAUUUGCCCACGGACA	2	1603
Neu2	0.183124124	UCGUAGGACAAACAAAUCU	2	1604
Neu2	0.109973778	AGCAUUGAGAUAGACCACC	2	1605
Neu2	0.436004637	UACAAGUUUACUCACUACC	2	1606
Neu2	0.191171874	GAUAUUUGCCCACGGACAG	2	1607
Neu2	0.444654675	UGCAGGACAGGGCAAGUCG	2	1608
Neu2	0.160950067	AGCCGUGUGACAUUAACCC	2	1609
Neu2	0.376798761	AUAGUGACAGCCGACACAC	2	1610
Neu2	0.284464194	GGAUAAUUUAUUAAGACUC	2	1611
Neu2	0.371159296	CUAGCAUUGAGAUAGACCA	2	1612
Neu2	0.171466235	AAUAGCGUCUCCUUCUGCA	2	1613
Neu2	0.492536463	UCCGCAAAGGCCAGCAGGG	2	1614

[00329] The specification is most thoroughly understood in light of the teachings of the references cited within the specification. The embodiments within the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan readily recognizes that many other embodiments are encompassed by the invention. All publications, patents, and sequences cited in this disclosure are incorporated by reference in their entirety. To the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material. The citation of any references herein is not an admission that such references are prior art to the present invention.

[00330] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following embodiments.

Appendix 1: nucleotide sequences of exemplary target genes from Chinese Hamster

Table 7

Protein Name	Gene Symbol	CHO number	SEQ ID No
sialidase	Neu2	CHO56727.1	1
α-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3-N-acetylgalactosaminide α-2,6-sialyltransferase 6	St6galnac 6	CHO57849.1	2
CMP-N-acetylneuraminic acid hydroxylase	Cmah	CHO69319.1	3
a1,3 galactosyltransferase	Abo	CHO52173.1	4
GDP-mannose 4,6-dehydratase	Gmds	CHO73348.1	5
GDP-4-keto-6-deoxy-D-mannose epimerase-reductase [FX epimerase]	Tsta3	CHO73003.1	6
solute carrier-35C1 [GDP-fucose transporter]	Slc35c1	CHO63353.1	7
solute carrier-35C2	Slc35c2	CHO73401.1	8
UDP-galactose-ceramide galactosyltransferase	Ugt8a	CHO55897.1*	9
O-linked N-acetylglucosamine transferase	Ogt	CHO71860.1	10
α-galactosidase	Gla	CHO64621.1	11
β-galactosidase	Glb1	CHO53916.1	12
α-1,3-galactosyltransferase	A3galt2	CHO71928.1*	13
mannosyl (α-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase	Mgat2	CHO68241.1	14
solute carrier family 35 (UDP-glucuronic acid/UDP-N-acetylgalactosamine dual transporter), member D1	Slc35d1	CHO51082.1	15
glycoprotein galactosyltransferase alpha 1, 3	Ggta1	CHO57755.1*	16
fucosyltransferase 8	Fut8	CHO70008.1	17

SEQ ID NO: 1: CHO56727.1

NNNN

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CTGGCTTTCCTCAGCCTGTTGGCAGGGCAGGTGGGGACAGTATATATAGAGGCTGCCATTCTGCATGTCGGTTGTCACTATGC
TAGTTTAACCTGCCTGTTTCCCCATGCCTAGTGTTTGAATGAGTATTAATAAAATATCCAACCCAGCCNNNNNNNNNNNNNN
NNNNNN

SEQ ID NO: 2: CHO57849.1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCGGCGGGGCCTGGGCGGCCAGAGCGGCG
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SEQ ID NO: 3: CHO69319.1
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SEQ ID NO: 7: CHO63353.1

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SEQ ID NO: 9: CHO55897.1

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NNNN

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SEQ ID NO: 11: CHO64621.1

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SEQ ID NO: 13: CHO71928.1

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SEQ ID NO: 15: CHO51082.1

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NNNN

NNNN

CTCTGGCTGAGGCCATCTANNNN

Appendix 2: nucleotide sequences of exemplary siRNAs

Table 8.1

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Neu2	CAACCAGGUAGUGAGUAAA	18	UUUACUCACUACCUGGUUG	19
	GGUGGUCUAUCUCAAUGCU	20	AGCAUUGAGAUAGACCACC	21
	GGGUUAAUGUCACACGGCU	22	AGCCGUGUGACAUUAACCC	23
	UGCAGAAGGAGACGCUAUU	24	AAUAGCGUCUCCUUCUGCA	25
	AGAUUUGUUUGUCCUACGA	26	UCGUAGGACAAACAAAUCU	27
	CUGAGAGGGUGGUCUAUCU	28	AGAUAGACCACCCUCUCAG	29
	CCUUUGUAUGACAAGCAGA	30	UCUGCUUGUCAUACAAAGG	31
	UGGUCUAUCUCAAUGCUAG	32	CUAGCAUUGAGAUAGACCA	33
	GGUCCAGGCACAAAGUCCU	34	AGGACUUUGUGCCUGGACC	35
	GGUAGUGAGUAAACUUGUA	36	UACAAGUUUACUCACUACC	37
	CGACUUGCCCUGUCCUGCA	38	UGCAGGACAGGGCAAGUCG	39
	GGGUGUGUACCUCAAUCAG	40	CUGAUUGAGGUACACACCC	41
	CCCUGCUGGCCUUUGCGGA	42	UCCGCAAAGGCCAGCAGGG	43
	GUAUGAGUCAAAUAACUAU	44	AUAGUUAUUUGACUCAUAC	45
	CAAGAUUGGGCCACAUUUG	46	CAAAUGUGGCCCAAUCUUG	47
	GAGGGUGGUCUAUCUCAAU	48	AUUGAGAUAGACCACCCUC	49
	CCUGCUGGUCCCUGCUUAU	50	AUAAGCAGGGACCAGCAGG	51
	GCUCCAGACUGGGGUUAAU	52	AUUAACCCCAGUCUGGAGC	53
	ACUAUGAAGAGAUUGUUUU	54	AAAACAAUCUCUUCAUAGU	55
	UGAGCAUGCAGAUUUGUUU	56	AAACAAAUCUGCAUGCUCA	57
	GCAUGUCGGUUGUCACUAU	58	AUAGUGACAACCGACAUGC	59
	GCUCAAAACACCCAUCUCU	60	AGAGAUGGGUGUUUUGAGC	61
	CAGGCACAAAGUCCUAACA	62	UGUUAGGACUUUGUGCCUG	63
	CCUAGUGUUUGAAUGAGUA	64	UACUCAUUCAAACACUAGG	65
	CCUCAAAGGCAGAUGCCUU	66	AAGGCAUCUGCCUUUGAGG	67
	CUCAAAGGCAGAUGCCUUA	68	UAAGGCAUCUGCCUUUGAG	69
	GCAAUUUGGGUGUCUGUAU	70	AUACAGACACCCAAAUUGC	71
	GCCUAGUGUUUGAAUGAGU	72	ACUCAUUCAAACACUAGGC	73
	GUUAAUGUCACACGGCUAU	74	AUAGCCGUGUGACAUUAAC	75
	GCUUAUGCCUAUCGGAAAC	76	GUUUCCGAUAGGCAUAAGC	77
	AGACAGAUGAGCAUGCAGA	78	UCUGCAUGCUCAUCUGUCU	79
	CUCUGAUCUACCUGUCAAA	80	UUUGACAGGUAGAUCAGAG	81
	GUGUUUGGUGCCCAGUGAU	82	AUCACUGGGCACCAAACAC	83
	GCGUCUGACCAAGACAGAU	84	AUCUGUCUUGGUCAGACGC	85
	GUUGUCACUAUGCUAGUUU	86	AAACUAGCAUAGUGACAAC	87
	CAAGGACCCUUUUCCUUUU	88	AAAAGGAAAAGGGUCCUUG	89
	GGAGACUAUGCUUACAGAA	90	UUCUGUAAGCAUAGUCUCC	91

	GGUUGUCACUAUGCUAGUU	92	AACUAGCAUAGUGACAACC	93
	UGGGGACAGUAUAUAUAGA	94	UCUAUAUAUACUGUCCCCA	95
	ACAACCAGGUAGUGAGUAA	96	UUACUCACUACCUGGUUGU	97
	GGCACAAAGUCCUAACAGU	98	ACUGUUAGGACUUUGUGCC	99
	CAUGUCCUUUGUAUGACAA	100	UUGUCAUACAAAGGACAUG	101
	UGAUCUACCUGUCAAAGCA	102	UGCUUUGACAGGUAGAUCA	103
	AGUGUUUGAAUGAGUAUUA	104	UAAUACUCAUUCAAACACU	105
	UGUCACUAUGCUAGUUUAA	106	UUAAACUAGCAUAGUGACA	107
	ACCCCACCUCAAAGGCAGA	108	UCUGCCUUUGAGGUGGGGU	109
	CCUGAUGGCUCCCCGCAAU	110	AUUGCGGGGAGCCAUCAGG	111
	GAGGAAGCUACAAUGCAGA	112	UCUGCAUUGUAGCUUCCUC	113
	CCUACAGACUCCCGGAAGA	114	UCUUCCGGGAGUCUGUAGG	115
	CCUUAUUGUUUCAGCCUCU	116	AGAGGCUGAAACAAUAAGG	117

Table 8.2

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
St6galnac6	CCACCUUCUAGAAACUUUA	118	UAAAGUUUCUAGAAGGUGG	119
	GGAAUGGGCCCCAAAUCAU	120	AUGAUUUGGGGCCCAUUCC	121
	CCUAGAUCUGGUUUUGUAA	122	UUACAAAACCAGAUCUAGG	123
	CAGUCUAAGACUCGGGUAC	124	GUACCCGAGUCUUAGACUG	125
	GAAUGGGCCCCAAAUCAUU	126	AAUGAUUUGGGGCCCAUUC	127
	CCCUCACUCUAUAUUGUAC	128	GUACAAUAUAGAGUGAGGG	129
	GGCUGGGGGUUAUCUCUUGU	130	ACAAGAGAUAACCCCAGCC	131
	GGGAUUUGGAAUCCUGUAU	132	AUACAGGAUUCCAAAUCCC	133
	GGGACAAAGAGGUCCUAUC	134	GAUAGGACCUCUUUGUCCC	135
	UAAUCAGGGUCUAAGCAAA	136	UUUGCUUAGACCCUGAUUA	137
	GCCUCAUGUUCCCUAACAU	138	AUGUUAGGGAACAUGAGGC	139
	CUGGCGUGCUAUAAUGACA	140	UGUCAUUAUAGCACGCCAG	141
	UGGCGUGCUAUAAUGACAU	142	AUGUCAUUAUAGCACGCCA	143
	CCCUGAAACGGUGUUCAUC	144	GAUGAACACCGUUUCAGGG	145
	GAAUCCUGUAUGGUUUAAU	146	AUUAAACCAUACAGGAUUC	147
	GACUCGGGUACUAUCCUUU	148	AAAGGAUAGUACCCGAGUC	149
	GGCUCUGGGCCGAACCAUU	150	AAUGGUUCGGCCCAGAGCC	151
	CUAGAAACUUUAGGGUAUU	152	AAUACCCUAAAGUUUCUAG	153
	CCGUAAGGGCAAUCACCAC	154	GUGGUGAUUGCCCUUACGG	155
	AUAUUGUACUUAAGUGUAA	156	UUACACUUAAGUACAAUAU	157
	GGGCCGAACCAUUUGACCU	158	AGGUCAAAUGGUUCGGCCC	159
	AGACUCGGGUACUAUCCUU	160	AAGGAUAGUACCCGAGUCU	161
	CAAAUCAUUUCCUCCUUAG	162	CUAAGGAGGAAAUGAUUUG	163
	CAACCAAUGUGUGAUCAUC	164	GAUGAUCACACAUUGGUUG	165

	ACAAAGAGGUCCUAUCACA	166	UGUGAUAGGACCUCUUUGU	167
	UAUUGUACUUAAGUGUAAU	168	AUUACACUUAAGUACAAUA	169
	GACAGAAAAUGGGGCAAACA	170	UGUUUGCCCCAUUUCUGUC	171
	GCUGGUUUACCAUGGUGAU	172	AUCACCAUGGUAAACCAGC	173
	CUAUAUUGUACUUAAGUGU	174	ACACUUAAGUACAAUAUAG	175
	AGAGACCCUCACUCUAUAU	176	AUAUAGAGUGAGGGUCUCU	177
	UCUAGAAACUUUAGGGUAU	178	AUACCCUAAAGUUUCUAGA	179
	UGCCAUACCACUACUAUGA	180	UCAUAGUAGUGGUAUGGCA	181
	GCCAGUUCAGGUUGGCUGG	182	CCAGCCAACCUGAACUGGC	183
	CCAGGUGCAACCAAUGUGU	184	ACACAUUGGUUGCACCUGG	185
	GGAGAGAGAUGAGUAGCAA	186	UUGCUACUCAUCUCUCUCC	187
	GCCCUGCACAGCAAGGUAA	188	UUACCUUGCUGUGCAGGGC	189
	GCAUGCAGCAGUUUGACGA	190	UCGUCAAACUGCUGCAUGC	191
	CAUGGUCCCUCCUGACUAU	192	AUAGUCAGGAGGGACCAUG	193
	CGGGCUGAGUGCACCAUCC	194	GGAUGGUGCACUCAGCCCG	195
	UCACUCUAUAUUGUACUUA	196	UAAGUACAAUAUAGAGUGA	197
	CUGAGUCAGUCUAAGACUC	198	GAGUCUUAGACUGACUCAG	199
	AGCAAAGUCAAUCAGGGUA	200	UACCCUGAUUGACUUUGCU	201
	GGAGUUCUAGAAGUUGUAG	202	CUACAACUUCUAGAACUCC	203
	GCAGCCUCCUGCGUGUCAU	204	AUGACACGCAGGAGGCUGC	205
	GCGUCGGCCUGUCAACCUC	206	GAGGUUGACAGGCCGACGC	207
	GGGUACUAUCCUUUCCCAA	208	UUGGGAAAGGAUAGUACCC	209
	CUGUAUGGUUUAAUCGGGU	210	ACCCGAUUAAACCAUACAG	211
	CUAGAUCUGGUUUUGUAAU	212	AUUACAAAACCAGAUCUAG	213
	CAGAGACCCUCACUCUAUA	214	UAUAGAGUGAGGGUCUCUG	215
	CUCUAUAUUGUACUUAAGU	216	ACUUAAGUACAAUAUAGAG	217

Table 8.3

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Cmah	AAUCUUAAUUAACAAUAUA	218	UAUAUUGUUAAUUAAGAUU	219
	GAAUUUGCUCUUAAAUCUU	220	AAGAUUUAAGAGCAAAUUC	221
	GCAUGUGCUUAGAGCAUAU	222	AUAUGCUCUAAGCACAUGC	223
	CUGUAUAACCCUAAAUUCA	224	UGAAUUUAGGGUUAUACAG	225
	GUGAGUAGAAAAUCUUAAA	226	UUUAAGAUUUUCUACUCAC	227
	GCGGGACCCUGAUAUAUAC	228	GUAUAUAUCAGGGUCCCGC	229
	CAAUAUAUGGGUGAGUAGA	230	UCUACUCACCCAUAUAUUG	231
	AUCUUAAUUAACAAUAUAU	232	AUAUAUUGUUAAUUAAGAU	233
	UCAAGAAUUUGCUCUUAAA	234	UUUAAGAGCAAAUUCUUGA	235
	UCAUAAAAUACUCAACACA	236	UGUGUUGAGUAUUUUAUGA	237
	AAGCAGACCUCAUUUAUAU	238	AUAUAAAUGAGGUCUGCUU	239
	GAGUAGAAAAUCUUAAAUU	240	AAUUUAAGAUUUUCUACUC	241

	GGUGAGUAGAAAAUCUUAA	242	UUAAGAUUUUCUACUCACC	243
	GCCAUAACUACCAUUAUUC	244	GAAUAAUGGUAGUUAUGGC	245
	GGGUCUCCCCUGCUUCGAU	246	AUCGAAGCAGGGGAGACCC	247
	GGUCAUAAAAUACUCAACA	248	UGUUGAGUAUUUUAUGACC	249
	CAUCUGUUUUGGAAUCAUU	250	AAUGAUUCCAAAACAGAUG	251
	GGAGACAAGCGAAUGGUAU	252	AUACCAUUCGCUUGUCUCC	253
	CAGAUAAAACUCCCUCUAA	254	UUAGAGGGAGUUUUAUCUG	255
	CUGGAAACACUUUCAUAGA	256	UCUAUGAAAGUGUUUCCAG	257
	GUGUCUUUUAUUCAGAGAU	258	AUCUCUGAAUAAAAGACAC	259
	GAAAAUUUACUGAGGAAUG	260	CAUUCCUCAGUAAAUUUUC	261
	GAGACAAGCGAAUGGUAUU	262	AAUACCAUUCGCUUGUCUC	263
	CCUGUAUAACCCUAAAUUC	264	GAAUUUAGGGUUAUACAGG	265
	CCAUCUGACUGGUUGGAGA	266	UCUCCAACCAGUCAGAUGG	267
	AUAACUACCAUUAUUCAUU	268	AAUGAAUAAUGGUAGUUAU	269
	CUUAAUUAACAAUAUAUGG	270	CCAUAUAUUGUUAAUUAAG	271
	CACACCUGUAUAACCCUAA	272	UUAGGGUUAUACAGGUGUG	273
	CCUGCUUCGAUAAACCUUU	274	AAAGGUUUAUCGAAGCAGG	275
	GGACCCUGAUAUAUACCAU	276	AUGGUAUAUAUCAGGGUCC	277
	AUAUAUGGGUGAGUAGAAA	278	UUUCUACUCACCCAUAUAU	279
	GUGCAAAGCAGACCUCAUU	280	AAUGAGGUCUGCUUUGCAC	281
	AAAGGUCAUAAAAUACUCA	282	UGAGUAUUUUAUGACCUUU	283
	CUUCGAUAAACCUUUCUGG	284	CCAGAAAGGUUUAUCGAAG	285
	UGAGUAGAAAAUCUUAAAU	286	AUUUAAGAUUUUCUACUCA	287
	GAUAAAACUCCCUCUAACA	288	UGUUAGAGGGAGUUUUAUC	289
	AGGUCAUAAAAUACUCAAC	290	GUUGAGUAUUUUAUGACCU	291
	GUAGUAUAGCAUGUGCUUA	292	UAAGCACAUGCUAUACUAC	293
	AGAAUUUGCUCUUAAAUCU	294	AGAUUUAAGAGCAAAUUCU	295
	CAAGGAAGGGAUCAAUUUU	296	AAAAUUGAUCCCUUCCUUG	297
	UGGGUGAGUAGAAAAUCUU	298	AAGAUUUUCUACUCACCCA	299
	AUUUACUGAGGAAUGGAAA	300	UUUCCAUUCCUCAGUAAAU	301
	ACAAGCGAAUGGUAUUUGA	302	UCAAAUACCAUUCGCUUGU	303
	GUUCCAACACACACACACA	304	UGUGUGUGUGUGUUGGAAC	305
	CGUUCCUAAUGCACUGUGA	306	UCACAGUGCAUUAGGAACG	307
	CCAUCUUGUAACGGUGAAU	308	AUUCACCGUUACAAGAUGG	309
	CCCUGAUAUAUACCAUCAU	310	AUGAUGGUAUAUAUCAGGG	311
	CAUAACUACCAUUAUUCAU	312	AUGAAUAAUGGUAGUUAUG	313
	ACACCUGUAUAACCCUAAA	314	UUUAGGGUUAUACAGGUGU	315
	GAUGGAAGGUCCGUUAAAU	316	AUUUAACGGACCUUCCAUC	317

Table 8.4

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.

Abo	GAACGAUGUUCUUGUCUUG	318	CAAGACAAGAACAUCGUUC	319
	AUCAGAAACUAAUAGCUAA	320	UUAGCUAUUAGUUUCUGAU	321
	AGAGCUAUUUGAACAAAUA	322	UAUUUGUUCAAAUAGCUCU	323
	GCAUGAUGAGAGCUAUUUG	324	CAAAUAGCUCUCAUCAUGC	325
	GGAACGAUGUUCUUGUCUU	326	AAGACAAGAACAUCGUUCC	327
	CCAUCAUGAAGAAGUUGAG	328	CUCAACUUCUUCAUGAUGG	329
	GGACAAGGCCAACGGCAUU	330	AAUGCCGUUGGCCUUGUCC	331
	GAACCAUCAGGCAAUCAGA	332	UCUGAUUGCCUGAUGGUUC	333
	CUAAUAGCUAAAUUCCUAU	334	AUAGGAAUUUAGCUAUUAG	335
	AGGCCUGCCAUGAAGCUAU	336	AUAGCUUCAUGGCAGGCCU	337
	CUGCUUUACCAUAAGCCAA	338	UUGGCUUAUGGUAAAGCAG	339
	ACACAAGGUCACCUACUAU	340	AUAGUAGGUGACCUUGUGU	341
	CCGAGAGGCCUUUACCUAU	342	AUAGGUAAAGGCCUCUCGG	343
	ACUAAUAGCUAAAUUCCUA	344	UAGGAAUUUAGCUAUUAGU	345
	UCAGAAACUAAUAGCUAAA	346	UUUAGCUAUUAGUUUCUGA	347
	GAAGAAGUUGAGAUAUGUG	348	CACAUAUCUCAACUUCUUC	349
	AGGCAAUCAGAAACUAAUA	350	UAUUAGUUUCUGAUUGCCU	351
	GAAACUAAUAGCUAAAUUC	352	GAAUUUAGCUAUUAGUUUC	353
	CCUACUAUGUCUUCACUGA	354	UCAGUGAAGACAUAGUAGG	355
	GAGCUAUUUGAACAAAUAC	356	GUAUUUGUUCAAAUAGCUC	357
	CCAUCAGGCAAUCAGAAAC	358	GUUUCUGAUUGCCUGAUGG	359
	GGGACACAAGGUCACCUAC	360	GUAGGUGACCUUGUGUCCC	361
	GGCUGUACCCAAGAACCAU	362	AUGGUUCUUGGGUACAGCC	363
	GAUGAGAGCUAUUUGAACA	364	UGUUCAAAUAGCUCUCAUC	365
	CCCAAGAACCAUCAGGCAA	366	UUGCCUGAUGGUUCUUGGG	367
	GCUAAAUUCCUAUUGGAGA	368	UCUCCAAUAGGAAUUUAGC	369
	GUUCUUGUCUUGACUCCUU	370	AAGGAGUCAAGACAAGAAC	371
	CGAUGUUCUUGUCUUGACU	372	AGUCAAGACAAGAACAUCG	373
	UCAGGCAAUCAGAAACUAA	374	UUAGUUUCUGAUUGCCUGA	375
	GUCAGUGCUAGAAGUGUAC	376	GUACACUUCUAGCACUGAC	377
	AGGUCACCUACUAUGUCUU	378	AAGACAUAGUAGGUGACCU	379
	UAGCUAAAUUCCUAUUGGA	380	UCCAAUAGGAAUUUAGCUA	381
	AAUACCUGCUUUACCAUAA	382	UUAUGGUAAAGCAGGUAUU	383
	ACCAUCAGGCAAUCAGAAA	384	UUUCUGAUUGCCUGAUGGU	385
	AGUAUGUGGUGUUCCUUAA	386	UUAAGGAACACCACAUACU	387
	UGGACAUGAAGUUCAGUGA	388	UCACUGAACUUCAUGUCCA	389
	AGUUGAGAUAUGUGGCUGU	390	ACAGCCACAUAUCUCAACU	391
	GAGAGCUAUUUGAACAAAU	392	AUUUGUUCAAAUAGCUCUC	393
	AAGCUAUGAUGGAGGACAA	394	UUGUCCUCCAUCAUAGCUU	395
	ACAAAUACCUGCUUUACCA	396	UGGUAAAGCAGGUAUUUGU	397
	CUGCCAUGAAGCUAUGAUG	398	CAUCAUAGCUUCAUGGCAG	399
	CCUGUGUGGCAUGAUGAGA	400	UCUCAUCAUGCCACACAGG	401
	CCAGCAGACCCAGUAUCAC	402	GUGAUACUGGGUCUGCUGG	403

	UGAACAAAUACCUGCUUUA	404	UAAAGCAGGUAUUUGUUCA	405
	UGAUGAGAGCUAUUUGAAC	406	GUUCAAAUAGCUCUCAUCA	407
	CCAGUAGGAACGAUGUUCU	408	AGAACAUCGUUCCUACUGG	409
	UGAAGAAGUUGAGAUAUGU	410	ACAUAUCUCAACUUCUUCA	411
	GUAGGAACGAUGUUCUUGU	412	ACAAGAACAUCGUUCCUAC	413
	AUUCCUAUUGGAGAGGACA	414	UGUCCUCUCCAAUAGGAAU	415
	GGCAUGAUGAGAGCUAUUU	416	AAAUAGCUCUCAUCAUGCC	417

Table 8.5

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Gmds	AUUUAAUACAGGUCGAAUU	418	AAUUCGACCUGUAUUAAAU	419
	UCAUUUAAUACAGGUCGAA	420	UUCGACCUGUAUUAAAUGA	421
	UCACAUACUCAUUUUACUU	422	AAGUAAAAUGAGUAUGUGA	423
	GUCGAAUUGAACAUUUAUA	424	UAUAAAUGUUCAAUUCGAC	425
	AGCUAAGAUUUACCUUGGA	426	UCCAAGGUAAAUCUUAGCU	427
	GGAAACAUGAAGUUGCACU	428	AGUGCAACUUCAUGUUUCC	429
	GAUGUAAAGAGACCGGCAA	430	UUGCCGGUCUCUUUACAUC	431
	UACAGGUCGAAUUGAACAU	432	AUGUUCAAUUCGACCUGUA	433
	GAAGCCCCGCGUUGCCUUU	434	AAAGGCAACGCGGGGCUUC	435
	CUAAGAUUUACCUUGGACA	436	UGUCCAAGGUAAAUCUUAG	437
	GGUCGAAUUGAACAUUUAU	438	AUAAAUGUUCAAUUCGACC	439
	AAUCAUCAAUGAAGUCAAA	440	UUUGACUUCAUUGAUGAUU	441
	AUUGAACAUUUAUAUAAGA	442	UCUUAUAUAAAUGUUCAAU	443
	CGAUCCAGUUCAUUUAAUA	444	UAUUAAAUGAACUGGAUCG	445
	CGGCAUUCUCUUCAAUCAU	446	AUGAUUGAAGAGAAUGCCG	447
	CUAUUGGAUUGUAGUGAAC	448	GUUCACUACAAUCCAAUAG	449
	CAUACUCAUUUUACUUGAA	450	UUCAAGUAAAAUGAGUAUG	451
	GUUUAAAAUCACAUACUCA	452	UGAGUAUGUGAUUUUAAAC	453
	GUACGAGGUCCAUGGAAUU	454	AAUUCCAUGGACCUCGUAC	455
	CCGGCAAAAUUCAUGUGAC	456	GUCACAUGAAUUUUGCCGG	457
	GAGACCGGCAAAAUUCAUG	458	CAUGAAUUUUGCCGGUCUC	459
	CCACUUCGCUUUUGUCAAA	460	UUUGACAAAAGCGAAGUGG	461
	AGCUAAUUUUGUUACUCGA	462	UCGAGUAACAAAAUUAGCU	463
	ACUUGUGGCCUUAUAAAUU	464	AAUUUAUAAGGCCACAAGU	465
	AAGACUUGUGGCCUUAUAA	466	UUAUAAGGCCACAAGUCUU	467
	CGGCGAUCCAGUUCAUUUA	468	UAAAUGAACUGGAUCGCCG	469
	CUAAUUUUGUUACUCGAAA	470	UUUCGAGUAACAAAAUUAG	471
	UUGAAAUUAUGUCACUAGA	472	UCUAGUGACAUAAUUUCAA	473
	GGACUUUGUCAUAGCUACU	474	AGUAGCUAUGACAAAGUCC	475
	ACUUUAUGCCUAUUGGAUU	476	AAUCCAAUAGGCAUAAAGU	477
	UAAUUUUGUUACUCGAAAA	478	UUUUCGAGUAACAAAAUUA	479

	GCUAAGGCCACUUCGCUUU	480	AAAGCGAAGUGGCCUUAGC	481
	CUUCUGGAUGCAAUUAAGA	482	UCUUAAUUGCAUCCAGAAG	483
	CGGUCAGUAGCUAAGAUUU	484	AAAUCUUAGCUACUGACCG	485
	AAUCAUGAGAGUCCUAGAA	486	UUCUAGGACUCUCAUGAUU	487
	GGAGCUAAUUUUGUUACUC	488	GAGUAACAAAAUUAGCUCC	489
	GGAUGCAAUUAAGACUUGU	490	ACAAGUCUUAAUUGCAUCC	491
	GUAGCUAAGAUUUACCUUG	492	CAAGGUAAAUCUUAGCUAC	493
	GCCCUAUGGAGCAGCCAAA	494	UUUGGCUGCUCCAUAGGGC	495
	CAAUCAUGAGAGUCCUAGA	496	UCUAGGACUCUCAUGAUUG	497
	GAUGUUACAAAAUGAUGAA	498	UUCAUCAUUUUGUAACAUC	499
	AGUUCAUAGUGUCCGUGAA	500	UUCACGGACACUAUGAACU	501
	GCGAUCCAGUUCAUUUAAU	502	AUUAAAUGAACUGGAUCGC	503
	CCAAACUUUAUGCCUAUUG	504	CAAUAGGCAUAAAGUUUGG	505
	CUACAAUCUUGGUGCCCAG	506	CUGGGCACCAAGAUUGUAG	507
	GUUCAUAGUGUCCGUGAAU	508	AUUCACGGACACUAUGAAC	509
	GGUCAGUAGCUAAGAUUUA	510	UAAAUCUUAGCUACUGACC	511
	UGUCACUAGACAACUUAAA	512	UUUAAGUUGUCUAGUGACA	513
	AGAGGAGCUAAUUUUGUUA	514	UAACAAAAUUAGCUCCUCU	515
	CUGAAAUACUACCGACCAA	516	UUGGUCGGUAGUAUUUCAG	517

Table 8.6

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Tsta3	GGGCCUACUUGCCUGAUUU	518	AAAUCAGGCAAGUAGGCCC	519
	CCAACAUUUUGCUUGUCUG	520	CAGACAAGCAAAAUGUUGG	521
	GGGGAAGUCACUUUUGAUU	522	AAUCAAAAGUGACUUCCCC	523
	CCCACAGCAGCAAUUUUGG	524	CCAAAAUUGCUGCUGUGGG	525
	GGGAAGUCACUUUUGAUUC	526	GAAUCAAAAGUGACUUCCC	527
	GCUGCAGUCUCAUCAUUUG	528	CAAAUGAUGAGACUGCAGC	529
	CCAUCUUGCUGCAAUGGUA	530	UACCAUUGCAGCAAGAUGG	531
	CCUAUUGAUGAAACAAUGA	532	UCAUUGUUUCAUCAAUAGG	533
	UGGGGAAGUCACUUUUGAU	534	AUCAAAAGUGACUUCCCCA	535
	CAAGCUUCGGGCCUACUUG	536	CAAGUAGGCCCGAAGCUUG	537
	GAGUAAUGGUUCAGCCUUG	538	CAAGGCUGAACCAUUACUC	539
	AUGCCAAGAGGAUGAUUGA	540	UCAAUCAUCCUCUUGGCAU	541
	CAACAAAGUCAGAUGGGCA	542	UGCCCAUCUGACUUUGUUG	543
	GGAAAGGCCAGACCAACAU	544	AUGUUGGUCUGGCCUUUCC	545
	CAUCCAUCUUGCUGCAAUG	546	CAUUGCAGCAAGAUGGAUG	547
	CAGUCUCAUCAUUUGCUUG	548	CAAGCAAAUGAUGAGACUG	549
	CGGAAUAUCAAAUACAACU	550	AGUUGUAUUUGAUAUUCCG	551
	ACAGUAUCCAGCAACCUGA	552	UCAGGUUGCUGGAUACUGU	553
	CCUUGACUGUUUGGGGUAC	554	GUACCCCAAACAGUCAAGG	555

	AUGAAACAAUGAUCCACAA	556	UUGUGGAUCAUUGUUUCAU	557
	CAUCAAUGACAACGUCCUG	558	CAGGACGUUGUCAUUGAUG	559
	GGCCUACUUGCCUGAUUUC	560	GAAAUCAGGCAAGUAGGCC	561
	CGGAGAGGAAUGGGUGUUU	562	AAACACCCAUUCCUCUCCG	563
	GGAGUACAAUGAAGUUGAG	564	CUCAACUUCAUUGUACUCC	565
	GCGGGAGUACAAUGAAGUU	566	AACUUCAUUGUACUCCCGC	567
	GGCCAAGAGUAAUGGUUCA	568	UGAACCAUUACUCUUGGCC	569
	AGUCAGAUGGGCAGUAUAA	570	UUAUACUGCCCAUCUGACU	571
	CCCCACAGCAGCAAUUUUG	572	CAAAAUUGCUGCUGUGGGG	573
	GCAAUUUUGGGUACUCGUA	574	UACGAGUACCCAAAAUUGC	575
	GGACCUCAUGACAACUUCA	576	UGAAGUUGUCAUGAGGUCC	577
	CUACUUGCCUGAUUUCCGU	578	ACGGAAAUCAGGCAAGUAG	579
	CGCAGAUGGCGCUGGCUUA	580	UAAGCCAGCGCCAUCUGCG	581
	GAUGAAACAAUGAUCCACA	582	UGUGGAUCAUUGUUUCAUC	583
	UUGAUUCAACAAAGUCAGA	584	UCUGACUUUGUUGAAUCAA	585
	GCUUCGGGCCUACUUGCCU	586	AGGCAAGUAGGCCCGAAGC	587
	CAAGAGGAUGAUUGACGUG	588	CACGUCAAUCAUCCUCUUG	589
	GCAGAACAGGGCCUACUUC	590	GAAGUAGGCCCUGUUCUGC	591
	CCAGGAGGAUCCUAGUGAC	592	GUCACUAGGAUCCUCCUGG	593
	CAGUAGGGCCCAUGUCCAU	594	AUGGACAUGGGCCCUACUG	595
	GAUGCAGCACAAACCCAAG	596	CUUGGGUUUGUGCUGCAUC	597
	CGGAAGUGAAGCAUGGGAC	598	GUCCCAUGCUUCACUUCCG	599
	CAGCAGCAAUUUUGGGUAC	600	GUACCCAAAAUUGCUGCUG	601
	CUGGUUCACCGACAACUAU	602	AUAGUUGUCGGUGAACCAG	603
	CCACGGAGGCAGUUCAUCU	604	AGAUGAACUGCCUCCGUGG	605
	AGUUCAUCUACUCACUGGA	606	UCCAGUGAGUAGAUGAACU	607
	GGAAGUCACUUUUGAUUCA	608	UGAAUCAAAAGUGACUUCC	609
	AAACCACGGAGGCAGUUCA	610	UGAACUGCCUCCGUGGUUU	611
	CAAUGAUCCACAAUGGUCC	612	GGACCAUUGUGGAUCAUUG	613
	CCCUACCAAUGUCUUUGGA	614	UCCAAAGACAUUGGUAGGG	615
	AUCAUUUGCUUGUCAAGAA	616	UUCUUGACAAGCAAAUGAU	617

Table 8.7

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Slc35c1	GUUGUAUGUGAUUUAAUAA	618	UUAUUAAAUCACAUACAAC	619
	CCUCAUAUAGGAACACUAG	620	CUAGUGUUCCUAUAUGAGG	621
	CGGCUUUGCCAUUGGCUAU	622	AUAGCCAAUGGCAAAGCCG	623
	GGCCCUGAGCAAUACUGUU	624	AACAGUAUUGCUCAGGGCC	625
	GGUCUUUAUCGGCAUGAUA	626	UAUCAUGCCGAUAAAGACC	627
	CCCCUGACCCACAAUGUAU	628	AUACAUUGUGGGUCAGGGG	629
	CCAUCUUGUUUUGGGAAAA	630	UUUUCCCAAAACAAGAUGG	631

	GGAAUAUGAUCUAAGAGGA	632	UCCUCUUAGAUCAUAUUCC	633
	GGGACAGAAGCACUCAGAC	634	GUCUGAGUGCUUCUGUCCC	635
	CCUCCAUCUCCAUGGUAUU	636	AAUACCAUGGAGAUGGAGG	637
	UGUAUGUGAUUUAAUAAUA	638	UAUUAUUAAAUCACAUACA	639
	GCAUGAUAAGUUUCAAUAA	640	UUAUUGAAACUUAUCAUGC	641
	GGCAUGAUAAGUUUCAAUA	642	UAUUGAAACUUAUCAUGCC	643
	GGGGUAAUGUCUGAUGUUU	644	AAACAUCAGACAUUACCCC	645
	GCUGGAUUGAUGAAGUGAU	646	AUCACUUCAUCAAUCCAGC	647
	GCAUCAUCAUUGGUGGUUU	648	AAACCACCAAUGAUGAUGC	649
	GCUCCUUUAAGCCUCAUAU	650	AUAUGAGGCUUAAAGGAGC	651
	CCCUGACCCACAAUGUAUC	652	GAUACAUUGUGGGUCAGGG	653
	GCCCUGGCAUGGUAGACUU	654	AAGUCUACCAUGCCAGGGC	655
	CCACAAUGUAUCAGGCACA	656	UGUGCCUGAUACAUUGUGG	657
	CAGACCACUUCCUUCUAUG	658	CAUAGAAGGAAGUGGUCUG	659
	GCAUCUGGCGCCUAACCUU	660	AAGGUUAGGCGCCAGAUGC	661
	CGUAAAUUUGGGGACAGAA	662	UUCUGUCCCCAAAUUUACG	663
	CCCUGGCAUGGUAGACUUC	664	GAAGUCUACCAUGCCAGGG	665
	GGGUGAGAAGAGUGCUAUU	666	AAUAGCACUCUUCUCACCC	667
	CCUACUUCCUACAAUCCCA	668	UGGGAUUGUAGGAAGUAGG	669
	GCGCCUAACCUUCUAUAAC	670	GUUAUAGAAGGUUAGGCGC	671
	GUCUUUAUCGGCAUGAUAA	672	UUAUCAUGCCGAUAAAGAC	673
	GGCGCCUAACCUUCUAUAA	674	UUAUAGAAGGUUAGGCGCC	675
	CUUCUAUAACAAUGUCAAU	676	AUUGACAUUGUUAUAGAAG	677
	AAUCCCAGGUUCUAAUGAA	678	UUCAUUAGAACCUGGGAUU	679
	CCUACCUGCUGCUCAAACA	680	UGUUUGAGCAGCAGGUAGG	681
	AGGACUCCAACGUAAAUUU	682	AAAUUUACGUUGGAGUCCU	683
	AUGACCUGUCAACCUCAAA	684	UUUGAGGUUGACAGGUCAU	685
	CUGGAUACCCCUAUCUUUG	686	CAAAGAUAGGGGUAUCCAG	687
	UUUUGUCCCUGUCAUAAAA	688	UUUUAUGACAGGGACAAAA	689
	CUAACCUUCUAUAACAAUG	690	CAUUGUUAUAGAAGGUUAG	691
	CCUUGGGGUAAUGUCUGAU	692	AUCAGACAUUACCCCAAGG	693
	GCCUCACUCAUGAGGGUUU	694	AAACCCUCAUGAGUGAGGC	695
	AUAACAAUGUCAAUGCCUG	696	CAGGCAUUGACAUUGUUAU	697
	AACCCUACCAGGAAUGGCA	698	UGCCAUUCCUGGUAGGGUU	699
	CUGGCGCCUAACCUUCUAU	700	AUAGAAGGUUAGGCGCCAG	701
	CAAUGUUCUUCUGUCCUAC	702	GUAGGACAGAAGAACAUUG	703
	GGACAAGCAACCUGAUGGU	704	ACCAUCAGGUUGCUUGUCC	705
	CUGAGUUGUAUGUGAUUUA	706	UAAAUCACAUACAACUCAG	707
	UCCCACUAUAGCUAGUGAA	708	UUCACUAGCUAUAGUGGGA	709
	GGGUCUUUCCUGGUAAUGU	710	ACAUUACCAGGAAAGACCC	711
	CCAUAUCAUCUCUGGGGUU	712	AACCCCAGAGAUGAUAUGG	713
	GCCUUAAGGGGCUGGAUUG	714	CAAUCCAGCCCCUUAAGGC	715
	CCGUGUUCAAUGUUCUUCU	716	AGAAGAACAUUGAACACGG	717

Table 8.8

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Slc35c2	AAUAAAUGGUGUUUGCUCA	718	UGAGCAAACACCAUUUAUU	719
	GACGAUGAUUAUUUUGUGA	720	UCACAAAAUAAUCAUCGUC	721
	GCAUCUUUAAGGAAGUCUG	722	CAGACUUCCUUAAAGAUGC	723
	UUUAUUUAUAGUUGAAGUA	724	UACUUCAACUAUAAAUAAA	725
	CCUCAGAGAGUAGAUUUAU	726	AUAAAUCUACUCUCUGAGG	727
	GGGUGAUCAGAUCAGCCUC	728	GAGGCUGAUCUGAUCACCC	729
	ACUGUACACAAUGACCAAA	730	UUUGGUCAUUGUGUACAGU	731
	CCAUCAGCCCCUUAGCCAC	732	GUGGCUAAGGGGCUGAUGG	733
	CCUCUUCAUGUUUACCUAU	734	AUAGGUAAACAUGAAGAGG	735
	GGCUCUGAGAGGCAGAUAA	736	UUAUCUGCCUCUCAGAGCC	737
	UCCUCAGAGAGUAGAUUUA	738	UAAAUCUACUCUCUGAGGA	739
	CGCUCUUUGCCGUAUUUGA	740	UCAAAUACGGCAAAGAGCG	741
	ACUGCUUCUCUAUAGGCAU	742	AUGCCUAUAGAGAAGCAGU	743
	GCUCUGAGAGGCAGAUAAA	744	UUUAUCUGCCUCUCAGAGC	745
	GGCCCUGGACGUCGCCUUU	746	AAAGGCGACGUCCAGGGCC	747
	CAACAAAUGGCUGACAAAG	748	CUUUGUCAGCCAUUUGUUG	749
	GGAGCUUUCUUUACAUCAC	750	GUGAUGUAAAGAAAGCUCC	751
	GUACACAAUGACCAAAUCA	752	UGAUUUGGUCAUUGUGUAC	753
	CUUCCUUGGCGGGAUUCUA	754	UAGAAUCCCGCCAAGGAAG	755
	GCUUCCACUUUCCCCUCUU	756	AAGAGGGGAAAGUGGAAGC	757
	GAUCUCAGGUUAUUGGGUU	758	AACCCAAUAACCUGAGAUC	759
	CGGGAUUCUAGCCUUUGGU	760	ACCAAAGGCUAGAAUCCCG	761
	CCCGCUCUUUGCCGUAUUU	762	AAAUACGGCAAAGAGCGGG	763
	UUACCUAUAAGUCCACACA	764	UGUGUGGACUUAUAGGUAA	765
	GGGCCUGGUCCUCCUCUAC	766	GUAGAGGAGGACCAGGCCC	767
	GGGUCUCUUCCCGCUCUUU	768	AAAGAGCGGGAAGAGACCC	769
	CGCCGGCAUCUUUAAGGAA	770	UUCCUUAAAGAUGCCGGCG	771
	CUACUACUGCUUCUCUAUA	772	UAUAGAGAAGCAGUAGUAG	773
	AUUUAUAGUUGAAGUAAUG	774	CAUUACUUCAACUAUAAAU	775
	CAUCCUUGGGGCCACACUA	776	UAGUGUGGCCCCAAGGAUG	777
	CUCCAUCGCCGGCAUCUUU	778	AAAGAUGCCGGCGAUGGAG	779
	GCCCCAGACCCCAUCCUUG	780	CAAGGAUGGGGUCUGGGGC	781
	GGCAGAUAAAUUUGACGUG	782	CACGUCAAAUUUAUCUGCC	783
	AUGUUUACCUAUAAGUCCA	784	UGGACUUAUAGGUAAACAU	785
	CAGCUGGACUGAUUACCUC	786	GAGGUAAUCAGUCCAGCUG	787
	CGUCCAGCUAACCAUGGGA	788	UCCCAUGGUUAGCUGGACG	789
	CAACUGGAGCUUUCUUUAC	790	GUAAAGAAAGCUCCAGUUG	791
	GAACCCUAUCGACACCAUG	792	CAUGGUGUCGAUAGGGUUC	793

	GACAAAGAGCUUCCACUUU	794	AAAGUGGAAGCUCUUUGUC	795
	AGGCAUGAGCACAAGGCAA	796	UUGCCUUGUGCUCAUGCCU	797
	GCAACAAUGACCAGUCCGG	798	CCGGACUGGUCAUUGUUGC	799
	GUUGAAGUAAUGAUUUCCU	800	AGGAAAUCAUUACUUCAAC	801
	ACAUCACUGUGUCACUGUA	802	UACAGUGACACAGUGAUGU	803
	GUUCCUCCUUGUGUCCAGA	804	UCUGGACACAAGGAGGAAC	805
	CAGAUAAAUUUGACGUGUG	806	CACACGUCAAAUUUAUCUG	807
	GUGCUCAGCUGGACUGAUU	808	AAUCAGUCCAGCUGAGCAC	809
	CCGCUCUUUGCCGUAUUUG	810	CAAAUACGGCAAAGAGCGG	811
	CUAACCAUGGGACAGUUUG	812	CAAACUGUCCCAUGGUUAG	813
	GGUGCUCAGCUGGACUGAU	814	AUCAGUCCAGCUGAGCACC	815
	CACCUGCUGGGUGAUCAGA	816	UCUGAUCACCCAGCAGGUG	817

Table 8.9

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Ugt8a	UGUUUAUUAUAGACAUUUU	818	AAAAUGUCUAUAAUAAACA	819
	UUGUUUAUUAUAGACAUUU	820	AAAUGUCUAUAAUAAACAA	821
	UUUUGUUUAUUAUAGACAU	822	AUGUCUAUAAUAAACAAAA	823
	UUUGUUUAUUAUAGACAUU	824	AAUGUCUAUAAUAAACAAA	825

Table 8.10

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Ogt	GCAUUAUCGACAUGCCUUG	826	CAAGGCAUGUCGAUAAUGC	827
	GCAGAAGAUUGUUAUAACA	828	UGUUAUAACAAUCUUCUGC	829
	GAUUCUUACUGCUAUUUAA	830	UUAAAUAGCAGUAAGAAUC	831
	GGAACUAGAUAACAUGCUU	832	AAGCAUGUUAUCUAGUUCC	833
	GGAUGAUAUAUAAUGAUAA	834	UUAUCAUUAUAUAUCAUCC	835
	GGAUGAUGGUACAAACUUC	836	GAAGUUUGUACCAUCAUCC	837
	CCAGGUAAGUAUAUAAGUA	838	UACUUAUAUACUUACCUGG	839
	CGGAUGAUAUAUAAUGAUA	840	UAUCAUUAUAUAUCAUCCG	841
	CCUAUAUCAGUCAUUUUAG	842	CUAAAAUGACUGAUAUAGG	843
	AGCAGUCAUCGAUUUUAAA	844	UUUAAAAUCGAUGACUGCU	845
	CAAUUGAGACGCAACCAAA	846	UUUGGUUGCGUCUCAAUUG	847
	GACGGAUGAUAUAUAAUGA	848	UCAUUAUAUAUCAUCCGUC	849
	GCACCUUGGCAAUUAAACA	850	UGUUUAAUUGCCAAGGUGC	851
	GCAUCGAUCUCAAAGCAUU	852	AAUGCUUUGAGAUCGAUGC	853
	CCUAUAGGGAUGAACUUUU	854	AAAAGUUCAUCCCUAUAGG	855
	GCAGAAGCCUAUUCGAAUU	856	AAUUCGAAUAGGCUUCUGC	857
	GGUAGGGAAUUGUAUUACU	858	AGUAAUACAAUUCCCUACC	859

	GAAGAAAAUUCGUGGCAAA	860	UUUGCCACGAAUUUUCUUC	861
	AAGUUAUUGAUAAUUAAGA	862	UCUUAAUUAUCAAUAACUU	863
	ACGGAUGAUAUAUAAUGAU	864	AUCAUUAUAUAUCAUCCGU	865
	UGAAGAAAUUGGUUAGUAU	866	AUACUAACCAAUUUCUUCA	867
	CCCCGUACCAUUAUUGUAA	868	UUACAAUAAUGGUACGGGG	869
	AGGAGAACCUAAUAUUCAA	870	UUGAAUAUUAGGUUCUCCU	871
	CAUUUAUGACAAUCGGAUA	872	UAUCCGAUUGUCAUAAAUG	873
	CUAUUUAAAACCUAAGCAA	874	UUGCUUAGGUUUUAAAUAG	875
	GAGAUUAACCAGCCGUUUU	876	AAAACGGCUGGUUAAUCUC	877
	GAUUUGGCUGGCAAUUCAU	878	AUGAAUUGCCAGCCAAAUC	879
	GAUGCUUACUCUAAUAUGG	880	CCAUAUUAGAGUAAGCAUC	881
	CCUAUAUCGCAAAGCAUUA	882	UAAUGCUUUGCGAUAUAGG	883
	GUACCUAAUAGCGUGCUUU	884	AAAGCACGCUAUUAGGUAC	885
	GGAAUUAGAGCGACUCUAU	886	AUAGAGUCGCUCUAAUUCC	887
	ACACCAGGGAUUUCUUAAU	888	AUUAAGAAAUCCCUGGUGU	889
	GGUUCCCCGUACCAUUAUU	890	AAUAAUGGUACGGGGAACC	891
	CACUGAUGCAUUAUAAGGA	892	UCCUUAUAAUGCAUCAGUG	893
	GGAAGCGAUUGAGCAUUAU	894	AUAAUGCUCAAUCGCUUCC	895
	CACUGUUCAUGGAUUAUAU	896	AUAUAAUCCAUGAACAGUG	897
	GAUUGAGCAUUAUCGACAU	898	AUGUCGAUAAUGCUCAAUC	899
	AAACAAACAAGCCGACAUA	900	UAUGUCGGCUUGUUUGUUU	901
	CCAACGAAACGUAUGCUUU	902	AAAGCAUACGUUUCGUUGG	903
	AGCAAGGCCUAAUAGACUU	904	AAGUCUAUUAGGCCUUGCU	905
	GUUUGUAGUGCUCUAUAUU	906	AAUAUAGAGCACUACAAAC	907
	CUGGCUUGUGUAUACUAUG	908	CAUAGUAUACACAAGCCAG	909
	GAACCGAUUUAGAAUACCU	910	AGGUAUUCUAAAUCGGUUC	911
	CCACAAGGAAUCUCUUAGA	912	UCUAAGAGAUUCCUUGUGG	913
	GGCUUGGAUAAAUAGACCUC	914	GAGGUCUAUUAUCCAAGCC	915
	CGCCUAUAUCGCAAAGCAU	916	AUGCUUUGCGAUAUAGGCG	917
	AUGUUUGCUUUCAGGUAUU	918	AAUACCUGAAAGCAAACAU	919
	GCCAAGGCAUGUUAUUUGA	920	UCAAAUAACAUGCCUUGGC	921
	GUUGCAGACGGAUGAUAUA	922	UAUAUCAUCCGUCUGCAAC	923
	CGUUAAAUUCUCCUGUGAC	924	GUCACAGGAGAAUUUAACG	925

Table 8.11

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Gla	CAAUUAUACAGAUAUCCAA	926	UUGGAUAUCUGUAUAAUUG	927
	CGACCACUCUCAAAUUUAG	928	CUAAAUUUGAGAGUGGUCG	929
	AGCCCAAUUAUACAGAUAU	930	AUAUCUGUAUAAUUGGGCU	931
	GUGAGUGGCCGCUUUAUAU	932	AUAUAAAGCGGCCACUCAC	933
	GCUUCGACUAGAAAGAUAA	934	UUAUCUUUCUAGUCGAAGC	935

	GUUUUGGGUACUAUGAUAU	936	AUAUCAUAGUACCCAAAAC	937
	CAGCUAGGCUUCUAUGAAU	938	AUUCAUAGAAGCCUAGCUG	939
	AGUACAGCUAGGCUUCUAU	940	AUAGAAGCCUAGCUGUACU	941
	GUCCUUGGCAGAUGGUUAU	942	AUAACCAUCUGCCAAGGAC	943
	CCCCGAGGGUAAACUUAAA	944	UUUAAGUUUACCCUCGGGG	945
	GCACUGGGAACGUUUCAUG	946	CAUGAAACGUUCCCAGUGC	947
	CUAGCUAAUUAUGUCCACA	948	UGUGGACAUAAUUAGCUAG	949
	GGGAGUUUUGGGUACUAUG	950	CAUAGUACCCAAAACUCCC	951
	GGAACGACCACUCUCAAAU	952	AUUUGAGAGUGGUCGUUCC	953
	UUUCAUAAGCCCAAUUAUA	954	UAUAAUUGGGCUUAUGAAA	955
	CAGGGGUACUGUUUUAGAA	956	UUCUAAAACAGUACCCCUG	957
	CCAGAUAUGUUAGUGAUUG	958	CAAUCACUAACAUAUCUGG	959
	GCUAGGAAUUUAUGCAGAU	960	AUCUGCAUAAAUUCCUAGC	961
	GCAACUUUGGCCUGAGUUG	962	CAACUCAGGCCAAAGUUGC	963
	GUUAUAAGUACAUGUCCUU	964	AAGGACAUGUACUUAUAAC	965
	GGUUAUAAGUACAUGUCCU	966	AGGACAUGUACUUAUAACC	967
	CCCGAGGGUAAACUUAAAA	968	UUUUAAGUUUACCCUCGGG	969
	CAAUAUUAUUGCAAUUACU	970	AGUAAUUGCAAUAAUAUUG	971
	AUAUCCAAUAUUAUUGCAA	972	UUGCAAUAAUAUUGGAUAU	973
	AUACAGAUAUCCAAUAUUA	974	UAAUAUUGGAUAUCUGUAU	975
	CGCCCCGAGGGUAAACUUA	976	UAAGUUUACCCUCGGGGCG	977
	CUAUGAUAUUGAUGCCCAG	978	CUGGGCAUCAAUAUCAUAG	979
	CCCUAGUCUUAGUUUUCUG	980	CAGAAAACUAAGACUAGGG	981
	UGCAGAUGUUGGGAAUAAA	982	UUUAUUCCCAACAUCUGCA	983
	CUUCGACUAGAAAGAUAAA	984	UUUAUCUUUCUAGUCGAAG	985
	CGCAGGUUAUGAAUACCUC	986	GAGGUAUUCAUAACCUGCG	987
	GACUCAAAGGGCCGACUUC	988	GAAGUCGGCCCUUUGAGUC	989
	AUAUGUUAGUGAUUGGCAA	990	UUGCCAAUCACUAACAUAU	991
	GUUGGAAUGACCCAGAUAU	992	AUAUCUGGGUCAUUCCAAC	993
	GCUUAACUCGGUAAACUGA	994	UCAGUUUACCGAGUUAAGC	995
	CCAAUUAUACAGAUAUCCA	996	UGGAUAUCUGUAUAAUUGG	997
	UAGAUCUGCUAAAAUUUGA	998	UCAAAUUUUAGCAGAUCUA	999
	GGGUAAACUUAAAAGCCCA	1000	UGGGCUUUUAAGUUUACCC	1001
	GCCCAAUUAUACAGAUAUC	1002	GAUAUCUGUAUAAUUGGGC	1003
	GCCCCGAGGGUAAACUUAA	1004	UUAAGUUUACCCUCGGGGC	1005
	GUACUGUUUUAGAAAGGAA	1006	UUCCUUUCUAAAACAGUAC	1007
	AAUAUUAUUGCAAUUACUG	1008	CAGUAAUUGCAAUAAUAUU	1009
	GACCCAGAUAUGUUAGUGA	1010	UCACUAACAUAUCUGGGUC	1011
	ACAGCUAGGCUUCUAUGAA	1012	UUCAUAGAAGCCUAGCUGU	1013
	CAGCACCUAGCUAAUUAUG	1014	CAUAAUUAGCUAGGUGCUG	1015
	UAUACAGAUAUCCAAUAUU	1016	AAUAUUGGAUAUCUGUAUA	1017
	GGCAGCUCCUCUACUCAUG	1018	CAUGAGUAGAGGAGCUGCC	1019
	GCUUUAUAUGAGACCCUUU	1020	AAAGGGUCUCAUAUAAAGC	1021

	AAUCCUGGUUGCAUCAUCA	1022	UGAUGAUGCAACCAGGAUU	1023
	UAUUAUUGCAAUUACUGGA	1024	UCCAGUAAUUGCAAUAAUA	1025

Table 8.12

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Glb1	GGCAGAGGCAUAAAUGAUU	1026	AAUCAUUUAUGCCUCUGCC	1027
	CCGUGACGUGGAAUAUUUC	1028	GAAAUAUUCCACGUCACGG	1029
	GUUUAAAGAUGUCCCAAAA	1030	UUUUGGGACAUCUUUAAAC	1031
	CUACUUUGCCUGUGAUUAU	1032	AUAAUCACAGGCAAAGUAG	1033
	GACAUUCAUCCAAGUUAAA	1034	UUUAACUUGGAUGAAUGUC	1035
	CGGAUACCUCGCUUCUAUU	1036	AAUAGAAGCGAGGUAUCCG	1037
	GCCGCAGGAUACCUUCAUU	1038	AAUGAAGGUAUCCUGCGGC	1039
	CCAAGUUCGCCUAUGGAAA	1040	UUUCCAUAGGCGAACUUGG	1041
	CCAAUUUUGCCUAUUGGAA	1042	UUCCAAUAGGCAAAAUUGG	1043
	CCACCACGAUGGACGCUUG	1044	CAAGCGUCCAUCGUGGUGG	1045
	CUUAACUUCAGUAUUCUCA	1046	UGAGAAUACUGAAGUUAAG	1047
	CCGAUGGGGGCGAAUGAAAA	1048	UUUUCAUUCGCCCCAUCGG	1049
	GAAUCUAUUGUUCUUCGAU	1050	AUCGAAGAACAAUAGAUUC	1051
	GAGCGAAACCGUGUGAUAU	1052	AUAUCACACGGUUUCGCUC	1053
	CCCACAGGUGACCUUGUUU	1054	AAACAAGGUCACCUGUGGG	1055
	GAACUAUGGCAGAGGCAUA	1056	UAUGCCUCUGCCAUAGUUC	1057
	GGCACAUUCUGACGACGUC	1058	GACGUCGUCAGAAUGUGCC	1059
	AGAAUCUAUUGUUCUUCGA	1060	UCGAAGAACAAUAGAUUCU	1061
	GCUACCCGCUGACAUUCAU	1062	AUGAAUGUCAGCGGGUAGC	1063
	GUGACGUGGAAUAUUUCAU	1064	AUGAAAUAUUCCACGUCAC	1065
	GCCAGGACAAUAUGAGUUU	1066	AAACUCAUAUUGUCCUGGC	1067
	AGUUUAAAGAUGUCCCAAA	1068	UUUGGGACAUCUUUAAACU	1069
	GGGGUGAACCCCAUUACAC	1070	GUGUAAUGGGGUUCACCCC	1071
	GCAAUGGCUCGAUGCCCAU	1072	AUGGGCAUCGAGCCAUUGC	1073
	CCAGGACAAUAUGAGUUUU	1074	AAAACUCAUAUUGUCCUGG	1075
	GGGUCAUCCGUGUCUCGAU	1076	AUCGAGACACGGAUGACCC	1077
	CUGCUUGGCUGCUAGAGAA	1078	UUCUCUAGCAGCCAAGCAG	1079
	ACCGUGACGUGGAAUAUUU	1080	AAAUAUUCCACGUCACGGU	1081
	GGGUUUGAUUUCUAACAUG	1082	CAUGUUAGAAAUCAAACCC	1083
	CCUACUUUGCCUGUGAUUA	1084	UAAUCACAGGCAAAGUAGG	1085
	GGUGGGACCAAUUUUGCCU	1086	AGGCAAAAUUGGUCCCACC	1087
	GGACCGUGACGUGGAAUAU	1088	AUAUUCCACGUCACGGUCC	1089
	GGACCUUUGAUCAAUUCUG	1090	CAGAAUUGAUCAAAGGUCC	1091
	CACCGAUGGGGCGAAUGAA	1092	UUCAUUCGCCCCAUCGGUG	1093
	AUGGCAGAGGCAUAAAUGA	1094	UCAUUUAUGCCUCUGCCAU	1095
	AGCCAGGACAAUAUGAGUU	1096	AACUCAUAUUGUCCUGGCU	1097

	CCUUCAUUCAGUUUCCUGG	1098	CCAGGAAACUGAAUGAAGG	1099
	GCAGUACGCACCCAUCUUG	1100	CAAGAUGGGUGCGUACUGC	1101
	AGGGUUUGAUUUCUAACAU	1102	AUGUUAGAAAUCAAACCCU	1103
	GAAGUAUUUUGCUCUUCGA	1104	UCGAAGAGCAAAAUACUUC	1105
	UCAUCCAAGUUAAACAGUA	1106	UACUGUUUAACUUGGAUGA	1107
	AGGUGGAUUUAUCAGGUGU	1108	ACACCUGAUAAAUCCACCU	1109
	CCGCAGGAUACCUUCAUUC	1110	GAAUGAAGGUAUCCUGCGG	1111
	CAGCCAGGACAAUAUGAGU	1112	ACUCAUAUUGUCCUGGCUG	1113
	AAUGGAGGGCCGAUUAUAA	1114	UUAUAAUCGGCCCUCCAUU	1115
	GGCACACCGCUUCCGUUAC	1116	GUAACGGAAGCGGUGUGCC	1117
	GGCUGCAGUACGCACCCAU	1118	AUGGGUGCGUACUGCAGCC	1119
	CAAUGACGUCCUUCUCUUC	1120	GAAGAGAAGGACGUCAUUG	1121
	GCUGUACCGAACAACACUU	1122	AAGUGUUGUUCGGUACAGC	1123
	UAAAUGAUUUUAAGGGUUU	1124	AAACCCUUAAAAUCAUUUA	1125

Table 8.13

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
A3galt2	CUGGCCAAGCAGGCGCCAA	1126	UUGGCGCCUGCUUGGCCAG	1127
	CAGCCCUUCCCUGGCCAAG	1128	CUUGGCCAGGGAAGGGCUG	1129
	GCCCUUCCCUGGCCAAGCA	1130	UGCUUGGCCAGGGAAGGGC	1131
	GCAGCCCUUCCCUGGCCAA	1132	UUGGCCAGGGAAGGGCUGC	1133
	CCUUCCCUGGCCAAGCAGG	1134	CCUGCUUGGCCAGGGAAGG	1135
	CCCUGGCCAAGCAGGCGCC	1136	GGCGCCUGCUUGGCCAGGG	1137
	UGGCCAAGCAGGCGCCAAC	1138	GUUGGCGCCUGCUUGGCCA	1139
	CCUGGCCAAGCAGGCGCCA	1140	UGGCGCCUGCUUGGCCAGG	1141
	AGCAGCCCUUCCCUGGCCA	1142	UGGCCAGGGAAGGGCUGCU	1143
	CCCUUCCCUGGCCAAGCAG	1144	CUGCUUGGCCAGGGAAGGG	1145
	CUUCCCUGGCCAAGCAGGC	1146	GCCUGCUUGGCCAGGGAAG	1147
	AGCCCUUCCCUGGCCAAGC	1148	GCUUGGCCAGGGAAGGGCU	1149
	UCCCUGGCCAAGCAGGCGC	1150	GCGCCUGCUUGGCCAGGGA	1151
	UUCCCUGGCCAAGCAGGCG	1152	CGCCUGCUUGGCCAGGGAA	1153

Table 8.14

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Mgat2	GAUAAUUAUUGAUUAGAAA	1154	UUUCUAAUCAAUAAUUAUC	1155
	CUUUCAAUGGGAUAUACAA	1156	UUGUAUAUCCCAUUGAAAG	1157
	ACACUGGCCUUAUACUUUU	1158	AAAAGUAUAAGGCCAGUGU	1159
	AGUACAGCAAGACUUAUUU	1160	AAAUAAGUCUUGCUGUACU	1161
	AUUGAAUAAAGGUAUAGGA	1162	UCCUAUACCUUUAUUCAAU	1163

	CAUUCUUAAAUAAUAACCA	1164	UGGUUAUUAUUUAAGAAUG	1165
	AAAUUUUAUUUAAAUCGCA	1166	UGCGAUUUAAAUAAAAUUU	1167
	ACCUCAAUGUAGAAAUACA	1168	UGUAUUUCUACAUUGAGGU	1169
	CAGAAGUUCCGUUUGUUAA	1170	UUAACAAACGGAACUUCUG	1171
	CCUCAAGCUCCGAGGAUUU	1172	AAAUCCUCGGAGCUUGAGG	1173
	AAGACUAUUAGAGUAUUGA	1174	UCAAUACUCUAAUAGUCUU	1175
	CAUAAAAUUCCCUAAUCAU	1176	AUGAUUAGGGAAUUUUAUG	1177
	CUUCAAUAUUUGACUGUAU	1178	AUACAGUCAAAUAUUGAAG	1179
	AGACACUUUCUGUACUUAU	1180	AUAAGUACAGAAAGUGUCU	1181
	AAACUGUCACAACAGUAAA	1182	UUUACUGUUGUGACAGUUU	1183
	CUGUGCUAUUGUGUUAAGA	1184	UCUUAACACAAUAGCACAG	1185
	UUUUCAUAAAAUUCCCUAA	1186	UUAGGGAAUUUUAUGAAAA	1187
	AGUUGAACUUCGAUCAGAU	1188	AUCUGAUCGAAGUUCAACU	1189
	GACUAUUAGAGUAUUGAUG	1190	CAUCAAUACUCUAAUAGUC	1191
	CCAGAAACUCUAGUUAUCA	1192	UGAUAACUAGAGUUUCUGG	1193
	CUUGUAACAGGUGUCCAAA	1194	UUUGGACACCUGUUACAAG	1195
	UUGAUAAUUAUUGAUUAGA	1196	UCUAAUCAAUAAUUAUCAA	1197
	CCCGGUUCUGCAAGUGUUC	1198	GAACACUUGCAGAACCGGG	1199
	UGUAUUUGGUUAAUAAGAA	1200	UUCUUAUUAACCAAAUACA	1201
	GCCCGGUUCUGCAAGUGUU	1202	AACACUUGCAGAACCGGGC	1203
	AGACUAUUAGAGUAUUGAU	1204	AUCAAUACUCUAAUAGUCU	1205
	CAAUUGAAUAAAGGUAUAG	1206	CUAUACCUUUAUUCAAUUG	1207
	GUUGGAAUGUAUUUGGUUA	1208	UAACCAAAUACAUUCCAAC	1209
	CCACCUUGCUGUGUCCGUA	1210	UACGGACACAGCAAGGUGG	1211
	UGAUAAUUAUUGAUUAGAA	1212	UUCUAAUCAAUAAUUAUCA	1213
	ACUUCUGGUCCGCAGAGAU	1214	AUCUCUGCGGACCAGAAGU	1215
	GGAGAUAUUAGGGACCAUG	1216	CAUGGUCCCUAAUAUCUCC	1217
	CCAGUGUCACUAUUGACUU	1218	AAGUCAAUAGUGACACUGG	1219
	CAAGAAAACAUGUAGGCCA	1220	UGGCCUACAUGUUUUCUUG	1221
	AAAACUGUCACAACAGUAA	1222	UUACUGUUGUGACAGUUUU	1223
	CCUAGUCAUCUUCAGCCAU	1224	AUGGCUGAAGAUGACUAGG	1225
	UCCCUAAUCAUUUUGCAGA	1226	UCUGCAAAAUGAUUAGGGA	1227
	GCUGAUUCCUGUGCUAUUG	1228	CAAUAGCACAGGAAUCAGC	1229
	CAGUUGAACUUCGAUCAGA	1230	UCUGAUCGAAGUUCAACUG	1231
	GUGGGUGGGGAGAUAUUAG	1232	CUAAUAUCUCCCCACCCAC	1233
	GGGAGAUAUUAGGGACCAU	1234	AUGGUCCCUAAUAUCUCCC	1235
	UAUUCAGUUAGUCUGAUUA	1236	UAAUCAGACUAACUGAAUA	1237
	ACACUUUCUGUACUUAUGA	1238	UCAUAAGUACAGAAAGUGU	1239
	AGGCCAAAUUCUCGCAAAC	1240	GUUUGCGAGAAUUUGGCCU	1241
	GCGACAAAGGAAGAACGAC	1242	GUCGUUCUUCCUUUGUCGC	1243
	CGCAAACCAAACAUCAUUG	1244	CAAUGAUGUUUGGUUUGCG	1245
	AAUUCAGUCUAUCUGCUUA	1246	UAAGCAGAUAGACUGAAUU	1247
	GAAGUUGAUCGAGUGCACA	1248	UGUGCACUCGAUCAACUUC	1249

	GGAUUACACUGGCCUUAUA	1250	UAUAAGGCCAGUGUAAUCC	1251
	GAAUAAAGGUAUAGGAACU	1252	AGUUCCUAUACCUUUAUUC	1253

Table 8.15

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Slc35d1	GCACAAAAGAAUAUAUUAA	1254	UUAAUAUAUUCUUUUGUGC	1255
	UGAAUUAAUUUAUAUCAGA	1256	UCUGAUAUAAAUUAAUUCA	1257
	CAUUCUGUUUACAAUGUUU	1258	AAACAUUGUAAACAGAAUG	1259
	GGUCUUUGGUGGAGAUUAU	1260	AUAAUCUCCACCAAAGACC	1261
	UGGGUGGGUUUUAAUUUAU	1262	AUAAAUUAAAACCCACCCA	1263
	GGAGAUUAUAUUUUCACAU	1264	AUGUGAAAAUAUAAUCUCC	1265
	CACAAAAGAAUAUAUUAAU	1266	AUUAAUAUAUUCUUUUGUG	1267
	CUAUAUUUUGGGAACCAAA	1268	UUUGGUUCCCAAAAUAUAG	1269
	UCACAUGGACAAAUUUUAU	1270	AUAAAAUUUGUCCAUGUGA	1271
	CCACUACCUCUACUAUAUU	1272	AAUAUAGUAGAGGUAGUGG	1273
	GACUGUAUUUGCAAUGAUU	1274	AAUCAUUGCAAAUACAGUC	1275
	CUUUCUUUUCUUUUCUUUU	1276	AAAAGAAAAGAAAAGAAAG	1277
	GGCCUAAAUAUAAGUAUUG	1278	CAAUACUUAUAUUUAGGCC	1279
	GUUCUGGGUGGGUUUUAAU	1280	AUUAAAACCCACCCAGAAC	1281
	CACAUGGACAAAUUUUAUU	1282	AAUAAAAUUUGUCCAUGUG	1283
	AACAGAAGUUAGAUUCGAA	1284	UUCGAAUCUAACUUCUGUU	1285
	UUAAUAACUUAUAUUGGAA	1286	UUCCAAUAUAAGUUAUUAA	1287
	CAGACUGGAAAAUGUUCUG	1288	CAGAACAUUUUCCAGUCUG	1289
	ACAGAAGUUAGAUUCGAAA	1290	UUUCGAAUCUAACUUCUGU	1291
	GCCUGGUGUAUUCUUACAU	1292	AUGUAAGAAUACACCAGGC	1293
	GAAGCCUGGUGUAUUCUUA	1294	UAAGAAUACACCAGGCUUC	1295
	GCUGGAAGCCUGGUGUAUU	1296	AAUACACCAGGCUUCCAGC	1297
	CAGAAAUGUACCUCGAAAG	1298	CUUUCGAGGUACAUUUCUG	1299
	UGGCCUAAAUAUAAGUAUU	1300	AAUACUUAUAUUUAGGCCA	1301
	UGGCCUGCUCUAUUACAAU	1302	AUUGUAAUAGAGCAGGCCA	1303
	AUAUAUUAAUAACUUAUAU	1304	AUAUAAGUUAUUAAUAUAU	1305
	UUUUCUUGGGGUAUUAAGA	1306	UCUUAAUACCCCAAGAAAA	1307
	GAAUUAAUUUAUAUCAGAC	1308	GUCUGAUAUAAAUUAAUUC	1309
	GUUGAAAGGACACAAAUGA	1310	UCAUUUGUGUCCUUUCAAC	1311
	UUUUAUUGGCCUAAAUAUA	1312	UAUAUUUAGGCCAAUAAAA	1313
	GAAAGAGCUGGGAAAGUAU	1314	AUACUUUCCCAGCUCUUUC	1315
	AUGCUAACUAUGUUUGAAU	1316	AUUCAAACAUAGUUAGCAU	1317
	AACUAUGUUUGAAUUAAUU	1318	AAUUAAUUCAAACAUAGUU	1319
	GAUCAAUGAUGUCCUGACA	1320	UGUCAGGACAUCAUUGAUC	1321
	CUUACAUCACUUUCACUGA	1322	UCAGUGAAAGUGAUGUAAG	1323
	GGUGUAUUCUUACAUCACU	1324	AGUGAUGUAAGAAUACACC	1325

	UUUAUUGGCCUAAAUAUAA	1326	UUAUAUUUAGGCCAAUAAA	1327
	AGAAGUUAGAUUCGAAAGA	1328	UCUUUCGAAUCUAACUUCU	1329
	GUGUAUUCUUACAUCACUU	1330	AAGUGAUGUAAGAAUACAC	1331
	GAAGGUUCUCCAUUCUGUU	1332	AACAGAAUGGAGAACCUUC	1333
	GAUCUGGAAGGAUAUGUUU	1334	AAACAUAUCCUUCCAGAUC	1335
	CCUAAAAUAUAAGUAUUGCU	1336	AGCAAUACUUAUAUUUAGG	1337
	CAGGAGAUGCACAAAAGAA	1338	UUCUUUUGUGCAUCUCCUG	1339
	GAGAUUAUAUUUUCACAUG	1340	CAUGUGAAAAUAUAAUCUC	1341
	AAUUUUAUUGGCCUAAAUA	1342	UAUUUAGGCCAAUAAAAUU	1343
	CGUGCUCACCAACUACAGA	1344	UCUGUAGUUGGUGAGCACG	1345
	GCCAGAGCUGGGUGGCAUG	1346	CAUGCCACCCAGCUCUGGC	1347
	CUGGAAGCCUGGUGUAUUC	1348	GAAUACACCAGGCUUCCAG	1349
	GGUUCUCCAUUCUGUUUAC	1350	GUAAACAGAAUGGAGAACC	1351
	UAUGUUUGAAUUAAUUUAU	1352	AUAAAUUAAUUCAAACAUA	1353

Table 8.16

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Ggta1	GGACGUGGAUCAAGUCUUU	1354	AAAGACUUGAUCCACGUCC	1355
	GGGAAGGCACUUAUGACAC	1356	GUGUCAUAAGUGCCUUCCC	1357
	CGUGGAUCAAGUCUUUCAA	1358	UUGAAAGACUUGAUCCACG	1359
	UGUGUGGGAAGGCACUUAU	1360	AUAAGUGCCUUCCCACACA	1361
	CUGCAUGGACGUGGAUCAA	1362	UUGAUCCACGUCCAUGCAG	1363
	GCAUGGACGUGGAUCAAGU	1364	ACUUGAUCCACGUCCAUGC	1365
	CGUGGAAGGCGCCGAUUGU	1366	ACAAUCGGCGCCUUCCACG	1367
	CGAUUGUGUGGGAAGGCAC	1368	GUGCCUUCCCACACAAUCG	1369
	GCACGAGGUCGACUUCCUC	1370	GAGGAAGUCGACCUCGUGC	1371
	UGGACGUGGAUCAAGUCUU	1372	AAGACUUGAUCCACGUCCA	1373
	CCAGCACGAGGUCGACUUC	1374	GAAGUCGACCUCGUGCUGG	1375
	GGCACUUAUGACACAGCUC	1376	GAGCUGUGUCAUAAGUGCC	1377
	UGGGAAGGCACUUAUGACA	1378	UGUCAUAAGUGCCUUCCCA	1379
	ACUCACAUUCUCAACCUCA	1380	UGAGGUUGAGAAUGUGAGU	1381
	GAGGUCGACUUCCUCUUCU	1382	AGAAGAGGAAGUCGACCUC	1383
	GUGGGAAGGCACUUAUGAC	1384	GUCAUAAGUGCCUUCCCAC	1385
	CCGUGGAAGGCGCCGAUUG	1386	CAAUCGGCGCCUUCCACGG	1387
	GGAAGGCACUUAUGACACA	1388	UGUGUCAUAAGUGCCUUCC	1389
	GGUUCUCAACCUGUGGGUC	1390	GACCCACAGGUUGAGAACC	1391
	GGCGCCGAUUGUGUGGGAA	1392	UUCCCACACAAUCGGCGCC	1393
	CGAGGUCGACUUCCUCUUC	1394	GAAGAGGAAGUCGACCUCG	1395
	CAUCCAGCACGAGGUCGAC	1396	GUCGACCUCGUGCUGGAUG	1397
	CCCCGUGGAAGGCGCCGAU	1398	AUCGGCGCCUUCCACGGGG	1399
	UCGACUUCCUCUUCUGCAU	1400	AUGCAGAAGAGGAAGUCGA	1401

	GGUCGACUUCCUCUUCUGC	1402	GCAGAAGAGGAAGUCGACC	1403
	UGUGGGAAGGCACUUAUGA	1404	UCAUAAGUGCCUUCCCACA	1405
	GACGUGGAUCAAGUCUUUC	1406	GAAAGACUUGAUCCACGUC	1407
	GUGUGGGAAGGCACUUAUG	1408	CAUAAGUGCCUUCCCACAC	1409
	CAUGGACGUGGAUCAAGUC	1410	GACUUGAUCCACGUCCAUG	1411
	CCGAUUGUGUGGGAAGGCA	1412	UGCCUUCCCACACAAUCGG	1413
	GAAGGCACUUAUGACACAG	1414	CUGUGUCAUAAGUGCCUUC	1415
	UCUGCAUGGACGUGGAUCA	1416	UGAUCCACGUCCAUGCAGA	1417
	GCGCCGAUUGUGUGGGAAG	1418	CUUCCCACACAAUCGGCGC	1419
	ACGUGGAUCAAGUCUUUCA	1420	UGAAAGACUUGAUCCACGU	1421
	UGGAAGGCGCCGAUUGUGU	1422	ACACAAUCGGCGCCUUCCA	1423
	AGCACGAGGUCGACUUCCU	1424	AGGAAGUCGACCUCGUGCU	1425
	GAAGGCGCCGAUUGUGUGG	1426	CCACACAAUCGGCGCCUUC	1427
	CGACUUCCUCUUCUGCAUG	1428	CAUGCAGAAGAGGAAGUCG	1429
	CUUCUGCAUGGACGUGGAU	1430	AUCCACGUCCAUGCAGAAG	1431
	CCCACAUCCAGCACGAGGU	1432	ACCUCGUGCUGGAUGUGGG	1433
	ACAUCCAGCACGAGGUCGA	1434	UCGACCUCGUGCUGGAUGU	1435
	AGGUCGACUUCCUCUUCUG	1436	CAGAAGAGGAAGUCGACCU	1437
	GAUUGUGUGGGAAGGCACU	1438	AGUGCCUUCCCACACAAUC	1439
	ACUUCCUCUUCUGCAUGGA	1440	UCCAUGCAGAAGAGGAAGU	1441
	ACGAGGUCGACUUCCUCUU	1442	AAGAGGAAGUCGACCUCGU	1443
	UCUUCUGCAUGGACGUGGA	1444	UCCACGUCCAUGCAGAAGA	1445
	GUGGAAGGCGCCGAUUGUG	1446	CACAAUCGGCGCCUUCCAC	1447
	CUACUCACAUUCUCAACCU	1448	AGGUUGAGAAUGUGAGUAG	1449
	UGUGGGUCGCGACCCCUUU	1450	AAAGGGGUCGCGACCCACA	1451
	CCUGUGGGUCGCGACCCCU	1452	AGGGGUCGCGACCCACAGG	1453

Table 8.17

Target gene	SenseSeq	SEQ ID No.	AntisSeq	SEQ ID No.
Fut8	GGUUCCUGGCGUUGGAUUA	1454	UAAUCCAACGCCAGGAACC	1455
	CAGGAAGAGUCCGUGUUUU	1456	AAAACACGGACUCUUCCUG	1457
	CUCUCCAAGAUUCUUGCAA	1458	UUGCAAGAAUCUUGGAGAG	1459
	GUCAUUUGGUUCGAGAUAA	1460	UUAUCUCGAACCAAAUGAC	1461
	UAUAGGUGGUCAUUUGGUU	1462	AACCAAAUGACCACCUAUA	1463
	UGAUCCUGGAUAUACACUU	1464	AAGUGUAUAUCCAGGAUCA	1465
	GUAAAAUAAUGUACUCACA	1466	UGUGAGUACAUUAUUUUAC	1467
	CUCUAAAGGUGUCAACAGA	1468	UCUGUUGACACCUUUAGAG	1469
	CAACCAGAUUGCAGUUUAU	1470	AUAAACUGCAAUCUGGUUG	1471
	CUCACACAUAACAUACAAA	1472	UUUGUAUGUUAUGUGUGAG	1473
	CUUCAACAUCAUAAUUCUA	1474	UAGAAUUAUGAUGUUGAAG	1475
	AGCGCUUAAAACAACAAAA	1476	UUUUGUUGUUUUAAGCGCU	1477

	CCCAUGCACAGUAAAAUAA	1478	UUAUUUUACUGUGCAUGGG	1479
	GAAAUUAGAAGGAAACGAA	1480	UUCGUUUCCUUCUAAUUUC	1481
	CUUUGUAAGAAUAACCACA	1482	UGUGGUUAUUCUUACAAAG	1483
	GAACUCUCCAAGAUUCUUG	1484	CAAGAAUCUUGGAGAGUUC	1485
	AGAUGACAUCUACUAUUUU	1486	AAAAUAGUAGAUGUCAUCU	1487
	CACUCAUCUUGGAAUCUCA	1488	UGAGAUUCCAAGAUGAGUG	1489
	CAGUAAAAUAAUGUACUCA	1490	UGAGUACAUUAUUUUACUG	1491
	CAUCAUAAUUCUAUGGGGA	1492	UCCCCAUAGAAUUAUGAUG	1493
	AGAUAGAACAGAGUUGAAA	1494	UUUCAACUCUGUUCUAUCU	1495
	GUACAAUGUUUCCAAUUCU	1496	AGAAUUGGAAACAUUGUAC	1497
	CCAGCGAACACUCAUCUUG	1498	CAAGAUGAGUGUUCGCUGG	1499
	GGCGUUGGAUUAUGCUCAU	1500	AUGAGCAUAAUCCAACGCC	1501
	GAAACAAGCUAGGAAUGAU	1502	AUCAUUCCUAGCUUGUUUC	1503
	CAUGGUACACGUUGAAGAA	1504	UUCUUCAACGUGUACCAUG	1505
	GAUAGAAACAGUCAAAUAC	1506	GUAUUUGACUGUUUCUAUC	1507
	UCACCAACCUCGAACUAAA	1508	UUUAGUUCGAGGUUGGUGA	1509
	AAUUCUUUUGGAUUUAGGA	1510	UCCUAAAUCCAAAAGAAUU	1511
	AUUAGUGAUAACUCUAUUU	1512	AAAUAGAGUUAUCACUAAU	1513
	GGUUCCUGUUUCCGUUUCU	1514	AGAAACGGAAACAGGAACC	1515
	CAGAAUUGGCGCUAUGCUA	1516	UAGCAUAGCGCCAAUUCUG	1517
	AUUCACUGCAAUCAACAGA	1518	UCUGUUGAUUGCAGUGAAU	1519
	CUUCAAACAUCCAGUUAUU	1520	AAUAACUGGAUGUUUGAAG	1521
	CUUGCAGAUCGACUCCUGA	1522	UCAGGAGUCGAUCUGCAAG	1523
	ACUCACACAUAACAUACAA	1524	UUGUAUGUUAUGUGUGAGU	1525
	UUAUGAAUUUAUUAGUGAU	1526	AUCACUAAUAAAUUCAUAA	1527
	CAGAAAGCUGGUAUGUAAU	1528	AUUACAUACCAGCUUUCUG	1529
	GAGCGCUUAAAACAACAAA	1530	UUUGUUGUUUUAAGCGCUC	1531
	AAUACAUGGUACACGUUGA	1532	UCAACGUGUACCAUGUAUU	1533
	UGUACUCACACAUAACAUA	1534	UAUGUUAUGUGUGAGUACA	1535
	CAUGAAAUCUUAAGGAGGA	1536	UCCUCCUUAAGAUUUCAUG	1537
	GCUGGUAUGUAAUAUCAAC	1538	GUUGAUAUUACAUACCAGC	1539
	GAAACAGUCAAAUACCCUA	1540	UAGGGUAUUUGACUGUUUC	1541
	ACUUCCAUUCUUUAGAUGA	1542	UCAUCUAAAGAAUGGAAGU	1543
	CUCACCAACCUCGAACUAA	1544	UUAGUUCGAGGUUGGUGAG	1545
	AACAUAUGGUUCAUUGACA	1546	UGUCAAUGAACCAUAUGUU	1547
	AUGAAUUUAUUAGUGAUAA	1548	UUAUCACUAAUAAAUUCAU	1549
	GAGAUGGAGUGUAAGAGAU	1550	AUCUCUUACACUCCAUCUC	1551
	CCAUCAUGUGGUUUACUGC	1552	GCAGUAAACCACAUGAUGG	1553

CLAIMS

What is claimed is:

1. A composition comprising an antibody or a fusion protein that comprises the Fc domain of an antibody,

   (a) wherein at least about 70% of the antibody or the fusion protein molecules comprise a complex N-glycan; and

   (b) wherein about 40% to about 100% of the N-glycans are afucosyl glycans.

2. The composition of claim 1, wherein said N-glycans comprise a mixture of glycoforms, and wherein

   i about 20% to about 100% of the G0 glycans are afucosyl G0 glycans; or

   ii about 1% to about 80% of the G1 and G1' glycans are afucosyl G1 and G1' glycans; or

   iii about 1% to about 80% of the G2 glycans are afucosyl G2 glycans; or

   iv a combination of (i), (ii), or (iii).

3. The composition of claim 1 or 2, wherein:

   i about 20% to about 100% of the G0 glycans are afucosyl G0 glycans; and

   ii about 1% to about 80% of the G1 and G1' glycans are afucosyl G1 and G1' glycans.

4. The composition of any one of claims 1-3, wherein:

   i about 20% to about 100% of the G0 glycans are afucosyl G0 glycans;

   ii about 1% to about 80% of the G1 and G1' glycans are afucosyl G1 and G1' glycans; and

   iii about 1% to about 80% of the G2 glycans are afucosyl G2 glycans.

5. The composition of any one of claims 1-4, wherein said N-glycan is linked to the Fc domain of the antibody or the fusion protein.

6. The composition of any one of claims 1-5, wherein said composition comprises an antibody and wherein said antibody is an IgG.

7. The composition of claim 6, wherein said IgG is IgG1.

8. The composition of any one of claims 5-7, wherein said composition comprises an antibody, and wherein said antibody is an anti-CD20 antibody.

9. The composition of claim 8, wherein the light chain of said antibody comprises a sequence that is at least 90% identical to the light chain of Rituximab.

10. The composition of claim 8 or 9, wherein the heavy chain of said antibody comprises a sequence that is at least 90% identical to the heavy chain of Rituximab.

11. The composition of any one of claims 8-10, wherein said composition has an increased binding affinity for an FcγRIII or increased antibody-dependent cellular cytotoxicity (ADCC) activity as compared to a composition comprising the same amount of the glycosylated anti-CD20 antibody in which less than 40% of the N-glycans are afucosyl glycans.

12. The composition of any one of claims 5-7, wherein said composition comprises an antibody, and wherein said antibody is an anti-EGFR antibody.

13. The composition of claim 12, wherein the light chain of said antibody comprises a sequence that is at least 90% identical to the light chain of Cetuximab.

14. The composition of claim 12 or 13, wherein the heavy chain of said antibody comprises a sequence that is at least 90% identical to the heavy chain of Cetuximab.

15. The composition of any one of claims 12-14, wherein said antibody has an increased binding affinity for an FcγRIII or increased antibody-dependent cellular cytotoxicity (ADCC) activity as compared to a composition comprising the same amount of the glycosylated anti-EGFR antibody in which less than 40% of the N-glycans are afucosyl glycans.

16. The composition of any one of claims 5-7, wherein said composition comprises an antibody, and wherein said antibody is an anti-HER2 antibody.

17. The composition of claim 16, wherein the light chain of said antibody comprises a sequence that is at least 90% identical to the light chain of Trastuzumab.

18. The composition of claim 16 or 17, wherein the heavy chain of said antibody comprises a sequence that is at least 90% identical to the heavy chain of Trastuzumab.

19. The composition of any one of claims 16-18, wherein said antibody has an increased binding affinity for an FcγRIII or increased antibody-dependent cellular cytotoxicity (ADCC) activity as compared to a composition comprising the same amount of the glycosylated anti-HER2 antibody in which less than 40% of the N-glycans are afucosyl glycans.

20. The composition of any one of claims 1-19, wherein at least about 40% of the glycans of said antibody or fusion protein do not comprise the α-Gal epitope.

21. The composition of any one of claims 1-20, wherein the glycans of said antibody or fusion protein are characterized by a total sialic acid content that contain no more than about 20% of N-glycolylneuraminic acid (Neu5Gc).

22. The composition of any one of claims 1-21, wherein one ore more of glycan molecules comprise N-acetylneuraminic acid (Neu5Ac).

23. A composition comprising a protein, wherein at least about 70% of the protein molecules comprise a glycan;

(a) wherein said protein is produced by a cell that is not a human, ape, or Old World monkey cell; and

(b) wherein at least about 40% of the glycosylated molecules do not comprise the α-Gal epitope.

24. The composition of claim 23, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated protein in which more than 40% of the glycosylated molecules comprise the α-Gal epitope.

25. The composition of claim 23 or 24, wherein said glycan is an N-glycan.

26. The composition of any one of claims 23-25, wherein said protein is an antibody.

27. The composition of claim 26 wherein said antibody is an IgG.

28. The composition of claim 27, wherein said IgG is IgG1.

29. The composition of any one of claims 26-28, wherein said antibody is an anti-CD20 antibody.

30. The composition of claim 29, wherein the light chain of said antibody comprises a sequence that is at least 90% identical to the light chain of Rituximab.

31. The composition of claim 29 or 30, wherein the heavy chain of said antibody comprises a sequence that is at least 90% identical to the heavy chain of Rituximab.

32. The composition of any one of claim 29-31, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated anti-CD20 antibody in which more than 40% of the glycosylated molecules comprise the α-Gal epitope.

33. The composition of any one of claims 26-28, wherein said antibody is an anti-EGFR antibody.

34. The composition of claim 33, wherein the light chain of said antibody comprises a sequence that is at least 90% identical to the light chain of Cetuximab.

35. The composition of claim 33 or 34, wherein the heavy chain of said antibody comprises a sequence that is at least 90% identical to the heavy chain of Cetuximab.

36. The composition of any one of claim 33-35, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount

of the glycosylated anti-EGFR antibody in which more than 40% of the glycosylated molecules comprise the α-Gal epitope.

37. The composition of any one of claims 26-28, wherein said antibody is an anti-HER2 antibody.

38. The composition of claim 37, wherein the light chain of said antibody comprises a sequence that is at least 90% identical to the light chain of Trastuzumab.

39. The composition of claim 37 or 38, wherein the heavy chain of said antibody comprises a sequence that is at least 90% identical to the heavy chain of Trastuzumab.

40. The composition of any one of claim 37-39, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated anti-HER2 antibody in which more than 40% of the glycosylated molecules comprise the α-Gal epitope.

41. The composition of any one of claims 23-25, wherein said protein is a fusion protein that binds to B7.

42. The composition of claim 41, wherein said fusion protein comprises the extracellular domain of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4).

43. The composition of claim 41 or 42, wherein said fusion protein comprises a sequence that is at least 90% identical to Abatacept.

44. The composition of any one of claim 41-43, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated fusion protein in which more than 40% of the glycosylated molecules comprise the α-Gal epitope.

45. A composition comprising a protein, wherein at least about 70% of the protein molecules comprise a glycan;

(a) wherein said protein is produced by a non-human cell; and

(b) wherein the glycans of said protein molecules are characterized by a total sialic acid content that contain no more than about 20% of N-glycolylneuraminic acid (Neu5Gc).

46. The composition of claim 45, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated protein in which the total sialic acid content of the glycans contain more than about 20% of Neu5Gc.

47. The composition of claim 45 or 46, wherein at least about 40% of the glycans do not comprise the α-Gal epitope.

48. The composition of any one of claims 45-47, wherein said glycans are N-glycans.

49. The composition of any one of claims 45-48, wherein said protein is an antibody.

50. The composition of claim 49 wherein said antibody is an IgG.

51. The composition of claim 50, wherein said IgG is IgG1.

52. The composition of any one of claims 49-51, wherein said antibody is an anti-CD20 antibody.

53. The composition of claim 52, wherein the light chain of said antibody comprises a sequence that is at least 90% identical to the light chain of Rituximab.

54. The composition of claim 52 or 53, wherein the heavy chain of said antibody comprises a sequence that is at least 90% identical to the heavy chain of Rituximab.

55. The composition of any one of claim 52-54, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated anti-CD20 antibody in which the total sialic acid content of the glycans contain more than about 20% of Neu5Gc.

56. The composition of any one of claims 49-51, wherein said antibody is an anti-EGFR antibody.

57. The composition of claim 56, wherein the light chain of said antibody comprises a sequence that is at least 90% identical to the light chain of Cetuximab.

58. The composition of claim 56 or 57, wherein the heavy chain of said antibody comprises a sequence that is at least 90% identical to the heavy chain of Cetuximab.

59. The composition of any one of claim 56-58, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated anti-EGFR antibody in which the total sialic acid content of the glycans contain more than about 20% of Neu5Gc.

60. The composition of any one of claims 49-51, wherein said antibody is an anti-HER2 antibody.

61. The composition of claim 60, wherein the light chain of said antibody comprises a sequence that is at least 90% identical to the light chain of Trastuzumab.

62. The composition of claim 60 or 61, wherein the heavy chain of said antibody comprises a sequence that is at least 90% identical to the heavy chain of Trastuzumab.

63. The composition of any one of claim 60-62, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount of the glycosylated anti-HER2 antibody in which the total sialic acid content of the glycans contain more than about 20% of Neu5Gc.

64. The composition of any one of claims 45-48, wherein said protein is a fusion protein that binds to B7.

65. The composition of claim 64, wherein said fusion protein comprises the extracellular domain of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4).

66. The composition of claim 64 or 65, wherein said fusion protein comprises a sequence that is at least 90% identical to Abatacept.

67. The composition of any one of claim 64-66, wherein said composition has reduced immunogenicity in human, as compared to a composition comprising the same amount

of the glycosylated fusion protein in which the total sialic acid content of the glycans contain more than about 20% of Neu5Gc.

68. The composition of any one of claims 45-67, wherein one or more of said glycan molecules comprise N-acetylneuraminic acid (Neu5Ac).

69. The composition of any one of claims 45-68, wherein said protein is an antibody or a fusion protein that comprises the Fc domain of an antibody, and wherein said glycan is an N-linked complex glycan.

70. The composition of claim 69, wherein about 40% to about 100% of the complex N-glycans are afucosyl glycans.

71. A method for producing a composition comprising an afucosylated glycoprotein, comprising:

(a) culturing a host cell in a large scale cell culture, wherein

i said host cell expresses the glycoprotein; and

ii said host cell comprises a target gene that encodes a protein that is selected from the group consisting of: GDP-fucose transporter (GFT), solute carrier-35C1 (SLC35C1), and solute carrier-35C2 (SLC35C2); and

(b) adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said target gene, and reduces or prevents the expression of said target gene.

72. The method of claim 71, wherein said host cell further comprises a target gene that encodes a protein that is selected from the group consisting of: GDP-mannose 4,6-dehydratase (GMD), GDP-4-keto-6-deoxy-D-mannose epimerase-reductase (FX), and Fucosyltransferase (Fut); and wherein step (b) further comprises:

adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to a target gene

that encodes a protein that is selected from the group consisting of: GDP-mannose 4,6-dehydratase (GMD), GDP-4-keto-6-deoxy-D-mannose epimerase-reductase (FX), and Fucosyltransferase (Fut), and wherein said RNA effector reduces or prevents the expression of said target gene.

73. A method for producing a composition comprising an afucosylated glycoprotein, comprising:

(a) culturing a host cell in a large scale cell culture, wherein

i said host cell expresses the glycoprotein; and

ii said host cell comprises at least two target genes that independently encode a protein that is selected from the group consisting of: GDP-mannose 4,6-dehydratase (GMD), GDP-4-keto-6-deoxy-D-mannose epimerase-reductase (FX), and Fucosyltransferase (Fut); and

(b) adding an effective amount of two or more RNA effector molecules to said large scale cell culture, wherein each of said RNA effector is substantially complementary to a target gene of (a), and reduces or prevents the expression of its target gene.

74. The method of any one of claims 71-73, wherein said RNA effector transiently reduces the expression of its target gene.

75. The method of any one of claims 71-74, further comprising harvesting said glycoprotein from said large scale culture.

76. The method of any one of claims 71-75, wherein said glycoprotein is an antibody or a fusion protein that comprises the Fc domain of an antibody.

77. The method of any one of claims 72-76, wherein at least one of the target genes encodes a fucosyltransferase.

78. The method of any one of claims 72-76, wherein the target gene is selected from the group consisting of: Fut8, GMD, and TSTA3.

79. The method of any one of claims 71-78, wherein said RNA effector molecule is an siRNA.

80. The method of any one of claims 71-78, wherein said RNA effector molecule is a shRNA.

81. The method of any one of claims 71-78, wherein said RNA effector molecule is an antisense molecule.

82. The method of any one of claims 71-78, wherein said RNA effector molecule comprises a sequence selected from the group consisting of SEQ ID NOs: 23-2358.

83. A method for producing a composition comprising a glycoprotein, comprising:

(a) culturing a host cell in a large scale cell culture, wherein

i said host cell expresses the glycoprotein; and

ii said host cell comprises a target gene that encodes an α1,3 galactosyltransferase; and

(b) adding an effective amount an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said target gene, and reduces or prevents the expression of the target gene.

84. The method of claim 83, wherein said glycoprotein has reduced immunogenicity in a human when compared to the same glycoprotein produced in the absence of said RNA effector molecule.

85. A method for producing a composition comprising a glycoprotein, comprising:

(a) culturing a host cell in a large scale cell culture, with the proviso that said host cell is not a human, ape, or Old World monkey cell, wherein

i said host cell expresses the glycoprotein; and

ii said host cell comprises a target gene that encodes a protein selected from the group consisting of ABO α1,3 galactosyltransferase and Ggta1; and

(b) adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said target gene, and reduces or prevents the expression of said target gene.

86. The method of any one of claims 83-85, wherein said RNA effector transiently reduces the expression of its target gene.

87. The method of any one of claims 83-86, further comprising harvesting said glycoprotein from said large scale culture.

88. The method of any one of claims 83-87, wherein said RNA effector molecule is an siRNA.

89. The method of any one of claims 83-87, wherein said RNA effector molecule is a shRNA.

90. The method of any one of claims 83-87, wherein said RNA effector molecule is an antisense molecule.

91. The method of any one of claims 83-87, wherein said RNA effector molecule comprises a sequence selected from the group consisting of SEQ ID NOs: 23-2358.

92. A method for producing a composition comprising a glycoprotein, comprising:

(a) culturing a host cell in a large scale cell culture, with the proviso that said host cell is not a human cell, wherein:

i said host cell expresses the glycoprotein; and

ii said host cell comprises a target gene that encodes CMP-N-acetylneuraminic acid hydroxylase (CMAH); and

(b) adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said gene, and reduces or prevents the expression of said target gene.

93. The method of claim 92, wherein said RNA effector transiently reduces the expression of its target gene.

94. The method of claim 92 or 93, further comprising harvesting said glycoprotein from said large scale culture.

95. The method of any one of claims 71-92, wherein said host cell further comprises a target gene that encodes a sialidase, and wherein step (b) further comprises: adding an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said gene, and reduces or prevents the expression of said target gene.

96. The method of claim 93, wherein said sialidase is NEU2 sialidase or α-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6 (ST6GALNAC6).

97. A method for producing a composition comprising a glycoprotein, comprising:

(a) culturing a host cell in a large scale cell culture, with the proviso that said host cell is not a human cell, wherein:

i said host cell expresses the glycoprotein; and

ii said host cell comprises a target gene that encodes a sialidase; and

(b) adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to said gene, and reduces or prevents the expression of said target gene.

98. The method of claim 97, wherein said sialidase is NEU2 sialidase or α-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide α-2,6-sialyltransferase 6 (ST6GALNAC6).

99. The method of claim 97 or 98, wherein said RNA effector transiently reduces the expression of its target gene.

100. The method of claim 97 or 98, further comprising harvesting said glycoprotein from said large scale culture.

101. The method of any one of claim 92-100, wherein said RNA effector molecule is an siRNA.

102. The method of any one of claim 92-100, wherein said RNA effector molecule is a shRNA.

103. The method of any one of claim 92-100, wherein said RNA effector molecule is an antisense molecule.

104. The method of any one of claim 92-100, wherein said RNA effector molecule comprises a sequence selected from the group consisting of SEQ ID NOs: 23-2358.

105. A method for producing a composition comprising a glycoprotein, comprising:

(a) culturing a host cell in a large scale cell culture, wherein

i said host cell expresses the glycoprotein; and

ii said host cell comprises a target gene that encodes a protein that is selected from the group consisting of: GDP-fucose transporter (GFT), solute carrier-35C1 (SLC35C1), solute carrier-35C2 (SLC35C2), MPDU1, and Ggta1; and

(b) adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to a target gene of (a), and reduces or prevents the expression of said target gene.

106. The method of claim 105, wherein said host cell further comprises a target gene that encodes a protein that is selected from the group consisting of: dolichyl-diphosphooligosaccharide-protein glycosyltransferase, UDP glycosyltransferase, UDP-Gal:βGlcNAcβ1,4-galactosyltransferase, UDP-galactose-ceramide galactosyltransferase, fucosyltransferase, protein O-fucosyltransferase, N-acetylgalactosaminyltransferase, O-GlcNAc transferase, oligosaccharyl transferase, O-linked N-acetylgrucosamine transferase, α-galactosidase, β-galactosidase, sialyltransferase, GMD dehydratase, FX epimerase, α-1,3-galactosyltransferase, mannosyl (α-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT1), MGAT4B, SLC35D1, ST6GALNAC6, and glucosamine (UDP-N-acetyl)-2-epimerase; and wherein step (b) further comprises:

adding an effective amount of an RNA effector molecule to said large scale cell culture, wherein said RNA effector is substantially complementary to a target gene that encodes a protein that is selected from the group consisting of: dolichyl-diphosphooligosaccharide-protein glycosyltransferase, UDP glycosyltransferase, UDP-Gal:βGlcNAcβ1,4-galactosyltransferase, UDP-galactose-ceramide galactosyltransferase, fucosyltransferase, protein O-fucosyltransferase, N-acetylgalactosaminyltransferase, O-GlcNAc transferase, oligosaccharyl transferase, O-linked N-acetylgrucosamine transferase, α-galactosidase, β-galactosidase, sialyltransferase, GMD dehydratase, FX epimerase, α-1,3-galactosyltransferase, mannosyl (α-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT1), MGAT4B, SLC35D1, ST6GALNAC6, and glucosamine (UDP-N-acetyl)-2-epimerase, and wherein said RNA effector reduces or prevents the expression of said target gene.

107. A method for producing a composition comprising a glycoprotein, comprising:

(a) culturing a host cell in a large scale cell culture, and wherein

i said host cell expresses the glycoprotein; and

ii said host cell comprises at least two target genes that independently encode a protein that is selected from the group consisting of: dolichyl-diphosphooligosaccharide-protein glycosyltransferase, UDP glycosyltransferase, UDP-Gal:βGlcNAcβ1,4-galactosyltransferase, UDP-

galactose-ceramide galactosyltransferase, fucosyltransferase, protein O-fucosyltransferase, N-acetylgalactosaminyltransferase, O-GlcNAc transferase, oligosaccharyl transferase, O-linked N-acetylgrucosamine transferase, α-galactosidase, β-galactosidase, sialyltransferase, GMD dehydratase, FX epimerase, α-1,3-galactosyltransferase, mannosyl (α-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase (MGAT1), MGAT4B, SLC35D1, ST6GALNAC6, and glucosamine (UDP-N-acetyl)-2-epimerase; and

(b) adding an effective amount of two or more RNA effector molecules to said large scale cell culture, wherein each of said RNA effector is substantially complementary to a target gene of (a), and reduces or prevents the expression of its target gene.

108. The method of any one of claims 105-107, wherein the target gene is selected from the group consisting of: FUT8, GMDS, TSTA3, ABO, CMAH, MGAT1, MGAT4B, SLC35D1, TSTA3, SLC35C1, SLC35C2, NEU2, and ST6GALNAC6.

109. The method of any one of claims 105-108, wherein said RNA effector transiently reduces the expression of its target gene.

110. The method of any one of claims 105-109, further comprising harvesting said glycoprotein from said large scale culture.

111. The method of any one of claims 71-110, wherein in step (b), the RNA effector, or at least one of the RNA effectors is added to the large scale cell culture two or more times before harvesting the glycoprotein.

112. The method of any one of claims 72-78, 95-96, and 106-111, wherein at least two of the RNA effectors are added simultaneously into the cell culture.

113. The method of any one of claims 72-78, 95-96, and 106-111, wherein at least two of the RNA effectors are added at different times into the cell culture.

114. The method of any one of claims 71-113, wherein step (b) comprises cultivating the cell culture for at least about 6 hours after the addition of the RNA effectors.

115. The method of any one of claims 71-114, wherein the expression level(s) of the target gene(s) is (are) reduced from about 10% to about 85%.

116. The method of any one of claims 71-78, 83-87, 92-100, and 105-115, wherein the RNA effector, or at least one of the RNA effectors comprises a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA:

(a) comprises a sense strand and an antisense strand that are substantially complementary to each other; and

(b) wherein said antisense strand comprises a region of complementarity that is substantially complementary to one of the target genes, and wherein said region of complementarity is from 10 to 30 nucleotides in length.

117. The method of any one of claims 71-78, 83-87, 92-100, and 105-116, wherein the RNA effector, or at least one of the RNA effectors is an siRNA.

118. The method of claim 117, wherein the siRNA is from 15 to 30 nucleotides in length.

119. The method of claim 117 or 118, wherein the siRNA is from 17 to 28 nucleotides in length.

120. The method of any one of claims 117-119, wherein the siRNA is from 19 to 23 nucleotides in length.

121. The method of any one of claims 71-120, wherein the RNA effector, or at least one of the RNA effectors is formulated with a lipid.

122. The method of any one of claims 71-121, wherein the RNA effector, or at least one of the RNA effectors comprises a modified nucleotide.

123. The method of any one of claims 71-122 wherein said host cell is a mammalian cell.

124. The method of claim 123, wherein the mammalian cell is a rodent cell selected from the group consisting of: BHK21 cell, BHK(TK-) cell, NS0 cell, Sp2/0 cell, EL4 cell, CHO cell, CHO-K1 cell, CHO-DUKX cell, CHO-DUKX B1 cell, CHO-DG44 cell, NIH/3T3 cell, 3T3-L1 cell, ES-D3 cell, H9c2 cell, C2C12 cell, YB2/0 and miMCD 3 cell.

125. The method of claim 71-84 and 97-124, wherein the host cell is a human cell.

126. The method of claim 125, wherein the human cell is selected from the group consisting of: SH-SY5Y cell, IMR32 cell, LAN5 cell, HeLa cell, MCFlOA cell, 293T cell, SK-BR3 cell, HuVEC cell, HuASMC cell, HKB-Il cell, hMSC cell, U293 cell, HEK 293 cell, PERC6® cell, Jurkat cell, HT-29 cell, LNCaP.FGC cell, A549 cell, MDA MB453 cell, HepG2 cell, THP-I cell, MCF7 cell, BxPC-3 cell, Capan-1 cell, DU145 cell, and PC-3 cell.

127. The method of claim 123, wherein the cell is selected from the group consisting of: PERC6 cell, HT-29 cell, LNCaP-FGC cell A549 cell, MDA MB453 cell, HepG2 cell, THP-1 cell, miMCD-3 cell, HEK 293 cell, HeLaS3 cell, MCF7 cell, Cos-7 cell, BxPC-3 cell, DU145 cell, Jurkat cell, PC-3 cell, EB66 and Capan-1 cell.

128. A method for producing a glycoprotein, comprising:

(a) culturing a host cell that expresses said glycoprotein in a large scale cell culture, wherein said host cell expresses target genes that are necessary for the glycosylation of said glycoprotein with two or more of N-glycolylneuraminic acid, fucose and galactose-α-1,3-galactose;

(b) adding two or more RNA effector molecules to said large scale cell culture, wherein each of said RNA effectors are substantially complementary to said target genes, and cultivating the large scale culture for a sufficient period of time such that the expression of the target genes are reduced or inhibited in said host cell, thereby producing a glycoprotein with reductions in two or more of N-glycolylneuraminic acid, fucose and galactose-α-1,3-galactose as compared to a glycoprotein expressed under the same conditions but in the absence of said two or more RNA effector molecules.

129. The method of claim 128, further comprising harvesting said glycoprotein from said large scale cell culture.

130. A composition comprising a glycoprotein produced according to any one of claims 71-129.

131. A cell comprising an RNA effector molecule substantially complementary to a target gene encoding a protein selected from the group consisting of: GDP-mannose 4,6-dehydratase (GMD), GDP-4-keto-6-deoxy-D-mannose epimerase-reductase (FX), GDP-fucose transporter (GFT), Fucosyltransferase (Fut), solute carrier-35C1 (SLC35C1), and solute carrier-35C2 (SLC35C2).

132. An RNA effector molecule substantially complementary to a target gene encoding a protein selected from the group consisting of: GDP-mannose 4,6-dehydratase (GMD), GDP-4-keto-6-deoxy-D-mannose epimerase-reductase (FX), GDP-fucose transporter (GFT), Fucosyltransferase (Fut), solute carrier-35C1 (SLC35C1), and solute carrier-35C2 (SLC35C2).

133. The RNA effector molecule of claim 132, wherein said RNA effector molecule is an siRNA.

134. The RNA effector molecule of claim 132, wherein said RNA effector molecule is a shRNA.

135. The RNA effector molecule of claim 132, wherein said RNA effector molecule is an antisense molecule.

136. The RNA effector molecule of claim 132, wherein said RNA effector molecule comprises a sequence selected from the group consisting of SEQ ID NO: 18-1553.

137. A cell comprising an RNA effector molecule substantially complementary to a target gene encoding ABO α-1,3 galactosyltransferase.

138. An RNA effector molecule substantially complementary to a target gene encoding ABO α-1,3 galactosyltransferase or Ggta1.

139. The RNAi effector agent of claim 138, wherein the RNAi effector agent comprises a sequence selected from the group consisting of: SEQ ID NO: 18-1553.

140. An RNA effector molecule substantially complementary to a target gene encoding CMP-N-acetylneuraminic acid hydroxylase (CMAH).

141. The RNA effector molecule of claim 140, wherein the RNAi effector agent comprises a sequence selected from the group consisting of: SEQ ID NO: 18-1553.

142. A cell comprising an RNA effector molecule substantially complementary to a target gene encoding CMP-N-acetylneuraminic acid hydroxylase (CMAH).

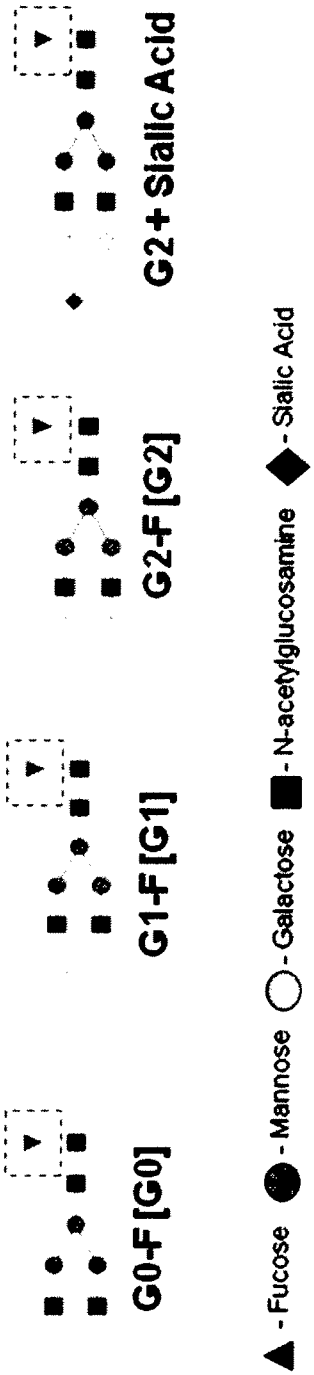

G0-F [G0]
G1-F [G1]
G2-F [G2]
G2+ Sialic Acid
- Fucose
- Mannose
- Galactose
- N-acetylglucosamine
- Sialic Acid

Figure 1

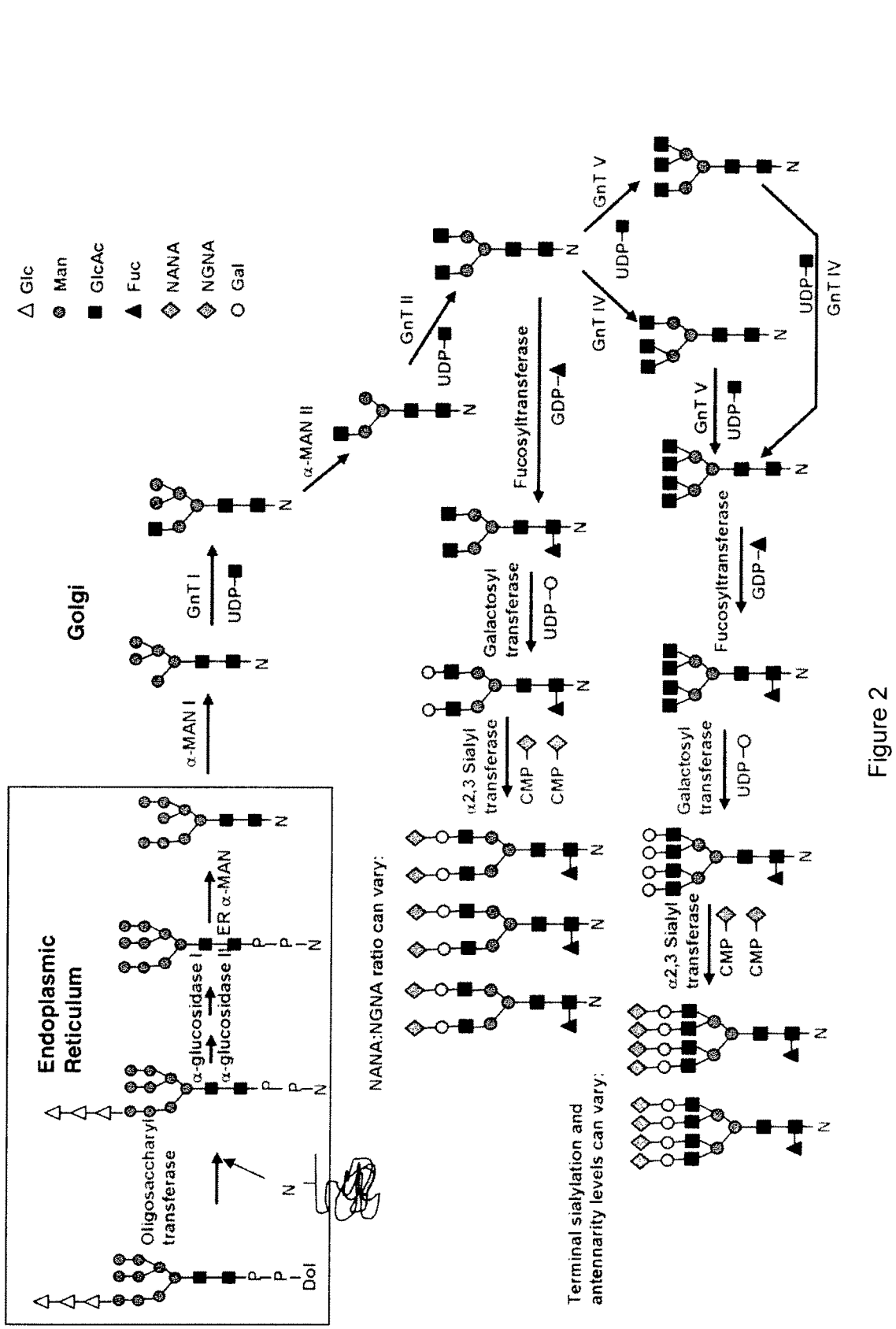


Figure 2

Hossler et al., Glycobiology vol. 19 no. 9 pp. 936–949, 2009

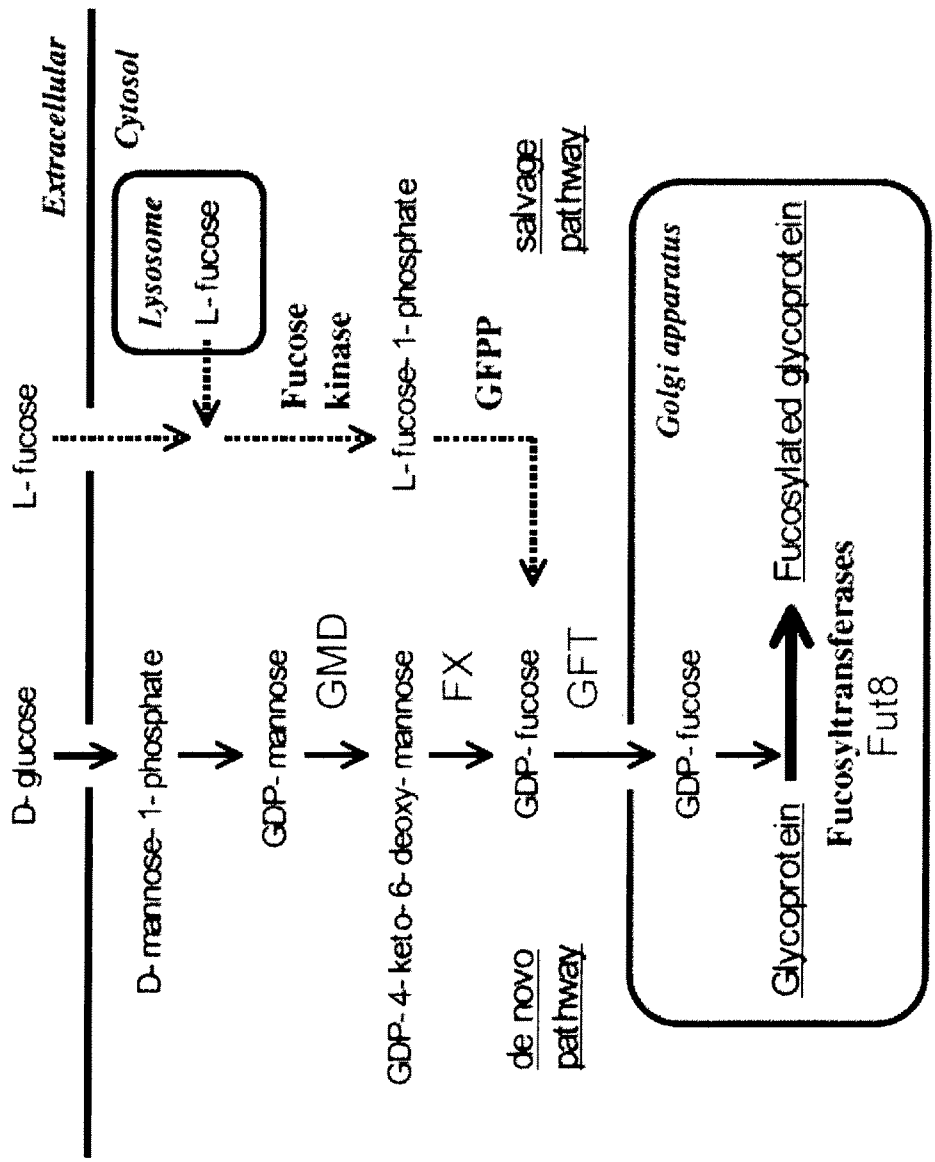

Extracellular
Cytosol
Lysosome
L-fucose
L-fucose
D-glucose
D-mannose-1-phosphate
GDP-mannose
GMD
GDP-4-keto-6-deoxy-mannose
FX
GDP-fucose
GFT
de novo pathway
Fucose kinase
L-fucose-1-phosphate
GFPP
salvage pathway
Golgi apparatus
GDP-fucose
Glycoprotein
Fucosyltransferases
Fut8
Fucosylated glycoprotein

Figure 3

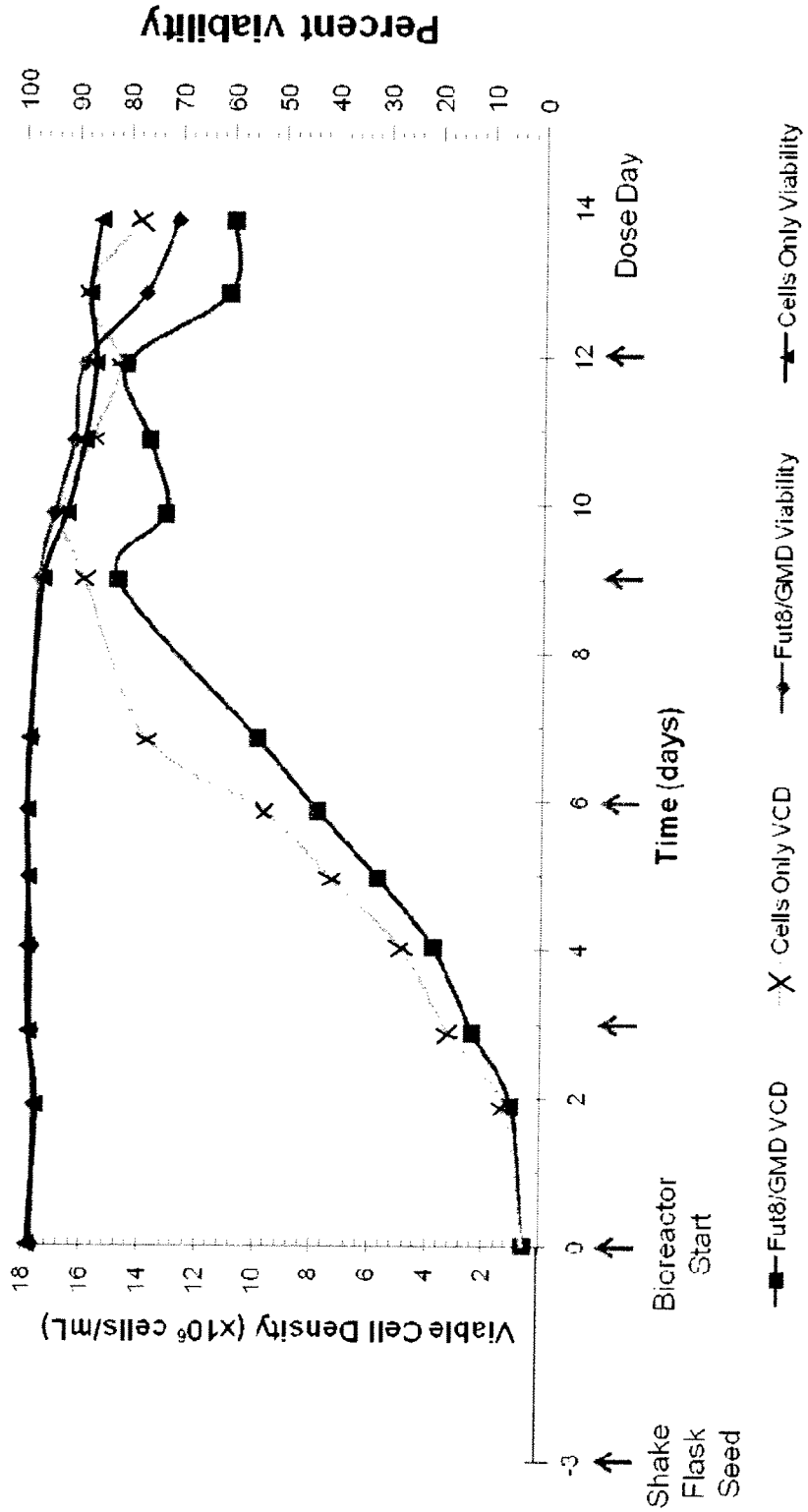

Percent viability
100
90
80
70
60
50
40
30
20
10
0
Viable Cell Density (x10⁶ cells/mL)
18
16
14
12
10
8
6
4
2
0
-3
Shake Flask Seed
Bicreactor Start
2
4
6
8
10
12
14
Dose Day
Time (days)
Fut8/GMD VCD
Cells Only VCD
Fut8/GMD Viability
Cells Only Viability

Figure 4

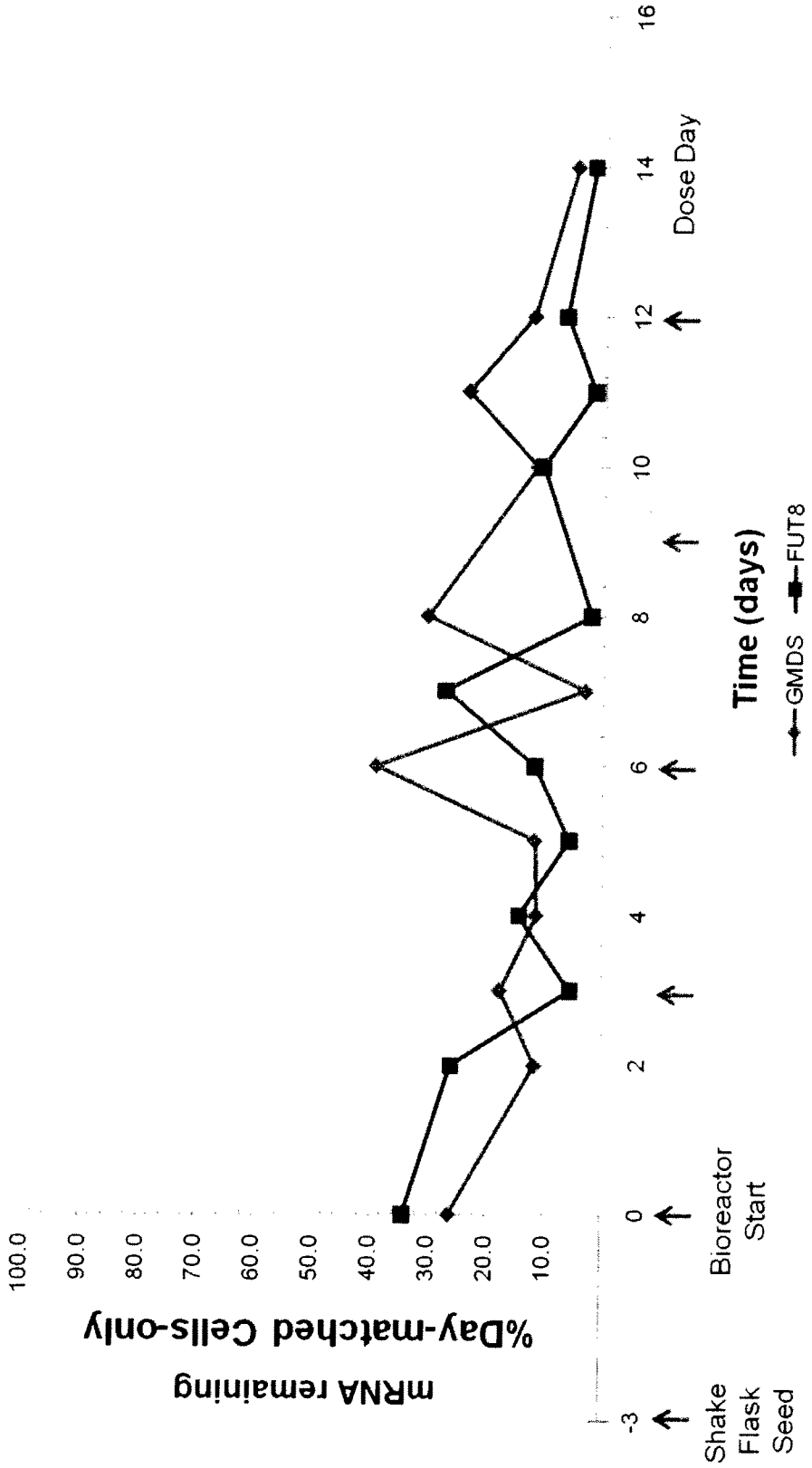

mRNA remaining
%Day-matched Cells-only
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
-3
0
2
4
6
8
10
12
14
16
Shake Flask Seed
Bioreactor Start
Dose Day
Time (days)
GMDS
FUT8

Figure 5

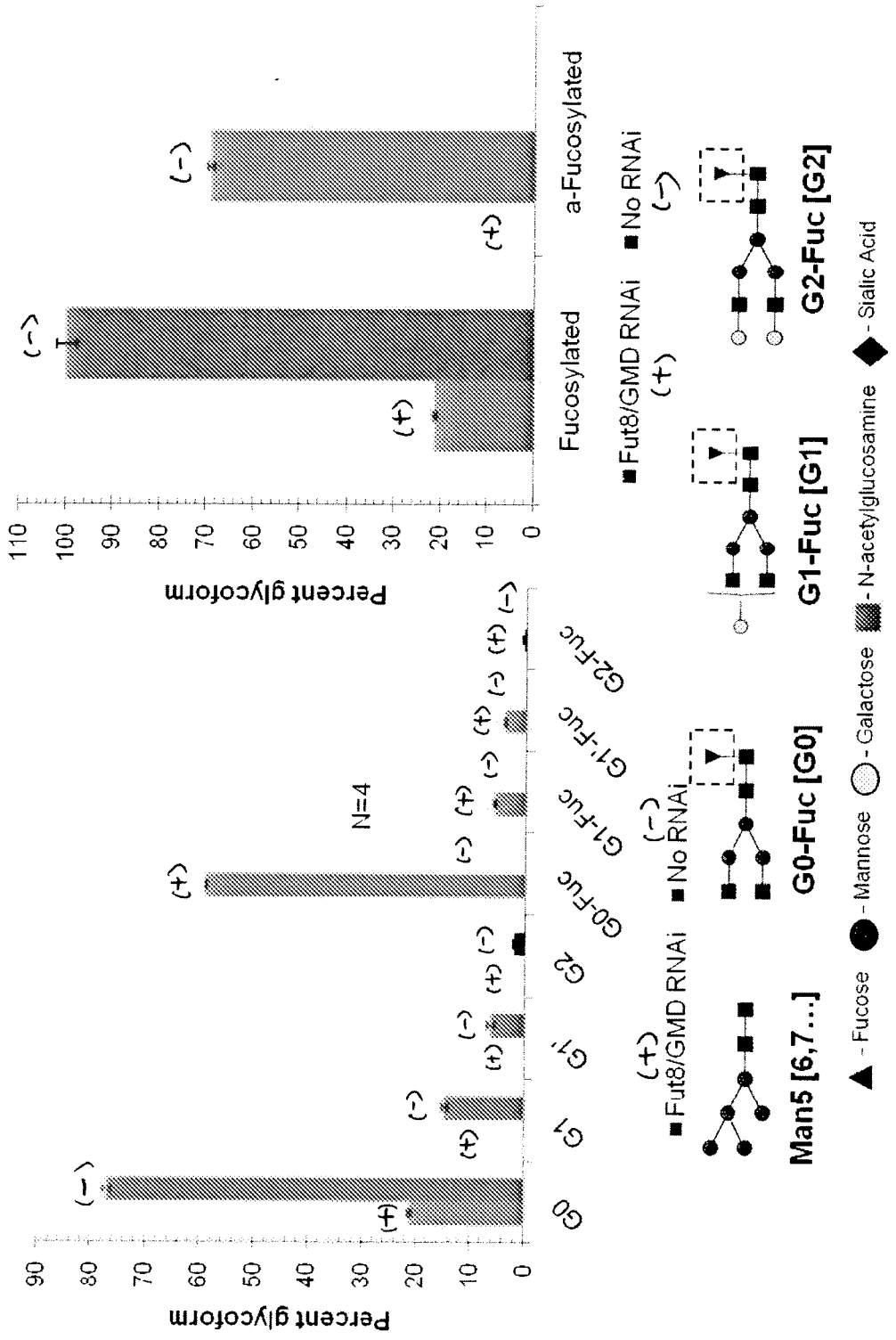

Percent glycoform
Fucosylated
a-Fucosylated
Fut8/GMD RNAi
No RNAi
Man5 [6,7...]
G0-Fuc [G0]
G1-Fuc [G1]
G2-Fuc [G2]
N=4
G0
G1
G1'
G2
G0-Fuc
G1-Fuc
G1'-Fuc
G2-Fuc
Fucose
Mannose
Galactose
N-acetylglucosamine
Sialic Acid

Figure 6

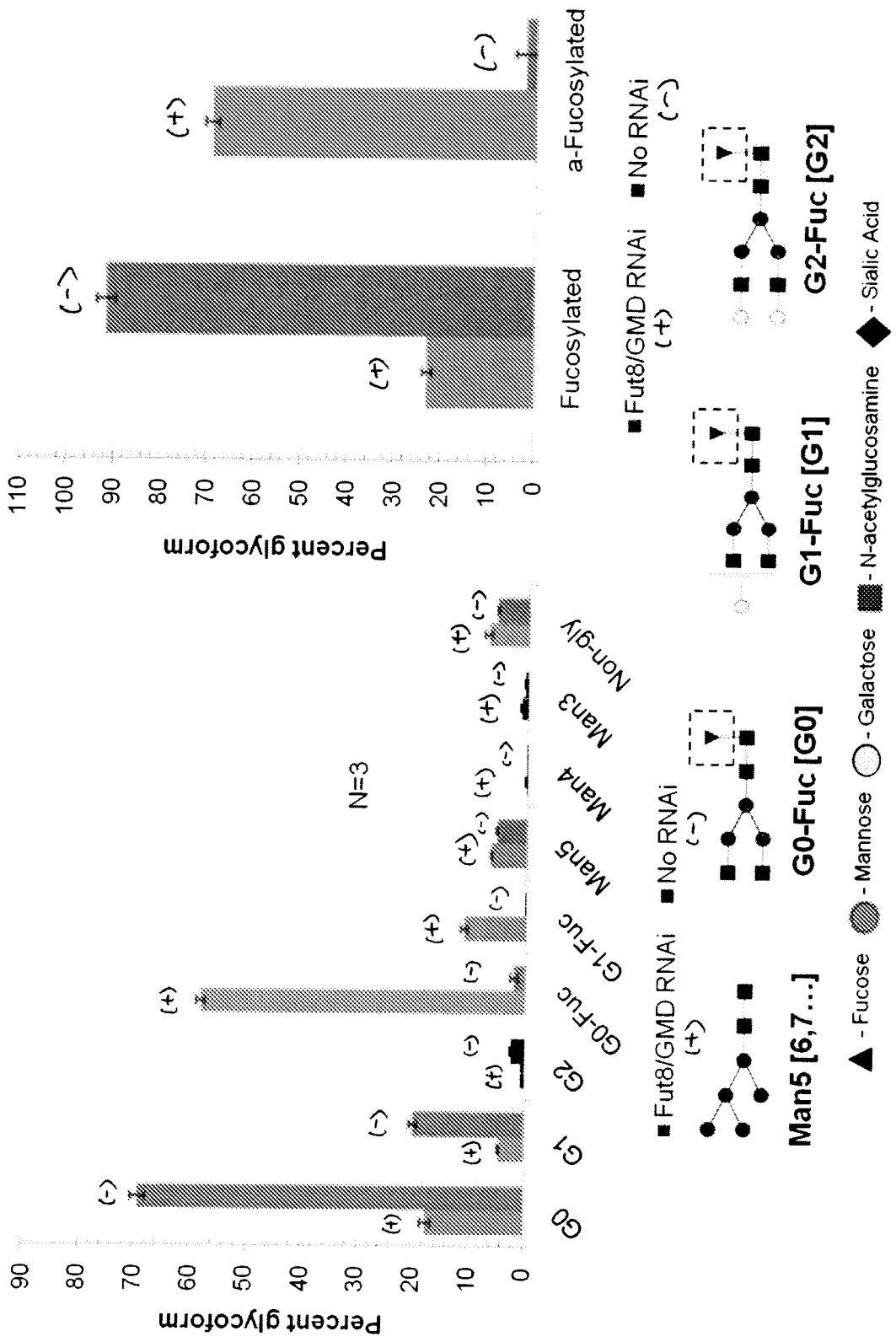

Percent glycoform
Fucosylated
a-Fucosylated
Fut8/GMD RNAi
No RNAi
N=3
G0
G1
G2
G0-Fuc
G1-Fuc
Man5
Man4
Man3
Non-gly
Man5 [6,7...]
G0-Fuc [G0]
G1-Fuc [G1]
G2-Fuc [G2]
- Fucose
- Mannose
- Galactose
- N-acetylglucosamine
- Sialic Acid

Figure 7

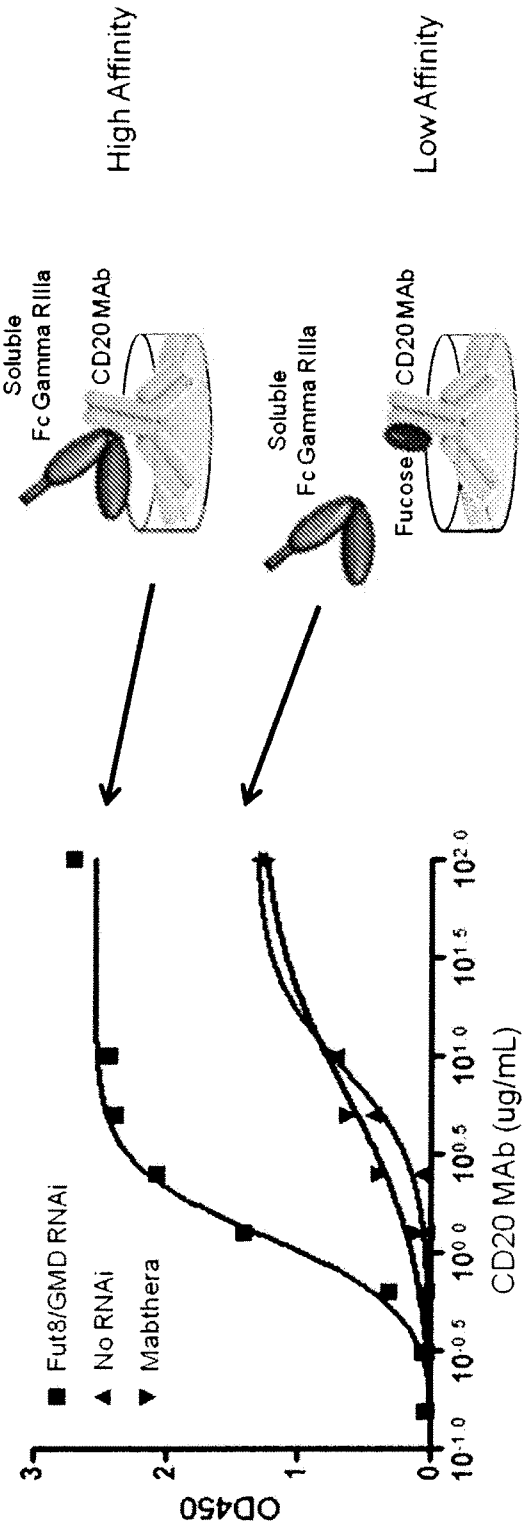

High Affinity
Low Affinity
Soluble
Fc Gamma RIIIa
CD20 MAb
Soluble
Fc Gamma RIIIa
Fucose
CD20 MAb
Fut8/GMD RNAi
No RNAi
Mabthera
OD450
0
1
2
3
10-1 0
10-0 5
100 0
100.5
101 0
101.5
102 0
CD20 MAb (ug/mL)

Figure 8

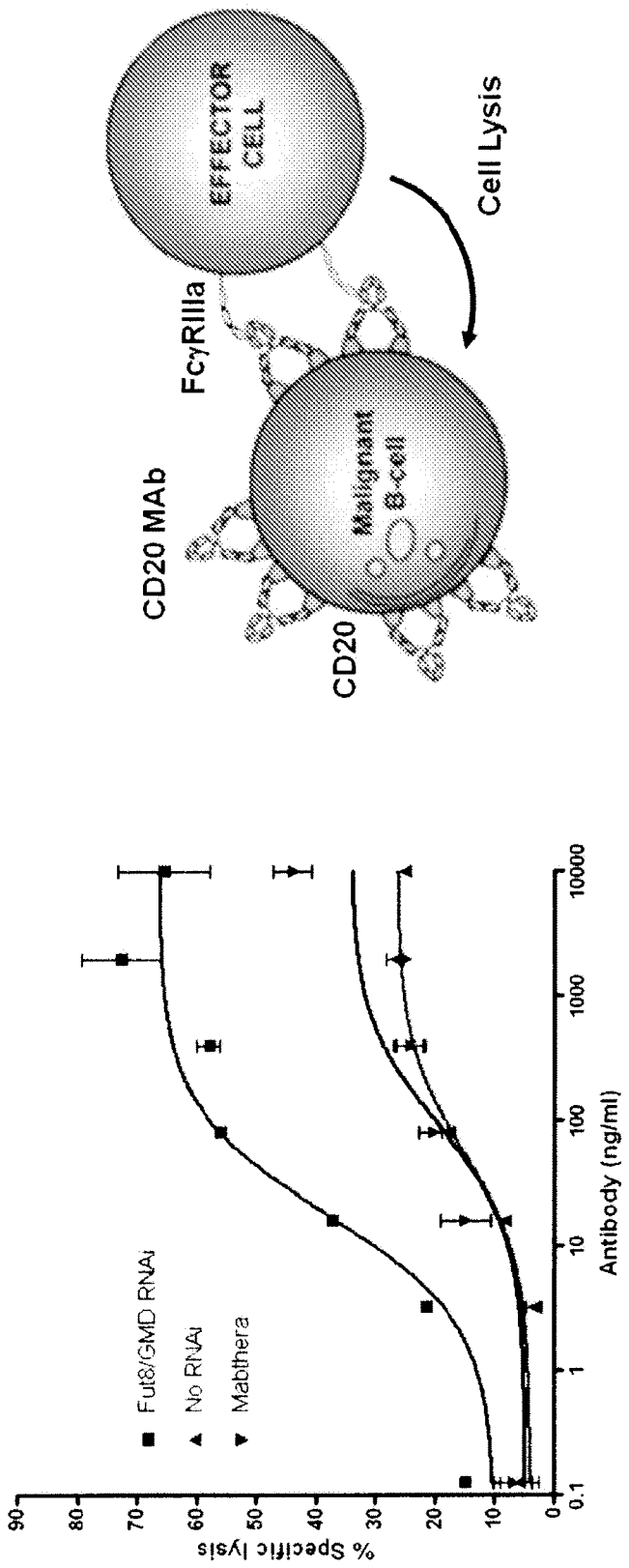

% Specific lysis
Antibody (ng/ml)
Fut8/GMD RNAi
No RNAi
Mabthera
EFFECTOR CELL
FcγRIIIa
CD20 MAb
CD20
Malignant B-cell
Cell Lysis

Figure 9