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[Continued on next page]

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

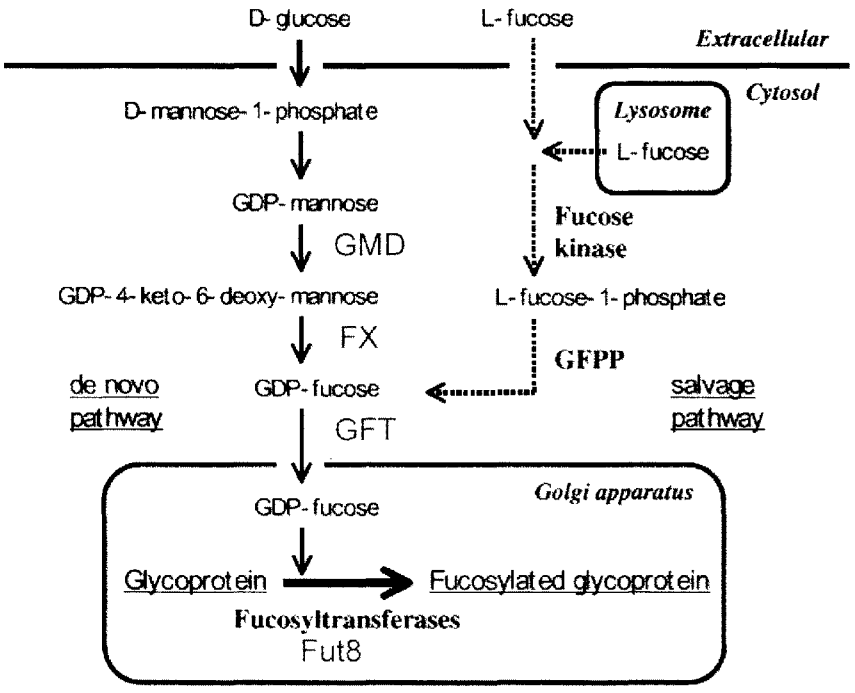

Figure 3

(57) **Abstract**: The invention generally relates to compositions and methods for producing glycoproteins that have alter glycan structure and improved properties. Tbe 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 tbe methods described herein have improved properties, such as improved effector activity, improved pharmacokinetic properties, reduced immunogenicity in humans and the like.

WO 2013/013013 A3

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

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395 (2013.01)
USPC - 424/137.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 39/395; C07K 16/00, 16/46, 19/00 (2013.01)
USPC - 424/133.1, 137.1; 530/387.1, 387.3, 388.1, 388.15

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

CPC - A61K 39/395, 39/39558; C07K 16/00, 16/283, 16/30

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, PubMed, Google Scholar, Google

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2011/0086050 A1 (PRESTA) 14 April 2011 (14.04.2011) entire document	1 --- 2
Y	US 2011/0053223 A1 (BAYER et al) 03 March 2011 (03.03.2011) entire document	2
Y	US 2010/0304436 A1 (CHEN et al) 02 December 2010 (02.12.2010) entire document	2
A	SZABO et al. 'Rapid high resolution characterization of functionally important monoclonal antibody n-glycans by capillary electrophoresis.' Anal Chem. 2011 July 1; 83(13): 5329–5336. Article retrieved from the internet. Retrieved on [07.01.2013]. entire document	1-3

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
08 January 2013	25 JAN 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/047346

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

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

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

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

3. ☒ Claims Nos.: 4-22, 26-44, 48-70, 75-82, 87-91, 95, 101-104, 109-127, 130
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

See Extra Sheet

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

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

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/047346

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claims 1-3 are drawn to a composition comprising an antibody or a fusion protein that comprises the Fe 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.

Group II: claims 23-25 are drawn to 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 alpha-Gal epitope.

Group III: claims 45-47 are drawn to 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).

Group IV: claims 71-74, 105, 106, 131-136 and 107, 108, 128, 129 (in part) are drawn to 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.

Group V: claims 83-86, 137, 138 and 107, 108, 128, 129 (in part) are drawn to a method tor 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 alpha-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.

Group VI: claims 92-94, 96, 140-142 and 107, 108, 128, 129 (in part) are drawn to 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 (i) said host cell is not a human cell, wherein: 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.

Group VII: claims 97-100 and 107, 108 (in part) are drawn to 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.

The inventions listed in Groups I-VII do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Group I, a composition comprising an antibody or a fusion protein that comprises wherein about 40% to about 100% afucosyl glycans, are not present in Groups II-VII; the special technical feature of Group II, a composition comprising a protein wherein at least about 40% of the glycosylated molecules do not comprise the alpha-Gal epitope, is not present in Groups I and III-VII; the special technical feature of Group III, a composition comprising a protein 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), are not present in groups I, III, and IV-VII; the special technical feature of Group IV, a method for producing a composition comprising an afucosylated glycoprotein and a host cell comprising 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), is not present in Groups I-III and V-VII; the special technical feature of Group V, a method tor producing a composition comprising a glycoprotein and a said host cell comprises a target gene that encodes an alpha-1, 3-galactosyltransferase, is not present in Groups I-IV, VI, and VII; the special technical feature of Group VI, a method for producing a composition comprising a glycoprotein, comprising a host cell comprising a target gene that encodes CMP-N-acetylneuraminic acid hydroxylase (CMAH), is not found in Groups I-V and VII; the special technical feature of Group VII, a method for producing a composition comprising a glycoprotein and a host cell wherein said host cell comprises a target gene that encodes a sialidase, is not present in Groups I-VI.

Further, Groups I-VII share the technical features of a composition comprising a protein, wherein at least about 70% of the protein molecules comprise a glycan, wherein said protein is produced by a cell that is not a human, ape, or Old World monkey cell; and a method for producing a glycoprotein comprising 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 are necessary for the glycosylation of said glycoprotein; 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.

However, these shared technical features do not represent a contribution over the prior art. Specifically, US 7,884,264 B2 to Dickey et al. disclose a composition comprising a protein, wherein at least about 70% of the protein molecules comprise a glycan (the production of a monoclonal antibody composition, wherein at least 90% or more of the intact antibody is represented by a single glycoform, Col. 3, Lns. 28-31), wherein said protein is produced by a cell that is not a human, ape, or Old World monkey cell (a glycoprotein produced in the transgenic plant, Col. 47, Lns. 62-63); and a method for producing a glycoprotein comprising culturing a host cell in a large scale cell culture (methods for altering the N-glycosylation pattern of homologous and heterologous polypeptides produced in a plant, particularly a plant that serves as an expression system for recombinant proteins of interest, Col. 10, Lns. 59-63), wherein (i) said host cell expresses the glycoprotein (a glycoprotein produced in the transgenic plant, Col. 47, Lns. 62-63); and (ii) said host cell comprises a target gene that encodes a protein that are necessary for the glycosylation of said glycoprotein (compositions comprising novel isolated polynucleotides and polypeptides encoding a Lemna minor alpha-1,3-fucosyltrausferase and beta-1,2-xylosyltransferase; recombinant nucleotide constructs that target expression of these two proteins, or expression of variants thereof, are also provided, as are plant cells, plant tissues, plants, and seeds comprising these recombinant constructs, Col. 3, Lns. 56-58; and (b) adding an effective amount an RNA effector molecule to said large scale cell culture (FucT or XylT inhibitory sequence can he a chemically synthesized or in vitro-produced small interfering RNA (siRNA) or microRNA (miRNA) that, when introduced into the host cell would directly, though transiently, inhibit FucT, XylT, or a combination thereof, by silencing expression of these target gene product(s), Col. 27, Lns. 59-65), wherein said RNA effector is substantially complementary to said target gene, and reduces or prevents the expression of the target gene (for antisense suppression, the expression cassette is designed to express an RNA molecule complementary to all or part of a messenger RNA encoding the FucT or XylT (target genes), Col. 31, Lns. 14-17; methods comprise the use of nucleotide constructs comprising one or more sequences that are capable of inhibiting expression of alpha-1,3-fucosyltransferase (FucT) and beta-1,2-xylosyltransferase (XylT) in a plant, Col. 10, Lns. 63-66; the phrase "capable of inhibiting" is used in the context of a polynucleotide inhibitory sequence, it is intended to mean that the inhibitory sequence itself exerts the inhibitory effect; or, where the inhibitory sequence encodes an inhibitory nucleotide molecule, for example hairpin RNA, miRNA, or double stranded RNA polynucleotides, Col. 18, Lns. 2-8).

The inventions listed in Groups I-VII therefore lack unity under Rule 13 because Groups I-VII do not share a same or corresponding special technical feature.