MABS
2018, VOL. 10, NO. 5, 693–711
https://doi.org/10.1080/19420862.2018.1466767

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REVIEW

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The “less-is-more” in therapeutic antibodies: Afucosylated anti-cancer antibodies with enhanced antibody-dependent cellular cytotoxicity

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ABSTRACT
Therapeutic monoclonal antibodies are the fastest growing class of biological therapeutics for the treatment of various cancers and inflammatory disorders. In cancer immunotherapy, some IgG1 antibodies rely on the Fc-mediated immune effector function, antibody-dependent cellular cytotoxicity (ADCC), as the major mode of action to deplete tumor cells. It is well-known that this effector function is modulated by the *N*-linked glycosylation in the Fc region of the antibody. In particular, absence of core fucose on the Fc *N*-glycan has been shown to increase IgG1 Fc binding affinity to the FcγRIIIa present on immune effector cells such as natural killer cells and lead to enhanced ADCC activity. As such, various strategies have focused on producing afucosylated antibodies to improve therapeutic efficacy. This review discusses the relevance of antibody core fucosylation to ADCC, different strategies to produce afucosylated antibodies, and an update of afucosylated antibody drugs currently undergoing clinical trials as well as those that have been approved.

ARTICLE HISTORY
Received 24 January 2018
Revised 5 April 2018
Accepted 16 April 2018

KEYWORDS
ADCC; Fc fucosylation; Fc gamma receptors; *in vivo* efficacy; therapeutic monoclonal antibodies

Introduction

Therapeutic antibodies represent the fastest growing group of biotherapeutics in recent years, both in the numbers of antibodies entering clinical trials and in global sales revenue.[1-4] Many monoclonal antibodies are used for treatment of various malignancies and autoimmune disorders. Anti-cancer antibodies target cancer cells by triggering effector functions such as antibody-dependent cellular cytotoxicity (ADCC) upon engagement of immune complexes with FcγRIIIa present on natural killer (NK) cells, or direct induction of tumor cell apoptosis through blocking the binding of pro-survival ligands or inhibiting signal receptor dimerization. NK cells are a type of lymphocyte, representing about 10% of total lymphocytes. Unlike B and T lymphocytes, which are the important components of the adaptive immune system, NK cells are a critical component of the innate immune system. The Fc region of monoclonal antibodies acts as an important bridge between adaptive and innate immune response. When the antigens expressed on the surfaces of cancer cells, virus-infected cells or invading pathogens are recognized by specific antibodies, the cells or pathogens become coated with the antibodies. The Fc region of the antibodies bound to these surfaces assists in the elimination of the targets via different mechanisms. Firstly, it can interact with the C1 molecule of the complement system and trigger the activation of classical pathway of the complement system. It can also recruit phagocytes via Fc receptors and activate the phagocytosis pathway and, as mentioned above, activate ADCC mediated by NK cells. Among these mechanisms, studies on rituximab and trastuzumab have suggested that ADCC is the key mechanism of action to eliminate cancer cells.[5-7]

The FcγRIII binds the Fc region of IgG1 antibodies by interacting with the hinge region and the CH2 domain.[8,9] This Fc-FcγRIII interaction is significantly affected by the glycan present at the conserved *N*-glycosylation site Asn297 (N297) in each of the CH2 domains.[10] Mutations in the CH2 domain that destroyed the conserved *N*-glycosylation motif and hence gave rise to aglycosylated Fc resulted in complete loss of binding to most FcγRs except FcγRI.[11] Several approaches have been utilized to increase the affinity between antibody and the FcγRIII. These include engineering the Fc region through amino acid mutations[12] and glycoengineering the Fc *N*-glycan to reduce core fucose.[13-15] It is now widely recognized that removal of the core fucose from Fc *N*-glycans represents the most effective approach to enhance ADCC activity.[14,15] A high-throughput study of the IgG glycome of three isolated human populations showed that most of the human plasma IgG antibodies are core fucosylated with levels of afucosylated IgG ranging from 1.3% to 19.3%, underlying the difference in ADCC efficacy of naturally occurring antibodies to protect against diseases.[16] Dramatic shifts in IgG glycan profile towards reduced galactosylation and fucosylation have been observed in human immunodeficiency virus (HIV)-specific antibodies and are associated with improved antiviral activity and HIV control.[17]

There are two FcγRIII genes in the human genome, one encodes FcγRIIIa and the other encodes FcγRIIIb. These two proteins share 97% homology at the amino acid level. While the

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Recent advances in animal model studies and clinical trials.

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transmembrane protein FcγRIIIa is expressed in most effector cells of the immune system, FcγRIIIb is exclusively expressed by neutrophils as a glycosylphosphatidylinositol (GPI)-anchored protein. FcγRIIIb is not known to play a role in ADCC, but it may play a role in phagocytosis of IgG-coated pathogens. Two common alleles of the FcγRIIIa gene encode two variants that differ at position 158, either a Val (V158) or a Phe (F158).[18,19] Between the two variants, FcγRIIIa-V158 has a higher affinity to human IgG1. For example, under similar experimental conditions, FcγRIIIa-V158 demonstrated an approximately 10-fold higher affinity for IgG than FcγRIIIa-F158.[20] Cells expressing the FcγRIIIa-V158 allele mediate ADCC more effectively.[19] In anti-epidermal growth factor receptor (EGFR) antibody-treated colorectal cancer patients, the clinical outcome was strongly associated with the FcγRIIIa polymorphisms. Better clinical outcomes have been observed in patients expressing high affinity FcγRIIIa variant (V158) when they were treated with anti-CD20 or anti-EGFR antibodies.[5,21-23]

Protein fucosylation in mammalian system

Fucose (6-deoxy-L-galactose) is a common component of many *N*- and *O*-linked glycans produced in mammalian cells. A total of 13 fucosyltransferases (FUT) that have been identified in the human genome transfer a fucose residue from GDP-fucose to an acceptor substrate.[24] FUT1 and FUT2 transfer the fucose residue to the terminal galactose and form an α1,2 linkage. FUT3 has both α1,3- and α1,4-fucosyltransferase activities responsible for the synthesis of Lewis^x- and Lewis^a-related structures. FUT4 to FUT7 and FUT9 to FUT11 are all α1,3-fucosyltransferases. These transferases are responsible for the synthesis of the ABH and the Lewis antigens.[25,26] Lewis-related tri- or tetra-saccharides play critical roles in leukocyte adhesion during inflammatory response and lymphocyte homing.[27] Based on the glycosidic linkages, the Lewis antigens can be divided into two types. Type I includes Lewis^a (Le^a), sialyl-Lewis^a (SLe^a) and Lewis^b (Le^b). Type II includes Lewis^x (Le^x), sialyl-Lewis^x (SLe^x) and Lewis^y (Le^y). Some of these Lewis antigens are found overexpressed on different types of cancer cells.[28,29] SLe^a or CA 19-9 (cancer antigen 19-9) is one of the commonly used tumor markers in clinics.[28,30] Lewis antigens may contribute to adhesion of cancer cells to vascular endothelium and promote hematogenous metastasis of cancer cells.[31,32] In the 1980s and early 1990s, many monoclonal antibodies were generated by whole-cell immunization of mice with different types of cancer cells. Many of these "anti-cancer" antibodies turned out to be specific for different Lewis antigens.[33-36] Unfortunately, the development of these antibodies into anti-cancer therapeutics has been quite challenging, because many Lewis antigens are also expressed in several types of normal tissues, particularly in the mucosa of human gastrointestinal tract in the form of *O*-linked glycans attached to the mucins.[37-48] For example, anti-Le^y antibodies showed strong side effects including nausea and vomiting in Phase 1 clinical studies because the expression of Le^y in the gastrointestinal tract.[33]

FUT8 is the only α1,6-fucosyltransferase that transfers fucose via an α1,6 linkage to the innermost *N*-acetylglucosamine on *N*-glycans for core fucosylation.[49] FUT8 is widely expressed in various tissues except in the liver, but it is significantly upregulated in hepatocellular carcinoma (HCC) tissues. Alpha-fetoprotein (AFP) is the most abundant plasma protein found in the human fetus. The level of AFP begins to decrease after birth and reaches very low levels in adults. Serum AFP level is elevated in people with HCC, and it has therefore been a reliable biomarker for HCC. However, the serum level of AFP also increases slightly in some patients with chronic liver diseases, which makes it difficult to diagnose HCC at its early stage when serum AFP level is still low. Since FUT8 is overexpressed in HCC patients and therefore the AFP in HCC patients is core-fucosylated, but the AFP is not core-fucosylated in patients with chronic liver diseases. Therefore, elevated levels of core-fucosylated AFP have been used as a more accurate tumor biomarker.[50,51] The other two fucosyltransferases are POFUT1 and POFUT2. They are *O*-fucosyltransferases that mediate the direct attachment of fucose to Ser or Thr residues of proteins in the ER.[52,53] *O*-fucosylation of Notch protein is essential for Notch signaling which plays an important role in the regulation of embryonic development.[54]

The substrate for fucosylation reactions, GDP-β-L-fucose (GDP-fucose), is synthesized in the cytoplasm through the *de novo* and the salvage pathway. The *de novo* pathway, which generates the majority of GDP-fucose, involves the conversion of GDP-mannose to GDP-fucose by GDP-mannose 4,6 dehydratase (GMD) and GDP-keto-6-deoxymannose 3,5-epimerase/4 reductase (also known as FX).[55] The salvage pathway, which accounts for only a small percentage of GDP-fucose production, utilizes free cytosolic fucose derived from degraded glycoproteins or glycolipids or exogenous fucose.[24] The GDP-fucose synthesized in the cytosol must be transported into the Golgi apparatus or the endoplasmic reticulum (ER) by specific transporters in order to serve as the substrate for fucosylation reactions. The Golgi GDP-fucose transporter (GFT), encoded by the *Slc35c1* gene, is a member of the solute carrier family 35 (SLC35).[56] GFT is responsible for transporting GDP-fucose from the cytosol into the Golgi. Mutations in the *Slc35c1* gene in humans lead to the development of leukocyte adhesion deficiency type II (LADII) or congenital disorder of glycosylation type IIc, characterized by severe immunodeficiency, mental retardation and slow growth.[57-60]

The effect of IgG core fucosylation on ADCC

The classic ADCC response is mediated by NK cells following the binding of the FcγRIIIa to the Fc region of antibody molecules. This binding triggers the NK cells to release cytokines and cytolytic agents that eventually kill the target cell. The ADCC activity is highly affected by the Fc *N*-glycan. In recombinant IgG therapeutics produced in Chinese hamster ovary (CHO) cells, the Fc *N*-glycans are heterogeneous biantennary complex type with a fucose residue attached to the core position. These *N*-glycans contain little to no sialic acid with zero (G0), one (G1) or two (G2) galactose residues. In the study by Shields *et al.*, humanized IgG1 antibodies expressed in CHO Lec13 cells demonstrated a 50-fold improvement in binding affinity to human FcγRIIIa compared to the same antibodies produced in wild type CHO cells.[14] Antibodies produced in Lec13 cells carry a significant amount of afucosylated *N*-glycans due to the mutated GMD gene in these cells.[61] Importantly, the

afucosylated IgG1 demonstrated significant improvement in ADCC *in vitro* using peripheral blood mononuclear cells (PBMCs) or NK cells in comparison to its fucosylated counterpart. Shinkawa *et al.* subsequently reported that the absence of fucose, but not the presence of galactose or bisecting GlcNAc, is critical for enhancing ADCC.[15] Another study also suggested that the removal of core fucose from antibodies was sufficient to achieve maximal ADCC activity.[62] It was shown that there was no significant difference in ADCC activity mediated by core fucose removal or amino acid mutations S229D/D298A/I332E, which was known to have higher binding affinity for FcγRIIIa.[12] In addition, no additive effect was observed on B-cell depletion activity of anti-CD20 IgG1 in human blood using a combination of these techniques.[62] Through the use of isothermal titration calorimetry, it was demonstrated that the IgG1-FcγRIIIa binding is driven by favorable binding enthalpy (ΔH), but opposed by unfavorable binding entropy change (ΔS).[63] Fucose removal enhanced the favorable ΔH leading to an increase in the binding constant of IgG1 for the receptor by a factor of 20–30 fold, suggestive of an increase in non-covalent interactions upon complexation.[63]

Molecular mechanisms to account for the enhanced affinity of afucosylated antibodies to FcγRIIIa

The first crystal structure of FcγRIII-IgG1-Fc complex was reported in 2000.[9] The FcγRIII used in the study was a soluble FcγRIIIb (sFcγRIIIb) produced in *E. coli* and the Fc was isolated from pooled human IgG1. The crystal structure revealed that the receptor is bound between the two CH2 domains and the hinge region asymmetrically through van der Waals contacts and hydrogen bonds. Only one *N*-glycan of the two CH2 domains makes contact with the receptor. The innermost GlcNAc residue of the Fc *N*-glycan was found to have the potential of forming hydrogen bonds with several amino acids of the FcγRIII. As the sFcγRIII preparation used in the study was unglycosylated, it was impossible to evaluate the impact of its *N*-linked glycan on the FcγRIII-Fc interaction. Nonetheless, the authors did highlight that Asn162 is a potential glycosylation site of FcγRIII that is close to a binding site and a larger carbohydrate moiety attached to this site may influence the affinity to IgG.[9] Indeed, a subsequent study revealed that, compared to the unglycosylated form of FcγRIII (by mutating Asn162 to Gln162), the glycosylated FcγRIII (Asn162) showed reduced affinity for native (fucosylated) IgG antibodies, while antibodies with or without the core fucose showed a similar affinity for unglycosylated FcγRIII.[20] However, when fucose-free antibody binds glycosylated FcγRIII (Asn162), the affinity increased significantly. The binding affinities of different glycoforms of IgG-FcγRIII pairs are in the following order: IgG-fucose-free/FcγRIIIa-Asn162 $>>$ IgG-native glycan/FcγRIIIa-Gln162 $>$ IgG-native glycan/FcγRIIIa-Asn162.[20] The authors concluded that the carbohydrate moieties of both FcγRIIIa and IgG are important for the interaction. An *N*-glycan needs to be attached to FcγRIIIa Asn162 and enhanced binding affinity can be achieved if the antibody is afucosylated.[20] In their proposed model, the fucose residue protrudes from the continuous surface of the Fc into open space, which prohibits close contact of the Fc receptor *N*-glycan core, thereby precluding additional productive interactions. Furthermore, the model predicts that only one of the two Fc-fucose residues needs to be absent for increased binding affinity toward FcγRIIIa.

Detailed X-ray crystallography studies on the Fc-FcγRIIIa complex confirmed this model. Ferrara *et al.* showed that a unique kind of carbohydrate–carbohydrate interaction coupled with increased number of newly formed hydrogen bonds and van der Waals contacts likely contribute to the increased binding affinity observed between afucosylated Fc and the Asn162-glycosylated receptor.[64] However, in the crystal structure of fucosylated Fc in complex with FcγRIIIa, the core fucose is oriented toward the second GlcNAc of the *N*-glycan attached to Asn162 and has to accommodate in the interface between the interacting glycan chains.[64] As a result, the whole oligosaccharide unit on Asn162 moves away from the Fc glycan, which leads to a weakened FcγRIIIa-IgG interaction.

Ferrara *et al.* demonstrated that the glycosylation at Asn162 of FcγRIII is not essential for the expression of the receptor; however, this glycosylation site is conserved among all FcγRIIIs (or the equivalent) in all mammals studied.[19] Furthermore, in all FcγRs, the regions that interact with the antibody are highly conserved, yet all other receptors lack this glycosylation site.[9] It is tempting to speculate that ADCC may be modulated by IgG core fucosylation because of the presence of the glycan at Asn162 of FcγRIII. Indeed, reduced core fucosylation of antibodies has been linked to enhanced immune response during an autoimmune disease and an infectious disease.[65,66]

Fc galactosylation and sialylation also modulate IgG1 interaction with FcγRIIIa, but to a significantly lesser extent

Recent studies have indicated that Fc galactosylation leads to increased FcγRIIIa binding, although to a significantly lesser extent compared to the removal of core fucose.[11,67-69] By carrying out enzymatic hyper-galactosylation across four batches of monoclonal antibodies produced from standard manufacturing processes in CHO cells, Thomann *et al.* demonstrated that hyper-galactosylation of antibody samples consistently leads to improvement in FcγRIIIa binding and ADCC.[68] However, addition of galactose to afucosylated antibodies did not confer additional improvements to ADCC efficacy, indicating that afucosylation remains the major determinant of ADCC activity. While afucosylation removes the steric hindrance for enhanced Fc-FcγRIIIa interaction, a more 'bulky' G2F *N*-glycan structure may help to keep the two CH2 domains of IgG Fc in a more open horseshoe conformation for FcγRIIIa to bind.[10] These observations are particularly important as recombinant therapeutic antibodies produced in CHO cells exhibit heterogeneity in terms of galactosylation, with G0F as the most abundant and G2F as the least abundant *N*-glycan. Improving the percentage of G2F can be achieved by over-expressing appropriate galactosyltransferases in CHO cells. In recombinant antibodies produced in CHO cells, only a small portion of the *N*-glycans is sialylated. On the contrary, a recent report showed that increased sialylation of the Fc *N*-glycan decreased ADCC if core fucose is present. However, in the absence of fucosylation, sialylation did not make any difference.[70] Therefore, core

fucosylation plays a much more significant role in modulating ADCC than galactosylation or sialylation.

Modulating FcγRIIIa interaction through Fc engineering

In addition to glycoengineering of the Fc *N*-glycan, various strategies have been performed to engineer the Fc domain to improve the ADCC effector function. Through alanine scanning mutagenesis of individual solvent-exposed residues on the human IgG1 Fc domain, residues involved in the binding site for human FcR were mapped.[12] IgG1 mutants with improved binding to FcγRIIIa – T256A, K290A, S298A, E333A, and K334A were identified. These Fc variants demonstrated up to 1-fold enhanced ADCC *in vitro*.[12]

With the use of computational structure-based design and high-throughput screening, a series of engineered Fc variants were generated.[71] These Fc variants of either single (S239D or I332E), double (S239D/I332E) or triple (S239D/I332E/A330L) mutations demonstrated up to 169-fold enhanced interaction with human FcγRIIIa.[71] The Fc variants also showed enhanced binding ratio between activating FcRγIIIa and inhibitory FcγRIIb of up to 9-fold. The double mutant (S239D/I332E) has been employed in the design of a humanized anti-CD19 antibody, XmAb5574, by Xencor. XmAb5574 was able to enhance ADCC activity against a wide range of B-lymphoma and leukemia cell lines and also that of patient-derived acute lymphoblastic leukemia and mantle cell lymphoma cells.[72] *In vivo*, it showed enhanced anti-tumor effect in mouse lymphoma xenograft over the wild type analogue.[72] XmAb5574 is currently in clinical trials against various forms of B cell lymphoma.

Functional genetic screen, through the use of yeast surface display, to identify Fc sites with enhanced binding to low affinity activating FcγRIIIa and reduced binding to the inhibitory FcγRIIb was performed.[73] An Fc variant 18 with several mutations (F243L/R292P/Y300L/V305I/P396L) was identified and demonstrated about 100-fold enhanced ADCC activity.[73] MGAH22, from Macrogenics, is a chimeric IgG1 anti-HER2 antibody, with similar affinity and specificity to trastuzumab, containing the engineered Fc domain (variant 18) except that V305I was replaced with L235V to reduce FcγRIIb binding.[74] MGAH22 showed enhanced affinity to both FcγRIIIa variants (F158 and V158), but decreased affinity to inhibitory FcγRIIb.[74] This translated into enhanced ADCC activity over the wild-type equivalent of MGAH22 antibody. *In vivo*, MGAH22 demonstrated enhanced anti-HER2 activity over HER2 positive tumor in transgenic mouse expressing the low affinity human FcγRIIIa F158 variant.[74] MGH22 is currently being evaluated in clinical studies of patients with HER2-positive cancers.

Strategies to produce afucosylated antibodies

Biosynthetic enzymes of GDP-fucose

CHO Lec13 cells are naturally defective in GDP-fucose formation due to a deficiency in endogenous GDP-mannose 4,6-dehydratase (GMD).[61] The enzyme is responsible for catalysing the first of three steps in the *de novo* GDP-fucose biosynthesis pathway. This has resulted in the application of Lec13 cells as the host cell line for the production of afucosylated antibodies.[14] However studies have shown that single clones isolated from Lec13 cells display a wide variety of fucosylation range, with most clones producing 50–70% fucosylated antibody when cultured to confluence in a static flask.[75] Further analysis revealed low-level expression of GMD at mRNA level as well as the presence of fucosylated oligosaccharides on cell surface using LCA-staining. Shields *et al.* also noted that the Lec13 cell line is not sufficiently robust to be utilized as a production cell line as expression levels of antibodies tested (anti-HER2Hu4D5 and anti-IgE HuE27) were lower than that produced in other CHO cells.[14] A GDP-keto-6-deoxymannose 3,5-epimerase/4 reductase (FX)-knockout CHO cell line that can be used to produce antibodies with completely afucosylated *N*-glycans was recently reported.[76]

Fucosyltransferase – FUT8

Shinkawa *et al.* employed rat hybridoma YB2/0 cells to produce humanized anti-human interleukin-5 receptor (IL-5R) IgG1 antibody (KM8399) and compared it against the same antibody produced in CHO cells (KM8404).[15] Although both antibodies showed similar levels of antigen binding, the ADCC activity of YB2/0-produced KM8399 was 50-fold higher than CHO-produced KM8404. Similar results were obtained when two other antibodies were produced in CHO cells and YB2/0 cells. Glycan analysis showed that lower level of core fucose in YB2/0 cells-produced antibodies was the main reason for the enhanced ADCC.[15] Analysis showed that YB2/0 cells have significantly lower levels of the Fut8 mRNA than CHO cells.

Another strategy to produce afucosylated antibodies involves inactivating the FUT8 gene. In the study by Yamane-Ohnuki *et al.*, the FUT8 gene in an anti-CD20 antibody-producing CHO DG44 cell line was targeted for disruption using sequential homologous recombination.[77] In the resultant cell line, both FUT8 alleles were knocked out from the FUT8 genomic region. The $FUT8^{-/-}$ cell line was shown to express completely afucosylated antibodies with a two-fold increase in ADCC compared to the same antibody produced in the parental cell line. The $FUT8^{-/-}$ cell line also demonstrated similar growth kinetics and productivity compared to the parental cell line when cultured in 1 L bioreactors. The FUT8 gene has also been targeted for inactivation using the zinc finger nuclease platform.[78] This also led to the production of completely afucosylated antibodies. Small interfering RNA (siRNA) was also used to target FUT8 in an antibody-producing CHO DG44 cell line, and stable clones that produced 60% afucosylated antibodies were isolated.[79]

GDP-fucose transporter (SLC35C1)

It has been shown that loss-of-function mutations in the Golgi GDP-fucose transporter (GFT) gene (*Slc35c1*) was able to eliminate fucosylation reactions that occur in the Golgi.[59,60] Our group inactivated the *Slc35c1* gene in CHO cells first by zinc-finger nucleases (ZFNs), followed by transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats-Cas9 (CRISPR-Cas9) techniques.[80,81] The mutant cells in the transfected pools were identified and isolated

by fluorescence-activated cell sorting (FACS) using fluorescently labelled fucose-specific *Aleuria aurantia* lectin (AAL).[80] CHO cells with inactivated *Slc35c1* gene have been named as CHO-gmt3 (CHO-glycosylation mutant3) cells. Mass spectrometry analyses demonstrated the complete lack of core fucose on *N*-glycans attached to the EPO-Fc fusion protein and IgG1 antibodies produced in the CHO-gmt3 cells.[80] The CHO-K1 transcriptome data have shown that among all Golgi fucosyltransferases, only FUT8 is expressed.[82] Therefore, inactivating *Fut8* or *Slc35c1* should have similar effects on CHO-K1 cells. A potential advantage of knocking out *Slc35c1* over *Fut8* is that it eliminates the potential complications caused by the gain-of-function mutations of fucosyltransferase found in LEC11 and LEC12 cells.[83] Using this approach, we have been able to establish stable $Slc35c1^{-/-}$ lines from several pre-existing antibody-producing CHO cell lines in less than two months. Our data showed that inactivation of the *Slc35c1* gene in the pre-existing antibody-producing CHO cell line does not alter cell growth rate, viable cell density and antibody productivity in serum-free suspension culture conditions.[80] This strategy has been used to produce afucosylated antibodies in a few recent studies.[84,85]

Generation of bisecting GlcNac

β-1,4-mannosyl-glycoprotein 4-β-*N*-acetylglucosaminyltransferase (GnT-III) is normally not expressed in CHO cells. GnT-III catalyzes the formation of a bisecting GlcNAc by attaching a GlcNAc in β1,4 linkage to the β-linked mannose of the trimannosyl core of *N*-glycans. It was shown that overexpression of GnT-III in CHO cells was able to reduce Fc core fucosylation. Ferrara *et al.* evaluated the overexpression of a series of Golgi resident enzymes in combination with GnT-III and showed that overexpression of GnT-III and Golgi α-mannosidase II (αManII) resulted in the highest level of bisecting and afucosylated glycans on IgG antibodies.[86] CHO cells that overexpress both GnT-III and αManII have been successfully used as the host cell line to produce anti-CD20 antibody GA101.

Expression of bacterial RMD in the cytosol of CHO cells to disrupt the GDP-fucose de novo pathway

In the *de novo* pathway of GDP-fucose biosynthesis in mammalian systems, GDP-mannose is first converted to GDP-4-keto-6-deoxy mannose (GKDM) by GDP-mannose-4,6-dehydratase. GKDM is eventually converted to GDP-fucose by several downstream enzymatic reactions. In bacteria, however, GKDM can be reduced to form GDP-rhamnose by a GDP-4-keto-6-deoxy mannose reductase (RMD).[87] GDP-rhamnose is a common component of bacterial cell surface glycans. Heterologous expression of bacterial RMD in the cytosol of CHO cells allowed the GDP-fucose *de novo* pathway to be efficiently bypassed and afucosylated IgG antibodies to be produced.[88] The dead-end product GDP-rhamnose is likely to inhibit the activity of GMD as a competitive inhibitor.

Biochemical inhibitors of fucosylation

To complement existing platforms that involve genetic engineering of cell lines for the production of afucosylated antibodies, Okeley *et al.* utilized small molecules to inhibit antibody fucosylation.[89] 2-fluorofucose and 5-alkynylfucose were shown to generate afucosylated monoclonal antibodies. The mechanism of action of these inhibitors is likely due to the depletion of intracellular GDP-fucose with a subsequent block of the *de novo* pathway or the inhibition of FUT8.

Plant cells as expression platforms

In addition to CHO cells, alternative expression platforms such as plants have also been reported for production of recombinant antibodies.[90] Unlike CHO cells, glycoproteins produced from plants lack α1,6-fucose, β1,4-galactose and α2,3-sialic acid. Plant *N*-glycans typically contains a $Man_3GlcNAc_2$ core modified with β1,2-xylose and α1,3-fucose. Large complex type *N*-glycans with mammalian Le^a structure containing α1,4-fucose and β1,3-galactose residues were sometimes observed.[91] Antibody *N*-glycans produced in plants are predominantly GnGnXF3 structures containing the unwanted residues β1,2-xylose and core α1,3-fucose.[92,93] These sugars are immunogenic to humans, and serum antibodies against core xylose and core α1,3-fucose have been detected in healthy human blood donors.[94] Strategies to overcome this immunogenicity include use of RNAi knockdown of α1,3-fucosyltransferase (FucT) and β1,2-xylosyltransferase (XylT) in plants[95,96] and FucT/XylT-knockout lines.[97,98] An afucosylated anti-CD30 monoclonal antibody with G0 structure was produced using glycoengineered aquatic plant *Lemna minor* and shown to have improved ADCC over the same CHO cell-produced antibody.[95] Anti-HIV 2G12 produced in XylT/FucT-knockdown *N. benthamiana* was found to be homogeneous G0 structures with terminal *N*-acetylglucosamine and lacking both xylose and α1,3-fucose residues.[96] Further glycoengineering in XylT/FucT knockdown *N. benthamiana* by expressing a modified human β1,4-galactosyltransferase was reported to produce anti-HIV monoclonal antibodies with fully β1,4-galactosylated *N*-glycans and improved virus neutralization potency.[99]

Chemoenzymatic remodelling strategy

Chemoenzymatic remodelling of antibodies represents another strategy for generating afucosylated antibodies. This chemical biology approach involves the use of an endo-β-*N*-acetylglucosamidase such as Endo S to remove the majority of *N*-glycans from antibodies, followed by treatment with an exoglycosidase such as fucosidase to remove the core fucose. The mono-GlcNac is then further extended by transglycosylation with Endo S-based glycosynthases in the presence of desialylated complex type glycan oxazoline, which serve as donor substrates to generate different homogenous afucosylated glycoforms.[100] However, this method is not cost effective for producing afucosylated therapeutic antibodies.

Enhanced ADCC activities by afucosylated antibodies in *in vivo* studies

The efficacy of numerous afucosylated antibodies have been investigated *in vivo* using animal models. The studies that have been published are compiled into Table 1. The diseases targeted

Table 1. Summary of glycoengineered antibodies that have been studied *in vivo* in animal models.

Name and format	Target	Fucosylation level	Method of glycoengineering	Result in *in vivo* model (murine/non-human primates)	Reference
BLX-300 (Rituximab) Chimeric IgG1	CD20	afucosylated	*Lemna* aquatic plant-based system with RNA silencing to eliminate the expression of plant specific xylosyl and fucosyl transferase genes	Temporal enhancement of B-cell depletion in cynomolgus monkeys in comparison to fucosylated rituximab during the first 72hrs at low doses	Gasdaska *et al.*[101]
Obinutuzumab/ GA101 Humanized IgG1	CD20	Reduced (<30%)	Coexpression with GnT III and α-ManII in CHO cells (GlycoMAb Technology)	Enhanced tumor inhibition of GA101 compared with rituximab in human lymphoma xenograft mouse models	Mossner *et al.*[102]
				Enhanced B-cell depletion in spleen and lymph nodes of cynomolgus monkeys over rituximab Enhanced tumor growth inhibition over rituximab in human-transformed follicular lymphoma RL model in SCID mice.	Dalle *et al.*[131]
Rituximab Chimeric IgG1	CD20	afucosylated	Commercial rituximab treated with endoglycosidase/fucosidase to generate GlcNAc-rituximab	Enhanced depletion of huCD20^{+} B cells in an FcγR-humanized mouse model over original rituximab	Li *et al.*[70]
GBR 401 Humanized IgG1	CD19	Reduced (~50%)	CHO cells with reduced fucosylation	Enhanced B-cell depletion over rituximab in xenografted SCID mouse	Breton *et al.*[107]
Inebilizumab/MEDI-551 Humanized IgG1	CD19	afucosylated	FUT8$^{-/-}$ CHO cells (Potelligent® Technology)	Enhanced B-cell depletion in huCD19/CD20 transgenic mouse over rituximab	Herbst *et al.*[105]
				A direct comparison between MEDI-551 and the fucosylated anti-CD19 in CD19^{+} Raji and Daudi cells lymphoma xenograft SCID mouse model only showed minor or insignificant improvement in tumor inhibition respectively	Ward *et al.*[104]
				Prolonged animal survival in SCID mice engrafted with human pre-B cells over afucosylated control IgG1	Matlawska-Wasowska *et al.*[106]
				Enhanced B-cell depletion over wild type counterpart in human CD19 transgenic mouse at lower doses	Gallagher *et al.*[129]
MDX-1342 Human IgG1	CD19	afucosylated	FUT8$^{-/-}$ CHO cells (Potelligent® Technology)	Dose-dependent enhancement of survival in murine B-cell lymphoma model with Ramos cells Enhanced B-cell depletion over fucosylated counterpart in cynomolgus monkeys	Cardarelli *et al.*[103]
Imgatuzumab /GA201/ RG7160 Humanized IgG1	EGFR	Reduced (~15%)	Coexpression with GnT III and α-ManII in CHO cells (GlycoMAb Technology)	Enhanced survival rate over fucosylated counterpart in mouse xenograft models displaying murine FcγRIV and over commercial Cetuximab in mouse xenograft models displaying murine FcγRIV and/or human FcγRIIIA.	Gerdes *et al.*[108]
ARGX-111 Human IgG1	c-MET	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Potent inhibition of c-Met-amplified tumor growth in MKN-45 xenograft mice	(a)
XGFR Bispecific antibody with EGFR (GA201) and IGF-1R (R1507) specificities	IGF-1R and EGFR	Reduced	Coexpression with GnT III and α-ManII in CHO cells (GlycoMAb Technology)	Improved survival rate of mice treated with XGFR over its fucosylated counterpart in intrasplenic colon carcinoma model in SCID beige mice	Schanzer *et al.*[110]

XGFR* Bispecific antibody with EGFR (GA201) and affinity-matured IGF-1R (R1507) – F13B5 specificities	IGF-1R and EGFR	Reduced	Coexpression with GnT III and α-ManII in CHO cells (GlycoMAb Technology)	Enhanced tumor inhibition over fucosylated bispecific orthotopic Mia-PaCa2 pancreatic cancer xenograft model in SCID mice	Schanzer *et al.*[130]
JNJ-61186372 Bispecific human IgG1	EGFR and c-Met	Reduced (<10%)	CHO cells with low level of fucose	Enhanced xenograft tumor inhibition in nude mouse over control and afucosylated IgG2 isotype	Grugan *et al.*[109]
Ifabotuzumab/KB004/IIIA4 Humanized IgG1	EPHA3	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Enhanced tumor growth inhibition over control antibody in DU145 or 22Rv1 xenograft mice	Vail *et al.*[132]
Bemarituzumab /FPA144 Humanized IgG1	FGFR2b	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Enhanced tumour growth inhibition in FGFR2-overexpressing gastric cancer xenograft mouse model over isotype control	(b)
Afucosylated Trastuzumab Humanized IgG1	HER2	afucosylated	FUT8$^{-/-}$ CHO cells (Potelligent® Technology)	Enhanced tumor growth inhibition over fucosylated trastuzumab in KPL-4 xenografts in human FcγRIIIα mice	Junttila *et al.*[133]
TrasGEX/ GT-MAB7.3-GEX/ Glycooptimized Trastuzumab-GEX Humanized IgG1	HER2	Reduced	Human glycoengineered production cell lines (GlycoExpress technology)	Potent Her2^{+} BT474 tumor growth inhibition in nude mice but exhibited similar tumor volume and number of regression between fucosylated and reduced fucosylated antibodies	(c)
Lumretuzumab /RG7116/ RO5479599/ GE-HuMAb-HER3 Humanized IgG1	HER3	Reduced	Coexpression with GnT-III and α-ManII in CHO cells (GlycoMAb Technology)	Enhanced survival of SCID-beige mice with A549-B34 NSCLC xenografts Enhanced tumor inhibition in A549 orthotopic mouse models over WT-huMab-HER3	Mirschberger *et al.*[134] (d)
Palivizumab-N Humanized IgG1	Respiratory syncytial virus (RSV)	afucosylated	Transgenic *N. benthamiana* with RNAi to eliminate the expression of plant specific xylosyl and fucosyl transferase genes	Enhanced RSV protection in rat models over fucosylated counterpart	Hiatt et al.,[114]
h-13F6 Chimeric IgG1	Heavily glycosylated mucin-like domain of EBOV glycoprotein (GP)	afucosylated	*N. benthamiana* plants with RNAi to eliminate the expression of plant specific xylosyl and fucosyl transferase genes	Enhanced anti-viral protection over CHO cells-produced fucosylated counterpart	Zeitlin et al.,[115]
LSEVh-LS-F Hexavalent fusion protein with IgG1 Fc	CD4 and HIV-1 gp120-binding sites	afucosylated	GDP-fucose transporter $^{-/-}$ CHO cells	Potent inhibition of SHIV infection in macaque models over PBS control Potent *in vivo* neutralization of an HIV-1 strain resistant to the broadly neutralizing antibodies VRC01 and 3BNC117 in humanized mouse	Bardhi *et al.*[84]
KM2760 Chimeric IgG1	CC chemokine receptor 4 (CCR4)	Reduced	YB2/0 cells	Enhanced anti-tumor activity over fucosylated counterpart in human PBMC-engrafted mouse model Significant anti-tumor activity in disseminated and non-disseminated Sezary syndrome (SS) and Mycosis fungoides (MF) mouse models Potent anti-tumor effect of KM2760 in NOG mice bearing adult T cell leukemia/lymphoma (ATLL) cells	Niwa et al.[135] Yano *et al.*[136] Ito et al.[137]

(*continued on next page*)

Table 1. (*Continued*)

Name and format	Target	Fucosylation level	Method of glycoengineering	Result in *in vivo* model (murine/non-human primates)	Reference
Mogamulizumab/ POTELIGEO/ KM0761 Humanized IgG1	CC chemokine receptor 4 (CCR4)	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Induced potent tumor growth inhibition and enhanced survival in ATLL tumor-bearing mice over vehicle control	Ishii *et al.*[138]
Benralizumab /MEDI-563 Humanized IgG1	IL-5Rα	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Efficient eosinophils depletion in non-human primates	Kolbeck *et al.*[123]
KHK2823 Humanized IgG1	IL-3Rα (CD123)	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Tumor growth inhibition of human AML cell line MOLM-13 grafted into nude rats compared to vehicle control Significant depletion of IL-3Rα-positive cells in the peripheral blood of cynomolgus monkeys	(e)
Low fucose Elotuzumab/ HuLuc63-LF Humanized IgG1	Signaling Lymphocyte Activation Molecule (SLAMF7, also called CS1)	Reduced	YB2/0 cells	Enhanced anti-tumor activity in OPM2 xenograft SCID mice model	Hsi *et al.*[139]
Afucosylated anti-CS1 Humanized IgG1	SLAMF7/ CS1	afucosylated	*Pichia pastoris*, which normally cannot produce GDP-fucose, is glycoengineered to eliminate fungal type glycans and to produce complex biantennary N-linked glycans	Enhanced anti-tumor efficacy in SCID mice xenograft tumor model over HEK293 produced fucosylated anti-CS1 antibody	Gomathinayagam *et al.*[140]
AK002 Humanized IgG1	Sialic acid immunoglobulin-like lectins 8 (Siglec-8)	afucosylated	FUT8$^{-/-}$ CHO cells (Potelligent® Technology)	Information not available	
BMS-986218 Human IgG1[f]	T-cell receptor cytotoxic T-lymphocyte-associated antigen 4 (CTLA4)	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Information not available	
Cusatuzumab/ARGX-110 Humanized IgG1	CD70, a member of TNF receptor ligand family	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Fucosylated version shown potent tumor growth inhibition in Raji xenograft mice [g]	Silence *et al.*[141]
DS-5573a Humanized IgG1	B7-H3, a member of B7 family	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	DS-5573a showed dose dependent and significant tumor inhibition in MDA-MB-231-bearing SCID mice	Nagase-Zembutsu *et al.*[142]
Gatipotuzumab/ PankoMab-GEX/ GT-MAB 2.5 GEX Humanized IgG1	Tumor specific glycoepitope of Muc1 (TA-Muc1)	Reduced	Human glycoengineered production cell lines (GlycoExpress technology)	Information not available	
GM102/ 3C23K Humanized IgG1	anti-mullerian Hormone Receptor II (AMHR2)	Reduced	YB2/0 cells (EMABling® version)	Inhibited tumor growth in COV434-MISRII tumor bearing mice while 2C23K-FcKO (could not bind FcRIIIα) did not reduce tumor growth	Estupina *et al.*[143]
GSK2831781/ IMP731 Humanized IgG1	Lymphocyte activation gene (LAG)-3	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Enhanced depletion of human LAG-3^{+} T cells in SCID mice over fucosylated antibody	(h)
KHK4083 Human IgG1	OX40	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Information not available	

OBT357/ MEN1112/ OX-001/OX-357 Humanized IgG1	Bst1/CD157	afucosylated	FUT8 $^{-/-}$ CHO cells (Potelligent® Technology)	Information not available	
SEA-CD40 Humanized IgG1	CD40	afucosylated	CHO cells. Use of modified sugars (fucosylation inhibitor, 2-fluorofucose) in culture media to inhibit fucosylation	Enhanced immune stimulating ability over parental dacetuzumab in xenograft tumor models	(i)
SEA-BCMA Humanized IgG1	BCMA (B-cell maturation antigen)	afucosylated	CHO cells. Use of modified sugars (fucosylation inhibitor, 2-fluorofucose) in culture media to inhibit fucosylation	Enhanced survival of SCID mice with tumor compared to control antibody	(j)
TRX518 Humanized IgG1	Glucocorticoid-induced TNF receptor (GITR)	aglycosylated [k]	Information not available	Information not available	

(a) Aftimos P. *et al.*, A Phase I, first-in-human study of ARGX-111, a monoclonal antibody targeting c-Met in patients with solid tumors, ASCO Poster 2015.
(b) Abigael T. *et al.*, FPA144: A therapeutic antibody for treating patients with gastric cancers bearing FGFR2 gene amplification, Proceedings: AACR Annual Meeting 2014.
(c) Goletz S. *et al.*, Patent Application US 2015/0166664 A1.
(d) Bossenmaier *et al.* GE-huMab-HER3, a novel humanized, glycoengineered HER3 antibody with enhanced ADCC and superior preclinical *in vitro* and *in vivo* efficacy, Proceedings: AACR 103rd Annual Meeting 2012.
(e) Akiyama T. et al., First Preclinical Report of the Efficacy and PD Results of KHK2823, a Non-Fucosylated Fully Human Monoclonal Antibody Against IL-3Rα, Blood, 2015 126:1349.
(f) BMS-986218 is a glycoengineered version of Ipilimumab, which is a human IgG1.
(g) ARGX-110 showed enhanced ADCC over fucosylated version *in vitro*. But only the fucosylated antibody was tested in the animal model.
(h) Written in Hamblin P.A. *et al.*, Anti-LAG-3 binding proteins, Patent application number WO2014140180 (A1) 2014.
(i) Gardai S.J. et al., SEA-CD40, a sugar engineered non-fucosylated anti-CD40 antibody with improved immune activating capabilities, Proceedings of the 106th Annual Meeting of the American Association for Cancer Research; 2015.
(j) Sussman *et al.*, BCMA antibodies and use of same to treat cancer and immunological disorder, Patent application publication number US 2017/0233484.
(k) This antibody is mutated such that it does not contain the conserved Fc *N*-glycosylation site.

by these antibodies include cancers, viral infections and inflammatory disorders.

CD20 is one of the most promising targets for B cell malignancies. The treatment of B cell malignancies has evolved significantly after the US Food and Drug Administration (FDA) approved the first anti-CD20 monoclonal antibody to treat non-Hodgkin's lymphoma (NHL) in 1997. Rituximab (Rituxan®), a type I chimeric IgG1, is currently the best-selling therapeutic monoclonal antibodies marketed for the treatment of B cell malignancies and rheumatoid arthritis. An afucosylated rituximab was evaluated in animal models, and it showed enhanced B-cell depletion in cynomolgus monkeys[101] and in human FcγR- and CD20-transgenic mice[70] compared with fucosylated rituximab. The next-generation anti-CD20 antibody obinutuzumab (GA101 or Gazyva®) is a type II humanized Fc glycoengineered antibody with improved efficacy. This antibody, with reduced fucosylation (<30%, according to the manufacturer), showed superior tumor inhibition in NHL xenograft SCID mice and B-cell depletion in cynomolgus monkeys over rituximab.[102]

CD19 is another B cell marker that has been targeted by monoclonal antibodies. CD19 is particularly important because it is present on malignant B cells that have lost CD20 expression upon repeated rituximab treatment. Several groups have developed anti-CD19 antibodies that are afucosylated.[103-107] These afucosylated antibodies generally showed enhanced B-cell depletion in murine and non-human primate models compared with the fucosylated counterparts. However, anti-CD19 monoclonal antibody MEDI-551 only showed a minor or insignificant improvement in tumor inhibition in $CD19^+$ Raji and Daudi cell lymphoma xenograft SCID mouse models.[104] The discrepancy in efficacy could be dependent on the level of CD19 on the target cell. In addition to ADCC, data suggested the importance of antibody-dependent cellular phagocytosis (ADCP) in MEDI-551-mediated B-cell depletion.[105,106]

Overexpressed receptor tyrosine kinases are frequently implicated as oncogenes in a wide range of cancers. Antibodies with reduced fucosylation against receptors like EGFR, insulin-like growth factor 1 receptor and c-Met have been generated and tested in murine models.[108-110] In addition to the anti-EGFR antibody imgatuzumab (GA201 or RG7160), bi-specific glycoengineered formats against two different receptors have also been developed.[109,110] In xenograft SCID mouse models, these afucosylated antibodies demonstrated enhanced tumor inhibition *in vivo*, which is probably dependent on their enhanced binding to FcγRIII on various effector cells.

Antibodies targeting several viruses that are associated with mortality have been developed as a possible means of passive immunization because no effective vaccines against these viruses are available yet. For example, respiratory syncytial virus (RSV) infection in high-risk young children and elderly is often associated with morbidity and mortality. Palivizumab, a humanized IgG1 against RSV, is suggested for preventive use in high-risk children where RSV can result in complications. Ebola virus (EBOV) is a single-stranded RNA virus that can cause hemorrhagic fever potentially leading to fatalities in humans.[111] It is one of the most virulent and infectious agents known. ZMapp, a cocktail of three monoclonal antibodies produced in plants against the glycoproteins of EBOV, has been successful in passive immunization in nonhuman primates.[112] HIV-1 is well known for its mortality and high rate of viral escape. Broadly neutralizing antibodies against HIV-1 gp120 have demonstrated efficacy in reducing viral load in animal studies and clinical trials.[113] Antibodies against RSV, EBOV and HIV have been glycoengineered to become afucosylated to further improve their anti-viral activity.[84,114,115] Enhanced binding to the FcγR by these afucosylated antibodies was correlated with enhanced efficacy in murine models.[114,115] For example, the afucosylated gp120-bispecific and hexavalent broadly neutralizing fusion protein – LSEVh-LS-F also showed potent inhibition of HIV-1 and simian-HIV infection in humanized mouse and macaque models through NK-cell mediated ADCC.[84]

In summary, the efficacies of the afucosylated antibodies have been tested in murine and non-human primates. The animal model data demonstrated enhanced *in vivo* efficacy, especially at lower doses, by the afucosylated antibodies. The exact *in vivo* mechanism of action can include a multitude of different effector functions (e.g., ADCC, ADCP). However, the significant improvement in ADCC by the afucosylated antibodies observed in the *in vitro* studies was often reduced in the animal models. This could be due to pharmacodynamic and pharmacokinetic effects, differences between the human and animal FcγR genotypes, and the characteristics and density of the antigens. Nevertheless, the enhanced efficacy and the tolerability of several of these glycoengineered drugs in animal studies supported progression into clinical trials.

Therapeutic afucosylated antibody drugs approved for market use and clinical trials

The encouraging results of the afucosylated antibodies in the animal models have led to their advancement into clinical trials. There are currently three afucosylated antibodies on the market and more than 20 are currently being evaluated in clinical trials (Table 2, source: https://clinicaltrials.gov/). We will discuss the approved drugs and a few selected differently glycoengineered antibodies.

Obinutuzumab (GA101 or Gazyva®) is the first glycoengineered therapeutic anti-CD20 antibody approved by FDA in 2013 for the combination treatment of patients with CLL and follicular lymphoma. Reduced fucosylation is achieved through the co-expression of GnT-III and αManII in CHO cells. The antibody demonstrated an enhanced binding affinity for FcγRIIIa and consequently, an increased ADCC activity.[102] Results from Phase1b/2 trials indicated that all patients with CLL experienced rapid and sustained removal of B cells in the peripheral blood.[116-119] In Phase 3 trials, GA101 in combination with chlorambucil prolonged overall survival significantly, as well as progression-free survival and increased the complete response rate.[120] In addition, this combination resulted in substantially increased time to next treatment.[121]

Mogamulizumab (POTELIGEO®) was first approved in 2012 in Japan for hematologic malignancies, and in 2014 for cutaneous T-cell lymphoma (CTCL). In November 2017, FDA granted it Breakthrough Therapy Designation status for the treatment of mycosis fungoides and Sézary syndrome in patients who have previously received at least one treatment.

Table 2. Current status of glycoengineered antibodies in clinical trials.

Antibody name and company	Target and format	Conditions	Current updates
Obinutuzumab/ GA101/ Gazyva [a]	CD20	Chronic lymphocytic leukemia ; Non-Hodgkin's lymphoma	Marketed
Roche	Humanized IgG1 with low fucose content	Various forms of B-cell associated lymphomas	Phase 1, 2, 3[b]
		Kidney Failure, Chronic	Phase 1
		Lupus Nephritis	Phase 2
Mogamulizumab/ POTELIGEO/KM0761[a]	CC chemokine receptor 4(CCR4) Humanized afucosylated IgG1	Relapsed or refractory CCR4-positive adult T-cell leukemia-lymphoma; Cutaneous T cell lymphoma ; Peripheral T-cell lymphoma	Marketed
Kyowa Hakko Kirin		Solid Tumors	Phase 1
		Advanced Solid Tumors	Phase 1
		Gastric Cancer, Esophageal Cancer, Lung Cancer, Renal Cancer	Phase 1
		Solid Tumor, Cancer, Carcinoma	Phase 1
		Diffuse Large B-Cell Lymphoma, Recurrent and/ Refractory Hodgkin Lymphoma, Recurrent and/ Refractory Non-Hodgkin Lymphoma	Phase 1 and 2
		Solid Tumor Cancer, Carcinoma, Hepatocellular Carcinoma	Phase 1 and 2
		Advanced Solid Tumors, Metastatic Solid Tumors	Phase 1 and 2
		Adult T-cell Leukemia-Lymphoma	Phase 2
		Cutaneous T-Cell Lymphoma	Phase 3
		HTLV-1 Associated Myelopathy	Phase 3
Benralizumab/MEDI-563/ Fasenra[a]	IL-5Rα	Asthma	Marketed
AstraZeneca	Humanized afucosylated IgG1	Eosinophilic Gastritis or Gastroenteritis	Phase 1 and 2
		Hypereosinophilic Syndrome	Phase 2
		Eosinophilic Chronic Rhinosinusitis	Phase 2
		Asthma	Phase 2, 3[c]
		Chronic Rhinosinusitis (Diagnosis), Nasal Polyps, Eosinophilia	Phase 2
		Moderate to Very Severe Chronic Obstructive Pulmonary Disease	Phase 3
		Nasal Polyposis	Phase 3
		Severe Prednisone Dependent Eosinophilic Asthma	Phase 3
		Chronic Idiopathic Urticaria	Phase 4
Inebilizumab/ MEDI-551	CD19	Scleroderma	Phase 1 (Completed)
MedImmune	Humanized afucosylated IgG1	Diffuse large B cell lymphoma	Phase 2 (Completed)
		Blood Cancer, Advanced B Cell Malignancies	Phase 1 and 2 (Completed)
		Multiple Sclerosis, Relapsing Forms	Phase 1 (Completed)
		Chronic lymphocytic leukemia	Phase 2 (Completed)
		Relapsed/Refractory Aggressive B-cell Lymphomas	Phase 1 and 2 (Completed)
		B-cell Malignancies	Phase 1
		Neuromyelitis Optica and Neuromyelitis Optica Spectrum Disorders	Phase 2 and 3
		Early Myeloma	Phase 1
		Multiple Myeloma	Phase 2
Ublituximab/ TG1101/ LFB-R603	CD20	Various forms of B-cell associated lymphomas	Phase 1 and 2 (Completed)
TG Therapeutics Inc	Chimeric IgG1, low fucose content	Chronic Lymphocytic Leukemia	Phase 1 (Completed)
		Chronic Lymphocytic Leukemia, Non-Hodgkin's Lymphoma	Phase 1, 2[c]
		Non-Hodgkin Lymphoma, B-cell Lymphoma, Waldenstrom's Macroglobulinemia, Marginal Zone Lymphoma, Chronic Lymphocytic Leukemia, Small Lymphocytic Lymphoma, Primary Central Nervous System Lymphoma	Phase 1
		Neuromyelitis Optica, Neuromyelitis Optica Spectrum Disorder	Phase 1
		Chronic Lymphocytic Leukemia, Mantle Cell Lymphoma	Phase 1 and 2
		Multiple Sclerosis	Phase 2
		Diffuse Large B-Cell, Lymphoma Follicular Lymphoma, Marginal Zone Lymphoma, Small Lymphocytic Lymphoma	Phase 2 and 3
		Chronic Lymphocytic Leukemia	Phase1, 2, 3[c]
		Relapsing Multiple Sclerosis (RMS)	Phase 2, 3[c]

(*continued on next page*)

Table 2. (*Continued*)

Antibody name and company	Target and format	Conditions	Current updates
Tomuzotuximab / CetuGEX™/ GT-MAB5.2 GEX Glycotope	EGFR Chimeric glyco-optimized (reduced fucosylated) IgG1	Solid Tumors Carcinoma, Squamous Cell of Head and Neck	Phase 1 Phase 2
Ifabotuzumab/KB004/IIIA4 Humanigen	EPHA3 Humanized afucosylated IgG1	Glioblastoma Myelodysplastic Syndrome (MDS) Myelofibrosis (MF)	Phase 1 Phase 1 (Suspended) Phase 2 (Suspended)
Bemarituzumab/ FPA144 Five Prime Therapeutics	FGFR2b Humanized afucosylated IgG1	Gastrointestinal Cancer, Metastatic Gastric Cancer Transitional Cell Carcinoma of the Genitourinary Tract (Bladder Cancer)	Phase 1 Phase 1
TrasGEX/ GT-MAB7.3-GEX/ Glycooptimized Trastuzumab-GEX Glycotope	HER2 Humanized glyco-optimized (reduced fucosylated) IgG1	Solid Tumors	Phase 1 (Completed)
Lumretuzumab /RG7116 /RO5479599/ GE-HuMAb-HER3 Roche	HER3 Humanized reduced fucosylated IgG1	Neoplasms Squamous Non-Small Cell Lung Cancer Breast Cancer	Phase 1 (Completed) Phase1 and 2 (Terminated) Phase 1 (Completed)
GSK2849330 GlaxoSmithKline	HER3 Humanized afucosylated IgG1/3	Cancer (Dose escalation study) Cancer (Immuno Positron Emission Tomography study)	Phase 1 (Completed) Phase 1 (Completed)
ARGX-111 Argenx	c-MET Human afucosylated IgG1	Cancer	Phase 1 (Completed)
Roledumab/ LFB R593 LFB Biotechnologies	Rhesus (Rh)D Human IgG1 with low fucose content	Rh disease	Phase 2 and 3
MEDI-570 MedImmune	ICOS Human afucosylated IgG1	Systemic lupus erythematosus Various stages and grades of T cell lymphoma	Phase 1 (Discontinued) Phase 1
Cusatuzumab/ ARGX-110 Argenx	CD70, a member of TNF receptor ligand family Humanized afucosylated IgG1	Acute Myeloid Leukemia, High Risk Myelodysplastic Syndrome Cancer Advanced Cancer	Phase 1 and 2 Phase 1 Phase 1 and 2
AK002 Allakos	Sialic acid immunoglobulin-like lectins (Siglec)-8 Humanized afucosylated IgG1	Healthy Indolent Systemic Mastocytosis Atopic Keratoconjunctivitis, Vernal Keratoconjunctivitis, Perennial Allergic Conjunctivitis Chronic Urticaria Actinic Keratosis	Phase 1 (Completed) Phase 1 Phase 1 Phase 2 Phase 2
BMS-986218 Bristol Myers Squibb	T-cell receptor cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) Afucosylated IgG1	Advanced Cancer	Phase 1 and 2
GM102/ 3C23K GamaMabs Pharma	anti-mullerian Hormone Receptor II (AMHR2) Humanized reduced fucosylated IgG1	Neoplasm, Gynecologic	Phase 1
GSK2831781/ IMP731 GlaxoSmithKline	Lymphocyte activation gene (LAG) – 3 Humanized afucosylated IgG1	Psoriasis	Phase 1

Gatipotuzumab/PankoMab-GEX/ GT-MAB 2.5 GEX Glycotope	Tumor specific glycoepitope of Muc1 (TA-Muc1) Humanized glyco-optimized (reduced fucosylated) IgG1	Solid Tumors Ovarian Epithelial Cancer, Recurrent Fallopian Tube Cancer, Primary Peritoneal Cancer	Phase 1 Phase 2
OBT357/MEN1112/OX-001/OX-357 Oxford BioTherapeutics, in collaboration with Menarini	Bst1/CD157 Humanized afucosylated IgG1	Recurrent Adult Acute Myeloid Leukemia, Acute Myeloid Leukemia, in Relapse	Phase 1
SEA-BCMA Unum Therapeutics and Seattle Genetics	BCMA (B-cell maturation antigen) Humanized afucosylated IgG1	Multiple Myeloma, Multiple Myeloma in Relapse, Refractory Multiple Myeloma	Phase 1
SEA-CD40 Seattle Genetics	CD40 Humanized afucosylated IgG1	Cancer and carcinomas	Phase 1
KHK2823 Kyowa Hakko Kirin Pharma	IL-3Rα / CD123 Humanized afucosylated IgG1	Acute Myeloid Leukemia, Myelodysplastic Syndrome	Phase 1
KHK4083 Kyowa Hakko Kirin Pharma	OX40 Humanized afucosylated IgG1	Dermatitis, Atopic Healthy Men and Subjects With Ulcerative Colitis Ulcerative Colitis, Digestive System Diseases, Colitis, Gastrointestinal Diseases, Inflammatory Bowel Diseases, Intestinal Diseases, Colonic Diseases, Autoimmune Disease, Abdominal Pain	Phase 1 Phase 1 Phase 2
TRX518 Leap Therapeutics	Glucocorticoid-induced TNF receptor (GITR) Humanized aglycosylated IgG1[(d)]	Unresectable Stage III or Stage IV Malignant Melanoma or Other Solid Tumor Malignancies Solid tumors	Phase 1 Phase 1

(a) Only ongoing clinical trials are listed for antibodies that are already on the market
(b) GA101 is being tested in numerous clinical trials for various forms of B-cell associated cancers
(c) Different clinical trials identifiers
(d) This antibody is mutated such that it does not contain the conserved Fc *N*-glycosylation site

The antibody is produced in FUT8-knockout CHO cells (Biowa Potelligent Technology) to achieve afucosylation. Mogamulizumab has demonstrated effectiveness against CTCL in Phase 2 randomized controlled trials.[122] Currently, it is in several clinical trials in combination with other drugs to target several forms of solid tumors. It is also in a Phase 3 clinical trial targeting human T-lymphotrophic virus 1 (HTLV1)-associated myelopathy.

Benralizumab (MEDI-563, Fasenra™) was approved by FDA in November 2017 for the treatment of severe eosinophil asthma. The antibody is produced in FUT8-knockout CHO cells (Biowa Potelligent Technology). It functions by blocking IL-5R signalling and ADCC-mediated depletion of IL-5R*α*-expressing eosinophils.[123] Benralizumab has completed seven Phase 3 studies for asthma treatment. Based on two published Phase 3 studies, benralizumab has reduced the annual exacerbation rate for patients having severe uncontrolled eosinophilic asthma despite treatment with medium to high dosage of inhaled corticosteroids and long-acting beta2-agonists.[124,125] Currently, it is being tested in several clinical trials against eosinophilic chronic rhinosinusitis and chronic obstructive pulmonary disease. A late-phase clinical trial is testing benralizumab for the treatment of patients with chronic allergic reaction to drugs or food, a condition known as chronic idiopathic urticaria, who are unresponsive to H1-antihistamines.

Ublituximab is a chimeric anti-CD20 IgG1 antibody produced in the YB2/0 cell line, which generates antibodies with low fucose and consequently higher ADCC.[126] Ublituximab has completed several Phase 1 and 2 clinical trials against B cell malignancies. In a Phase 1/2 clinical trial in patients with B cell NHL or CLL previously treated with rituximab, ublituximab was well tolerated and efficacious.[127] Currently, it is in several clinical trials in combination with other drugs for treatment of patients with CLL. It is also being tested in combination with the drug teriflunomide for safety and efficacy in patients with relapsing multiple sclerosis in Phase 3 clinical trials.

TrasGEX (GT-MAB7.3-GEX, Glycooptimized Trastuzumab-GEX) was developed by Glycotope. It is a humanized anti-HER2 IgG1 that is glycoengineered through the GlycoExpress Technology, which yields antibodies with humanized and optimized glycosylation pattern. It has completed a Phase 1 trial for dose-escalating and pharmacokinetic analysis in patients with HER2-positive cancers. In a female patient with metastatic $HER2^{+}$ colorectal cancer against which all other options failed, the use of TrasGEX resulted in a 10-fold to 140-fold enhanced ADCC.[128]

SEA-CD40 is a humanized afucosylated anti-CD40 IgG1 developed by Seattle Genetics. The antibody is produced by the sugar-engineered antibody (SEA) technology to eliminate the fucose sugar group to enhance the ADCC activity. The SEA technology involves the use of modified sugars (fucosylation inhibitor, 2-fluorofucose) to inhibit fucosylation during cell culture. Currently, it is in a Phase 1 trial for a range of patients with cancer such as Hodgkin disease, non-small cell lung cancer and melanoma.

Concluding remarks

ADCC is one of the critical effector functions triggered when a therapeutic antibody is used to eliminate target cells. Antibodies specific for CD20 and CD19 have been used to treat B cell malignancies by triggering ADCC. Anti-CD20 antibody rituximab has also been used to deplete B cells in rheumatoid arthritis patients. Antibodies specific for EGFR have been used to target EGFR-positive tumors. Elevated levels of eosinophils in certain severe asthma patients can be removed by antibodies against IL-5R*α* on eosinophils. Studies have shown that antibodies can eliminate HIV- or influenza virus-infected cells by the same mechanism. As discussed in this article, removal of fucose from all these antibodies has significantly improved their ADCC activity in *in vitro* and *in vivo* studies.

The enhanced ADCC activity by afucosylated antibodies was discovered by *in vitro* binding analyses and cell-based ADCC assays. The initial *in vitro* observations have been confirmed in *in vivo* animal models and clinical studies. The enhanced affinity is the result of a unique carbohydrate-carbohydrate interaction between the *N*-glycan of the IgG and the *N*-glycan of the Fc*γ*RIIIa at Asn162. This is the first example where two glycans from two binding partners interact and the carbohydrate-carbohydrate interaction significantly modulates the binding affinity between the two proteins. Because of this novel phenomenon, various approaches have been utilized to target the fucosylation machinery of the host cell lines. A more economically effective approach involves the glycoengineering of mammalian cell lines to produce afucosylated antibodies. As of today, at least 35 glycoengineered antibodies, with their Fc fucose partially or completely removed, have been investigated in animal models (Table 1), 26 of them have been studied in clinical trials and 3 have been approved for use in clinical practice (Table 2). We expect that more afucosylated antibodies will enter clinical trials and subsequently be approved for clinical use.

Abbreviations

AFP	Alpha-fetoprotein
αManII	α-mannosidase II
ADCC	antibody-dependent cellular cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
EGFR	epidermal growth factor receptor
FUT	fucosyltransferase
GFT	GDP-fucose transporter
GKDM	GDP-4-keto-6-deoxy mannose
GnT-III	β-1,4-mannosyl-glycoprotein 4-β-N-acetylglucosaminyltransferase
GMD	GDP-mannose 4,6-dehydratase
HCC	hepatocellular carcinoma
IL-5R	interleukin-5 receptor
NK cells	natural killer cells

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by A*STAR BMRC Strategic Positioning Fund. The authors would like to thank Mr. Ryan Haryadi and Dr. Irene Kiess for careful review of the manuscript.

Funding

Agency for Science, Technology and Research (A*STAR), Singapore.

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