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To cite this article: Natasha A. Pereira, Kah Fai Chan, Pao Chun Lin & Zhiwei Song (2018) The “less-is-more” in therapeutic antibodies: Afucosylated anti-cancer antibodies with enhanced antibody-dependent cellular cytotoxicity, mAbs, 10:5, 693-711, DOI: 10.1080/19420862.2018.1466767

To link to this article: https://doi.org/10.1080/19420862.2018.1466767

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Accepted author version posted online: 07 May 2018. Published online: 07 May 2018.

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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.

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 FcγRII.11 Several approaches have been utilized to increase the affinity between antibody and the FcγRIII. These include engineering the Fc region through amino acid mutations12 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
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 FcγRIIa-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 Lewisα- and Lewisβ-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 Lewisa (Lea), sialyl-Lewisα (SLeα) and Lewisβ (Leb). Type II includes Lewisα (Lea), sialyl-Lewisβ (SLeb) and Lewisβ (Leb). Some of these Lewis antigens are found overexpressed on different types of cancer cells.28,29 SLeα 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-Leβ antibodies showed strong side effects including nausea and vomiting in Phase 1 clinical studies because the expression of Leβ 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.51 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 FcyRIIa.63 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-FcyRIIa 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 FcyRIIa**

The first crystal structure of FcyRIII-IgG1-Fc complex was reported in 2000.8 The FcyRIII used in the study was a soluble FcyRIIIb (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 FcyRIII. As the sFcγRIII preparation used in the study was unglycosylated, it was impossible to evaluate the impact of its N-linked glycan on the FcyRIII-Fc interaction. Nonetheless, the authors did highlight that Asn162 is a potential glycosylation site of FcyRIII 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 FcyRIII (by mutating Asn162 to Gln162), the glycosylated FcyRIII (Asn162) showed reduced affinity for native (fucosylated) IgG antibodies, while antibodies with or without the core fucose showed a similar affinity for unglycosylated FcyRIII.20 However, when fucose-free antibody binds glycosylated FcyRIII (Asn162), the affinity increased significantly. The binding affinities of different glycoforms of IgG-FcyRIII pairs are in the following order: IgG-fucose-free/FcyRIIa-Asn162 >> IgG-native glycan/FcyRIIa-Gln162 >> IgG-native glycan/FcyRIIa-Asn162.20 The authors concluded that the carbohydrate moieties of both FcyRIIa and IgG are important for the interaction. An N-glycan needs to be attached to FcyRIIIa 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 FcyRIIa.

Detailed X-ray crystallography studies on the Fc-FcyRIIa 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 FcyRIIa, 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 FcyRIIa-IgG interaction.

Ferrara et al. demonstrated that the glycosylation at Asn162 of FcyRIII is not essential for the expression of the receptor; however, this glycosylation site is conserved among all FcyRIIIs (or the equivalent) in all mammals studied.19 Furthermore, in all FcyRs, 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 FcyRIII. 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 FcyRIIa, but to a significantly lesser extent**

Recent studies have indicated that Fc galactosylation leads to increased FcyRIIa binding, although to a significantly lesser extent compared to the removal of core fucose.61,67-69 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 FcyRIIIa 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-FcyRIIa 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 FcyRIIa 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. 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.

With the use of computational structure-based design and high-throughput screening, a series of engineered Fc variants were generated. These Fc variants of either single (S239D or I332E), double (S239D/I332E) or triple (S239D/I332E/A330L) mutations were identified and demonstrated up to 169-fold enhanced interaction with human FcγRIIIa. The Fc variants also showed enhanced binding ratio between activating FcγRIIIa 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. In vivo, it showed enhanced anti-tumor effect in mouse lymphoma xenograft over the wild type analogue. 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. An Fc variant 18 with several mutations (F243L/R292P/Y300L/V305I/P396L) was identified and demonstrated about 100-fold enhanced ADCC activity. 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. MGAH22 showed enhanced affinity to both FcγRIIIa variants (F158 and V158), but decreased affinity to inhibitory FcγRIIB. 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. 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). 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. 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. 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. 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.

**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). 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. 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. 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. 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.

**GDP-fucose transporter (SLC35C1)**

It has been shown that loss-of-function mutations in the Golgi GDP-fucose transporter (GFT) gene (Slc35c1) were able to eliminate fucosylation reactions that occur in the Golgi. 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. 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). 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. The CHO-K1 transcriptome data have shown that among all Golgi fucosyltransferases, only Fut8 is expressed. 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. 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. This strategy has been used to produce afucosylated antibodies in a few recent studies.

**Generation of bisecting GlcNac**

β-1,4-mannosyl-glycoprotein 4-β-N-acetylgalcosaminyltransferase (GnT-III) is normally not expressed in CHO cells. GnT-III catalyzes the formation of a bisecting GlcNac by attaching a GlcNac in β-1,4 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. 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). 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. 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. 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. 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 Manα1,3GlcNAc2 core modified with β1,2-xylose and α1,3-fucose. Large complex type N-glycans with mammalian Lea structure containing α1,4-fucose and β1,3-galactose residues were sometimes observed. Antibody N-glycans produced in plants are predominantly GnGnXF3 structures containing the unwanted residues β1,2-xylose and core α1,3-fucose. 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. Strategies to overcome this immunogenicity include use of RNAi knockdown of α1,3-fucosyltransferase (FucT) and β1,2-xylotransferase (XylT) in plants and Fut8/XytT-knockout lines. An afucosylated anti-CD30 monoclonal antibody with G0 structure was produced using glycoengineered aquatic plant *Lemma minor* and shown to have improved ADCC over the same CHO cell-produced antibody. Anti-HIV 2G12 produced in XylT/FucT-knockdown *N. benthamiana* was found to be homogeneous G0 structures with terminal N-acetylgalcosamine and lacking both xylose and α1,3-fucose residues. Further glycoengineering in XytT/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.

**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-acetylgalcosaminidase 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. 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.
### 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-ManI in CHO cells (GlycoMAb Technology) | Enhanced tumor inhibition of GA101 compared with rituximab in human lymphoma xenograft mouse models | Mossner et al. 102 |
| Rituximab Chimeric IgG1 | CD20 | afucosylated | Commercial rituximab treated with endoglycosidase/fucosidase to generate GlcNAc-rituximab | Enhanced depletion of huCD20⁺ B cells in an FcR⁺-humanized mouse model over original rituximab | Li et al. 103 |
| 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. 104 |
| Inebilizumab/MEDI-551 Humanized IgG1 | CD19 | afucosylated | FUT8−/− CHO cells (Potelligent® Technology) | Enhanced B-cell depletion in huCD19/CD20 transgenic mouse over rituximab | Ward et al. 105 |
| 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 | Cardarelli et al. 106 |
| Imagatumumab/GA201/ RG7160 Humanized IgG1 | EGFR | Reduced (~15%) | Coexpression with GnT III and α-ManI in CHO cells (GlycoMAb Technology) | Enhanced survival rate over fucosylated counterpart in mouse xenograft models displaying murine FcγRII and over commercial Cetuximab in mouse xenograft models displaying murine FcγRII and/or human FcγRIIIA. | Gerdes et al. 107 |
| 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-ManI 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. 108 |
| Antibody | Glycoengineering Technology | Reduced Glycoform | Reduced (≤ 10%) Glycoform | Tumor Inhibition Impact |
|----------|-----------------------------|-------------------|--------------------------|------------------------|
| IgG1-IR  | GlycoMAb Technology         | CHO cells         | CHO cells with low level of fucose | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| EGFR     | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| IGF-1R   | Potelligent Technology      | Reduced          | Reduced (≤ 10%)           | Enhanced tumor inhibition over control antibody in xenograft models |
| Name and format | Target | Fucosylation level | Method of glycoengineering | Result in in vivo model (murine/non-human primates) | Reference |
|-----------------|--------|-------------------|----------------------------|-----------------------------------------------|-----------|
| Mogamulizumab/POTELIGEO/ KMO761 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 | Ishi et al. 138 |
| Benralizumab/MEDI-563 Humanized IgG1 | IL-5Ra | afucosylated | FUT8 −/− CHO cells (Potelligent® Technology) | Efficient eosinophils depletion in non-human primates | Kolbeck et al. 123 |
| KHK2823 Humanized IgG1 | IL-3Ra (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-3Ra-positive cells in the peripheral blood of cynomolgus monkeys |  |
| 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. 40 |
| AK002 Humanized IgG1 | Sialic acid immunoglobulin-like lectins 8 (Siglec-8) | afucosylated | FUT8 −/− CHO cells (Potelligent® Technology) | Information not available |  |
| BMS-986218 Human IgG1 | 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 | 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 |
| GSX281781/ 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 |  |
| KHK4083 Human IgG1 | OX40 | afucosylated | FUT8 −/− CHO cells (Potelligent® Technology) | Information not available |  |
| MABS 701 | Bst1/CD157 | afucosylated | FUTB−/− CHO cells (Potelligent® Technology) | Information not available |
|---------|------------|--------------|---------------------------------------------|---------------------------|
| SEA-CD40 | 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 | 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 | 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) Bosseenmaier et al. GE-huMab-HER3, a novel humanized, glycoengineered HER3 antibody with enhanced ADCC and superior preclinical \textit{in vitro} and \textit{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 \textit{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 1 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 and in human FcγR- and CD20-transgenic mice 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 (BOEV) 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. 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 vitro 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 FcγRIIa 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.
| Antibody name and company | Target and format | Conditions | Current updates |
|---------------------------|------------------|------------|----------------|
| **Obinutuzumab/ GA101/ Gazyva (a)** | CD20 Humanized IgG1 with low fucose content | Chronic lymphocytic leukemia; Non-Hodgkin’s lymphoma | Marketed Phase 1, 2, 3<sup>(b)</sup> |
| Roche | | Various forms of B-cell associated lymphomas | Phase 1 |
| | | Idiopathic Lupus Erythematosus | Phase 2 |
| | | Kidney Failure, Chronic | |
| | | Lupus Nephritis | |
| **Mogamulizumab/ POTELIGEO/KM0761 (a)** | CC chemokine receptor 4 (CCRA4) Humanized afucosylated IgG1 | Relapsed or refractory CCRA-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/or Refractory Hodgkin Lymphoma, Recurrent and/or 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 Humanized afucosylated IgG1 | Asthma | Marketed |
| AstraZeneca | | Eosinophilic Gastritis or Gastroenteritis | Phase 1 and 2 |
| | | Hypereosinophilic Syndrome | Phase 2 |
| | | Eosinophilic Chronic Rhinosinusitis | Phase 2 |
| | | Asthma | Phase 2, 3<sup>(b)</sup> |
| | | 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 (a)** | CD19 Humanized afucosylated IgG1 | Scleroderma | Phase 1 (Completed) |
| MedImmune | | Diffuse large B cell lymphoma | Phase 2 (Completed) |
| | | Blood Cancer, Advanced B Cell Malignancies | (Completed) |
| | | Multiple Sclerosis, Relapsing Forms | Phase 1 (Completed) |
| | | Chronic lymphocytic leukemia | Phase 2 (Completed) |
| | | Relapsed/Refractory Aggressive B-cell Lymphomas | (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/ TGI101/ LFB-R603** | CD20 Chimeric IgG1, low fucose content | Various forms of B-cell associated lymphomas | Phase 1 and 2 (Completed) |
| TG Therapeutics Inc | | Chronic Lymphocytic Leukemia | Phase 1 (Completed) |
| | | Chronic Lymphocytic Leukemia, Non-Hodgkin’s Lymphoma | (Completed) |
| | | 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 and 2 |
| | | Chronic Lymphocytic Leukemia, Mantle Cell Lymphoma | Phase 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 | Phase 1, 2, 3<sup>(b)</sup> |
| | | Relapsing Multiple Sclerosis (RMS) | Phase 2, 3<sup>(b)</sup> |

(continued on next page)
| Antibody name and company | Target and format | Conditions | Current updates |
|---------------------------|-------------------|------------|----------------|
| Tomuzotuximab / CetuGEXTM/Glycotope | EGFR Chimeric glyco-optimized (reduced fucosylated) IgG1 | Solid Tumors Carcinoma, Squamous Cell of Head and Neck | Phase 1 Phase 2 |
| Ilabotuzumab/KB004/I1A4 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 /RG5479599/GE-HuMAb-HER3 Roche | HER3 Humanized reduced fucosylated IgG1 | Neoplasms Squamous Non-Small Cell Lung Cancer Breast Cancer | Phase 1 (Completed) Phase 1 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 Argex | c-MET Human afucosylated IgG1 | Cancer | Phase 1 (Completed) |
| Rolodumab/ LFB R593 LFB Biotechnologies | Rhesus (RhD) 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 Argex | 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 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 |
| GSK281781/ IMP731 GlaxoSmithKline | Lymphocyte activation gene 3 (LAG – 3) Humanized afucosylated IgG1 | Psoriasis | Phase 1 |
| Antibody | Description | Indications | Phase |
|----------|-------------|-------------|-------|
| Gatipotuzumab/PankoMab-GEX GT-MAB 2.5 GEX | 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 |
| OB1T357/MBN1112/OX-001/OX-357 | 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-3Rz / 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 2 |
| TRX518 Leap Therapeutics | Glucocorticoid-induced TNF receptor (GITR) Humanized aglycosylated IgG1 | Unresectable Stage III or Stage IV Malignant Melanoma or Other Solid Tumor Malignancies | 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. 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 eosinophilic 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-expressing eosinophils. 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. 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. 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. 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 Glycootope. 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.

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.

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-5Rα 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 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 FcyRIIa 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**

| Abbreviation | Full Form |
|--------------|-----------|
| 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 |
| GMD          | 4-β-N-acetylgalcosaminyltransferase |
| 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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