Improved Effector Functions of a Therapeutic Monoclonal Lewis Y-Specific Antibody by Glycoform Engineering

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Abstract
The aim of the present study was to produce glycosylation variants of the therapeutic Lewis Y-specific humanized IgG1 antibody IGN311 to enhance cell-killing effector function. This was achieved via genetic engineering of the glycosylation machinery of the antibody-producing host. Antibody genes were transiently cotransfected with acetyl-glycosaminyltransferase-III genes into human embryonic kidney-EBV nuclear antigen cells. A control wild-type antibody, IGN311wt, was expressed in the same host using identical expression vectors, but without cotransfection of genes for acetyl-glycosaminyltransferase-III expression. Both expression products were purified to homogeneity and characterized. The glycoengineered expression product (IGN312-Glyco-I) showed a remarkably homogenous N-linked glycosylation pattern consisting of one major hybrid-type, nonfucosylated and agalactosylated form carrying a bisecting GlcNAc-group. Wild-type expression product (IGN311wt) on the other hand was glycosylated by a multitude of different core-fucosylated complex-type structures of variable degrees of galactosylation. Target affinity of the glycoengineered antibody as well as heavy and light chain assembly were not affected by acetyl-glycosaminyltransferase-III expression. In vitro experiments showed a ~10-fold increase of antibody-dependent cellular cytotoxicity of the glycoengineered antibody using different Lewis Y-positive target cancer cell lines (SK-BR-3, SK-BR-5, OVCAR-3, and Kato-III). Complement-mediated cytotoxicity of IGN312-Glyco-I was 0.4-fold reduced using SK-BR-5 as target cell line. The reduction of complement activation could be prevented and even converted into a slight increase of activity by using a different molecular-biological approach directing the glycosylation towards increased levels of complex N-linked oligosaccharides of bisected, nonfucosylated type, as a result of cotransfection of mannosidase II together with acetyl-glycosaminyltransferase-III. (Cancer Res 2005; 65(17): 7934-41)

Introduction
Hybridoma technology (1) initially enabled the production of monoclonal antibodies and their therapeutic use for passive immunotherapy (2–7). For a long period of time, the major bottleneck for monoclonal antibody programs was large-scale production. Actually, product concentrations of high yield clones reach up to 3 g/L in industrial fed-batch fermentation systems by using recombinant expression systems together with sophisticated gene amplification methods (8–13). This spectacular recent increase in productivity may, however, have attained its limit due to cellular physiology (14–16) and in addition, the need to apply high amounts of antibodies, even if these antibodies are chimeric or completely humanized antibodies, can lead to dose-dependent side effects. It could therefore be important to find ways to improve the efficacy of available clinical antibodies.

The goal of the current study was to increase potency of the therapeutic humanized monoclonal antibody IGN311 with the aim to lower the minimal required therapeutic dose (17), and the potential to reduce dose related side effects as well as cost of goods. For this purpose the cell line producing the antibody IGN311 was genetically modified to produce human IgG1 with an engineered glycosylation pattern. IGN311 has been tested successfully in a Rhesus monkey toxicology study, subsequently in a dose escalation phase I trial and is currently under investigation in a phase Ib clinical trial. It targets Lewis Y, a type 2 blood group related oncofetal carbohydrate antigen expressed on nearly 70% of human epithelial carcinomas (18–20). Most normal tissue do not express this blood group associated antigen, except for epithelial cells in the gastrointestinal tract (21–23). Immunohistologic expression analysis in normal adult tissues using tissue arrays showed that Lewis Y is localized mainly cytoplasmatically in these tissues so that anti-Lewis Y antibodies administrated i.v. will not be able to bind to these cells. In contrast, similar studies showed that on tumor cell tissue expression of Lewis Y can be both cytoplasmatically as well as on the surface of the cells (24, 25).3 Only membranous Lewis Y is accessible for passive antibody therapy. For successful clinical treatment with anti-Lewis Y antibodies the fine-specificity of the respective antibody is of importance. Especially the potential cross-reactivity with the more commonly expressed Lewis X antigen and H-type 2 structures on normal tissues or neutrophilic cells may be responsible for observed side effects in other trials with anti-Lewis Y antibodies (26, 27). In general, with IGN311, no side effects like neutropenia, leukopenia, gastrointestinal toxicity or cutaneous allergies (28) were observed and IGN311 showed a good safety profile even in the absence of premedication. Only mild nausea and vomiting was seen in the highest dose during the infusion period, which was easily treated and stopped after finishing the infusion.

In vitro, IGN311 is able to interfere with tumor cell growth by at least three different pathways: antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC; ref. 29), and by inhibition of signal transduction through Lewis

3 G. Sauter, Basel, personal communication.
Y-glycosylated growth factor receptors such as the epidermal growth factor-receptor (30). Based on the clinical observations with IGN311, we expect that enhancing the effector functions of the antibody may lead to an even more pronounced clinical efficacy without changing the side effect profile. It is possible, e.g., to enhance ADCC activity of antibodies by changing the glycosylation of the antibody from a typical core fucosylated complex type to a structure carrying a bisecting Glc-Nac (N-acetylgalactosamine) group and lacking for core fucosylation (refs. 31, 32; Fig. 1). This leads to an increased affinity to FcγRIII (CD16) expressed on natural killer cells, granulocytes, and monocytes (33–35).

Here we show that indeed the lytic potential of a monoclonal antibody can be improved by coexpression of GnT-III transferase (Glyco-I glycoengineering approach). Once an oligosaccharide is bisected by the action of GnT-III transferase, it is no more a suitable substrate for consecutive glycosylation enzymes, especially for Golgi-mannosidase II, galactosidases, and fucosyl transferases (36). N-Linked hybrid oligosaccharide structures as result of incomplete mannoscleavage may show reduced complement activation. The ratio of bisected complex-type to bisected hybrid-type oligosaccharides on the antibody Fc region essentially depends on the sequential action and timing of glycosylation enzymes and could therefore be controlled by coexpression and defined spatial localization of Golgi mannosidase II (Man II) gene together with GnT-III (acetyl-glucosaminyl transferase III) gene in the antibody-producing cell, as shown in a second Glyco-II glycoengineering approach.

Materials and Methods

Cell lines. Tumor cell lines A-431 (37), OVCAR-3 (38), SK-BR-3 (39), and Kato-III (40) as well as human embryonic kidney line (HEK-293) EBV nuclear antigen (EBNA) were purchased from American Type Culture Collection (Manassas, CA). SK-BR-5 was kindly provided by Novartis Research Center Vienna.

Production of glycoengineered antibodies and controls. Glycoengineered versions of IGN311 are named IGN312 and were expressed transiently in HEK-293 EBNA cells. Genes encoding variable regions of IGN311 were amplified from the cell line 392-WCR by PCR. Variable region genes were joined to human IgG, constant region genes and subsequently inserted into one mammalian expression vector each. All vectors for heavy and light chain and for recombinant expression of FcγRIII together with Man-II with the identical IGN311 expression vector into EBNA cells. This time, GnT-III was transfected with its autologous localization domain, which localizes the expression product more towards the trans-Golgi cisternae.

Determination of N-linked oligosaccharide profiles. Oligosaccharides were enzymatically released from the antibodies by N-glycosidase digestion (PNGaseF, QA-Bio, San Mateo, CA) at 0.05 mU/μg protein in 2 mmol/L Tris (pH 7) for 3 hours at 37°C. A fraction of the PNGaseF-treated sample was subsequently digested with endoglycosidase H (EndoH, Roche, Basel, Switzerland) at 0.8 mU/μg protein and incubated for 3 hours at 37°C. The released oligosaccharides were incubated in mild acid (150 mmol/L acetic acid) prior to purification through a cation exchange resin (AG50W-X8 resin, hydrogen form, 100-200 mesh, Bio-Rad, Reinach, Switzerland). The oligosaccharide samples were then analyzed with dHB as matrix using an Autoflex matrix-assisted laser desorption ionization-time of flight (MALDI-TOF; Bruker Daltonics, Faellanden, Switzerland) in positive ion mode. For the assignment of an oligosaccharide structure to each peak, endoglycosidase H was used due to its specificity. It digests most hybrid and high mannose, but not complex oligosaccharides. On EndoH digestion, the peaks at m/z 1,664 and 1,810 Da shift to 1,460 Da, confirming the increase in bisected, nonfucosylated (1,664 Da) and their fucosylated (1,810 Da) versions.

SDS-PAGE. Integrity, molecular weight, and potential degradation products of purified expression product were analyzed by SDS-PAGE using a Novex electrophoresis system (Invitrogen, United Kingdom) on NuPAGE 4% to 12% Bis-Tris gels according to the instructions of the manufacturer. Gels were silver-stained.

Determination of charge distribution. Charge form-distribution was analyzed by isoelectric focusing. Samples were loaded on an isoelectric focusing gel (pH 3 to 10; Invitrogen), and separated according to the instructions of the manufacturer. Gels were silver-stained.

Figure 1. N-Glycosylation pathways. The classical glycosylation pathway leads to complex type core fucosylated oligosaccharide structures with a variable amount of galactosylation (IGN311wt). The presence of GnT-III transferase leads to bisected oligosaccharide chains which inhibit further fucosylation or cleavage of mannosic residues (strike-through arrows) resulting in hybrid-type bisected oligosaccharide structures (Glyco-I type). Balanced and concerted action of Man II followed by GnT-III (dark arrows) leads to bisected complex type nonfucosylated oligosaccharides (Glyco-II type). Respective masses (Da) of oligosaccharides are indicated.
Determination of binding specificity. Binding activity of the expression products was analyzed in a specific sandwich ELISA by incubating antibody samples in serial dilutions (from 100 pg to 1 µg/mL) in microtiter wells coated with the monoclonal anti-idiotypic antibody MAA383 (41). After blocking with 5% FCS and washing, bound expression product was determined by reaction with a goat-immunoglobulin peroxidase conjugate specific for human IgG, IgM, and IgA (Zymed, CA) and stained with o-phenylenediamine/hydrogen peroxide. Optical densities (492 nm) were plotted versus logarithm of the antibody concentration (ng/mL) and fitted using a sigmoidal four-parameter fit using GraphPad Prism 4 software. EC$_{50}$ (50% effective concentration) values were calculated and used for quantification.

Determination of complement-dependent cytotoxicity. Complement mediated cell lysis activity was tested in triplicates in a $^{51}$Cr-release assay using the Lewis Y-positive SK-BR-5 breast cancer cell line as target. Target cells were incubated for 1 hour with 100 µCi of $^{51}$Cr, washed twice with medium, and plated at a density of 20 × 10$^4$ cells per well into a 96-well microplate together with a serial dilution of the sample to be analyzed (100 ng to 50 µg/mL) and complement-active serum from a volunteer donor. The plate was incubated for 1 hour at 37°C in a CO$_2$ incubator. Supernatants were collected and counted for released $^{51}$Cr (Cs). Values for spontaneous release (Sr) and maximum release (Mr) were measured after incubation of representative samples with medium alone and with detergent (SDS), respectively. Complement-mediated cytotoxicity was calculated as the percentage of cell lysis by the formula 100 × (Cs – Sr) / (Mr – Sr) and was plotted against the logarithm of antibody concentration (ng/mL) and fitted using a sigmoidal four-parameter fit using GraphPad Prism 4 software. EC$_{50}$ values were calculated and used for quantification. Samples with negative lyses data were set to 0%.

Determination of antibody-dependent cellular cytotoxicity. ADCC was tested in triplicates in a $^{51}$Cr-release assay using various Lewis Y-positive cancer cell lines as target cells (SK-BR-3, SK-BR-5, Kato-III, and OVCAR-3). Target cells were incubated for 1 hour with 100 µCi of $^{51}$Cr, washed, and plated at a density of 25 × 10$^3$ cells per well into 96-well microplates. Effector cells (peripheral blood mononuclear cells from a healthy volunteer donor) were freshly prepared and added to the target cells to achieve E/T ratios of 40:1 together with serial dilutions (100 pg to 1 µg/mL) of the antibody sample to be analyzed. After incubation at 37°C for 16 hours in a CO$_2$ incubator, cell supernatants were collected and counted for released $^{51}$Cr (Cs). Values for spontaneous release (Sr) and maximum release (Mr) were measured after incubation of representative samples with medium alone and with detergent (SDS), respectively. Cytotoxicity was calculated as percentage of cell lysis by the formula 100 × (Cs – Sr) / (Mr – Sr). The percentage cytotoxicity was plotted against the logarithm of the antibody concentration (ng/mL) and fitted using a sigmoidal four-parameter fit using GraphPad Prism 4 software. EC$_{50}$ values were calculated and used for quantification.

**Results**

Transient Expression Product Glyco-I and Glyco-II
IGN312-Glyco-I and Glyco-II were expressed transiently, respectively, by cotransfection of GnT-III transferase alone (fused to Man II localization domain) or by cotransfection of GnT-III transferase together with Man II. Clones with highest GnT-III activity were selected and expression products were purified to homogeneity.

**N-Linked Glycosylation Pattern**
N-Linked oligosaccharide profiles of the expression products were compared with the one of IGN311wt by MALDI-TOF analysis after deglycosylation and purification of the saccharide fractions. Results are shown in Fig. 2. IGN311wt (A) showed the typical N-linked oligosaccharide pattern of IgG molecules expressed in murine cell lines consisting of three predominant core fucosylated complex-type forms which were agalactosylated (1,485 Da), monogalactosylated (1,647 Da), and digalactosylated (1,810 Da). In contrast, the glycoengineered version IGN312-Glyco-I (B) showed a completely different and remarkably homogenous pattern, which was composed of only two structures. The predominant pattern was a nonfucosylated hybrid-type structure carrying a bisecting GlcNAc residue, containing uncleaved mannose residues (1,664 Da). A second, much less abundant structure was core-fucosylated and also contained a bisecting GlcNAc residue (1,810 Da). The glycosylation pattern of IGN312-Glyco-II (C) on the other hand, differed significantly from the one of Glyco-I and IGN311wt. In contrast to the homogenous pattern of Glyco-I, oligosaccharide distribution consisted of a predominant complex type form without fucose or galactose groups, carrying a bisecting Glc-Nac group (1,543 Da, with expected enhanced ADCC and enhanced CDC). Minor represented oligosaccharide structures were the core-fucosylated version of the predominant saccharide (1,689 Da) and also core fucosylated (1,486 Da) and a-fucosylated (1,340 Da) versions of a complex type a-galactosylated structure without bisecting Glc-Nac group. No hybrid structures could be detected as expected due to the enhanced mannosidase II activity.

Protein Analyses
SDS-PAGE analysis of the expression products IGN311wt, IGN312-Glyco-I, and IGN312-Glyco-II is shown in Fig. 3A. Under nonreducing conditions, all samples showed exactly the same protein bands in the range of ~150 kDa corresponding to the expected molecular weight of intact IgG. Under reducing conditions, protein bands of ~50 and 25 kDa could be detected, corresponding to IgG heavy and light chains, respectively. No differences between the expression products could be found. No degradation products or aggregates were detected. The glycoengineered versions of IGN311 were intact and correctly assembled IgG. Isoelectric focusing analysis is shown in Fig. 3B. Comparison of IGN312 glycovariants and IGN311wt showed exactly the same band distribution between a pH of 7.8 and 8.0. The same predominant charge form is visible for all expression products. Changes in glycosylation pathway obviously had no impact on antibody charge distribution, indicating unchanged charged oligosaccharides.

**Antibody Specificity**
Antibody specificity of the glycoengineered products was analyzed by its antigen binding activity in an anti-idiotypic ELISA. Dilution curves are displayed graphically in Fig. 4A and B (IGN312-Glyco-I and IGN312-Glyco-II, respectively) in comparison with IGN311wt. No significant differences between curves of both glycoengineered products and IGN311wt were observed. The modified glycosylation pattern of IGN312 did not influence the affinity for the target antigen.

**Effector Functions**
Cell lysis potency was studied in vitro using four different Lewis Y-positive tumor cell lines, SK-BR-3, SK-BR-5, OVCAR-3, and Kato-III, as target cells with a broad Lewis Y antigen density distribution. A Lewis Y-negative cell line was used as control. Expression of Lewis Y was checked prior to the lysis experiments by fluorescence-activated cell sorting. Expected wide-ranging antigen densities were confirmed: SK-BR-3, 1,803 mean fluorescence intensity; OVCAR-3, 361 mean fluorescence intensity, and Kato III, 55 mean fluorescence intensity.

**Complement-dependent cytotoxicity.** Figure 5 shows the results of a CDC analysis using SK-BR-5 cells as a target cell line. Data series were fitted using a sigmoid four-parameter fit (goodness of fit, $R^2 > 0.99$). EC$_{50}$ values of 535 ± 4, 806 ± 7, and
$1.375 \pm 9$ ng/mL were calculated for IGN312-Glyco-II, IGN311wt, and IGN312-Glyco-I, respectively. The lysis potential of IGN312-Glyco-I via complement activation was $\sim 0.4$-fold reduced in comparison to IGN311wt. In contrast, IGN312-Glyco-II showed a 1.5-fold enhanced activity in comparison to IGN311wt, indicating that cotransfection of GnT-III together with Man II indeed could at least restore CDC activity, probably by reducing the amount of hybrid structures in N-linked oligosaccharide pattern.

**Antibody-dependent cellular cytotoxicity.** Results are shown in Fig. 6. IGN312-Glyco-I was compared with IGN311wt on three cell lines: Kato III, OVCAR-3, and SK-BR-3 (Fig. 6A). Data series were fitted using a sigmoid four-parameter model. EC$_{50}$ of IGN311wt and IGN312-Glyco-I were calculated and represented graphically (Fig. 6B). IGN312-Glyco-I shows a 6- to 11-fold improved lytic activity on investigated cell lines: on SK-BR-3, EC$_{50}$ values of 7.5 and 49.4 ng/mL were calculated for IGN312-Glyco-I and IGN311wt, respectively, on OVCAR-3, expressing Lewis Y at lower density, values of 7.7 and 87.2 ng/mL could be found. On Kato-III, the cell line with the lowest Lewis Y density, EC$_{50}$ values were 153.4 and 1,165 for Glyco-I and IGN311wt, respectively. It should be noted that the glycoengineered antibody reached a higher maximal level of toxicity relative to the unmodified antibody. Therefore, an EC$_{50}$ comparison in the classical sense is not possible. A direct correlation between Lewis Y antigen density measured by fluorescence-activated cell sorting analysis (geometric mean fluorescence intensity) could be observed both for the glycoengineered and for the wild-type expression products. Cell lines expressing the target antigen Lewis Y at lower densities required higher amounts of IgG to induce the same lysis. The Lewis Y-negative cell line could not be lysed by the antibodies.
A direct comparison of glycoengineered expression products IGN312-Glyco-I and IGN312-Glyco-II to IGN311wt was done on SK-BR-5 cells (Fig. 6C). EC50 values of 25, 5, and 3 ng/mL were calculated for IGN311wt, IGN312-Glyco-I, and IGN312-Glyco-II, respectively. As expected, no significant differences between the lyses curves of both glycoengineered variants IGN312-Glyco-I and Glyco-II were observed. An ~8-fold enhanced lytic activity in comparison to IGN311wt was calculated with SK-BR-5 as a target cell line, but again, the glycoengineered antibody achieves a higher maximal lysis compared with the unmodified antibody.

Discussion

In the present study, we show that by selectively altering the glycosylation pattern of a humanized IgG1 antibody, the lytic potential of this antibody can be enhanced >10-fold without affecting the affinity and specificity of the antibody. The parent antibody used for this study was IGN311, a monoclonal antibody against Lewis Y, currently under investigation in a phase Ib clinical trial. IGN311 may interfere with tumor cell growth by classical effector functions like CDC and ADCC, but also through inhibition of signal transduction by Lewis Y glycosylated growth factor receptors (30). Different technologies to enhance IgG potency by increasing effector functions have been reported: Changing binding affinity to Fc receptors by altering amino acid sequences (42) or by glycoengineering the carbohydrate component of the Fc-part, particularly reducing core fucosylation (32, 43–45) in a direct or indirect manner. Two glycoengineering strategies have been described recently: Umana et al. (31) overexpressed GnT-III in antibody-producing cells leading to increased levels of bisected, nonfucosylated oligosaccharides. Yamane-Ohnuki et al. (46) achieved a reduction of core fucosylation by recombinant antibody expression in Chinese hamster ovary cells lacking core-fucosyl transferase activity, whereas Mori et al. (47) maximized effector functions of expressed antibodies using fucosyl transferase specific short interfering RNA. Both strategies resulted in an increased ADCC. We have chosen to coexpress GnT-III transferase in the producer cell line. GnT-III transferase leads to the addition of a bisecting GlcNAc residue which has an important influence on multiple subsequent enzymatic glycosylation reactions in the Golgi complex of the cell, especially on the inhibition of Golgi α-mannosidase II, α1,6 fucosyltransferase or galactosyl-transferases. Our data show that a specific change of the glycosylation pattern of an IgG1 antibody by overexpression of GnT-III transferase in the antibody-producing cell line has a significant impact on Fc-mediated effector functions, particularly ADCC, and to a lesser degree, CDC. GnT-III transferase was spatially distributed for this
purpose along the Golgi-apparatus by fusing Man II localization domain to the catalytic domain of GnT-III. The antibody characteristics such as antigen-binding affinity, its assembly and its stability were not affected by these modifications. Enhancing effector functions of this antibody may therefore improve tumor cell destruction without increasing cross reactivity–related side effects.

An interesting additional effect of this glycoengineering strategy is the inhibition of mannose cleavage caused by the presence of a bisecting GlcNAc-group and by sterical hindrance of Golgi Man-II, which leads to increased levels of bisected, hybrid type oligosaccharides. These forms seem to be related to a moderate reduction of complement activation (Fig. 1). By cotransfecting the GnT-III transferase (with its autologous localization domain directing the enzyme towards the trans-Golgi cisternae) together with Man-II, the CDC activity could be recovered and even slightly enhanced in IGN312-Glyco-II, as summarized in Fig. 1. The concerted action of Man II followed by GnT-III was enabled by specifically localizing the corresponding enzymes in the Golgi and represents a requirement for accessing to mainly complex type bisected oligosaccharides (36). Depending on the clinical need, fine-tuning of complement activation and a reduced CDC activity, found for Glyco-I strategy, may be preferred because it has been reported that CDC may be responsible for the severe "first-dose side effects," as seen with rituximab in lymphoma patients (48).

Another interesting side effect shown in this study is a significant reduction of glycoform complexity of glycoengineered antibodies, especially for Glyco-I. A reason for this effect could be the presence of a bisecting-GlcNAc group which inhibits several subsequent glycosylation steps (32). This leads to IgG molecules glycosylated with one major glycoform (in our case bisected; a-galacto and a-fucosylated) in contrast to normal immunoglobulins whose N-linked oligosaccharides are composed of at least three major forms of different degrees of galactosylation and a multitude of less abundant forms (Fig. 2). Molar ratios of these complex structures would depend mainly on multiple factors described as "culture and host conditions" (e.g., pH, medium, stress factors, temperature, cell density, culture growth-phase) and the control of their synthesis is challenging and necessary, because each glycoform may have a different biological activity, especially in the case of effector functions of monoclonal antibodies (49). A homogenous glyco-profile, consisting of one reproducible and stable major oligosaccharide form, may reduce product complexity and simplify analytic measures for quality control.

This technology may represent an important step towards more potent and therapeutic antibodies and may also allow therapeutic dose reduction with a potential positive impact on production economics. An inverse correlation between target antigen (Lewis Y) expression density and required EC_{50} concentrations was shown in

![Figure 5](https://example.com/figure5.png)

**Figure 5.** CDC analysis (chromium release) on SK-BR-5 target cells. CDC of IGN312-Glyco-I (light gray curve), IGN312-Glyco-II (black curve) and IGN311wt (gray curve) were compared. Data series were fitted using a four-parameter sigmoidal fit (goodness of fit, R^2 > 0.99). Lyses potential was evaluated at EC_{50}: IGN311 wild-type, 806 ± 70 ng/mL; IGN312-Glyco-I, 1,375 ± 86 ng/mL; IGN312-Glyco-II, 535 ± 38 ng/mL.

![Figure 6](https://example.com/figure6.png)

**Figure 6.** ADCC lysis experiments: A, comparison of IGN312-Glyco-I to IGN311wt on Kato III (black curves), on OVCAR-3 (gray curves), and on SK-BR-3 (light gray curves). B, graphical display of EC_{50} values. C, comparison of IGN312-Glyco-I and IGN312-Glyco-II (black curves) and IGN311wt (gray curve) on SK-BR-3. Data series were fitted using a four-parameter sigmoidal fit (goodness of fit, R^2 > 0.99 for all cases). Lyses potential was evaluated at EC_{50}: IGN311 wild-type, 49.4, 87.2 and 1,165 ng/mL; IGN312-Glyco-I, 7.5, 7.7, and 153.4 ng/mL on SK-BR-3, OVCAR-3 and Kato-III cells, respectively; IGN311 wild-type, 25 ± 7 ng/mL; IGN312-Glyco-I, 5 ± 3 ng/mL, and IGN312-Glyco-II, 3 ± 3 ng/mL on SK-BR-5 cells.
ADCC experiments meaning that cells with lower antigen density would require higher amounts of IgG to induce the same lysis. The observed shift to lower EC_{50} values as observed for the glycoengineered antibody would therefore target at the same antibody concentrations a much broader spectrum of Lewis Y-positive tumor cells with regard to their antigen density. These findings may be of clinical relevance due to highly variable target antigen expression on tumor cells, tumor tissues, or derived metastases.

A limitation of classical antibody-based therapies has been reported to be related to a functional polymorphism of FcyRII receptor on effector cells. The FcyRII-158V isofrom, which is present in low frequency in the population shows good affinity to both natural and glycoengineered antibodies (43, 50). Whereas the predominant isofrom FcyRII-158F, in contrast only shows high affinity to glycoengineered antibodies and not to classical antibodies. Glycoengineering should therefore dramatically increase the number of clinical responders to passive antibody therapies based on lytic effector functions.

The issue of tolerability and toxicity of glycoengineered antibodies has thus far not been investigated. Although the glycoengineered antibodies show no change in affinity for the target antigen, it cannot be excluded at this stage that unwanted cross-reactivity with normal tissue is not also enhanced by the modification of the antibody. Our ADCC experiments with lower antigen density-expressing cell lines indicate that the glycoengineered antibodies were able to lyse these cells more potently than the parent antibody, still this doesn't mean that normal tissue with probably much lower Lewis Y expression is also affected at the same range. The aim of producing a more potent antibody was to lower the minimally effective clinical dose, which should compensate for possible side effects on normal tissue. However, first data addressing this very important issue can only be obtained from in vivo studies in suitable animal species with a similar target antigen expression on normal tissue (for Lewis Y, this could be in primates and dogs; ref. 51), whereas conclusive data will only come from the first phase I studies in human subjects.

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