Modulating Cytotoxic Effector Functions by Fc Engineering to Improve Cancer Therapy

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Summary
In the last two decades, monoclonal antibodies have revolutionized the therapy of cancer patients. Although antibody therapy has continuously been improved, still a significant number of patients do not benefit from antibody therapy. Therefore, rational optimization of the antibody molecule by Fc engineering represents a major area of translational research to further improve this potent therapeutic option. Monoclonal antibodies are able to trigger a variety of effector mechanisms. Especially Fc-mediated effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC) are considered important in antibody therapy of cancer. Novel mechanistic insights into the action of monoclonal antibodies allowed the development of various Fc engineering approaches to modulate antibodies’ effector functions. Strategies in modifying the Fc glycosylation profile (Fc glyco-engineering) or approaches in engineering the protein backbone (Fc protein engineering) have been intensively evaluated. In the current review, Fc engineering strategies resulting in improved ADCC, ADCP and CDC activity are summarized and discussed.

Introduction

Monoclonal antibodies have improved significantly the therapeutic options of patients and are standard in the treatment of cancer today [1–4]. However, a considerable number of patients still do not benefit from antibody therapy and relapse remains a serious issue. Thus, further optimization of the antibody molecule to achieve a higher therapeutic efficacy represents a major area in current translational research.

New avenues for the generation of rationally designed ‘fit-for-purpose’ antibodies were opened by a better understanding of antibody effector functions and their relative contribution to the antibody’s therapeutic efficacy. Effector functions of antibodies are complex, and antibodies are able to kill cancer cells by various mechanisms in vitro (fig. 1). These include direct induction of cell death by receptor cross-linkage or blockade of receptor-ligand interactions, complement-dependent cytotoxicity (CDC), and recruitment of effector cells for antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) by engagement of activating Fcγ receptors (FcγRs). In particular, as revealed in various animal models, Fc-mediated effector cell recruitment and functions such as ADCC or ADCP were suggested to play crucial roles for tumor-targeting antibodies. For example, the antibodies rituximab or trastuzumab lost their therapeutic activity in genetically modified mice either lacking expression of activating FcγR or being defective in FcγR signaling, while their efficacy was enhanced in FcγRIIb knocked-out mice [5, 6]. Moreover, analysis of isotype switch variants with different ratios of affinities to activating and inhibitory FcγR (A:I ratio), revealed that antibodies with a higher A:I ratio had superior therapeutic activity [7]. However, the role of distinct FcγR is complex, as FcγRIIB binding promoted the pro-apoptotic activities of agonistic antibodies targeting members of the tumor necrosis factor receptor superfamily [8–11]. The importance of FcγR engagement was also sup-
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The presence of the oligosaccharide attached to the asparagine residue at position 297 (N297) in IgG1 antibodies is critical for Fc-attached oligosaccharide to manipulate Fc-mediated antibody effector functions. IgG antibodies typically contain a glycan (black) attached to amino acid N297 in each heavy chain (dark grey, heavy chain, light grey, light chain). The N-297 attached glycans contain a core structure comprising N-acetylgalactosamine (GlcNAc) and mannose (Man) residues, which can be extended by fucose (Fuc), a bisecting GlcNAc and galactose (Gal) and sialic acid (NANA) residues.

Further complexity derives from the fact that complement may deposit and anaphylatoxin formation were shown to enhance therapy [20, 21].

In patients, clear evidence is lacking. How -

certain mouse models C1q knockout resulted in loss of therapeutic activity [18, 19] pointing to an important role of CDC. The functional consequences of FcγRIIa-H131 engagement are not yet understood in detail. The presence of the oligosaccharide attached to the asparagine residue at position 297 (N297) in IgG1 antibodies is critical for Fc-attached oligosaccharide to manipulate Fc-mediated antibody effector functions. IgG antibodies typically contain a glycan (black) attached to amino acid N297 in each heavy chain (dark grey, heavy chain, light grey, light chain). The N-297 attached glycans contain a core structure comprising N-acetylgalactosamine (GlcNAc) and mannose (Man) residues, which can be extended by fucose (Fuc), a bisecting GlcNAc and galactose (Gal) and sialic acid (NANA) residues.

Further complexity derives from the fact that complement may deposit and anaphylatoxin formation were shown to enhance therapy [20, 21].

In certain mouse models C1q knockout resulted in loss of therapeutic activity of rituximab at least in indi-

The functional consequences of FcγRIIa-R131 engagement are not yet understood in detail. In addition, the relative contribution of selected effector cell populations (i.e. NK cells, monocytes, macrophages, granulocytes), has not been completely clarified [15–17].

Whereas the observations outlined above indicate that efficient FcR engagement determines the efficacy of therapeutic antibodies in patients, the role of complement has not strictly been proven. In certain mouse models C1q knockout resulted in loss of therapeutic activity. FcγRIIIa-V158 binds the IgG1 Fc domain with higher affinity compared to FcγRIIIa-F158, resulting in stronger ADCC activity [14]. Thus, C3b deposition triggered by target cell-bound antibodies was shown to hinder the interaction of the antibody with FcγRIIIa on NK cells, resulting in diminished ADCC [24]. Therefore, efficient complement fixation is beneficial when induc-

In conclusion, available experimental evidence may suggest that only a selected repertoire of effector functions is available depending on target antigen characteristics, tumor type, tumor burden, tumor location, antibody isotype, and availability of distinct components of the patients’ immune system [27–29].

Based on these observations, antibody Fc engineering strategies were developed with the aim to further improve the performance of therapeutic antibodies by enhancing the antibody’s ability to induce CDC or to trigger effector cell-mediated killing [30–32]. In addition, other modifications were established to abolish selected Fc functions, which for example may allow reducing unwanted side effects. Here, different Fc engineering technologies to improve ADCC and CDC activity of human IgG1 antibodies are summarized.
The presence of the oligosaccharide attached to the asparagine residue at position 297 (N297) in IgG1 antibodies is critical for binding to FcγR and the complement factor C1q. While non-glycosylated antibodies displayed significant structural changes in the CH2 domain [33] leading to a closed conformation hindering binding to FcγR or C1q, antibodies with a monosaccharide attached to the Fc domain still mediated effector functions via FcγR [34]. The impact of Fc glycosylation on antibodies’ ADCC activity was supported using CAMPATH-H1 expressed in different cell lines resulting in distinct glycosylation profiles [35]. Based on these and other related observations, controlled alteration of the Fc-attached oligosaccharide to manipulate Fc-mediated antibody functions became one major issue in antibody engineering [36]. In particular, a reduced content of fucose or sialic acid was correlated with improved ADCC activity (fig. 2; table 1) [37, 38]. Based on these findings, various strategies have been developed allowing rational engineering of the glycosylation profile to boost ADCC.

For reduction in core fucosylation different technologies are available. The most straightforward approach is the use of cell lines, such as the Lec13 CHO mutant with reduced capacity to incorporate fucose in the antibody-attached oligosaccharide [39]. In addition, generation of completely non-fucosylated antibodies was achieved by knockout of the FUT8 gene encoding the α1,6-fucosyltransferase (FUT8® technology) [40].

In an alternative approach, overexpression of N-acetylglucosaminyltransferase III (GnT III) led to the attachment of a bisecting GlcNAc residue (GlycoMAb® technology), directly resulting in improved FcR binding [41]. Importantly, the bisected oligosaccharide is not a suitable substrate for α1,6-FucT, resulting in non-fucosylated antibody species [42]. By applying this strategy, an anti-neuroblastoma antibody was glyco-engineered. The 20-fold improvement in NK cell-mediated ADCC correlated with the relative content of bisected, non-fucosylated oligosaccharides [43]. Similar results were observed for a glyco-engineered rituximab variant [41] and a CD19 antibody [44]. Subsequently, alternative strategies have been developed to reduce core fucosylation in mammalian cell lines (table 1) [40, 41, 43, 45]. Mainly mammalian cell lines have been used in the production of glyco-engineered antibodies, but also non-mammalian expression hosts such as yeasts and plants genetically modified to allow production of antibodies with defined carbohydrate structures might be an option. Besides engineering the expression host, also chemo-enzymatic modification has been used for rational remodeling of Fc-bound N-glycans. Although antibodies with well-defined carbohydrate structures and optimized effector functions can be realized, a more complex production process may generate additional costs [46].

From a mechanistic point of view, Ferrara and colleagues [47] unraveled that high affinity interaction of human IgG1 with FcγRIIa is due to reciprocal action between the receptor carbohydrate and areas of the antibody’s Fc part that are only accessible when fucose is lacking. Importantly, co-crystal structures of FcγRIIa and non-fucosylated Fc domains suggested that the N-glycan structure at the asparagine residue at position 162 (N162) of FcγRIIa interacts with the Fc-bound carbohydrate structure [48, 49]. FcγRIIIa and FcγRIIB are the only human FcR which are glycosylated at this position, explaining the selective increase in the antibodies’ affinity for FcγRII [47–49].

 Besides the obvious impact of fucose levels on FcγRIIIa-triggered NK cell-mediated ADCC, also the cytolytic and phagocytic activities of neutrophils expressing the highly homologous FcγRIIb isofrom is modulated by lack of core fucosylation. While phagocytosis of target cells coated with non-fucosylated antibodies was improved [50], in another study impaired neutrophil-mediated ADCC was observed after removal of fucose [51, 52].

Besides fucose, also other sugar residues in the Fc-attached oligosaccharide were shown to modulate ADCC activity. Recently, hypergalactosylation of IgG1 was correlated with enhanced ADCC activity [53], although the effects were less pronounced compared to removal of fucose. Also, incorporation of a bisecting sugar residue by enzymatic remodeling resulted in enhanced ADCC activity [54]. Scallon and colleagues [55] demonstrated that for selected antibodies a higher level of sialic acid resulted in reduced ADCC activity, while for other antibody specificities differences in sialic acid levels had no impact on FcγRIIa binding but unexpectedly on antigen binding capacity. Expression of modified sialidase A in host cell lines resulted in expression of non-sialylated monoclonal antibodies, consistently demonstrating stronger ADCC activity than sialylated counterparts [56].

### Table 1. Approaches to manipulate antibody fucosylation

<table>
<thead>
<tr>
<th>Technique</th>
<th>Fucosylation level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified/mutated cell line (YB02, LEC13)</td>
<td>variable</td>
<td>[35, 37]</td>
</tr>
<tr>
<td>N-acetylgalcosaminyltransferase III (GnT III) overexpression</td>
<td>variable</td>
<td>[43]</td>
</tr>
<tr>
<td>Co-expression of heterologous GDP-6-deoxy-D-lyxo-4-hexulose reductase</td>
<td>variable</td>
<td>[84]</td>
</tr>
<tr>
<td>FUT8 gene knockout</td>
<td>afucosylated</td>
<td>[40]</td>
</tr>
<tr>
<td>FUT8 siRNA knockdown</td>
<td>afucosylated</td>
<td>[85]</td>
</tr>
<tr>
<td>Antibody expression in the presence of the glycosidase inhibitor kifunensine</td>
<td>variable</td>
<td>[45]</td>
</tr>
<tr>
<td>Zinc-finger nuclease mediated knockdown of GDP-fucose transporter gene</td>
<td>afucosylated</td>
<td>[86]</td>
</tr>
</tbody>
</table>

**Enhancing ADCC and ADCP Activity**

**Glyco-Engineering**

The presence of the oligosaccharide attached to the asparagine residue at position 297 (N297) in IgG1 antibodies is critical for binding to FcγR and the complement factor C1q (fig. 2). While non-glycosylated antibodies displayed significant structural changes in the CH2 domain [33] leading to a closed conformation hindering binding to FcγR or C1q, antibodies with a monosaccharide attached to the Fc domain still mediated effector functions via FcγR [34]. The impact of Fc glycosylation on antibodies’ ADCC activity was supported using CAMPATH-H1 expressed in different cell lines resulting in distinct glycosylation profiles [35]. Based on these and other related observations, controlled alteration of the Fc-attached oligosaccharide to manipulate Fc-mediated antibody functions became one major issue in antibody engineering [36]. In particular, a reduced content of fucose or sialic acid was correlated with improved ADCC activity (fig. 2; table 1) [37, 38]. Based on these findings, various strategies have been developed allowing rational engineering of the glycosylation profile to boost ADCC.

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**Protein Engineering**

Exchanging amino acids directly in the FcγR binding site within the antibody’s Fc domain was a logical strategy to manipu-
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The gain in FcγRIIIa affinity for selected protein-engineered human IgG1 molecule enhanced ADCC activity with NK cells, especially when the low-affinity FcγRIIIa-F158 allele was expressed homozygously. Although many of the described amino acid exchanges enhanced affinity to FcγR, these variants often differed in their affinities to other FcγRs. Lazar and colleagues [39] generated an Fc variant (S239D-I332E) with enhanced FcγRIIIa binding affinity which also displayed a higher affinity to FcγRIIa and FcγRIIb (fig. 3A; table 2). Antibodies harboring the S239D-I332E amino acid modification were more efficient in inducing NK cell-mediated ADCC than native IgG1 molecules. Impressively, this variant even triggered stronger ADCC than the triple Fc variant described by Shields and colleagues [57]. With regard to rituximab, the S239D-I332E variant showed improved ADCC activity with NK cells and a modest enhancement in ADCP by macrophages. In contrast, CDC activity was not affected. In non-human primates this S239D-I332E rituximab variant depleted half of the circulating B cells at an approximately 50-fold lower dose level than the non-engineered IgG1 counterpart [59]. The S239D-I332E modification was also applied to enhance ADCC in other antibodies targeting CD20 [60].

Table 2. Selected engineered IgG1-Fc variants with enhanced ADCC activity

<table>
<thead>
<tr>
<th>Variant</th>
<th>FcγRIIIa binding</th>
<th>FcγRIIa binding</th>
<th>FcγRIIb induction</th>
<th>X-fold reduction in EC50 value</th>
<th>Complement activation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>–</td>
<td>+</td>
<td>[57]</td>
</tr>
<tr>
<td>S298A-E333A-K334A</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
<td>10–100 n.d.a.</td>
<td>[57]</td>
<td></td>
</tr>
<tr>
<td>S239D-I332E</td>
<td>↑↑↑</td>
<td>↑</td>
<td>↑↑↑</td>
<td>10–100 n.d.a.</td>
<td>[59]</td>
<td></td>
</tr>
<tr>
<td>S239D-I332E-A330L</td>
<td>↑↑↑</td>
<td>↑</td>
<td>↑↑</td>
<td>10–100</td>
<td>[59]</td>
<td></td>
</tr>
<tr>
<td>L235V-F243L-R292P-Y300L-P396L</td>
<td>↑↑↑</td>
<td>↑</td>
<td>↑↑</td>
<td>10–100</td>
<td>n.d.a.</td>
<td>[60, 83]</td>
</tr>
</tbody>
</table>

EC50 = Effective concentration 50 %; ↑ = enhanced activity/binding compared to wt; ↓ = reduced activity/binding compared to wt; n.d.a. = no data available.

Laforet and colleagues [57] exchanged all solvent exposed amino acids in the Fc domain of human IgG1 to alanine and thereby identified various Fc domain variants with altered FcγR binding profiles. In this pioneering approach a triple variant (S298A-E333A-K334A) was identified, which exerted a higher affinity to FcγRIIIa but showed diminished binding to FcγRIIa and FcγRIIb. This modification resulted in enhanced ADCC activity with NK cells, especially when the low-affinity FcγRIIIa-F158 allele was expressed homozygously. Subsequently, a variety of Fc variants with increased FcγRIIIa binding profile and increased ADCC activity were discovered by following different experimental strategies [58–60]. Although many of the described amino acid exchanges enhanced affinity to FcγRIIIa, these variants often differed in their affinities to other FcγRs. These data may further suggest that a certain threshold in FcγR engagement has to be overcome to reach maximal NK cell-mediated ADCC. This threshold is already overcome by selected protein-engineered Fc variants or by non-fucosylated Fc glycans, which are found in different isotypes and may account for improved FcγR binding.

Selected engineered IgG1-Fc variants with enhanced ADCC activity

Fig. 3. Fc protein-engineering strategies for enhanced ADCC or CDC.

A Antibody model of a human IgG1 molecule engineered by exchanging selected amino acid positions. Yellow: amino acid substitutions introduced in Margetuximab (MacroGenics); [62]. Purple: amino acid substitutions introduced in MOR208, Xmab-5574 (Xencor, Morpholinos); [59]. B Antibody model of an antibody molecule engineered by exchanging larger amino acid stretches between the IgG1 and IgA1 isotypes (IgGA cross-isotype) [65]. C IgG1 molecule with marked E345 position. Antibodies harboring amino acid exchanges at this (or other) positions are expressed as monomeric IgG1 molecules but form hexamers at the cell surface of target cells after antigen binding [73].

EC50 = Effective concentration 50 %; ↑ = enhanced activity/binding compared to wt; ↓ = reduced activity/binding compared to wt; n.d.a. = no data available.
to engineer a CD19 antibody [61, 62]. While the corresponding native CD19 IgG1 antibody had no B-cell depleting activity in monkeys, the Fc-optimized variant efficiently eliminated peripheral B cells [63]. The S239D–I332E variant was recently proven to enhance ADCC activity also in CD33 or CD133 antibodies targeting AML tumor cells [64].

While many Fc protein engineering approaches are based on substitution of selected individual amino acids, alternative strategies have been developed by exchanging larger amino acid stretches between different isoforms. These so-called cross-isotypes demonstrated interesting profiles of effector functions. For example, Kelton and colleagues [65] reported the engineering of an IgGA ‘cross-isotype’ antibody which combines selected effector functions of both IgG and IgA (fig. 3B). IgGA binds to FcαRI, FcγRI and FcγRIIa, while no binding to FcγRIIa was observed. A trastuzumab IgGA potently activated both neutrophils and macrophages and mediated higher CDC than IgG1 or IgA antibodies.

**Combining Glyco- and Protein Engineering**

Most engineering strategies focus on either introducing amino acid exchanges in the protein backbone or in modifying the Fc-bound glycosylation profile. Recently, also combined Fc engineering approaches have been described. The ADCC-optimized S298A–E333A–K334A Fc variant was glyco-engineered by expression in Lec13 cells to reduce fucose content. This double-engineered variant displayed improved NK cell-mediated ADCC activity [66]. Yet, in a similar approach employing S298D–I332E, S239D–I332E–A330L Fc variants, improved ADCC activity was not observed, although the affinity of FcγRIIa binding was 10-fold increased [67, 68]. The gain in FcγRIIa affinity for selected protein-engineered and glyco-engineered variants may therefore be based on different mechanisms. While protein–protein interactions are improved by amino acid substitutions, stronger interactions between Fc- and FcR-attached carbohydrates may account for improved FcR binding. These data may further suggest that a certain threshold in FcγRIIa engagement has to be overcome to reach maximal NK cell-mediated ADCC. This threshold is already overcome by selected protein-engineered Fc variants or by non-fucosylated Fc parts. However, these findings need to be confirmed in a more complex in vivo situation.

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**Table 3.** Selected engineered IgG1–Fc variants with enhanced CDC activity

<table>
<thead>
<tr>
<th>Variant</th>
<th>ID</th>
<th>Clq fold binding</th>
<th>CDC (fold) potency</th>
<th>ADCC (fold) potency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>[69]</td>
</tr>
<tr>
<td>K326W</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>[69]</td>
</tr>
<tr>
<td>K326W–E333S</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>[69]</td>
</tr>
<tr>
<td>S267E–H268F–S324T</td>
<td>EFT 47</td>
<td>6.9</td>
<td>0.045</td>
<td>[70]</td>
<td></td>
</tr>
<tr>
<td>S267E–H268F–S324T–G236A–I332E</td>
<td>EFT + AE</td>
<td>23</td>
<td>1.2</td>
<td>[70]</td>
<td></td>
</tr>
<tr>
<td>IgG1/IgG3 chimera</td>
<td>113F</td>
<td>7.1</td>
<td>3.7</td>
<td>1</td>
<td>[70, 71]</td>
</tr>
<tr>
<td>E345K (hexamer variant)</td>
<td>1</td>
<td>&gt;10</td>
<td>2–5</td>
<td>[74]</td>
<td></td>
</tr>
<tr>
<td>E430G (hexamer variant)</td>
<td>1</td>
<td>&gt;10</td>
<td>2–5</td>
<td>[74]</td>
<td></td>
</tr>
</tbody>
</table>

n.d.a. = No data available.

**Enhancing CDC Activity**

Protein engineering could also be applied to improve IgG2 antibodies’ capacity to trigger CDC (table 3). Several studies investigated the impact of selected amino acid substitutions in the CH2 domain on complement activation [69, 70]. Idusogie and colleagues [69] identified amino acids K326 and E333 to be critical in Clq binding, Moore et al. [70] analyzed a variety of Fc variants thereby unraveling S267, H268 and S324 to be important for effective CDC. Variants carrying K326W or E333S substitutions resulted in enhanced CDC activity. However, CDC was not further enhanced by their combination although it resulted in a 5-fold higher Clq binding capacity compared to variants carrying the individual substitutions. Interestingly, whereas variants carrying either K326W or E333S substitutions lacked significant ADCC activity, the double-mutant K326A–E333A demonstrated enhanced CDC activity, while ADCC activity was unaffected. A roughly 7-fold improvement in CDC activity was reached by exchanging three amino acids (S267E–H268F–S324T). This triple-mutant displayed substantially diminished ADCC activity, which could be restored by introducing two additional amino acid exchanges G236A and I332E, resulting in wild type-like ADCC activity and an even further enhanced CDC activity. An alternative approach was developed by Natsume and colleagues [71] by designing mixed IgG1/IgG3 Fc variants with significantly enhanced CDC activity.

Most CDC-optimized Fc variants were designed to more potently trigger CDC while maintaining FcγR-mediated ADCC activity. Recently, Lee and colleagues [26] described novel Fc mutations enabling selective binding to Clq without concomitant engagement of Fcγ receptors. The engineered Fc domains were introduced into the rituximab and rituximab Fab molecules thereby unraveling S267, H268 and S324 to be important for effective CDC. Variants carrying K326W or E333S substitutions lacked significant ADCC activity, the double-mutant K326A–E333A demonstrated enhanced CDC activity, while ADCC activity was unaffected. A roughly 7-fold improvement in CDC activity was reached by exchanging three amino acids (S267E–H268F–S324T). This triple-mutant displayed substantially diminished ADCC activity, which could be restored by introducing two additional amino acid exchanges G236A and I332E, resulting in wild type-like ADCC activity and an even further enhanced CDC activity. An alternative approach was developed by Natsume and colleagues [71] by designing mixed IgG1/IgG3 Fc variants with significantly enhanced CDC activity.

Even though less well established, also glyco-engineering may be applied to modulate CDC activity of therapeutic antibodies. For
example, Quast and colleagues [72] showed that high levels of terminal sialic acid significantly impaired CDC activity mediated by rituximab.

**Dual ADCC and CDC Optimization**

As outlined above, various engineering strategies aim at individually improving selected effector mechanisms, such as ADCC or CDC. The rational choice of an engineering strategy for clinical application is not trivial, because the relative contribution of individual effector mechanism to the therapeutic activity of monoclonal antibodies in vivo is not fully understood. Therefore, concomitant enhancement of different effector functions might be desirable [30].

Natsume and colleagues [71] addressed this problem by reducing the fucosyl level in the background of IgG1/IgG3 mixed isotype variants. These variants demonstrated improved CDC activity compared to IgG1 and IgG3 variants, and interestingly enhanced NK cell-mediated ADCC activity. Wirt and colleagues [73] developed a similar approach by generating non-fucosylated variants of the EFTAE-Fc variant described by Moore and colleagues [70]. The EFTAE variant displays significantly improved C1q binding, resulting in stronger CDC-mediated lysis of tumor cells. The derived non-fucosylated variant, in addition to improved CDC activity, also demonstrated enhanced NK-cell mediated ADCC, due to high-affinity FcyRIIfa binding.

Based on the finding that IgG antibodies can organize into hexamers on the cell surface after antigen binding, thereby building optimal docking sites for C1q, de Jong and colleagues [74, 75] translated this natural concept to design antibodies with improved effector functions. Mutations that enhanced hexamer formation and complement activation by IgG1 antibodies were identified (fig. 3C). Especially Fc domains with E345K or E430G amino acid substitutions potently enhanced CDC activity and interestingly also improved ADCC in the background of selected antibodies.

Finally, in certain clinical settings, in which interactions with immune cells or complement can lead to adverse events, Fc-mediated functions may be undesirable. Thus, in sharp contrast to typical tumor targeting antibodies, antibodies which exclusively rely on target antigen binding or neutralization of receptors, such as certain immune checkpoint blockers, antibodies harboring immunological silent Fc domains may be advantageous. Non-immune stimulatory Fc domains were generated using different approaches [76]. This type of ‘Fc silencing’ technology has recently been applied to the anti-PD-L1 antibody atezolizumab.

**Perspectives of Fc-Engineered Antibodies in Clinical Applications**

Until 2017, three Fc engineered antibodies have received approval for clinical use in cancer therapy (table 4). Two of them are typical tumor targeting antibodies that were optimized for enhanced effector cell receptor binding by Fc glyco-engineering: mogamulizumab, which is specific for C-C chemokine receptor type 4 (CCR4) and which has been approved for the treatment of adult T-cell leukemia/lymphoma (ATLL) in Japan in 2012, contains a non-fucosylated Fc domain generated by applying the Potelligent™ technology [77]. Obinutuzumab, which has received approval by the Food and Drug Administration (FDA) in the treatment of chronic lymphocytic leukemia (CLL) in 2016, is a low-fucose CD20 antibody that was generated by GlycoMAB™ technology [78]. In contrast, the third approved antibody, atezolizumab, which targets PD-L1 and which is employed for immune checkpoint blockade by blocking interaction of PD-L1 with its inhibitory receptor PD-1 on T cells, contains an aglycosylated Fc domain and lacks Fc receptor and complement binding [79]. This was achieved by replacing the N-glycosylation site asparagine 298 by alanine. Atezolizumab has been approved by the FDA for treatment of patients with locally advanced or metastatic urothelial carcinoma as a monotherapy [80].

<table>
<thead>
<tr>
<th>Antibody (company)</th>
<th>Trade name</th>
<th>Antigen</th>
<th>Engineering strategy</th>
<th>Indication</th>
<th>Year of approval</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mogamulizumab (Kyowa Hakko Kirin)</td>
<td>Poteligeo</td>
<td>CCR4</td>
<td>non-fucosylated (host: FUT8-deficient CHO; Potelligent™ technology)</td>
<td>ATLL</td>
<td>2012</td>
<td>[77]</td>
</tr>
<tr>
<td>Obinutuzumab (Roche)</td>
<td>Gazyva</td>
<td>CD20</td>
<td>low fucose (GNT III over-expression; GlycoMAB™ technology)</td>
<td>CLL</td>
<td>2013</td>
<td>[78]</td>
</tr>
<tr>
<td>Atezolizumab (Roche)</td>
<td>Tecenriq</td>
<td>PD-L1</td>
<td>non-glycosylated (AA substitution: N298A)</td>
<td>urothelial carcinoma</td>
<td>2016</td>
<td>[79]</td>
</tr>
</tbody>
</table>

CC4 = C-C chemokine receptor type 4; PD-L1 = programmed cell death 1 ligand 1; FUT8 = α(1,6)-fucosyltransferase; GNT III = N-acetylglucosaminyltransferase III; AA = amino acid; ATLL = adult T-cell leukemia/lymphoma; CLL = chronic lymphocytic leukemia.

*Country-specific approval (Japan).
Table 5. Examples of Fc-engineered antibodies in advanced stages of clinical development

<table>
<thead>
<tr>
<th>Antibody (Company)</th>
<th>Antigen</th>
<th>Engineering strategy</th>
<th>Indication</th>
<th>Status</th>
<th>ClinicalTrials.gov Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obinutuzumab (Roche)</td>
<td>CD20</td>
<td>low fucose (host: YB2/0; EMAAb® technology)</td>
<td>CLL, DLBCL</td>
<td>phase III</td>
<td>NCT02612311, NCT02492711</td>
</tr>
<tr>
<td>Margetuximab (MacroGenics)</td>
<td>HER2</td>
<td>AA substitutions: L235V/F243L/R292P/Y300L/F396L</td>
<td>breast cancer</td>
<td>phase III</td>
<td>NCT02793583</td>
</tr>
<tr>
<td>Talacotuzumab, [NJ]-56022473 (CSL, Jansen Biotech)</td>
<td>CD123</td>
<td>AA substitutions: 239D/I332E (Xmab® technology)</td>
<td>AML</td>
<td>phase II/III</td>
<td>NCT02472145</td>
</tr>
<tr>
<td>MOR208, Xmab-5574 (Xencor, MorphoSys)</td>
<td>CD19</td>
<td>AA substitutions: S239D/I332E (Xmab® technology)</td>
<td>DLBCL, CLL</td>
<td>phase II/III</td>
<td>NCT02763319</td>
</tr>
<tr>
<td>Inebilizumab, MEDI-551 (MedImmune LLC)</td>
<td>CD19</td>
<td>non-fucosylated (host: FUT8-deficient CHO; Potelligent® technology)</td>
<td>DLBCL, CLL</td>
<td>phase II</td>
<td>NCT01453205, NCT01466153</td>
</tr>
<tr>
<td>BI 836826 (Boehringer Ingelhein)</td>
<td>CD37</td>
<td>AA substitutions: S239D/I332E (Xmab® technology)</td>
<td>CLL</td>
<td>phase II</td>
<td>NCT02624492</td>
</tr>
<tr>
<td>CetuGEX (Glycotope GmbH)</td>
<td>EGFR</td>
<td>reduced fucosylation, optimized galactosylation and degree of branching; GEX® platform</td>
<td>squamous cell carcinoma of the head and neck</td>
<td>phase II</td>
<td>NCT02012960</td>
</tr>
<tr>
<td>PankoMab-GEX (Glycotope GmbH)</td>
<td>TA-MUC1</td>
<td>reduced fucosylation, optimized galactosylation and degree of branching; GEX® platform</td>
<td>ovarian cancer</td>
<td>phase II</td>
<td>NCT01899599</td>
</tr>
<tr>
<td>Lumretuzumab (Roche)</td>
<td>HER3</td>
<td>Low fucose (GNT III over-expression; GlycoMab® technology)</td>
<td>NSCLC</td>
<td>phase II</td>
<td>NCT020204345</td>
</tr>
</tbody>
</table>

FUT8 = α1,6-fucosyltransferase; GNT III = N-acetylgalcosaminyltransferase III; AA = amino acid; CLL = chronic lymphocytic leukemia; DLBCL = diffuse large B-cell lymphoma; AML = acute myeloid leukemia; NSCLC = non-small cell lung cancer. *Country-specific approval (Japan).

Conclusions

In the last two decades, monoclonal antibodies have revolutionized the therapy of cancer, although not all patients benefit. Currently, new generations of engineered antibodies are frequently entering the clinic with rationally designed effector functions. While strategies in improving antibodies’ Fc affinity to selected FcγRs to improve immunotherapy of cancer is widely accepted, the concept of enhancing complement activation is still under debate and to our knowledge CDC-optimized antibody variants have not been tested in the clinic to date. While a variety of engineered Fc variants have been described, detailed comparisons between larger panels of protein-engineered and/or glyco-engineered Fc variants with distinct profiles of effector functions are missing. Especially when analyzed in carefully designed in vivo models, such data may allow to unravel the relative contribution of selected effector cell populations to successful antibody therapy and to identify Fc variants with the highest potential in a given clinical application. Together with a more detailed understanding in the development and progression of diseases, the outlined technologies may allow the design of antibodies with tailor-made effector functions that are optimally suited for application in a given clinical situation.
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Disclosure Statement

The authors declare no conflicts of interest.
Modulating Cytotoxic Effector Functions by Fc Engineering to Improve Cancer Therapy


