Advances in Therapeutic Fc Engineering – Modulation of IgG-Associated Effector Functions and Serum Half-life

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Today, monoclonal immunoglobulin gamma (IgG) antibodies have become a major option in cancer therapy especially for the patients with advanced or metastatic cancers. Efficacy of monoclonal antibodies (mAbs) is achieved through both its antigen-binding fragment (Fab) and crystallizable fragment (Fc). Fab can specifically recognize tumor-associated antigen (TAA) and thus modulate TAA-linked downstream signaling pathways that may lead to the inhibition of tumor growth, induction of tumor apoptosis, and differentiation. The Fc region can further improve mAbs’ efficacy by mediating effector functions such as antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cell-mediated phagocytosis. Moreover, Fc is the region interacting with the neonatal Fc receptor in a pH-dependent manner that can slow down IgG’s degradation and extend its serum half-life. Loss of the antibody Fc region dramatically shortens its serum half-life and weakens its anticancer effects. Given the essential roles that the Fc region plays in the modulation of the efficacy of mAb in cancer treatment, Fc engineering has been extensively studied in the past years. This review focuses on the recent advances in therapeutic Fc engineering that modulates its related effector functions and serum half-life. We also discuss the progress made in aglycosylated mAb development that may substantially reduce the cost of manufacture but maintain similar efficacies as conventional glycosylated mAb. Finally, we highlight several Fc engineering-based mAbs under clinical trials.

Keywords: antibody Fc region, ADCC, CDC, ADCP, serum half-life, aglycosylated antibody, FcRn, cancer therapy

INTRODUCTION

Monoclonal antibodies (mAbs) can target tumors through specific recognition of tumor-associated antigens and subsequent recruitment of effector elements including macrophages, dendritic cells, natural killer (NK) cells, T-cells, and the complement pathway components (1). Such recruitments are achieved by interactions among the immunoglobulin gamma (IgG)-crystallizable fragment (Fc) and the immune cell receptors like Fcγ receptors (FcγRs) and the complement protein C1q of the complement system (2–4). These interactions lead to the activation of immune cells for enhanced antibody-dependent cellular cytotoxicity (ADCC)/antibody-dependent cell-mediated phagocytosis (ADCP), formation of the membrane attack complex, and more efficient presentation of antigen
to the dendritic cells (1). Through a recycling mechanism, the neonatal Fc receptor (FcRn) prolongs the half-life of mAbs in a pH-dependent interaction with the Fc region (5). The schematic of overall IgG structure and its binding regions with FcγRs, C1q, and FcRn is depicted in Figure 1.

The FcγRs, consisting of FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) classes, are heterogeneous in terms of their cellular expression and Fc binding affinities (1, 6). FcγRI binds to the Fc region with $K_D \approx 10^{-8}$–$10^{-9}$ M and is expressed on mononuclear phagocytes, dendritic cells, and IFN-γ-activated neutrophils (1, 7, 8). FcγRII binds to the Fc region with relatively lower affinity ($K_D \approx 10^{-7}$ M) and exists in five isoforms; among them, activating (FcγRIIa, harboring an immunoreceptor tyrosine-based activation motif on neutrophils) or inhibitory (FcγRIIb, harboring an immunoreceptor tyrosine-based inhibitory motif predominantly on B-lymphocytes) are critical for immune regulation (1, 7). FcγRIII, expressed in two isoforms, binds the Fc region with the lowest affinities ($K_D \approx 10^{-5}$ M) (1, 7). Among these, FcγRIIa has a moderate Fc binding allele (V158) and a low binding allele (F158), and is expressed on NK cells, macrophages, and T-cell subsets and activates NK and T cell-mediated ADCC response (1, 6, 7); FcγRIIIb is exclusively present on neutrophils and lacks signal generation capacity (1, 7).

Crystal structures of Fc in complex with FcγRI (9) (Figure 2A), Fc in complex with FcγRII (10) (Figure 2B), and Fc in complex with FcγRIII (11) (Figure 2C) reveal that the FcγRs’ interaction sites on Fc are all located within the lower hinge-upper heavy chain constant domain 2 (CH2). Furthermore, the binding affinity of Fc region to FcγRs also varies with the IgG subclasses (12).

The C1q is a multisubunit protein of the complement system (3). It uses one of its six heads to establish a low-affinity ($\sim 10^{-6}$ M) interaction with the lower hinge-upper CH2 domain of the Fc region (3). Crystal structure of the human C1q head revealed that it is assembled with a heterotrimer globular architecture (14). Though the molecular basis of how the C1q head recognizes the Fc region is not known at the atomic resolution, Schneider and Zacharias (15) proposed a working model of C1q in complex with Fc based on known experimental data, docking, and molecular dynamics simulation. According to this model, upon initial weak interaction between the C1q head and the Fc region, IgGs can aggregate while recognizing “multiple epitopes” on the antigen surface and thus give many C1q molecules an opportunity to bind to their Fc regions, which enhance the “cumulative affinity” to $\sim 10^{-9}$ M (3, 15). This leads to the deposition of complement component 3 (C3b) on the target cell and ultimate formation of the membrane attack complex that disrupts the lipid bilayer of the target cell, promotes cytolysis, and completes complement dependent cytotoxicity (CDC) (16, 17).

The pharmacokinetic profiles of antibodies vary among subclasses and are related to the structural features of the Fc region (18, 19). It is known that the serum half-life of IgG subclasses (IgG1, IgG2, and IgG4) is ~23 days as compared to 2–6 days for IgG3 and other Ig classes (18, 19). The Fc region spanning the interface of CH2 and CH3 domains interacts with the FcRn in the placenta, liver, mammary glands, and adult intestine to regulate IgG homeostasis and deliver maternal IgG across the placenta to the fetus (5). This interaction is favored by an acidic environment of the endosome after IgG is pinocytosed.
FIGURE 2 | Crystal structures illustrating crystallizable fragment (Fc) interactions with FcγRs and FcRn. Representative structures are shown for (A) Fc–FcγRI cocrystals [PDB: 4W4O (9)], (B) Fc–FcγRII cocrystals [PDB: 3RY6 (10)], (C) Fc–FcγRIII cocrystals [PDB: 1T89 (11)], and (D) Fc–FcRn cocrystals [PDB: 1I1A (13)] with β2 microglobulin (β2M) domain shown in cyan. The Fc region and FcγRs are represented by gray and yellow color, respectively (A–D). N297 glycans within the CH2 domain are shown in stick model. The critical binding regions are highlighted in the upper part of each panel; region from the Fc fragment in green, region from the FcγRs, FcRn, and β2M in red. The lower part of each panel shows the detailed residues, which are involved in the interactions between Fc and its binding partners.
and thus IgG is protected from lysosomal degradation (20). The endocytosed IgG is then recycled to the cell surface and released into the blood stream at an alkaline pH, thereby maintaining the sufficient IgG serum half-life for proper immune functions and desired therapeutic efficacies (20). Recently, the endothelial and hematopoietic cells are identified as the major sites associated with FcRn expression and their critical role in IgG homeostasis (20–23).

Based on site-directed mutagenesis of Fc region and design of a hybrid Fc heterodimer harboring one half of Fc wild type (WT) and one half of Fc mutant, Kim and coworkers identified the key Fc residues involved in the FcRn interaction and proposed a preliminary model that one Fc hinge homodimer bound with two FcRn molecules (24, 25). Shortly thereafter, Burmeister and coworkers reported high resolution crystal structure of FcRn alone at 2.2 Å and low resolution crystal structure of Fc in complex with FcRn at 6.5 Å (26, 27). Structural analysis and biophysical data confirmed that one Fc homodimer binds with two FcRn molecules (26–29). Later, Martin and coworkers reported a high resolution crystal structure of Fc in complex with FcRn at 2.8 Å (13). This structure clearly shows the key residues involved in Fc and FcRn interactions and reveals the pH-dependent binding mechanism (13) (Figure 2D).

Evidence demonstrates the presence of oligosaccharides, attached to the N297 residue within the CH2 domain Asn-X-Ser/Thr glycosylation motif of Fc region, is essential in maintaining the Fc conformation and mediating its interactions with FcyRs (FcγRI, FcγRIIa, FcγRIIb, and FcγRIIIa) and C1q, but not FcRn (30–41). The glycan moiety is formed by two N-linked biantennary oligosaccharide chains consisting of a core heptasaccharide [N-acetylglucosamine (GlcNAc) and mannose (Man)] but occurrence of other residues like terminal N-acetyleneuraminic acid, galactose (Gal), bisecting N-acetylglucosamine (GlcNAc), and fucose (Fuc) have also been reported (1, 42, 43). Additionally, 5–17 and 2–7% of IgG structures could be monosialylated and disialylated, respectively (1, 44). This impacts a significant complexity and heterogeneity to therapeutic IgG molecules when expressed in mammalian cells, which can affect the therapeutic profile of IgG (30). On the other hand, the presence of bisected N-acetylgalactosamine structures in rituximab, a purer glycoform with lesser heterogeneity, leads to an efficient engagement of FcγRIII and increases ADCC activity against CD20+ cells by up to 20-fold (30, 45). Similarly, non-fucosylated glycoform of Herceptin produced in engineered Chinese hamster ovary (CHO) cell line (LEC13) can enhance ADCC via FcγRIII engagement by up to 50-fold (30, 46).

However, mAb-associated glycan heterogeneity poses several key challenges (30, 33, 45–51) including (1) difficulties in developing therapeutic mAbs with glycan composition similar to naturally occurring human IgG1, (2) difficulties in controlling glycan heterogeneity, (3) lengthier development time to construct cell lines producing glycan homogeneity, (4) lengthier IgG production time and higher manufacturing cost in mammalian cells as compared to that in E. coli or yeast-based expression systems, (5) dominance of particular glycoforms that can affect effector functions of IgG molecules, and (6) difficulties in separating various glycoforms generated from mammalian cells. Alternatively, development of aglycosylated mAbs with similar efficacy as glycosylated counterpart but lower manufacturing cost has attracted great efforts in the past decade.

In this review, we focus on the recent progress in therapeutic Fc engineering-associated effector functions (ADCC, ADCP, and CDC) and pharmacokinetics. The mutations known to induce profound effects on Fc interaction with FcyRs, C1q, and FcRn are summarized (see Table 1). We also briefly describe the advances in aglycosylated mAb development. Finally, we highlight clinical trials of several mAbs developed from relevant Fc engineering.

**Table 1** | Tabulation of the Fc mutations known to mediate a profound effect on antibody effector functions and immunoglobulin gamma homeostasis.

| Fc type          | Mutation                          | Target | Functional | Reference |
|------------------|-----------------------------------|--------|------------|-----------|
| Hu-IgG2-Glyco    | K326W/E333S                       | C1q    | Yes        | (52)      |
| Mu-IgG2b-Glyco   | E235L                             | FcγRI  | Yes        | (2)       |
| Hu-IgG3-Glyco    | E235Y                             | FcγRII | Yes        | (33)      |
| Hu-IgG1-Glyco    | S239D, I332E, S239D/I332E, and S239D/I332E/A330L | FcγRIIa | Yes | (6, 53–55) |
| Hu-IgG1-Glyco    | G236A, G236A/I332E, S239D/I332E, and G236A/S239D/I332E | FcγRIIa > FcγRIIIa > FcγRI | Yes | (56) |
| Hu-IgG1-Glyco    | L235V/F243L/R292P/Y300L/P396L      | FcγRII | Yes | (57, 58) |
| Hu-IgG1-Glyco    | P238D/L332E                       | FcγRIIb | NA | (59) |
| Hu-IgG1-IgA-Glyco| IgGA (many motifs)                 | FcγRIs + FcαRI | Yes | (60) |
| Hu-IgG1-Glyco    | F243L/R292P/Y300L and F243L/R292P/Y300L/P396L | FcγRIIa/FcγRIIa | Yes | (29) |
| Hu-IgG1-Aglyco   | S298G/T299A                       | FcγRII | NA | (61) |
| Hu-IgG1-(c-Fuc)  | F234L                             | FcγRII | Yes | (62) |
| Hu-IgG1-Aglyco   | E382V/M428I                       | FcγRII | Yes | (63) |
| Hu-IgG1-Aglyco   | Q296R/L328W/A330V/P331V/I332Y     | FcγRII | Yes | (64) |
| Hu-IgG1-Aglyco   | M428L/N434S                       | FcγRII | Yes | (65) |
| Hu-IgG1-Glyco    | M252Y/S254T/T256E and H433K/N434F/Y436H | FcRn | Yes | (66, 67) |
| Hu-IgG1-Glyco    | N343A/E380A                       | FcRn   | Yes | (68) |
| Hu-IgG1-Glyco    | M252Y/S254T/T256E                 | FcRn   | Yes | (69) |
| Hu-IgG1-Glyco    | T250F/M428L                       | FcRn   | Yes | (70) |
| Hu-IgG1-Aglyco   | Q296R/L328W/A330V/P331V/I332Y     | FcRn   | Yes | (64) |

Hu, Human; Mu, Murine; Glyco, Glycosylated; Aglyco, Aglycosylated; Fuc, Fucose; NA, not available.
MODULATION OF EFFECTOR FUNCTIONS BY Fc ENGINEERING

To develop more effective antibodies with desired ADCC, ADCP, and CDC activities, various strategies including site-directed mutagenesis, alanine scanning, structure-based computational design, and directed evolution technologies are employed.

The Fc amino acid residues that confer improved binding to FcγRs/C1q and enhanced immune response were initially characterized by site-directed mutagenesis studies. The earliest described mutations were discovered by scanning residues to isolate non-binders while focusing on the conserved residues. Fc residues (E318, K320, and L322) in the mouse IgG2b-Fc region were identified as the C1q binding site (3). However, the relevance of E318 and K320 was challenged in human Fc–C1q interaction (71). Novel residues (D270, K322, P329, and P331) were proposed for normal C1q binding on human Fc (71). This finding underscores the interspecies differences in such molecular interactions that may show a different effect in preclinical models. Furthermore, an IgG1 isotype of rituximab carrying K326W/E333S mutations was shown to have fivefold more binding to C1q (52) and the same motif, when transferred to the IgG2 isotype (poor complement activator) of rituximab, increased the cell lysis by fivefold (52). Next, a single mutation from E to L at position 235 of the mouse IgG2b–Fc region proposed it to be the “major determinant” for FcγRI binding (with ~100-fold increased affinity to human monocyte FcγRII) (2). Additionally, using a mouse–human chimeric antibody, amino acids at position 234 and 237 were shown to mainly influence the interaction with FcγRII. Based on these observations, FcγRI and FcγRII were proposed to recognize an overlapping but non-identical site on the Fc region (35).

Alanine scanning mutagenesis of selected Fc residues resulted in many variants with altered binding to specific FcγRs, which was also reflected in their ability to promote ADCC. Activating FcγRIIIa mutations improved ADCC by 100% (68). Furthermore, mutants based on the activating or suppressing effect on FcγRs were categorized into different classes. Among these, IgG1 mutations A327Q/P329A (interact with FcγRI), D265A/S267A/H268A/D270A/K326A/S337A (interact with FcγRIIa), and T256A/K290A/S298A/E333A/K334A (interact with FcγRIIIa) promoted high-affinity interactions (68).

Computational optimization of the Fc region by creating a single (S239D or I332E), double (S239D/I332E), and triple mutations (S239D/I332E/A330L) improved the affinity against human FcγRIIIaV158F/V158I engagement (57). The same Fc motif was applied to the MGA271 mAb (anti-CD276), which targets B7-H3+ tumor cells and resulted in an increased binding to FcγRIIIa, enhanced ADCC, and potent antitumor activity in a renal cell carcinoma/bladder cancer xenograft mouse model (58). Recently, the immune activating potential of IgA via FcγRII engagement was exploited by developing IgG and IgA hybrid molecules "IgGA" through substituting α1 loop residues of CH1/2/3 region with CH2/3 (60). The "IgGA" hybrid trastuzumab mediated an enhanced ADCC/ADCP activity against Her2 overexpressing cells and destroyed up to 50% SKBr3 breast cancer cells (via ADCC) and MDA-MB-453 cells (via ADCP) (60). Similarly, "IgGA" hybrid rituximab lysed ~70% of the CD20+ calcine-AM-loaded Raji tumor cells when compared to the WT counterparts (60).

A negative selection strategy was applied using yeast surface display to enrich Fc mutants exhibiting selective high affinity to FcγRIIIa (29). Among these isolates, F243L was predicted to make a direct contact with the carbohydrate portion, which can “influence sialylation and affect the quaternary structure” of the Fc domain (29). Additionally, R292P partially reduced the binding to FcγRIIa, while Y300L, in combination with other mutations (F243L/R292P/V305I/P396L), showed an ~10-fold less Ki0, 100-fold enhanced ADCC activity, and potency in a xenograft mouse model of ovarian and breast cancer (29). Furthermore, an Fc variant with three changes (F243L/R292P/V300L) was also effective in increasing the rate of cytolyis by ~100-fold (29). In another report, human IgG1 Fc variants were generated by an error-prone PCR and ribosome display to select high-affinity aglycosylated binders to human FcγRIIIa using a solution phase method (62). The isolated Fc mutant (F243L) lacked Fuc residues in most oligosaccharide chains and exhibited an improved FcγRIIIaV158I binding.

In addition, “shuffled variants” of anti-CD20/CD57 antibody were constructed by grafting the CH1/hinge and CH3 carboxy-terminal of IgG1 into the Fc of IgG3 to retain both the ADCC activity from IgG1 and the CDC activity from IgG3 (72). It is known that IgG1 is the most potent ADCC activator, while IgG3 has highest potency to recruit complement system (72). Therefore, IgG1 and IgG3 Fc regions can complement one another to maximize the immune effector response. These variants with chimeric CH regions showed ~25–60% increase in ADCC and CDC activity compared to WT of IgG1 and IgG3 molecules (72). Furthermore, the CDC activity of humanized anti-CD20 IgG1 (ocrelizumab) was increased by ~23-fold while retaining normal IgG1 ADCC by combining a triple mutant (S267E/H268F/S324T) with earlier reported G236A/I332E in the CH2 domain (73).

Multiple mutations (L235V/F243L/R292P/Y300L/P396L) in the trastuzumab Fc region (MGAH22) increased the potency against low Her2-expressing cells via low-affinity FcγRIIIaV158I engagement (57). The same Fc motif was applied to the MGA271 mAb (anti-CD276), which targets B7-H3+ tumor cells and resulted in an increased binding to FcγRIIIa, enhanced ADCC, and potent antitumor activity in a renal cell carcinoma/bladder cancer xenograft mouse model (58). Recently, the immune activating potential of IgA via FcγRII engagement was exploited by developing IgG and IgA hybrid molecules "IgGA" through substituting α1 loop residues of CH1/2/3 region with CH2/3 (60). The "IgGA" hybrid trastuzumab mediated an enhanced ADCC/ADCP activity against Her2 overexpressing cells and destroyed up to 50% SKBr3 breast cancer cells (via ADCC) and MDA-MB-453 cells (via ADCP) (60). Similarly, "IgGA" hybrid rituximab lysed ~70% of the CD20+ calcine-AM-loaded Raji tumor cells when compared to the WT counterparts (60).

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Incorporated into anti-CD19/CD40 mAbs (53, 54). Furthermore, it was shown that a change from glycine to alanine at residue 236 can shift the immune balance toward activating FcγRIIa relative to inhibitory FcγRIIb (56). The coupling of G236A to either I332E or S239D/I332E had dual beneficial effect as these mutants not only improve FcγRIIa:FcγRIIb ratio but also enhance binding to FcγRIIIa by ~6- to 31-fold (56). These mutants had significantly improved NK cell-mediated ADCC and macrophage-mediated ADCP activity (56).

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binding and enhanced ADCC as compared to WT Fc (62). Recently, an anti-EGFR antibody (S239D/I332E) was noted to elicit mononuclear cell-mediated ADCC via FcγRIIa engagement and at the same time showed impaired polymorphonuclear cell (PMN)-mediated ADCC due to the engagement of FcγRIIb, a highly homologous isoform to FcγRIIa (53). The inability of FcγRIIb to activate immune signaling in such a scenario can be overcome by imparting high-affinity binding to FcγRIIa, which can enhance both NK cell- and PMN mediated ADCC (35). These observations highlight that Fc engineering toward related FcRs needs to be tailored specifically to achieve desirable immune effects.

MODULATION OF ANTIBODY PHARMACOKINETICS BY Fc ENGINEERING

Along with the efforts to engineer Fc regions for enhanced effector functions, attempts have been made to improve antibody pharmacokinetics. Clearly, enhanced Fc–FcRn interaction at acidic pH can extend IgG’s serum half-life and positively regulate its homeostasis, which may benefit patients by greater therapeutic efficacy, less frequent dosing, and lower cost burden. Alanine scanning and display/directed evolution are commonly used techniques to identify favorable Fc mutants that can strengthen Fc–FcRn pH-dependent interactions.

A human Fc variant (N434A), isolated by alanine scanning of all solvent-exposed residues, showed fourfold increased binding to FcRn at pH 6.0 (68), which later, when studied in cynomolgus monkeys, showed a twofold extension of IgG serum half-life confirming the modulation of pharmacokinetics (74). Such an interaction at pH 6.0 prolongs IgG availability in serum, which correlates with the therapeutic effectiveness as demonstrated by the improved antitumor activity of IgG-Fc mutant (M428L/N434S from the CH3 domain) in a human FcRn transgenic mouse model (65).

A phage displayed antibody library approach was employed with the aim of isolating high-affinity binders against FcRn at pH 6.0. Random mutations were created at residues T252, T254, and T256, which are proximal to the IgG–FcRn interaction site, and binders were selected in a solution phase against FcRn at pH 6.0 and eluted by PBS at pH 7.4 (66). The Fc mutants generated in this manner were able to bind both human and rat FcRn with high affinity at pH 6.0; however, these variants (M252Y/S254T/T256E from the CH2 domain and H433K/N434F/Y436H from the CH3 domain) also showed tighter binding to mouse FcRn at pH 7.4 that decreased the serum IgG concentration in a mouse model (67). Yeung and coworkers (74) reported a similar observation that an Fc mutant (N434W), though possessing an ~80-fold enhanced affinity to FcRn at both acidic and neutral pH, did not stabilize serum IgG due to the loss in pH selectivity. These results suggest that high-affinity binding to the receptor at neutral pH can compromise the “beneficiary effect” of increased affinity at pH 6.0. Later, a humanized anti-respiratory syncytial virus antibody (MEDI-524) with a triple mutant Fc (M252Y/S254T/T256E from the CH2 domain) was reported to have a 10-fold increase in a pH-dependent way toward FcRn and about 4-fold improvement in serum half-life in cynomolgus monkey (69). Similarly, a high-affinity Fc variant (T250R/M428L) bound FcRn selectively at pH 6.0 and accounted for a 2.8-fold lesser degradation of serum IgG2 (70) and IgG1 (75) in rhesus monkey. Accumulating studies highlight that to prolong IgG serum half-life, pH-dependent FcRn affinity has to be maintained.

Of note, Greveys and coworkers recently analyzed known IgG-Fc mutants, which show enhanced FcRn pH-dependent affinity and extended serum half-life (M252Y/S254T/T256E from the CH2 domain and M428L/N434S from the CH3 domain), for their effects on ADCC, ADCP, and CDC (65, 69, 76). Surprisingly, they found that both mutants showed reduced effector functions with regards to ADCC, ADCP, and CDC. More interestingly, they found one previously known mutant (H433K/N434F from the CH3 domain), which showed reduced FcRn pH-dependent affinity and shortened serum half-life, displayed enhanced effector functions in ADCP, CDC, and ADCC (76, 77). These findings highlight that, though the interaction region of Fc with FcγRs and C1q (lower hinge region–CH2 domain) is distant from its interaction with FcRn (CH2–CH3 domain interface), it is still possible that these mutants can trigger a long range effect to other part of the Fc region in an unknown mechanism.

AGLYCOSYLATED Fc TO OVERCOME GLYCAN HETEROGENEITY

The demand for therapeutic antibodies is high, and it has been estimated that 8,000 kg of clinical grade mAbs were produced in 2013 (78). Currently, ~50% of the clinical grade biologics are produced in mammalian cells like CHO, mouse myeloma cell lines NSO and SP2/0 (30, 78–80).

However, the inherent glycan heterogeneity of mAbs when expressed in mammalian cell systems can cause high production cost and variations in mAb functions from batch to batch. Recently, great efforts have been invested in developing aglycosylated mAbs as an alternative. Ideally, aglycosylated mAbs would be as efficient as or even better than its glycosylated peers in mediating effector functions for antigen or target cell clearance.

To develop aglycosylated IgGs as alternatives, Sazinsky and coworkers constructed three small subsets of saturation substitutions covering the Asn-X-Ser/Thr glycan motif of the Fc C1′/E loop and displayed these libraries on the yeast cell surface (61). After selection against FcγRIIa by fluorescence-activated cell sorting (FACS), a double Fc mutant isolate (S298G/T299A) in an aglycosylated form showed threefold stronger binding as compared to the WT and variants with single mutation, which indicates that the glycosylation of N297 is not a strict requirement for the interaction of Fc with FcγRIIa (61). In contrast, the indispensable role of asparagine at position 297 was demonstrated in the backdrop of N297Q, N297D, or N297A mutation, which abolished the double mutant (S298G/T299A) binding to FcγRIIa (61). Moreover, this dual mutant is functional in vivo in that murine platelet clearance is as efficient as that of WT mAb. Based on the modeling of Fc dual mutants in complex with FcγRIIa, the N297 residue of an aglycosylated IgG can
make a hydrogen bond with the S126 residue of FcγRIIa (61, 81). Furthermore, such interaction may be strengthened by a bridging water molecule present in an unbound FcγRII crystal (61, 81). However, this dual mutant showed 10-fold reduced FcγRI binding and no binding to both FcγRIII and CIq proteins. The inability of aglycosylated mAbs to bind with CIq and thus activate CDC can limit their application in treating hospital acquired microbial infections among cancer patients where complement activity plays an important role (82).

In another major breakthrough, bacterial display and FACS was used to isolate Fc variants displaying increased binding and specificity to FcγRI (63). One such aglycosylated variant called “Fc5” (E382V/M428I) was incorporated into trastuzumab. The trastuzumab-Fc5 variant bound selectively to the FcγRI with nanomolar range affinity and promoted monocyte-derived dendritic cell-dependent lysis of SkBr3 breast cancer cells that over-express Her2. (63). The three-dimensional structure of human aglycosylated Fc domain suggests a greater “conformational flexibility of the CH2-CH3 domain interface,” as compared to the glycosylated counterpart (83). Additional mutations (Q295R/L328W/A330V/P331Y) in trastuzumab-Fc5 variant (63) increased the affinity for FcγRI by ~120-fold and retained pH-dependent FcRn binding and function (64). Other mutants were also reported with specific binding to FcγRI and without compromising the pH-dependent FcRn binding (84).

It is worth mentioning that removal of appended glycans, though does not affect IgG’s solubility, binding affinities to FcγRs, and in vivo half-life but often compromises IgG related CDC, lowers its thermostability and increases its aggregation at the low pH (85–87).

### Fc ENGINEERING-BASED mAbs UNDER CLINICAL TRIALS

A number of mAbs harboring various modifications in the Fc region are being investigated in different clinical trial stages (see Table 2 for Fc engineering-based mAbs being tested in clinics). These molecules broadly fall into three categories (1) with enhanced effector response to treat cancer and infectious diseases, (2) capable of inhibiting immune activation to treat inflammatory diseases, and (3) new class of aglycosylated mAbs with either inert or active-immune function.

The Fc variants capable of inducing enhanced ADCC are being tested in many antibody candidates. Anti-CD37 antibody (BI836826; Boehringer) against B cell malignancies is currently under phase-1 trial for the treatment of chronic lymphocytic leukemia (CLL) (6, 88, 89). This is a mouse–human chimeric antibody, which targets tetraspanin CD37 and shows high pro-apoptotic activity against malignant B cells via enhanced ADCC. Using human CD37 transgenic mice, a single dose of BI836826 was demonstrated to reduce peripheral B cells (88) and efficacious in suppressing tumor growth in Ramos mouse model of human B-cell lymphoma (88). Furthermore, therapeutic efficacy of a surrogate Fc-engineered antibody against macaque CD37 has also been demonstrated in cynomolgus monkey (88). A fully humanized anti-CD123 antibody (JNJ-56022473; Janssen R & D) targeting overexpressed interleukin-3 receptor α-chain is being tested in acute myeloid leukemia patients (89–91). The Fc fragment of JNJ-56022473 has been engineered for enhanced NK cell-mediated ADCC. The molecule efficiently reduced the growth of the patient-derived acute myelogenous leukemia xenografts in bone marrow and peripheral organs and increased the survival in animal models (91). Similarly, a humanized anti-CD30 antibody (XmAb2513; Xencor) with enhanced binding to FcγRIIa is being evaluated in the treatment of CD30+ Hodgkin’s lymphoma (HL) patients who had previously received two or more therapies (89, 92, 93). It has been shown to be safely administered and biologically active in relapsed, refractory HL subjects and reduces tumor in a majority of patients (92, 93). Another interesting molecule is an anti-CD19 antibody (XmAb5774; Xencor), which induces potent NK cell-mediated ADCC/ADCP response against CLL (89, 96). The antibody is known to get internalized in primary CLL cells and induces a modest toxicity (96).

Antibodies harboring Fc mutations that can suppress the immune response are being tested for the treatment of inflammatory diseases. The immunosuppressive version of anti-CD19 antibody (XmAb5871; Xencor) binds inhibitory FcγRIIB with ~430-fold enhanced affinity and efficiently depletes CD19+ B-cells in systemic lupus erythematosus (SLE) patients (90, 94). The depletion of CD19+ B-cells correlates with the strong inhibition

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**Table 2 | Fc-engineered antibody candidates under clinical evaluation.**

| Antibody | Target | Fc modification | Disease | Clinical development | Company | Reference |
|----------|--------|----------------|--------|----------------------|---------|-----------|
| BI836826 | CD37   | NA             | CLL    | Phase-1              | Boehringer | (6, 88, 89) |
| JNJ56022473 | CD123 | NA             | AML    | Phase-2              | Janssen R & D | (89–91) |
| XmAb2513 | CD30   | NA             | Hodgkin/large cell lymphoma | Phase-1 | Xencor, Inc. | (89, 92, 93) |
| XmAb5871 | CD19   | S267E/L328F    | SLE    | Phase-1              | Xencor, Inc. | (80, 94) |
| XmAb7195 | IgE    | S267E/L328F    | Allergic diseases | Phase-1 | Xencor, Inc. | (95) |
| XmAb5774 | CD19   | S239D/I328E    | CLL    | Phase-1              | Xencor, Inc. | (89, 96) |
| TRX4     | CD3    | N297A          | Type-1 diabetes mellitus (autoimmune) | Phase-3 | GSK/Tolerx | (48, 49, 97) |
| Onartuzumab | MET | N297A       | NSCLC/gastroesophageal cancer | Phase-3 | Roche | (48, 98, 99) |
| ALD518   | IL-6   | N297A          | RA/NMSCL/oral mucositis | Phase-2 | Ailer | (48, 49, 100) |
| TRX518   | GITR   | N297A          | Malignant melanoma | Phase-1 | Tolerx | (48, 49, 101) |

CLL, chronic lymphocytic leukemia; AML, acute myeloid leukemia; SLE, systemic lupus erythematosus; NSCLC, non-small cell lung cancer; RA, rheumatoid arthritis; NA, not available.

Source: https://clinicaltrials.gov/.
of B-cell receptor-induced calcium mobilization among healthy volunteers and SLE patients (94). The same Fc fragment has been engineered into a humanized anti-IgE antibody (XmAb7195; Xencor) for the treatment of allergies (95). This antibody prevents the binding of IgE to its high-affinity IgE receptor (FcεRI) that is present on basophils and mast cells and is useful in the treatment of allergic asthma (95). The XmAb7195 has 5- and ~430-fold higher affinity for human IgE and FcγRIIb, respectively, and therefore is effective in inhibiting IgE production and plasma cell differentiation (95).

Finally, aglycosylated IgG molecules have recently been shown to have therapeutic properties (48, 49, 61, 63), and a few of them are undergoing clinical testing. An anti-CD3 antibody (TRX4; Tolerx) incorporating the N297A mutation suppresses pathogenic T-cells in type-1 diabetes (T1D) patients (48, 49, 97) and is being evaluated in phase-3 trials. The antibody downregulates pathogenic T-cells while restoring the normal activity of T-regulatory cells and thereby inhibits autoimmune mediated T1D (97).

It is widely accepted that hepatocyte growth factor (HGF) binding to receptor tyrosine kinase MET aggravates malignancy in a variety of cancers (98). Therefore, an aglycosylated anti-MET antibody (Onartuzumab; Roche) is being evaluated in phase-3 trials and ameliorated NSCLC-related anemia and cachexia (99). Similarly, an aglycosylated mAb (ALD518; Alder) targeting IL-6 is being tested in phase-2 studies and ameliorated NSCLC-related anemia and cachexia (100). Another aglycosylated mAb (TRX518; Tolerx) is currently in phase-1 trials to treat malignant myeloma (48, 49, 101). TRX518 mAb recognizes the glucocorticoid-induced tumor necrosis factor receptor on regulatory and effector T-cells, B-cells, NK cells, and antigen-presenting cells to enhance effector T-cell response and inhibits T-regulatory cell-mediated suppression (101). Furthermore, the efficacy of TRX518 mAb in reducing tumor burden and increased survival rates has been demonstrated in mouse and non-human primate models (101).

These studies highlight that Fc engineering has played important roles in developing antibodies with desirable properties and functions, and the ongoing clinical studies can give valuable information on the efficacy of the Fc-engineered mAbs, as compared to their existing peers.

**FINAL REMARKS**

Crystallizable fragment engineering has made substantial progress in the identification of new mutant(s) that can enhance effector functions and improve pharmacokinetics of mAbs for cancer treatment. Two major notions have emerged during the past decades’ efforts. First, the ratio of human activating FcγRs (FcγRI, FcγRIIa, and FcγRIIb) and inhibitory FcγR (FcγRIIb) has to be taken into account during Fc engineering design. Beneficial effect can be achieved when Fc mutant(s) show higher selectivity and binding affinity toward activating FcγRs, as compared to the inhibitory FcγR. Second, the synergistic effects of improved ADCC, ADCP, and CDC of Fc region could increase the potency in cancer treatment.

Excitingly, aglycosylated mAb, expressed in bacteria and yeast, have been found to possess similar properties as glycosylated mAb with regards to FcγRs binding and serum half-life. However, efforts are still needed to improve its thermostability, solubility, and its binding affinity to C1q and CDC activity before it can really compete with its glycosylated peer for cancer therapy. On the other hand, the inability of aglycosylated mAbs to bind to C1q may have beneficial effects when CDC activity is not required such as for treatment of autoimmune diseases where CDC is chronically and pathologically activated (102). Similarly, aglycosylated mAbs may have advantages over glycosylated counterparts when only selective activation of FcγRs is desired such as activation of FcγRI (63) or FcγRIIa (84) to stimulate tumor cell killing.

**AUTHOR CONTRIBUTIONS**

DW conceived the topic; AS and DW wrote the manuscript; and DW revised the manuscript.

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**REFERENCES**

1. Jefferis R, Lund J, Pound JD. IgG-Fc-mediated effector functions: molecular definition of interaction sites for effector ligands and the role of glycosylation. ImmunoL Rev (1998) 163:59–76. doi:10.1111/j.1600-065X.1998.tb01188.x
2. Duncan AR, Woof JM, Partridge LJ, Burton DR, Winter G. Localization of the binding site for the human high-affinity Fc receptor on IgG. Nature (1988) 332:563–4. doi:10.1038/332563a0
3. Duncan AR, Winter G. The binding site for C1q on IgG. Nature (1988) 332:738–40. doi:10.1038/332738a0
4. Rayner LE, Hui GK, Gor J, Heenan RK, Dalby PA, Perkins SJ. The solution structures of two human IgG1 antibodies show conformational stability and accommodate their C1q and FcgammaR ligands. J Biol Chem (2015) 290:8420–38. doi:10.1074/jbc.M114.631002
5. Ghetie V, Ward ES. Multiple roles for the major histocompatibility complex class I-related receptor FcRn. Annu Rev Immunol (2000) 18:739–66. doi:10.1146/annurev.immunol.18.1.739
6. Lazar GA, Dang W, Karki S, Vafa O, Peng JS, Hyun L, et al. Engineered antibody Fc variants with enhanced effector function. Proc Natl Acad Sci U S A (2006) 103:4005–10. doi:10.1073/pnas.0508123103
7. Ghirlando R, Ward ES. Multiple roles for the major histocompatibility complex class I-related receptor FcRn. Annu Rev Immunol (2000) 18:739–66. doi:10.1146/annurev.immunol.18.1.739
8. Dewald B, Li S, Zhang H, Liu J, Shi X, Vafa O. Engineered FcγRs bind with longer half-life and are more potent in ADCC. J Immunol (2015) 195:1285–300. doi:10.4049/jimmunol.1500255
9. Dewald B, Li S, Zhang H, Liu J, Shi X, Vafa O. Engineered FcγRs bind with longer half-life and are more potent in ADCC. J Immunol (2015) 195:1285–300. doi:10.4049/jimmunol.1500255
10. Dewald B, Li S, Zhang H, Liu J, Shi X, Vafa O. Engineered FcγRs bind with longer half-life and are more potent in ADCC. J Immunol (2015) 195:1285–300. doi:10.4049/jimmunol.1500255
25. Kim JK, Tsen MF, Ghetie V, Ward ES. Identifying amino acid residues that influence plasma clearance of murine IgG1 fragments by site-directed mutagenesis. *J Mol Biol* (2001) 276:46974–77. doi:10.1006/jmbi.2000.5020

26. Brohns P, Iamnisch R, England F, Mancardi DA, Fernandez N, Jorieux S, et al. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. *Blood* (2009) 113:3716–25. doi:10.1182/blood-2008-09-177954

30. Wright A, Morrison SL. Effect of glycosylation on antibody function: implications for genetic engineering. *Trends Biotechnol* (1997) 15:26–8. doi:10.1016/S0167-7799(96)01062-7

31. Wright A, Morrison SL. Effect of glycosylation on antibody function: implications for genetic engineering. *Trends Biotechnol* (1997) 15:26–8. doi:10.1016/S0167-7799(96)01062-7

32. Krapp S, Mimura Y, Jefferis R, Huber R, Sondermann P. Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. *J Mol Biol* (2003) 325:979–86. doi:10.1016/S0022-2836(02)01250-0

33. Jefferis R. Recombinant antibody therapeutics: the impact of glycosylation on mechanism of action. *Trends Pharmacol Sci* (2009) 30:356–62. doi:10.1016/j.tips.2009.04.007

34. Jefferis R, Lund J. Interaction sites on human IgG-Fc for FcgammaR: current models. *Immunol Lett* (2002) 82:57–65. doi:10.1016/S0165-2478(02)00019-6

35. Lund J, Winter G, Jones PT, Pound JD, Tanaka T, Walker MR, et al. Human Fcgamma RI and Fcgamma RIIA interact with distinct but overlapping sites on human IgG. *J Immunol* (1991) 147:2657–62.

36. Lund J, Yoshiyuki T, Noriko T, Sarmay G, Yoji A, Jefferis R. A protein structural change in aglycosylated IgG3 correlates with loss of huFcR1 and huFcR1I1 binding and/or activation. *Mol Immunol* (2009) 45:1145–53. doi:10.1016/j.molimm.2008.04.003

37. Medesan C, Radu C, Kim JK, Ghetie V, Ward ES. Localization of the site of the IgG molecule that regulates maternofoetal transmission in mice. *J Immunol* (1996) 156:2533–6. doi:10.1064/ji.1996.156.6.2533

38. Mimura Y, Sondermann P, Ghirlanda R, Lund J, Young S, Goodall M, et al. Role of oligosaccharide residues of IgG1-Fc in Fc-gamma RII binding. *J Biol Chem* (2006) 251:4539–47. doi:10.1016/j.jbc.2005.12.004

39. Radaev S, Sun PD. Recognition of IgG by Fcgamma receptor. The role of FcgammaRII binding and the binding of peptide inhibitors. *J Biol Chem* (2001) 276:16478–83. doi:10.1016/S0022-2501(00)00019-6

40. Roopenian DC, Aiklesh S. FcRn: the neonatal Fc receptor comes of age. *Proc Natl Acad Sci U S A* (2015) 112:1111–6. doi:10.1073/pnas.1502981112

41. Walker MR, Lund J, Thompson KM, Jefferis R. Aglycosylation of human IgG1 and IgG3 monoclonal antibodies can eliminate recognition by human cells expressing FcgammaRII and/or FcgammaRII. *Biochem J* (1989) 259:347–53. doi:10.1042/bj0390347

42. Jefferis R, Lund J, Mizutani H, Nakagawa H, Kawazoe Y, Arata Y, et al. A comparative study of the N-linked oligosaccharides structures of human IgG subclass proteins. *Biochem J* (1990) 268:529–37. doi:10.1042/bj026529a

43. Raju TS. Terminal sugars of Fc glycans influence antibody effector functions. *Curr Opin Biotechnol* (2015) 31:94–101. doi:10.1016/j.coi.2014.09.001

44. Radaev S, Sun PD. Recognition of IgG by Fcgamma receptor. The role of FcgammaRII binding and the binding of peptide inhibitors. *J Biol Chem* (2001) 276:16478–83. doi:10.1016/S0022-2501(00)00019-6

45. Davies J, Jiang L, Pan LZ, LaBarre MJ, Anderson D, Reff M. Expression of GalT and IIIN3 in a recombinant anti-CD20 CHO production cell line: cell expression of antibodies with altered glycoforms leads to an increase in ADCC through FcgammaRII. *Biochem J* (2008) 413:121–7. doi:10.1042/bj20080082
50. Raju TS. Assessing Fc glycan heterogeneity of therapeutic recombinant monoclonal antibodies using NP-HPLC. Methods Mol Biol (2013) 988:169–80. doi:10.1007/978-1-62703-3275_10

51. Kuhota T, Nowa R, Satoh M, Akinaga S, Shitara K, Hanai N. Engineered therapeutic antibodies with improved effector functions. Cancer Sci (2009) 100:1566–72. doi:10.1111/j.1349-7006.2009.01222.x

52. Iudsogloz EE, Wong PY, Presta LG, Gazzano-Santoro H, Topal K, Utsch M, et al. Engineered antibodies with increased activity to recruit complement. J Immunol (2001) 166:2571–5. doi:10.4049/jimmunol.166.4.2571

53. Horton HM, Bernstein MJ, Pong E, Pemp M, Karki S, Chu SY, et al. Fc-engineered antibodies: a high-throughput biophysical analysis and data visualization of conformational stability of an IgG1 monoclonal antibody after deglycosylation. MAbs (2011) 2:519–27. doi:10.4161/mabs.2.2.11158

54. Alsenaidy MA, Jung ST, Kang TH, Monzingo AF, Georgiou G. Revisiting the role of tumor-associated carbohydrates in the FcγRIIa interaction: mechanistic insights into the role of sialylation. J Immunol (2008) 182:7663–71. doi:10.4049/jimmunol.0804182

55. Kelton W, Mehta N, Charab W, Lee J, Lee CH, Kojima T, et al. IgG1A: a novel Fc-engineered monoclonal antibody with selectively enhanced FcγRIIb binding over both FcγRIIa(R131) and FcγRIIa(H131). Protein Eng Des Sel (2013) 26:589–98. doi:10.1093/protein/gzt022

56. Jerne K, Nystrom JL, Gorlatov S, Zhang W, Yang Y, Huang L, Burke S, et al. Anti-tumor activity and toxicokinetics analysis of MGAH22, an anti-HER2 monoclonal antibody with enhanced FcγRβ binding properties. Breast Cancer Res (2011) 13:R123. doi:10.1186/bcr3069

57. Loo D, Alderson RF, Chen FZ, Huang L, Zhang W, Gorlatov S, et al. Development of an Fc-enhanced anti-B7-H3 monoclonal antibody with potent antitumor activity. Clin Cancer Res (2012) 18:3834–45. doi:10.1158/1078-0432.CCR-12-0715

58. Stewart R, Thom G, Levens M, Guler-Gane G, Holgate R, Rudd PM, et al. Engineered antibody Fc variant with selectively enhanced FcgammaIIb binding over both FcgammaIIa(R131) and FcgammaIIa(H131). Protein Eng Des Sel (2013) 26:589–98. doi:10.1093/protein/gzt022

59. Wehe GS, Coggin SJ, Stehling ME, Lien S, et al. IgG1a: a novel Fc-engineered antibody in murine and human systems have implications for therapeutic antibodies. J Immunol (2008) 182:7663–71. doi:10.4049/jimmunol.0804182

60. Moore GL, Chen H, Karki S, Lazar GA. Engineered Fc variant antibodies with enhanced ability to recruit complement and mediate effector functions. MAbs (2010) 2:181–9. doi:10.4161/mabs.2.2.11158

61. Yeung YA, Leabman MK, Marvin JS, Qiuf J, Adams CW, Lien S, et al. Engineered human IgG1 affinity to human neonatal Fc receptor: impact of affinity improvement on pharmacokinetics in primates. J Immunol (2009) 182:7663–71. doi:10.4049/jimmunol.0804182

62. Hinton PR, Xiong JM, Johls MG, Tang MT, Keller S, Tuuruhni N, et al. Engineered antibodies of IgG1/IgG3 mixed isotype with enhanced cytotoxic activities. Cancer Res (2008) 68:3863–72. doi:10.1158/0008-5472.147.11.08.008

63. Sazinsky SL, Ott RG, Silver NW, Tidor B, Ravetch JV, Wittrup KD. Aglycosylated immunoglobulin G1 variants productively engage activating Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 antibodies with improved binding to the Fc gamma R. J Biol Chem (2002) 277:6599–604. doi:10.1074/jbc.M00943200

64. Alsenaidy MA, Kim JH, Majumdar R, Weis DD, Joshi SB, Tolbert TJ, et al. Engineered human IgG1 antibody against lymphoma and leukemia. Cancer Res (2008) 68:3863–72. doi:10.1158/0008-5472.114.114.1178

65. Grevy A, Bern M, Foss S, Bratlie DB, Moen A, Gunnarsen KS, et al. Fc engineering of human IgG1 for altered binding to the neonatal Fc receptor affects Fc effector functions. J Immunol (2015) 194:5497–508. doi:10.4161/jimmunol.1401218

66. Vaccaro C, Bawdon R, Wanjie S, Ober RJ, Ward ES. Divergent activities of an engineered antibody in murine and human systems have implications for therapeutic antibodies. Nat Biotechnol Bioprocess Eng (2013) 24:671–8. doi:10.1093/protein/gzr015

67. Athari A, Frost D, Suthar KS, Brobbel R, Sanders DA, Brogden RN, et al. Improved binding to the Fc gamma R. J Biol Chem (2002) 277:6599–604. doi:10.1074/jbc.M00943200

68. Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, et al. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem (2002) 277:6599–604. doi:10.1074/jbc.M00943200

69. Alsenaidy MA, Nystrom JL, Gorlatov S, Zhang W, Yang Y, Huang L, Burke S, et al. Increasing the affinity of a human IgG1 for the neonatal Fc receptor (FcRn). J Biol Chem (2006) 281:23514–24. doi:10.1074/jbc.M604292200

70. Hinton PR, Johls MG, Xiong JM, Hanestad K, Bullock C, et al. Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem (2004) 279:6213–6. doi:10.1074/jbc.M300470200

71. Naso MF, Tam SH, Scallon BJ, Raju TS. Engineering host cell lines to reduce terminal sialylation of secreted antibodies. Nat Biotechnol (2002) 116:3004–12. doi:10.1038/jimmunol.166.4.2571

72. Naso MF, Tam SH, Scallon BJ, Raju TS. Engineering host cell lines to reduce terminal sialylation of secreted antibodies. Nat Biotechnol (2002) 116:3004–12. doi:10.1073/pnas.00197X

73. Karki S, Mcdonald M, Hinton P, Bratlie DB, Monzingo AF, Georgiou G. Engineered human IgG1 antibody restores neutrophil-mediated cytotoxicity. MAbs (2010) 2:181–9. doi:10.4161/mabs.2.2.11158

74. Ecker DM, Jones SD, Levine HL. The therapeutic monoclonal antibody anti-CD40 antibody enhances multiple effector functions and exhibits potent anti-tumor activity and in vivo antitumor activity against hematologic malignancies. J Immunol (2001) 166:3004–12. doi:10.1074/jimmunol.166.4.2571
and high proapoptotic activity for treatment of B-cell malignancies. Blood (2011) 118:4159–68. doi:10.1182/blood-2011-04-351932
89. Sondermann P, Szymkowski DE. Harnessing Fc receptor biology in the design of therapeutic antibodies. Curr Opin Immunol (2016) 40:78–87. doi:10.1016/j.coi.2016.03.005
90. Horton HM, Chu SY, Ortiz EC, Pong E, Cemerski S, Leung IW, et al. Antibody-mediated coengagement of FcgammaRIIb and B cell receptor complex suppresses humoral immunity in systemic lupus erythematosus. J Immunol (2011) 186:4223–33. doi:10.4049/jimmunol.1003412
91. Lee EM, Yee D, Busfield SJ, McManus JF, Cummings N, Vairo G, et al. Efficacy of an Fc-modified anti-CD123 antibody (CSL362) combined with chemotherapy in xenograft models of acute myelogenous leukemia in immunodeficient mice. Haematologica (2015) 100:914–26. doi:10.3324/haematol.2014.113092
92. Blum KA, Smith M, Fung H, Zalevsky J, Combs D, Ramies DA, et al. Phase I study of an anti-CD30 Fc engineered humanized monoclonal antibody in Hodgkin lymphoma (HL) or anaplastic large cell lymphoma (ALCL) patients: safety, pharmacokinetics (PK), immunogenicity, and efficacy. J Clin Oncol (2009) 27:8531.
93. Kumar A, Blum KA, Fung HC, Smith MR, Foster PA, Younes A. A phase I dose-escalation study of XmAb(R) 2513 in patients with relapsed or refractory Hodgkin lymphoma. Br J Haematol (2015) 168:902–4. doi:10.1111/ bjh.13152
94. Chu SY, Vostiar I, Karki S, Moore GL, Lazar GA, Pong E, et al. Inhibition of B cell receptor-mediated activation of primary human B cells by coengagement of CD19 and FcgammaRIIb with Fc-engineered antibodies. Mol Immunol (2008) 45:3926–33. doi:10.1016/j.molimm.2008.06.027
95. Chu SY, Horton HM, Pong E, Leung IW, Chen H, Nguyen DH, et al. Reduction of total IgE by targeted coengagement of IgE B-cell receptor and FcgammaRIIb with Fc-engineered antibody. J Allergy Clin Immunol (2012) 129:1102–15. doi:10.1016/j.jaci.2011.11.029
96. Awan FT, Lapalombella R, Trotta R, Butcher JP, Yu B, Benson DM Jr, et al. CD19 targeting of chronic lymphocytic leukemia with a novel Fc-domain-engineered monoclonal antibody. Blood (2010) 115:1204–13. doi:10.1182/blood-2009-06-229039
97. Aromon R, Gottlieb PA, Christiansen JS, Donner TW, Bosi E, Bode BW, et al. Low-dose otelixizumab anti-CD3 monoclonal antibody DEFEND-1 study: results of the randomized phase III study in recent-onset human type 1 diabetes. Diabetes Care (2014) 37:2746–54. doi:10.2337/dc13-0327
98. Merchant M, Ma X, Maun HR, Zheng Z, Peng J, Romero M, et al. Monovalent antibody design and mechanism of action of onartuzumab, a MET antagonist with anti-tumor activity as a therapeutic agent. Proc Natl Acad Sci U S A (2013) 110:E2987–96. doi:10.1073/pnas.1302725110
99. Park HI, Yoon HW, Jung ST. The highly evolvable antibody Fc domain. Trends Biotechnol (2016) 34:895–908. doi:10.1016/j.tibtech.2016.04.005
100. Bayliss TJ, Smith JT, Schuster M, Dragnev KH, Rigas JR. A humanized anti-IL-6 antibody (ALD518) in non-small cell lung cancer. Expert Opin Biol Ther (2011) 11:1663–8. doi:10.1517/14712598.2011.627850
101. Rosenzweig M, Ponte I, Apostolou I, Doty D, Guild I, Slavonic M, et al. Development of TRX518, an aglycosyl humanized monoclonal antibody (Mab) agonist of huGITR. J Clin Oncol (2010) 28:e13028.
102. Melis JR, Strumane K, Ruuls SR, Beurskens FJ, Schuurman J, Parren PW. Complement in therapy and disease: regulating the complement system with antibody-based therapeutics. Mol Immunol (2015) 67:117–30. doi:10.1016/j.molimm.2015.01.028

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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