



Recent Achievements and Challenges in Prolonging the Serum Half-Lives of Therapeutic IgG Antibodies Through Fc Engineering

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Abstract

Association of FcRn molecules to the Fc region of IgG in acidified endosomes and subsequent dissociation of the interaction in neutral pH serum enables IgG molecules to be recycled for prolonged serum persistence after internalization by endothelial cells, rather than being degraded in the serum and in the lysosomes inside the cells. Exploiting this intracellular trafficking and recycling mechanism, many researchers have engineered the Fc region to further extend the serum half-lives of therapeutic antibodies by optimizing the pH-dependent IgG Fc–FcRn interaction, and have generated various Fc variants exhibiting significantly improved circulating half-lives of therapeutic IgG antibodies. In order to estimate pharmacokinetic profiles of IgG Fc variants in human serum, not only a variety of in vitro techniques to determine the equilibrium binding constants and instantaneous rate constants for pH-dependent FcRn binding, but also diverse in vivo animal models including wild-type mouse, human FcRn transgenic mouse (Tg32 and Tg276), humanized mouse (Scarlet), or cynomolgus monkey have been harnessed. Currently, multiple IgG Fc variants that have been validated for their prolonged therapeutic potency in preclinical models have been successfully entered into human clinical trials for cancer, infectious diseases, and autoimmune diseases.

1 pH-Dependent Interaction of IgG and Neonatal Fc Receptor

Among therapeutic antibodies containing engineered Fc variants for improved serum half-life, Ultomiris[®] (ravulizumab-cwvz) was first approved by the US FDA in 2018, and was in the spotlight because it has fourfold longer frequency of administration compared with Soliris[®] (eculizumab) targeting the same indication. A main reason leading to this excellent serum persistence of the drug is that the engineered Fc region of Ultomiris[®] recirculates more

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Key Points

The pH-dependent interaction between the Fc region of IgG and FcRn is crucial for enhanced circulating half-life of an IgG antibody, and various Fc variants with enhanced pH-selective binding profile have been developed through rational design or a combinatorial approach.

To characterize IgG antibody Fc variants, equilibrium dissociation constants and instantaneous rate constant for Fc–FcRn interaction at both endosome and serum pH conditions have been analyzed, and a cell-based assay system that mimics serum recycling of IgG has been developed.

For the prediction of improved serum persistence of IgG antibody Fc variants in humans, various in vivo animal models such as transgenic mice (Tg32/Tg276/scarlet) and cynomolgus monkeys have been utilized.

Recently, Fc-engineered therapeutic antibodies with prolonged circulating half-lives have been validated in human clinical trials for various indications.

effectively via interaction with human neonatal Fc receptor (hFcRn). The importance of the IgG–FcRn interaction has been demonstrated in a mouse study, where the circulating half-life of mouse IgG1 antibody is only 1.4 days in FcRn-deficient mice (mFcRn^{-/-}), compared with a half-life of 9 days in mFcRn^{-/+} and mFcRn^{+/+} mice [1]. The recycling of IgG antibodies depends on the pH-dependent interaction with FcRn in particular. FcRn in the acidified endosomes binds with IgG antibodies that have been transported into the endosome by the pinocytosis mechanism, thereby preventing degradation of the antibodies via the lysosomal pathway. Because these antibodies show non-detectable binding affinity with FcRn at the neutral pH of the serum, they readily dissociate in the blood for re-circulation (Fig. 1a) [2]. These biological properties of antibodies are closely related to not only serum recycling but also transcytosis, a mechanism of antibodies or albumin for passing through the cells [2–4]. The CH2–CH3 interface region of the IgG Fc region binds to FcRn, which is a heterodimer consisting of an α -chain and a β -2 microglobulin (β_2m) (Fig. 1b) [5]. Although it is well known that the α -chain of FcRn interacts directly with IgG Fc, β_2m also affects the structural stability and function of FcRn via a non-covalent assembly with the α -chain [4, 6], explaining that β_2m -deficient mice show abnormally low serum levels of IgG [6–8]. To facilitate pH-dependent binding with strong binding at slightly acidic pH values (pH 5.5–6.5) and rapid dissociation at the physiological pH (pH 7.0–7.5), Ile253, Ser254, His310, His433,

His435, and Tyr436 amino acid residues in the CH2–CH3 interface region of Fc are known to be extremely important for binding with FcRn (Fig. 1b) [5, 9–11]. In particular, the His residues show pKa values in the range of 6.0–6.5 [12] and the imidazole side chains of the His residues undergo protonation to form a salt bridge with acidic residues on FcRn (Glu115 and Asp130), resulting in non-covalent bonding between Fc and FcRn that can act as a sensitive pH-dependent switch [2, 13]. In addition, the CH2–CH3 interface in the Fc region of human IgG1 contains a highly conserved Met252 (on the CH2 domain) and Met428 (on the CH3 domain) [14], and these Met residues form methionine sulfoxide (MetO) when oxidized, which significantly alters the structure of the Fc region and has been reported to impair Fc–FcRn interactions [15, 16]. The effects of each Met residue on FcRn binding have been analyzed using site-directed mutagenesis and Met252 oxidation was found to have a greater negative effect on FcRn binding than Met428 oxidation [14, 17].

There are numerous strategies aimed at improving the serum half-lives of short-lived therapeutic proteins using the FcRn interaction-mediated recycling mechanism such as either direct fusion or indirect targeting of pH-dependent FcRn binding molecules like Fc domain or albumin [10, 18–26]. Considering that Fc-engineered therapeutic antibody was first approved by the US FDA [27], and the emerging significance of serum persistence of IgG antibodies for viral diseases [28, 29] or cancer [30] therapy, we focused

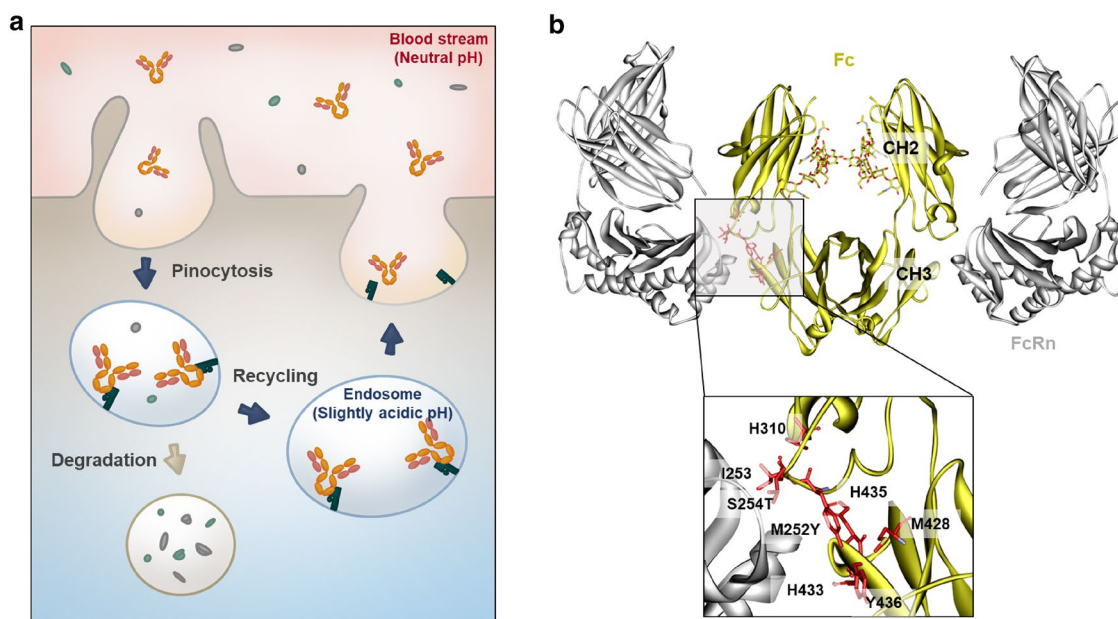


Fig. 1 Interaction between IgG Fc and the neonatal Fc receptor. **a** Recycling of serum IgG by pH-dependent binding with FcRn. **b** Complex structure of human FcRn and human IgG Fc variant (M252Y/S254T/T256E) (PDB: 4N0U). The key amino acid residues

of human Fc region that highly contribute to the pH-dependent interaction with human FcRn were annotated in the crystal structure. The numbers of amino acid were denoted based on the Kabat EU numbering system

on Fc engineering studies to enhance circulating half-lives of IgG antibodies via modulating the FcRn binding. In this review, we present Fc variants engineered to extend the serum half-lives of IgG antibodies by amino acid modification for superior pH-dependent interaction with FcRn, the analytical methods to assess the pharmacokinetic profiles of the IgG Fc variants using in vitro and in vivo models including a variety of transgenic mouse (Tg32, Tg276, and scarlet mouse) models and a cynomolgus monkey model, and the latest human clinical trials to evaluate the utility of the half-life—extending Fc variants applied to therapeutic IgG antibodies in a clinical area for various indications such as immune disorders, viral diseases, or cancer.

2 Fc Variants with Improved FcRn-IgG Interaction for Half-Life Extension

Understanding the structure and characteristics of FcRn–IgG interactions has provided a basis for engineering works to improve the serum half-lives of IgG antibodies [11, 31, 32]. Zalevsky et al. used a combination of high-throughput protein screening and rational design methods to discover the M428L/N434S (LS) variant, which has an 11-fold higher hFcRn binding affinity at pH 6.0 compared with the wild-type (Table 1) [30]. The authors introduced the LS variant into two model antibodies (bevacizumab and cetuximab) and confirmed 4.3-fold and 4.8-fold prolonged serum half-lives in an hFcRn transgenic (Tg) mouse model and 3.2-fold and 3.1-fold longer serum half-lives in a cynomolgus monkey model, respectively. Furthermore, they performed a xenograft study using an hFcRn/Rag1^{-/-} mouse model to investigate whether the enhanced pharmacokinetic properties also improved efficacy and found that anticancer antibodies with the half-life—extending Fc variant remarkably increased inhibition of tumor cell growth. In addition to improving the serum half-lives of IgG antibodies, the LS variant showed improved IgG transfer efficiency from blood to mucosa by hFcRn, which led to higher concentrations in the mucosa and improved prophylactic protection against primate SHIV infection through the mucosa [28, 29, 33]. Borghi et al. utilized the LS mutant to understand maternal transfer of the IgG antibody to the fetus and found that only interaction with FcRn determines the transfer of maternal antibodies [34]. This differs from a previous report by Jennewein et al., in which only maternal antibodies with a specific glycan were selectively transferred to the fetus by an FcγRIIIa-mediated mechanism [35].

Dall'Acqua et al. [36] used phage display to discover the M252Y/S254T/T256E (YTE) variant, which showed 10-fold higher binding affinity with hFcRn (Table 1). The MEDI-524 with the YTE variant, an antibody targeting respiratory syncytial virus (RSV), showed an almost 3.7-fold

longer serum half-life in a cynomolgus monkey model [37]. Oganessian et al. reported the crystal structure for the complex of the YTE variant and hFcRn, offering a molecular understanding of the interactions between human IgG antibodies and hFcRn [38].

Yeung et al. used phage display to discover N434A and N434W variants (Table 1) [39]. In that paper, the authors analyzed the correlation between the in vitro FcRn binding affinity and in vivo pharmacokinetics using the N434W variant, which showed improved binding affinity at both pH 6.0 and pH 7.4, and the N434A variant, which showed improved binding affinity at pH 6.0 but similar binding affinity to wild-type at pH 7.4. In a cynomolgus monkey model, the N434A variant showed a 2.3-fold longer half-life but the half-life of the N434W variant was not improved. These findings reassert the importance of pH-dependent FcRn binding by demonstrating that significantly increased binding affinity at pH 7.4 does not increase the half-life of antibodies, even with a greatly improved binding affinity at pH 6.0.

More recently, multiple Fc variants identified by various rational or combinatorial approaches have been reported (Table 1) [40]. Mackness et al. constructed a library with saturation point mutations at the amino acids critical for interactions with FcRn and used biolayer interferometry (BLI) to screen candidate human Fc variants. Next, the authors additionally analyzed the binding affinity for hFcRn and identified T256D/T307Q (DQ), T256D/T307W (DW), and M252Y/T256D (YD) exhibiting 2.1-fold, 2.1-fold, and 2.4-fold improved serum half-lives compared with wild-type Fc in a cynomolgus monkey model, respectively. Booth et al. discovered T307Q/Q311V/A378V and T256D/H286D/T307R/Q311V/A378V variants using a structure-based design, and the antibody Fc variants showed 3.1-fold and 3.9-fold longer half-lives, respectively, compared with the counterpart antibody with wild-type Fc in a cynomolgus monkey model (Table 1) [41]. Lee et al. discovered L309D/Q311H/N434S (DHS) by displaying full-length IgG on the bacterial inner membrane [42] and verified a 4.1-fold longer serum half-life in a humanized mouse model [Scarlett (hFcRn^{+/-} hβ2m^{+/-} hFcγR^{+/-} hIgG1^{+/-}, κ^{+/-})] (Table 1, Fig. 2c) [43]. In the study, the DHS variant exhibiting a lower binding affinity at pH 7.4 showed an improved serum half-life compared with the LS and YTE variants, which maintained a certain level of binding affinity with FcRn at pH 7.4.

In this section, we describe various engineered Fc variants exerting enhanced pH-dependent FcRn binding and prolonging circulating half-lives of IgG antibodies. LS and YTE variants have been adopted for therapeutic IgG antibodies, and their therapeutic utility resulting from enhanced pharmacokinetic profiles have been validated in human clinical trials. In addition, studies using N434A, N434W, and several recently reported Fc variants revealed that not only

Table 1 List of engineered Fc variants with enhanced binding to human FcRn

Mutations ^a	Model antibody (target antigen)	Fold increase of affinity at pH 6.0 (vs wild-type Fc)	Affinity at pH 7.4	Fold increase of half-life (vs wild type Fc)		References
				Tg mouse (mouse strain)	Cynomolgus monkey	
M428L/N434S	Bevacizumab (VEGF)	~ 11.28	Low	~ 4.3 (Tg 276 hemizygote)	~3.2	[30]
	Cetuximab (EGFR)			~ 4.8 (Tg 276 hemizygote)	~3.1	
M252Y/S254T/T256E	MEDI-524 (RSV)	~ 11	Low		~3.7	[36, 37]
N434A	Anti-hBSR (hBSR)	~ 4	Low		~2.3	[39]
N434W		~ 80	High		~1.5	
T256D/T307Q	mAb2 (Unknown)	~ 10	Low	~ 2.1 (Tg 32 homozygote)	~2.1	[40]
T256D/T307W		~ 14	Low	~ 1.7 (Tg 32 homozygote)	~2.1	
M252Y/T256D		~ 25	Low	~ 1.5 (Tg 32 homozygote)	~2.4	
T307Q/Q311V/A378V	Motavizumab (RSV)	~ 12.4	Low	~ 10 (Tg 276 homozygote)	~3.1	[41]
T256D/H286D/T307R/Q311V/A378V		~ 12.4	Low	~ 9.4 (Tg 276 homozygote)	~3.9	
L309D/Q311H/N434S	Trastuzumab (Her2)	~ 4.1	Very low	~ 5.8 (Humanized mouse ^b)		[43]

The data in the table represent the results reported in the literature. It should be noted that the pharmacokinetic profiles are highly variable depending on antigen type and animal model

^aThe amino acid residue numbers of the mutations are represented based on the Kabat EU numbering system

^bScarlett (hFcRn^{K1} hβ2m^{K1} hFcγR^{K1} hIgG1, κ^{K1}) mouse model

EGFR epidermal growth factor, *hBSR* human B-cell surface receptor, *RSV* respiratory syncytial virus, *VEGF* vascular endothelial growth factor

enhancing FcRn binding affinity at slightly acidic pH but also reducing its affinity at neutral pH is essential to determine whether serum half-life is prolonged or not.

3 In Vitro/in Vivo Models to Predict and Analyze the Serum Half-Life of IgG Fc Variants

Many researchers have isolated Fc variants using library screening methods based on a display system or site-directed mutagenesis studies on amino acid residues that have been reported to be critical for the Fc–FcRn interaction. In addition, various methods have been developed to predict the pharmacokinetic profiles of the IgG antibody containing the identified Fc variants. One such method for predicting the serum half-life in vitro is to measure the quantitative binding affinity with FcRn both at pH 6.0 and pH 7.4. However, because one hIgG homodimer binds with two hFcRn molecules, the quantitative rate constant and equilibrium dissociation constant can differ depending on the measurement method. To solve this problem, Abdiche et al. reported

a method to more precisely quantify the FcRn–Fc binding affinity using a surface plasmon resonance (SPR) biosensor [44]. Souders et al. reported a method of screening for candidate Fc variants enabling an improved serum half-life of IgG. In the work, they used BLI to measure the instantaneous binding rate, rather than the equilibrium dissociation constant, at pH 6.0 and pH 7.4 [45]. In addition to methods that quantitatively measure the binding affinity, there are methods that use cells to screen Fc variants expected to show a longer half-life. Grevys et al. developed a cell-based assay that mimics antibody recycling in the body and demonstrated that the results of using the assay for previously reported Fc variants were in strong agreement with a previous study [46].

To develop drugs based on the Fc variants verified in various in vitro studies, the pharmaceutical industry has been using the cynomolgus monkey model, a primate with 97% sequence identity with hFcRn and similar expression levels in tissue [47, 48]. The cynomolgus monkey model has been commonly used in assessments of antibody pharmacokinetics because it shares 90–93% genetic homology with humans and has a similar genetic background. Moreover,

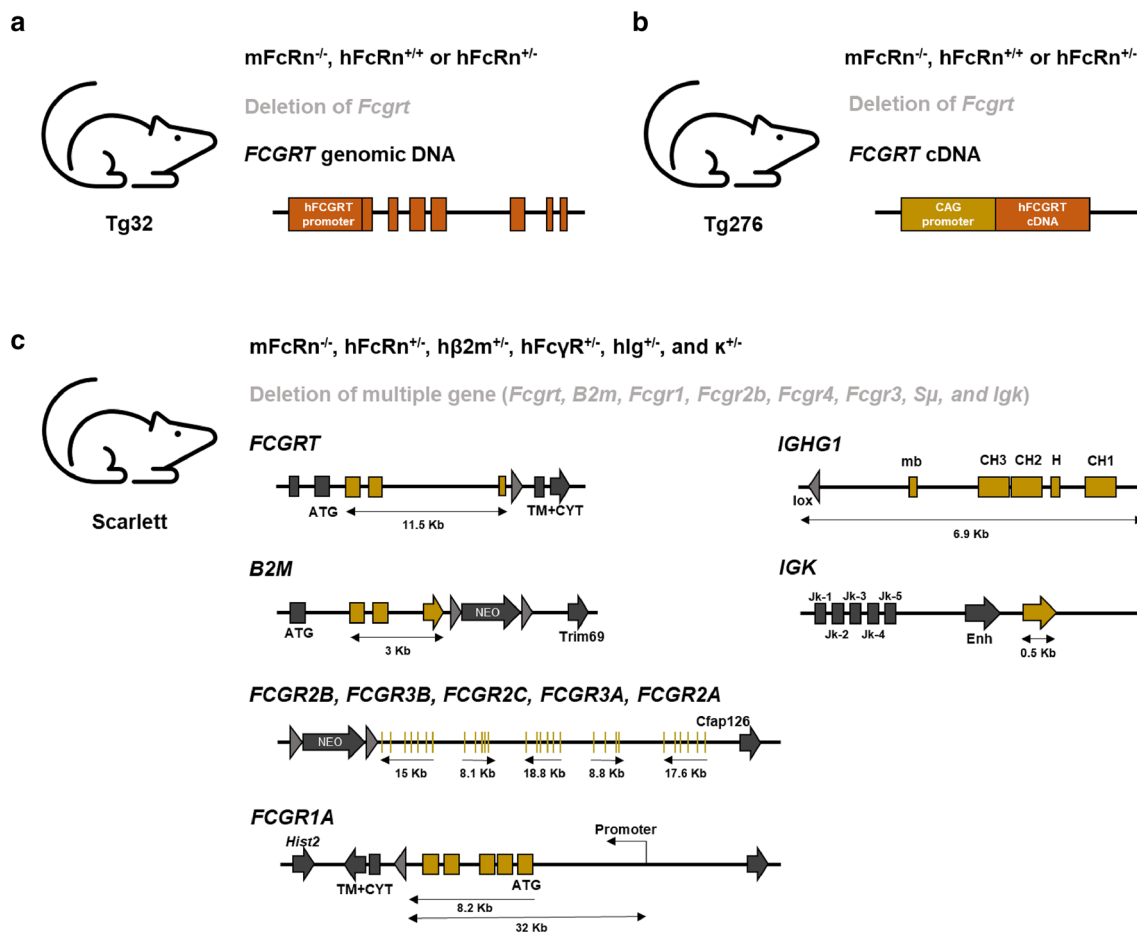


Fig. 2 In vivo mouse models that have been used for pharmacokinetic profile analysis of Fc-engineered IgG antibodies. **a–c** Schematic representations of gene structures for mouse models: Tg32 (**a**), Tg276 (**b**), and Scarlett (**c**). *B2M* mouse β-2 microglobulin gene, *FCGR1A* human Fcγ receptor I gene, *FCGR2A* human Fcγ receptor IIa gene, *FCGR2B* human Fcγ receptor IIb gene, *FCGR2C* human Fcγ receptor IIc gene, *FCGR3A* human Fcγ receptor IIIa gene, *FCGR3B* human

Fcγ receptor IIIb gene, *FCGRT* human neonatal Fc receptor gene, *IGHG1* human immunoglobulin heavy constant gamma 1 gene, *IGK* human immunoglobulin kappa gene, *B2m* mouse β-2 microglobulin gene, *Fcgr1* mouse Fcγ receptor I gene, *Fcgr2b* mouse Fcγ receptor IIb gene, *Fcgr3* mouse Fcγ receptor III gene, *Fcgr4* mouse Fcγ receptor IV gene, *Fcgrt* mouse neonatal Fc receptor gene, *Igk* mouse immunoglobulin kappa gene, *Sμ* S region of heavy chain gene

several studies have reported allometric scaling when analyzing the correlations between antibody pharmacokinetic profiles in cynomolgus monkey models and in humans [49–51]. However, because cynomolgus monkey models are too expensive to be used as a screening tool for Fc variants, the half-lives have been assessed using mouse models, which allow researchers to predict the pharmacokinetics in humans while being relatively inexpensive. Caution is required when using a normal mouse model to analyze the pharmacokinetics of antibody Fc variants, since human IgG shows a stronger binding affinity for mFcRn than for hFcRn and the Fc variants show different binding characteristics between hFcRn and mFcRn [52]. Most studies use the hFcRn Tg mouse model provided by the Jackson Laboratory to assess the improved serum half-lives of Fc-engineered therapeutic antibodies [53]. This model was created by disrupting the

Fcgrt (mFcRn) gene and inserting the *FCGRT* (hFcRn) gene in C57BL/6J(B6) mice (Fig. 2a and b). This model can be divided into Tg32 and Tg276 lines depending on the type of *FCGRT* gene, where Tg32 contains human *FCGRT* genomic DNA and Tg276 contains human *FCGRT* cDNA [53, 54]. Generally, the Tg276 mouse model generates lower serum half-life of an IgG antibody and exhibits subtle differences among the serum half-lives among IgG antibody Fc variants more sensitively compared with the Tg32 mouse model [53, 55]. On the other hand, the Tg32 mouse model has similar tissue distribution of hFcRn to that of humans in contrast to the Tg276 mouse model, of which the hFcRn is expressed at high levels in all tissues [48]. In addition, the correlation of serum half-lives of IgG antibodies between Tg32 mice and cynomolgus monkeys has been validated, and the mouse model has been used to predict pharmacokinetic profiles of

therapeutic antibodies in humans in various studies [56]. Based on the results reported so far, the Tg32 mouse model is preferred over the Tg276 mouse model for predicting the serum half-lives of IgG antibodies in humans.

Recently, for a pharmacokinetic profile analysis, Lee et al. employed Scarlett mice (hFcRn^{+/-} hβ2m^{+/-} hFcγR^{+/-} hIgG1^{+/-}, κ^{+/-} mice), which added hIgG1 heavy chains and human kappa light chains in a Marlene mice model (hFcRn^{+/-} hβ2m^{+/-} hFcγR^{+/-} mice) possessing human FcγR genes (*FCGR1A*, *FCGR2A*, *FCGR2B*, *FCGR2C*, *FCGR3A*, and *FCGR3B*), *FCGRT*, and *B2M* (human β2m gene) (Fig. 2c). The authors used the Scarlett mouse model to assess the half-lives of previously developed YTE and LS variants and their own DHS Fc variant. The DHS Fc variant showed a 1.5-fold longer half-life compared with previously reported Fc variants (YTE and LS) that are currently undergoing clinical trials for multiple antibodies [43].

4 Clinical Applications

4.1 Immunological Disorders

For Fc variants that have been proven to prolong the circulating half-life of the IgG antibody, several clinical studies are being conducted for various antigen-specific antibodies (Table 2). Of these, the most commonly used Fc variant is Xencor's LS variant (M428L/N434S), and as of September 20, 2020, there are eight antibodies using this platform that are in clinical trials. Ultomiris[®] (Ravulizumab-cwvz; administered at 8-week intervals), which was developed by Alexion, is an antibody targeting complement component 5 (C5). This antibody was approved by the US FDA in December 2018 for a phase III clinical trial [27] after demonstration of its safety and efficacy compared with eculizumab (administered at 2-week intervals), which is used to treat paroxysmal nocturnal hemoglobinuria (PNH). MEDI5117 is an example of a monoclonal antibody developed from a half-life-extended antibody using the YTE platform [57], and was developed by WuXi-Medimmune (joint venture of Medimmune and Wuxi Apptec) to treat rheumatoid arthritis (RA). This Fc-engineered therapeutic antibody was developed with a target antigen of interleukin-6 (IL-6), which is a key mediator causing pain and inflammation in various pathologic states [57], and is currently undergoing phase I clinical trials in the US and Europe.

4.2 Cancer

For anti-cancer therapy, three bispecific antibodies using Xtend[™] technology (XmAb20717: PD-1 × CTLA-4, XmAb23104: PD-1 × ICOS, and XmAb22841: CTLA-4 ×

LAG-3) are in phase I clinical trials targeting solid tumors. These are bispecific antibodies that can prevent tumor-reactive T-cell downregulation and reactivate T cells, restoring the immune response in the tumor microenvironment (TME) by targeting appropriate combinations of programmed cell death protein-1 (PD-1), cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), inducible T-cell co-stimulator (ICOS), or lymphocyte activation gene-3 (LAG-3). In clinical applications, these antibodies are intended to not only selectively bind T cells that highly express the immune checkpoint targets, but also to reduce the need for combination therapy. Additionally, the serum half-lives were greatly improved by introducing Xtend[™], which enables enhanced therapeutic effects and reduced frequency of administration of antibodies [58–60].

4.3 Viral Infectious Diseases

Clinical studies are being conducted on antibodies containing half-life extension Fc variants to treat viral diseases. In particular, VIR-7831 (GSK4182136), which was developed by Vir Biotechnology and GlaxoSmithKline (GSK), used Xtend[™] as a therapeutic antibody against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The antibody has been confirmed to neutralize the SARS-CoV-2 live virus by strongly binding to its spike protein (K_D value of S309-IgG1 and SARS-CoV-2 spike: 1.5×10^{-11} M) [61] and is currently in a phase II/III clinical trial. Other antiviral antibodies being developed by Vir Biotechnology with Xtend[™] include VIR-2482 and VIR-3434. VIR-2482 is an influenza A-neutralizing mAb that binds to the conserved region of hemagglutinin (HA) and neutralizes all major strains since the Spanish flu in 1918 (H1N1), which suggests the feasibility of using the antibody for a universal prophylactic vaccine, overcoming the limitations of current influenza vaccines, where the antibody response is dependent on individual seasonal antigens. In addition, because the serum half-life is increased, a single dose can last the entire influenza season of around 5–6 months. VIR-3434 is a monoclonal neutralizing antibody for chronic hepatitis B virus (HBV) infections. The company, Vir, claims that VIR-3434 prevents all ten HBV genotypes from entering hepatocytes and reduces the levels of virions and subviral particles in the blood. Also, they assert that the experimental results using a mouse model indicate that virions or subviral particles captured by VIR-3434 could stimulate maturation of dendritic cells (DCs) by an interaction between Fc domain of VIR-3434 and FcγRs on immature DCs, and ultimately it could have a vaccine-like property by generating HBV-specific T cells. Published preclinical and clinical data on VIR-3434 containing engineered Fc is eagerly awaited. Other antiviral antibodies include VRC01LS and elipovimab (GS-9722) for HIV, and nirsevimab (MEDI8897)

Table 2 Clinical status of therapeutic antibodies with extended serum half-lives

Name	Target	Format	Primary indication	Clinical phase*
XmAb20717	PD-1 × CTLA-4	Bispecific/Xtend	Oncology	I (NCT03517488)
XmAb23104	PD-1 × ICOS	Bispecific/Xtend	Oncology	I (NCT03752398)
XmAb22841	CTLA-4 × LAG-3	Bispecific/Xtend	Oncology	I (NCT03849469)
VRC01LS	CD4-binding site on gp120 of HIV-1	mAb/Xtend	HIV	I (NCT02599896)
Elipovimab (GS-9722)	V3 glycan motif on gp120 of the HIV	mAb/Cytotoxic/Xtend	HIV	I [62, 76, 77]
VIR-2482	Influenza A HA	mAb/Xtend	Influenza A	I/II (NCT04033406)
VIR-3434	Conserved region of HBsAg	mAb/Xtend	Chronic HBV infection	I (NCT04423393)
MEDI 5117	IL-6	mAb/YTE	RA	I (NCT01559103)
VIR-7831 (GSK4182136)	SARS-CoV-2	mAb/Xtend	COVID-19	II/III (NCT04545060)
Nirsevimab (MEDI8897)	RSV F protein	mAb/YTE	RSV infection	III (NCT03979313)
Ravulizumab (Ultomiris®)	C5	mAb/Xtend	PNH, aHUS	Approved (NCT03056040)

aHUS atypical hemolytic uremic syndrome, CTLA-4 cytotoxic T-lymphocyte-associated antigen-4, HA hemagglutinin, HBV hepatitis B virus, HIV human immunodeficiency virus, ICOS inducible T-cell co-stimulator, LAG-3 lymphocyte activation gene-3, PD-1 programmed cell death protein-1, PNH paroxysmal nocturnal hemoglobinuria, RA rheumatoid arthritis, RSV respiratory syncytial virus

*ClinicalTrials.gov identifiers are included in parentheses. The identifier for elipovimab is not listed on the website

for RSV [62]. VRC01LS and elipovimab, which were developed by the NIH and Gilead Science, respectively, utilize Xtend™ and are in human phase I clinical trials. VRC01LS shows an approximately fourfold longer serum half-life than the counterpart antibody with wild-type Fc (VRC01) and showed similar neutralizing activity in serum to VRC01 during 48 weeks of a phase I clinical trial [63, 64]. Elipovimab is derived from the HIV-neutralizing antibody PGT121 [65, 66] and consists of the Fab region, which is engineered to lower the immunogenicity and improve the stability at low pH, and the Fc region, which is engineered for prolonged serum half-life using Xtend™ and for enhanced binding affinity to FcγRIIIa (158Val/Phe) and FcγRIIa (131His/Arg) by introducing FcγR binding enhancing mutations [30, 67, 68]. Through these modifications, the antibody was designed to show not only HIV-neutralizing activity, but also enhanced effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) by natural killer (NK) cells and antibody-dependent cellular phagocytosis (ADCP) by macrophages [62]. Nirsevimab, an mAb targeting RSV fusion (F) glycoprotein that mediates virus entry through fusion of the viral membrane with the host cell membrane [69], was developed in collaboration with AstraZeneca and Sanofi Pasteur using the YTE platform [70]. MEDI8897

containing the YTE variant is currently in a phase III clinical trial. In a phase Ib/IIa trial in healthy preterm infants (32–35 weeks), MEDI8897 showed a very long serum half-life ($t_{1/2}$) of 62.5–72.9 days. The antibody reached the 90% effective concentration target level (EC_{90}) in 87% of the 50-mg dose group and showed a more than fourfold higher RSV-neutralizing antibody level. Thus, the half-life-extended anti-RSV-neutralizing antibody MEDI8897 was shown to offer protection from RSV for a typical 5-month season with a single intramuscular (IM) dose (50 mg) [71].

5 Discussion

Human IgG antibodies show much longer serum half-lives (around 3 weeks) compared with other therapeutic proteins due to recycling via interactions with hFcRn. Because the feasibility of research on half-life-extending Fc variants and the clinical utility of using therapeutic antibodies with the developed Fc variant have been well demonstrated, many pharmaceutical companies and research institutes are making huge efforts to prolong the antibody half-lives by improving the pH-dependent FcRn binding profiles [40, 41, 43].

Considering the very narrow range of pH difference between the endosome (pH 5.5–6.0) and serum (pH ~7.4), it is extremely difficult to fine tune the Fc–FcRn interaction for very high pH selectivity, which enables generation of Fc variants for dramatically improved serum half-lives of therapeutic antibodies [39]. However, accumulated structural insights and directed evolution techniques equipped with various cutting-edge high throughput screening platforms have allowed isolation of Fc variants with enhanced pH-dependent FcRn binding capability. As research on various engineered Fc variants has been actively conducted, the requirements that Fc variants must satisfy for an improved half-life have been suggested. Borrok et al. proposed that the half-life of the IgG antibody does not increase or even decrease when the affinity with FcRn at the pH of the Fc variant (7.4) is above a certain level [72]. To verify this hypothesis and to determine the threshold level of the FcRn binding affinity at pH 7.4, which is necessary for increasing the serum half-life of the IgG antibody, a variety of engineered Fc variants with various pH-dependent FcRn binding profiles should be discovered and the pharmacokinetic profiles of antibodies containing these Fc variants should be analyzed. Although the immunogenicity issue caused by the use of engineered Fc variants has not been reported for therapeutic antibodies that are approved or in human clinical trials, there are several experimental reports that some Fc variants could bind to rheumatoid factor [40, 43]. Therefore, thorough evaluation of potential immunogenicity for IgG antibodies containing Fc variants is necessary in the clinical development stage.

The major factor that determines whether or not the half-life is improved is the pH-dependent binding between Fc and FcRn, but there are other factors that affect the serum half-life of IgG, such as the isoelectric point (pI) of the antibody and self-interaction between antibodies, which should definitely be considered [73–75]. For this reason, to evaluate the half-life of an antibody introduced with an Fc variant, a pharmacokinetic analysis using *in vivo* models (Tg32 mice, Tg276 mice, humanized mice, cynomolgus monkeys, etc.) is crucial, as well as simply analyzing the FcRn binding affinities and physicochemical properties of the Fc-engineered antibodies. Since these animal model studies are costly and time consuming, *in vitro* analysis methods that can predict the *in vivo* half-lives of IgG Fc variants at reduced cost are in high demand, and seminal works related to this have been reported [45, 46]. However, the *in vitro* analysis methods developed to date have limitations in that they cannot take into account transport phenomena during IgG antibody circulation in the blood vessels of the body and during ADME (absorption, distribution, metabolism, and excretion). Therefore, to predict more precisely the pharmacokinetic profile of a variety of IgG Fc variants with reduced time and costs, it is absolutely necessary to develop *in vitro* analysis systems that

consider the critical parameters related to IgG circulation in an *in vivo* environment.

6 Conclusion

Isolation of various engineered Fc variants with endosome pH-selective FcRn binding capability and understanding of the parameters that affect the *in vivo* serum half-life will enable tailoring of the serum persistence of therapeutic antibodies. Therapeutic antibodies with improved half-lives not only lead to reduced patient inconvenience resulting from frequent injections, but also to enhanced therapeutic efficacy as an additional effect of improved serum persistence in the body [28–30, 33]. In addition, the developed Fc variants can be applied to develop monoclonal antibodies as prophylactic vaccines against novel infectious microorganisms such as SARS-CoV-2.

Declarations

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