Abstract: The constant region of the immunoglobulin (IG) or antibody heavy gamma chain is frequently engineered to modify the effector properties of the therapeutic monoclonal antibodies. These variants are classified in regards to their effects on effector functions, antibody-dependent cytotoxicity (ADCC), antibody-dependent phagocytosis (ADCP), complement-dependent cytotoxicity (CDC) enhancement or reduction, B cell inhibition by the coengagement of antigen and FcγR on the same cell, on half-life increase, and/or on structure such as prevention of IgG4 half-IG exchange, hexamerisation, knobs-into-holes and the heteropairing H-H of bispecific antibodies, absence of disulide bridge inter H-L, absence of glycosylation site, and site-specific drug attachment engineered cysteine. The IMGT engineered variant identifier is comprised of the species and gene name (and eventually allele), the letter ‘v’ followed by a number (assigned chronologically), and for each concerned domain (e.g., CH1, h, CH2 and CH3), the novel AA (single letter abbreviation) and IMGT position according to the IMGT unique numbering for the C-domain and between parentheses, the Eu numbering. IMGT engineered variants are described with detailed amino acid changes, visualized in motifs based on the IMGT numbering bridging genes, sequences, and structures for higher order description.

Keywords: IMGT; immunogenetics; immunoinformatics; immunoglobulin (IG); antibody; system biology; bioengineering; allotypes; variants; effector properties

1. Introduction

The adaptive immune response, acquired by jawed vertebrates (or gnathostomata) more than 450 million years ago and found in all extant jawed vertebrate species from fish to humans, is characterized by a remarkable immune specificity and memory, which are the properties of the B and T cells because of the extreme diversity of their antigen receptors [1]. The antigen receptors of the adaptive immune response [1,2] comprise the immunoglobulins (IG) or antibodies of the B cells and plasmocytes [3,4] and the T cell receptors (TR) of the T cells [5]. The IG recognizes antigens in their native (unprocessed) form, whereas the TR recognizes processed antigens, which are presented as peptides through its highly polymorphic major histocompatibility (MH, in humans HLA for human leucocyte antigens) proteins [6]. Immunoglobulins (IG) or antibodies serve a dual role in immunity. First, they both recognize antigens on the surface of foreign bodies such as bacteria and viruses, and second, they trigger elimination mechanisms such as cell lysis and phagocytosis to rid the body of these invading cells and particles [4]. IMGT®, the international ImMunoGeneTics information system® (https://www.imgt.org) (accessed on 11 October 2022) [1], was created in 1989 by Marie-Paule Lefranc in Montpellier, France, Laboratoire d’ImmuGénétique Moléculaire (LIGM) des Prof G. and M-P. Lefranc (Université de Montpellier and CNRS) to manage the huge diversity of the IG and TR repertoires. For the first time, immunoglobulin (IG) or antibody and T cell receptor (TR) variable (V),
diversity (D), joining (J) and constant (C) genes were officially recognized as ‘genes’ and conventional genes [1,3,5,7–10]. Through its creation, IMGT® marks the advent of a new science, immunoinformatics, which emerged at the interface between immunogenetics and bioinformatics [1]. As an ontology and system, IMGT® bridges genes, sequences and structures of the antigen receptors to better understand their functions. Focusing on the constant region of the IgG, a standardized definition of engineered variants of therapeutic antibodies is provided based on the IMGT concepts.

2. An Ontology and a System to Bridge Genes, Sequences and Structures to Functions

IMGT®, the international ImMunoGeneTics information system® (Figure 1) [1,11–21], is an integrated system for the genes, sequences and structures of the IG or antibodies, TR and MH of the adaptive immune responses of the jawed vertebrates, as well as other proteins of the IG superfamily (IgSF) [22] and MH superfamily (MhSF) of vertebrates and invertebrates [23].

Figure 1. IMGT® is the international ImMunoGeneTics information system® (https://www.imgt.org) [11–21]. The IMGT web resources (>25,000 pages, the IMGT Marie-Paule page) are not shown. IMGT/mAb-DB, the interface for therapeutic monoclonal antibodies and fusion proteins for immune applications (FPIA), has been available online since 4 December 2009 and IMGT/HighV-QUEST portal for the next generation sequencing (NGS) high-throughput sequence analysis since 22 November 2010 (with permission from M-P.Lefranc and G. Lefranc, LIGM, Founders of IMGT® from the international ImMunoGeneTics information system® (https://www.imgt.org)).
Immunoinformatics [1] builds and organizes molecular immunogenetics knowledge to be managed and shared in IMGT®. IMGT® comprises seven databases [24–30], 17 tools [31–50] and more than 25,000 pages of web resources (Table 1). IMGT® databases are specialized in sequences (i.e., IMGT/LIGM-DB [24,25]), genes and alleles (IMGT/GENE-DB [26]), two-dimensional (2D) structures (IMGT/2Dstructure-DB) and three-dimensional (3D) structures (IMGT/3Dstructure-DB) [27–29], whereas the IMGT/mAb-DB [30] interface allows the querying of therapeutic monoclonal antibodies (IG, mAb), fusion proteins for immunological applications (FPIA), composite proteins for clinical applications (CPCA) and related proteins (RPI) of therapeutic interest (with links to amino acid sequences in IMGT/2Dstructure-DB, and if available, to 3D structures in IMGT/3D structure-DB). The IMGT® tools include: (1) For nucleotide sequence analysis, IMGT/V-QUEST [31–36] and the integrated IMGT/JunctionAnalysis [37,38] and IMGT/Automat [39,40] tools, and for next generation sequencing, the high-throughput version IMGT/HighV-QUEST [36,41–45] and the downloadable IMGT/StatClonotype [46,47] package (which allows for statistical pairwise analysis of the diversity and expression of the IMGT clonotypes (AA) [43] and repertoire comparisons in adaptive immune responses); (2) for genomic analysis, IMGT/LIGMotif [48] (which allows for the identification and description of new genes in genomic sequences); (3) for amino acid sequence analysis per the domain, IMGT/DomainGapAlign [28,49,50]; and (4) for graphical representations of the domains, the IMGT/Collier-de-Perles tool [51] (e.g., IMGT Colliers de Perles of the variable (V), constant (C) and groove (G) domains). IMGT® Web resources (the IMGT Marie-Paule page) comprise the IMGT Repertoire (IG and TR, MH and RPI), IMGT Scientific chart, IMGT Education (IMGT Lexique, Aide-mémoire (amino acid physicochemical properties [52], splicing sites) and tutorials, etc.

Table 1. The IMGT databases, tools and web resources (‘The IMGT Marie-Paule Page’) for sequences, genes and structures.

| IMGT Databases | IMGT Tools | IMGT Web Resources | 'The IMGT Marie-Paule Page' |
|----------------|------------|---------------------|-----------------------------|
| **Sequences**  |            |                     |                             |
| IMGT/LIGM-DB [24,25] | IMGT/V-QUEST [31–36] | Standardized keywords and labels [53,54] |
| IMGT/PRIMER-DB | IMGT/JunctionAnalysis [37,38] | Standardized labels [55–58] |
| IMGT/CLL-DB | IMGT/Automat [39,40] | IMGT Repertoire (IG and TR, MH, RPI |
|                | IMGT/HighV-QUEST [36,41–45] | Alignments of alleles |
|                | IMGT/StatClonotype [46,47] | Protein displays |
|                | IMGT/PhyloGene | Tables of alleles |
|                | IMGT/Allele-Align | CDR-IMGT lengths |
|                |                     | Allotypes [59,60] |
|                |                     | Isotypes, etc. |
| **Genes**      | IMGT/GENE-DB [26] | Gene and allele nomenclature | [1–5,7,10,61–63] |
|                | IMGT/LIGMotif [48] | Chromosomal localizations |
|                | IMGT/LocusView | Locus representations |
|                | IMGT/GeneView | Locus description |
|                | IMGT/GenSearch | Gene exon/intron splicing sites |
|                | IMGT/CloneSearch | Gene tables |
|                | IMGT/GenInfo | Potential germline repertoires |
|                |                     | Lists of genes |
|                |                     | Correspondence between nomenclatures |
| **Structures** | IMGT/2Dstructure-DB | IMGT unique numbering per domain | [64–72] |
| IMGT/3Dstructure-DB [27–29] | IMGT/DomainGapAlign [28,49,50] | 2D Colliers de Perles (IG and TR, MH, RPI |
| IMGT/mAb-DB [30] | IMGT/DomainDisplay | [51,73–77] |
|                | IMGT/StructuralQuery | IMGT classes for amino acid |
|                | IMGT/Collier-de-Perles [51] | physicochemical properties [52] |
|                |                     | IMGT Colliers de Perles reference profiles [52] |
|                |                     | 3D representations |
The bridging of genes, structures and functions is based on the IMGT-ONTOLOGY axioms and concepts from which were generated the IMGT Scientific chart rules [78–82] (Table 2): CLASSIFICATION for the IMGT standardized gene and allele nomenclature [1–5,7–10,61–63], IDENTIFICATION for IMGT standardized keywords and keyword abbreviations (e.g., clonotype, paratope and epitope, variant, Fc receptor and FcR) [53,54], DESCRIPTION for IMGT standardized labels [55–58] (e.g., complementarity determining region (CDR)-IMGT (CDR1-IMGT to CDR3-IMGT) [57] and framework region (FR-IMGT) (FR1-IMGT to FR4-IMGT) [58]), NUMEROTATION for the IMGT unique numbering [64–72] and the IMGT Colliers de Perles [51,73–77]. IMGT positions per domain are used in Protein displays, Alignments of alleles, CDR-IMGT lengths, Allotypes [59,60] sections of the IMGT Repertoire, and to number amino acids involved in paratope/epitope (antigen receptor V-domains/target interactions [83]) (Table 1) and in effector properties (antigen receptor C-domain/effector binding proteins [6]).

Table 2. IMGT-ONTOLOGY axioms, concepts and IMGT Scientific chart rules.

| IMGT-ONTOLOGY Axioms and Concepts | IMGT Scientific Chart Rules |
|-----------------------------------|-----------------------------|
| IDENTIFICATION [54]               | Concepts of identification [53] |
|                                   | Standardized keywords [53,54] |
|                                   | (e.g., clonotype, paratope, epitope, variant, Fc receptor, FcR) (1). |
| DESCRIPTION [56]                  | Concepts of description [55] |
|                                   | Standardized labels and annotations [55–58] (e.g., CDR-IMGT [57], FR-IMGT [58], antibody description [84]) |
| CLASSIFICATION [63]               | Concepts of classification [62] |
|                                   | Reference sequences |
|                                   | Standardized IG and TR gene nomenclature (group, subgroup, gene, allele) [1–5,7–10,61–63] (1). |
| NUMEROTATION [64]                 | Concepts of numerotation [65–72] |
|                                   | IMGT unique numbering for |
|                                   | V- and V-LIKE domains [65–67] |
|                                   | C- and C-LIKE domains [68] |
|                                   | G- and G-LIKE domains [69] |
|                                   | IMGT Colliers de Perles [73–77] |
| ORIENTATION                       | Concepts of orientation |
|                                   | Chromosome orientation |
|                                   | Locus orientation |
|                                   | Gene orientation |
|                                   | DNA strand orientation |
|                                   | Domain beta-strand orientation |
| OBTENTION                         | Standardized origin |
|                                   | Standardized methodology |

Keyword use versus gene name nomenclature for defining a receptor: in this paper, this concerns the related proteins of immune interest (RPI) such as the Fc receptor’s gamma. Owing to the diversity and multiplicity of these receptors, and in the absence of standardized sequence characterization in functional analysis, these receptors are usually identified with keywords, for example for Homo sapiens, FcyR, FcyRI, FcyRII, FcyRIII and so on. However, it should be noted that, when there is no ambiguity as to the interactive chain involved, the HGNC gene name should be used (FCGR1A, FCGR2A, FCGR2B, FCGR2C, FCGR3A and FCGR3B). This rule is applied in this paper for the neonatal Fc receptor (FcRn), which is made of the interactive Fc gamma receptor and transporter (FCGRT) chain that is associated with B2M.

ImGT standards have been used since 2006 in the description of the therapeutic antibodies published in the World Health Organization’s (WHO) International Nonproprietary Names (INN) programme [84–86]. Since 2003, IMGT® has been widely used in the analysis of therapeutic antibodies for humanization and/or engineering [4,11,13,87–96].

3. Immunoglobulin IgG Receptor, Chains, Domains and Amino Acids

The Homo sapiens’ IgG1-kappa (Figure 2) is taken as an example (Table 3) because it is the most represented subclass in therapeutic antibodies.
3. Immunoglobulin IgG receptor, Chains, Domains and Amino Acids

The Homo sapiens IgG1-kappa (Figure 2) is taken as an example (Table 3) because it is the most represented subclass in therapeutic antibodies.

Figure 2. Immunoglobulin IgG1. The structure is that of the antibody b12, an IgG1-kappa, and so far is the only complete human IG crystallized (PDB code: 1hzh, from IMGT® https://www.imgt.org, IMGT/3Dstructure-DB). H-GAMMA-1 and L-KAPPA (used for the chains), VH, CH1, CH2, CH3, V-KAPPA and C-KAPPA (for the domains) are written in capital letters as they are IMGT standardized labels (DESCRIPTION) [1]. This first 3D-structure of a complete Homo sapiens IG shows the expected Y shape with the two Fragment antigen binding (Fab) arms (one L-KAPPA light chain (V-KAPPA-C-KAPPA) paired to the VH-CH1 of each H-GAMMA-1 heavy chain) and the Fragment crystallisable (Fc), made of the paired hinge-CH2-CH3 of the two H-GAMMA-1 heavy chains. The figure also shows the relative position, in space, of the L-KAPPA relative to the VH-CH1 in each Fab (in the front on the left hand side, and the back right hand side). The sequences of the two H-GAMMA1 chains (colored in purple and dark blue for a better visibility) are identical and the sequences of the two L-KAPPA chains (colored in orange and green for a better visibility) are identical (with permission from M-P. Lefranc and G. Lefranc, LIGM, Founders of IMGT®, the international ImMunoGeneTics information system®, https://www.imgt.org).

Table 3. The immunoglobulin IgG1 receptor, chain and domain structure labels and correspondence with sequence labels. IMGT standardized labels are in capital letters. They are shown with the example Homo sapiens IgG1-kappa.

| Receptor | Chain | Domain Type | Domain | Region ¹ |
|----------|-------|-------------|--------|---------|
| IG-GAMMA-1_KAPPA | H-GAMMA-1 | V | VH | V-D-J-REGION |
|          |       | C | CH1 | C-REGION ² |
|          |       | C | CH2 |        |
|          |       | C | CH3 |        |
| L-KAPPA | V     | V-KAPPA | V-J-REGION |
|          | C     | C-KAPPA | C-REGION |

¹ The VH-domain (or V-D-J-REGION) and the VL-domain (V-KAPPA or V-LAMBDA) (or V-J-REGION) are encoded by rearranged V-(D)-J genes, whereas the remainder of the chain is the C-REGION (encoded by a C gene). The C-REGION comprises one C-domain (C-KAPPA or C-LAMBDA) for the L chain, or several C-domains (CH) for the H chain. ² The heavy chain C-REGION also includes the HINGE-REGION, and for membrane IG (mIG), the CONNECTING-REGION (CO), TRANSMEMBRANE-REGION (TM) and CYTOPLASMIC-REGION (CY); for secreted IG (sIG), the C-REGION includes CHS instead of CO, TM and CY.
In the IMGT system, the C-domain includes the C-DOMAIN of the IG and of the TR [1] and the C-LIKE-DOMAIN of the IgSF other than IG and TR [22]. The C-domain description of any receptor, any chain and any species is based on the IMGT unique numbering for the C-domain (C-DOMAIN and C-LIKE-DOMAIN) [68]. A C-domain (Figure 3) comprises about 90–100 amino acids and is made up of seven antiparallel beta strands (A, B, C, D, E, F and G), linked by beta turns (AB, DE and EF), a transversal strand (CD) and two loops (BC and FG), and forms a sandwich of two sheets [ABED] [GFC]. A C-domain has a topology and a three-dimensional structure that is similar to that of a V-domain [67], but without the C’ and C” strands and the C’C” loop, which is replaced by a transversal CD strand [68]. The lengths of the strands and loops (Table 4) are visualized in the IMGT Colliers de Perles on one layer and two layers (Figure 3).

Figure 3. IG constant (C) domain. (A) 3D structure ribbon representation with the IMGT strand and loop delimitations. (B) IMGT Collier de Perles on two layers with hydrogen bonds. The IMGT Colliers de Perles on two layers show, in the forefront, the GFC strands, and in the back, the ABED strands (located at the interface CH1/CL of the IG), linked by the CD transversal strand. The IMGT
Collier de Perles with hydrogen bonds (green lines online, only shown here for the GFC sheet) is generated by the IMGT/Collier de Perles tool [51] integrated in the IMGT/3Dstructure-DB, from experimental 3D structure data. (C) IMGT Collier de Perles on two layers from IMGT/DomainGapAlign [28,49,50]. (D) IMGT Colliers de Perles on one layer. Amino acids are shown in the one-letter abbreviation. All proline (P) are shown online in yellow. IMGT anchors are represented by squares. Hatched circles are IMGT gaps according to the IMGT unique numbering for the C-domain [68]. Positions with bold (online red) letters indicate the four conserved positions that are common to a V-domain and to a C-domain: 23 (1st-CYS), 41 (CONSERVED-TRP), 89 (hydrophobic), 104 (2nd-CYS), and position 118, which is only conserved in V-DOMAIN. The identifier of the chain to which the CH-domain belongs is 1n0x_H (from the Homo sapiens b12 Fab, in IMGT/3Dstructure-DB, https://www.imgt.org) [27–29]. The 3D ribbon representation was obtained using PyMOL and “IMGT numbering comparison” of 1n0x_H (CH1) from IMGT/3Dstructure-DB (https://www.imgt.org) [27–29].

Table 4. C-domain strands, turns and loops, IMGT positions and lengths, based on the IMGT unique numbering for C-domain (C-DOMAIN and C-LIKE-DOMAIN) [68]. (With permission from M-P. Lefranc and G. Lefranc, LIGM, Founders of IMGT® , the international ImMunoGeneTics information system®, https://www.imgt.org).

| C Domain Strands, Turns and Loops a | IMGT Position b | Lengths c | Characteristic IMGT Residue@Position d |
|-----------------------------------|----------------|-----------|--------------------------------------|
| A-STRAND                          | 1–c15          | 15 (14 if gap at 10) |                                      |
| AB-TURN                           | 15.1–15.3      | 0–3       |                                      |
| B-STRAND                          | 16–26          | 11        | 1st-CYS 23                           |
| BC-LOOP                           | 27–31          | 10 (or less) |                                      |
|                                   | 34–38          |           |                                      |
| C-STRAND                          | 39–45          | 7         | CONSERVED-TRP 41                     |
| CD-STRAND                          | 45.1–45.9     | 0–9       |                                      |
| D-STRAND                          | 77–84          | 8 (or 7 if gap at 82) |                                      |
| DE-TURN                           | 84.1–84.7      | 0–14      |                                      |
|                                   | 85.1–85.7      |           |                                      |
| E-STRAND                          | 85–96          | 12        | hydrophobic 89                      |
| EF-TURN                           | 96.1–96.2      | 0–2       |                                      |
| F-STRAND                          | 97–104         | 8         | 2nd-CYS 104                          |
| FG-LOOP                           | 105–117        | 13 (or less, or more) |                                      |
| G-STRAND                          | 118–128        | 11 (or less) |                                      |

- IMGT labels (concepts of description) are written in capital letters (no plural) [55,56].
- based on the IMGT unique numbering for C-domain (C-DOMAIN and C-LIKE-DOMAIN) [68].
- in number of amino acids (or codons).
- IMGT Residue@Position is a given residue (usually an amino acid) or a given conserved property amino acid class, at a given position in a domain, based on the IMGT unique numbering [68].

There are six IMGT anchors in a C-domain (four of them identical to those of a V-domain): Positions 26 and 39 (anchors of the BC loop), 45 and 77 (by extension, anchors of the CD strand as there is no C'-C" loop in a C-domain [68]), and 104 and 118 (anchors of the FG loop). A C-domain has five characteristic amino acids at given positions (positions with bold (online red) letters in the IMGT Colliers de Perles). Four of them are highly conserved and hydrophobic [52] and are common to the V-domain: 23 (1st-CYS), 41 (CONSERVED-TRP), 89 (hydrophobic) and 104 (2nd-CYS). These amino acids contribute to the two major features shared by the V and C-domains: The disulfide bridge (between the two cysteines 23 and 104) and the internal hydrophobic core of the domain (with the side chains of tryptophan W41 and amino acid 89). The fifth position, 118, is diverse and is characterized as being an FG loop anchor. In the IMGT system, the C-domains (C-DOMAIN and C-
LIKE-DOMAIN) are delimited considering the exon delimitation, whenever appropriate, allowing the integration of strands A and G, which do not have structural alignments.

The 20 usual amino acids (AA) have been classified in eleven IMGT physicochemical classes [52] (IMGT® https://www.imgt.org, IMGT Education > Aide-mémoire > Amino acids) (Figure 4).

| 'Volume' classes | 'Hydrophobic' | Neutral | 'Hydrophilic' |
|------------------|--------------|---------|--------------|
| in Å³             |              |         |              |
| Very large       | 189-228      | W        |              |
| Large            | 162-174      | I, L     | K, R         |
| Medium           | 138-154      | V, C     | H, E, Q      |
| Small            | 108-117      | A, S     | D, N         |
| Very small       | 60-90        |          |              |

Figure 4. IMGT physicochemical classes of the 20 usual amino acids (AA) [52] (with permission from M-P. Lefranc and G. Lefranc, LIGM, Founders of IMGT®, the international ImMunoGeneTics information system®, https://www.imgt.org).

4. IGHG, IGKC and IGLC2 Engineered Variants

One hundred and fourteen IGHG engineered variants have been defined by their IMGT gene nomenclature, the IMGT unique numbering for C-domain [68] and IMGT motifs in domain strands and/or loops (Table 4, Figure 3), with corresponding Eu positions [97] (IMGT https://www.imgt.org, IMGT Scientific chart > Correspondence between C numberings > Correspondence between the IMGT unique numbering for C-DOMAIN, the IMGT exon numbering, the EU and Kabat numberings: Human IGHG [97,98] https://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html) (Supplementary Table S1). The IGKC and IGLC2 engineered variants involved in the structure have also been defined similarly by their IMGT gene nomenclature, the IMGT unique numbering for the C-domain [68] and IMGT motifs in the domain strands and/or loops (Table 4), with corresponding Eu positions [97] (IMGT https://www.imgt.org, IMGT Scientific chart > Correspondence between C numberings > Correspondence between the IMGT unique numbering for the C-DOMAIN, the IMGT exon numbering, the EU and Kabat numberings: Human IGKC [97,98].

The correspondence between the IMGT unique numbering and the Eu positions are provided here in a horizontal format for the IGHG1 CH1, hinge, CH2 and CH3-domains (Figure 5), and hinges of IGHG1, IGHG2, IGHG3 and IGHG4 (Figure 6), and by extension to the alignment of IGKC and IGLC2 with IGHG1 CH1 (Figure 7).
Correspondence between the Homo sapiens IGHG1 amino acid sequence, based on the IMGT unique numbering for the C-domain [68] and the Eu positions (shown vertically) from 118 to 445 [97].

(A) IGHG1 CH1, CH2 and CH3. The standardized presentation of the IMGT unique numbering on the top two lines [68] can be obtained using IMGT/DomainGapAlign [28,49,50], the IMGT reference tool for constant C-domain amino acid sequence analysis. The IMGT unique numbering for the CH1, CH2 and CH3 is shown on the first horizontal line with additional IMGT positions (by comparison to the V-domain IMGT unique numbering [67]) on line two. Amino acids at these additional positions are highlighted in bold. The Eu numbers are read vertically (on three lines top to down) at each position below the amino acid sequence. For example, the first amino acid of the Homsap IGHG1 CH1 is A1.4 (read G1, and going left, K1.1, T1.2, S1.3 and A1.4) and corresponds to Eu 118 (below A, read one top line, one second line and eight third line). The last amino acid of CH1 is a V, at position IMGT 121 (3 dots after 118), and corresponds to Eu 215 (below V, read two top line, one second line and five third line). The first amino acid of the Homsap IGHG1 CH2 A1.6 corresponds to Eu 231, whereas the last one, K, at position IMGT 125 (7 dots after 118), corresponds to Eu 340. The first amino acid of the Homsap IGHG1 CH3 G1.4 corresponds to Eu 341, whereas the last one, P, at position IMGT 125, corresponds to Eu 445. The first amino acid of the CH1, hinge, CH2 and CH3 results from the splicing. The four conserved amino acids of the C-DOMAIN C23, W41, hydrophobic 89 and C104 are highlighted in colors (C23 and C104 in pink, W41 and hydrophobic 89 (V, L) in blue). The four AA and IMGT positions C23, W41, hydrophobic 89 and C104 correspond, respectively, to Eu 144, 158, 186 and 200 in CH1, 261, 277, 306 and 321 in CH2, and 367, 381, 410 and 425 in CH3. The CH2 asparagine N84.4 of the N-glycosylation site corresponds to Eu 297 (colored in green). The amino acids of the C-domain BC-LOOP and FG-LOOP (Table 4) are highlighted in bold and brown color. (B) Homsap IGHG1 hinge. The hinge IMGT 1 to 15 corresponds to Eu 216 to 230. Cysteines (C) and prolines (P) with Eu positions are highlighted in pink and yellow, respectively. (Drawn by Marie-Paule Lefranc and Gérard Lefranc, LIGM, Founders and Authors of IMGT®, the international ImMunoGeneTics information system®, https://www.imgt.org, Copyright 2022.)
Figure 6. Correspondence between the *Homo sapiens* IGHG1, IGHG2, IGHG3 (4 exons) and IGHG4 IMGT numbering with the IGHG1 Eu positions. The top line indicates the IMGT numbering for the IGHG1, IGHG2 and IGHG4 hinges and for the four exons (H1 to H4) of the IGHG3 hinge. The Eu numbers are read vertically (on three lines top to down) at each position below the amino acid sequence. Dashes indicate the positions that are absent in the Eu numbering. Cysteines (C) and prolines (P) with Eu positions are highlighted in pink and yellow, respectively. (Drawn by Marie-Paule Lefranc and Gérard Lefranc, LIGM, Founders and Authors of IMGT®, the international ImMunoGeneTics information system®, https://www.imgt.org, Copyright 2022).

Figure 7. Correspondence between the *Homo sapiens* IGKC, IGLC2 and IGHG1 CH1 sequences, based on the IMGT unique numbering [68] and the Eu positions [97]. The first amino acid of each sequence results from the splicing. The IGHG1 CH1 chosen as the CH representative is from Figure 5A. The IMGT unique numbering is shown on the top horizontal line one with additional IMGT positions on line two. Amino acids at these additional positions (by comparison to the V-domain IMGT unique
numbering [67]) are highlighted in bold in the Homsap IGKC, IGLC2 and IGHG1 CH1 sequences. The Eu numbers are read vertically (on three lines top to down) at each position below the amino acid sequences. For example, the first amino acid of IGKC R1.4 corresponds to Eu 108, that of IGLC2 G1.5 to Eu 107, and that of IGHG1 CH1 A1.4 to Eu 118, the last amino acid of IGKC C126 corresponds to Eu 214, that of IGLC2 S215 to ‘deduced Eu position 215’ and that of IGHG1 CH1 V at position IMGT 121 corresponds to Eu 215. The four conserved amino acids of the C-DOMAIN C23, W41, hydrophobic 89 and C104 are highlighted in colors (C23 and C104 in pink, W41 and hydrophobic 89 (L, V) in blue). The four AA and IMGT positions C23, W41, hydrophobic 89 and C104 correspond, respectively, to Eu 134, 148, 179, 194 for IGKC and IGLC2 and to Eu 144, 158, 186 and 200 in IGHG1 CH1. The amino acids of the C-domain BC-LOOP and FG-LOOP (Table 4) are highlighted in bold and brown color. (Drawn by Marie-Paule Lefranc and Gérard Lefranc, LIGM, Founders and Authors of IMGT®, the international ImMunoGeneTics information system®, https://www.imgt.org, Copyright 2022.)

Standardized characterization has become a necessity, owing to the increasing number of engineered antibodies of effector properties [99,100] and/or various formats. Based on the IMGT Scientific chart rules, we propose a standardized IMGT nomenclature of engineered variants involved in effector properties (ADCC, ADCP and CDC), half-life and structure of therapeutical monoclonal antibodies. The standardized variant characterization comprises (1) the IMGT engineered Fc variant name (e.g. G1v1), (2) the IMGT variant definition (for each amino acid (AA) change: domain, AA in the one-letter abbreviation [52] and its position in the IMGT unique numbering for C domain [68], e.g. CH2 P1.4, (3) the IMGT amino acid changes on the IGHG CH domain with the Eu numbering between parentheses (e.g., CH2 E1.4 > P (233)), (4) the Eu numbering variant (e.g., E233P), (5) the IMGT motif positions according to the IMGT unique numbering [68], followed between parentheses, by the Eu numbering, motif with AA before and after the AA change in bold (e.g., IGHG1 CH2 1.6–3 (231–239) APHELGGPS > APPELLGGPS; underlined amino acids in the motif correspond to additional positions in the IMGT unique numbering for the C-domain [68,70–72], e.g., APELLG and APPLLG which correspond to 1.6, 1.5, 1.4, 1.3, 1.2 and 1.1), and (6) information from the literature regarding ‘property and function’.

These properties and functions have allowed to classify the IMGT engineered variants in 19 types (#1 to #19) corresponding to four categories. The first category ‘Effector’ refers to the variants that affect the effector properties: ADCC reduction #1 (Table 5), ADCC enhancement #2 (Table 6), ADCP and CDC enhancement #3 (Table 7), CDC enhancement #4 (Table 8), CDC reduction #5 (Table 9), ADCC and CDC reduction #6 (Table 10), B cell inhibition by the coengagement of antigen and FcγR on the same cell #7 (Table 11), knock out CH2 84.4 glycosylation #8 (Table 12), the second category ‘Half-life’ refers to the variants that affect (most of them increasing) the half-life #9 (Table 13), the third one ‘Protein A’ refers to the abrogation of binding to protein A #10 (Table 14), the fourth one ‘Structure’ refers to variants that affect the stability or structure of monospecific, bispecific or multispecific antibodies and include: formation of additional bridge stabilizing CH2 in the absence of N84.4 (297) glycosylation #11 (Table 15), prevention of IgG4 half-IG exchange #12 (Table 16), hexamerisation #13 (Table 17), knobs-into-holes and the enhancement of heteropairing H-H of bispecific antibodies #14 (Table 18), suppression of inter H-L and/or inter H-H disulfide bridges #15 (Table 19), site-specific drug attachment #16 (Table 20), enhancement of heteropairing H-L of bispecific antibodies #17 (Table 21), control of half-IG exchange of bispecific IgG4 #18 (Table 22), reducing acid-induced aggregation #19 (Table 23).
Table 5. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in antibody-dependent cellular cytotoxicity (ADCC) reduction (Effector #1).

| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes With the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function |
|----------------------------------|----------------------------------|-------------------------------------------|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------|----------------------|----------------------|
| G1v1                             | CH2 P1.4                         | CH2 E1.4 > P (233)                        | E233P                           | IGHG1 CH2 1.6–3 (231–239) APLELLGGPS > APELLAGGPS                                                                                   | ADCC reduction.     | Prevents FcγRI binding [101] |
|                                  |                                 |                                           |                                 |                                                                                                                                                                                   |                      |                      |
| G1v2                             | CH2 V1.3                         | CH2 L1.3 > V (234)                        | L234V                           | IGHG1 CH2 1.6–3 (231–239) APLELLGGPS > APELLAGGPS                                                                                   | ADCC reduction.     | Decreases FcγRI binding [101] |
| G1v3                             | CH2 A1.2                         | CH2 L1.2 > A (235)                        | L235A                           | IGHG1 CH2 1.6–3 (231–239) APLELLGGPS > APELLAGGPS                                                                                   | ADCC reduction.     | Prevents FcγRI binding [101] |
| G1v5                             | CH2 W109                         | CH2 K109 > W (326)                        | K326W                           | IGHG1 CH2 FG 105–117 (322–323) KVSNKA..LPAPI > KVSNWA..LPAPI                                                                         | ADCC reduction [102] | CDC enhancement. Increases C1q binding [102] |
| G1v47                            | CH2 delG1.1                      | CH2 G1.1 > del (326)                      | G236del                         | IGHG1 CH2 1.6–3 (231–239) APLELLGGPS > APELLAGGPS                                                                                   | ADCC reduction.     | Eliminates binding to FcγRI, FcγRIIA, FcγRIIIA [103] |
| G1v50                            | CH2 P1.4 V1.3 A1.2 delG1.1       | CH2 E1.4 > P (233), L1.3 > V (234), L1.2 > A (235), G1.1 > del (236) | E233P, L234V, L235A, G236del | IGHG1 CH2 1.6–3 (231–239) APLELLGGPS > APELLAGGPS                                                                                   | ADCC reduction.     | Decreases FcgammaR binding (G2-like motif). [Combines G1v1, v2, v3 and v47] |
| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes With the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function |
|--------------------------------|-----------------------------------|-------------------------------------------------|---------------------------------|-------------------------------------------------------------------------------------------------|------------------|------------------|
| G1v52                          | CH2 R1.L, R113                     | CH2                                             | CH2  | CH2 G1.1 > R (231) | IGHTG CH2 G1.1 > R (231) | ADCC reduction. Abrogates Fcγ receptor binding |
|                                |                                   |                                                 | L113 > R (328) | L113 > R (328) | L328R, D275R, GRLR |                                                                                               |
|                                |                                   |                                                 | G236R, L328R | G236R, L328R | APELLGGRPS > APELLGGRPS |                                                                                               |
|                                |                                   |                                                 | APELLGGRPS 1.1 > R (231–239) | APELLGGRPS 1.1 > R (231–239) | KVSNA.K.PAPI > KVSNA.K.PAPI |                                                                                               |
|                                |                                   |                                                 | FG 105–117 (322–332) | FG 105–117 (322–332) |                                                                                               |
| G1v66                          | CH2 A27                           | CH2                                             | CH2  | CH2 D27 > A       | IGHG1 CH2 23–31 (261–269) | ADCC reduction. Reduces FcγR binding. |
|                                |                                   |                                                 | D27 > A                     | D27 > A                     | CVVVVAVSHF > CVVVVAVSHF |                                                                                               |
|                                |                                   |                                                 | D265A                        | D265A                        |                                                                                               |
| G1v67                          | CH2 S27                           | CH2                                             | CH2  | CH2 D27 > S       | IGHG1 CH2 23–31 (261–269) | ADCC reduction. Reduces FcγR binding. |
|                                |                                   |                                                 | D27 > S                      | D27 > S                      | CVVVVAVSHF > CVVVVAVSHF |                                                                                               |
|                                |                                   |                                                 | D265S                        | D265S                        |                                                                                               |

Engineered amino acid changes are in bold in the IMGT variants (red before the change, green after the change). The motif is in yellow and shown before and after the AA change(s). Amino acids of the motifs at additional positions in the IMGT unique numbering for C-domain [68] (by comparison to the V-domain IMGT unique numbering [67]) are underlined. Alias variant names found in the literature are written in blue in column 4 ‘Amino Acid Changes with the Eu Positions’. The background color indicates a reduction (pink color) or an enhancement (green color) of the involved effector ‘Property and Function’. For other ‘Property and Function’, background colors refer to structure (yellow), half-life (pale blue color) or protein A (pale orange).
Table 6. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in antibody-dependent cellular cytotoxicity (ADCC) enhancement (Effector #2).

| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3D |
|----------------------------------|-----------------------------------|-------------------------------------------------------------------------------|------------------------------------------|-------------------------------------------------------------------------------------------------|--------------------------|--------------------------|----|
| G1v6                             | CH2  
A85.4,  
A118,  
A119 | CH2  
S85.4 > A (298),  
E118 > A (333),  
K119 > A (334) | S298A,  
E333A,  
K334A | IGHG1 CH2  
84.1–85.1 (294–301)  
EQYNSTYR >  
EQYNA TYR  
FG 105–117, 118, 119 (322–334)  
KVSNKA.LPAPIE >  
KVSNKA.LPAPIIA  
ADCC enhancement.  
Increases FcγRIIIa binding [104] | | | |
| G1v7                             | CH2  
D3,  
E117 | CH2  
S3 > D (239),  
I117 > E (332) | S239D,  
I332E  
DE | IGHG1 CH2  
1.6–3 (231–239)  
APELLGGPS >  
APELLGGPD  
FG 105–117 (322–332)  
KVSNKA.LPAPIE >  
KVSNKA.LPAPIIA  
ADCC enhancement.  
Increases FcγRIIIA binding [105] | | | |
| G1v8                             | CH2  
D3,  
L115,  
E117 | CH2  
S3 > D (239),  
A115 > L (330),  
I117 > E (332) | S239D,  
A330L,  
I332E  
DLE, 3M | IGHG1 CH2  
1.6–3 (231–239)  
APELLGGPS >  
APELLGGPD  
FG 105–117 (322–332)  
KVSNKA.LPAPIE >  
KVSNKA.LPAPIIA  
ADCC enhancement.  
Increases FcRIIIA binding [105]  
Decreases FcγRIIB binding [105]  
3D [106] | | | |
| G1v9                             | CH2  
L7,  
P83,  
L85.2,  
I88,  
CH3  
L83 | CH2  
F7 > I (243),  
R83 > P (292),  
Y85.2 > L (300),  
V88 > I (305)  
CH3  
P83 > L (396) | F243L,  
R292P,  
Y300L,  
V305I,  
P396l  
PLIL | IGHG1 CH2  
6–10 (242–246)  
LPFPK >  
LPKK  
83–88  
(292–305)  
EQYNSTYR[VVS] >  
EQYNSTL[VVS]  
CH5.83-84.4 (396–401)  
PVLSD >  
IVLSD  
ADCC enhancement.  
100% increase. [107] | | | |
| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3D |
|---------------------------------|-----------------------------------|--------------------------------------------------|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|-----------------|-----------------|-----|
| G1v10                           | CH2                               | Y1.3, Q1.2, W1.1, M3, D30, E34, A85.4 | L1.3 > Y (234), L1.2 > Q (235), G1.1 > W (236), S3 > M (239), H30 > D (268), D34 > E (270), S85.4 > A (286) | L234Y, L235Q, G236W, S239M, H268D, D270E, S298A | IGHG1 CH2 1.6–3 (231–239) | APELLLGFPS > APELYQWGPML27–31,34 (265–270) | ADCC enhancement. Increases FcyIIIA binding [108] >2000-fold (F158), >1000-fold (V158) in the association of G1v10 and G1v11 [108] |
| G1v11                           | CH2                               | E34, D109, M115, E119                  | D34 > E (270), K109 > D (326), A115 > M (330), K119 > E (334) | D270E, K326D, A330M, K334E | IGHG1 CH2 27–31,34 (265–270) | DVSHEED > DVSHEED | FG 105–117,118,119 (322–334) | ADCC enhancement. Increases FcyIIIA binding [108] >2000-fold (F158), >1000-fold (V158) in the association of G1v10 and G1v11 [108] |
| G2v1                            | CH2                               | L1.3, L1.2, G1.1                      | V1.2 > L (234,235), A1.1 > G (236) | V235LL, A236G | IGHG2 CH2 1.6–3 (231–239) | APPYAGFPS > APPYAGFPS | ADCC enhancement. Confers FcyRI binding (WT does not show any binding capacity) [101] |
| G4v1                            | CH2                               | L1.3                                | F1.3 > L (234) | F234L | IGHG4 CH2 1.6–3 (231–239) | APEFLGGFPS > APEFLGGFPS | ADCC enhancement. Increases FcyRI affinity [101] |
| Mus musculus                    | CH2                               | L1.2                                | E1.2 > L (235) | E235L | IGHG2B CH2 1.6–3 (231–239) | APNLELLGGFPS > APNLELLGGFPS | ADCC enhancement. Increases FcyRI affinity [109] |
| G2Bv1                           | CH2                               | L1.2                                | F1.3 > L (234) | F234L | IGHG2B CH2 1.6–3 (231–239) | APNLELLGGFPS | ADCC enhancement. Increases FcyRI affinity [109] |
Table 7. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) enhancement (Effector #3).

| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3D |
|-------------------------------|-----------------------------------|-------------------------------------------------|--------------------------------------|--------------------------------------------------------------------------------|-----------------|-----------------|-----|
| G1v12                         | CH2                               | CH2                                             | A1.1 > A (236), S3 > D (239), A115 > L (330), I117 > E (332) | G236A, S239D, A330L, I332E | GASDALIE | ADCC enhancement. Increases FcγRIIIA binding [110] | ADCC enhancement. Increases FcγRIIIA binding [110] | 5d4q, 5d6d |
| G1v13                         | CH2                               | CH2                                             | A1.1 > A (236), S3 > D (239), I117 > E (332) | G236A, S239D, I332E | GASDIE, ADE | ADCC enhancement. Increases FcγRIIIA binding [111] | ADCC enhancement. Increases FcγRIIIA binding (70×fold)Increases FcγRIIA/FcγRIIB binding ratio (15-fold) [111] | |
| G1v45                         | CH2                               | CH2                                             | A1.1 > A (236), A115 > L (330), I117 > E (332) | G236A, A330L, I332E | GAALIE | ADCC enhancement Increases FcγRIIIA binding | ADCC enhancement NK cell activation | |
Table 8. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in complement-dependent cytotoxicity (CDC) enhancement (Effector #4).

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function |
|-----------------------------|-----------------------------------|-------------------------------------------------|---------------------------------|-------------------------------------------------------------------------------------------------|---------------------|---------------------|
| G1v5                        | CH2 W109                          | CH2 K109 > W (326)                              | K326W                           | IGHG1 CH2 FG 105–117 (322–332) KVSNKAKLPAPI > KVSNWAILPAPI                                       | CDC enhancement. | Increases C1q binding [102] |
|                            |                                   |                                                 |                                 |                                                                                                 |                     | ADCC reduction [102]. |
| G1v15                       | CH2 S118                          | CH2 E118 > S (333)                              | E333S                           | IGHG1 CH2 FG 105–117,118 (322–333) KVSNKAKLPAPI > KVSNWAILPAPI                                 | CDC enhancement. | Increases C1q binding [102] |
|                            |                                   |                                                 |                                 |                                                                                                 |                     |                     |
| G1v16                       | CH2 W109, S118                    | CH2 K109 > W (326), E118 > S (333)             | K326W, E333S                    | IGHG1 CH2 FG 105–117,118 (322–333) KVSNKAKLPAPI > KVSNWAILPAPI                                 | CDC enhancement. | Increases C1q binding [102] |
|                            |                                   |                                                 |                                 |                                                                                                 |                     |                     |
| G1v17                       | CH2 E29, F30, T107                | CH2 S29 > E (267), H30 > F (268), S107 > T (324) | S267E, H268E, S324T             | IGHG1 CH2 FG 105–117 (322–332) KVSNKAKLPAPI > KVSNWAILPAPI                                     | CDC enhancement. | Increases C1q binding [112] |
|                            |                                   |                                                 |                                 |                                                                                                 |                     |                     |
| G1v18                       | CH3 R1, G109, Y120                | CH3 E1 > R (345), E109 > G (430), S120 > Y (440) | E345R, E430G, S440Y            | IGHG1 CH3 1.4–2 (341–346)                                                                                                      | CDC enhancement. | Increases C1q binding [113]. |
|                            |                                   |                                                 |                                 |                                                                                                 |                     | The triple mutant IgG1-005-RGY (IGHG1v18) form IgG1 hexamers [113] |
|                            |                                   |                                                 |                                 |                                                                                                 |                     | Favors IgG1 hexamerization. |
## Table 8. Cont.

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function |
|-----------------------------|-----------------------------------|--------------------------------------------------------------------------|----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|---------------------------|--------------------------|
| G1v35                       | CH2 E29                           | CH2                                                                      | S29 > E (267)                          | S267E SER                                                        | CDC enhancement. Increases C1q binding [112] | Binds to FCGRT and FcγRIIB, but not to other FcγR in a mouse model [114]. |
| G1G3v1                      | CH2 Q38, K40, F85.2                | CH2                                                                      | K38 > Q (274), N40 > K (276), Y85.2 > F (300) | K274Q, N276K, Y300F chimere G1–G3 (1)                             | CDC enhancement. Increases C1q binding [115]. | |
| G4v2                        | CH2 P116                          | CH2                                                                      | S116 > P (331)                          | S331P                                                           | CDC enhancement [116]. (G1-, G2-, G3-like). | |

(1) The chimeric chain is the IGHG1*01 CH1-hinge—IGHG3*01 CH2-CH3. Amino acids Q38, K40 (CH2) and F85.2 (CH3) are from IGHG3*01. The changes are shown in comparison to the IGHG1*01 amino acids at the same positions as K38, N40 (CH2) and Y85.2 (CH3).
Table 9. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in complement-dependent cytotoxicity (CDC) reduction (Effector #5).

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|-----------------------------|------------------------------------|-----------------------------------------------------------------|----------------------------------------|---------------------------------------------------------------------------------|----------------------|
| G1v8                        | CH2 D3, L115, E117                  | CH2 S3 > D (239), A115 > L (330), I117 > E (332)                  | S239D, A330L, I332E DLE               | IGHG1 CH2 1.6–3 (231–239) APELLGGPS > APELLGPD FG 105–117 (322–332) KVSNKA..LPAPI > KVSNKA..LPAPI | CDC reduction. Ablates CDC [105] |
| G1v19                       | CH2 A34                             | CH2 D34 > A (270)                                                | D270A                                  | IGHG1 CH2 34–41 (270–277) APEVKFNW > APEVKFNW                                  | CDC reduction. Reduces C1q binding [117] |
| G1v20                       | CH2 A105                            | CH2 K105 > A (322)                                               | K322A                                  | IGHG2B CH2 100–110 KEFKCKVNNKD > KEFKCKVNNKD                                  | CDC reduction. Reduces C1q binding [119] |
| Mus musculus G2Bv2          | CH2 A101                            | CH2 E101 > A (318)                                               | E318A (2)                              | IGHG2B CH2 100–110 KEFKCKVNNKD > KEFKCKVNNKD                                  | CDC reduction. Reduces C1q binding [119] |
| Mus musculus G2Bv3          | CH2 A103                            | CH2 K103 > A (320)                                               | K320A (2)                              | IGHG2B CH2 100–110 KEFKCKVNNKD > KEFKCKVNNKD                                  | CDC reduction. Reduces C1q binding [119] |
| Mus musculus G2Bv4          | CH2 A105                            | CH2 K105 > A (322)                                               | K322A (2)                              | IGHG2B CH2 100–110 KEFKCKVNNKD > KEFKCKVNNKD                                  | CDC reduction. Reduces C1q binding [119] |

(2) Mus musculus IGHG2B CH2 E101, K103 and K105 form a common core in the interactions of IgG and C1q [119].
| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3. 3D and Property and Function |
|-------------------------------|-----------------------------------|-------------------------------------------------|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------|----------------|----------------|----------------|
| G1v4                          | CH2                               | CH2                                             | P114 > A (329)                    | P329A) IGHG1 CH2 FG 105–117 (322–332)KVSNKA.LPAPI > KVSNA.LAAPI | ADCC reduction. Reduces FcγR binding [117] | CDC reduction. Reduces C1q binding [117] |
| G1v14                         | CH2                               | A1.3, A1.2, A1                                  | CH2                              | L1.3 > A (234), L1.2 > A (235), L234A, L235A | IGHG1 CH2 1.6–3 (231–239)APELGPS > APEAAGGPS | ADCC reduction. Reduces FcγR binding [118,120] | CDC reduction. Reduces C1q binding [118,120] |
| G1v14-1                       | CH2                               | A1.3, A1.2, A1                                  | CH2                              | L1.3 > A (234), L1.2 > A (235), G1 > A (237), L234A, L235A, G237A | IGHG1 CH2 1.6–3 (231–239)APELGPS > APEAAGGPS | ADCC reduction. Reduces FcγR binding. | CDC reduction. Reduces C1q binding. |
| G1v14-4                       | CH2                               | A1.3, A1.2, A114                                | CH2                              | L1.3 > A (234), L1.2 > A (235), P114 > A (329) | IGHG1 CH2 1.6–3 (231–239)APELGPS > APEAAGGPS FG 105–117 (322–332)KVSNKA.LPAPI > KVSNA.LAAPI | ADCC reduction. Reduces FcγR binding. | CDC reduction. Reduces C1q binding. |
| G1v14-48                      | CH2                               | A1.3, A1.2, R113                                | CH2                              | L1.3 > A (234), L1.2 > A (235), L113 > R (328) | IGHG1 CH2 1.6–3 (231–239)APELGPS > APEAAGGPS FG 105–117 (322–332)KVSNKA.LPAPI > KVSNA.LAAPI | ADCC reduction. Reduces FcγR binding. | CDC reduction. Reduces C1q binding. |
| G1v14-49                      | CH2                               | A1.3, A1.2, G114                                | CH2                              | L1.3 > A (234), L1.2 > A (235), P114 > G (329) | IGHG1 CH2 1.6–3 (231–239)APELGPS > APEAAGGPS FG 105–117 (322–332)KVSNKA.LPAPI > KVSNA.LGAPI | ADCC reduction. Reduces FcγR binding [121] | CDC reduction. Reduces C1q binding [121] |
| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3. 3D and Property and Function |
|--------------------------------|----------------------------------|-------------------------------------------------|---------------------------------|--------------------------------------------------------------------------------|-----------------|-----------------|-----------------|
| G1v14-67                       | CH2 A1.3, A1.2, S27              | CH2 L1.3 > A (234), L1.2 > A (235), D27 > S (265) | L234A, L235A, D265S             | IGHG1 CH2 1.6–3 (231–239) APELGGPS > APELGGPS CVVVVSVSHE > CVVVVSVSHE | ADCC reduction. Reduces FcγRI binding [121]. | CDC reduction. Reduces C1q binding [121]. | Combines Homsap G1v14 and G1v67 (G1 CH2 S27). |
| G1v23                          | CH2 E1.2                         | CH2 L1.2 > E (235)                               | L235E                          | IGHG1 CH2 1.6–3 (231–239) APELGGPS > APELGGPS FG 105–117 (322–332) KVSNKALPAPI > KVSNKALPAPI | ADCC reduction. Reduces FcγRI binding [122] | CDC reduction. Reduces C1q binding [122] | |
| G1v38                          | CH2 S108, F113                   | CH2 N108 > S (325), L113 > F (328)               | N325S, L328F                  | IGHG1 CH2 FG 105–117 (322–332) KVSNKALPAPI > KVSNKALPAPI | ADCC reduction. Abrogates FcγRIII binding, increases FcγRII binding, retains FcγRI high affinity binding [123] | CDC reduction. Abrogates C1q binding. | |
| G1v39                          | CH2 F1.3, E1.2, S116             | CH2 L1.3 > F (234), L1.2 > E (235), P116 > S (331) | L234F, L235E, P331S FES, TM   | IGHG1 CH2 1.6–3 (231–239) APELGGPS > APELGGPS FG 105–117 (322–332) KVSNKALPAPI > KVSNKALPAPI | ADCC reduction. Reduces Fcγ effector properties [124] (2) | CDC reduction. Reduces C1q binding [122] | 3D 3c2s |
| G1v40                          | CH2 A1.3, A1.2, S116             | CH2 L1.3 > A (234), L1.2 > A (235), P116 > S (331) | L234A, L235A, P331S            | IGHG1 CH2 1.6–3 (231–239) APELGGPS > APELGGPS FG 105–117 (322–332) KVSNKALPAPI > KVSNKALPAPI | ADCC reduction. Reduces FcγRI binding. | CDC reduction. Reduces C1q binding. | |
| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3. 3D and Property and Function |
|---------------------------------|-----------------------------------|---------------------------------------------------------------------|------------------------------------------|---------------------------------------------------------------------------------|------------------|------------------|----------------------|
| G1v41                           | CH2 F1.3, E1.2                    | CH2 L1.3 > F (234), L1.2 > E (235)                                  | L234F, L235E FE                         | IGHG1 CH2 1.6–3 (231–239) APELGGPS > APELGGPS                                   | ADCC reduction.  | Reduces FcyR binding | CDC reduction. Reduces C1q binding [122] |
| G1v43                           | CH2 A1.3, E1.2, A1                | CH2 L1.3 > A (234), L1.2 > E (235), G1 > A (237)                     | L234A, L235E, G237A                     | IGHG1 CH2 1.6–3 (231–239) APELGGPS > APELGGPS                                   | ADCC reduction.  | Reduces FcyR binding | CDC reduction. Reduces C1q binding |
| G1v48                           | CH2 R113                          | CH2 L113 > R (328)                                                  | L328R                                   | IGHG1 CH2 FG 105–117 (322–332) KVSNKA_LPAPI > KVSNKA_LPAPI                    | ADCC reduction.  | Reduces FcyR binding | CDC reduction. Reduces C1q binding |
| G1v49                           | CH2 G114                          | CH2 P114 > G (329)                                                  | P329G                                   | IGHG1 CH2 FG 105–117 (322–332) KVSNKA_LPAPI > KVSNKA_LPAPI                    | ADCC reduction.  | Reduces FcyR binding | CDC reduction. Reduces C1q binding [121] |
| G1v51                           | CH2 K29                           | CH2 S29 > K (267)                                                   | S267K                                   | IGHG1 CH2 27–31 (265–269) DVSHK > DVSHK                                       | ADCC reduction.  | Reduces FcyR binding | CDC reduction. Reduces C1q binding |
| G1v53                           | CH2 F1.3, Q1.2, Q105              | CH2 L1.3 > F (234), L1.2 > Q (235), K105 > Q (322)                  | L234F, L235Q, K322Q, FQQ               | IGHG1 CH2 1.6–3 (231–239) APELGGPS > APELGGPS                                   | ADCC reduction.  | Reduces FcyR binding | CDC reduction. Reduces C1q binding |
| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3. 3D and Property and Function |
|---------------------------------|------------------------------------|---------------------------------------------------------------------------------|------------------------------------------|--------------------------------------------------------------------------------|--------------------------|--------------------------|--------------------------|
| **G1v53, G1v21**                |                                    | CH2                                                                              | L1.3 > F (234), L1.2 > Q (235), K105 > Q (322), M15.1 > Y (252), S16 > T (254), T18 > E (256) | IGHE1 CH2 1.6-3 (231–239) APELLGGS > APELQGPS 15-18 (251–256) LMLSRT > LYTRE FG 105-117 (322–332) KVSNKA.LPAPI > QVSNKA.LPAPI | ADCC reduction. Reduces FcγR binding [125] (G1v53) | CDC reduction. Reduces C1q binding [125] (G1v53) | Extends half-life [125] (G1v21). |
| **G1v59**                       |                                    | CH2                                                                              | L1.3 > S (234), L1.2 > T (235), G1.1 > R (236) | IGHE1 CH2 1.6-3 (231–239) APELLGGS > APELQGPS | ADCC undetectable. Abrogates FcγR binding [126] | CDC undetectable. Abrogates C1q binding [126] |                       |
| **G1v60**                       |                                    | CH2                                                                              | A115 > S (330), P116 > S (331) | PG 105-117 (322–332) KVSNKA.LPAPI > QVSNKA.LPAPI | ADCC reduction. Reduces FcγR binding. | CDC reduction. Reduces C1q binding. |                       |
| **G1v63**                       |                                    | CH2                                                                              | P2 > S | IGHE1 CH2 1.6-3 (231–239) APELLGGS > APELQGPS | ADCC reduction. Reduces FcγR binding. | CDC reduction. Reduces C1q binding. |                       |
| **G1v65**                       |                                    | CH2                                                                              | E1.4 > del, L1.3 > del, L1.2 > del | IGHE1 CH2 1.6-3 (231–239) APELLGGS > AP−CCGS | ADCC reduction. Reduces FcγR binding. | CDC reduction. Reduces C1q binding. |                       |
| **G1v70**                       |                                    | S1, S11, S14, CH2                                                              | C5 > S (220), C11 > S (226), C14 > S (226) | EPKSSDKTHTPPCP > EPKSSDKTHTPSSP IGHE1 CH2 1.6-3 (231–239) APELLGGS > APELQGPS | ADCC reduction. Reduces FcγR binding. | CDC reduction. Reduces C1q binding. | Combines G1v63 with G1v37 (no H-L), G1v61 (no H-H h11) and G1v62 (no H-H h14). |
### Table 10. Cont.

| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3. 3D and Property and Function |
|-------------------------------|----------------------------------|------------------------------------------------------------------------------|------------------------------------------|-------------------------------------------------------------------------------------------------|--------------------------|--------------------------|-----------------------------|
| **G2v2**                      |                                  | CH2                                                                           | CH2                                      | CH2                                                                                             | ADCC reduction.         | CDC reduction.           |                              |
|                               |                                  | Q30,                                                                          | H30 > Q (268),                          | H268Q,                                                                                            | Reduces FcγR binding    |                         |                             |
|                               |                                  | L192,                                                                         | V92 > L (309),                          | V309L,                                                                                            |                         |                         | [127]                       |
|                               |                                  | S115,                                                                         | A115 > S (330),                         | A330S,                                                                                            |                         |                         |                             |
|                               |                                  | S116                                                                           | P116 > S (331)                          | P331S IgG2m4                                                                                     |                         |                         |                             |
|                               |                                  |                                                                               |                                          |                                                                                                  | ADCC reduction.         |                         |                             |
|                               |                                  |                                                                               |                                          |                                                                                                  | Reduces FcγR binding    |                         |                             |
|                               |                                  |                                                                               |                                          |                                                                                                  |                         |                         | [127]                       |
| **G2v3**                      |                                  | CH2                                                                           | A1.2,                                    | A1.2 > A (235), A1 > A (237), P2 > S (238), H30 > A (268), H268A, V92 > L (309), A115 > S (330), P116 > S (331) | ADCC reduction.         | CDC reduction.           |                              |
|                               |                                  |                                                                               | S2,                                      | V235A,                                                                                            | Reduces FcγR binding    |                         |                             |
|                               |                                  |                                                                               | A30,                                      | G237A,                                                                                            |                         |                         | [124]. Undetectable ADCC and V1 ADCP [124] |
|                               |                                  |                                                                               | LY2,                                      | P238S,                                                                                            |                         |                         |                              |
|                               |                                  |                                                                               | S115,                                     | H268A,                                                                                            |                         |                         | [128]. Undetectable CDC [124] |
|                               |                                  |                                                                               | S116,                                     | V309L,                                                                                            |                         |                         |                              |
| **G2G4v1**                    | (1)                              | CH2                                                                           | CH2                                      | CH2                                                                                             | ADCC reduction.         | CDC reduction.           |                              |
|                               |                                  |                                                                               | E1.4 > del,                              | E1.4 > del (233),                                                                                  | Reduces FcγR binding    |                         |                             |
|                               |                                  |                                                                               | P1.3,                                    | P1.3 > P (234),                                                                                   |                         |                         | [128]                       |
|                               |                                  |                                                                               | V1.2,                                    | V1.2 > V (235),                                                                                   |                         |                         |                              |
|                               |                                  |                                                                               | A1.1,                                    | G1.1 > A (226),                                                                                   |                         |                         |                              |
| **G4v3**                      |                                  | CH2                                                                           | CH2                                      | CH2                                                                                             | ADCC reduction.         | CDC reduction.           |                              |
|                               |                                  |                                                                               | E1.2                                      | E1.2 > E (235),                                                                                   | Reduces FcγR binding    |                         |                             |
|                               |                                  |                                                                               |                                           |                                                                                                  |                         |                         | [122]                       |
|                               |                                  |                                                                               |                                           |                                                                                                  |                         |                         |                              |
| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3. 3D and Property and Function |
|---------------------------------|-----------------------------------|------------------------------------------------------------------------|-----------------------------------|------------------------------------------------------------------------------------------------|-------------------|-------------------|-------------------|
| **G4v3**<br>**G4v5**            | h P10, CH2 E1.2                    | h S10 > P (228) CH2 L1.2 > E (235)                                    | S228P,                            | IGHG4 h 1–12 (216–230) ESKYGPPCPSCP > ESKYGPPCPSCP CH2 L1.6–3 (231–239) APEFGGPS > APEFGGPS | ADCC reduction. | CDC reduction. | Prevents IgG4 half-IG exchange [129] (G4v5). |
| **G4v3-49**                     | CH2 E1.2 G114                      | CH2 L1.2 > E (235) P114 > G (329)                                     | L235E P329G LEPG                  | IGHG4 CH2 1.6–3 (231–239) APEFGGPS > APEFGGPS FG 105–117 (322–332) KVSNKA_LPAPI > KVSNKA_LGAPI | ADCC reduction. | CDC reduction. | Reduces C1q binding [121]. |
| **G4v3-49**<br>**G4v5**         | h P10, CH2 E1.2                    | h S10 > P (228) CH2 L1.2 > E (235) P114 > G (329)                    | L235E P329G SPLEPG                | IGHG4 h 1–12 (216–230) ESKYGPPCPSCP ESKYGPPCPSCP CH2 1.6–3 (231–239) APEFGGPS > APEFGGPS FG 105–117 (322–332) KVSNKA_LPAPI > KVSNKA_LGAPI | ADCC reduction. | CDC reduction. | Reduces C1q binding [121] (G4v3-49). |
| **G4v4**                        | CH2 A1.3, A1.2                     | CH2 F1.3 > A (234), L1.2 > A (235)                                   | F234A L235A FALA                 | IGHG4 CH2 1.6–3 (231–239) APEFGGPS > APEAAGGPS | ADCC reduction. | CDC reduction. | Reduces C1q binding [120]. |
| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3. 3D and Property and Function |
|--------------------------------|----------------------------------|-------------------------------------------|----------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------|---------------------|-----------------------------|
| G4v4                           |                                  | h                                        | S10 > P (228)                    | S228P; ESKYGPPCPSCP > ESKYGPPCPSCP                                      | ADCC reduction. | CDC reduction. | Prevents IgG4 half-IG exchange [129] (G4v5). |
|                                |                                  | A1.3, A1.2                               | CH2 F1.3 > A (234), L1.2 > A (235) | F234A, L235A IgG4ProAlaAla                                                |                     |                     |                             |
|                                |                                  | CH2                                       |                                 |                                                                                   |                     |                     |                             |
|                                | G4v5                             |                                            |                                 |                                                                                   |                     |                     |                             |
| G4v7                           |                                  | CH2                                       |                                 |                                                                                   |                     |                     |                             |
|                                | delE1.4, P1.3, V1.2,             |                                            |                                 |                                                                                   |                     |                     |                             |
|                                | A1.1                             |                                            |                                 |                                                                                   |                     |                     |                             |
|                                | G4v49                            | CH2                                       | P114 > G (329)                  | P329G                                                                         | ADCC reduction. | CDC reduction. |                             |
|                                |                                  |                                            |                                 |                                                                                   |                     |                     |                             |
|                                |                                   |                                            |                                 |                                                                                   |                     |                     |                             |
|                                |                                   |                                            |                                 |                                                                                   |                     |                     |                             |
| Canis lupus familiaris G2v1    |                                  | CH2                                       |                                 |                                                                                   |                     |                     |                             |
|                                |                                  | A1.3, A1.2                               | M1.3 > A (234), L1.2 > A (235), | M234A, L235A IgG4ProAlaAla                                                | ADCC reduction. | CDC reduction. |                             |
|                                |                                  | G1                                       | G1 > A (237)                    | G237A                                                                         |                     |                     |                             |
|                                |                                   |                                            |                                 |                                                                                   |                     |                     |                             |
|                                |                                   |                                            |                                 |                                                                                   |                     |                     |                             |
| Canis lupus familiaris G2v2    |                                  | CH2                                       |                                 |                                                                                   |                     |                     |                             |
|                                |                                  | A1.3, A1.2                               | M1.3 > A (234), L1.2 > A (235), | M234A, L235A IgG4ProAlaAla                                                | ADCC reduction. | CDC reduction. |                             |
|                                |                                  | G114                                      | P114 > G (329)                  | P329G                                                                         |                     |                     |                             |
|                                |                                   |                                            |                                 |                                                                                   |                     |                     |                             |

(1) The monoclonal antibody is eculizumab. The heavy chain is the chimeric IGHG2*01 CH1-hinge—IGHG4*01 CH2-CH3. The CH2 and CH3 are from IGHG4*01, except for the CH2 positions 1.6-1.1 (APFPA) with del 1.4 and amino acids P1.3, V1.2 and A1.1 being from IGHG2*01. The changes are shown in comparison to the IGHG4*01 amino acids at the same positions as E1.4, F1.3, L1.2 and G1.1.
Table 11. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in the B cell inhibition by the coengagement of antigen and FcγR on the same cell (Effector #7).

| IMGT Engineered Fc Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|---------------------------------|----------------------------------|------------------------------------------------------------------|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------|----------------------|
| G1v25                            | CH2                              | CH2 S<sup>29</sup> → E (<sup>267</sup>)<br> L<sup>113</sup> → F (<sup>328</sup>) | S<sup>267</sup>E<br> L<sup>328</sup>F | IGHG1 CH2 27–31 (265–269)<br> DVSHE<br> DVHE<br> FG 105–117 (322–332)<br> KVSNKA_LPAPI<br> KVSNKA_LPAPI | Increases FcγRIIB binding (400-fold) [130]<br> Inhibits by downstream ITIM signaling in B cells [131] |

Table 12. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in the knock out CH2 84.4 glycosylation (Effector #8).

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|------------------------------|----------------------------------|------------------------------------------------------------------|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------|----------------------|
| G1v29                        | CH2                              | CH2 N<sup>84.4</sup> → A (<sup>297</sup>)                        | N<sup>297</sup>A | IGHG1 CH2 83–86<br> REEQYNSTYRVV<br> REEQYAGSTYRVV | ADCC reduction. Reduces FcγR binding [132] |
| G1v30                        | CH2                              | CH2 N<sup>84.4</sup> → G (<sup>297</sup>)                        | N<sup>297</sup>G | IGHG1 CH2 83–86<br> REEQYNSTYRVV<br> REEQYAGSTYRVV | ADCC reduction. Reduces FcγR binding [132] |
| G1v36                        | CH2                              | CH2 N<sup>84.4</sup> → Q (<sup>297</sup>)                        | N<sup>297</sup>Q | IGHG1 CH2 83–86<br> REEQYNSTYRVV<br> REEQYAGSTYRVV | ADCC reduction. Reduces FcγR binding |
| G4v36                        | CH2                              | CH2 N<sup>84.4</sup> → Q (<sup>297</sup>)                        | N<sup>297</sup>Q | IGHG1 CH2 83–86<br> REEQYNSTYRVV<br> REEQYAGSTYRVV | ADCC reduction. Reduces FcγR binding |
| Canis lupus familiaris       | G2v29                            | CH2 N<sup>84.4</sup> → A (<sup>297</sup>)                        | N<sup>297</sup>A | IGHG1 CH2 83–86<br> REEQYNSTYRVV<br> REEQYAGSTYRVV | ADCC reduction. Reduces FcγR binding |
Table 13. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in half-life increase (Half-life #9).

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|------------------------------|-----------------------------------|--------------------------------------------------------------------------------|----------------------------------------|--------------------------------------------------------------------|----------------------|
| G1v21                        | CH2                               | CH2                                                                            | M15.1 > Y (252), S16 > T (254), T18 > E (256) | M252Y, S254T, T256E                                               | Half-life increase   |
|                              | Y15.1, T16, E18                   |                                                                                |                                        | **YTE**                                                            | Enhances FCGRT binding at pH 6.0 [133,134] (1) |
| G1v22                        | CH2                               | CH2                                                                            | M15.1 > Y (252), S16 > T (254), T18 > E (256) | M252Y, S254T, T256E                                               | Half-life increase   |
|                              | Y15.1, T16, E18, CH3              |                                                                                |                                        | **YTE**                                                            | Enhances FCGRT binding at pH 6.0 [134] |
|                              | K113, F114, H116                  |                                                                                |                                        | **YTE**                                                            |                      |
| G1v24                        | CH3                               | CH3                                                                            | M107 > L (428), N114 > S (434)          | M428L, N434S                                                       | Half-life increase   |
|                              | L107, S114                        |                                                                                |                                        | **YTE**                                                            | Enhances FCGRT binding at pH 6.0 (11-fold increase in affinity) [135] (2) |
| G1v42                        | CH2                               | CH2                                                                            | T14 > Q (250), M107 > L (428)           | T250Q, M428L                                                       | Half-life increase   |
|                              | Q14, CH3 L107                     |                                                                                |                                        | **YTE**                                                            | Enhances FCGRT binding at pH 6.0 [134] |
| G1v46                        | CH3                               | CH3                                                                            | H113 > K (433), N114 > F (434)          | H433K, N434F                                                       | Half-life increase   |
|                              | K113, F114                        |                                                                                |                                        | **YTE**                                                            | Enhances FCGRT binding at pH 6.0. |
Table 13. Cont.

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|------------------------------|-----------------------------------|--------------------------------------------------|-----------------------------------------|---------------------------------------------------------------------------------------------------------------|----------------------|
| G2v4                         | CH2Q14                            | CH2 T14 > Q (250)                                  | T250Q                                   | IGHG2 CH2 13–18 (249–256) DTLMISRT > DQLMISRT                                                                 | Half-life increase |
|                              |                                   |                                                  |                                         |                                                                                                                | Enhances FCGRT binding at pH 6.0 [136] |
| G2v5                         | CH3L107                           | CH3 M107 > L (428)                                 | M428L                                   | IGHG2 CH3 FG 105–117 (426–437) SVMHEA.LHNHYT > SVLHEA.LHNHYT                                                  | Half-life increase |
|                              |                                   |                                                  |                                         |                                                                                                                | Enhances FCGRT binding at pH 6.0 [136] |
| G2v6                         | CH2Q14, CH3L107                   | CH2 T14 > Q (250)                                  | T250Q M428L                            | IGHG2 CH2 13–18 (249–256) DTLMISRT > DQLMISRT                                                                 | Half-life increase |
|                              |                                   | CH3 M107 > L (428)                                 |                                         |                                                                                                                | Enhances FCGRT binding at pH 6.0 [136] |
| G2v8-1                       | CH2A93                            | CH2 H93 > A (310)                                  | H310A                                   | IGHG2 CH2 89–96 (306–313) LTVVHQDWD > LTVVADQDW                                                             | Abrogates FCGRT binding at pH 6.0 |
|                              |                                   |                                                  |                                         |                                                                                                                | (G2v8 any amino acid replacement of H93 except cystein) [137]. Number 1 of G2v8-1 is for A |
| G3v1                         | CH3H115                           | CH3 R115 > H (435)                                 | R435H                                   | IGHG3 CH3 FG 105–117 (426–437) SVMHEA.LHNHFT > SVMHEA.LHNHFT                                               | Half-life increase |
|                              |                                   |                                                  |                                         |                                                                                                                | Extends half-life [138] |
| G4v21                        | CH2Y15.1, T16, E18                | CH2 M15.1 > Y (252), S16 > T (254), T18 > E (256) | M252Y, S254T, T256E YTE                | IGHG4 CH2 13–18 (249–256) DTLMISRT > DTLYTIRE                                                               | Half-life increase |
|                              |                                   |                                                  |                                         |                                                                                                                | Enhances FCGRT binding at pH 6.0 [134] |
### Table 13. Cont.

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|------------------------------|-----------------------------------|-----------------------------------------------------------------------------|-----------------------------------------|--------------------------------------------------------------------------------|----------------------|
| G4v22                        | CH2                                 | CH2                                                                         | S16>T (254), V91>P (308)                | S254T, V308P                                                                     | Half-life increase   |
|                              |                                    | CH3                                                                         | N114>A (434)                           | N434A                                                                           |                      |
|                              | T16, P91, A114                       |                                                                            |                                         |                                                                                  |                      |
| G4v24                        | CH3                                 | CH3                                                                         | M107>L (428), N114>S (434)             | M428L, N434A                                                                     | Half-life increase   |
|                              |                                    |                                                                            |                                         |                                                                                  |                      |
|                              | L107, S114                          |                                                                            |                                         |                                                                                  |                      |

(1) Ten-fold increase at pH 6.0 [134] and four-fold increases half-life in a cynomolgus pK study [140]. The T16->E amino acid change provides two novel salt bridges between the Fc and BM2 of FCGRT IMGT/3Dstructure-DB: 4n0f, 4n0u [137]. A change of IGHG1 CH2 His H93 (310) into any other amino acid (excluding Cys) leads to an undetectable binding to FCGRT (FcRn) at pH 6.0 [137]. (2) An increased reduction in tumor burden in human FCGRT (FcRn) transgenic tumor-bearing mice treated with an anti-EGFR or an anti-VEGF antibody [135]. From the 3D structure, it is postulated that N114>S (434) allows for additional hydrogen bonds with FCGRT (FcRn) [137] IMGT/3Dstructure-DB: 4n0f, 4n0u.

### Table 14. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in the abrogation of binding to Protein A (Protein A #10).

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino acid changes on IGHG CH domain (Eu numbering between parentheses) | Amino acid changes with the Eu positions | Motif identifiable in gene and domain with positions according to the IMGT unique numbering and with Eu positions between parentheses | Property and function |
|------------------------------|-----------------------------------|-----------------------------------------------------------------------------|-----------------------------------------|--------------------------------------------------------------------------------|----------------------|
| G4v8                         | CH3                               | CH3                                                                         | H115>R (435), Y16>F (436), L125>P (445) | H435R, Y436F, L445P                                                           | Abrogates binding to Protein A |
Table 15. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in the formation of additional bridge stabilizing CH2 in the absence of N84.4 (297) glycosylation (Structure #11).

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|-----------------------------|----------------------------------|-----------------------------------------------------------------|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------|----------------------|
| G1v54                        |                                   | CH2                                                             | R83 > C (292), R83 > C (292), N84.4 > A (297), R83 > C (292), N84.4 > G (297), V85 > C (302) | IGHG1 CH2 83–86 REEQYNSTYRVV > CEEQYSTYRCV (v29), REEQYNSTYRVV > CEEQYSTYRCV (v30), REEQYNSTYRVV > CEEQYSTYRCV (v36) | Stabilizes CH2 in the absence of N84.4 (297) glycosylation |
| G1v54-29                     |                                   | CH2                                                             | R83 > C (292), R83 > C (292), N84.4 > A (297), R83 > C (292), N84.4 > G (297), V85 > C (302) | IGHG1 CH2 83–86 REEQYNSTYRVV > CEEQYSTYRCV (v29), REEQYNSTYRVV > CEEQYSTYRCV (v30), REEQYNSTYRVV > CEEQYSTYRCV (v36) | Stabilizes CH2 in the absence of N84.4 (297) glycosylation |
| G1v54-30                     |                                   | CH2                                                             | R83 > C (292), R83 > C (292), N84.4 > A (297), R83 > C (292), N84.4 > G (297), V85 > C (302) | IGHG1 CH2 83–86 REEQYNSTYRVV > CEEQYSTYRCV (v29), REEQYNSTYRVV > CEEQYSTYRCV (v30), REEQYNSTYRVV > CEEQYSTYRCV (v36) | Stabilizes CH2 in the absence of N84.4 (297) glycosylation |
| G1v54-36                     |                                   | CH2                                                             | R83 > C (292), R83 > C (292), N84.4 > A (297), R83 > C (292), N84.4 > G (297), V85 > C (302) | IGHG1 CH2 83–86 REEQYNSTYRVV > CEEQYSTYRCV (v29), REEQYNSTYRVV > CEEQYSTYRCV (v30), REEQYNSTYRVV > CEEQYSTYRCV (v36) | Stabilizes CH2 in the absence of N84.4 (297) glycosylation |
Table 16. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in the prevention of IgG4 half-IG exchange (Structure #12).

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|-----------------------------|-----------------------------------|-----------------------------------------------------------------------------|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|----------------------|
| G4v5                        | h P10                             | h S10 > P (228)                                                             | S228P                                   | IGHG4 h 1–12 (216–230) ESKYGPPCPSCP > ESKYGPPCP CP (G1-like)                                                                  | Prevents in vivo and in vitro IgG4 half-IG exchange [129] |
| G4v6                        | CH3 K88                           | CH3 R88 > K                                                                 | R409K                                   | IGHG1 CH3 85.4–89 (404–410) GSFFLYSKL > GSFFLYSLK                                                                           | Reduces IgG4 half-IG exchange [141] |

Table 17. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in hexamerisation (Structure #13).

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|-----------------------------|-----------------------------------|-----------------------------------------------------------------------------|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|----------------------|
| G1v34                       | CH3 G109                          | CH3 E109 > G (430)                                                          | E430G                                   | IGHG1 CH3- FG 105–117 (426–437) SVMHEA.LHNHYT > SVMHGA.LHNHYT                                                               | Favors IgG1 hexamerisation by increased intermolecular Fc-Fc interactions after antigen binding on the cell surface |
Table 18. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in knobs-into-holes and the enhancement of heteropairing H-H of bispecific antibodies (Structure #14).

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|-----------------------------|-----------------------------------|-------------------------------------------------------------------------|------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------|----------------------|
| G1v26                       | CH3 T22 > Y (366)                  | T366Y                                                                   | IGHG1 CH3 20–26 (364–370) SLTCLVK > SLYCLVK | Knob of knobs-into-holes G1v26 knob/G1v31 hole interactions between the CH3 of the two different gamma1 chains [142]          |
| G1v31                       | CH3 Y86 > T (407)                  | Y407T                                                                   | IGHG1 CH3 85.4–89 (404–410) GSFLYSKL > GSFLTSKL | Hole of knobs-into-holes G1v26 knob/G1v31 hole interactions between the CH3 of the two different gamma1 chains [142] (G1v26 knob/G1v31 hole) |
| G1v32                       | CH3 W22 T22 > W (366)              | T366W                                                                   | IGHG1 CH3 20–26 (364–370) SLTCLVK > SLCWCLVK | Knob of knobs-into-holes G1v32 knob/G1v33 hole interactions between the CH3 of the two different gamma1 chains                   |
| G1v33                       | CH3 T22 > S (366), L24 > A (368), Y86 > V (407) | T366S, L368A, Y407V                                                      | IGHG1 CH3 20–26 (364–370) SLTCLVK > SLSCAVK 85.4–89 (404–410) GSFLYSKL > GSFLVSKL | Hole of knobs-into-holes G1v32 knob/G1v33 hole interactions between the CH3 of the two different gamma1 chains                 |
| G1v68                       | CH3 T6 > V (350), T22 > L (366), K79 > L (392), T81 > W (394) | T350V, T366L, K392L, T394W                                                | IGHG1 CH3 3–9 (347–353) QVYLTPP > QVYLPP 20–26 (364–370) SLTCLVK > SLLCLVK 77–83 (390–396) NYKTTP > NYLTWP | Enhances, with G1v69, the heteropairing H-H of bispecific antibodies                                                             |
### Table 18. Cont.

| IMGT Engineered Variant Name | IMGT Engineered Variant Definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | Property and Function |
|-----------------------------|-----------------------------------|-----------------------------------------------------------------------------|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|----------------------|
| G1v69                       | CH3                               | CH3                           | T350V                               | IGHG1 CH3 3–9 (347–353)                                                             | Enhances, with G1v68, the heteropairing H-H of bispecific antibodies |
|                             | V6, Y7, A85.1, V86                | T6 > V (350)                  | L351Y                               | QVYTILPP > QVYVYPP                                                                     |
|                             |                                   | L7 > Y (351)                  | F405A                               | IGHG1 CH3 85.4–89 (404–410)                                                        |
|                             |                                   | F85.1 > A (405)               | Y407V                               | GSFLYSKL > GSFAVLYSKL                                                                 |
|                             |                                   | Y86 > V (407)                 |                                     |                                                                                     |

### Table 19. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in the suppression of inter H-L and/or inter H-H disulfide bridges (Structure #15).

| IMGT Variant Name | IMGT Variant Description | IMGT Amino Acid Changes on IGHG CH Domain with Eu Numbering between Parentheses | Eu Numbering Variant | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering | Property and Function |
|-------------------|--------------------------|---------------------------------------------------------------------------------|----------------------|-----------------------------------------------------------------------------------------------|----------------------|
| G1v37             | h S5                     | h C5 > S (220)                                                                 | C220S                | IGHG1 h 1–15 (216–230) EPK5DKTHTCPPPCP > EPK5DKTHTCPPPCP                                    | No disulfide bridge inter H-L |
| G1v61             | h S11                    | h C11 > S (226)                                                                | C226S                | IGHG1 h 1–15 (216–230) EPK5DKTHTCPPPCP > EPK5DKTHTCPPPCP                                    | No disulfide bridge inter H-H h 11 |
| G1v62             | h S14                    | h C14 > S (229)                                                                | C229S                | IGHG1 h 1–15 (216–230) EPK5DKTHTCPPPCP > EPK5DKTHTCPPPCP                                    | No disulfide bridge inter H-H h 14 |
Table 20. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in site-specific drug attachment (Structure #16).

| IMGT Variant Name | IMGT Variant Description | IMGT Amino Acid Changes on IGHG CH Domain with Eu Numbering between Parentheses | Eu Numbering Variant | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering | Property and Function |
|-------------------|--------------------------|---------------------------------------------------------------------------------|----------------------|--------------------------------------------------------------------------------|----------------------|
| G1v27             | CH2 C3                   | CH2 S3 > C (329)                                                               | S239C                | IGHGI CH2 1.6–4 (231–240) APELLGGPSV > APELLGGPCV                              | Site-specific drug attachment engineered cysteine |
|                   |                          |                                                                                 |                      |                                                                                |                      |
| G1v28             | CH2 C(3°4)               | CH2 (3°4)C(239°240)                                                           | C(239°240)           | IGHGI CH2 1.6–4 (231–240) APELLGGPSV > APELLGGPSCV                           | Site-specific drug attachment engineered cysteine |
|                   |                          |                                                                                 |                      |                                                                                |                      |
| G1v44             | CH3 C122                 | CH3 S122 > C (442)                                                            | S442C                | IGHGI CH3 118–125 (438–445) QKSLSSLP > QKSLCSSP                             | Site-specific drug attachment engineered cysteine |
|                   |                          |                                                                                 |                      |                                                                                |                      |
| G1v55             | CH3 C123                 | CH3 L123 > C (443)                                                            | L443C                | IGHGI CH3 118–125 (438–445) QKSLSSLP > QKSLCSSP                             | Site-specific drug attachment engineered cysteine |
|                   |                          |                                                                                 |                      |                                                                                |                      |
| G1v56             | CH2 F85.2 CH3 F85.2      | CH2 Y85.2 > F (pAMF)                                                          | Y300F                | IGHGI CH2 84.1–85.1 (294–301) EQYNSTFR > EQYNSTFR                            | Modified phenylalanine for conjugation (produced in Escherichia coli, non glycosylated) |
|                   |                          |                                                                                 |                      |                                                                                |                      |
|                   |                          |                                                                                  |                      |                                                                                |                      |
| G1v64             | CH2 C36                  | CH2 E36 > C                                                                    | E272C                | IGHGI CH2 34–41 (270–277) DPEVKFNW > DPCVKFNW                               | Conjugation site-specific engineered cysteine |

*pAMF* Modified phenylalanine for conjugation (produced in *Escherichia coli*, non glycosylated)
Table 21. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in the enhancement of hetero pairing H-L of bispecific antibodies (Structure #17).

| IMGT Variant Name | IMGT Variant Description | IMGT Amino Acid Changes on IGHG CH Domain with Eu Numbering between Parentheses | Eu Numbering Variant | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering | Property and Function |
|-------------------|--------------------------|---------------------------------------------------------------------------------|----------------------|--------------------------------------------------------------------------------|----------------------|
| **G1v57**         | CH1                      | CH1 K26 > E (147), K119 > E (213)                                               | K147E, K213E         | IGHG1 CH1 23–26 (144–147) CLV > CLVE 118–121 (212–215) DRKV > DEKV          | Enhances, with KCv57, the hetero pairing H-L of bispecific antibodies |
|                   | E26, E119                |                                                                                  |                      |                                                                                  |                      |
| **KCv57**         | IGKC                     | IGKC E12 > R, Q13 > K                                                           | E123R, Q124K         | IGKc 10–15 (121–126) SDEFQLK > SDRK1K                                         | Enhances, with G1v57, the hetero pairing H-L of bispecific antibodies |
|                   | R12, K13                 |                                                                                  |                      |                                                                                  |                      |
| **G1v58**         | CH1                      | CH1 F5 > C (126), hC5 > V (220)                                                 | F126C, C220V         | IGHG1 CH1 1.4–15 (118–136) ASTKGPSVFPLAPSSKSTS > ASTKGPSVCPLAPSSKSTS          | Alternative interchain cysteine mutations to enhance, with LC2v58, heteropairing of bispecific antibodies |
|                   | C5, hV5                  |                                                                                  |                      |                                                                                  |                      |
|                   |                          |                                                                                  |                      |                                                                                  |                      |
| **LC2v58**        | IGLC                     | IGLC S10 > C (121), C126 > V (214)                                             | S121C, C214V         | GOKKAAPSVTFLPPSSEELQ > GOKKAAPSVTFLPPSSEELQ                                   | Alternative interchain cysteine mutations to enhance, with G1v58, heteropairing of bispecific antibodies |
|                   | C10, V126                |                                                                                  |                      |                                                                                  |                      |
Table 22. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in the control of half-IG exchange of bispecific IgG4 (Structure #18).

| IMGT Variant Name | IMGT Variant Description | IMGT Amino Acid Changes on IGHG CH Domain with Eu Numbering between Parentheses | Eu Numbering Variant | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering | Property and Function |
|-------------------|--------------------------|--------------------------------------------------------------------------------|----------------------|-----------------------------------------------------------------------------------------|----------------------|
| G4v10             | CH3 L85.1, K88           | CH3 F85.1 > L (405), R88 > K (409)                                             | F405L, R409K         | IGHG1 CH3 85.4–92 (402–413) GSFLYSLTVD > GSFLYYSKLTVD                                | Control of half-IG exchange of bispecific IgG4 |

Table 23. IMGT nomenclature, Eu positions and IMGT motif of engineered Fc variants involved in reducing acid-induced aggregation (Structure #19).

| IMGT Engineered Fc Variant Name | IMGT Engineered Variant definition | IMGT Amino Acid Changes on IGHG CH Domain (Eu Numbering between Parentheses) | Amino Acid Changes with the Eu Positions | Motif Identifiable in Gene and Domain with Positions According to the IMGT Unique Numbering and with Eu Positions between Parentheses | 1. Property and Function | 2. Property and Function | 3. Property and Function |
|---------------------------------|-----------------------------------|--------------------------------------------------------------------------------|------------------------------------------|--------------------------------------------------------------------------------|-------------------------|-------------------------|-------------------------|
| G2v7                            | CH2 Y85.2, L92, A339              | CH2 F85.2 > Y (300), V92 > L (309), T339 > A (339)                              | F300Y, V309L, T339A                      | IGHG2 CH2 85.4–92 (300–309) STYRVSSLTVV > STYRVSSLTVV                             | Low ADCC Low FcγR binding [143] | Low CDC Low C1q binding [143] |                        |
In the tables, the different columns correspond to the items of the standardized variant characterization detailed above. Engineered amino acid changes are in bold in the IMGT variants (red before the change, green after the change. The motif is in yellow and shown before and after the AA change(s).

The variants involved in antibody-dependent cellular cytotoxicity (ADCC) reduction include nine Homo sapiens IGHG1 variants, which comprise: G1v1 [1], G1v2 [1], G1v3 [1], G1v5 [6], G1v47 [37], G1v50 (the variant G1v50 is a variant combining the G1v1, G1v2, G1v3 and G1v47 amino acid changes), G1v52 ‘GRLR’, G1v66 and G1v67 (Table 5).

The variants involved in antibody-dependent cellular cytotoxicity (ADCC) enhancement include nine variants, of which six Homo sapiens IGHG1 variants: G1v6 [3], G1v7 ‘DE’ [4], G1v8 ‘DLE’ ‘3M’ [4] [25], G1v9 [14], G1v10 [15] and G1v11 [15]; one Homo sapiens IGHG2 variant: G2v1 [1]; one Homo sapiens IGHG4 variant: G4v1 [1]; and one Mus musculus IGHG2B variant: Musmus G2Bv1 [5] (Table 6).

The variants involved in antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) enhancement include three Homo sapiens IGHG1 variants: G1v12 ‘GASDALIE’ [26], G1v13 ‘GASDIE’ ‘ADE’ [16] and G1v45 ‘GAALIE’ (Table 7).

The variants involved in complement-dependent cytotoxicity (CDC) enhancement include 8 variants, of which seven Homo sapiens IGHG1 variants: G1v5 [6], G1v15 [6], G1v16 [6], G1v17 ‘EFT’ [18], G1v18 [19], G1v35 ‘SE’ [18,27] and the chimeric G1G3v1 [17], and one IGHG4 variant: G4v2 [8] (Table 8).

The variants involved in complement-dependent cytotoxicity (CDC) reduction include six variants, of which three Homo sapiens IGHG1 variants: G1v8 ‘DLE’ [4], G1v19 [2] and G1v20 [2,39]; and three Mus musculus IGHG2B variants: Musmus G2Bv2 [7], Musmus G2Bv3 [7] and Musmus G2Bv4 [7] (Table 9).

The variants involved in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) reduction include 32 variants and four variant associations, of which 22 Homo sapiens IGHG1 variants: G1v4 [2], G1v14 ‘LALA’ [21,39], G1v14-1, G1v14-4, G1v14-48, G1v14-49 ‘LALAPG’ [40], G1v14-67, G1v23 [20], G1v38 [35], G1v39 ‘FES’ ‘TM’ [20,24], G1v40, G1v41 [20,24], G1v43, G1v48, G1v49 [40], G1v51, G1v53 ‘FQQ’, G1v59 [41], G1v60, G1v63, G1v65, G1v70 and one association G1v53-G1v21 ‘FQQ-YTE’ [38]; three Homo sapiens IGHG2 variants: G2v2 ‘IgG2m4’ [23], G2v3 ‘G2sigma’ [24] and the chimeric G2G4v1 [22]; five Homo sapiens IGHG4 variants: G4v3 ‘LE’ [20], G4v3-49 ‘LEPG’ [40], G4v4 ‘FALA’ [21], G4v7, G4v49 [40] and three associations G4v3-G4v45 ‘SPEL’ [12,20], G4v3-49-G4v5 ‘SPELP’ [40] [12] and G4v4-G4v5 ‘lgG4ProAlaAla’ [12,24] and two Canis lupus familiaris IGHG2 variants: CanlupfamG2v1 and CanlupfamG2v2 (Table 10).

The variants involved in B cell inhibition by coengagement of antigen and FcγR on the same cell include one Homo sapiens IGHG1 variant: G1v25 [33,34] (Table 11).

The variants involved in knock out CH2 84.4 glycosylation include five variants, of which three Homo sapiens IGHG1 variants: G1v29 [42], G1v30 [42], G1v36; one Homo sapiens IGHG4 variant: G4v36; and one Canis lupus familiaris IGHG2 variant: CanlupfamG2v2 (Table 12).

The variants involved in half-life increase or decrease include 13 variants, 12 of them increase half-life, of which five Homo sapiens IGHG1 variants: G1v21 ‘YTE’ [9,29–32], G1v22 [30], G1v24 [32], G1v42 [30] and G1v46; three Homo sapiens IGHG2 variants: G2v4 [10], G2v5 [10] and G2v6 [10]; one Homo sapiens IGHG3 variant: G3v1 [11]; three Homo sapiens IGHG4 variants: G4v21 ‘YTE’ [30], G4v22 [36] and G4v24. One variant G2v8-1 abrogates binding to FCGRT (FcRn) (Table 13).

The variants involved in abrogation of binding to Protein A include one Homo sapiens IGHG4 variant: G4v8 (Table 14).

The variants involved in formation of additional bridge stabilizing CH2 in the absence of N84.4 (Eu 297) glycosylation include four Homo sapiens IGHG1 variants: G1v54, G1v54-29, G1v54-30 and G1v54-36 (Table 15).
The variants involved in prevention of IgG4 half-IG exchange include two *Homo sapiens* IGHG4 variants: G4v5 [12] and G4v6 [13] (Table 16).

The variants involved in hexameration include one *Homo sapiens* IGHG1 variant: G1v34 (Table 17).

The variants involved in knobs-into-holes and enhancement of heteropairing H-H of bispecific antibodies include six *Homo sapiens* IGHG1 variants: G1v26 knob [28] and G1v31 hole [28], G1v32 knob and G1v33 hole, G1v68 and G1v69 (Table 18).

The variants involved in suppression of inter H-L and/or inter H-H disulfide bridges includes three *Homo sapiens* IGHG1 variants: G1v37, G1v61 and G1v62 (Table 19).

The variants involved in site-specific drug attachment include six *Homo sapiens* IGHG1 variants: G1v27, G1v28, G1v44, G1v55, G1v56 and G1v64 (Table 20).

The variants involved in enhancement of hetero pairing H-L include two *Homo sapiens* IGHG1 variants: G1v57 used in association with *Homo sapiens* IGKC variant: KCv57, and G1v58, used in association with *Homo sapiens* IGLC2 variant: LC2v58 (Table 21).

The variants involved in control of half-IG exchange of bispecific IgG4 antibodies include one *Homo sapiens* IGHG4 variant: G4v10 (Table 22).

The variants involved in reducing acid-induced aggregation include one *Homo sapiens* IGHG2 variant: G2v7 (Table 23).

Two variants have been assigned to two properties belonging to different types and are therefore found in two tables, G1v5 (Tables 5 and 8) and G1v8 (Tables 6 and 9).

Supplementary Table S2 provides the variants of Tables 5–23 in an alphanumeric order of the IMGT engineered variants involved in the effector properties (ADCC, ADCP and CDC), half-life and structure of the therapeutical monoclonal antibodies.

5. Conclusions

The therapeutic monoclonal antibody engineering field is the most promising in the medical field. A standardized analysis of IG genomic and expressed sequences, structures and interactions is crucial for a better molecular understanding and comparison of the mAb specificity, affinity, half-life, Fc effector properties and potential immunogenicity. IMGT has provided the concepts for the IG loci description of newly sequenced genomes [2], antibody structure/function characterization [4], antibody engineering (single chain Fragment variable (scFv), phage displays, combinatorial libraries) and antibody humanization (chimeric, humanized and human antibodies). IMGT® standardization allows the repertoire analysis and antibody humanization studies to move to novel, high-throughput methodologies with the same high-quality criteria. The CDR-IMGT lengths are now required for mAb INN applications and are included in the WHO-INN definitions (84–86). The characterization of the IGHG engineered variants for effector properties, half-life increase, and new structures of bi- and multi-specific antibodies brings a new level of standardized information in the comparative analysis of therapeutic antibodies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antib11040065/s1, Table S1: Correspondence between the IMGT unique numbering for C-DOMAIN, the IMGT exon numbering, the EU and Kabat numberings: Human IGHG [97,98] https://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnumber.html; Table S2: IMGT nomenclature (alphanumeric order) of engineered variants involved in effector properties (ADCC, ADCP and CDC), half-life and structure of therapeutical monoclonal antibodies.

Author Contributions: Conceptualization, methodology, validation, investigation, data curation, writing, review and editing, visualization, ontology, M.-P.L. and G.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data is contained within the article or Supplementary material.

Acknowledgments: We thank Souphatta Sasorith, Melissa Cambon and Karima Cherouali for their contribution in the early phase of this work.
26. Giudicelli, V.; Chaume, D.; Lefranc, M.-P. IMGT/GENE-DB: A comprehensive database for human and mouse immunoglobulin and T cell receptor genes. *Nucleic Acids Res.* 2005, 33, D256–D261. [CrossRef]

27. Kaas, Q.; Ruiz, M.; Lefranc, M.-P. IMGT/3Dstructure-DB and IMGT/StructuralQuery, a database and a tool for immunoglobulin, T cell receptor and MHC structural data. *Nucleic Acids Res.* 2004, 32, D208–D210. [CrossRef]

28. Ehrenmann, F.; Kaas, Q.; Lefranc, M.-P. IMGT/3Dstructure-DB and IMGT/DomainGapAlign: A Database and a Tool for Immunoglobulins or Antibodies, T Cell Receptors, MHC, IgSF and MhcSF. *Nucleic Acids Res.* 2010, 38, D301–D307. [CrossRef]

29. Ehrenmann, F.; Lefranc, M.-P. IMGT/3Dstructure-DB: Querying the IMGT Database for 3D Structures in Immunology and Immunoinformatics (IG or Antibodies, TR, MH, RPL, and FPIA). *Cold Spring Harb. Protoc.* 2011, 6, 750–761. [CrossRef]

30. Poiron, C.; Wu, Y.; Ginestoux, C.; Ehrenmann, F.; Duroux, P.; Lefranc, M.-P. IMGT/mAb-DB: The IMGT® database for therapeutic monoclonal antibodies. In Proceedings of the 11èmes Journées Ouvertes de Biologie, Informatique et Mathématiques (JOBIM), Montpellier, France, 7–9 September 2010.

31. Giudicelli, V.; Chaume, D.; Lefranc, M.-P. IMGT/V-QUEST, an Integrated Software for Immunoglobulin and T Cell Receptor V-J and V-D-J Rearrangement Analysis. *Nucleic Acids Res.* 2004, 32, W435–W440. [CrossRef]

32. Giudicelli, V.; Lefranc, M.-P. Interactive IMGT on-line tools for the analysis of immunoglobulin and T cell receptor repertoire. In *New Research on Immunology*; Vesikler, B.A., Ed.; Nova Science Publishers Inc.: New York, NY, USA, 2005; pp. 77–105.

33. Brochet, X.; Lefranc, M.-P.; Giudicelli, V. IMGT/V-QUEST: The Highly Customized and Integrated System for IG and TR Standardized V-J and V-D-J Sequence Analysis. *Nucleic Acids Res.* 2008, 36, W503–W508. [CrossRef]

34. Giudicelli, V.; Lefranc, M.-P. IMGT® standardized analysis of immunoglobulin rearranged sequences. In *Immunoglobulin Gene Analysis in Chronic Lymphocytic Leukemia*; Ghia, P., Rosenquist, R., Davi, F., Eds.; Wolters Kluwer Health Italy: Milano, Italy, 2008; Chapter 2; pp. 33–52.

35. Giudicelli, V.; Brochet, X.; Lefranc, M.-P. IMGT/V-QUEST: IMGT standardized analysis of the immunoglobulin (IG) and T cell receptor (TR) nucleotide sequences. *Cold Spring Harb. Protoc.* 2011, 6, 695–715. [CrossRef]

36. Alamyar, E.; Duroux, P.; Lefranc, M.-P.; Giudicelli, V. IMGT® tools for the nucleotide analysis of immunoglobulin (IG) and T cell receptor (TR) V-(D)-J repertoire, polymorphisms, and IG mutations: IMGT/V-QUEST and IMGT/HighV-QUEST for NGS. In *Immunogenetics*, Christiansen, F., Tait, B., Eds.; Methods in Molecular Biology; Humana Press: New York, NY, USA, 2012; Chapter 32; Volume 882, pp. 569–604. [CrossRef]

37. Yousfi Monod, M.; Giudicelli, V.; Chaume, D.; Lefranc, M.-P. IMGT/JunctionAnalysis: The first tool for the analysis of the immunoglobulin and T cell receptor complex V-J and V-D-J JUNCTIONs. *Bioinformatics* 2004, 20, i379–i385. [CrossRef]

38. Giudicelli, V.; Lefranc, M.-P. IMGT/JunctionAnalysis: IMGT standardized analysis of the V-J and V-D-J Rearrangements of the immunoglobulins (IG) and T cell receptors (TR). *Cold Spring Harb. Protoc.* 2011, 6, 716–725. [CrossRef]

39. Giudicelli, V.; Protat, C.; Lefranc, M.-P. The IMGT strategy for the automatic annotation of IG and TR cDNA sequences: IMGT/Automat. In Proceedings of the European Conference on Computational Biology (ECCB 2003), Data and Knowledge Bases, Institut National de Recherche en Informatique et en Automatique, Poster DKB_31, ECCB 2003, Paris, France, 7–9 September 2003; pp. 103–104.

40. Giudicelli, V.; Chaume, D.; Jabado-Michaloud, J.; Lefranc, M.-P. Immunogenetics sequence Annotation: The strategy of IMGT based on IMGT-ONTOLOGY. *Stud. Health Technol. Inform.* 2005, 116, 3–8.

41. Alamyar, E.; Giudicelli, V.; Duroux, P.; Lefranc, M.-P. IMGT/HighV-QUEST: A high-throughput system and web portal for the analysis of rearranged nucleotide sequences of antigen receptors—High-throughput version of IMGT/V-QUEST. In Proceedings of the 11èmes Journées Ouvertes de Biologie, Informatique et Mathématiques (JOBIM), Montpellier, France, 7–9 September 2010.

42. Alamyar, E.; Giudicelli, V.; Li, S.; Duroux, P.; Lefranc, M.-P. IMGT/HighV-QUEST: The IMGT® web portal for immunoglobulin (IG) or antibody and T cell receptor (TR) analysis from NGS high throughput and deep sequencing. *Immunome Res.* 2012, 8, 26.

43. Li, S.; Lefranc, M.-P.; Miles, J.J.; Alamyar, E.; Giudicelli, V.; Duroux, P.; Freeman, J.D.; Corbin, V.; Scheerlinck, J.-P.; Frohman, M.A.; et al. IMGT/HighV-QUEST paradigm for T cell receptor IMGT clonotype diversity and next generation repertoire immunoprofiling. *Nat. Commun.* 2013, 4, 2333. [CrossRef]

44. Giudicelli, V.; Duroux, P.; Lavoie, A.; Aouinti, S.; Lefranc, M.-P.; Kossida, S. From IMGT-ONTOLOGY to IMGT/HighV-QUEST for NGS immunoglobulin (IG) and T cell receptor (TR) repertoires in autoimmune and infectious diseases. *Autoimmun. Infect. Dis.* 2015, 1, 1–15.

45. Giudicelli, V.; Duroux, P.; Kossida, S.; Lefranc, M.-P. IG and TR single chain Fragment variable (scFv) sequence analysis: A new advanced functionality of IMGT/ V-QUEST and IMGT/HighV-QUEST. *BMC Immunol.* 2017, 18, 35. [CrossRef]

46. Aouinti, S.; Malouche, D.; Giudicelli, V.; Kossida, S.; Lefranc, M.-P. IMGT/HighV-QUEST statistical significance of IMGT clonotype (AA) diversity per gene for standardized comparisons of next generation sequencing immunoprofiles of immunoglobulins and T cell receptors. *PloS ONE* 2015, 10, e0142353, https://doi.org/10.1371/journal.pone.0142353. eCollection 2015. Correction: *PloS ONE* 2016, 11, e0146702. [CrossRef]

47. Aouinti, S.; Giudicelli, V.; Duroux, P.; Malouche, D.; Kossida, S.; Lefranc, M.-P. IMGT/StatClonotype for pairwise evaluation and visualization of NGS IG and TR IMGT clonotype (AA) diversity or expression from IMGT/HighV-QUEST. *Front. Immunol.* 2016, 7, 339. [CrossRef]

48. Lane, J.; Duroux, P.; Lefranc, M.-P. From IMGT-ONTOLOGY to IMGT/LIGMotif: The IMGT® standardized approach for immunoglobulin and T cell receptor gene identification and description in large genomic sequences. *BMC Bioinform.* 2010, 11, 223. [CrossRef]
49. Ehrenmann, F.; Lefranc, M.-P. IMGT/DomainGapAlign: IMGT standardized analysis of amino acid sequences of Variable, Constant, and Groove domains (IG, TR, MH, IgSF, MhSF). Cold Spring Harb. Protoc. 2011, 6, 737–749. [CrossRef]

50. Ehrenmann, F.; Lefranc, M.-P. IMGT/DomainGapAlign: The IMGT® tool for the analysis of IG, TR, MH, IgSF and MhSF domain amino acid polymorphism. In Immunogenetics; Christiansen, F., Tait, B., Eds.; Methods in Molecular Biology; Humana Press: New York, NY, USA, 2012; Chapter 33; Volume 882, pp. 605–633. [CrossRef]

51. Ehrenmann, F.; Giudicelli, V.; Douroux, P.; Lefranc, M.-P. IMGT/Collier de Perles: IMGT Standardized representation of Domains (IG, TR, and IgSF Variable and Constant Domains, MH and MhSF Groove Domains). Cold Spring Harb. Protoc. 2011, 6, 726–736. [CrossRef]

52. Pommi, C.; Levadoux, S.; Sabatier, R.; Lefranc, G.; Lefranc, M.-P. IMGT standardized criteria for statistical analysis of immunoglobulin V-REGION amino acid properties. J. Mol. Recognit. 2004, 17, 17–32. [CrossRef]

53. Lefranc, M.-P. From IMGT-ONTOLOGY IDENTIFICATION axiom to IMGT standardized keywords: For immunoglobulins (IG), T cell receptors (TR), and conventional genes. Cold Spring Harb. Protoc. 2011, 6, 604–613. [CrossRef]

54. Lefranc, M.-P. IMGT-ONTOLOGY, IDENTIFICATION axiom. In Encyclopedia of Systems Biology; Dubitzky, W., Wolkenhauer, O., Cho, K.-H., Yokota, H., Eds.; Springer: New York, NY, USA, 2013. [CrossRef]

55. Lefranc, M.-P. From IMGT-ONTOLOGY DESCRIPTION axiom to IMGT standardized labels: For immunoglobulin (IG) and T cell receptor (TR) sequences and structures. Cold Spring Harb. Protoc. 2011, 6, 614–626. [CrossRef]

56. Lefranc, M.-P. IMGT-ONTOLOGY, DESCRIPTION axiom. In Encyclopedia of Systems Biology; Dubitzky, W., Wolkenhauer, O., Cho, K.-H., Yokota, H., Eds.; Springer: New York, NY, USA, 2013. [CrossRef]

57. Lefranc, M.-P. Complementarity Determining Region (CDR-IMGT). In Encyclopedia of Systems Biology; Dubitzky, W., Wolkenhauer, O., Cho, K.-H., Yokota, H., Eds.; Springer: New York, NY, USA, 2013. [CrossRef]

58. Lefranc, M.-P. Framework Region (FR-IMGT). In Encyclopedia of Systems Biology; Dubitzky, W., Wolkenhauer, O., Cho, K.-H., Yokota, H., Eds.; Springer: New York, NY, USA, 2013. [CrossRef]

59. Jefferis, R.; Lefranc, M.-P. Human immunoglobulin allotypes: Possible implications for immunogenicity. mAbs 2009, 1, 332–338. [CrossRef]

60. Lefranc, M.-P.; Lefranc, G. Human Gm, Km and Am allotypes and their molecular characterization: A remarkable demonstration of polymorphism. In Immunogenetics; Christiansen, F., Tait, B., Eds.; Humana Press: New York, NY, USA, 2012; Chapter 34; Volume 882, pp. 635–660. [CrossRef]

61. Lefranc, M.-P. Nomenclature of the human immunoglobulin heavy (IGH) genes. Exp. Clin. Immunogenet. 2001, 18, 100–116. [CrossRef]

62. Lefranc, M.-P. From IMGT-ONTOLOGY CLASSIFICATION axiom to IMGT standardized gene and allelic nomenclature: For immunoglobulins (IG) and T cell receptors (TR). Cold Spring Harb. Protoc. 2011, 6, 627–632. [CrossRef]

63. Lefranc, M.-P. IMGT-ONTOLOGY, CLASSIFICATION Axiom. In Encyclopedia of Systems Biology; Dubitzky, W., Wolkenhauer, O., Cho, K.-H., Yokota, H., Eds.; Springer: New York, NY, USA, 2013. [CrossRef]

64. Lefranc, M.-P. IMGT-ONTOLOGY, NUMEROTATION axiom. In Encyclopedia of Systems Biology; Dubitzky, W., Wolkenhauer, O., Cho, K.-H., Yokota, H., Eds.; Springer: New York, NY, USA, 2013. [CrossRef]

65. Lefranc, M.-P. Unique database numbering system for Immunoglobulins, T cell receptors and Ig-like domains. Immunology 1999, 7, 132–136.

66. Lefranc, M.-P.; Pommié, C.; Ruiz, M.; Giudicelli, V.; Foulquier, E.; Truong, L.; Thouvenin-Contet, V.; Lefranc, G. IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. Dev. Comp. Immunol. 2003, 27, 55–77. [CrossRef]

67. Lefranc, M.-P.; Pommié, C.; Kaas, Q.; Duprat, E.; Bosc, N.; Guiraudou, D.; Jean, C.; Ruiz, M.; Da Piedade, I.; Rouard, M.; et al. IMGT unique numbering for immunoglobulin and T cell receptor constant domains and Ig superfamily C-like domains. Dev. Comp. Immunol. 2005, 29, 185–203. [CrossRef]

68. Lefranc, M.-P.; Duprat, E.; Kaas, Q.; Tranne, M.; Thiriot, A.; Lefranc, G. IMGT unique numbering for MHC groove G-DOMAIN and MHC superfamily (MhcSF) G-LIKE-DOMAIN. Dev. Comp. Immunol. 2005, 29, 917–938. [CrossRef]

69. Lefranc, M.-P. IMGT unique numbering for the Variable (V), Constant (C), and Groove (G) domains of IG, TR, MH, IgSF, and MhSF. Cold Spring Harb. Protoc. 2011, 6, 633–642. [CrossRef]

70. Lefranc, M.-P. IMGT unique numbering. In Encyclopedia of Systems Biology; Dubitzky, W., Wolkenhauer, O., Cho, K.-H., Yokota, H., Eds.; Springer: New York, NY, USA, 2013; pp. 952–959. [CrossRef]

71. Lefranc, M.-P. Immunoinformatics of the V, C and G domains: IMGT® definitive system for IG, TR and IgSF, MH, and MhSF. In Immunoinformatics: From Biology to Informatics, 2nd ed.; De, R.K., Tomar, N., Eds.; Methods in Molecular Biology; Humana Press: New York, NY, USA, 2014; Volume 1184, pp. 59–107. [CrossRef]

72. Ruiz, M.; Lefranc, M.-P. IMGT gene identification and Colliers de Perles of human immunoglobulins with known 3D structures. Immunogenetics 2002, 53, 857–883.

73. Kaas, Q.; Lefranc, M.-P. IMGT Colliers de Perles: Standardized sequence-structure representations of the IgSF and MhcSF superfamily domains. Curr. Bioinform. 2007, 2, 21–30. [CrossRef]

74. Kaas, Q.; Ehrenmann, F.; Lefranc, M.-P. IG, TR and IgSF, MHC and MhcSF: What do we learn from the IMGT Colliers de Perles? Brief. Funct. Genom. Proteom. 2007, 6, 253–264. [CrossRef]
103. Brinkhaus, M.; Douwe, R.G.J.; Bentlage, A.E.H.; Temming, A.R.; de Taeye, S.W.; Tammes, M.; Gerritsen, J.; Mok, J.Y.; Brasser, G.; Ligthart, P.C.; et al., Glycine 236 in the lower hinge region of human IgG1 differentiates FcγR from Complement effector function. *J. Immunol.* 2020, 205, 3456–3467. [CrossRef]

104. Shields, R.L.; Namenuk, A.K.; Hong, K.; Meng, Y.G.; Rae, J.; Briggs, J.; Xie, D.; Lai, J.; Stadlen, A.; Li, B.; et al. High resolution mapping of the binding site on human FcG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. *J. Biol. Chem.* 2001, 276, 6591–6604. [CrossRef]

105. Lazar, G.A.; Dang, W.; Karki, S.; Vafa, O.; Peng, J.S.; Hyun, L.; Chan, C.; Chung, H.S.; Ivai, A.; Yoder, S.C.; et al. Engineered antibody Fc variants with enhanced effector function. *Nat. Protoc.* 2006, 103, 4005–4010. [CrossRef]

106. Oganesyan, V.; Damschroder, M.M.; Leach, W.; Wu, H.; D’AllaQua, W.F. Structural characterization of a mutated, ADCC-enhanced human Fc fragment. *Mol. Immunol.* 2008, 45, 1872–1882. [CrossRef]

107. Stavenhagen, J.B.; Gorlatov, S.; Taullion, N.; Rankin, C.T.; Li, H.; Burke, S.; Huang, L.; Vijh, S.; Johnson, S.; Bonvini, E.; et al. Fc optimization of therapeutic antibodies enhances their ability to kill tumor cells in vitro and controls tumor expansion in vivo via low-affinity activating Fc gamma receptors. *Cancer Res.* 2007, 67, 8882–8890. [CrossRef]

108. Mimoto, F.; Igawa, T.; Kuramochi, T.; Katada, H.; Kadono, S.; Kamikawa, T.; Shida-Kawazoe, M.; Hattori, K. Novel asymmetrically engineered antibody Fc variant with superior FcγR binding affinity and specificity compared with afucosylated Fc variant. *mAbs* 2013, 5, 229–236. [CrossRef]

109. Duncan, A.R.; Woof, J.M.; Burton, D.R.; Winter, G. Localization of the binding site for the human high-affinity Fc receptor on IgG. *Nature* 1988, 332, 563–564. [CrossRef] [PubMed]

110. Ahmed, A.A.; Keremane, S.R.; Vielmetter, J.; Bjorkman, P.J. Structural characterization of GASDALIE Fc bound to the activating Fc receptor FcγRIIa. *J. Struct. Biol.* 2016, 191, 78–89. [CrossRef] [PubMed]

111. Tao, M.H.; Smith, R.I.; Morrison, S.L. Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation. *J. Exp. Med.* 1993, 178, 661–667. [CrossRef]

112. Idusogie, E.E.; Presta, L.G.; Gazzano-Santorio, H.; Totpal, K.; Wong, P.Y.; Utzsch, M.; Meng, Y.G.; Mukkiren, M.G. Mapping of the C1q binding site on rituximab, a chimeric antibody with a human IgG1 Fc. *J. Immunol.* 1997, 161, 4178–4184. [CrossRef] [PubMed]

113. Hezareh, M.; Hessell, A.J.; Jensen, R.C.; van de Winkel, J.G.; Parren, P.W. Effector function activities of a panel of mutants of a mouse model recapitulating human FcγR. *J. Biol. Chem.* 2014, 289, 2517–2527. [CrossRef] [PubMed]

114. Smith, P.; DiLillo, D.J.; Bournazos, S.; Li, F.; Ravetch, J.V. Mouse model recapitulating human FcγRIIa. *J. Immunol.* 2016, 197, 15309–15318. [CrossRef] [PubMed]

115. Natsume, A.; In, M.; Takamura, H.; Nakagawa, T.; Shiz, Y.; Kitajima, K.; Wakiuti, M.; Ohta, S.; Satoh, M.; Shitara, K.; et al. Engineered antibodies of IgG1/IgG3 mixed isotype with enhanced cytokytic activities. *Cancer Res.* 2008, 68, 3863–3872. [CrossRef]

116. Tao, M.H.; Smith, R.I.; Morrison, S.L. Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation. *J. Biol. Chem.* 1993, 268, 15309–15318. [CrossRef] [PubMed]

117. An, Z.; Forrest, G.; Moore, R.; Cukan, M.; Haytko, P.; Huang, L.; Vitelli, S.; Zhao, J.Z.; Lu, P.; Hua, J.; et al. IgG2m4, an engineered antibody isotype with reduced Fc function. *mAbs* 2009, 3, 572–579. [CrossRef]
128. Rother, R.P.; Rollins, S.A.; Mojzik, C.F.; Brodsky, R.A.; Bell, L. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. Nat. Biotechnol. 2007, 25, 1256–1264. [CrossRef] [PubMed]
129. Labrijn, A.F.; Builisse, A.O.; van den Bremer, E.T.; Verwilligen, A.Y.; Bleeker, W.K.; Thorpe, S.J.; Killestein, J.; Polman, C.H.; Aalberse, R.C.; Schuurman, J.; et al. Therapeutic IgG4 antibodies engage in Fab-arm exchange with endogenous human IgG4 in vivo. Nat. Biotechnol. 2009, 27, 767–771. [CrossRef] [PubMed]
130. Chu, S.Y.; Vostiar, I.; Karki, S.; Moore, G.L.; Lazar, G.A.; Pong, E.; Joyce, P.F.; Szymkowski, D.E.; Desjarlais, J.R. 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, 3926–3933. [CrossRef] [PubMed]
131. Szili, D.; Cserhalmi, M.; Bankó, Z.; Nagy, G.; Szymkowski, D.E.; Sármay, G. Suppression of innate and adaptive B cell activation pathways by antibody coengagement of FcγRIIb and CD19. mAbs 2014, 6, 991–999. [CrossRef] [PubMed]
132. Leabman, M.K.; Meng, Y.G.; Kelley, R.F.; DeForge, L.E.; Cowan, K.J.; Iyer, S. Effects of altered FcγR binding on antibody pharmacokinetics in cynomolgus monkeys. mAbs 2013, 5, 896–903. [CrossRef] [PubMed]
133. Robbie, G.J.; Criste, R.; Dall’acqua, W.F.; Jensen, K.; Patel, N.K.; Losonsky, G.A.; Griffin, M.P. A novel investigational Fc-modified humanized monoclonal antibody, motavizumab-YTE, has an extended half-life in healthy adults. Antimicrob. Agents Chemother. 2013, 57, 6147–6153. [CrossRef] [PubMed]
134. Dall’Acqua, W.F.; Woods, R.M.; Ward, E.S.; Palaszynski, S.R.; Patel, N.K.; Brewah, Y.A.; Wu, H.; Kiener, P.A.; Langermann, S. Increasing the affinity of a human IgG1 for the neonatal Fc receptor: Biological consequences. J. Immunol. 2002, 169, 5171–5180. [CrossRef] [PubMed]
135. Zalevsky, J.; Chamberlain, A.K.; Horton, H.M.; Karki, S.; Leung, L.W.; Sproule, T.J.; Lazar, G.A.; Roopenian, D.C.; Desjarlais, J.R. Enhanced antibody half-life improves in vivo activity. Nat. Biotechnol. 2010, 28, 157–159. [CrossRef] [PubMed]
136. Hinton, P.R.; Johlfs, M.G.; Xiong, J.M.; Hanestad, K.; Ong, K.C.; Bullock, C.; Kelley, S.; Tang, M.T.; Tso, J.Y.; Vásquez, M.; et al. Engineered human IgG antibodies with longer serum half-lives in primates. J. Biol. Chem. 2004, 279, 6213–6216. [CrossRef] [PubMed]
137. Oganesyan, V.; Damshroder, M.M.; Cook, K.E.; Li, Q.; Gao, C.; Wu, H.; Dall’Acqua, W.F. Structural insights into neonatal Fc receptor-based recycling mechanisms. J. Biol. Chem. 2014, 289, 7812–7824. [CrossRef] [PubMed]
138. Stapleton, N.M.; Andersen, J.T.; Stemerding, A.M.; Bjarnarson, S.P.; Verheul, R.C.; Gerritsen, J.; Zhao, Y.; Kleijer, M.; Sandlie, I.; de Haas, M.; et al. Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. Nat. Commun. 2011, 2, 599. [CrossRef] [PubMed]
139. Zhang, J.; Huang, Y.; Xi, G.; Zhang, F. HX008: A humanized PD-1 blocking antibody with potent antitumor activity and superior pharmacologic properties. mAbs 2020, 12, 1724751. [CrossRef] [PubMed]
140. Dall’Acqua, W.F.; Kiener, P.A.; Wu, H. Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). J. Biol. Chem. 2006, 281, 23514–23524. [CrossRef] [PubMed] [PubMed]
141. Labrijn, A.F.; Rispen, T.; Meesters, J.; Rose, R.J.; den Bleeker, T.H.; Loverix, S.; van den Bremer, E.T.; Neijssen, J.; Vink, T.; Laster, I.; et al. Species-specific determinants in the IgG CH3 domain enable Fab-arm exchange by affecting the noncovalent CH3-CH3 interaction strength. J. Immunol. 2011, 187, 3238–3246. [CrossRef] [PubMed]
142. Ridgway, J.B.; Presta, L.G.; Carter, P. ‘Knobs-into-holes’ engineering of antibody CH3 domains for heavy chain heterodimerization. Protein Eng. 1996, 9, 617–621. [CrossRef] [PubMed]
143. Saito, S.; Namisaki, H.; Hiraishi, K.; Takahashi, N.; Iida, S. Engineering a human IgG2 antibody stable at low pH. Protein Sci. 2020, 29, 1186–1195. [CrossRef] [PubMed]