Insights into the IgG heavy chain engineering patent landscape as applied to IgG4 antibody development

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ABSTRACT
Despite being the least abundant immunoglobulin G in human plasma, IgG4 antibodies are used therapeutically when weak effector functions are needed. The increase in engineered IgG4-based antibodies on the market led us to study the patent landscape of IgG4 Fc engineering, i.e., patents claiming modifications in the heavy chain. Thirty-seven relevant patent families were identified, comprising hundreds of IgG4 Fc variants focusing on removal of residual effector functions (since IgG4s bind to FcγRI and weakly to other FcγRs), half-life enhancement and IgG4 stability. Given the number of expired or soon to expire major patents in those 3 areas, companies developing blocking antibodies now have, or will in the near future, access to free tools to design silenced, half-life extended and stable IgG4 antibodies.

Introduction
Although the least abundant of the 4 IgG subclasses naturally found in human plasma,1 IgG4 antibodies comprise the second largest subclass of approved monoclonal antibody (mAb) products behind IgG1. Among the 80 approved biopharmaceuticals containing a human IgG Fc portion (70 mAbs and 10 fusion proteins), 58 are indeed based on IgG1, and 15 are based on IgG4, historically relying on a desire to avoid target cell killing and immune activation,2 while maintaining a plasmatic half-life as long as that of IgG1.

At one time, IgG4 were thought to be unable to bind to cellular Fc receptors and to induce antibody-dependent cell-mediated cytoxicity (ADCC), and it was considered the worst subclass at promoting complement-induced target cell lysis.3–5 A direct comparison of IgG4 and IgG1 versions of Campath (anti-CD52 alemtuzumab) in a clinical study,9 however, showed that Campath-G4 depleted target cells in most patients, but to a lesser extent than Campath-G1.9,10 IgG4 were initially thought to bind to FcγRI only,11 but study results indicate they also bind to FcγRIIA, FcγRIIB, FcγRIIC, and FcγRIIIA, especially the V158 allotype, although less than IgG1.12,13 Indeed, the use of IgG4 does not guarantee the absence of immune activation, since cross-linking by FcγRIIB may have been critical for the “cytokine storm” observed in volunteers during the tragic TGN1412 (anti-CD28 superagonist IgG4) Phase 1 trial.

Pharmacological differences between IgG1 and IgG4 subclasses result from structural differences between the γ1 and γ4 heavy chains. However, depending on allotypes (genetic variants), comparison between these two isotypes can be misleading. Here, we use γ1 and γ4 to refer to the chains encoded by the IGHG1*01 and IGHG4*01 alleles, respectively. The differences are mainly located in and around the hinge (Figure 1a,b) and can also affect the downstream processing during bioproduction. For example, the purification process can contribute to aggregation, but to a different extent according to the subclass. Indeed, IgG4 have been showed to be less stable than IgG1s at low pH conditions.19,20 In particular, the serine at position 228 is specific to γ4 and is one of the key amino-acids playing a role in the stability of IgG4s. A study has shown that S228P substitution resulted in a homogenous IgG4 when analyzed by SDS-PAGE.21 It was later demonstrated that this substitution could block the half-IgG exchange phenomenon usually known as Fab-arm exchange (Figure 2).22,23

Other γ1/γ4 differences are located in the heart of the CH3 and at its C-terminal side (Figure 1a,b). It was shown that not only the hinge region but also the CH3 domain contribute to Fab-arm exchange,25 and particularly the arginine residues at position 409,24 which is polymorphic since replaced by a lysine in the IGHG4*03 allele. The role of IgG4 Fab-arm exchange has been interpreted as a type of posttranslational modification, which could serve as an additional mechanism for generating anti-inflammatory activity.23 This exchange prevents IgG4s from bridging two membrane antigens or forming immune complexes with soluble antigens since exchanged IgG4 become bispecific, and thus functionally monovalent. This phenomenon does not seem to cause safety issues, based on the results for natalizumab (anti-α4 integrin), reslizumab (anti-IL-5) or ibalizumab (anti-CD4), which are wild-type approved IgG4 mAbs known to undergo Fab-arm exchange with endogenous IgG4.22

Of the 15 approved IgG4-based therapeutics (Table 1), 12 are modified in their constant heavy chain, while only 14 of 50 approved IgG1-based therapeutics harbor constant heavy chain modifications. Moreover, most of the IgG4 antibodies currently in Phase 3 bear constant heavy chain modifications (Table 1). The increase of engineered IgG4 constant heavy chain-based products arriving on the market, notably the anti-PD1

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antibodies, led us to study the US and European patent landscape regarding technologies optimizing classical antagonist IgG4 antibodies. We discuss areas where companies are free to operate (or not), the relevance of different technologies, and what could be the standard IgG4 blocking antibody constant region format in the future.

Patent analysis

Description of the patent corpus

A corpus of patent families integrating the structural and functional description of IgG4 constant heavy chain modifications was established by using relevant keywords, cooperative patent classification codes or citing patents in the Orbit© patent database (Questel, France). The claims sections were carefully examined to build a patent corpus referring to the main engineering improvements of antagonist IgG4 antibodies. The areas of improvements were: 1) reduction of effector functions, 2) half-life modulation, 3) stability, and 4) downstream processes. Because issues like half-life modulation or effector function reductions are not only related to IgG4, the corpus includes broader patents, covering any polypeptide comprising an IgG framework. Here, we define broad claims as main claims covering any polypeptide comprising at least an IgG4-Fc with the disclosed technology. Many applications describe variants that were not, or are no longer, protected by a patent. Close examination of the details of what has been described and what has been claimed is generally necessary because the patent landscape is always evolving. New family patents need to be monitored, but also applications or new applications in current patent families. Indeed, continuation applications may be issued later and what has not been claimed in an earlier application could be claimed in a later application of the same family in order to obtain the full protection extent of the parent application. For example, Xencor filed several continuations in part from a 2003 parent application (WO2004029207) and the last application from this family was issued in 2018.

As of June 2019, the corpus comprised 40 patent families incorporating claims encompassing IgG4 framework

![Figure 1. Primary structural differences between γ4 and γ1 heavy chains. (a) Three dimensional structure of pembrolizumab highlighting the amino-acid differences between γ4 and γ1 heavy chains: pembrolizumab differs from wild-type IgG4 by one amino-acid at position 228 in the hinge region, a proline replacing a serine residue (S228P, shown in pink but masked in part by other residues). The rotated CH2 of pembrolizumab is shown in green cartoon. The two glycans are indicated in orange, showing the external exposition of the glycan from the rotated domain. IgG4 presents two isoallotypes (single nucleotide polymorphisms in the IGHG4 gene) at position 309 in CH2 with either a leucine or a valine, and at position 409 in the CH3 with either an arginine or a lysine, shown in purple spheres. The positions which have different amino-acid between γ4 and γ1 heavy chains are shown in blue sphere for CH1 (131; 133; 137; 138; 196; 199; 203; 214), in red spheres for hinge region (217; 219; 220; 224; 225; 228), in green spheres for CH2 (234; 258; 274; 296; 327; 330; 331), and in yellow spheres for CH3 (355; 356; 358; 409; 419; 445). All of these residues were superimposed on the pembrolizumab structure (PDB: 5DK3) using PyMOL Molecular Graphics System, version 1.7.4 (Schrödinger). (b) Amino-acid sequence comparison between γ1 and γ4 hinge regions. Nucleotide and amino-acid differences in γ4 compared to γ1 are shown in red. Cysteines indicating disulfide bridges are shown in bold. The nucleotide alignment has enabled us to number amino acids at positions Y219 and G220, which were not numbered in the original Eu numbering. γ4 hinge region differ by 6 amino-acids from γ1 hinge region. The γ4 hinge has a three amino-acid deletions (shown in gray), with only two disulfide bridges, while γ1 has three. The missing one, C220 bridges the light chain to the γ1 heavy chain; in IgG4, this inter-chain bridge involves C131 in the CH1.)
modifications. Patents in the same restricted family (determined by Orbit) were grouped according to the first international publication number (if existing) of the family (Table 2).

**Patents related to IgG effector function reduction**

One of the first applications regarding modulation of IgG properties (WO8907142) was filed in 1989 by Columbia University. The idea was to swap the domains of different IgG subclasses in order to obtain antibodies with desired properties. Although a European patent was granted in 1996, Celltech filed an opposition and the patent was eventually revoked in 2006.

The first application (WO9428027) claiming a silenced IgG4 (with the L235E substitution) was filed in 1993 by Arch Development Corporation. However, the US and European patents main claims are quite restrictive since they refer to an anti-CD3 antibody with this mutation, and not to any antibody having this mutation. The example section of other applications (WO9429351, WO9526403) contain IgG4 variants with mutations (L235A, F234A or G237A). However, we did not find any patent with a broad claim covering any IgG (hence IgG4)
comprising a single mutation at either of these positions to abolish effector functions. Cellect biotech filed an application (WO9526403) in 1994 with a broad claim regarding reduction of complement activation, but no patent was granted. Indeed, several publications\(^\text{10,25}\) had already identified the binding sites associated with effector functions. In 2000, Genentech filed an application (WO200042072) comprising hundreds of single variants that later resulted in dozens of patents. For the most part, the patents relate to FcγR binding enhancement, but they also contain examples of mutations reducing the binding to all FcγR, e.g., D265A, which is covered by a patent (US7332581) in the family; however, its legal status is uncertain. These patents are due to expire in 2020. In 2003, Xencor filed several applications from their parent application (WO2003029207) comprising hundreds of Fc variants modulating affinity to FcγR. Among them, they obtained US patents claiming, for example, the L328 substitution decreasing ADCC, or the A330R mutation decreasing binding to FcγRIIA. Some have been opposed, including the EP2364996B1 patent covering the F243L mutation.

Another way to abolish effector functions is to mutate residues in or close to the N-glycosylation site,\(^\text{26,27}\) as claimed in WO2005018572. However, although references to aglycosylated IgG4 may be found in patents, we did not find any aglycosylated IgG4 mAb in development.

From the early 1990s to the early 2000s, publications and patent applications disclosed most of the interesting single mutations reducing effector functions. Companies are thus seeking to patent mutation combinations in order to freely exploit their own silenced mAbs.

Alexion developed a technology to abolish effector functions by joining an IgG2 (up to T260) with the end of an IgG4 Fc (Figure 3), giving a molecule with very weak binding to C1q and Fcγ receptors. This is the format of the anti-C5 marketed antibodies eculizumab (Soliris\(^\text{\*}\)) and ravulizumab (ULTOMIRIS\(^\text{\*}\)). Alexion withdrew their 2005 application (WO2005007809) describing this format, probably due to their prior publication in 1997, which compromised any claim of novelty.\(^\text{28}\) However, to our knowledge, this format (albeit not covered by a patent) has not yet been used by other companies, at least for mAbs in clinical development (except eculizumab biosimilars). However, patent applications comprising this kind of IgG2/IgG4 format\(^\text{29}\) have been issued, including the WO2015140591 application by the Norwegian University of Science and Technology and the

### Table 1. Biopharmaceuticals with an IgG4-Fc fragment that are approved or in late-stage clinical development.

| Antibody          | Target | Society                  | Format | Fc modifications (Eu) | Approval year or phase reached in the US |
|-------------------|--------|--------------------------|--------|-----------------------|------------------------------------------|
| Gemtuzumab        | CD33   | Wyeth/Pfizer/Celltech/UCB| IgG4   | S228P                 | 2000, withdrawn in 2010, approved in 2017 |
| ozogamicin        |        |                          |        |                       |                                          |
| Natalizumab       | VLA-4  | Biogen                   | IgG2   | No mutation           | 2006                                     |
|                   |        |                          | IgG4   |                       | 2007                                     |
| Eculizumab        | C5     | Alexion                  | IgG2/IgG4 |                       | 2018                                     |
|                   |        |                          | IgG4   |                       | 2014                                     |
| Dulaglutide       | GLP-1 R| Lilly                    | IgG4 fusion |                       |                                          |
|                   |        |                          |        |                       |                                          |
| Nivolumab         | PD1    | BMS                      | IgG4   | S228P                 | 2015                                     |
| Pembrolizumab     | PD1    | Merck                    | IgG4   | S228P                 | 2015                                     |
| Ixekizumab        | IL-17  | Lilly                    | IgG4   | S228P                 | 2016                                     |
| Reslizumab        | IL-5   | Teva Celltech/UCB        | IgG4   | No mutation           | 2016                                     |
| Emicizumab        | Factor IX | Chugai Roche      | IgG4   | S228P, K196Q, S228P   | 2017                                     |
|                   | Factor X |                          |        | F296Y, E356K, R409K   |                                          |
| Inotuzumab        | CD22   | Pfizer/Celltech/UCB      | IgG4   | S228P                 | 2017                                     |
|                  |        |                          |        |                       |                                          |
| Dupilumab         | IL-4Ra | Regeneron/Sanofi          | IgG4   | S228P                 | 2017                                     |
| Ibitalizumab      | CD4    | TaiMed Tanox             | IgG4   | No mutation           | 2018                                     |
|                   |        |                          |        |                       |                                          |
| Cemiplimab        | PD-1   | Regeneron/Sanofi          | IgG4   | S228P                 | 2018                                     |
| Galcanezumab      | CCR5   | Ligand                   | IgG4   | S228P, F234A, L235A   | 2018                                     |
|                   |        |                          |        |                       |                                          |
| Ravulizumab       | C5     | Alexion                  | IgG2/IgG4 |                       | 2018                                     |
|                   |        |                          | IgG4   |                       | 2018                                     |
| Andecaliximab     | MMP-9  | Gilead                    | IgG4   | S228P                 | III                                      |
|                   |        |                          |        |                       |                                          |
| Tralokinumab      | IL-13  | MedImmune                | IgG4   | No mutation           | III                                      |
|                   |        |                          |        |                       |                                          |
| Cannelizumab      | PD-1   | Jiangsu HengRui          | IgG4   | S228P                 | III                                      |
|                   |        |                          |        |                       |                                          |
| Spartializumab    | PD-1   | Novartis                 | IgG4   | S228P, K447> del      | III                                      |
|                   |        |                          |        |                       |                                          |
| Tiselizumab       | PD-1   | BeGene/Celgene           | IgG4   | S228P, E233P, F234V, L235A, D265A, R409K | III                                      |
|                   |        |                          |        |                       |                                          |
| Mirikizumab       | IL-2   | Lilly                    | IgG4   | S228P, F234A, L235A, K447> del | III                                      |
|                   |        |                          |        |                       |                                          |
| Evinacumab        | ANGPT-L3 | Regeneron/Bayer       | IgG4   | S228P                 | III                                      |
|                   |        |                          |        |                       |                                          |
| CS1001            | PD-L1  | Cstone Pharmaceuticals   | IgG4   | No sequence found     | III                                      |
|                   |        |                          |        |                       |                                          |
| Lebrizumab        | IL-13  | Roche/Genentech          | IgG4   | S228P                 | III                                      |
|                   |        |                          |        |                       |                                          |
| Fasimunab         | NGF    | Regeneron/Sanofi          | IgG4   | S228P                 | III                                      |
|                   |        |                          |        |                       |                                          |
| Crenezumab        | A4B2 and | Roche Genentech        | IgG4   | S228P                 | III                                      |
|                   |        | A4B4                     |        |                       |                                          |
|                   |        |                          |        |                       |                                          |
| Leronimab         | CCR5   | CytoDyn                  | IgG4   | No mutation           | II/III                                   |
|                   |        |                          |        |                       |                                          |
| Dostarlimab       | PD-1   | AnaptysBio/Tesaro, Inc.  | IgG4   | S228P                 | III                                      |
|                   |        |                          |        |                       |                                          |
| Narsoplimab       | MASP-2 | Omeros Corporation       | IgG4   | S228P                 | III                                      |
|                   |        |                          |        |                       |                                          |
| Rozanolizumab     | Fcγn   | UCB                      | IgG4   | S228P                 | III                                      |
|                   |        |                          |        |                       |                                          |
| Olokizumab        | IL-6   | R-Pharm                   | IgG4   | S228P                 | III                                      |
|                   |        |                          |        |                       |                                          |
| Sutimlimab        | CS1    | R-Pharm                   | IgG4   | S228P, L235E          | III                                      |
|                   |        |                          |        |                       |                                          |
| Relationib        | LAG-3  | Medarex                  | IgG4   | S228P, K447> del      | II/III                                   |

Marketed antibodies are in gray cases; “These variants also include a deletion of the lysine at position 447 (and emicizumab also a deletion at position 446), abrogating the heterogeneity at the C-terminal end; “In the anti-factor IX chain: K196Q, S228P, F296Y, E356K, R409K, H435R, L445P, removal of G446 and K447, in the anti-factor X chain: A196Q, S228P, F296Y, R409K, K439E, L445P and removal of G446 and K447.\(^\text{\*}\)
Table 2. Applications related to IgG4 heavy chain modifications addressed in the text

| Family Application Numbera | Main claimb | Main Applicant | Filing year | US patent (if any) expiration date | EU patent (if any) expiration date |
|-----------------------------|-------------|----------------|-------------|-----------------------------------|-----------------------------------|
| **Effector Functions Reduction** | | | | | |
| WO200907142 | Replacement of hinge, CH2, or CH3 with the ones from other subclasses | UNIV COLUMBIA/ BECTON DICKINSON MACROGENICS/ JANSEN CELLTECH | 1989 | REVOKED | |
| WO9428027 | Replacement of L235 and F234 to abrogate effector functions | | | | |
| WO9429351 | Antibodies with reduced effector functions, including an IgG4 with L235E substitutions | | | | |
| WO9526403 | Stabilized (S228P) antibody against E-selectin with weak effector functions (L235A) | | | | |
| WO200042072 | Multiple mutations (main claims are sometimes very broad since neither amino acid substituted nor functions effect are specified) | GENENTECH | 2000 | GRANTED 2020 | GRANTED 2020 |
| WO2000429207/ US20060024298 | At least positions 328, A330R, T299A | XENCOR | 2003 | GRANTED 2024 | |
| WO2005018572 | Aglycosylated antibody (T299A,C) | BIOGEN ALEXION | 2004 | GRANTED 2024 | LAPSED 2024 |
| WO2005007809 | An antibody composed of a portion of IgG2 (until T260) fused to IgG4 to abrogate effector functions with S228P/L235E/P329G | CENTOCOR/ JANSEN | 2011 | GRANTED 2035 | |
| WO2011066501 | Multiple mutation and domain swapping | MERCK and Co | 2011 | GRANTED 2032 | |
| WO2011149999 | Multiple mutation and domain swapping | ROCHE | 2012 | GRANTED 2032 | GRANTED 2032 |
| WO201211087 | Multiple mutation and domain swapping | REGENERON GSK | 2014 | GRANTED 2034 | GRANTED 2034 |
| WO2017079369 | Multiple mutations | XENCOR | 2016 | | |
| **Half-Life Enhancement** | | | | | |
| WO200042072 | N434, T307 substitution | GENENTECH | 2000 | GRANTED 2020 | GRANTED 2020 |
| WO200600919 | M252Y/S254T/T256E mutations and several other mutation combinations | MEDIMMUNE | 2001 | GRANTED 2022 | GRANTED 2021 |
| WO2004035752 | T250Q/E and M428L/F combination | ABBOT | 2003 | GRANTED 2024 | GRANTED 2023 |
| WO2006053301 | IgG4, V308W, V308Y, V308F | XENCOR | 2005 | GRANTED 2026 | GRANTED 2026 |
| WO2007114319 | Isoelectic point modification | CHUGAI | 2007 | | REVOKED |
| WO2009058492 | M252Y/M428L, D259I/V308F, N434S | XENCOR | 2007 | GRANTED 2030 | GRANTED 2028 |
| WO2009086320 | M428L/N434S | XENCOR | 2008 | | |
| WO2010045193 | Several mutations | GENENTECH | 2009 | PENDING | |
| WO2010016180 | Multiple mutation combination | LFB | 2009 | GRANTED 2030 | |
| US2010024454 | M428L/N434s, T307Q/N434s, M428L/V308F, and Q311V/N434s | XENCOR | 2010 | GRANTED 2028 | |
| WO2012016627 | Mutation combination lowering isoelectric point level | XENCOR and Co | 2011 | GRANTED 2031 | |
| WO2013074598 | Mutations already known in combination with mutations in position F243/V264 | MERCK and Co | 2013 | | |
| GB201302878 | H433K/N434F | ARGEXN | 2013 | | |
| WO2013016360 | E258F/V264T | BIDATLA | 2014 | | |
| US20140294812 | Multiple mutation combination | XENCOR | 2014 | | |
| WO2015175874 | Modified at positions 432 and 437 | MEDIMMUNE | 2015 | | |
| WO2017158426 | Mutation combinations at positions 311, 428, 434, 435 and 438. | University of Oslo | 2017 | | |
| WO2018052556 | No mutation claimed (only list of properties), multiple combinations mutations in dependent claims like T256D/Q311V/A378V | Visterra | 2017 | | |
| KR101792191, KR101792205, KR20180113717, KR20180113907, KR20180113904, US2019010343 | Multiple mutation combinations | University of Kookmin | 2017 | | |
| **Downstream Processing** | | | | | |
| WO2006033386 | S228P/L235E/R409K,T,M,L | KYOWA HAKKO KIRIN | 2005 | GRANTED 2025 | GRANTED 2025 |
| WO2008145142 | R409K,T,M,L and hinge without CPC motif | GENMAB | 2008 | | |
| WO2009041613 | G466del/K447del | CHUGAI | 2009 | GRANTED 2029 | LAPSED 2029 |
| WO2010085682 | Multiple mutations reducing the aglycosylation-induced loss of thermal stability | BIOGEN | 2010 | | |
| WO2010067385 | IgG4 stabilized with mutations K370Q/E and optionally R409X/L309X | GENMAB | 2010 | GRANTED 2029 | GRANTED 2029 |
| WO2012022982 | C131 and upper modifications | UCB | 2010 | | |
| US2017029521 | K370 substitution | GENMAB | 2016 | | |
| WO2018065389 | Mutations in the region between Kabat residues 203 and 256 reducing binding to HCP | GSK | 2017 | | |
| WO2018119380 | Substitution at positions 197 or 217/220/(224 or 225) | BMS | 2017 | | |

a The table is organized around parent applications (generally PCT).
b Main claims in the table are the most relevant claims regarding an IgG4 blocking antibody. Note that the precise content of claims and the number of granted patents may differ between the US and Europe.
WO2011066501 application by Centocor. In the latter, it was originally an IgG2-based format with IgG4 point mutations, but Janssen (formerly Centocor) eventually obtained a patent (US10053513B2) concerning a mutated IgG4 (S228P, F234A, L235A, G237A, and P238S) from the same WO2011066501 patent family.

Filed in 2011, Merck and Co.’s application WO2011149999 described sialylated Fc-polypeptides comprising the F243A/V264A mutation combination. Although this mutation combination is related to an IgG1 framework in the example section and in the claims of the granted patent, these mutations could be useful, if they result in a more reduced binding to Fcγ receptors, with an IgG4 framework.

P329G substitution combined with L234A and L235A, in the case of IgG1, or S228P and L235E, in the case of IgG4, has been shown to further decrease effector functions by disrupting a sandwich proline motif within the Fc/Fcγ receptor interface. Surprisingly, IgG1 antibodies bearing these mutations were more silenced than IgG4. Roche started to exploit this technology through an IgG1 framework (cergutuzumab amunaleukin (anti-CEA immunocytokine)). In 2015, Roche was granted a US patent from the family application WO2012130831 for any polypeptide comprising an Fc, including this mutations combination. In 2017, Xencor also claimed the P329G substitution, but they apparently abandoned their application (US2017166655). In 2014, Regeneron applied for claims related to antibodies with reduced effector functions and was granted both US and European patents from the WO2014121087 family. Although the main claim covers any antibody, the protection is narrow since it covers several heavy chain modifications, which are not independent from each other. Surprisingly, the main claim in the European patent specifies that the antibody can bind with higher affinity (although weakened by the mutations) to FcγRIIA than to FcγRIIB. GlaxoSmithKline’s 2016 application WO2017079369 described new IgG2 Fc and IgG4 Fc mutation combinations, especially the E233P/F234A/L235A/G236del/G237A mutation combination for IgG4.

**Patents related to IgG4 half-life modulation**

Half-life is critical for any IgG subclass used as a therapeutic, since its extension could help decrease the amount dosed or the spacing of administrations, for example. Therefore, having a patent portfolio regarding technologies applicable to any kind of polypeptide containing an Fc portion is of the utmost importance. Given the large number of family patents issued in the last decades, a non-exhaustive list of documents will be discussed hereafter.

Genentech was among the first applicants to obtain patents regarding mutations that enhance the binding of human antibodies to FcRn. From the WO200042072 patent family, they obtained three patents, covering the 307, 380, and 434 positions. For example, N434A, N434H or T307A/E380A/N434A improved half-life in animal models with N434A being probably the best variant. However, the patent claiming a mutation at position 434 only covers IgG1 and is deemed to expire in 2020.

Another pioneer, Medimmune (now AstraZeneca) has probably the most interesting patent portfolio. In 2001, from the WO20060919 application family, Medimmune obtained a US patent (US7083784) with broad main claims covering modifications at eight positions 252/254/256/309/311/433/434/436. This patent covers the well-known M252Y/S254T/T256E (YTE) mutation combination, which increases the serum half-life of antibodies in cynomolgus monkeys by nearly 4-fold and increases the half-life of motavizumab up to 100 days in humans. Seven other patents from this family were granted, extending protection to other mutation combinations located or near the Fc/FcRn interface (308/311/385/386/389/428). The European patents are different since the main claim in patent EP1355919B1 protects the single 252 position (Y,F,W,T modifications) and the main claim of the EP2354149B1 patent only protects the 433K/434F/436H combination. This patent family is deemed to expire in 2022.

From the WO2004035752 application family, Abbott was granted both a US and a European patent regarding any antibody comprising the T250Q/E and M428L/F substitutions. Antibodies of different subclasses and different CDRs including the mutation combination T250Q/M428L (QL) showed a near 2-fold IgG half-life enhancement in rhesus macaques.

Xencor has many broad patents from several application families, with both US and European patents for: 1) the N434S, 308W, 308Y and 308F mutations (WO2006053301 family, expires in 2026), 2) the M252Y/M428L and D259I/V308F combination (WO2009058492), and 3) their well-known M428L/N434S (LS) (WO2009086320) mutation combination reported as having 4-fold extended half-life in humans (deemed to expire in 2028). Xencor’s patent portfolio also includes patents covering the T307Q/N434S, M428L/V308F, and Q311V/N434S combinations.

In 2009, LFB was granted a US patent (WO2010106180) regarding 12 mutation combinations enhancing FcRn binding. Surprisingly, several mutations are not located at the Fc/FcRn interface, but some of them, such as prolines at positions 228 and 230 could have distant effects on the FcRn binding site. However, given the differences between the hinge regions of IgG1 and IgG4, it is unclear whether the effect of these mutations would be the same for IgG1 variants. Monnet et al. evaluated the pharmacokinetics of 6 mutants in huFcRn transgenic mice and showed they had half-lives up to 2.8-fold superior to the wild type, but we did not find any in vivo data for humans administered antibodies with these mutations.

In 2009, Genentech filed a patent application (WO2010045193) to protect mutation combinations for positions already described in earlier applications, notably combinations comprising the T307Q/N434A mutations, which results in a 25-day half-life in cynomolgus monkeys. Despite documents questioning novelty, US and European applications have not been abandoned yet.

Other organizations have tried to apply for different Fc mutations enhancing the binding to FcRn, such as Bioatla in 2013 (WO2013163630). They issued several applications with different mutations, for example, the E258F/V427T mutation combination in the (US20180186863) application. However, there is no description of their mutants in their application; the example section only states that the tested variants have a 2- to 80-fold FcRn binding at acidic pH compared to the...
wild type Fc and normal binding at neutral pH. Visterra, University of Oslo (WO2017158426), University of Kookmin (KR101792191 multiple applications) and Macrogenics (US20190010243) also found new variants with enhanced properties. For example, Visterra\(^{40}\) (WO2018052556) designed new variants with enhanced half-life while retaining good effector functions (although this feature is not relevant for a blocking antibody).

Serum half-life could also be decreased by lowering the isoelectric point\(^{41}\) via substitution of positively charged amino acids for ones with negative charges. For example, Chugai was granted a broad European patent (EP2006381) regarding serum half-life modulation through any modification in the variable region that alters the isoelectric point. Alexion, followed by others, opposed the patent and Chugai’s patent was eventually revoked, but Chugai appealed the decision. From the WO2021016227 parent application, Xencor also has one EU (EP3029066) and two US (US697641 and US9605061) patents covering isoelectric point modification in the constant regions, but they are restricted to particular mutation combinations. Other applications, such as recently issued US2018022965, claim mutation combinations modulating half-life, but it is still too soon to know if these claims will be maintained.

**Patents related to stabilization and downstream processing**

Celltech researchers had published in 1992\(^{21}\) that a single S228P substitution (proline being present at this position in all IgG subclasses but IgG4) in the hinge region was sufficient to abolish heterogeneity of human IgG4 antibodies. In 1994, Celltech filed a patent application (WO9526403) for an antibody against E-selectin, comprising the L235E substitution that decreases effector functions and the S228P substitution abolishing the formation of half antibody molecules. However, to our knowledge, this well-known mutation has never been protected as such. Indeed, we did not find any patent containing a broad claim regarding the S228P substitution that would encompass any IgG4 mAbs and yet, this discovery has had a major impact on the development of IgG4-derived antibodies. This mutation is nearly always included in newly developed IgG4 antibodies. For example, the companies that developed tabalumab (anti-BAFF) or relatlimab (anti-LAG-3) had planned to incorporate this mutation since their patents claimed or mentioned S228P as an embodiment of the invention. Although this mutation solved the main problem of IgG4 stability, companies tried to patent alternative solutions.

IgG4 stabilization patents moved from focusing on the hinge to the CH3 in 2006, with a Kyowa application (WO2006033386) addressing the issue of IgG4 aggregation under low pH conditions by mutating R409. In the examples section, they demonstrated that S228P/L235E/R409K variants showed less of a tendency to aggregate at low pH than S228P/L235E variants. Both US and European patents were granted with broad claims regarding mutation combinations for inhibiting IgG4 aggregation. Genmab’s application (WO2008145142) in 2008 described an identical mutation reducing Fab-Arm exchange,\(^{42}\) but an opposition was filed against their European patent, mainly due to the prior Kyowa patent. Genmab’s patent was maintained, although amended, because of its reference to the hinge region in the claim, and because the opposition division made a distinction between the Fab-arm exchange phenomena and the aggregation process. The opposition division acknowledged that the novel and inventive step was Genmab’s demonstration that the 409 mutation alone (hence not necessarily with the S228P) could reduce Fab-arm exchange. Made one year later, Genmab’s application (WO2010063785) for other stabilizing substitutions such as K370Q/E (among others) was granted both US and European patents. In 2016, they also filed an application (US20170292521) for any substitution at position 370, but limited to polypeptides comprising a CXPC or CPXC (not S228P) sequence in the hinge region. We did not find any patent covering the S228P/R409K mutation combination, which could combine both advantageous effects. As a matter of fact, companies had already considered using this format for further developments. For instance, in the example section of application WO2018127586A1, Elsaly Biotech tested this mutation combination for their anti-CD160 mAb. Other companies like Calypso considered this format in the embodiment of their EP2985295A1 application, concerning their anti-MMP9 mAb.

In 2010, Biogen filed a patent application (WO2010085862) for a technology involving aglycosylated, stabilized IgG4 with IgG1 CH3 domains. The idea was to engineer a silent IgG with numerous mutations reducing the aglycosylation-induced loss of thermal stability. They described numerous mutations, for example at positions 297, 299, 307, 309, 399, 409 and 427, as well as valine substitutions (in 240, 262, 264, and 266) to hydrophobic amino acids associated with greater bulk in a hydrophobic patch. However, they withdrew the application.

The S228P is not the only mutation that can both modulate disulfide bond formation and stabilization of the molecule.\(^{43}\) In 2010, UCB filed an application (WO2010022982) and was granted a European patent for any IgG4 with a substitution at position 131 associated with a substitution of any amino acid to cysteine in the upper hinge. They generated numerous IgG4 variants with increased Fab thermal stability and reduced product heterogeneity by modifying the upper hinge in addition with the S228P mutation. However, it is not clear whether these new mutations give a real advantage compared to the sole S228P mutation.

Chugai was granted a European patent from the WO2009041613 family, which covers the reduction of IgG4 C-terminal heterogeneity\(^{44}\) by deleting G446 and K447. According to their sequence listing and their claims, the protection applies to any IgG4 having the S228P mutation. Although Chugai has a patent on C-terminal heterogeneity reduction by deleting both G446 and K447 amino acids, deletion of only K447 is already used in some antibodies, such as blosozumab (anti-sclerostin), dupilumab (Dupixent\(^{*}\), anti-IL-4Ra), emebetuzumab (anti-cMet), ixekizumab (Taltz\(^{\#}\), anti-IL-17). This mutation taken alone does not seem to be covered by any patent. However, according to Chugai patent WO2009041613, its double deletion could further diminish heterogeneity compared to the sole K447 deletion.

According to application WO2018119380 filed by Bristol-Myers Squibb in 2017, S228P-stabilized IgG4 can cause undesirable bio-analytical and bioprocessing behaviors. In the example section, they showed that S228P IgG4 tends to elute
as a double peak on CEX-HPLC, probably caused by two binding conformations in the column. To limit this behavior, mutations have been introduced into the heavy chain. The main claim is the substitution of lysine at position 196 by any other amino acid or substitution at positions 217/220/(224 or 225).

In addition, Tran et al. showed that IgG4 were more prone than IgG1 to bind to individual host cell proteins such as phospholipase B-like 2. In 2017, GlaxoSmithKline filed an application (WO2018065389) covering mutations in the heavy chain (more specifically to the hinge region) that abolish the binding to this protein, and more broadly to host cell proteins. This application is in an early stage, and it’s unlikely the very broad main claim will be granted, since numerous documents have questioned novelty.

**Developments and perspectives**

Although IgG4 have weak effector functions, their binding to FcγRs show that they are not totally devoid of immune activities. Patent applicants have focused on this issue since the early 1990s, and, for example, dozens of patent families describe variants in the CH2 with modified effector functions. However, few applications include *in vitro* comparison with mutations already patented, making it difficult to assess the pros and cons of a particular mutation. For example, few data are available about the effects of a given mutation on the yield and quality of the antibody production. In the context of IgG4 development, freedom to operate regarding reducing effector functions is not of great concern, as there are several technologies not covered by any patent. Only particular mutations or mutation combinations are protected by broad claims (like Roche’s patent from WO2012130831) and the advantage over well-established technologies that are freely available for use remains to be demonstrated.

In contrast, the mutations that increase half-life in animal models the most (YTE, QL and LS) are still covered by patents. At least 3 antibodies containing the YTE mutations, suvratoxumab (MEDI4893, anti-*Staphylococcus aureus* alpha toxin), MEDI8897 (anti-RSV) and MEDI5117 (anti-IL-6), are or were in development, and at least 5 antibodies containing the LS (Xtend technology) mutation are in development or approved, including Alexion’s ravulizumab (Ultomiris*, anti-C5), according to Xencor’s pipeline. Because the patents of Medimmune (now AstraZeneca) and Xencor are broad, it is difficult to assess which mutations can be freely used and which cannot. However, the YTE mutation is deemed to fall in the public domain in 2022, and QL and LS in 2024 and 2028, respectively. Currently, an antibody with the YTE mutation combination seem to have the most notably improved pharmacokinetic properties, as validated in a human trial, although new variants also have good pH6.0/pH7.0 ratio binding. Moreover, YTE mutations seem to reduce effector functions, which could be of interest for pure antagonistic IgG mAbs. As pointed out by Borok et al., further enhancement in half-life may be difficult, and other ways, in addition to FcRn binding modulation, may be required to improve the half-life of an antibody.

In addition to incorporating technologies already freely available, companies could start the development of antibodies comprising mutations deemed to fall in the public domain in the near future. Under the research privilege ("Bolar exemption"), studies and trials that provide data needed for marketing approval do not infringe patents. For example, efgartigimod (human Fc targeting FcRn) comprises YTE in its combination of mutations, but there is no patent infringement as long as the product reaches the market after expiry of patents covering one of those mutations (for example, the EP1355919B1 patent covering the M252Y/W/F/T). Given the patent landscape and scientific purpose, companies can choose to incorporate modifications or not in the constant region of an antibody. In the context of a blocking IgG4 antibody,
it is tempting to design the “perfect” antibody optimized in 3 aspects: reduced effector function, increased half-life, and improved downstream processing. Hence, future IgG4 blocking antibodies may well comprise YTE, silencing mutations, R409K and K447del (Figure 4). This is already the case, in part, for several antibodies containing the K447del and/or F234A/L235A mutations in addition to S228P, such as mirikizumab (anti-IL-23, Phase 3), galcanezumab (Emgality™, anti-CGRP, approved) and embetuzumab (anti-cMet, Phase 2), which were developed by Eli Lilly. It is possible that too many mutations could impair stability, yield or cause immunogenicity. However, several highly mutated IgG4 antibodies (e.g., tislelizumab, emicizumab, ravulizumab) have advanced to Phase 3 or were approved, as have many other antibodies that are of different subclasses. Our data suggest that the number of mutated IgG4 will increase, as will the number of mutations per antibody.

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The authors declare no competing financial interests that are directly relevant to the content of this article.

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