Antibody Isotypes for Tumor Immunotherapy

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Introduction

During the evolution of antibody repertoires, the emergence of highly variable antigen binding regions is accompanied by the development of limited sets of constant heavy chains [1]. These constant heavy chains determine the antibody isotype (in humans IgA, IgD, IgE, IgG, and IgM), which can be further classified for some isotypes (e.g. in humans as IgG1 to IgG4, IgA1 and IgA2) [2, 3]. Many of these antibody isotypes are polymorphic in different populations – creating antibody allotypes. Employment of the limited set of constant heavy chains endows the variable antigen binding regions with particular sets of effector functions. These effector functions are recruited e.g. by interactions with other soluble proteins (e.g. C1q of the complement cascade) or with specialized receptors on immune effector cells. In addition to classical Fc receptors for IgA, IgE and IgG [4–6], a receptor for IgM [7], a common receptor for IgA and IgM (FcαR) [8], and a family of Fc receptor-like molecules (FcRL) have been distinguished [9].

Antibodies of various isotypes and their respective receptors on effector cells constitute complex networks, which link the adaptive and innate immune systems. These networks are partially conserved between different species, even though also many critical differences have evolved [10]. These species specific characteristics are important in determining the pharmacokinetic properties of the respective antibodies, while the recruitment of effector functions, antibody isotypes also display considerable differences in their pharmacokinetic properties (see below), which may determine their suitability for particular clinical applications.

All human isotypes consist of heterodimers of heavy and light chains, which are typically paired by differently arranged disulfide bonds (fig. 1). Both light and heavy chains contain one variable domain (V(L) and v(H), respectively). Light chains contain one constant domain (C(L)), while the number of constant domains for heavy chains is four for IgE and IgM (cH1–4) and three for all other isotypes (cH1–3). The different heavy chains govern functional and innate immune systems.

References

[1]... [10]...
chains is four for IgE and IgM (ch1–4) and three for all other iso-
domains (chL), while the number of constant domains for heavy
main (vL and vH, respectively). Light chains contain one constant
domain (vL), which are typically paired by differently arranged disulfide
bonds (fig. 1). Both light and heavy chains contain one variable do-
main (vl and vh, respectively). Light chains are usually attached to
interchain disulfide bridges (fig. 1), which are often more exposed (e.g. in
IgA).

The number of N-glycosylation sites for each antibody isotype
was described previously [11, 12]. IgG1 antibodies are almost
completely N-glycosylated, whereas IgG2, IgG3, and IgG4 contain
only one N-glycosylation site (N297, which is rather buried in the
protein structure), other isotypes, such as IgE antibodies, only occur
as mono- or dimeric forms. In contrast to the other isotypes, IgM
antibodies solely exist as multimers, primarily as pentamers. N-glycosylation sites are depicted by ▶. O-glycosylation
sites are marked by ▼.

### Antibody Isotypes for Tumor Immunotherapy

**IgG1**

Almost 30 years ago Brüggemann et al. [15] analyzed different human antibody isotypes and subclasses for their potential to activate
complement to mediate complement-dependent cytotoxicity (CDC) and to recruit effector cells for antibody-dependent cellular
cytotoxicity (ADCC) against human target cells. Based on their observations, IgG1 appeared as the most promising antibody isotype
tumor immunotherapy. In addition to these and many other in
vitro results, human IgG1 antibodies were also effective in mouse
models, since human IgG1 binds well to activating murine Fcγ
receptors on effectors cells. Apart from its promising effector func-
tions, IgG1 antibodies were demonstrated to interact well with the
human, but also with the murine neonatal Fc receptor (FcRNI).

Binding to FcRNI protects IgG1 molecules from degradation and
thereby extends their serum half-life compared to non-FcRn-bind-
ing isotypes [16]. Additionally, human IgG1 antibodies

![Fig. 1. Schematic representation of antibody isotypes and subclasses.](image-url)
columns), and development of specific storage formulations for increased stability. These characteristics allowed the establishment of Good Manufacturing Practice (GMP) to obtain optimal therapeutic agents. From an economical point of view, these industrial procedures contributed to the prominent role of IgG1 antibodies in the clinic [17]. Importantly, human IgG1 antibodies are often used as backbone for Fc engineering strategies which aim to further improve effector functions, stability, or pharmacokinetic properties of therapeutic antibodies [18, 19].

From the initial clinical studies, it took almost 10 years until rituximab as the first therapeutic antibody for oncological treatment received FDA approval in 1997. Rituximab is a chimeric CD20-targeting antibody, which paved the way for the development of so far almost 20 chimeric, humanized or human IgG1 monoclonal antibodies being approved for different oncological indications during the following two decades (table 1). When ipilimumab was approved for the treatment of metastatic melanoma in 2011, the era for the so-called immune checkpoint blockers began [20]. However, also antibodies against these types of antigens may prove effector functions, stability, or pharmacokinetic properties of therapeutic antibodies [18, 19].

**Table 1. List of approved monoclonal antibodies for tumor therapy and their isotype/subclass**

| Antibody        | Tradename     | Target  | Format  | Indication               | Year of Approval |
|-----------------|---------------|---------|---------|--------------------------|-----------------|
| Rituximab       | MabThera      | CD20    | chimeric IgG1 | non-Hodgkin’s lymphoma     | 1997            |
| Trastuzumab      | Herceptin     | HER2    | humanized IgG1 | breast cancer              | 1998            |
| Alectumumab     | Campath       | CD32    | humanized IgG1 | chronic lymphocytic leukemia | 2001            |
| Bevacizumab     | Avastin       | VEGF    | chimeric IgG1 | colorectal cancer          | 2004            |
| Cetuximab       | Erbitux       | EGFR    | chimeric IgG1 | colorectal cancer          | 2004            |
| Ofatumumab      | Arzerra       | CD20    | human IgG1 | chronic lymphocytic leukemia | 2009            |
| Ipilimumab      | Yervoy        | CTLA-4  | human IgG1 | metastatic melanoma        | 2011            |
| Pertuzumab      | Perjeta       | HER2    | humanized IgG1 | breast cancer              | 2012            |
| Obinutuzumab    | Gazyva        | CD20    | humanized IgG1 | chronic lymphocytic leukemia | 2013            |
| Ramucirumab     | Carameta      | VEGFR2  | human IgG1 | gastric cancer             | 2014            |
| Daratumumab     | Darzalex      | CD38    | human IgG1 | multiple myeloma           | 2015            |
| Elotuxumab      | Empliciti     | CS1     | humanized IgG1 | multiple myeloma           | 2015            |
| Dinutuximab     | Unituxin      | GD2     | chimeric IgG1 | neuroblastoma              | 2015            |
| Necitumumab     | Portrazza     | EGFR    | human IgG1 | non-small cell lung cancer | 2015            |
| Olaratumab      | Lartruvo      | PDGFRα  | human IgG1 | soft tissue sarcoma        | 2016            |
| Atezolizumab    | Tecemix       | PD-L1   | human IgG1 | urothelial carcinoma       | 2016            |
| Panitumumab     | Vectibx       | EGFR    | human IgG2 | colorectal cancer          | 2006            |
| Nivolumab       | Opdivo        | PD1     | human IgG4 | melanoma                   | 2014            |
| Pembrolizumab   | Keytruda      | PD1     | humanized IgG4 | melanoma                   | 2014            |

HER2 = Human epidermal growth factor receptor 2; VEGF = vascular endothelial growth factor; EGFR = epidermal growth factor receptor; CTLA-4 = cytotoxic T-lymphocyte-associated protein 4; VEGFR2 = vascular endothelial growth factor receptor 2; GD2 = ganglioside G2; PDGFRα = platelet-derived growth factor receptor α; PD-L1 = programmed death-ligand 1; PD-1 = programmed cell death protein 1.

**IgG2**

The human IgG2 isotype is predominantly selected when neutralization of antigens (e.g. soluble cytokines) or inhibition of receptor-ligand interactions are targeted, while Fc-mediated effector functions (e.g. ADCC and CDC) appear undesired. Currently, the epidermal growth factor receptor (EGFR) antibody panitumumab is the only approved IgG2 antibody for cancer immunotherapy. However, with the emergence of immune checkpoint blockade as therapeutic principle, many more IgG2 antibodies are currently in clinical trials – with some of them short before approval [21].

IgG2 has limited C1q binding activity, but can trigger CDC at high target antigen and high antibody concentrations [22]. Furthermore, human IgG2 is only capable of binding to FcRIIa (CD32a), but not to other activating Fcγ receptors. Importantly, human IgG2 binding to FcRIIa is significantly affected by a functional single nucleotide polymorphism (SNP) in this receptor, leading to a single amino acid change at position 131 (histidine or arginine). This polymorphism impacts the functional activity of human IgG2 antibodies. Thus, the high affinity variant (131-His) of FcRIIa was demonstrated to induce anti-CD3-IgG2-mediated T-cell activation and proliferation after cross-linking via myeloid cell engagement [23]. Furthermore, the human IgG2 EGFR antibody panitumumab was demonstrated to mediate ADCC by myeloid effector cells against EGFR-positive tumor cells [24]. A randomized phase III study showed that panitumumab was non-inferior to the EGFR IgG1 antibody cetuximab in regard to overall survival [25]. Further studies need to address the impact of the FcRIIa polymorphism on the efficacy and potential toxicity of human IgG2 antibodies in cancer immunotherapy across different target antigens.

Recently, White et al. [26] analyzed the activity of immune stimulatory CD40 monoclonal antibodies of IgG2 isotype. In contrast to other IgG subclasses, IgG2 antibodies displayed Fc receptor binding than their IgG1 counterparts. Additionally, IgG2 antibodies exhibited strong Fc-mediated effector functions in vitro. For example, the human IgG2 antibody ipilimumab was demonstrated to mediate ADCC by myeloid effector cells against EGFR-positive tumor cells [24]. A randomized phase III study showed that panitumumab was non-inferior to the EGFR IgG1 antibody cetuximab in regard to overall survival [25]. Further studies need to address the impact of the FcRIIa polymorphism on the efficacy and potential toxicity of human IgG2 antibodies in cancer immunotherapy across different target antigens.
Antibody Isotypes for Tumor Immunotherapy

**IgG4**

IgG4 is commonly regarded as a non-activating antibody isotype in immunotherapy. Experiments performed by Brüggemann et al. [15] displayed the low activity of IgG4 to induce CDC as well as ADCC. Nevertheless, antibodies of the IgG4 subclass bind to activating Fcγ receptors – in particular to FcγRI (CD64). While the affinity of IgG4 to FcγRI was similar to that of IgG1 and IgG3, the affinities for FcγRII (a/b/c) and FcγRIIIa (CD16a) were lower [11]. Thus, a human IgG4 antibody against CD20 was able to induce ADCC against human B cells by engaging mononuclear effector cells [13].

In contrast to other antibody isoforms, the biology of IgG4 is characterized by a unique process called Fab arm exchange [33]. During this process half-molecules are formed – consisting of one heavy and one light chain, which are able to recombine with other half-molecules. Thereby, natural monovalent bispecific antibodies are formed, which may explain the biology and pathophysiology of IgG4 in health and disease [34]. This Fab arm exchange was also documented to occur with a therapeutic IgG4 antibody, which exchanged Fab arms with natural IgG4 antibodies from the serum [35]. Fc engineering studies demonstrated that this Fab arm exchange can be prevented by a serine 228 proline (S228P) mutation, which stabilizes the IgG4 hinge and which is employed in most currently approved or evaluated therapeutic IgG4 antibodies. For example, two monoclonal IgG4 antibodies (pembrolizumab and nivolumab) were recently approved for immune checkpoint blockade. Both of these programmed cell death protein 1 (PD-1) monoclonal antibodies are mediating their therapeutic efficacy by blocking PD-1 and programmed death-ligand 1/2 (PD-L1/PD-L2) interactions, leading to increased anti-tumor T-cell responses [20]. Since PD-1 antibodies, in contrast to PD-L1 antibodies, were shown to mediate their efficacy independently from Fcγ receptors [36], IgG4 appeared to be a reasonable IgG subclass for this therapeutic strategy.

While uncontrolled Fab arm exchange constituted a problem for IgG4 antibodies as therapeutic agents, it also opened up a new way to design bispecific antibodies. Introduction of two different matching mutations (K409R/F405L) into the Fc backbone of two parental IgG1 antibodies targeting different antigens resulted in the controlled formation of bispecific antibodies. These mutations are located in the CH3 domains and are responsible for the efficient and directed Fab arm exchange between the two parental IgG1 antibodies [37].

**IgA Antibodies**

Despite the success of therapeutic antibodies of the IgG isotype, research on alternative antibody isotypes for tumor immunotherapy continues. Antibodies of the IgA isotype were particularly effective in recruiting myeloid effector cells (monocytes/macrophages and granulocytes) for ADCC [38–40] while natural killer cells (NK cells) are not activated by IgA antibodies. Physiologically,

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**Antibody Isotypes for Tumor Immunotherapy**

| Antibody | Isotype | Target | Format | Indication | Year of Approval |
|----------|---------|--------|--------|-----------|-----------------|
| Nivolumab | Opdivo | PD1 human IgG4 | melanoma | 2014 |
| Olaratumab | Lartruvo | PDGFRα human IgG1 | soft tissue sarcoma | 2016 |
| Necitumumab | Portrazza | EGFR human IgG1 | non-small cell lung cancer | 2015 |
| Pertuzumab | Perjeta | HER2 humanized IgG1 | breast cancer | 2012 |
| Ofatumumab | Arzerra | CD20 human IgG1 | chronic lymphocytic leukemia | 2009 |
| Cetuximab | Erbitux | EGFR chimeric IgG1 | colorectal cancer | 2004 |
| Alemtuzumab | Campath | CD52 humanized IgG1 | chronic lymphocytic leukemia | 2001 |
| Rituximab | MabThera | CD20 chimeric IgG1 | non-Hodgkin’s lymphoma | 1997 |

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**IgG3**

The interest in IgG3 as a therapeutic antibody isotype was stimulated following observations that an anti-HIV-specific IgG3 response was correlated with improved disease control and longer survival [28]. Antibodies of the IgG3 subclass were long known to exhibit strong Fc-mediated effector functions in vitro. For example, hapten-directed IgG3 antibodies were able to induce ADCC as well as CDC very effectively [15]. Interestingly, IgG3 antibodies showed an increased ability to induce CIq binding and were more effective in Fc receptor binding than their IgG1 counterparts. Detailed analyses of IgG antibodies and their ability to activate the complement system demonstrated the superior efficacy of complement activation by the IgG3 isotype, particularly when epitope densities were lower [22, 29]. Furthermore an IgG3 version of cetuximab was able to activate complement in contrast to the parental IgG1 antibody – with CD55 being the main regulator of IgG3-induced complement activation [30].

Despite these promising biological activities, no IgG3 antibody has entered the clinic so far. This is probably explained by manufacturing issues: IgG3 antibodies cannot be purified by protein A chromatography, have a tendency to form aggregates, and carry O-linked glycans in their extended hinge region. Furthermore, most IgG3 allotypes are not recycled by FcRn – leading to a serum half-life of approximately 7 days for IgG3, compared to 21 days for other IgG isotypes. However, Stapleton et al. [31] characterized an IgG3 allotypic variant, which contains an amino acid exchange at position 435 (arginine to histidine), which lead to effective FcRn transport and increased serum half-life.

Recently, Bournezos et al. [32] generated an engineered bispecific antibody for broad HIV neutralization. For improvement of the neutralization efficacy, the hinge region of an IgG3 was grafted onto the backbone of an IgG1 antibody. To further increase the flexibility of this molecule, all but the lowest two cysteines in the hinge region were exchanged by serine, thereby leading to an open conformation of this new molecule. In comparison to the parental unmodified antibody, the IgG3 open-hinge molecule was significantly more effective in HIV-1 neutralization. Further studies need to address the impact of these hinge engineering approaches for the generation of novel antibodies, especially under the aspect of neutralization or Fc-mediated effector functions.
IgA antibodies are the first line of immune defense against pathogens at mucosal surfaces [41]. Two different isotypes – IgA1 and IgA2 – are characterized in men (fig. 1), which share many key characteristics with IgG antibodies. However, IgA antibodies differ in the number of glycosylation sites, the length of their hinge regions, and the number and position of disulfide bridges within the molecules (fig. 1). Furthermore, IgA can form dimeric and secretory isoforms. Dimeric IgA is produced by mucosal plasma cells by connecting the tailpiece cysteines of two monomeric IgA molecules covalently with the so-called joining (J) chain. Dimeric IgA can then bind to the polymeric immunoglobulin receptor (pIgR) on the basolateral surface of mucosal epithelial cells. Bound dimeric IgA is then transported by transcytosis through epithelial cells to the luminal site of mucosal surfaces, where secretory IgA is released by proteolytic cleavage of pIgR. Thus, secretory IgA consists of J-chain-connected dimeric IgA and the associated secretory component (SC), which is an extracellular part of the pIgR [41].

In addition to their potent activity in recruiting myeloid effector cells for ADCC, IgA antibodies against CD20 triggered CDC against lymphoma cells [40]. Fab-mediated effector functions of monomeric IgA antibodies were similar to IgG antibodies, but were enhanced when dimeric IgA antibodies were compared with monomeric monoclonics [38]. In vivo experiments in different xenogenic and syngeneic human FcRIR transgenic mouse models demonstrated significant anti-tumor activity of an EGFR-directed human IgA2 antibody, although its serum half-life was short [42]. Additional studies suggested that the short serum half-life was triggered by asialo-glycoprotein receptor (ASGPR)-mediated elimination of the therapeutic antibody in the liver. To overcome this limitation, an Fc-engineered IgA2 molecule was developed, which demonstrated improved stability and a longer serum half-life translating into higher in vivo efficacy of the engineered compared to the parental IgA molecule [43]. Also IgA antibodies against human epidermal growth factor receptor 2/neu (HER2/neu) or CD20 demonstrated in vitro and in vivo efficacy [40, 44]. Despite these promising preclinical activities, IgA antibodies have currently not been introduced into clinical studies.

IgM

Like other immunoglobulin isotypes, monomeric IgM molecules are composed of heavy and light chain heterodimers, which are covalently connected via disulfide bridges. However, serum IgM antibodies are predominantly pentameric molecules which are interconnected by the J-chain (fig. 1). As described for IgA, binding of pentameric IgM to the pIgR and its transport through epithelial cells leads to the formation of secretory IgM at mucosal surfaces. Nevertheless, IgM antibodies are mostly found as pentameric IgM in the circulation. IgM is produced by either B1 lymphocytes as ‘natural antibodies’ without being exposed to antigenic stimuli or by B2 lymphocytes after immunization as a defense mechanism against invading pathogens. Natural antibodies recognize a variety of pathogenic molecules such as nucleic acids, lipids and proteins, which are phylogenetically conserved and which were not encountered previously. Thus, IgM antibodies close the gap that arises after the first contact of potential pathogens and the first adaptive response of the immune system [, 45]. IgM antibodies and their pentameric structure are ideal activators of the complement system. Recently, Michaelis and colleagues [46] showed not only that serum and pentameric IgM are potent CDC inducers but also that secretory IgM, which is transported via transcytosis to mucosal tissues, induced comparable levels of CDC. Furthermore, IgM-induced effector functions do not seem to be influenced by the association of the molecule with either J-chain or SC. The Fc receptor for IgM is called FcµR and is found exclusively on lymphocytes (B, T and NK cells) in men and only on B cells in mice. FcµR shows a unique immunoreceptor tyrosine-based inhibition motif (ITIM) and immunoreceptor tyrosine-based activation motif (ITAM) pattern suggesting that the receptor may have the ability to act as a dual signal transmitter [7]. However, rather little is known about the IgM FcR and its role in immunity.

Only few tumor-directed IgM antibodies have been moved into clinical trials. For example, a human IgM antibody (called PAT-SM6) is directed against a tumor-specific variant of the glucose-regulated protein 78 (GPR78) / heat shock 70 kDa protein 5 (HSPA5). This antibody-mediated induction of apoptosis and, to a lower extent, CDC against multiple myeloma cells and was evaluated in a phase I trial regarding safety and tolerability in patients with relapsed or refractory multiple myeloma. PAT-SM6 demonstrated good tolerability and modest activity in this phase I study [47]. Another monoclonal IgM antibody, which was introduced into a phase I trial, was MORAb-028. This GD2 antibody was administered intra-tumorally in patients with metastatic melanoma (NCT01123304).

IgE

IgE antibodies are commonly associated with allergic or parasitic diseases. Monomeric IgE antibodies bind with high affinity to FcεRI, which is predominantly expressed on mast cells, basophils, monocytes, and macrophages making them favorable effector cell populations for IgE-mediated tumor growth control. Upon antigen binding, FcεRI is cross-linked and triggers degranulation and mediator release to induce acute allergic reactions or tumor cell killing. Complexed or target-bound IgE can bind to the low-affinity IgE receptor FcεRII (CD23), which is expressed on dendritic cells, macrophages, and eosinophils. Unlike for IgG antibodies, inhibitory Fc receptors have not been described for IgE. Compared with IgG1, IgE has a short serum half-life of 1.5 days, whereas in tissues the half-life is prolonged to approximately 2 weeks due to binding to Fce receptor-expressing immune cells (reviewed in [48]).

Bridging allergy and cancer IgE antibodies were demonstrated to contribute to the natural immune surveillance of tumors. For example, elevated IgE levels were observed in serum samples of pancreatic cancer patients. Some of these IgE antibodies were found to be tumor antigen-specific and induced ADCC in panec-
IgE antibodies are commonly associated with allergic or para-
immune diseases. Monomeric IgE antibodies bind with high affinity to
IgE receptors FcεRI (CD23), which is expressed on dendritic cells,
diater release to induce acute allergic reactions or tumor cell kill
in vivo. For example, Karagiannis and colleagues [50] investigated
this goal.

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Antibodies are proteins that are produced by the immune system in response to foreign antigens. There are several types of antibodies, each with different properties and functions. In the context of tumor immunotherapy, the choice of antibody isotype can be critical for the success of the treatment.

**References**

1. Cooper MD: The early history of B cells. Nat Rev Immunol 2015;15:191–197.
2. Jeffers R: Isotype and glycoform selection for antibody therapeutics. Arch Biochem Biophys 2012;526:159–366.
3. Vidalgonzalez G, Dikkers G, Baepens T: IgG subclasses and allotopes: from structure to effector functions. Front Immunol 2014;5:520.
4. Monteiro BC, Van De Winkel JG: IgA Fc receptors. Annu Rev Immunol 2003;21:177–204.
5. Nimmerjahn F, Raveche JY: Divergent immunoglobulin G subclass activity through selective Fc receptor binding. Science 2003;305:1510–1512.
6. Nimmerjahn F, Kinet JP: Fc receptors. Annu Rev Immunol 1991;9:457–492.
7. Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang DW, Jones D, Nishioka N, Miyawaki T, Benoit LF, Sanders SK, Honjo K: The long elusivity IgM Fc receptor, FμR. J Clin Immunol 2014;34(suppl 1):S35–45.
8. Kinch JP, Louna P: FcγR Jr. Single member or first born in the family? Nat Immunol 2000;1:371–372.
9. Maltais LJ, Lovering RC, Tararin AV, Coloman M, Raveche JY, Dallá-Ferro R, Burrows PD, Cooper MD, Davis RS: New nomenclature for Fc receptor-like molecules. Nat Immunol 2006;7:431–432.
10. Akula S, Mohammedamin S, Hellman L: Fc receptors for immunoglobulins and their appearance during vertebrate evolution. PLoS One 2010;5:e9603.
11. Bruns P: Properties of mouse and human IgG receptors and their contribution to disease models. Blood 2012;119:5640–5649.
12. Overdijk MB, Verpooren S, Ortiz Biausse A, Vink T, Leusen JH, Bleeker WK, Parren PW: Crossstalk between human IgG isotypes and murine effector cells. J Immunol 2012;188:3430–3438.
13. Hogarth PM, Anania KC, Wines RD: The FcγR of humans and non-human primates and their interaction with IgG: Implications for induction of inflammation, resistance to infection and the use of therapeutic monoclonal antibodies. Curr Top Microbiol Immunol 2014;382:321–352.
14. Beers SA, Glennie MJ, White AL: Influence of immunoglobulin isotype on therapeutic antibody function. Blood 2016;127:1097–1101.
15. Kretschmer A, Valerius T, Klausz K, Otte A, Lutz S, Kretschmer A, Valerius T, Klausz K, Otte A, Gramatikis M, Pfeiff M, Kehrer C: An Fc double-engineered CD20 antibody with enhanced CDC and ADCC activity. Transfus Med Hemother 2017;44:430–326.
16. Hogarth PM, Anania KC, Wines RD: The FcγR of humans and non-human primates and their interaction with IgG: Implications for induction of inflammation, resistance to infection and the use of therapeutic monoclonal antibodies. Curr Top Microbiol Immunol 2014;382:321–352.
17. Hogarth PM, Anania KC, Wines RD: The FcγR of humans and non-human primates and their interaction with IgG: Implications for induction of inflammation, resistance to infection and the use of therapeutic monoclonal antibodies. Curr Top Microbiol Immunol 2014;382:321–352.
18. Wurm FM: Production of recombinant protein therapeutics in cultured mammalian cells. Nat Biotechnol 2004;22:1393–1398.
19. Carpenter P: Potent antibody therapeutics by design. Nat Rev Immunol 2006;6:343–357.
20. Wirt T, Roskopf S, Böser T, Eichholz K, Kahrs A, Lutz S, Kretschmer A, Valerius T, Klausz K, Otte A, Gramatikis M, Pfeiff M, Kehrer C: An Fc double-engineered CD20 antibody with enhanced CDC and ADCC activity. Transfus Med Hemother 2017;44:430–326.
21. Reichert JM: Antibodies to watch in 2017. MAbs 2017;9:167–181.
22. Garred P, Michelsen TE, Aase A: The IgG subclass pattern of complement activation depends on epitope density and antibody and complement concentration. Scand J Immunol 1989;30:379–382.
23. von Carmen PW, Warmerdam P, Boeck LC, Aris J, Westerdal NA, Vlag A, Capel J, Aarden LA, van de Winkel JG: On the interaction of IgG subclasses with the low affinity FcγRII (CD16) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG. J Clin Invest 1992;90:1537–1546.
24. Schneider-Merck T, Lammerts van Bueren J, Berger S, Roosen K, van Borkel PH, Derer S, Beyer T, Lohse S, Bleeker WK, Pfeiff M, Parren PW, van de Winkel JG, Valerius T, Dechant M: Human IgG2 antibodies against epidermal growth factor receptor effectively trigger antibody-dependent cellular cytotoxicity but, in contrast to IgG3, only by cells of myeloid lineage. J Immunol 2010;184:512–520.
25. Price TJ, Perrote M, Kan TW, Li J, Cascina S, Ruff P, Suroosh AA, Thomas A, Tuulidun S, Zhang K, Mura-gapan S, Siddhu R: Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECT-CT): A randomised, multicentre, open-label, non-inferiority phase 3 study. Lancet Oncol 2014;15:569–579.
26. White AL, Chan HT, French RR, Williams J, Mockridge CI, Bughlanan A, Pentfold CA, Booth SJ, Dodbby A, Polak ME, Potter EA, Arden-Jones MR, Verbeek JS, Johnson PW, Al-Shamkhani A, Cragg MS, Beers SA, Glennie M: Confirmation of the human immunoglobulin G2 hinge imparts superagonistic properties to immunostimulatory anticancer antibodies. Cancer Cell 2015;27:138–148.
27 Konitzer JD, Sieron A, Wacker A, Enenkel B: Refactoring rituximab into human IgG2 and IgG4 isotypes dramatically improves apoptosis induction in vitro. PLoS One 2015;10:e0145633.

28 Banerjee K, Klaus PJ, Sanders RW, Pereryza F, Michael E, Lu M, Walko BD, Moore JP: IgG subclass profiles in infected HIV type 1 controllers and chronic progressors and in uninfected recipients of Env vaccines. AIDS Res Hum Retroviruses 2010;26:445–458.

29 Rosner T, Dreyer S, Kellner C, Dechant M, Lohse S, Vidarsson G, Peipp M, Valerius T: An IgG3 switch variant of rituximab mediates enhanced complement-dependent cytotoxicity against tumour cells with low CD20 expression levels. Br J Haematol 2013;163:282–286.

30 Rosner T, Lohse S, Peipp M, Valerius T, Derer S: Epidermal growth factor receptor targeting IgG3 triggers complement-mediated lysis of decay accelerating factor expressing tumour cells through the alternative pathway amplification loop. J Immunol 2014;193:1485–1495.

31 Stapleton NM, Andersen JT, Stenmark AM, Bjarne Rubin SL, Prendergast GF, Gertruds J, Zhao Y, Kinjo M, Sandlie I, de Haas M, Jonsdottir I, van der Schoot CR, Vidarsson G: Competition for FcRN-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. Nat Commun 2011;2:599.

32 Bourouzaa S, Gazumyan A, Seaman MS, Nussenzen MC, Ravetch JV: Bicpecific anti-HIV-1 antibodies with enhanced breadth and potency. Cell 2016;165:1594–1602.

33 Schuurman J, Grays UF, Labrin I, Ruuls S, Parren PW: Opening the door to innovation. MAbs 2014;6:1594–1620.

34 van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, den Bleker TH, Wirgin L, Yin Y, Aarden LA, De Baets ME, van de Winkel JG, Aalberse RC, Parren PW: Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science 2007;317:1534–1537.

35 Labrijn AF, Buissje AO, van den Bremer ET, Verwijlen, gen AT, Bleker WK, Thorpe SJ, Kellner J, Valerius T, Derer S, Rosenwald A, Meulenbroek LK, Jansen JH, Neder- mond E, Kortschaker A, Klaus K, Mogensdinger U, Derer S, Kellner C, Schewe D, Sondermann P, Tiswi S, Kolarich D, Peipp M, Liew LP, Boon L, Burke D, Biele J, Bialek J, Van de Hulst F, Aalberse RC, Parren PW: Therapeutic IgG4 antibodies engage in Fab-arm exchange with endogenous human IgG4 in vivo. Nat Biotechnol 2009;27:767–771.

36 Dahan R, Segal E, Engelhardt J, Selby M, Korman AI, Ravetch JV: FcRys modulate the anti-tumor activity of antibodies targeting the PD-1/PD-L1 axis. Cancer Cell 2015;28:285–295.

37 Labrin IA, Meesters JJ, de Groot HE, van den Bremer ET, Nauts J, van Kampen MD, Struikmans E, Verploegen S, Koudur A, Groen M, van Beek JH, van den Winkel JG, Schuurman J, Parren PW: Efficient generation of stable bispecific IgG1 by controlled Fab-arm exchange. Proc Natl Acad Sci U S A 2013;110:5154–5159.

38 Dechant M, Beyer T, Schneider-Merck T, Weißen W, Peipp M, van de Winkel JG, Valerius T: Effector mechanisms of recombinant IgA antibodies against epidermal growth factor receptor. J Immunol 2007;179:2936–2943.

39 Huls G, Heijnen IAFM, Cmueto G, van der Linden J, Boel E, van de Winkel JG, Lu M, van der Zwaluw J, Ueberberg T: Antitumor immune effector mechanisms recruited by phage display-derived fully human IgG1 and IgA1 monoclonal antibodies. Cancer Res 1999;59:5778–5784.

40 Lohse S, Loew S, Kortschaker A, Jansen JHM, Meyer S, Ten Broeke T, Rosner T, Dechant M, Derer S, Klauss K, Kellner C, Schwabehc R, French RR, Tipton TRW, Cragg MS, Dohme MM, Peipp M, Leunen JF, van de Winkel JG, Valerius T: Effector mechanisms of IgA antibodies against CD20 include recruitment of myeloid cells for anti-body-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity. Br J Haematol 2017;179:2832–2843.

41 Woof JM, Russell MW: Structure and function relationships in IgA. Mucosal Immunol 2011;4:590–597.

42 Borus P, Lahou S, Nedermond M, Jansen JH, van Tetering G, Dechant M, Peipp M, Royle L, Liu P, Boon L, van Roon P, Nedermond M, Bleeker WK, Parren PW, van de Winkel JG, Valerius T, Leunen JH: IgA FGR antibodies mediate tumour killing in vivo. EMBO Mol Med 2013;5:1213–1226.

43 Labrijn AF, Meyer S, Meulenbroek LK, Jansen JH, Nedermond M, Kortschaker A, Klaus K, Mogensdinger U, Derer S, Rosner T, Kellner C, Liesche D, Sondermann P, Tiswi S, Kolarich D, Peipp M, Leunen JH, Valerius T: An anti-EGFR IgA that shows improved pharmacokinetics and myeloid effector cell engagement in vivo. Cancer Res 2016;76:403–417.

44 Rouwendal JG, van der Lee MM, Meyer S, Reinders KR, Schutten J, de Roo G, Egging DF, Leunen JH, Borus P, Wubler M, Verheijden GF, Dokter WH, Timmers M, Utbik R: A comparison of anti-HER2 IgA and IgG1 in vivo efficacy is facilitated by higl N-glycan sialylation of the IgA. MAbs 2018;10:74–86.

45 Gromatova C, Vas J, Silverman GA: Protective roles of natural IgM antibodies. Front Immunol 2012;3:66.

46 Michaelen TE, Emilsen S, Sandin RH, Granerud BK, Boe H, Oile S, Sandlie J: Human secretory IgA antibodies activate human complement and offer protection at mucosal surface. Scand J Immunol 2017;85:43–50.

47 Rosner T, Dreyer S, Naviaux RC, Czarnik F, Valerius T: Induction of stable bispecific IgG1 by controlled Fab-arm exchange. Proc Natl Acad Sci U S A 2013;110:5145–5150.

48 Raatsch L, Duehl JJ, Castro IC, Dubilevic V, Chatterjee M, Knop S, Hensel F, Rosenwald A, Einsele H, Topp MS, Brandlein S: GRP78-directed immunotherapy in relapsed or refractory multiple myeloma – results from a phase 1 trial with the monoclonal immunoglobulin M antibody PAT. SMJ Haematologica 2015;100:377–384.

49 Joseph DH, Spicer JP, Karagiannis P, Gould HJ, Karagiannis SN: IgM immunotherapy: a novel concept with promise for the treatment of cancer. MAbs 2014;6:54–72.

50 Fu SK, Pierre J, Smith-Norowitz TA, Hagler M, Bowse W, Pincus MR, Mueller CM, Zenilman ME, Bluth MH: Immunoglobulin E antibodies from pancreatic cancer patients mediate antibody-dependent cell-mediated cytotoxicity against pancreatic cancer cells. Clin Exp Immunol 2008;153:401–409.

51 Karagiannis SN, Brache MG, Hunt J, McClaskley N, Beavis RL, Beavis AJ, Fear DJ, Thompson RG, East N, Burke F, Moore RJ, Dombrowicz D, Balkwill FR, Gould HJ: IgG1 antibody-dependent immunotherapy of solid tumors: cytotoxic and phagocytic mechanisms of eradication of ovarian cancer cells. J Immunol 2007;179:2832–2843.

52 Leus J, Daniels-Wells TR, Penichet ML: IgM immunotherapy against cancer. Curr Top Microbiol Immunol 2015;388:109–149.