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Title: Harnessing the immune system via FcγR function in immune therapy: A pathway to next-gen mAbs.

Running title: *FcγR and mAb immune Therapy*

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Abstract

The human FcγRs interact with antigen-complexed IgG ligands to both activate and modulate a powerful network of inflammatory host-protective effector functions that are key to the normal physiology of immune resistance to pathogens. More than 100 therapeutic monoclonal antibodies (mAbs) are approved or in late stage clinical trials, many of which harness the potent FcγR-mediated effector systems to varying degrees. This is most evident for antibodies targeting cancer cells inducing antibody-dependent killing or phagocytosis but is also true to some degree for the mAbs that neutralise or remove small macromolecules such as cytokines or other immunoglobulins. The use of mAb therapeutics has also revealed a "scaffolding" role for FcγR which, in different contexts, may either underpin the therapeutic mAb action such as immune agonism or may trigger catastrophic adverse effects. The still unmet therapeutic need in many cancers, inflammatory diseases or emerging infections such as SARS-CoV-2, requires increased effort on the development of improved and novel mAbs. A more mature appreciation of the immunobiology of individual FcγR function and the complexity of the relationships between FcγRs and antibodies is fuelling efforts to develop more potent "next-gen" therapeutic antibodies. Such development strategies now include focused glycan or protein engineering of the Fc to increase affinity and/or tailor specificity for selective engagement of individual activating FcγRs or the inhibitory FcγRIIb or alternatively, for the ablation of FcγR interaction altogether. This review touches on recent aspects FcγR and IgG immunobiology and its relationship to the present and future actions of therapeutic mAbs.

INTRODUCTION

The regulatory approval of the first therapeutic monoclonal antibodies, or mAbs, in the 1980s ushered in the modern era of immune therapy. Since then, mAbs have become one of the most clinically successful therapeutic modalities across a diverse array of diseases. They have revolutionised the treatment of chronic inflammatory diseases and of some cancers including otherwise incurable malignancies.[1] They are commercially important and in 2017, five mAbs collectively grossed $45.6 billion in sales, placing them in the top ten drugs globally.[2] MAb development is expanding rapidly with over 100 mAbs approved for clinical use or in late stage clinical trials and over 600 in various stages of clinical development.[1]

The therapeutic actions of mAbs can take many forms - neutralisation of the target such as cytokines in autoimmune disease, clearance of the target such as virus in infection or IgE in allergy, induction of innate effector cell activation that leads to target destruction by direct killing or the induction of apoptosis, and the induction of adaptive immunity. Most therapeutic mAbs are IgG in origin and the heavy chain subclass determines many of their biological properties including their long plasma half-life[3], complement activation which is important in the action of some cytotoxic mAbs[4-6] and importantly engagement by their Fc region with specific cell surface receptors, called FcγR, the subject of this review.

In normal homeostatic immunity, there is a balance between IgG immune complex activation of proinflammatory responses through the activating-type FcγRs - which leads to the destruction of opsonised pathogens - and of the modulation of these destructive effector responses by the inhibitory-type FcγR thereby avoiding injury to the host. Thus, therapeutic mAbs powerfully exploit these opposing activities, making them versatile drugs whose therapeutic potency can be improved by specific engineering of Fc:FcγR interactions.[7]

Many therapeutic mAbs depend, to varying degrees, on FcγR function (Figure 1, Table 1) for their mechanism-of-action (MOA) and/or their pharmacokinetic properties. For some mAbs interaction with FcγR is central to their MOA, such as the destruction of a target cell by antibody dependant cell-mediated cytotoxicity (ADCC) (Figure 1a) or antibody dependant cell-mediated phagocytosis (phagocytosis or ADCP) (Figure 1b). This also includes, mAbs that may harness the inhibitory action of FcγRIIb to modulate the proinflammatory responses of immunoreceptor tyrosine activation motif (ITAM)-dependent receptor signalling complexes (Figure 1c). For other mAbs,

FcγR may play a secondary role, such as the removal or "sweeping" of all immune complexes formed by cytokine or virus-specific neutralizing antibodies or of opsonised fragments of lysed target cells which, in antigen presenting cells may also feed the antigen into the antigen presentation pathways (Figure 1d). Additionally, FcγR, particularly FcγRIIb (Figure 1e), are also key participants in the MOA of immune stimulating agonistic mAbs or apoptotic mAbs by acting as a scaffold for the additional cross-linking of mAbs already bound to a cellular target thereby inducing a signal in the target cell.

This review focusses on the cell-based effector functions that arise from the interaction of IgG with the classical human leukocyte FcγR.[7] Although beyond the scope of this review, it should be noted that the IgG-Fc portion dictates other aspects of an antibody's biology, including its serum half-life mediated by FcRn,[3] the activation of complement C1[8], antiviral protection via the intracellular receptor TRIM21[9] and interactions with the Fc receptor-like, FcRL family.[10]

HUMAN FcγR GENERAL PROPERTIES.

The human leukocyte receptors fall into two functional groups – pro-inflammatory, activating-type receptors (FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa and FcγRIIIb a.k.a CD64, CD32a, CD32c, CD16a, and CD16b, respectively) and the anti-inflammatory, inhibitory-receptor group (FcγRIIb, CD32b) which was the first immune checkpoint described.

These FcγR are high avidity sensors of immune complexes which initiate, and then modulate, cell responses. In the context of normal immune physiology, opsonised target molecules can engage various FcγR and induce a spectrum of effector responses which can be harnessed by many therapeutic mAbs (Figure 1, Table 1). These responses are not mutually exclusive and one therapeutic mAb may initiate various responses via different FcγR and via different cell types.

Understanding the importance of cell-based effector functions in the MOA of therapeutic mAbs requires an appreciation of FcγR biology (Tables1,2,3) which also underpins future efforts to tailor new mAbs for the exploitation specific effector responses. In this review we address only key aspects of the extensive knowledge of the human leukocyte FcγR family as it relates to effector functions. A number of other reviews more comprehensivly explore FcγR biology physiology, biochemistry, genetics and structure.[7, 11-14] Notwithstanding the recognised differences between the immunobiology of human FcγR and of rodents or nonhuman primates, animal models of FcR

effector function *in vivo* have helped shape the strategies for the development of current therapeutic mAbs and are well reviewed.[12, 15] Furthermore, humanised FcγR models will provide even greater insights into the future.[16]

FcγR expression on haemopoietic cells

The tissue distribution of the human leukocyte FcγR is well documented and reviewed comprehensively elsewhere.[7, 11, 17] In the context of effector functions harnessed by therapeutic mAbs, several aspects of the cellular distribution (Table 2) should be emphasised.

FcγR expression profiles differ between cell lineages but almost all mature human leukocytes, and platelets, express at least one FcγR (Table 2). It should also be appreciated that the cellular expression levels and receptor diversity as described below is also influenced by the activation state of the cells, anatomical location and the cytokine environment which modulates FcγR expression particularly for FcγRI and FcγRIIb.[18] For example resting monocyte subpopulations may express only FcγRIIa but activated macrophages FcγRI, FcγRIIa and FcγRIIIa and/or FcγRIIb.[7]

Thus, specific characteristics of leukocyte FcγR expression are summarised as follows:

FcγRI is not usually expressed until induced by cytokines such as IFN-γ on monocytes, neutrophils, macrophages, microglial cells in the brain, dendritic cells and mast cells. Its sensitivity to IFN-γ suggests its *in vivo* activity is closely tied to immune activation events. and mouse studies have suggested that it has a critical role early in immune responses.[19, 20] Its role in the MOA of antibodies may vary with anatomical location.[21]

FcγRIIa is expressed only in primates and shows the broadest expression of all FcγRs, being present on all innate leukocytes. It is also present also on platelets but a role in effector functions is not established but it is important in certain immune thrombocytopenias. A polymorphic form of this receptor is the only human receptor for human IgG2. This together with its limited species expression and unique ITAM-containing cytoplasmic tail (reviewed in ref .[11] suggests a unique function in human leukocytes. Interestingly, polymorphism of the receptor is associated with systemic lupus erythematosus (SLE) and resistance to gram-negative organisms.[11] A rare, hyper responsive form is a risk factor for neutrophil driven anaphylaxis in Ig replacement therapy.[22]

FcγRIIc is an activating FcγR whose expression is regulated Single Nucleotide Polymorphism (SNP) that permits expression in approximately 20% of humans and in whom it is present at low levels on NK cells and B cells.[11] It has arisen by gene duplication/recombination resulting in an extracellular region related to FcγRIIb, which binds IgG4, but with an ITAM-containing cytoplasmic tail related to the activating receptor FcγRIIa, thus providing IgG4 with an activation receptor pathway and confers a new biology of IgG4 in these individuals. Its low frequency in the population may also confound *in vivo* mAb clinical testing or use but as yet there is no evidence for this.

FcγRIII forms are two highly related gene products, FcγRIIIa and FcγRIIIb. The FcγRIIIa is expressed on NK cells and professional phagocytes, particularly macrophages. It is only recently apparent that FcγRIIIa is expressed on neutrophils, albeit at low levels, but plays a role in their function.[23] FcγRIIIb is unique to humans and unlike other FcγR it is attached to cell membrane via a glycophosphatidyl anchor. It is expressed, predominantly and abundantly, on human neutrophils.[7] Its effector function depends in part on its co-expression with FcγRIIa. The lack of FcγRIIIb on macaque neutrophils appears to be compensated for by an increase in FcγRIIa expression.[15]

FcγRIIb are the inhibitory-type FcγR and arise from a single gene. They lack intrinsic pro-inflammatory signalling and are instead immune checkpoints. They provide feed-back regulation by antibodies, in the form of immune complexes, to inhibit B cell activation by specific antigen. They also control activating–type FcγR function on innatet cells. Two major splice variant forms of FcγRIIb exist with differential tissue expression profiles. FcγRIIb1 preferentially expressed on B lymphocytes contains a 20 amino acid cytoplasmic insertion necessary for membrane retention and co-capping with the BCR. FcγRIIb2 is the predominant inhibitory receptor found on basophils and neutrophils, as well as subpopulations of mast cells, dendritic cells, some monocytes macrophages. FcγRIIb2 lacks the cytoplasmic insertion of FcγRIIb1 and consequently can internalise rapidly including with the activating FcR when they are co-cross-linked.[11] It is not clear which form is present on human T cells.

One additional comment on tissue distribution is that FcγR expression on T cells has been difficult to establish unequivocally. However, there is increasing evidence that T lymphocyte populations express FcγR. Some γδ T cells express FcγRIIIa and αβ T cells reportedly express FcγRIIa,

FcγRIIb, or FcγRIIIa but the significance with respect to effector function mediated by antibody is presently unclear.[24-28]

Expression on non-haemopoietic cells

The immunobiolgy of FcγR is studied and understood almost exclusively in the context of hematopoietic cell function but relatively recent investigations have identified and explored FcγR expression on non-haemopoietic cells. These studies suggest important roles in normal immune function and in the MOA of some therapeutic mAbs. The most extensively characterised receptor expression is FcγRIIb which is expressed on follicular DC, airway smooth muscle and liver endothelium. Its abundant expression on liver sinusoidal endothelial cells (LSEC), is estimated to represent the majority of *in vivo* FcγRIIb expression.[17, 29-31] As FcγRIIb lacks intrinsic pro-inflammatory signalling function its role on these non-haemopoitic cells, involves immune complex handling without the danger of, or the need for, induction of local tissue destructive inflammatory responses. On LSEC its major role appears to be immune complex sweeping, a process of removal of small immune complexes such as opsonised virus or macromolecules.[17] This scavenging role by FcγRIIb on LSEC can be exploited in principle by mAbs forming small soluble complexes with their targets such as anti-viral, anti-cytokine or similar antibodies.

FcγR Activating or inhibitory signalling.

Effector functions that are initiated via the activating-type FcγR, occur by signalling via the immunoreceptor tyrosine activation motif (ITAM) pathway of immune receptors. This well characterised pathway is used by B and T cell antigen receptors (BCR and TCR), the IgE receptor FcεRI and IgA receptor FcαRI (reviewed extensively in[7, 11, 32]) Induction of an activating signal requires the aggregation of activating FcγR by immune complexes, or by antigen in the case of the B cell antigen receptor (BCR). This aggregation at the cell membrane results in specific tyrosine phosphorylation of the ITAM by src kinases, thus initiating the activation cascade.[32-34]

The inhibitory-type, FcγRIIb1 and FcγRIIb2, whose expression is cell-lineage restricted, modulate the ITAM signalling of the BCR or the activating-type FcγRs respectively.[11] Their function is dependent on the immunoreceptor tyrosine inhibition motif (ITIM) in their cytoplasmic tail.[32, 33] This checkpoint action requires that FcγRIIb are co-aggregated with the tyrosine phosphorylated ITAM-signalling receptor complex which results also in ITIM tyrosine phosphorylation and

consequential recruitment of lipid or protein tyrosine phosphatases that powerfully dampen the ITAM induced cell activation.

FcγR DEPENDANT EFFECTOR RESPONSES.

Not all opsonised targets are equal: size, distance, valency and Fc geometry affect potency.

To understand the immunobiology of FcγR effector responses particularly in the therapeutic mAb context, it is important to appreciate that the quality and potency of such effector responses is greatly affected by the nature of the IgG immune complex and/or the state of potential effector cells.

First, opsonisation, *per se,* of a target is not necessarily sufficient to ensure FcγR interaction in a way that initiates an effector response. Although it is the the IgG Fc that interacts with and clusters the FcγR to induce a response, the nature of the Fab interaction with its epitope can strongly influence the likelihood or potency of FcγR effector responses by influencing the density of appropriately presented Fc portions.[35] and also the size of the immune complex.[36] Furthermore, the display/orientation and geometry of the Fc portions, as a consequence of the Fab interaction with the target epitope, can result in effector responses such as ADCC that differ substantially in potency, presumably because the orientation of the Fc makes FcγR engagement more, or less, accessible.[37, 38]

Second, in innate effector cells at rest, the largely linear actin cytoskeleton and the extracellular glycosaminoglycan glycocalyx regulate function by interacting with large glycoproteins, such as CD44, arranging these into ordered "picket" fences.[39, 40] These corral receptors, including the FcγRs and sterically inhibit their interaction with ligands. Upon cell activation, cytoskeletal remodelling is associated with the loss of the receptor corrals allowing FcγRs and other receptors to freely diffuse, engage ligand, cluster and signal.[39] The influence of such surface constraints on receptors and effector cell function helps explain some of the observed epitope distance requirements for optimal mAb function.[39, 41] which were apparent in a comparative study of ADCC and ADCP.[42] ADCC was optimal when the epitope was displayed close, 0.3nm "flush" or 1.5 nm, to the target membrane where close conjugation of effector and target by the mAb presumably facilitates the delivery of pore forming proteins to the target membrane as required by

ADCC. Interestingly complement-dependant cytotoxicity which also utilises pore forming proteins for its cytotoxicity has similar distance constraints. By contrast ADCP, was poor for epitopes displayed 'flush' (within ~ 0.3 nm) of a target cell membrane but activity was restored when the epitope was displayed 1.5 nm off the membrane.[42]

Although the action of agonistic/antagonistic mAbs is mechanistically distinct to those eliciting cytotoxicity and ADCP, the distance segregation between target and FcγR$^+$ cells is also important. Indeed the membrane proximal epitopes of CD28 and CD40 are important for the FcγR function in the complex MOA of these mAbs.[43, 44]

Clearly, the effects of immune complex valency, Fc density, presentation and geometry together with FcγR organisation in the cell membrane suggest that the development of mAbs to certain targets will be heavily influenced by the context of use. Thus, improved mAb potency may not necessarily be achieved by engineering of the Fc polypeptide or its glycan alone. A more function oriented approach early in mAb selection and development by, for example, application of rapid screening technologies that select for effector potency[34], followed by Fc engineering may be more productive.

ADCC and phagocytosis.

ADCC and ADCP are the most widely appreciated FcγR-dependent effector functions (Figure1a, b) and are respectively mediated primarily via FcγRIIIa on NK cells and professional phagocytes such as macrophages. These effector functions, particularly NK cell ADCC, are believed to be major components of the MOA of cytotoxic therapeutic mAbs use in cancer therapy. In addition ADCP can also occur via FcγRIIa and FcγRI[45] but the extent to which cytotoxic anti-cancer therapeutic mAbs depend on these for their MOA in patients is unclear. The improvement in clinical utility of mAbs engineered for selectively increased FcγRIII binding suggests that FcγRIIa and FcγRI may be less important *in vivo* in cell killing effects but perhaps are more important in other aspects of therapeutic efficacy - see below.

Inhibition of cell activation by FcγRIIb.

FcγRIIb is an immune checkpoint[46, 47] and its splice variants are potent modulators of ITAM-dependent signalling (Figure1c). This modulatory function occurs only when FcγRIIb is co-

aggregated with an ITAM signalling receptor. Thus, B cell activation is modulated by the simultaneous binding of antigen, in the immune complex, to the BCR and the Fcs in the immune complex, to the FcγRIIb1 on the same cell. In innate leukocytes, the activating-type FcR i.e. FcγRI, FcγRIIa, FcγRIIc, FcγRIII as well as the high affinity IgE receptor, FcεRI, and the IgA receptor, FcαRI are all modulated by immune complex co-engagement with FcγRIIb2. The inhibitory function contributes to the MOA of therapeutic antibodies that target cell-activating molecules where the target cells also express the inhibitory FcγRIIb such as the BCR (below).

Sweeping: Clearance of small immune complexes.

The removal of immune complexes in humans depends primarily on the complement receptor pathway and to a lesser degree the FcγR. Among the FcγR it has been widely believed that immune complex removal only occurs by the phagocytosis/endocytosis by activating-type FcγR. Surprisingly, the inhibitory FcγRIIb which lacks intrinsic activating function, rapidly "sweeps" away small complexes from the circulation, (Figure 1d).[48, 49] A major tissue involved in the clearance is likely to be the liver sinusoidal endothelial cells (LSEC) where FcγRIIb is expressed abundantly in mice and humans. This role is potentially important in resistance to viruses and toxins but may also be key to optimal performance of therapeutic IgG mAbs whose primary MOA is believed to be only neutralisation of soluble macromolecules for example cytokines or IgE.

FcγR uptake of antigen:antibody complexes and shaping the immune response.

Monoclonal antibody therapy is a form of passive immunisation. Indeed, longer term vaccine-like or vaccinal immunity has been demonstrated in anti-CD20 treated mice via FcγRIIa[50 51] and *in vitro* recall memory responses from CD20 treated patients.[52] Although this is dependent on FcγR and anti-CD20, the mechanism by which long term anti-tumour response is established is still unclear.

None-the less the active involvement of FcγR in the enhancement of antigen-specific immunity by uptake of immune complexes through FcγR is historically well documented in experimental systems where FcγR bind immune complexes and thereby feed antigens into the antigen presentation pathways.[53] This has been demonstrated *in vivo* for small immune complexes via human FcγRI on human antigen presenting cells[54] and in mice.[19] Similarly the capacity of FcγRIIb to bind and rapidly internalise antigen:antibody complexes suggests it too may significantly

influence the feeding antigen into professional antigen presenting cells of hematopoietic origin such as dendritic cells and possibly B lymphocytes.

Whilst not classical MHC-dependant antigen presentation, FcγRIIb on the stroma-derived follicular dendritic cells influences antibody immunity by recycling antigen:antibody complexes to the cell surface for presentation of intact antigen to B cells.[55]

Although somewhat speculative, FcγRIIb rapid internalisation/sweeping of complexes by the abundant LSEC, which interact with lymphocytes and can present antigen.[56] may have a significant role in shaping immune responses.

Scaffolding of cell-bound mAbs by FcγR$^+$ cells.

FcγR-expressing cells can be critical, but passive, participants in the MOA of some mAbs (Figure 1e). In FcγR scaffolding, IgG mAb molecules that have opsonised the cell surface of a target cell are additionally crosslinked by their Fc portions engaging the FcγRs that are arrayed on the surface of a second cell. This "super-crosslinking" of the target-bound mAb by the FcγR lattice or "scaffold" on the adjacent cell greatly exceeds the target crosslinking by the mAb alone, *thereby inducing a response in the target cell*. Scaffolding was originally identified as the basis of T cell mitogenesis induced by anti-CD3 mAb.[57, 58] The CD3 mAbs alone were poor mitogens but the "super cross-linking" of the T cell-bound CD3 mAb by the membrane FcγR on adjacent cells, particularly by monocytes, induced rapid T cell expansion and cytokine secretion but did not require activation of FcγR expressing cells.[57] Regrettably, FcγR scaffolding came to prominence and clinical relevance because of its causal role in the catastrophic adverse events resulting from the administration of anti-CD3[57] and anti-CD28 (TGN1412)[59] mAbs.

None-the-less FcγR scaffold-based induction of intracellular responses in a target cell can also lead to beneficial therapeutic effects. Such examples are the induction of apoptotic death in a target cell, which is likely part of the MOA of Daratumumab in multiple myeloma[60] or the controlled agonistic expansion of cells, for example, via CD40 mAb agonism.[43]

IgG SUBCLASSES: SPECIFICITY AND AFFINITY FOR FcγR.

Most FcγR (Table 2), are weak, low-affinity receptors (affinities in the micromolar range) for IgG Fc irrespective of whether the IgG is uncomplexed, monomeric IgG or when it is complexed with

antigen i.e an immune complex. The very *avid*, binding of immune complexes to an effector cell surface that, displays an array of FcγR molecules, is the result of the *collective* contributions of the *low-affinity* interactions of each Fc of the IgGs in the complex with an FcγR. This *avidity* effect is necessary as the FcγR operate *in vivo* in environments of high concentrations of un-complexed IgG (normally 3 – 12 g/L). Thus, the avid multi-valent binding of the complex out competes un-complexed, monomeric IgG. The notable exception to this is the enigmatic FcγRI. This receptor shows high, nanomolar affinity for un-complexed monomeric IgG and thus would be expected to be constantly occupied *in vivo* by the normal circulating monomeric IgG. However, IgG dissociation permits engagement with immune complexes. Furthermore FcγRI it is not expressed, or expressed poorly on resting cells requiring interferon-γ for induction of its expression, presumably at sites of inflammation.

Although the human IgG heavy chain constant domains have greater than 90% identity, key amino acid differences confer each subclass with unique structural and functional properties. IgG1 (and IgG3) are "universal" ligands, binding to all FcγRs. Formal measurement of the weak, micromolar K_D, interactions of the low affinity receptors with monomeric IgG1 also revealed differing affinities between the low affinity FcγR, with inhibitory FcγRIIb generally lowest affinity and the FcγRIII higher, sometimes referred to as a "moderate" affinity receptor.[7, 61]

The strength of IgG1 interaction can also be affected by FcγR polymorphism and in the context of therapeutic mAbs, variation in FcγRIIIa is particularly important. The most common and possibly clinically significant polymorphism is phenylalanine /valine variation at position 158 in the IgG binding site wherein FcγRIIIa-F^{158} binds IgG1 less well than the FcγRIIIa-V^{158} form.

IgG4 and IgG2 have more restricted FcγR specificity. IgG4 has low affinity ($K_A \sim 2x10^5\ M^{-1}$) for the inhibitory FcγRIIb but is also a high affinity ligand for FcγRI ($K_A \sim 4x10^8\ M^{-1}$). IgG2 exhibits a highly restricted specificity, showing functional activity with only one polymorphic form of FcγRIIa (binding affinity $K_A \sim 4.5x10^5\ M^{-1}$) which is permitted by the presence of histidine at position 131 of its IgG binding site. This FcγRIIa–H^{131} form is expressed in approximately 70% of the population whereas IgG2 has no functional activity on the other common allelic form, FcγRIIa-R^{131} which contains arginine at positon 131.[11, 61]

IgG SUBCLASSES: STRUCTURE AND PROPERTIES.

The molecular basis of IgG and FcγR interactions.

The extracellular regions of the FcγR are structurally similar. Each low affinity FcγR has two ecto-domains, while the high affinity FcγRI has a third domain but this is not directly involved in IgG binding.[62]

The interaction between the IgG subclasses and the FcγR is most comprehensively defined for human IgG1 by both X-ray crystallographic[7, 62, 63] and mutagenesis structure/function analyses.[64-66] These studies defined key regions of the IgG sequence required for interaction with their FcγRs.

Crystallographic analyses of the human IgG1-Fc complexed with FcγRI, FcγRIII or FcγRIII show that these interactions are similar in topology, and asymmetric in nature. The second extracellular domain of the FcγR inserts between the two heavy chains. Here it makes contacts with the lower hinge of both H-chains and with residues of the adjacent BC loop of one CH2 domain and the FG loop of the other. The N-linked glycan at asparagine 297 (N^{297}) of the heavy chain is essential to the structural integrity of the IgG-Fc by affecting the spacing and conformation of the CH2 domains. Indeed, its removal ablates FcγR binding.[67] Of particular relevance to therapeutic mAb development is that the normal low affinity IgG interaction with FcγRIIIa is profoundly increased by the removal of the core fucose from the N^{297} Fc-oligosaccharide.[68]

No crystallographic data is available for IgG2 or IgG4 Fc in complex with FcγR, but mutagenesis studies of the Fc and the FcγR revealed general similarity, but with critical differences, in the interaction of these subclasses with their cognate FcγR.

Unique features of the IgG2 and IgG4 subclasses.

In IgG1, the stable interaction of the two heavy chains results from the combined effects of stable covalent inter H-chain disulphide bonds and strong non-covalent interaction of the two CH3 domains (Table 3). In stark contrast, in IgG2 and IgG4 the interaction of the CH3 domains of each H-chain is weak. Residues 392, 397, and 409 (Eu numbering) profoundly affect the stability of these interactions. The difference at position 409 (R^{409} in IgG4, K^{409} in IgG1) confers a 100-fold decrease in stability in the IgG4 H:H-chain interaction compared to IgG1 (Table 3).[69]

Furthermore, the core hinge of IgG4 differs from IgG1 at position 228 (P^{228} in IgG1, S^{228} in IgG4) resulting in unstable inter-heavy chain disulphide bonds. This, together with the destabilising

amino acids in the CH3, confers the unique property of half-antibody (Fab arm) exchange between different IgG4 antibodies[69] thereby creating monovalent, bispecific IgG4 antibodies *in vivo*.[69, 70] The similarly unstable interactions between the CH3 domains in IgG2 are conferred by the interface residue M^{397}, however the stable inter-H chain disulphide bonds of the core and upper hinge prevent half-molecule exchange (Table 3).[69]

Additionally, IgG2 uniquely has three disulphide bond conformers (Table 3). The distinct conformers are formed with when 1) each light chain is attached to the Cys^{131} residue of CH1 in the heavy chain (IgG2-A conformer); 2) both light chains attach to the upper hinge (IgG2-B) or 3) one light chain is attached to the CH1 Cys^{131} and one to the upper hinge of the other H chain (IgG2-AB).[71] This results in distinct positioning of the Fabs relative to the Fc portions in the different conformers which has implications for the interaction with antigen and the capacity of IgG2 to crosslink target molecules in the absence of FcγR binding, for example in an agonistic mAb setting.[72]

It should also be noted that IgG3 has not been used in therapeutic mAbs despite its unique biology. The main impediment to its use are its physico-chemical properties such as susceptibility to proteolysis and propensity to aggregate that present challenges to industry-scale production and stability but protein engineering is attempting to overcome these hurdles.[73]

THERAPEUTIC ANTIBODY DESIGN – IMPROVING mAb POTENCY

Many factors affecting FcγR dependant responses *in vivo* come into play during mAb therapy. The experience of three decades of clinical use of mAbs taken together with our extensive, albeit incomplete, knowledge of IgG and FcγR structure and immunobiology provides a war chest for the innovative development of new and highly potent mAbs through the manipulation of their interaction with the FcγR.

Therapeutic mAb engineering strategies are directed by many factors including the biology of the target, the nature of the antigen, the desired MOA, and possibly the anatomical location of the therapeutic effect.[21] and thus to optimise potency for a desired response, the context of use is critical.

The nature of the IgG isotype.

Different capabilities for the recruitment and activation of the different immune effector functions are naturally found in the Fc regions of the human IgG subclasses. Thus, to achieve a desired MOA, the different IgG subclasses are important starting points for the selection and engineering of the optimal mAb Fc. IgG1 is, in many ways, a proinflammatory or "effector-active" subclass, as it can initiate the complement cascade and is a "universal" FcγR ligand.[74] Notwithstanding it is also a ligand for the inhibitory FcγRIIb, IgG1 elicits proinflammatory responses through all the activating-type FcγR including ADCC, ADCP and cytokine release.

Because of their more restricted FcγR binding profile IgG2 and IgG4 have offered some choice in potentially avoiding FcR effector function without the need for Fc engineering. They have been used as the backbone for therapeutic mAbs either because recruitment of patients' effector functions was unlikely to be necessary for the primary MOA of the mAb or is possibly detrimental to the desired therapeutic effect.[75] However, the use of these unmodified "inert" subclasses is not without consequences and underscores the need for Fc engineering to modify FcγR interactions - See below "*Attenuating and ablating FcγR related functions of IgG***"**.

Thus, the choice of IgG subclass for therapeutic mAb engineering is an important first step for engineering of novel mAbs of improved specificity, potency and safety.

Fc Engineering for Enhanced Anti-Cancer Therapeutics

IgG1 is the pre-dominant subclass used in the development of cytotoxic mAbs where induction of an activation-type response, ADCC or phagocytosis, is considered desirable.[45, 76, 77]

Cytotoxic mAb cancer therapeutics can control disease progression by one or more mechanisms. Their MOA include direct induction of apoptotic cell death of the cancer cell (anti-CD20, anti-CD52) or by blocking receptor signalling (anti-HER2, anti-EGFR). They may also harness FcγR-effector functions, including ADCC in the tumour microenvironment.[78] The approved mAbs, Rituximab (anti-CD20), Trastuzumab (anti-HER2), and Cetuximab (anti-EGFR) are formatted on a human IgG1 backbone and all require activating-type FcγR engagement for optimal therapeutic activity.[79, 80] This presents an example where context of therapeutic use is critical for therapeutic mAb design. IgG1 antibodies bind both the activating FcγR e.g. FcγRIIIa, and the inhibitory FcγRIIb. Thus in environments where effector cells co-express FcγRIIb together with FcγRI, FcγRIIa and FcγIIIa, possibly a tumour infiltrating macrophage, therapy with an IgG1 anti-cancer

cell mAb will be compromised by the ITIM action of FcγRIIb on the ITAM signalling of the activating FcγR as they would be co-engaged on the effector cell by the mAb bound to the target cell.. This leads to reduced therapeutic mAb potency. Thus, the relative contributions of activating (A) and the inhibitory (I) FcγR to the response by an effector cell, the A:I ratio, which may be an important determinant in clinical outcome of therapeutic mAb activity[76, 81, 82] i.e. the higher the A:I ratio, the greater the pro-inflammatory response induced by the therapeutic mAb or conversely the lower the A:I ratio the greater the inhibition or dampening of the proinflammatory response.

Thus, the challenge for the development of more potent FcγR effector mAbs is to overcome three major obstacles. First, improving activation potency by selectively enhancing interaction with activating type FcγR, particularly FcγRIIIa due to its predominant role in ADCC-mediated killing of tumour cells. Second, reducing binding interactions with the inhibitory FcγRIIb. These two approaches improve the FcγR A:I ratio of cytotoxic IgG1 mAbs. Third, overcoming the significant affinity difference in the interaction with the main FcγRIII allelic forms of FcγRIIIa-V^{158} and FcγRIIIa-F^{158} refs [76, 83, 84] which appears to be an important source of patient variability in responses to therapeutic mAb treatment of cancer.

At the time of writing, some mAbs with improved potency are coming into clinical use. Their improved action has been achieved by modifying the N-linked glycan or the amino acid sequence of the heavy chain Fc (Table 4).

Modification of the Fc glycan. The typical complex N-linked glycan attached to N^{297} of the heavy chain includes a core fucose.[85] Antibodies that lack this fucose have ~50-fold improved binding to FcγRIIIa and FcγRIIIb and importantly retain the weak, low affinity binding to the inhibitory FcγRIIb. Furthermore this glycoengineering increased binding affinity of the modified IgG1 mAb for both FcγRIIIa V^{158} F^{158} allelotypes.[86-88] Afucosyl versions of the tumour targeting mAbs such as anti-HER2, anti-EGFR, and anti-CD20 had greater anti-tumour effects and increased survival[68, 88, 89] which is a reflection of the greatly increased, and selective, FcγRIII binding. Compared to their unmodified counterparts, the afucosyl mAbs showed dramatic improvement of FcγRIII related effector responses such as stronger NK cell-mediated ADCC, or enhanced neutrophil mediated phagocytosis through FcγRIIIb and/or FcγRIIIa.[23] However certain neutrophil functions via FcγRIIa may be compromised.[90, 91]

There are six afucosylated antibodies in late-stage clinical trials or approved for treatment (Table 4). Notable is Obinutuzumab, an afucosyl anti-CD20 mAb which nearly doubles progression-free survival in chronic lymphocytic leukaemia patients as compared to the fucose-containing Rituximab.[68] This dramatic improvement in clinical utility reinforces the value of glycan engineering specifically and of Fc engineering generally in anti-cancer treatments.

Mutation of the Fc amino acids. Alteration of the amino acids in the heavy chain Fc can alter IgG specificity and affinity for activating FcγRs. The anti-CD19 antibody MOR208 (XmAb-5574) is currently in Phase III trials for the treatment of chronic lymphocytic leukemia.[92] It contains two mutations in its IgG1 Fc, $S^{329}D$ and $I^{332}E$, which increases affinity to FcγRIIIa, particularly the "lower-affinity" FcγRIIIa F^{158} allele. The mAb shows increased FcγRIII-mediated ADCC and phagocytosis *in vitro*, and reduced lymphoma growth in mouse models.

Margetuximab is an ADCC-enhanced IgG1 Fc engineered variant of the approved anti-HER2 mAb Trastuzumab in Phase III for HER2-expressing cancers.[66, 93] Alteration of five amino acid ($L^{235}V$, $F^{243}L$, $R^{292}P$, $Y^{300}L$, and $P^{396}L$) enhanced binding to FcγRIIIa which also had the additional effect of decreasing binding to the inhibitory FcγRIIb and thereby increased its A:I FcγR ratio. This was apparent when compared to unmodified Trastuzumab, Margetuximab showed enhanced ADCC against $HER2^{+}$ cells *in vitro* and demonstrated superior anti-tumour effects in a HER2-expressing tumour model in mice.

The anti-CD20 Ocaratuzumab is an Fc engineered IgG1 mAb in late stage clinical trials for the treatment of a range of cancers, including Non-Hodgkin Lymphoma and chronic lymphocytic leukaemia (CLL).[94] Two Fc mutations $P^{247}I$ and $A^{339}Q$ conferred ~20-fold increase in binding to the both major allelic variants of FcγRIIIa and elicited 6-fold greater ADCC than unmodified IgG1.

Thus, the engineering of the Fc domain or glycan for improved FcγRIIIa binding is a powerful tool to create more potent and clinically effective anti-cancer mAbs.

Attenuating and ablating FcγR related functions of IgG.

There are circumstances where binding to FcγR is unnecessary or undesirable in the MOA of a therapeutic mAb. Unmodified IgG irrespective of its subclass or intended therapeutic effect has the potential to engage an FcγR which may lead to suboptimal therapeutic performance or to

unexpected and catastrophic consequences.[57, 59] Clearly reducing or eliminating FcγR interactions, where they are not required for therapeutic effect, may be desirable. Indeed, this had been addressed by the choice of IgG subclass or by modifying the Fc region. Indeed to date, most efforts in Fc engineering mAbs that have translated to an approved drug have focused on the reduction or elimination of FcγR interactions (Table 4).

One approach to minimise interactions with the activating FcγR has been the use of IgG4 or IgG2 backbones, which show a more restricted specificity for the activating FcγR and consequently have been traditionally, and simplistically, viewed as "inert" IgG subclasses. Notwithstanding the unexpected, and FcγR-dependant, severe adverse reaction induced by the IgG4 TGN1412 mAb, the IgG4 or IgG2 backbones have been successfully used in many settings. Indeed, checkpoint inhibitors, such as mAbs targeting CTLA-4 or the PD-L1/PD-1 interaction for the suppression of inhibitory signals that contribute to immune tolerance in the tumour microenvironment, are formatted on an IgG4 backbone. Pembrolizumab, Nivolumab, and Cemiplimab are all anti-PD-1 antibodies currently used for cancer therapy and have been formatted on an IgG4 backbone[95-97] with a stabilised core hinge ($S^{228}P$) to prevent half-IgG4 exchange. Similarly, the checkpoint inhibitor Tremelimumab is an anti-CTLA-4 antibody formatted on an IgG2 backbone to avoid potential ADCC killing of target cells.[98]

However, the use of IgG2 and IgG4 as "inert" subclasses is problematic. Both bind to the activating receptors FcγRIIa-H^{131} and FcγRI respectively (Table 2) and initiate effector functions such as neutrophil activation and apoptosis induction.[75, 99] Interestingly, in experimental systems, crosslinking of anti-PD-1 IgG4-based mAb by FcγRI, switched its activity from blocking to activatory.[100] Moreover, IgG4 binds to FcγRIIb, which may scaffold the therapeutic mAb. Whilst scaffolding may be beneficial in some contexts for example in immune agonism[43] it can be disastrous and unexpected in others as it was for the anti-CD28 TGN1412 mAb.[59] Thus, the IgG2 and IgG4 subclasses are not be the optimum choice for "FcR-inactive" mAbs so modifying the Fc is a more direct approach.

The complete removal of the heavy chain N-linked glycan is well known to ablate all FcγR binding by dramatically altering the Fc conformation.[36, 67, 101, 102] Atezolizumab, an IgG1 anti-PD-L1 checkpoint inhibitor mAb, utilises this strategy and eliminates FcγR and also complement activation.[103]

Modification to the Fc amino acid sequence of the FcγR-contact regions can also be used to reduce FcγR binding. A widely used modification of IgG1 is the substitution of leucine 234 and 235 in the lower hinge sequence (L^{234} L^{235} G^{236} G^{237}) with alanine ($L^{234}A$ $L^{235}A$). It is often referred to as the "LALA mutation" and effectively eliminates FcγR binding by >100fold[104, 105] and are used in Teplizumab and Spesolimab (Table 4).

A separate strategy has used combinations of amino acid residues from the FcγR binding regions of IgG2 and IgG4, which have restricted FcγR specificity, together with other binding-inactivating mutations. The lower hinge amino acids of the IgG1 mAbs Durvalumab (anti-PD-L1) and Anifrolumab (anti-interferon α receptor) (Table 4) were modified to mimic the lower hinge of IgG4 ($L^{234}F$). They additionally incorporated $L^{235}E$ also in the lower hinge and $P^{331}S$ in the F/G loop of the CH2 domain to ablate FcγR binding by disrupting two major FcγR contact sites.[7] and also coincidently decreasing C1q activation.[106]

IgG4 mAbs have been similarly engineered to eliminate its interaction with FcγRI and FcγRIIb. The IgG4 anti-PD-1 antibody Tislelizumab has had its FcγR contact residues in the lower hinge E^{233}, F^{234}, L^{235} substituted with the equivalent residues of IgG2 P, V, A ($E^{233}P$, $F^{234}V$, $L^{235}A$) as well as the additional $D^{265}A$ mutation which disrupts a major FcgR contact in CH2. It also has substitutions in the core hinge $S^{228}P$ and the CH3 $L^{309}V$, $R^{409}K$ to stabilise the H-chain disulphides and CH3 interactions respectively and thereby preventing half-Ig exchange characteristic of natural IgG4. Collectively these modifications create a stable IgG4 with no FcγR binding nor complement activation.[107]

Thus, Fc engineering is an effective way to remove FcγR effector functions and may be preferable to using unmodified IgG2 or IgG4 backbones have a more restricted repertoire of FcγR interactions but which are still able to induce certain effector functions.

Improving FcγRIIb interactions.

Preferential or specific Fc engagement of FcγRIIb over the activating FcγR offers several potential therapeutic advantages for new mAbs in distinct therapeutic settings.

Improved recruitment of FcγRIIb ITIM-dependant inhibitory function. Harnessing the physiological inhibitory function of FcγRIIb by mAbs that target ITAM receptors has the potential to shut down ITAM dependent signalling pathways of major importance in antibody

pathologies.[32, 108] Such ITAM signalling receptors include the BCR complex on B cells which is active in SLE, the FcεRI on basophils and mast cell subsets in allergies or the activating-type FcγR on a variety of innate leukocytes in antibody-mediated tissue destruction. In such scenarios, the ITAM signalling receptor complex that is targeted by the therapeutic mAb *must be co-expressed* on the cell surface with the inhibitory FcγRIIb. This permits co-engagement ITAM signalling receptor by the Fab of the mAb and inhibitory FcγRIIb by its Fc which is the critical requirement in the inhibitory MOA for such therapeutic mAbs (Figure 1).

Obexelimab (aka XmAb5871) (Table 4) currently in early clinical testing in inflammatory autoimmune disease is an IgG1 mAb that targets CD19 of the B cell antigen receptor complex.[109] It contains two Fc modifications, $S^{267}E$ and $L^{328}F$ (a.k.a. "SELF" mutations) that selectively increased FcγRIIb binding 400 fold to ~1nM resulting powerful suppression of BCR signalling and the proliferation of primary B cells.[109]

The anti-IgE mAb Omalizumab is an IgG1 mAb, approved for the treatment of allergic disorders.[110, 111] A similar but Fc engineered IgG1 mAb XmAb7195, currently in early clinical testing, contains the affinity-enhancing SELF modifications.[112] Both mAbs sterically neutralise the interaction between IgE and its high-affinity receptor FcεRI to prevent basophil and mast cell activation.[113, 114] However, XmAb7195 exhibited more efficient removal (sweeping –see below) of circulating IgE and also inhibited B cell IgE production, presumably by binding to the IgE BCR on the B cell surface and co-clustering with FcγRIIb via its affinity-enhanced Fc domain.[112] Thus, XmAb7195's selective modulation of IgE production by IgE^+ B cells in addition to its enhanced clearance of IgE may offer significantly improved therapeutic benefits in allergy therapy beyond simple IgE neutralisation.[112] Note also the 'SELF' mutations have also been used in agonistic mAbs (see below).

One cautionary note is that the arginine 131 (R^{131}) of the IgG binding site in FcγRIIb is critical for the enhanced affinity binding of 'SELF' mutated Fcs but it is also present in the activating-type "high responder" FcγRIIa-R^{131}. Thus, antibodies modified with 'SELF' have very high affinity binding to FcγRIIa-R^{131} [ref 115] with a potentially increased risk of FcγRIIa-dependent complications in patients expressing this allelic form but none have been reported in clinical trial. However, an alternative set of six Fc mutations, termed "V12" ($P^{238}D$, $E^{233}D$, $G^{237}D$, $H^{268}D$, $P^{271}G$, and $A^{330}R$), potently enhance FcγRIIb binding without increasing FcγRIIa-R^{131} interaction.[115]

Enhancing the Sweeping of Small Immune complexes. The expression of FcγRIIb on LSEC and its action in the 'sweeping" or removal of small immune complexes has opened up new possibilities for the application of FcγRIIb–enhancing modifications.[17] Antibodies or Fc fusion proteins, whose primary MOA is the neutralisation of soluble molecules such as IgE or cytokines are particularly attractive candidates for this approach. Proof-of-concept for this strategy has been demonstrated in experimental models.[48] Indeed, this may be a significant component of the rapid disappearance of IgE from the circulation of patients treated with the anti-IgE XmAb7195 containing the FcγRIIb enhancing 'SELF' modifications, as described above.

Immune agonism through FcγR scaffolding. Agonistic mAbs induce responses in target cells by stimulating signalling of their molecular target. Typically this is to either enhance anti-tumour immunity by engaging co-stimulatory molecules on antigen-presenting cells or T cells (i.e. CD40, 4-1BB, OX40) or to promote apoptosis by engaging death receptors on cancer cells (i.e. DR4, DR5, Fas).[116]

The role of FcγR in the action of these types of mAbs appears to be primarily as a scaffold. FcγRIIb is often the predominate receptor involved and the extent of its involvement is complex. In the case of CD40, the degree of FcγRIIb scaffolding potency is linked to the epitope location of the targeting mAb with greater potency seen for membrane proximal epitopes.[43, 117] It is also noteworthy that depending on the epitope location, the scaffolding of anti-CD40 mAbs may convert antagonist mAbs to agonistic.

Engineering of the IgG1 Fc region for enhanced and/or specific binding to FcγRIIb can greatly improve agonistic function.[72, 118-120] Such mutations induced significantly greater agonistic activity in an anti-DR5 model through increased induction of apoptotic death and decreased tumour growth compared to unmodified IgG1.[121] The 'SELF' modifications that dramatically and selectively increase affinity for FcγRIIb, have also been used to enhance immune agonism in an anti-OX40 model.[122]

The incorporation into IgG1 of the "V12" Fc mutations which specifically enhance FcγRIIb interaction 200-fold, conferred enhanced agonistic activity of an anti-CD137 antibody and an anti-OX40 mAb.[115, 122]

FUTURE ENGINEERING STRATEGIES

Monoclonal antibodies are potent therapeutics in a number of chronic or once incurable diseases. However, there is still extensive unmet clinical need as well as considerable room for improvement in many existing therapeutics.

Further understanding of how antibody structure affects FcγR function is essential for future development of more potent and effective mAbs. Already, engineering of the IgG Fc and its glycan has proved a potent and effective approach for increasing the clinical effectiveness, functional specificity and safety of therapeutic mAbs and is an emerging pathway to the development of the "next-gen" therapeutics.

Future directions in the development and use of therapeutic antibodies should increasingly mimic normal protective antibody responses, which are polyclonal and elicited in the context of innate receptor engagement which includes the FcR as well as other powerfully responsive systems including the toll-like receptors and complement receptors. Furthermore, the mixed subclass nature of these normal antibody responses suggest circumstances may arise in therapeutic strategies where there is value in having distinctly modified Fcs for the nuanced engagement of different FcγR family members. Treatments comprising multiple mAbs and immune stimulants are under investigation in infectious disease for neutralisation coverage of variant strains, indeed such an approach may be most effective in emerging infections disease such as SARS-CoV-2 infection. The use of multiple mAbs tailored for distinct effector functions and targeting different epitopes will maximise the opportunity for cocktailing of effector functions in different types of diseases. Indeed, in a small but contemporary example outside of infectious disease, the FDA approved combination in adenocarcinoma therapy uses a cocktail of two mAbs, Pertuzumab and Trastuzumab, against Her2.[123]

Rather than one type of Fc to conquer all, the combined use of appropriately selected mAbs whose individual components are enhanced for the engagement of different FcγR members may utilise multiple components of the spectrum of effector responses on offer by the immune system. Such 'next-gen' biologics will begin to realise the full potential of FcγR-mediated antibody immune therapeutics and offer transformational change for the treatment of intractable and incurable diseases.

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Conflict of interest

NONE

Figure 1 Legend.

Graphical representation of the FcγR effector functions. (a), Natural Killer cell Antibody Dependant Cell-mediated Cytotoxicity via FcγRIIIa. (b), Antibody Dependant Cell-mediated Phagocytosis, and/or trogocytosis of large immune complexes, by professional phagocytes via activating FcγR such as FcγRIIIa and FcγRIIa. Biological sequelae include the destruction of the ingested complexes which may also feed antigen into antigen presentation pathways of APC. (c), Inhibition of cell activation by FcγRIIb. The ITAM-mediated signalling of B cell antigen receptors (left) or of activating FcγR (right) on innate immune cells such as macrophages and basophils, is inhibited by IgG Fc mediated co-crosslinking of these activating receptors with the inhibitory FcγRIIb. This leads to phosphorylation of the FcγRIIb ITIM and consequently recruits the phosphatases that modulate the ITAM driven signalling responses leading to diminished cell responses. (d), Sweeping or internalisation of small immune complexes leading to their removal and, in APC, to enhanced immune responses. (e), Scaffolding in which the FcγR play a passive role. Typically involving FcγRIIb, no signal is generated in the effector cell but "super cross-linking" of the opsonising antibody by the FcγR on one cell generates a signal in the adjacent target cell for example induction of apoptosis or activation in agonistic expansion of cells and /or their secretion of cytokines. In extreme cases this leads to life-threatening cytokine storm.

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Table 1 FcγR responses relevant to therapeutic mAbs

FcγR mediated *MOA*	*Effector Responses*	*Action*	*Dominant receptor*
Activation	• ADCC • ADCP, trogocytosis • Antigen presentation	• Direct killing of target cell • Direct killing of target cell • Vaccine-like immunity post-mAb therapy	• FcγRIIIa; • FcγRIIIa, FcγRIIa • FcγRIIa, FcγRI, FcγRIIIa
Inhibition	• Reduce B cell proliferation or innate cell activation by antibody complexes	• Inhibition of ITAM cell activation i.e. BCR, activating-type FcRi.e.FcγR, FcεRI, FcαRI. NOTE the FcγRIIb must be co-cross-linked with the ITAM activating receptor.	• FcγRIIb
Sweeping	• internalisation	• Removal of small immune complexes	• FcγRIIb
Scaffolding	• Target agonism or apoptosis	• Passive "super-crosslinking" of mAb on opsonised target cell e.g.CD40, CD28, CD20 by FcγR on an adjacent cell	• FcγRIIb; also FcγRIIa, FcγRI?

Table 2: Properties of FcγR.

Receptor	Affinity	IgG specificity	Cell distribution
FcγRI	High	IgG1, IgG3, IgG4	IFN-γ induced on monocytes, neutrophils, macrophages, dendritic cells subpopulations; mast cells.
FcγRIIa	low	IgG1, IgG3, IgG2 (limited to only FcγRIIa-H^{131} form, ~70% people)	All leukocytes and platelets except T and B lymphocytes.
FcγRIIc†	low	IgG1, IgG3, IgG4	NK cells.
FcγRIIIa	Low	IgG1, IgG3. Binding avidity reduced by Phe at position 158	NK cells, macrophages, subpopulation of circulating monocytes, myeloid dendritic cells, neutrophils-at very low levels
FcγRIIIb	low	IgG1, IgG3,	Neutrophils
FcγRIIb	low	IgG1, IgG3, IgG4	B lymphocytes, some monocytes (can be upregulated); basophils; eosinophils?; Plasmacytoid and myeloid dendritic cells; NK cells only of individuals with FcγRIIIb CNV. Airway smooth muscle, LSEC, placenta, follicular DC,

† Expressed in 20% of people.

Table 3 Unique features of IgG subclass Fc and hinge

IgG subclass	FcγR specificity	Light chain attachment	Hinge characteristics	Fc stability	Comment
IgG1	All FcγR	Upper hinge	Light chain attachment Stable core hinge	Stable	Fc is >100X times more stable than IgG4 and IgG2.
IgG2	FcγRIIa His^{131}	CH1 of Fab and/or upper hinge	Stable core hinge with additional inter H–chain disulphide bonds in the upper hinge.	Unstable CH3:CH3	Alternative light chain attachment creates distinct conformers. Unlike IgG4, the CH3:CH3 instability does not lead to half molecule exchange due to stable core and upper hinge disulfides.
IgG4	FcγRI, FcγRIIb, FcγRIIc	CH1 of Fab	Labile core hinge	Unstable CH3:CH3	Combined instability of core hinge and CH3:CH3 permits half molecule exchange

Table 4 Fc or hinge engineered mAbs approved or in advanced clinical development.

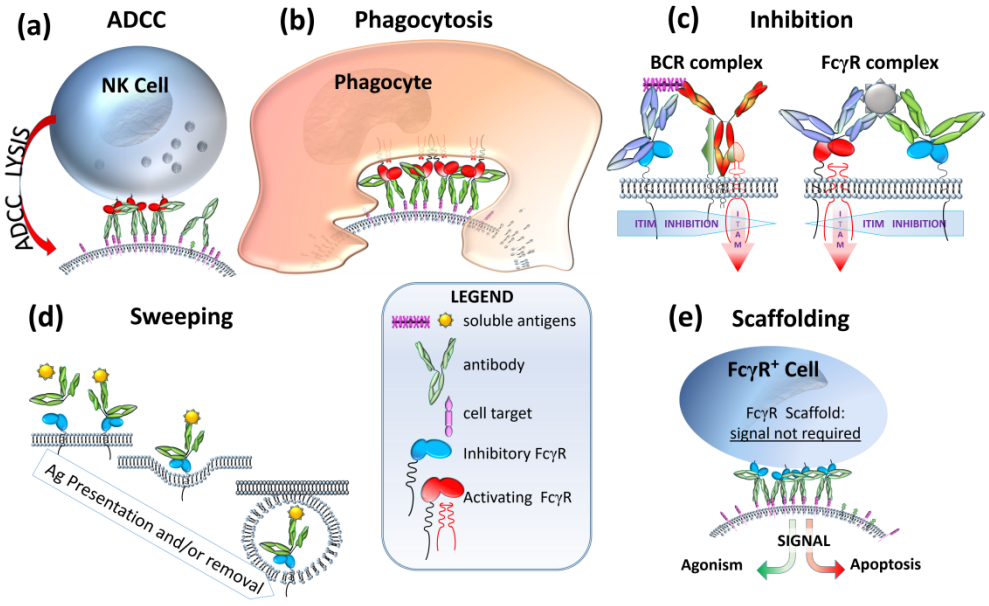
mAb Name	Target	IgG Backbone	Fc modification	Effect on mAb	Therapy Area	Most Advanced Development Stage
Andecaliximab	Matrix Metalloproteinase 9 (MMP9)	IgG4	$S^{228}P$	Stabilise core-hinge	Oncology	Phase III
Anifrolumab	Interferon Alpha/Beta Receptor 1	IgG1	$L^{234}F$; $L^{235}E$; $P^{331}S$	Mimic IgG4 hinge and its CH2/F/G loop; plus ablate FcγR binding	Immunology	Phase III
Atezolizumab	PD-L1	IgG1	Aglycosylated ($N^{297}A$)	Ablate FcγR binding	Oncology	Marketed
Benralizumab	Interleukin 5	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Respiratory Dermatology; Ear Nose Throat Disorders; Gastrointestinal; Hematology;	Marketed

					Immunology;	
Durvalumab	PD-L1	IgG1	$L^{234}F$; $L^{235}E$; $P^{331}S$	Mimic IgG4 hinge and its CH2 F/G loop; plus, ablate FcγR binding	Oncology	Marketed
Evinacumab	Angiopoietin Related Protein 3	IgG4	$S^{228}P$	Stabilise core-hinge	Metabolic Disorders	Phase III
Inebilizumab	CD19	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Central Nervous System; Oncology	Phase III
Ixekizumab	Interleukin 17A	IgG4	$S^{228}P$	Stabilise core hinge	Dermatology; Immunology; Musculoskeletal Disorders	Marketed
Margetuximab	HER2	IgG1	$F^{243}L$; $L^{235}V$; $R^{292}P$; $Y^{300}L$; $P^{396}L$	Selectively enhance FcγRIII interaction	Oncology	Phase III
Mogamulizumab	C-C Chemokine Receptor Type 4	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Central Nervous System; Oncology	Marketed

	(CCR4)					
***Tafasitamab* (*MOR208* Xmab-5574)**	CD19	IgG1	$S^{239}D$; $I^{332}E$	Selectively enhance FcγRIII interaction	Oncology	Phase III
Nivolumab	PD-1	IgG4	$S^{228}P$	Stabilise core hinge	Infectious Disease; Oncology	Marketed
Obinutuzumab	CD20	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Immunology; Oncology	Marketed
Ocaratuzumab	CD20	IgG1	$P^{247}I$; $A^{339}Q$	Selectively enhance FcγRIII interaction	Oncology	Phase III
Pembrolizumab	PD-1	IgG4	$S^{228}P$	Stabilise core hinge	Infection; Oncology	Marketed
Roledumab	Rhesus D	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Hematological Disorders	Phase III
Spesolimab (BI-655130)	IL-36R	IgG1	$L^{234}A$; $L^{235}A$	Ablate FcγR binding	Gastrointestinal; Immunology	Phase III
Teplizumab	CD3	IgG1	$L^{234}A$; $L^{235}A$	Ablate FcγR binding	Metabolic Disorders	Phase II

Tislelizumab	PD-1	IgG4	$S^{228}P$; $E^{233}P$; $F^{234}V$; $L^{235}A$; $D^{265}A$; $L^{309}V$; $R^{409}K$	Stabilise core hinge; Mimic IgG2 lower hinge for restricted FcγR specificity; Ablate FcγR binding; Stabilise CH3 interaction	Oncology	Phase III
Toripalimab (JS 001)	PD-1	IgG4	$S^{228}P$	Stabilise core hinge	Oncology	Phase III
Ublituximab	CD20	IgG1	Afucosylated	Selectively enhance FcγRIII interaction	Central Nervous System; Oncology	Phase III

Figure 1



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