Review

Fc-gamma receptors: Attractive targets for autoimmune drug discovery searching for intelligent therapeutic designs

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Autoantibody immune complexes (ICs) mediate pathogenesis in multiple autoimmune diseases via direct interference with target function, complement fixation, and interaction with Fc-gamma receptors (FcγRs). Through high avidity interactions, ICs are able to crosslink low affinity FcγRs expressed on a wide variety of effector cells, leading to secretion of pro-inflammatory mediators and inducing cytotoxicity, ultimately resulting in tissue injury. Given their relevance in numerous autoimmune diseases, FcγRs have been considered as attractive therapeutic targets for the last three decades. However, a limited number of investigational drug candidates have been developed targeting FcγRs and only a few approved therapeutics have been associated with impacting FcγRs. This review provides a historical overview of the different therapeutic approaches used to target FcγRs for the treatment of autoimmune and inflammatory diseases.

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1. Introduction

Fc-gamma receptors (FcγRs) are a conserved family of cell-surface glycoproteins that recognize the Fc domain of IgG molecules and are expressed by multiple cells and tissues, including most immune cells [1–3]. Through interaction with the Fc domain of IgGs, FcγRs link the adaptive humoral immune response to the effector functions of the cells in the innate immune system, including macrophages, dendritic cells, neutrophils, natural killer cells, and mast cells [4]. The expression of FcγRs varies by cell type and depends on the activation state of the immune cells as well as individual genetic variations in the genes encoding these receptors [2,4].

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In humans, there are six FcγRs, which differ in function, affinity, and distribution across different cell types. Signaling by FcγRs requires cross-linking of receptors, such as caused by binding to immune complexes. All but one of the FcγRs activate immune responses, typically through direct or indirect association with immunoreceptor tyrosine-based activation motif (ITAM) signaling domains [4,5]. In contrast, a single receptor, FcγRIIB, inactivates immune responses through an immunoreceptor tyrosine-based inhibitory motif (ITIM) signaling domain [6]. FcγRI high affinity (nanomolar Kd) towards the Fc portion of immunoglobulins, but the rest of the FcγRs have affinities in the micromolar range and are therefore considered low affinity receptors. Due to this low affinity, these receptors bind strongly to IgG immune complexes (ICs) containing multiple Fc domains [7]. This provides cells with the ability to distinguish between monomeric IgG and immune complexes, and thereby sense when antibody production requires further activation or down-regulation.

ICs mediate inflammatory mechanisms through their engagement of Fcγ receptors expressed in a variety of immune cells. For example, this IC–FcγR interaction can lead to cellular phagocytosis, neutrophil degranulation, cytokine release from myeloid cells, platelet activation, and antigen cross presentation, which may lead to T- and B-cell activation [4]. FcγRs bind the IgG-Fc domain within the N-terminal region of the CH2 domain, near the antibody hinge region, and the interaction depends on the IgG isotype and IgG-Fc glycosylation, providing further points of regulation and specificity in the immune response [4,8,9].

Despite promising research, progress towards the development of therapeutics targeting the FcγR family for autoimmune and inflammatory diseases has been slow, reflecting the complexity of the FcγR biology and competing development interest in soluble mediators and T cell targets. Interest in FcγRs resurfaced in light of proposals that the efficacy of intravenous immunoglobulin (IVIg) is mediated in part through FcRs [1,10]. Transgenic and humanized animal models have now been developed than can partially offset the challenge of generating proof of mechanism, dose estimation, and toxicity signals [11,12]. Consequently, a number of candidate therapeutics have been brought into early development, including agents that enhance regulatory signaling through the inhibitory FcγR, that inhibit the impact of signaling through stimulatory FcγR, or that block effector functions. In this review, we provide an overview of the evolution of drugs and drug candidates targeting FcγRs for the treatment of autoimmune and inflammatory diseases. To simplify the review, we divide the discussion of the therapeutic agents into five categories: IVIg and IgG-Fc fragments, inhibitors of activating FcγRs, modulators of inhibitory FcγRIB, multivalent IgG-Fcs, and small-molecule inhibitors.

2. Intravenous immunoglobulins and Fc-fragments

IVIg is a complex, heterogeneous mixture of predominantly human polyclonal immunoglobulin G (IgG) originally developed as a replacement therapy for the treatment of agammaglobulinemia. However, following a serendipitous observation by Imbach in 1980 that IVIg increased platelet counts in idiopathic thrombocytopenic purpura (ITP) patients, IVIg has become the treatment option for several autoimmune and inflammatory diseases. In a phase 2 study in patients with stable chronic ITP, a single dose of MDX-33 produced marked immunomodulation, with down-regulation of monocyte CD64 for >6 days after dosing [22]. The primary toxicities were chills, fever, headache, and muscle ache [22]. In a phase 2 study in patients with stable chronic ITP, a single dose of MDX-33 transiently elevated platelet counts [23]. Adverse events (AEs) were generally mild and similar to those reported in phase 1 testing. Although these data were generally positive, MDX-33 did not show sufficient promise, and development was discontinued [24].

The murine monoclonal antibody H197, which binds to two epitopes of FcγRI, was evaluated in a patient with refractory ITP. Clinical improvement and down-modulation of monocyte expression of FcγRI was observed but no alteration in platelet counts occurred [25]. No further studies are reported.

3. Therapeutic candidates targeting activating FcγRs

Inhibition of FcγR-mediated cellular activation has long been proposed as an attractive approach to inhibit pro-inflammatory mechanisms and cellular/tissue damage in autoimmune diseases. However, designing optimal therapeutic antagonists has proved challenging due to the complexity of the FcγR system. For example, multiple FcγRs may be involved in concomitant and redundant pro-inflammatory signaling pathways. This redundancy makes it challenging to target one specific FcγR with selective monoclonal antibodies to inhibit broad pro-inflammatory processes. High sequence homology among different FcγRs, including activating and inhibitory receptors such as FcγRIIa, FcγRIIb, and FcγRIIIb, has also complicated the development of specific anti-FcγRs antibodies. Furthermore, broad interactions between immune complexes and different FcγRs are drivers of pro- and anti-inflammatory events. However, the specific structural determinants controlling these interactions are poorly understood, thus making it challenging to design broad antagonists of IC-driven FcγR activation.

Fc-gamma receptors are expressed in humans, including FcγRI, FcγRIIa, FcγRIIb, and FcγRIIIa [11]. Targeting the activating FcγRIIa with an antibody was the first example of a promising therapeutic approach for an autoimmune disease, and over the years, several specific antibodies targeting the activating FcγRs have been developed and subjected to preclinical and clinical testing (Table 1).

3.1. Anti-FcγRI

A humanized version of the murine H22 monoclonal antibody, MDX-33 (Medarex), binds to and downregulates FcγRI on human peripheral monocytes [21]. In a phase 1 study in healthy volunteers (N = 17), MDX-33 produced marked immunomodulation, with down-regulation of monocyte CD64 for >6 days after dosing [22]. The primary toxicities were chills, fever, headache, and muscle ache [22]. In a phase 2 study in patients with stable chronic ITP, a single dose of MDX-33 transiently elevated platelet counts [23]. Adverse events (AEs) were generally mild and similar to those reported in phase 1 testing. Although these data were generally positive, MDX-33 did not show sufficient promise, and development was discontinued [24].

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3.2. Anti-FcγRIIa

A potential role for monoclonal antibodies directed against FcγRIIa in autoimmune disorders has also been investigated [26]. An in vitro study using engineered Fc variant antibodies demonstrated enhanced macrophage phagocytosis of tumor cells [26]. Although to date, no such antibodies have entered clinical trials. TTI-314 (Trillium
Table 1
Therapeutics and therapeutic concepts targeting FcγRs for the treatment of autoimmune and inflammatory diseases.

<table>
<thead>
<tr>
<th>Class</th>
<th>Therapeutic candidate</th>
<th>Product</th>
<th>Stage of development</th>
<th>Summary: benefits/limitations</th>
</tr>
</thead>
</table>
| **Intravenous immunoglobulins and Fc-fragments** | • Intravenous immunoglobulin | IVIg (multiple commercial products available) | • 1980: pilot study in ITP (N = 6) [13,14]  
• 1981: pilot study in ITP (N = 13) [14]  
• 1980s–90s: multiple reports and clinical trials in autoimmune diseases [105]  
• 1984: IVIg approved by the FDA  
• 2016: 9 products currently approved by the FDA for various indications, including ITP, Kawasaki syndrome, and neuropathy [106] | • Platelet count increased in ITP  
• No severe adverse reactions  
• Non-specific |
| **Targeting activating FcγRs** | • Anti-FcγRI | MDX-33 (humanized H22 monoclonal antibody) | • 1997: phase 1 in HV (N = 17) [22]  
• 2000: phase 2 in ITP (N = 30) [23]  
• 2003: reported to be discontinued [24] | • Evidence of immunomodulation  
• Transient increases in platelet counts  
• AEs included chills, fever, headache, muscle ache |
| | • Anti-FcγRIIb | H197 (murine monoclonal antibody) | • 2003: case study in ITP (N = 1) [25]  
• No further reports | • Evidence of immunomodulation  
• No alteration in platelet counts  
• AE of monocytopenia  
• No published data |
| | • Anti-FcγRIIa | TTI-314 | • 2013: discontinued during preclinical development [27] | • Generally short-term increased platelet counts  
• Repeated dosing resulted in attenuated response  
• Patients developed HAMAs  
• 2 patients died from ARDS  
• AEs included chills, fever, nausea and vomiting |
| | • Soluble FcγRIIb | GMA161 (humanized and chemically modified version of 3G8)  
scFv–HSA fusion protein | • 2006: pilot/phase 1 in ITP (N = 4; terminated early due to low enrollment) [30,33,34]  
• 2011: reported to be discontinued [35]  
• 2016: preclinical data only [36] | • Short-lived increased platelets  
• Transient WBC decreases  
• AEs included chills, fever, and vomiting  
• Shows promise in murine ITP model  
• Potential for reduced toxicity  
• Unclear  
• Ameliorated symptoms in 75% of ITP patients and 40% of SLE patients  
• AEs included infusion site reactions and WBC decrease  
• No anti-drug antibodies reported  
• Unclear  
• May be able to overcome resistance in B-cell malignancies  
• Effect in autoimmune diseases is unexplored  
• Reduced disease activity levels in 33% of RA patients  
• AEs included nausea, vomiting, diarrhea  
• Serious infusion-related reaction and venous thrombosis  
• Appears to be in development for subjects with allergies  
• Potential for efficacy in SLE  
• Favorable safety profile in non-human primates  
• May be able to increase treatment efficacy  
• Not yet tested in humans |
| | • Anti-FcγRIIb | hu286/MCA 321  
SM01/BAX 1810 | • 2014: preclinical data only [37]  
• 2012: phase 1b/2a in ITP (N = 36) [40]  
• 2014: phase 2a in SLE (N = 51) [42]  
• 2016: phase 2 in IgA nephropathy (withdrawn) [44]  
• Development ongoing  
• 2007: preclinical data only [51]  
• 2016: reported to be discontinued [52]  
• 2015: preclinical data only [47] | • Unclear  
• AEs included chills, fever, and vomiting  
• Shows promise in murine ITP model  
• Potential for reduced toxicity  
• No published data  
• No anti-drug antibodies reported  
• Unclear  
• May be able to overcome resistance in B-cell malignancies  
• Effect in autoimmune diseases is unexplored  
• Reduced disease activity levels in 33% of RA patients  
• AEs included nausea, vomiting, diarrhea  
• Serious infusion-related reaction and venous thrombosis  
• Appears to be in development for subjects with allergies  
• Potential for efficacy in SLE  
• Favorable safety profile in non-human primates  
• May be able to increase treatment efficacy  
• Not yet tested in humans |
| | • Forced co-localization of BCR and FcγRIIb | XmA5871 | • 2015: phase 1b/2a in RA (N = 57) [55]  
• Development ongoing  
• 2016: phase 1 in HV (N = 72) [58] | • AEs included infusion site reactions and WBC decrease  
• Phase 3 results in ITP available in 2016  
• Safety profile may have impacted on efficacy in RA  
• Phase 3 results in ITP available in 2016 |
| **Recombinant multimeric IgG-Fc** | • Stradomer | GL-2045 | • 2015: preclinical data only [72]  
• Development ongoing  
• 2016: preclinical data only [80] | • Oral administration  
• Efficacy in animal models of RA  
• AEs include postural dizziness  
• Oral administration  
• Efficacious in ITP and lymphoma  
• Mixed results from RA  
• AEs include GI-related toxicities, fatigue, cytopenias, hypertension, and liver enzyme abnormalities  
• Up to 7% of RA patients experienced serious AEs, and 3 patients died  
• Safety profile may have impacted on efficacy in RA  
| | • SIF | M230 | • Development ongoing  
• 2016: phase 1 in HV (N = 48) [60] | • Oral administration  
• Efficacy in animal models of RA  
• AEs include postural dizziness  
• Oral administration  
• Efficacious in ITP and lymphoma  
• Mixed results from RA  
• AEs include GI-related toxicities, fatigue, cytopenias, hypertension, and liver enzyme abnormalities  
• Up to 7% of RA patients experienced serious AEs, and 3 patients died  
• Safety profile may have impacted on efficacy in RA  
| | • Hexamer | HexaGard | • 2016: awaiting commercialization [79]  
• 2006: phase 1 in HV (N = 35) [83] | • Oral administration  
• Efficacy in animal models of RA  
• AEs include postural dizziness  
• Oral administration  
• Efficacious in ITP and lymphoma  
• Mixed results from RA  
• AEs include GI-related toxicities, fatigue, cytopenias, hypertension, and liver enzyme abnormalities  
• Up to 7% of RA patients experienced serious AEs, and 3 patients died  
• Safety profile may have impacted on efficacy in RA  
| | • Syk inhibitors | R406 | • Oral administration  
• Efficacy in animal models of RA  
• AEs include postural dizziness  
• Oral administration  
• Efficacious in ITP and lymphoma  
• Mixed results from RA  
• AEs include GI-related toxicities, fatigue, cytopenias, hypertension, and liver enzyme abnormalities  
• Up to 7% of RA patients experienced serious AEs, and 3 patients died  
• Safety profile may have impacted on efficacy in RA  
| | • R788/fostamatinib | • 2006: phase 1 in HV (N = 100) [84]  
• 2008–2015: phase 2 in RA (4 studies, N = 1144) [86–89]  
• 2009: phase 2 in ITP (N = 16) [82]  
• 2010: phase 2 in NHL and CLL (N = 68) [85]  
• 2014: phase 3 in RA (2 studies, N = 1241) [90,91]  
• 2014: development in RA terminated  
• 2015: orphan drug designation for ITP [92]  
• 2016: phase 3 in ITP underway (3 studies) [93–95] | • Oral administration  
• Efficacy in animal models of RA  
• AEs include postural dizziness  
• Oral administration  
• Efficacious in ITP and lymphoma  
• Mixed results from RA  
• AEs include GI-related toxicities, fatigue, cytopenias, hypertension, and liver enzyme abnormalities  
• Up to 7% of RA patients experienced serious AEs, and 3 patients died  
• Safety profile may have impacted on efficacy in RA  

(continued on next page)
Therapeutics) was in preclinical development for disorders including systemic lupus erythematosus (SLE), RA, and ITP, but the program was discontinued in 2013 [27].

3.3. Anti-FcyRIIlra

In small studies and a case report, the mouse monoclonal anti-FcyRIIa antibody (3G8) demonstrated increases in platelet counts in patients with ITP [28–31]. The case report discussed a patient who was intravenously infused with two doses of 3G8 (25 mg and 42 mg). Doses were administered a week apart [28]. After receiving the first dose the number of platelets increased to 22,000 mm$^3$ in 6 h and continued to rise to a peak of 305,000 mm$^3$ until receiving the second dose [28]. In one of the small studies, patients (N = 7) were infused with an initial dose of 3G8 25 mg. In the first week, subsequent infusions were given to patients whose platelet counts did not respond to the initial infusion. Five patients had significant increases in platelet counts; 4 of these required just a single infusion [29]. In another study, 11 patients with low platelet counts after splenectomy were infused with 3G8 25 mg, with 55% (6/11) patients responding to treatment [30]. In another study, an HIV-positive patient with refractory ITP was infused with 3 doses (25 mg, 25 mg, and 50 mg) of 3G8 in 8 days [31]. The patient’s platelet count initially lowered and then rebounded to a higher level with each injection, although the 50 mg dose demonstrated an increase of up to 60,000 mm$^3$ [31]. In each of these studies, the improvements were generally not sustained after infusion (i.e., platelet counts decreased in the weeks following treatment), and the antibody proved suboptimal for further clinical development due to significant immunogenicity. Several patients developed high titers of human anti-mouse antibodies, 2 patients with pre-existing lung conditions died shortly after infusion from acute respiratory distress syndrome, and fever, chills, and vomiting were commonly reported [28–31].

A second-generation humanized anti-FcyRIIa antibody (GMA161, Genzyme/MacroGenics) was subsequently engineered, with the Fc portion devoid of carbohydrates, in an attempt to reduce binding to Fc receptors, minimize neutrophil and monocyte depletion, and limit cytokine release [32]. An open-label, single ascending-dose study in patients with ITP who had failed splenectomy and previous therapies was terminated early due to low enrollment [33]. Two of the first 4 patients who received GMA161 had short-lived increases in platelet counts and decreases in white blood cells [30,34]. As with 3G8, AEs included fever, chills, and vomiting [30,34]. A subsequently published non-clinical evaluation study for GMA161 in human transgenic mouse models containing both FcyRIIa and FcyRIIib suggested atypical hypersensitivity reactions mediated by platelet activating factor (PAF) [32], and development was discontinued [35].

More recently, novel approaches have emerged to leverage the therapeutic efficacy of anti-FcyRIIa antibodies while circumventing their original limitations of suboptimal efficacy and high toxicity. Because modifications to the Fc region of GMA161 did not improve toxicity, some researchers focused on development of a monovalent Fab fragment for ITP therapy. One example is the anti-FcyRIIa–Fab–albumin fusion protein, scFv–HSA, which retained the ability to inhibit the IgG–FcyRIIa interaction in vitro [36]. A murine version of scFv–HSA has demonstrated efficacy in ITP mouse models, suggesting that AEs associated with previous anti-FcyRIIa antibodies may be overcome by monovalent Fab–Y blockade [36].

Other research has focused on building antibodies with enhanced binding capabilities to FcyRIIa, using glyco-engineering techniques to remove fucose moieties from the Fc region of the antibody [37]. This approach appears to induce higher rates of cellular toxicity in vitro [37] and has proven beneficial in the development of several anti-cancer therapeutics. It remains unclear whether such modifications will prove useful for the clinical treatment of autoimmune diseases.

4. Therapeutic candidates targeting inhibitory FcyRIIib

FcyRIIib is the primary inhibitory/regulatory Fc receptor in humans, and the primary FcyR expressed on B cells [1]. Activation of B cells by specific antigens through the B-cell receptor (BCR) leads to activation, proliferation, differentiation, specific antibody production, and initiation of a regulatory process that involves FcyRIIib expression [1]. When sufficient antibody is generated to engage most of the antigen in immune complexes (IC), IC co-localizes the BCR and FcyRIIib on antigen-specific B cells, leading to cell cycle arrest and regulation of inflammatory mediators, including IL-4, LPS, and BAFF [38]. Multiple lines of evidence indicate that dysfunction of FcyRIIib-dependent B-cell regulation contributes to failure of peripheral tolerance in systemic lupus (SLE), lupus nephritis (LN), chronic inflammatory demyelinating neuropathy (CIDP), and other autoimmune diseases, and that restoration of function may reduce production of autoantibodies and ameliorate disease [1,38,39]. Several novel approaches to therapeutic targeting of FcyRIIib are currently under investigation (Table 1).

4.1. Soluble FcyRIIib

A recombinant, soluble, human FcyRIIib (SM101; Baxalta [formerly SuppreMol]) is thought to compete with membrane-bound FcyR in the binding of ICs, resulting in down-modulation of the immune response [40]. SM101 has received orphan drug status for ITP in the US and Europe [41]. In ITP patients, treatment with SM101 resulted in sustained increases in platelet counts [40]. Treatment-related AEs included leukopenia/neutropenia and infusion site reaction and no anti-
drug antibodies were detected [40]. Promising results were also reported in a study of patients with SLE ± LN: 39% (n = 18) and 36% (n = 22) of patients responded to treatment with 6 and 12 mg/kg SM101, respectively, vs 18% (n = 11) of placebo-treated patients [42]. No safety signals attributable specifically to SM101 were reported [42]. SM101 remains in early clinical development [43], although a phase 2 trial in patients with immunoglobulin A nephropathy was recently withdrawn [44], and no other trials are ongoing.

4.2. Anti-FcγRIlb

SM201 (Baxalta [formerly SuppreMol]), a monoclonal antibody specific to FcγRIlb, which targets activated B cells but does not affect resting B cells, is in development. This would potentially allow down-modulation of autoreactive B cells in autoimmune diseases, while retaining a functional memory response [45]. SM201 is in preclinical development, as are several other anti-FcγRIlb antibodies (SM211 and SM301) [43,46].

In preclinical studies, the FcγRIlb-blocking antibody BI-1206 (Biovint) was evaluated in chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) FhγRIlb-transgenic mice models [47]. The CLL model utilized primary human CLL cells that proliferated in clusters, whereas the MCL model involved immunodeficient mice engrafted with Jeko or primary MCL cells to model human conditions [47]. A tumor-suppressing effect that was able to overcome resistance to treatment with the CD20-specific antibody rituximab by preventing drug destruction was observed [47]. BI-1206 is scheduled to enter phase 1/2 clinical testing shortly to evaluate BI-1206 alone or in combination with rituximab for B-cell malignancy [48,49]. BI-1206 has not been evaluated in autoimmune disease.

hu2B6-3.5 ([MacroGenics]) is a humanized monoclonal antibody specific to FcγRIlb, capable of directing cytotoxicity against mononuclear cells in vitro and able to reduce tumor growth rates in mouse xenograft models of human B-cell lymphoma [50,51]. It was hypothesized that such antibodies could be used to aggregate FcγRIlb to generate a pro-apoptotic signal independent of the BCR: to date, no clinical data have been generated [52], and the company is developing dual affinity antibodies such as MGD010 (discussed later).

4.3. Forced co-localization of BCR and FcγRIlb

One potential mechanism which could be exploited for therapeutic intervention in autoimmune diseases is the forced co-localization of BCR and FcγRIlb independent of cognate antigen. Researchers have produced a number of antibodies targeting B cells which have been engineered to bind selectively, and with high affinity, to FcγRIlb.

XmAb5871 (Xencor) contains an Fc domain with high affinity for FcγRIlb combined with a humanized Fv domain that recognizes human CD19 [38]. This antibody targets all CD19 B cells, blocking BCR-induced activation and suppressing the immune response in severe combined immunodeficiency mice [38]. Preclinical data also suggest a role in the treatment of RA [53,54]. In RA patients, XmAb5871 10 mg/kg reduced disease activity levels; AEs included nausea, vomiting, infusion-related reactions, and venous thrombosis [55]. Development of this product for the treatment of moderate to severe RA and IgG4-related disease is ongoing [56].

XmAb7195 (Xencor) contains a mutated Fc domain with high affinity for FcγRIlb, combined with a humanized Fv domain that engages only IgE, effectively sequestering IgE [57]. This antibody is currently in phase 1 trials in healthy volunteers and patients with allergies [58].

MGD010 (MacroGenics) is a bi-specific molecule that co-ligates the inhibitory FcγRIlb and the BCR component CD79B on B cells. In ex vivo samples from patients with autoimmune disorders, MGD010 blocked B-cell activation, and in animal models, it was able to inhibit humoral immune response [59]. MGD010 entered clinical development in 2015, and a phase 1 study in healthy volunteers is ongoing [60,61].

5. Indirect modulation of FcγRIlb

Indirect modulation of the inhibitory FcγRIlb has emerged as one of the proposed anti-inflammatory mechanisms of action of IVIg [1,2,10]. Clinical studies have shown a relationship between the levels of Fc-sialylation in circulating antibodies and diseases severity as well as an increase in Fc-sialylation upon treatment with IVIg [62–64]. More importantly, in a series of studies in animal models of autoimmune diseases, it was demonstrated that Fc-sialylation serves as an anti-inflammatory modulator through indirect upregulation of FcγRIlb [65–67]. These findings led to the development of a tetra-Fc-sialylated IVIg therapeutic candidate which exhibited a significant enhancement of anti-inflammatory activity over IVIg across different animal models of autoimmune diseases [68]. This candidate is currently in preclinical development [69].

6. Recombinant oligomeric IgG-Fc

Commercial IVIg preparations contain a small fraction of higher order IgG structures (i.e., dimers and aggregates) which have been proposed to be important for IVIg anti-inflammatory activity [70]. These observations have stimulated the development of recombinant oligomeric Fc proteins as potential replacement to IVIg [71]. Stradomer (GL-2045) generated by Gliknik/Pfizer [72] is a drug candidate consisting of a mixture of heterogeneous IgG-Fc oligomeric structures (which prevented the onset of ITP) [73] and ameliorated symptoms in other autoimmune diseases including collagen-induced arthritis [73], experimental autoimmune neuritis [74], and autoimmune myasthenia gravis [75]. This drug candidate is in preclinical development, and in 2015, it received orphan drug status in the US for the treatment of chronic inflammatory demyelinating polyradiculoneuropathy [76]. Hexameric Fc proteins with altered effector functions were also generated by Richard Pleass as potential alternatives to IVIg [77,78] although these proteins have been shown to activate complement pathways [77]. These proteins have yet to advance to the clinic, although researchers claim that it should be more effective and less expensive to produce than IVlg [79].

More recently, we have introduced the concept of selectively antagonizing the activating FcyR system without activating pro-inflammatory pathways. Through novel insights on the modulation of the FcγR pathways, a trivalent-IgG-Fc recombinant product candidate, M230 was designed (manuscript in preparation). M230 selectively immune-modulates the FcγR system and has been shown to have 40-fold greater potency than IVIg in animal models of autoimmune diseases such as arthritis and immune thrombocytopenia. This drug candidate is currently in preclinical development [80].

7. Small-molecule inhibitors

As an alternative to engineered antibodies, there is also great interest in the development of orally administered, small-molecule agents targeting key protein kinases involved in downstream FcyR signaling and immune regulation (Table 1).

7.1. Syk inhibitors

One potential target for autoimmune therapy is spleen tyrosine kinase (Syk), a non-receptor tyrosine protein kinase critical for B-cell activation and FcyR signaling [81]. In ITP, autoantibodies to platelet glycoproteins crosslink FcyRs on macrophages, which results in the recruitment of Syk, and ultimately leads to the phagocytic engulfment of antibody-opsonized platelets. Because Syk has a central role in FcyR-mediated signal transduction and propagation of the inflammatory response, it is thought that blocking Syk enzymatic activity may reduce phagocytic macrophage activity and ameliorate platelet destruction [82]. Syk also likely plays key roles in RA, SLE, multiple sclerosis, and

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allergies as demonstrated in FcγR-deficient mouse and animal models [81].

R406 (Rigel Pharmaceuticals) is an orally available, potent inhibitor of FcγR signaling which primarily targets Syk [83]. In mouse models of arthritis, R406 inhibited paw inflammation and reduced joint damage [83]. R406 was well-tolerated in the 27 healthy volunteers that received it (N = 35) and postural dizziness was the only reported AE [83]. The company is focusing further development on a prodrug of R406 (R788), which was engineered to improve bioavailability for potential oral administration [84].

Fostamatinib disodium (R788; Rigel Pharmaceuticals), like R406, was investigated in phase 2 studies in ITP, lymphoma/lungroma, and RA. Each of these studies evaluated different treatment regimens in terms of dose and duration of treatment in order to assess fostamatinib efficacy. In patients (N = 16) with chronic, refractory ITP, fostamatinib induced sustained improvement in platelet count in 50% (8/16) of patients [82]. In heavily pre-treated and relapsed patients with non-Hodgkin lymphoma or CLL (N = 68), fostamatinib treatment resulted in overall response rates of 10% for patients with follicular lymphoma and 55% for patients with small lymphocytic leukemia/CLL [85]. Results from 4 RA studies were mixed. Two reported significantly greater (57–65%) American College of Rheumatology 20% (ACR20) responses with fostamatinib than with placebo (p < 0.01) [86,87]. However, in RA patients (N = 219) who had failed biologic therapy, no difference from placebo was observed [88], although a higher proportion of patients in the R788 group was receiving prednisone (42% vs 31%) and failed to respond to ≥ 3 biologic therapies (21% vs 12%) at baseline in this study [88]. In a study in patients with RA not currently receiving DMARDs (N = 279), fostamatinib was superior to placebo but statistically significantly inferior to adalimumab [89]. Common toxicities were gastrointestinal (diarrhea, nausea, vomiting), fatigue, cytopenias, hypertension, and alterations in liver enzymes [82,85–89].

Two phase 3 RA studies, OSIKA-1 (N = 918) and OSIKA-3 (N = 323), showed greater ACR20 responses but no difference in radiographic progression compared to placebo [90,91]. Significantly more patients treated with fostamatinib achieved ACR20 at week 24 compared with placebo: 49.0% for fostamatinib 100 mg twice daily for 52 weeks vs 34.2% for placebo (95% CI 8–22%, p < 0.001) and 44.4% for fostamatinib 100 mg twice daily for 4 weeks vs 34.2% for placebo (95% CI 3–18%, p = 0.0006) [90] in the OSIKA-1 study and 36.2% for fostamatinib 100 mg twice daily for 24 weeks vs 21.1% for placebo (p = 0.004) in the OSIKA-3 study [91]. The safety profile was generally consistent with the phase 2 studies, although a large proportion of patients (up to 7%) reported serious AEs, and three deaths occurred [90,91]. Based on these results, development in RA was halted [90,91]. In 2015, Rigel received orphan drug designation from the FDA for fostamatinib in ITP [92]. Three phase 3 clinical studies are currently underway in patients with ITP [93–95], and results are expected later in 2016.

Rigel also began development of another Syk inhibitor, R112, for the treatment of allergies. Initially, R112 was reported as effective in rapid reduction of symptoms of allergic rhinitis [96], but a second phase 2 study failed to show a statistically significant difference from placebo [97]. Development was subsequently halted.

GS-9973 (entsolentinib; Gilead), an inhibitor of Syk with high selectivity against a panel of 359 kinases tested in the KINOMEScan® platform (DiscoverX Corporation, Fremont, CA) [98], is in phase 2 development for hematologic malignancies [99]. A second Syk inhibitor from Gilead, GS-9878, is in phase 1 development for autoimmune disorders; no data are available to date [99].

Cerdelagnil (Portola Pharmaceuticals), a dual inhibitor of Syk and Janus kinase (Jak) family kinases, was reported to suppress inflammation and autoantibody generation in a rat model of arthritis [100]. Clinical development has been in B-cell leukemia and lymphoma, although proof-of-concept studies in autoimmune disease are also planned [100, 101].

PR72761 (Portola Pharmaceuticals) is a potent and selective inhibitor of Syk, which may be efficacious in treating allergies. No preclinical or clinical data have been reported thus far [102].

Another selective Syk inhibitor, SKI-O-703 (Genosco), will begin phase 1 development for autoimmune diseases including ITP, RA, and SLE [103]. No further information on this molecule has been made available.

### 7.2. Pyrazole derivatives

Pyrazole derivatives have been shown to effectively inhibit FcγR-mediated phagocytosis. Research is ongoing to produce derivatives with increased solubility and potency for further development in a clinical environment [104].

#### Take-home messages

- Significant unmet needs still exists in the management of autoimmune diseases.
- Given their central role in modulating the inflammatory state of a wide variety of immune cells and tissues, FcγRs remain attractive therapeutic targets for numerous autoimmune diseases.
- Although no therapeutics specifically targeting FcγRs are currently approved for the treatment of autoimmune diseases, continued progress in the characterization of the molecular and cellular basis of the FcγR biology is promoting the development of new and promising therapeutic designs.

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