Biologic response modifiers for granulomatosis with polyangiitis (Wegener's): from promise to reality

Advances in the treatment of granulomatosis with polyangiitis (GPA; formerly Wegener's granulomatosis) have resulted in marked improvements in patient outcomes. While severe GPA was originally described as progressive and invariably fatal, the use of immunosuppressive therapy has transformed the disease into a manageable, chronically relapsing illness. However, standard immunosuppressive therapies are associated with numerous side effects and significant treatment failures. In an effort to provide safer and more effective treatments for patients with GPA, several alternative therapies have been investigated in recent years, most notably among biologic response modifiers. The use of biologic response modifiers in GPA has had mixed results. The efficacy of rituximab in GPA is now firmly established, while inhibitors of TNF-α have not shown clear benefits. Investigations into other biologic response modifiers in GPA remain preliminary, but hold promise for a future in which the treatment of GPA achieves further improvements in outcomes with fewer side effects.

KEYWORDS: alemtuzumab ■ ANCA-associated vasculitis ■ B-cell activating factor ■ biologic response modifiers ■ CTLA-4 antigen ■ granulomatosis with polyangiitis ■ rituximab ■ TNF-α ■ Wegener's granulomatosis

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The antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) comprise a group of three heterogeneous syndromes: granulomatosis with polyangiitis (GPA; formerly Wegener's granulomatosis [WG]), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA; formerly Churg–Strauss syndrome) [1]. These three entities share the histopathologic feature of small-vessel vasculitis; that is, inflammation and necrosis of blood vessel walls. In addition, the majority of patients with AAV have ANCA detectable in the serum at the time of initial presentation [2–4].

GPA is the most common AAV in European and North American populations, with an incidence of 8–10 cases per million per year [5–7]. GPA characteristically involves necrotizing granulomatous inflammation, affecting the upper and lower respiratory tract, which distinguishes it from MPA. The GPA syndrome does not include asthma or eosinophilia, which are defining characteristics of EGPA [1]. GPA also differs from MPA and EGPA on the basis of its associated ANCA. Two types of ANCA are of clinical significance in patients with vasculitis: ANCA causing a cytoplasmic immunofluorescence pattern on ethanol-fixed neutrophils and reacting with proteinase 3 (PR3-ANCA), and ANCA causing a perinuclear immunofluorescence pattern on ethanol-fixed neutrophils and reacting with myeloperoxidase (MPO-ANCA) [8]. PR3-ANCA occurs in the vast majority of patients with GPA, while MPO-ANCA occurs far less frequently. By contrast, MPO-ANCA is the predominant type of ANCA in patients with both MPA and EGPA.

Untreated, severe GPA follows a progressive course with an invariably fatal outcome [9]. The use of cyclophosphamide (CYC) in combination with glucocorticosteroids (GCS) heralded a major therapeutic breakthrough in GPA, transforming the disease to a manageable, chronically relapsing illness [9,10]. Treatment with CYC and GCS has remained the standard therapy for remission induction in patients with GPA for decades. However, not all patients respond satisfactorily to CYC and GCS. Approximately 10% of patients do not achieve remission with these medications, and up to half of the patients who initially achieve remission relapse within the first 3–5 years. Moreover, long-term and repeated use of CYC is associated with substantial toxicity, which is dependent on the cumulative dose applied over the patient's disease course [11–14].

The search for less toxic alternatives to CYC led to the introduction of methotrexate (MTX) as the standard for remission induction in patients with GPA with limited or nonsevere disease manifestations [15,16]. The use of CYC in GPA has been further curtailed by the realization that CYC does not need to be continued

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for years, but can be replaced after 3–6 months of remission induction therapy by MTX or azathioprine (AZA) for more long-term remission maintenance [17,18].

Biologic response modifiers (BRMs) provide an alternative therapeutic modality in the treatment of GPA. BRMs exert targeted effects on specific immune pathways with the goal of reducing maladaptive inflammation [19]. By modulating the actions of the immune system, BRMs have proven useful in the management of a variety of autoimmune diseases, including autoimmune vasculitides. Among the agents in clinical use, BRMs act via depletion of B lymphocytes, inhibition of cytokine signaling and interference with T-lymphocyte activity. As summarized in Table 1, several BRMs have been investigated in GPA with the goal of improving treatment outcomes or limiting treatment-associated toxicities. This review aims to provide a perspective on the current knowledge about the treatment of GPA with BRMs by summarizing their actions, theoretical benefits and clinical outcomes.

Therapy directed against B lymphocytes

Rationale for targeting B lymphocytes in GPA

B lymphocytes have been implicated in the pathogenesis of GPA for more than two decades [20,21]. B lymphocytes are essential for the production of ANCA, which is probably a key contributor to the pathogenesis of the disease. In addition, the frequency of activated B lymphocytes has been found to be associated with both disease activity and severity [22]. B lymphocytes have been proposed as the primary target of the therapeutic effects of CYC in AAV and, thus, targeting B lymphocytes with BRMs held particular promise for GPA [20,21,23].

Table 1. Summary of biologic response modifiers investigated in granulomatosis with polyangiitis.

Target	Name	Proposed mechanisms
B lymphocytes		
CD20	Rituximab	B-lymphocyte depletion via antibody-dependent cellular cytotoxicity, complement-mediated lysis, apoptosis and sensitization to cytotoxic agents or steroids
Cytokines		
TNF-α	Etanercept, infliximab and adalimumab	Inhibition of inflammatory cascade via reduced induction of proinflammatory cytokines
BAFF	Belimumab	Reduced survival of autoreactive B lymphocytes
T lymphocytes		
CD80/CD86/ CTLA-4	Abatacept	Inhibition of T-lymphocyte activation via reduced costimulatory CD28 signaling
CD52	Alemtuzumab	T-lymphocyte depletion via complement-mediated cell lysis, cell-mediated cytotoxicity and apoptosis

BAFF: B-cell activating factor; CTLA: Cytotoxic T-lymphocyte-associated antigen.

Rituximab

Rituximab (RTX) is a chimeric monoclonal antibody directed against the cell-surface protein CD20, a 297-amino acid phosphoprotein with four transmembrane domains, which is selectively expressed on cells of B-lymphocyte lineage [24,25]. Binding of RTX to CD20 causes the death of the target cell. The exact mechanisms of RTX-mediated cell death remain unclear, but *in vitro* studies have demonstrated that RTX induces antibody-dependent cellular cytotoxicity via macrophages and natural killer cells, complement-mediated B-lymphocyte lysis, apoptosis and sensitization to cytotoxic agents or steroids [26–31]. Following treatment with RTX, circulating B lymphocytes remain undetectable in the peripheral blood for approximately 6–12 months [32,33]. However, despite depletion in the circulating peripheral blood, B lymphocytes may persist in the bone marrow, spleen, lymph nodes and pathologic inflammatory tissue [34].

The first published use of RTX in GPA was reported in 2001 [23]. A 66-year-old male diagnosed with GPA in 1994 had developed CYC toxicity, precluding its further use, while prednisone in combination with AZA or mycophenolate mofetil failed to restore remission during a disease flare. This history prompted the use of RTX on a compassionate-use basis under the hypothesis that remission could be induced by B-lymphocyte depletion, with consequent removal of ANCA. The treatment regimen consisted of four weekly RTX doses at 375 mg/m^2, accompanied by a prednisone taper. This dosing regimen had been approved by the US FDA in 1997 for the use in relapsed or refractory, low-grade or follicular B-cell non-Hodgkin's lymphoma. Remission was achieved quickly in this patient and prednisone could be discontinued.

Following the success of this index case, RTX was used on a compassionate basis in an additional ten patients with severe refractory AAV, nine of whom had GPA [33]. All of these patients received RTX as four weekly doses of 375 mg/m^2, in combination with oral prednisone for remission induction. While the majority received intravenous methylprednisolone

and three patients underwent plasma-exchange immediately prior to receiving the first RTX dose, all other immunosuppressive agents had been discontinued. Clinical remission was induced in all patients and adverse effects were minimal.

Building on this favorable experience, Keogh *et al.* conducted an open-label pilot trial of RTX in conjunction with a strictly protocolized oral glucocorticoid-tapering regimen [35]. Ten patients with active, severe, refractory AAV – all of whom had PR3-ANCA – were treated with RTX. All patients achieved remission by 3 months and, again, adverse effects were minimal. Subsequently, at least 20 additional case series and uncontrolled studies have been published describing the use of RTX in patients with refractory AAV, including 171 patients with GPA [36]. Most of these studies describe treatment success, with an excellent overall rate of complete remission. Two subsequent randomized controlled trials have firmly established the efficacy of RTX in GPA [37,38]. **Table 2** provides a comparison of these two trials.

The RAVE trial was a multicenter randomized, double-blind, double placebo-controlled trial that compared the efficacy and safety of RTX with CYC for remission induction in severe AAV [37]. A total of 197 patients were enrolled, including 147 with GPA. All subjects were positive for ANCA. The patients were randomized to receive RTX (four weekly intravenous doses of 375 mg/m^2) or CYC (2 mg/kg/day orally). In addition, all patients received GCS treatment, consisting of one to three daily doses of 1000 mg of intravenous methylprednisolone, followed by a protocolized tapering regimen of prednisone. By the end of month 5, prednisone was completely discontinued. Once remission was achieved between months 3 and 6, patients receiving CYC were switched to maintenance therapy with AZA, while patients on RTX were switched to an AZA placebo. The study's primary end point was disease remission, defined as a Birmingham Vasculitis Activity Score/WG (BVAS/WG) of 0, in the absence of GCS therapy at month six. Patients were stratified by ANCA type across the two treatment arms, and randomization resulted in patients with GPA being equally distributed. In total, 63 (64%) of the patients in the RTX arm versus 52 (53%) in the CYC arm achieved the primary end point, which met criteria for noninferiority ($p < 0.0001$), but not superiority ($p = 0.09$) for the RTX group. The ANCA type did not affect the primary comparison when examined in a logistic regression model and, among the subset of patients with GPA, the achievement of the primary end point was also similar between the two treatment groups (RTX: 63%, CYC: 50%; $p = 0.11$). Among the 101 patients with severe relapses upon enrollment, RTX was more efficacious than CYC, with 67% of the RTX group achieving the primary end point, as compared with 42% of the CYC group ($p = 0.01$). The number of total adverse events, serious adverse events or nondisease-related adverse events was also similar between the RTX and CYC groups. On extended follow-up, the rates of sustained remission remained similar between the two groups at 12 and 18 months, and no unexpected safety issues were detected [39].

Another major randomized controlled study RITUXVAS was initiated [38]. RITUXVAS was an open-label, two-group, parallel-design, randomized trial involving 44 patients with newly diagnosed AAV. All patients were positive for ANCA and all had evidence of renal involvement, as evidenced by biopsy demonstrating necrotizing glomerulonephritis or by urinalysis demonstrating hematuria or red cell casts. In total, 22 of the patients had GPA. The patients were randomized 3:1 to receive a RTX-based regimen or standard therapy. Prior to enrolling in the study, 25% of the patients underwent plasma exchange. After enrollment, all patients received a standard glucocorticoid regimen, including intravenous methylprednisolone. The 33 patients randomized to the RTX group received RTX (four weekly intravenous doses of 375 mg/m^2) with two intravenous CYC pulses given concomitantly with the first and third RTX infusion. The 11 patients randomized to the control group received intravenous CYC pulses for 3–6 months followed by AZA administration. The primary outcomes were sustained remission (defined as BVAS of 0 for 6 months) and rates of severe adverse events at 12 months. The frequency of sustained remission was similar in the two treatment groups (76% in the RTX group vs 82% in the control group; $p = 0.68$). The incidence rates of severe adverse events were also similar (1.00 per patient-year in the RTX group vs 1.10 per patient-year in the control group; $p = 0.77$) and 18% of the patients died in both groups. On extended follow-up, rates of relapse, death and end-stage renal disease were similar between the treatment groups at 2 years [40].

Both RAVE and RITUXVAS included the frequency of disease relapses as important secondary outcomes. In RITUXVAS, 15% of the patients in the RTX group and 10% in the control group had a relapse during the 12-month

Table 2. Comparison of two randomized controlled trials investigating rituximab in antineutrophil cytoplasmic antibody-associated vasculitis.

Trial feature	**RAVE** [37]	**RITUXVAS** [38]
Trial design	Randomized, double-blind, double-dummy, noninferiority trial	Randomized, open-label, two-group, parallel-designed trial
Patients enrolled (n)	197	44
Disease-associated inclusion criteria	ANCA-positive, severe AAV, either newly diagnosed (49%) or relapsing (51%)	ANCA-positive, newly diagnosed severe AAV with renal involvement
AAV subtype	GPA: 148 patients MPA: 48 patients Undetermined: one patient	GPA: 22 patients MPA: 16 patients Renal-limited vasculitis: six patient
Induction therapy in RTX group	RTX plus GCS	RTX plus CYC plus GCS
Induction therapy in CYC/control group	CYC plus GCS	CYC plus GCS
Maintenance therapy in RTX group	Placebo	Low-dose GCS
Maintenance therapy in CYC/control group	AZA	AZA plus low-dose GCS
Primary end points	Remission (absence of disease activity) plus completion of GCS taper at 6 months	• Sustained remission (absence of disease activity for at least 6 months) • Severe adverse events
Main outcomes	Primary end point achieved in 64% in RTX group and 53% in CYC group (RTX noninferior; $p < 0.0001$)	• Sustained remission achieved in 76% of RTX group and 82% of control group (RTX not superior; $p = 0.68$) • Incidence rates of severe adverse events per patient-year: 1.00 in RTX group and 1.10 in control group ($p = 0.77$)
Disease flares	Reported per patient-month, according to severity: • Severe flares: 0.011 in RTX group and 0.018 in CYC group ($p = 0.30$) • Limited flares: 0.023 in RTX group and 0.027 in CYC group ($p = 0.81$)	Reported as frequency of relapse during the study period: 15% in the RTX group and 10% in the control group ($p = 0.70$)
Outcomes in subgroup with severe relapsing AAV at enrollment	Primary end point achieved in 67% in RTX group and 42% in CYC group (RTX superior; $p < 0.01$)	Not tested
Outcomes in subgroup with GPA	Achievement of the primary end point was similar between the two treatment groups (RTX group 63% and CYC group 50%; $p = 0.11$)	Not reported

AAV: Antineutrophil cytoplasmic antibody-associated vasculitis; ANCA: Antineutrophil cytoplasmic antibody; AZA: Azathioprine; CYC: Cyclophosphamide; GCS: Glucocorticosteroid; GPA: Granulomatosis with polyangiitis; MPA: Microscopic polyangiitis; RTX: Rituximab.

follow-up period (p = 0.70). In RAVE, flare rates at 6 months were also similar between the groups. Furthermore, during extended follow-up of the RAVE cohort, neither the frequency nor the severity of disease flares differed between the treatment groups at 18 months [39].

The results of RAVE and RITUXVAS have established RTX as the first proven effective and safe alternative to CYC in the management of severe GPA. Based on the data from the RAVE trial, the FDA approved RTX in combination with GCS for remission induction in newly diagnosed and relapsing severe GPA and MPA. In addition, in demonstrating superiority of RTX over CYC in patients presenting with severe relapses, the results of the RAVE trial have strongly suggested that RTX be considered the preferred agent for such patients [41].

Nonetheless, several questions remain regarding the use of RTX in GPA. First, long-term outcomes, including potential delayed-onset adverse effects, have not been fully established. Second, both RAVE and RITUXVAS restricted their study populations to ANCA-positive patients with severe disease. To date, only anecdotal reports and a single case series have suggested that RTX may also be effective in ANCA-negative patients or those with limited GPA [42,43]. Third, it remains unclear which remission maintenance treatment, if any, newly diagnosed patients should receive following a successful first remission induction with RTX. Fourth, questions remain regarding the significance of a reconstituted B-lymphocyte population or a rising ANCA titer in predicting disease relapse following treatment with RTX [43,44]. Finally, the role of RTX itself in maintenance therapy has yet to be established. In recent years, four retrospective studies have examined the use of RTX for remission maintenance, with all four concluding that RTX may safely and effectively prevent disease relapses in GPA [44–47]. Initial results from the MAINRITSAN study, the first prospective, randomized controlled trial to investigate the use of RTX for maintenance therapy, have also been promising [48]. In MAINRITSAN, 114 patients with newly diagnosed or relapsing AAV (including 86 with GPA) who had achieved remission with a conventional regimen were randomized to receive RTX or AZA for maintenance. Patients randomized to the RTX arm were given 500-mg RTX infusions on days 1 and 15, then 5.5 months later, and again every 6 months, for a total of five doses over 18 months. Patients in the AZA arm received AZA for 22 months at an initial dose of 2 mg/kg/day. The primary end point was major relapse rate at 28 months. Initial results have found RTX to be superior to AZA for remission maintenance, with a major relapse rate of 3.6% in the RTX arm and 27.1% in the AZA arm. This issue will be re-examined in the forthcoming RITAZAREM trial, which will investigate RTX versus AZA for remission maintenance in relapsing AAV [201].

Despite these questions, RTX is now well established as a key agent in the armamentarium of treatment options for GPA. RTX is an apparently safe and proven effective alternative to CYC in the management of severe GPA and it is the preferred agent for the treatment of severe relapses.

Therapy directed against cytokines

TNF-α inhibition

Rationale for targeting TNF-α in GPA

TNF-α is a key mediator in the inflammatory cytokine network. While a variety of proinflammatory cytokines are detected in inflamed tissues, TNF-α serves an integral role in the development of the inflammatory cascade. For example, in cultured synovial cells drawn from patients with rheumatoid arthritis, TNF-α has been shown to be the dominant inducer of multiple proinflammatory cytokines, including IL-1, IL-6, IL-8 and GM-CSF [49]. Conversely, in patients with rheumatoid arthritis, disruption of TNF-α signaling by means of inhibitory antibodies results in the rapid reduction in circulating levels of many of these same cytokines [50]. Given its primary role in the inflammatory cascade, TNF-α has served as an important therapeutic target in a number of chronic inflammatory conditions. In fact, TNF-α blockade has become a well-established therapeutic strategy in rheumatoid arthritis, juvenile rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, psoriasis and psoriatic arthritis [51]. Given the clear benefits of TNF-α inhibitors in other chronic inflammatory disorders, it was logical to consider their utility in AAV.

Early investigations into TNF-α in AAV suggested the potential for a key pathogenic role. Excess TNF-α production was demonstrated within glomeruli affected by ANCA-associated glomerulonephritis and from $CD4^+$ T lymphocytes from patients with GPA [52,53]. In addition, serum levels of TNF-α receptor in patients with GPA correlates with disease activity [54].

Experience with TNF-α inhibitors in GPA

Several TNF-α inhibitors have been approved for use in chronic inflammatory disorders. These

Table 3. Inhibitors of TNF-α in antineutrophil cytoplasmic antibody-associated vasculitis: a review of key studies and case series.

Study (year)	Investigational drug	Study design	Control group	Patients (n), diagnosis	Main findings	Ref.
Stone *et al.* (2001)	Etanercept	Prospective, open-label pilot study	None	25 GPA	Etanercept was well tolerated; may reduce disease activity and provide steroid-sparing effect	[55]
WGET Research Group (2005)	Etanercept	Randomized, placebo-controlled trial	Placebo plus standard therapy	180 GPA	No clinical benefit derived from addition of etanercept to standard therapy; increased risk of solid malignancy in etanercept group	[56]
Lamprecht *et al.* (2002)	Infliximab	Case series	None	Six GPA	Remission induced in five out of six patients with refractory GPA	[64]
Bartolucci *et al.* (2002)	Infliximab	Prospective, open-label pilot study	None	Seven GPA Two RA-associated vasculitis One cryoglobulinemia	All patients realized persistent improvement at 6 months	[68]
Booth *et al.* (2004)	Infliximab	Prospective, open-label study	None	19 GPA 13 MPA	28 out of 32 patients achieved disease remission; severe infections occurred in seven out of 32 patients	[70]
Morgan *et al.* (2011)	Infliximab	Prospective, open-label, nonrandomized trial	Standard therapy	22 GPA 11 MPA	No clinical benefit derived from addition of infliximab to standard therapy	[71]
de Menthon *et al.* (2011)	Infliximab	Prospective, randomized trial	RTX	17 GPA	Results favor RTX for induction and maintenance of remission	[72]
Laurino *et al.* (2010)	Adalimumab	Prospective, open-label study	None	Nine GPA Five MPA	Adalimumab was well tolerated; may provide steroid-sparing effect	[76]

GPA: Granulomatosis with polyangiitis; MPA: Microscopic polyangiitis; RTX: Rituximab.

agents differ in their molecular configurations and precise mechanisms of action, as will be described below. To date, clinical trials in GPA have been performed with etanercept, infliximab and adalimumab. Features of the key studies are summarized in Table 3.

Etanercept

Etanercept is a fusion protein that consists of two extracellular p75 TNF receptors linked to the Fc portion of human IgG1. Etanercept functions as a potent TNF-α inhibitor by binding circulating TNF-α and rendering it biologically inactive.

Stone *et al.* published the first study to investigate the use of etanercept in GPA [55]. This study consisted of a 6-month open-label trial in which etanercept was added to standard therapies. In total, 20 patents with GPA who were experiencing either disease flares or persistent disease activity were enrolled in the study at two US academic centers. The subjects were treated with etanercept at a dose of 25 mg twice weekly, in addition to conventional immunosuppressive therapies, which were utilized in accordance with disease severity. The standard therapies included GCS, CYC, MTX and AZA. The authors found that etanercept was well tolerated, as no instances of serious adverse effects were experienced by any of the patients during the 6 months of follow-up. In addition, there were indications that the addition of etanercept had resulted in reduced disease activity and had provided a steroid-sparing effect. At the end of the study, the mean BVAS/WG score among all patients had declined by 3 points ($p < 0.001$), and among the 14 patients for whom the addition of etanercept was the only medication change upon study entry, the mean BVAS/WG score had declined by 3.14 points ($p < 0.001$). As for etanercept's potential steroid-sparing effect, the mean daily prednisone dose for all patients in the trial decreased from 19 mg at study entry to 7.4 mg at the end of the 6-month trial period ($p = 0.023$).

In light of these promising findings, a randomized, placebo-controlled trial of the use of etanercept in GPA was conducted by the WGET Research Group [56]. In this study, 180 patients with active GPA (experiencing either newly diagnosed disease or a flare of existing, previously-quiescent disease) were randomized to receive etanercept or placebo, in addition to standard therapy consisting of GCS plus either CYC or MTX. The dose of etanercept was 25 mg twice weekly for all patients. The choice of standard therapy was made on the basis of the severity of GPA manifestations. Those with disease that posed an immediate threat to either the patient's life or vital organ function (severe disease) were treated with CYC and GCS, whereas those with limited (nonsevere) disease received MTX and GCS. The standard therapies were adjusted by established protocols on the basis of disease activity.

The trial's primary outcome was sustained disease remission, defined as a BVAS/WG of zero for at least 6 months. After a mean duration of 27 months follow-up, there was no significant difference in the rate of sustained remission between the etanercept and placebo groups (69.7 vs 75.3%; p = 0.39). In addition, there were no significant differences between the two groups in the time to achieve sustained remission, the frequency of disease flares, the average disease activity or the quality of life. The majority of patients in both groups experienced disease flares and significant adverse events. However, of the six solid malignancies observed during the study period, all occurred among patients in the etanercept group (p = 0.01).

The disparity in the frequency of solid malignancies prompted further analyses. Based on a gender- and age-matched normal population in the NIH Surveillance Epidemiology and End Results (SEER) database, 1.9 solid malignancies would have been expected in the etanercept group during the study period. Therefore, the six observed solid malignancies give a standardized incidence ratio of 3.1 (95% CI: 1.2–6.8; p = 0.01) [57]. A post-trial follow-up study was conducted to further investigate the relationship between etanercept therapy and solid malignancy [58]. Follow-up data were available for 153 of the 180 patients who comprised the original WGET cohort, with 77 of the patients (50.3%) having received etanercept during the study. During a median follow-up time of 43 months from the common closeout date, 13 new solid malignancies were detected, with eight in the etanercept group and five in the placebo group (p = 0.39). Comparison with gender- and age-matched normal populations from the SEER database showed that the increased risk of solid malignancy in the etanercept group persisted during the follow-up period (standardized incidence ratio: 3.92, 95% CI: 1.69–7.72), but that the risk of solid malignancy in the placebo group was increased to a similar degree (standardized incidence ratio: 2.89; p = 0.6). Thus, the increased risk of solid malignancy during long-term follow-up could not be solely attributed to etanercept exposure. Nonetheless, these data suggest that etanercept should be avoided in patients with GPA, particularly among those whose risk of developing malignancy is already elevated [59].

The negative findings of WGET, together with concerns regarding the risk of inducing solid malignancy, ended the enthusiasm for the use of etanercept in GPA. Its use is currently not recommended for either induction or maintenance of disease remission in GPA.

Infliximab

Despite the WGET study's negative findings with regard to the use of etanercept, there remained a rationale for the use of other TNF-α inhibitors in GPA. The biologic effects of infliximab, a chimeric monoclonal antibody to TNF-α comprised of mouse variable and human constant regions, differ in some respects from those of etanercept, despite the fact that the primary action of both agents is to block TNF-α signaling. Unlike etanercept, which only binds soluble TNF, infliximab also binds TNF that is expressed on cell surface membranes, thereby inducing apoptosis in target cells *in vitro* [60,61]. It is unclear whether this difference has significance *in vivo*, but it has been established that there are instances in which infliximab is effective while etanercept is not. For example, in Crohn's disease and sarcoidosis – both of which share the feature of granulomatous inflammation with GPA – treatment with infliximab is beneficial, even though treatment with etanercept is not [62,63].

Early reports and uncontrolled studies regarding the use of infliximab in GPA suggested the possibility that infliximab may succeed where etanercept had failed. Lamprecht *et al.* reported the first use of infliximab in GPA in a case series of six patients [64]. All six of these patients had GPA refractory to treatment with CYC and GCS. Following the addition of infliximab to standard therapy, five of the patients achieved sustained remission. Other authors later reported

similarly positive results with infliximab as a rescue therapy in refractory GPA [65–67].

Subsequent uncontrolled studies also suggested a possible beneficial effect of infliximab. Bartolucci *et al.* reported the successful use of infliximab in an open-label pilot study of ten patients with systemic vasculitis refractory to GCS and receiving at least one standard immunosuppressant [68]. Seven of these patients had GPA; all seven experienced complete or partial remission 6 weeks after initiating infliximab, and all had persistent improvement at 6 months. In a related long-term follow-up, involving many of the same patients, infliximab continued to be useful beyond 2 years of treatment initiation [69]. Separately, in a prospective, open-label, multicenter trial, Booth *et al.* studied the use of infliximab in 32 patients with AAV, including 19 patients with GPA and 13 patients with MPA [70]. None of the subjects had immediately life-threatening disease manifestations at study entry. All patients received infliximab in addition to standard therapy, consisting of either CYC and prednisolone or the patient's existing regimen. Mean follow-up was 16.8 months. A total of 28 out of the 32 patients (88%) achieved disease remission, with an overall mean time to remission of 6.4 weeks. Although severe infections occurred in seven of the 32 patients (22%) and were associated with one death, these results were considered promising.

However, further investigations that have included separate treatment arms have not fulfilled the promise that infliximab showed in the early case reports and uncontrolled studies. In a single-center, open-label, nonrandomized, prospective study, Morgan *et al.* investigated two contemporaneous cohorts of patients with active AAV who differed on the basis of whether they received infliximab in addition to standard remission-induction therapy [71]. A total of 33 patients with AAV, 22 of whom had GPA, were followed for 1 year. All patients received standard immunosuppression consisting of prednisolone plus CYC, mycophenolate mofetil or AZA, and those with life- or organ-threatening disease underwent plasma exchange. In addition, 16 of the patients received infliximab, dosed at 5 mg/kg at study entry and at weeks 2, 6 and 10. The primary outcome was time to clinical remission, which did not differ between the two cohorts. Other clinical outcomes included disease activity and episodes of relapse, neither of which differed between the cohorts. The authors concluded that the study did not identify any obvious clinical benefit from the addition of infliximab to standard therapy for AAV.

Finally, in a prospective, randomized, multicenter trial, de Menthon *et al.* compared infliximab with RTX in GPA [72]. In total, 17 patients with GPA refractory or intolerant to treatment with glucocorticoids and standard immunosuppressants were randomized to receive either infliximab or RTX in addition to their ongoing regimen. The primary end point was complete or partial remission at 12 months. Although the results of this small study should be interpreted with caution, there was a trend favoring RTX and *post hoc* analysis of long-term follow-up showed that RTX performed better in terms of induction and maintenance of remission. In addition, among five patients in the infliximab group who failed to respond to the anti-TNF therapy, four were subsequently treated successfully with RTX.

On the basis of these comparison studies, enthusiasm for the use of infliximab in GPA has sharply diminished. Despite the initial promise, infliximab currently is not recommended as a standard component of either induction or maintenance therapy in GPA and is generally only considered for salvage therapy.

Adalimumab

Similar to infliximab, adalimumab is a monoclonal blocking antibody to TNF-α. However, while infliximab is a chimeric antibody that requires intravenous administration, adalimumab is a fully humanized, recombinant IgG1 antibody that is delivered subcutaneously. Adalimumab's ease of administration and reduced potential for immunogenicity have made it an attractive alternative to infliximab [73]. In Crohn's disease, adalimumab is effective for the induction and maintenance of remission, and retains efficacy in some patients who have lost response or become intolerant to infliximab [73–75].

To date, only one uncontrolled study has investigated the use of adalimumab in AAV. Laurino *et al.* conducted an open-label, prospective study of 14 patients with acute flares of AAV, nine of whom had GPA [76]. The patients were treated with a 3-month course of adalimumab, dosed at 40 mg every 2 weeks. In addition, they received induction CYC and prednisolone, followed by maintenance AZA or mycophenolate mofetil along with a reducing dose of prednisolone. The study found that adalimumab was well tolerated and that the rate of remission at 3 months was similar to historic controls, but with a reduction in exposure to prednisolone. These results have yet to be confirmed in randomized controlled trials. Similar to the other TNF inhibitors, adalimumab does

not currently have a defined role in the treatment of GPA.

B-cell-activating factor inhibition

Rationale for targeting B-cell-activating factor in GPA

B-cell-activating factor (BAFF; also called B-lymphocyte stimulator) is a member of the TNF family and plays an essential role in B-cell development and survival, and in immunoglobulin production [77]. BAFF has emerged as a potential therapeutic target in autoimmune diseases on the basis of mechanistic considerations, findings in animal models and observations in patients.

Based on its function, BAFF may play a crucial role in the maintenance of autoimmunity. BAFF acts primarily as a B-lymphocyte survival factor. Autoantigen-binding B cells appear to have increased dependence on BAFF for their survival compared with nonautoreactive B cells [78,79]. Animal models have suggested that excess BAFF may itself induce autoimmunity. For example, transgenic mice with overexpression of BAFF have been shown to develop a lupus-like disease followed by a Sjögren-like disease [80]. In human studies, patients with a variety of rheumatologic conditions have been found to have elevated serum BAFF levels [81]. The targeting of BAFF via belimumab, a human IgG-1λ monoclonal antibody that binds to and neutralizes soluble BAFF, has been shown to be effective and safe in systemic lupus erythematosus [82,83].

BAFF may also emerge as a therapeutic target in GPA. Serum BAFF levels are increased in patients with GPA compared with healthy controls and, among GPA patients, treatment with GCS is associated with lower serum BAFF levels [84–86]. Serum BAFF levels have not been shown to positively correlate with either ANCA titers or GPA disease activity, but indirect evidence suggests that elevated BAFF levels in patients with GPA may perpetuate an inflammatory cycle and may contribute to disease relapse under certain conditions. Holden *et al.* showed that treating neutrophils with PR3-ANCA results in the release of bioactive BAFF [87]. Further experiments demonstrated that the BAFF-rich supernatants from neutrophils treated with PR3-ANCA promote B-cell survival *in vitro*. These findings suggest the possibility of an inflammatory cycle linking ANCA and BAFF, whereby ANCA-activated neutrophils that arrive at an inflammatory site release BAFF and directly promote the survival of ANCA-producing B cells already *in situ*.

Other studies suggest the possibility that BAFF facilitates reconstitution of B-cell populations and autoimmune disease relapse following B-cell depletion therapy, such as RTX. In patients with rheumatoid arthritis, serum BAFF levels increase soon after RTX infusion and remain above baseline until B cells return to the peripheral blood [88]. In patients with GPA, serum BAFF levels also significantly increase above baseline following initiation of RTX treatment [87]. Increased serum BAFF levels may themselves contribute to the reconstitution of B-cell populations. For example, in Sjögren's syndrome, higher serum BAFF levels following RTX infusion correlate with shorter durations of B-cell depletion [89]. It is conceivable that the high serum BAFF levels that follow RTX infusion promote the survival of small numbers of B cells, including autoreactive B lymphocytes [90].

Given the potential for BAFF to perpetuate ANCA-associated inflammation and to facilitate the reconstitution of B-cell populations following therapy, BAFF is a potential target for future therapeutic investigations in GPA. Anti-BAFF therapy could conceivably serve either as an adjunct treatment in active GPA disease (to help break the cycle of inflammation) or as a secondary agent used in conjunction with B-cell depleting mediations, such as RTX, to amplify and extend their effects [90].

Experience with BAFF inhibitors

The only BAFF antagonist currently approved by the FDA is belimumab, the human monoclonal antibody used in systemic lupus erythematosus, which is specific to soluble BAFF only. A Phase III trial to investigate belimumab in combination with AZA for remission maintenance in GPA and MPA is currently being planned (BREVAS) [202]. Three other BAFF antagonists undergoing clinical investigation are: atacicept, a receptor fusion protein; blisibimod, a 'peptibody' or fusion protein consisting of the Fc portion of IgG and a peptide sequence that binds BAFF with high affinity; and tabalumab, a monoclonal antibody that binds both soluble and membrane-bound BAFF. None of these agents currently has a role in the treatment of GPA.

Therapy directed against T lymphocytes

Rationale for targeting T-lymphocyte activation in GPA

T-cell activation provides yet another potential therapeutic target in GPA. T cells are frequently

found in the granulomatous lesions that define the disease [91]. In addition, serum markers of T-cell activation, such as soluble IL-2 receptor and soluble CD30, are increased in GPA and associated with disease activity [92,93]. Abnormalities in T-cell activation may facilitate the loss of self-tolerance that is central to the pathogenesis of GPA.

Full activation of T cells requires at least two signals from an antigen-presenting cell (APC) [94]. The first signal is elicited by binding of the antigen-specific T-cell receptor to an APC's MHC peptide complex. The second signal is delivered by binding of a T-cell costimulatory receptor to a ligand on the same APC. One important T-cell costimulatory receptor is CD28, which binds to CD80 or CD86 on APCs [95]. Binding of both the T-cell receptor and CD28 results in T-cell proliferation and proinflammatory cytokine release. Conversely, binding of the T-cell receptor without engaging the costimulatory CD28 results in suboptimal activation, renders the T cell poorly responsive to subsequent stimulus and may initiate apoptosis.

Following activation, T cells express cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), which serves to dampen the immune response by inhibiting costimulatory signaling via the CD28 pathway [96]. CTLA-4 is a high-avidity receptor for both CD80 and CD86, binding these ligands 500–2500-times as avidly as CD28. Therefore, abnormalities in CTLA-4 expression result in a disruption in the balance of T-cell activation. Genetic association studies have demonstrated that GPA is associated with particular polymorphisms in the *CTLA-4* gene [97–103], which may reduce the efficacy of expressed CTLA-4. Reduced CTLA-4 activity could, in turn, partly account for the increased T-cell activation observed in GPA. Therapeutically supplementing the activity of CTLA-4 therefore provides a potential treatment strategy in GPA.

Abatacept

Abatacept is a soluble fusion protein, comprised of the extracellular domain of human CTLA-4 and a fragment of the Fc domain of human IgG1, modified to prevent complement fixation [96]. Abatacept serves to supplement the activity of CTLA-4 and thereby modulate T-cell activation. Similar to CTLA-4, abatacept competitively inhibits CD28, reducing the frequency of T-cell costimulatory signaling [104]. It has been approved for use in patients with rheumatoid arthritis who have had an inadequate response to anti-TNF-α therapy [105].

Investigations into the use of abatacept in GPA remain preliminary. A recent report describes an open-label trial of abatacept in 20 patients with mild relapsing GPA [106]. These patients were treated with abatacept at a dose of 10 mg/kg intravenously on days 1, 15 and 29, and monthly thereafter, in addition to their standard therapies. Abatacept was well tolerated and was associated with disease remission and discontinuation of prednisone in a high percentage of patients. These promising findings suggest that abatacept (or perhaps the next generation agent belatacept) warrants further study in the treatment of GPA, particularly for remission maintenance.

Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody against CD52, which consists of human IgG1 constant and variable framework regions together with murine complementarity-determining regions. The target CD52 antigen is widely expressed by B lymphocytes, T lymphocytes, monocytes, macrophages and natural killer cells; however, the predominant effect of alemtuzumab is prolonged T-lymphocyte depletion [107]. Thus, while treatment with abatacept aims to modulate T-lymphocyte activity, the use of alemtuzumab is directed toward depleting and altering T-lymphocyte populations [108]. Alemtuzumab's only FDA-approved indication is in the treatment of B-cell chronic lymphocytic leukemia, but it has drawn interest in T-lymphocyte-driven diseases, including multiple sclerosis, rheumatoid arthritis and Behçet disease [107–110].

To date, only one report describes long-term follow-up of the use of alemtuzumab in AAV. Walsh *et al.* describe 71 patients with AAV who were treated with alemtuzumab at a single center between 1991 and 1999, with a mean follow-up of 5 years [111]. A total of 63 of these patients had GPA, and all of them had either chronically relapsing or life-threatening disease, despite standard treatment. Prior to receiving alemtuzumab, all immunosuppressive medications (except prednisolone) were discontinued. Alemtuzumab was administered intravenously on five consecutive days at doses of 4, 10, 40, 40 and 40 mg/day. Clinical remission was achieved in 46 of the patients (65%), while an additional 14 patients (20%) had clinically significant improvement. However, high rates of relapse, severe infections and malignancy were also observed.

In order to build on this experience, a prospective, open-label, randomized, multicenter

study is ongoing to characterize the clinical response and severe adverse event rate associated with alemtuzumab for the treatment of relapsing or refractory AAV (ALEVIATE [203]). Outside of an investigational context, alemtuzumab currently has no role in the treatment of GPA.

Conclusion & future perspective

In recent years, several BRMs have been investigated as alternative or adjunctive therapies in GPA. While multiple BRMs are useful in a variety of other autoimmune diseases, to date, RTX is the only BRM that has been proven to be an effective and safe addition to the armamentarium of GPA therapies. Based on data from the RAVE trial, RTX in combination with GCS has been approved by the FDA for remission induction in severe GPA. In addition, given the RAVE trial's finding of superiority of RTX to CYC in patients presenting with severe relapses, it has become the preferred therapy for such patients. Investigations into BRMs that target B-lymphocyte stimulation (such as belimumab) and T-lymphocyte activation or depletion (such as abatacept and

Executive summary

Rationale for targeting B lymphocytes in granulomatosis with polyangiitis

- B lymphocytes are essential for the production of antineutrophil cytoplasmic antibody, which is considered a key contributor to the pathogenesis of granulomatosis with polyangiitis (GPA).
- The frequency of activated B lymphocytes is associated with both disease activity and severity.

Rituximab in GPA

- Rituximab is a chimeric monoclonal antibody directed against CD20, a protein expressed on the surface of cells of B-lymphocyte lineage.
- Two randomized controlled trials (RAVE and RITUXVAS) have firmly established the efficacy of rituximab for remission induction in severe GPA.
- Rituximab is the preferred treatment for severe relapses in GPA.

Rationale for targeting TNF-α in GPA

- TNF-α is a key mediator of the inflammatory cascade, serving as the dominant inducer of multiple proinflammatory cytokines.
- TNF-α blockade is a well-established therapeutic strategy in multiple autoimmune diseases.

Etanercept in GPA

- Etanercept is a fusion protein that incorporates TNF receptor domains and serves to bind and inactivate circulating TNF-α.
- The WGET study, a randomized placebo-controlled trial, found no clinical benefit from the addition of etanercept to standard therapy in the treatment of GPA.
- Patients in WGET who were treated with etanercept were found to have an increased risk of solid malignancy.

Infliximab in GPA

- Infliximab is a chimeric monoclonal antibody against TNF-α.
- No clear benefit has been shown to derive from the addition of infliximab to standard therapy in the treatment of GPA.

Adalimumab in GPA

- Adalimumab is a fully humanized, recombinant monoclonal antibody against TNF-α.
- Investigations remain preliminary, but adalimumab may provide a steroid-sparing effect in GPA.

Rationale for targeting B-cell-activating factor in GPA

- B-cell-activating factor serves an essential role in B-cell development and survival, and in immunoglobulin production; it may also be crucial to the maintenance of autoimmunity.

Experience with B-cell-activating factor inhibitors

- The use of B-cell-activating factor inhibitors in GPA remains investigational.

Rationale for targeting T-lymphocyte activation in GPA

- Markers of T-lymphocyte activation are elevated in GPA and are associated with disease activity.

Abatacept in GPA

- Abatacept is a soluble fusion protein that incorporates the extracellular domain of CTLA-4 and serves to inhibit T-lymphocyte activation.
- Investigations remain preliminary, but abatacept has been well tolerated and associated with disease remission in a high percentage of patients.

Alemtuzumab in GPA

- Alemtuzumab is a humanized monoclonal antibody against CD52, which induces prolonged T-lymphocyte depletion.
- Investigations remain preliminary and a prospective, open-label, randomized, multicenter study is ongoing.

alemtuzumab) have only been preliminary with regard to their use in AAV, and these agents do not yet have a role in the standard treatment of GPA. Anti-TNF-α agents, including etanercept, infliximab and adalimumab, have not shown clear benefits in GPA.

As our understanding of autoimmunity improves in the years ahead, we should expect new BRMs to come to light and expand our treatment options in GPA. We can hope that future studies will allow us to predict which patients will derive the greatest benefit from the various agents. Clearly, the potential for BRMs in the treatment of GPA is only beginning to be realized.

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▪ **The only published report on the long-term follow-up of alemtuzumab in AAV describes a promising clinical response, although high rates of relapse, severe infections and malignancy were also observed.**

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