

# Treatment of ANCA-Associated Vasculitis: New Therapies and a Look at Old Entities

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**Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a small-vessel vasculitis that primarily comprises 2 clinical syndromes: granulomatosis with polyangiitis and microscopic polyangiitis. Cyclophosphamide and glucocorticoids have traditionally been used for induction of remission. However, more recent studies have shown that rituximab is as effective as cyclophosphamide for induction therapy in patients with newly diagnosed severe AAV and superior for patients with relapsing AAV. There is also accumulating evidence indicating a potential role of rituximab for maintenance therapy in AAV. In this article, we will review the evidence supporting the various treatment choices for patients with AAV.**

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**Key Words:** ANCA-associated vasculitis, Cyclophosphamide, Rituximab

## Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) refers to a multisystem small-vessel vasculitis that is composed of 3 heterogeneous clinical syndromes including microscopic polyangiitis (MPA), granulomatosis polyangiitis (GPA, formerly Wegener's granulomatosis), and eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome).<sup>1</sup> AAV primarily affects the kidneys, lungs, and peripheral nervous system and is characterized by the presence of necrotizing lesions in small vessels. In most patients ANCA is present in the serum at the time of initial diagnosis.<sup>2-4</sup> The 2 major patterns seen by indirect immunofluorescence when sera of patients containing ANCA are incubated with ethanol-fixed neutrophils include cytoplasmic ANCA (C-ANCA) and perinuclear ANCA (P-ANCA) patterns.<sup>5</sup> The C-ANCA pattern represents diffuse granular staining in the cytoplasm that is seen mostly in patients with GPA and is caused by antibodies against proteinase-3 (PR3).<sup>6</sup> On the other hand, the P-ANCA pattern is most commonly seen in patients with MPA and represents perinuclear staining. In contrast to the C-ANCA pattern, the P-ANCA pattern can be caused by various different antibodies targeting cationic

granule constituents that rearrange around the negatively charged nucleus under ethanol fixation conditions. Because only antibodies against myeloperoxidase (MPO) causing a P-ANCA pattern are of interest in the context of AAV, confirmation of MPO specificity by enzyme-linked immunosorbent assay is mandatory in the diagnostic evaluation of patients suspected of having AAV.<sup>6</sup>

Immunosuppressive therapy is indicated in all patients with active AAV. Without therapy, the disease follows a progressive course and results in vital organ failure with fatal outcomes once there is evidence of kidney involvement.<sup>7</sup> The choice of therapy may depend on disease activity, but treatment typically consists of 2 phases: (1) a remission induction phase, with the goal of therapy being the interruption of active inflammation to prevent tissue damage and allow recovery; and (2) a remission maintenance phase, in which therapy aims to prevent relapses, ideally without the use of glucocorticoids. In this article, we will review standard induction therapy as well as new therapeutic approaches with the use of rituximab. We will also review the role of rituximab for maintenance therapy.

## Remission Induction Therapy

### Cyclophosphamide

Before the introduction of high-dose glucocorticoids in combination with cyclophosphamide (CYC), AAV was considered to be fatal once there was evidence of kidney involvement.<sup>7</sup> In 1973, the first series of 15 patients who were treated with CYC (2 mg/kg per day) in combination with prednisone was published.<sup>8</sup> In this study, 13 of the 15 patients achieved remission. Follow-up studies of larger cohorts observed for longer periods of time provided similar data with a 75% rate of complete remission and 80% rate of survival.<sup>9,10</sup> The continued use of corticosteroids plus CYC changed AAV from a uniformly fatal disease to a chronic and relapsing condition. However, CYC-based therapy is associated with significant short- and long-term toxicities including

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bone marrow suppression (2%); infection (46%); malignancies, particularly bladder cancer (2.8%); and infertility (57%).<sup>10</sup> In an attempt to lower the exposure to the cumulative dose of CYC, intravenous (IV) CYC pulse therapy was suggested.

The European Vasculitis Study Group (EUVAS) conducted a randomized controlled trial (CYCLOPS Trial—Randomized Trial of Daily Oral vs Pulse Cyclophosphamide as Therapy for AAV) to evaluate the efficacy of IV CYC given as 3 IV pulses of CYC, 15 mg/kg, given 2 weeks apart, followed by pulses at 3-week intervals (15 mg/kg IV or 5 mg/kg orally on 3 consecutive days, at the physician's discretion) until remission, and then for another 3 months vs oral CYC given at a dose of 2 mg/kg per day, until remission, followed by 1.5 mg/kg per day for another 3 months.<sup>11</sup> There were no differences in the rate or time to remission between the 2 treatment arms (88.1% in the IV group vs 87.7% in the oral group). There was a higher reported rate of leukopenia in the oral CYC arm compared with the IV CYC arm. However, there was no difference in the rate of serious infections or any other adverse events between the 2 arms.<sup>11</sup> The relapse rate was numerically higher in the IV CYC group (19%) compared with the oral CYC group (9%). This difference did not reach statistical significance, and the study was not powered to evaluate the effect of the intervention on relapse. However, the long-term follow-up of the CYCLOPS trial did show a significantly higher rate of relapse in the IV arm (39.5%) compared with the oral arm (20.8%) ( $P = .029$ ).<sup>12</sup> There were no differences in kidney function, survival, or adverse events between the 2 treatment arms.<sup>12</sup>

CYCLOPS showed that oral and IV CYC are possible options for induction therapy. However, providers should be aware that IV CYC is not superior to the oral formulation, and, when used for the same duration of therapy, it may be associated with a higher rate of relapse. In our practice, we prefer the oral formulation because necessary dose adjustments can be implemented promptly in the event of leukopenia or severe infection. In comparison, the bone marrow effects of IV CYC pulses are longer lasting, and, once administered, their effect cannot be easily reversed. However, we do consider IV CYC in patients in whom fertility is a concern, in those who may have issues with compliance, or in those who experience severe nausea with the use of oral CYC.

Although treatment with CYC induced remission in most patients, the optimal duration of therapy was unclear. In an open-label trial by Langford and colleagues, in which patients were switched to methotrexate therapy after induction was achieved by CYC, the median duration to remission was 3 months,<sup>13</sup> indicating that duration of therapy with CYC could be shortened. Similar results were obtained from the trial by EUVAS in which patients were treated with 3 months of CYC (2 mg/kg per day) and prednisolone, and then, once remission was achieved, they were randomized to either azathioprine (2 mg/kg per day) or continuation of CYC at a lower dose of CYC (1.5 mg/kg per day) for a total of 12 months. The relapse rate was no different between the azathioprine and the CYC arm (15.7% vs 13.7%).<sup>14</sup> This confirmed that CYC use could be limited to the duration required to induce remission, which is typically 3 to 6 months and does not need to be extended beyond 6 months. Consequently, after remission has been achieved, CYC should be discontinued and replaced with a less toxic alternative such as azathioprine.

#### CLINICAL SUMMARY

- Rituximab is as effective as Cyclophosphamide in inducing remission in severe AAV.
- Rituximab is the preferred agent when treating patients with relapsing AAV and in patients where fertility is a concern.
- Rituximab monotherapy is effective as maintenance therapy in AAV.
- MMF and high-dose corticosteroids may be a treatment option for patients who are MPO-ANCA positive and have mild renal impairment.

#### Side-Effect Profile of CYC

##### Bone Marrow Suppression and Infection

CYC is associated with significant short- and long-term side effects, and strategies to lower the associated morbidity and mortality should be implemented. One of the

most common side effects of CYC is bone marrow suppression, leukopenia, and subsequently an increased risk of infection.<sup>14</sup> Therefore, it is recommended that all patients on daily oral CYC therapy have a complete blood count (CBC) performed at least every 2 weeks (ideally weekly) while on CYC. Dose adjustments should be made to maintain a total white blood cell count above 3500/mm<sup>3</sup> and an absolute neutrophil count above 1500/mm<sup>3</sup>. If patient is receiving IV CYC, then CBC should be performed before each dose (on average once every 2 weeks). In addition, to prevent infection with *Pneumocystis jiroveci*, all patients should receive prophylaxis with either sulfamethoxazole/trimethoprim or an alternative agent.<sup>15</sup> We recommend that the oral dose of CYC be reduced by 25% in patients over the age of 60 years or creatinine more than 2.5 mg/dL to 1.5 mg/kg per day for induction therapy. For example, a 62-year-old male patient with a serum creatinine of 3 mg/dL who weighs 80 kg should receive no more than 125 mg of oral CYC per day (1.5 mg/kg per day).

## Malignancy

The risk of malignancy with CYC is substantial with the hazard of neoplasia roughly correlated to the cumulative dose and duration of cytotoxic therapy.<sup>16-18</sup> Faurschou and colleagues have investigated the incidence of malignancies associated with CYC exposure in a cohort of 293 patients with GPA.<sup>19</sup> The risk of malignancy was not increased for patients who never received CYC or for patients treated with cumulative CYC doses of 36 g or less.<sup>19</sup> In contrast, high risks of leukemia (standardized incidence ratio 59.0, 95% confidence interval 12-172) and bladder cancer (standardized incidence ratio 9.5, 95% confidence interval 2.6-24) were observed for patients treated with cumulative CYC doses more than 36 g. These data would suggest that a patient who weighs 80 kg and is treated with CYC at a dose of 2.5 mg/kg per day for 6 months would exceed this threshold. Because AAV is a remitting and relapsing disease with the potential for significant cumulative CYC dosing, there is a substantial risk of late-occurring malignancies.

The increased risk of transitional urothelial carcinoma and bladder cancer is related to the effect of acrolein (metabolite of CYC) on the urothelium.<sup>20</sup> Patients who receive oral CYC should be advised to take the dose all at once in the morning and to have adequate fluid intake throughout the day to keep the urine dilute and to minimize the contact time between acrolein and the urothelium. Patients can also be treated with 2-mercaptoethanesulphonate, which binds to acrolein and can lower the exposure. 2-Mercaptoethanesulphonate is easier to use in patients receiving IV CYC because it can be dosed on the day the patient receives IV CYC. All patients should have a biannual evaluation with urinalysis for detection of hematuria in addition to urine cytology. Should there be evidence of hematuria or abnormal urine cytology, this should be further pursued with cystoscopy.

## Infertility

The other major concern limiting CYC utility is related to its gonadal toxicity. Data indicate that ovarian failure is seen in female patients of any age receiving a cumulative dose of as little as 28 g of CYC.<sup>21,22</sup> In addition, these authors found that the age at the onset of therapy was an additional independent factor associated with sterility.<sup>21,22</sup> Although male infertility is harder to assess, studies have demonstrated that doses above 7.5 g/m<sup>2</sup> of CYC can result in permanent oligospermia.<sup>23</sup> Therefore, all patients of child-bearing age should be advised on the potential side effect of infertility, and other therapies for induction therapy should be considered. Therapy with testosterone in males and gonadotrophin-releasing hormone such as leuprolide in females may be of some benefit.<sup>24,25</sup> Female and male patients should be offered cryopreservation of ova and sperms before treatment with CYC.

## Rituximab

As noted previously, introduction of CYC significantly improved the outcome of patients with AAV. However, despite the high rate of initial remission, follow-up studies with extended timelines revealed that close to half of the patients who responded to the initial therapy relapsed in the first 3 to 5 years, and treatment was associated with significant morbidity in up to 42% of patients, offsetting the benefits achieved from treatment with CYC.<sup>26</sup> In addition, approximately 10% of patients do not respond satisfactorily to standard treatment. These observations indicated the need for a more effective and less toxic alternative.

### Pathogenic Role of B Lymphocytes

Emerging evidence suggested a critical role for B lymphocytes in the pathogenesis of autoimmune diseases, including AAV.<sup>27</sup> First, B lymphocytes are the precursors for short-lived plasma cells, which are thought to be source of ANCA that have been implicated in the pathogenesis of AAV.<sup>28-30</sup> Second, the number of activated B lymphocytes seems to correlate with disease severity and activity.<sup>31</sup> Third, B lymphocytes have been identified in the granulomatous lesions of patients affected with GPA close to plasma cells and PR3-positive cells where selection and affinity maturation of autoreactive B lymphocytes may occur.<sup>32</sup> In fact, the beneficial effect of CYC has been attributed to its effect on B lymphocytes.<sup>33</sup>

### Rituximab for Induction Therapy

On the basis of this evidence, rituximab, a chimeric monoclonal antibody against CD20+ B cells, was initially used in treating a patient with AAV who was failing therapy with CYC and prednisone.<sup>34</sup> Several small prospective studies produced further evidence of its efficacy.<sup>35-39</sup> These studies were followed by 2 major randomized controlled trials in 2010 and examined the efficacy of rituximab compared with CYC for remission induction in patients with severe AAV.<sup>40,41</sup>

The first was the Rituximab in ANCA-Associated Vasculitis (RAVE) trial, a randomized, double-blind, double placebo-controlled, noninferiority trial that evaluated the efficacy of rituximab to CYC in inducing complete remission by 6 months in patients with severe AAV.<sup>40</sup> Patients with newly diagnosed or relapsing disease and positive PR3- or MPO-ANCA and severe disease were eligible for the study. Severe disease was defined as vital organ involvement by AAV that posed an immediate threat to the function of that organ or the patient's life.<sup>42</sup> A total of 197 patients with severe AAV (GPA to MPA ratio of 3:1) were enrolled in the study and were randomized 1:1 to receive either rituximab at a dose of 375 mg/m<sup>2</sup> once weekly for 4 weeks or daily CYC at a dose of 2 mg/kg. All patients also received



methylprednisolone (MTP) at a dose of 1 to 3 g IV followed by a protocolized tapering regimen of oral prednisone aimed at complete discontinuation of corticosteroids by 5 months. After achieving remission at months 3 to 6, patients in the CYC arm were switched to azathioprine at a dose of 2 mg/kg per day whereas those in the rituximab arm were switched to placebo. Sixty-four percent of patients in the rituximab vs 53% in the CYC arm achieved the primary endpoint of complete remission defined as a Birmingham Vasculitis Activity Score for Wegener's Granulomatosis (BVAS/WG) of 0 and complete discontinuation of corticosteroids by month 6, which met the noninferiority criterion ( $P < .001$ ) and almost reached superiority ( $P = .09$ ). Among patients with relapsing disease, rituximab was more effective compared with CYC, with 67% reaching the primary endpoint compared with 42% in the CYC arm ( $P = .01$ ). The superiority persisted after adjusting for ANCA type and clinical site ( $P = .03$ ). There was no difference in the rate of adverse events between the 2 arms. It is important to note that some of the long-term adverse events associated with CYC, which are primarily dependent on the cumulative dose of CYC (eg, malignancy or infertility), may not occur until after the trial period.<sup>43</sup>

Results from another randomized controlled trial, the Rituximab versus Cyclophosphamide in ANCA-Associated Renal Vasculitis trial, were reported in the same issue of the *New England Journal of Medicine*.<sup>41</sup> In this trial, 44 patients were randomly assigned in a 3:1 ratio to receive either rituximab at a dose of 375 mg/m<sup>2</sup> weekly for 4 weeks plus 2 IV CYC pulses given with the first and third dose of rituximab or 3 to 6 months of IV pulse CYC followed by azathioprine. Both groups received standardized glucocorticoid doses. The primary endpoint results, which included the rate of sustained remission (absence of disease activity [BVAS of 0] for at least 6 months) (76% in rituximab vs 82% in CYC) and severe adverse events (42% in rituximab vs 36% in CYC), were similar between the 2 groups.

On the basis of the RAVE trial results, the U.S. Food and Drug Administration (FDA) approved the use of rituximab for induction therapy in the treatment of severe GPA or MPA in April 2011, and many regulatory agencies across the globe subsequently followed suit. Although there was no difference in the rate of remission in patients with newly diagnosed AAV, rituximab proved to be superior to CYC in the treatment of patients with relapsing disease. It is important to note that in the RAVE trial patients who were assigned to the rituximab arm did not receive any active maintenance therapy whereas those in the CYC arm were maintained on azathioprine after achieving remission. The lack of requiring maintenance therapy in the rituximab group is a potential advantage. It should also be emphasized that patients who have received rituximab and remain B-cell-depleted are immunosuppressed.

## Side-Effect Profile of Rituximab

### Infusion Reactions

The cumulative data suggest that rituximab overall has a favorable side-effect profile. Infusion-related adverse events are well recognized and can occur in up to 18% of patients treated with rituximab for the first time.<sup>44</sup> Acute infusion reactions are typically mild and include rhinitis, fevers, chills, rigors, urticaria, rash, and cough; less commonly they can include angioedema, bronchospasm, or hypotension.<sup>44</sup> The incidence of these acute reactions decreases after the second infusion of rituximab.<sup>44</sup> Pretreatment before receiving rituximab infusion may minimize these infusion-related reactions. At our institution, patients receive 100 mg IV of MTP in addition to 650 mg of oral acetaminophen and 25 to 50 mg of oral diphenhydramine before administration of rituximab. Slowing the rate of infusion may also help reduce the rate of infusion-related reactions.

### Infections

Hepatitis B reactivation is a concern when treating patients with rituximab. Most reports of reactivation are in patients who are chronic carriers<sup>45</sup>; however, reactivation of hepatitis B in patients with resolved hepatitis B has been reported.<sup>46</sup> Depletion of B cells may aid hepatitis B virus to escape recognition by the immune system and contribute to viral replication. Screening of high-risk patients for hepatitis B before treatment with rituximab is advised. If the patient is a chronic carrier and receives rituximab, then serial monitoring of liver enzymes, hepatitis B viral DNA, and treatment with antiviral prophylaxis is recommended.<sup>47</sup>

The most feared complication of rituximab is the risk of progressive multifocal leukoencephalopathy (PML). A few isolated cases of PML have been in reported patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and vasculitis after receiving rituximab.<sup>48</sup> Most, if not all, patients had other factors of immunosuppression in addition to rituximab. In addition, the risk of PML associated with rituximab treatment for AAV should be carefully weighed in light of multiple reports of cases of PML in patients who have been treated for AAV with other immunosuppressive agents.<sup>49-51</sup> Likewise, there are multiple reports of patients with SLE or other autoimmune diseases that have developed PML when treated with various other immunosuppressive agents (most commonly CYC) in the absence of rituximab.<sup>52-54</sup> This suggests that the degree of immunosuppression and the underlying disease condition play a role in the development of PML rather than any particular agent.

Patients treated with rituximab are at increased risk for opportunistic infections while B cells are depleted. One of the potential opportunistic infections is *P. jiroveci*,

and prophylaxis against pneumocystis is recommended in all patients until their B cells are reconstituted.<sup>55</sup>

### Late-Onset Neutropenia

Late-onset neutropenia (LON) is another long-term side effect of rituximab therapy that has been reported in up to 6% of patients with underlying autoimmune diseases up to 5 months after therapy.<sup>56,57</sup> The time of LON seems to coincide with reconstitution of B cells, and decreased granulopoiesis during this time has been proposed as a potential mechanism.<sup>58</sup> LON is often self-limiting with rapid recovery, but it may be associated with fever and increased risk of infection and hospitalization. The risk of infection may be heightened in patients who also have hypogammaglobulinemia (see below),<sup>57</sup> and treatment with colony stimulating factor may be required.<sup>56</sup> In the study by Besenda and colleagues, 6 of the 8 patients with LON were retreated with rituximab and none had recurrence of neutropenia, indicating that re-treatment with rituximab after an episode of LON may be safe in this population.<sup>57</sup>

### Hypogammaglobulinemia

Hypogammaglobulinemia has been reported in patients with AAV treated with rituximab. The risk is highest in patients who receive rituximab therapy after treatment with CYC or are on maintenance rituximab therapy.<sup>57,59,60</sup> Whether hypogammaglobulinemia results in increased risk of infection remains unanswered; however, patients who have hypogammaglobulinemia with LON may be at increased risk of infection,<sup>56</sup> and treatment of hypogammaglobulinemia and neutropenia may be warranted in such cases.

### Pulmonary Toxicity

Pulmonary toxicity is a rare but potentially fatal complication of rituximab therapy that has been more recently recognized.<sup>61-63</sup> In the largest systemic review to date, a total of 121 patients with interstitial lung disease (ILD) associated with rituximab therapy were identified from the review of literature.<sup>63</sup> Of these, most were treated for lymphoma as part of combination chemotherapy, and only 24.7% received rituximab as a monotherapy. Rituximab-associated ILD was seen more commonly in men and primarily in the fifth and sixth decade of life. The average cumulative dose before onset of symptoms was 1500 mg/m<sup>2</sup>. The mean time to onset of respiratory symptoms or radiographic findings after the last rituximab infusion was 30 days. Hypoxemia was present in all patients, with dyspnea, fever, and nonproductive cough constituting the most common symptoms. The most common radiological finding was diffuse bilateral lung infiltrates with evidence of pulmonary inflammation on lung biopsies. The radiographic findings were present in some cases in the absence of any symptoms. Of the 121

patients, rituximab-associated ILD was fatal in 18 (14.9%). The most common therapy included discontinuation of rituximab and initiation of glucocorticoids. It is important to note that when evaluating patients with pulmonary symptoms who have been treated with rituximab, an infectious etiology should be considered and ruled out first.

### Thrombocytopenia

Thrombocytopenia has been reported after the use of rituximab.<sup>64-66</sup> This side effect is commonly seen in patients who are treated for lymphoma who have a high tumor burden.<sup>66,67</sup> The onset is typically within the first 24 hours after administration of rituximab and may be preceded by infusion-related symptoms. The underlying mechanism is not well known, but the presence of soluble CD20 antigen on the platelets and subsequent destruction by rituximab has been proposed.<sup>64</sup> The outcome is overall good, and platelets reconstitute within weeks after administration of rituximab.<sup>66</sup>

### Pregnancy

Another concern with the use of rituximab is its effect on women of child-bearing age and the fetus. Overall, the data regarding use of rituximab during pregnancy are limited, and rituximab is currently considered a Category C drug by the FDA, which indicates that animal studies have revealed an adverse event, but human studies are lacking and the drug may be given only if the potential benefits outweigh the potential risks.<sup>68</sup> Rituximab can easily cross the placenta if administered in the second and third trimester of pregnancy.<sup>69</sup> In the largest series evaluating the effect of rituximab on pregnancy by Chakravarty and colleagues, a total of 253 pregnancies related to rituximab were identified from the Rituximab Global Safety Database, of which 153 had known outcomes.<sup>70</sup> Of these, 90 resulted in live births, 61 resulted in abortion (elective and therapeutic), 1 resulted in still birth, and 1 resulted in maternal death. Of the 90 live births, 68 were full term and 22 were premature. Of the 90 live births, 11 had hematologic abnormalities (B-cell depletion, lymphopenia, anemia, and thrombocytopenia), 3 had congenital anomalies (club foot, cardiac anomaly, and Turner syndrome), and 4 had neonatal infections (fever, neonatal bronchiolitis, cytomegalovirus hepatitis, and chorioamnionitis). Of the 90 live births, 41 patients received rituximab for a nonhematologic autoimmune-related disease (RA, SLE, and mixed connective tissue disease). It is important to emphasize that the underlying disease and other therapies are major confounders in interpreting the effect of rituximab during pregnancy; in fact, in the study by Chakravarty and colleagues of the 70 pregnancies with available data, more than half were exposed to other teratogenic medications such as MTX and mycophenolate mofetil (MMF).

Pendergraft and colleagues recently reported on the outcomes of 8 pregnancies in patients treated with rituximab for AAV.<sup>71</sup> Of 22 women under the age of 40 who were treated with rituximab, a total of 6 women had 8 pregnancies. Of the 8 pregnancies, there was only 1 miscarriage at 15 weeks of gestation with the same patient having had a successful pregnancy previously after rituximab infusion. Of the 6 patients who carried out the pregnancies, only 1 had progressive airway disease that responded to prednisone. On the basis of these data, the use of rituximab in patients with AAV appears to be associated with low rate of adverse effects. Because data related to pregnancy outcomes remain limited, exposure to rituximab during pregnancy is currently not recommended, and patients should be counseled to avoid pregnancy up to 12 months after exposure to rituximab.<sup>72</sup>

### Immunization

There are limited data available on the safety and efficacy of nonlive vaccines after therapy with rituximab. Most data are from patients with RA treated with rituximab.<sup>73,74</sup> These studies suggest that treatment with rituximab lowers the immune response to pneumococcal (a T-cell-independent antigen) and influenza vaccination, but it has no effect on tetanus toxoid vaccine (T-cell-dependent antigen).<sup>73,74</sup> Therefore, it is recommended that patients receive nonlive vaccines 4 weeks before receiving rituximab.<sup>72</sup> Currently, there are no data available on the safety of live virus vaccination after rituximab therapy; therefore, immunization with live virus vaccine is not recommended.<sup>72</sup>

### Corticosteroids

Corticosteroids are part of the standard induction therapy and are used in combination with CYC or rituximab. There is no set approach to dosing of corticosteroids, but in our practice we adopt the glucocorticoid dosing that was used in the RAVE trial, which consists of 1 to 3 doses of 1000 mg IV MTP followed by prednisone at a dose of 1 mg/kg per day, which is then tapered over 5 months.<sup>40</sup>

### Plasma Exchange

Plasma exchange (PLEX) has been used for the last several decades when treating patients with AAV with the potential benefit of removing ANCA antibodies, activated lymphocytes, and other excess physiological factors such as cytokines, all of which may play a role in disease activity.<sup>75</sup> The evidence supporting the use of PLEX has been lacking.<sup>76</sup> We currently use PLEX for treatment of patients with severe kidney disease (creatinine > 5.0 mg/dL or requiring dialysis), and in patients with pulmonary hemorrhage requiring ventilatory support. The PEXIVAS (Plasma Exchange and Glucocorticoid Dosing in the Treatment of ANCA Vasculitis) study

is currently underway, and it is the largest randomized controlled trial to date. PEXIVAS is powered to detect a moderate effect of PLEX in patients with AAV presenting with an estimated glomerular filtration of less than 50 mL/minute per 1.73 m<sup>2</sup> or with alveolar hemorrhage.<sup>77</sup> This study will help address the role of PLEX in patients with AAV.

### MMF

The use of MMF in GPA or relapsing/resistant disease has been disappointing.<sup>78-80</sup> However, MMF has been shown to be effective when used primarily in patients with MPA who have mild to moderate kidney involvement.<sup>81,82</sup> In a prospective open-label trial by Silva and colleagues, 17 consecutive patients with positive MPO-ANCA and mild to moderate kidney involvement (creatinine  $\leq$  3.0 mg/dL) were treated with MMF (starting at 750 mg twice daily and titrated up to 1000 mg twice daily as tolerated) for a total of 18 months.<sup>81</sup> Patients also received glucocorticoids in the form of 1 to 3 g of IV MTP followed by a tapering regimen of prednisone in addition to MMF. Those with a diagnosis of GPA or evidence of pulmonary hemorrhage or creatinine greater than 3.0 mg/dL were excluded. Most patients (58%) were newly diagnosed. The primary outcome of remission at 6 months was achieved in 13 patients (76%) and was sustained up to 18 months in 12 patients (70%). Ten patients experienced a side effect, which was primarily gastrointestinal upset followed by leukopenia. The rate of remission in this population is similar to that achieved by CYC.

The only randomized controlled trial comparing the efficacy of MMF to CYC has been by Hu and colleagues in a Chinese population in which 35 patients were randomly assigned to receive either MMF or IV CYC.<sup>82</sup> Most patients (97%) had a diagnosis of MPA, and those with serum creatinine of 5.6 mg/dL or greater were excluded. The remission rate achieved in those treated with MMF was 77.8%, which is similar to the previous studies. Unfortunately, the follow-up was restricted to 6 months, and no information regarding the long-term effect of MMF or rate of relapse is available. At this point, MMF may be considered as an induction agent only in patients with MPA and MPO-ANCA positivity who have mild to moderate kidney involvement and no evidence of any life-threatening organ damage.

### Remission Maintenance Therapy

Remission maintenance after induction therapy is the next step in the management of patients with AAV. The goal of therapy is to maintain remission while lowering the risk of cumulative disease-associated damage and treatment-associated toxicities. The choice of therapy, timing of transition from induction agent to maintenance agent, and duration of therapy have been debated and



the subjects of randomized controlled trials for the last couple of decades. There is now sufficient evidence to suggest that transitioning from CYC after 3 to 6 months of therapy to alternative agents is safe.<sup>13,14</sup> MTX and azathioprine are viable options, and the choice may depend on the side-effect profile and tolerability of the drug by the individual patient.<sup>14,83-85</sup> MMF has been found to be associated with a higher relapse rate than azathioprine.<sup>86</sup> More recently, rituximab has also been used for maintenance therapy, the evidence for which is reviewed below.

### ***Conventional Agents for Remission Maintenance***

The efficacy of azathioprine for remission maintenance was compared to MTX in a randomized controlled trial.<sup>85</sup> The study was designed to test the hypothesis that MTX was associated with fewer side effects compared with azathioprine. The primary outcome was the rate of serious adverse events resulting in death or discontinuation of therapy. A total of 126 newly diagnosed patients who had achieved remission with CYC and glucocorticoids were randomized to receive either azathioprine (2 mg/kg per day) or MTX (0.3 mg/kg/week with increasing dose to 25 mg/week) for a total of 12 months and then tapered over 3 months. There were no differences in the rate of relapse between the groups (36% in azathioprine arm vs 33% in MTX arm) or the rate of adverse events or serious adverse events (11% in azathioprine vs 19% in MTX,  $P = .21$ ). Consequently, azathioprine and MTX are equally effective for maintenance therapy, and the choice may be determined by patient-specific factors. Azathioprine is the more suitable choice for patients with any degree of kidney insufficiency because MTX can result in bone marrow toxicity in this population.

Small observational studies have suggested that MMF may be efficacious for maintenance of remission in patients with AAV.<sup>87-89</sup> The EUVAS study evaluated the use of MMF (2000 mg/day) compared to azathioprine (2 mg/kg/day) in a randomized controlled trial of 156 newly diagnosed patients who achieved remission after induction with CYC and glucocorticoids.<sup>86</sup> Patients were followed for a median of 39 months with the primary endpoint of relapse-free survival. The relapse rate was significantly higher in the MMF group (55%) compared with the azathioprine group (37.5%), and there were no differences in the rate of adverse events. On the basis of the results of this study, MMF is now considered a second-line agent for remission maintenance in AAV that may be useful for patients who cannot tolerate MTX or azathioprine.

### ***Rituximab for Remission Maintenance***

Several retrospective studies have evaluated the use of rituximab as a remission maintenance agent in patients with refractory or chronically relapsing AAV.<sup>90,92</sup> Rhee and colleagues evaluated the use of rituximab in 39

patients with AAV who were either in complete or partial remission. All patients received rituximab at initiation and were treated preemptively every 4 months (1 g dose) for a total of 1 year.<sup>90</sup> Disease control was achieved in all 39 patients. At 1 year of follow-up, the percentage of patients requiring cytotoxic agents or prednisone was reduced significantly.

Smith and colleagues also evaluated the use of rituximab for maintenance therapy. In this retrospective study, 73 patients with vasculitis (95% ANCA positive) were included. Twenty-eight patients received rituximab for induction therapy and then further rituximab therapy at the time of clinical relapse (Group A). Forty-five patients received rituximab induction therapy at the beginning and then preemptively every 6 months for a total of 2 years (Group B). Nineteen patients who relapsed (originally from Group A) received rituximab induction therapy again and then routinely every 6 months for 4 years (Group C).<sup>91</sup> More than 90% of patients in all groups achieved remission at 1 year. However, at 2 years of follow-up, those patients who were treated preemptively (Group B and C) had much lower rates of relapse (11-12%) compared with those who were treated based on clinical recurrence of disease (73%). In addition, the time to relapse was much shorter in Group A compared with those who received rituximab preemptively (Group B and C). The authors did not find an association between the ANCA titers or the B-cell count and the relapses, and they suggested that routine retreatment with rituximab may be the only strategy in treating patients with relapsing AAV. This was in contrast to the study by Cartin-Ceba and colleagues, who evaluated the role of long-term rituximab therapy in patients with refractory GPA.<sup>92</sup> Thirty-four percent of rituximab courses were given to treat relapses and 66% were given preemptively based on B-cell reconstitution or an increase in ANCA titers. In this study, reconstitution of B cells accompanied or preceded the rise in serum ANCA levels and was associated with subsequent relapses (except in 1 ANCA-negative patient). Relapses were not observed in the absence of B cells or before increases in PR3-ANCA levels. These findings described by Cartin-Ceba and colleagues are more in line with findings from the RAVE trial, as discussed below.

The 18-month follow-up analysis of the RAVE trial recently showed that a single course of rituximab was as effective as conventional immunosuppressive therapy consisting of CYC followed by azathioprine to induce and maintain remission over a period of 18 months in severe AAV.<sup>93</sup> In the rituximab arm, patients who completed the prednisone taper by 6 months and remained in remission received only placebo from 6 to 18 months. On the other hand, patients in the CYC arm were switched to azathioprine for maintenance therapy after achieving remission for a total of 18 months. The primary comparison at 12 and 18 months included the percentage of patients who did not have a relapse, who had completed steroid taper by 6 months, and who had

a BVAS/WG score of 0. In the rituximab group, 48% of patients maintained complete remission at 12 months compared with 39% in CYC group ( $P = .22$ ). At 18 months, 39% in the rituximab group vs 33% in the CYC group maintained remission ( $P = .32$ ). There were no differences in the rate and duration of remission or the number and severity of relapses between the 2 groups. The mean time to relapse in the rituximab group was 176 days vs 142 days in the CYC group ( $P = .16$ ). There were no major differences in the overall rate of adverse events between the 2 groups.

Among patients who entered the trial with a severe disease relapse, 49% of patients in the rituximab group vs 24% in the CYC group remained in complete remission at 12 months ( $P = .009$ ). At 18 months, 37% in the rituximab group vs 20% in the CYC group remained in complete remission ( $P = .06$ ). Thus, the superiority of rituximab over conventional immunosuppression for relapsing patients was maintained at 12 months but not at 18 months. In the rituximab arm, relapses were rare before reconstitution of B cells and increases in ANCA levels. The fact that the superiority of rituximab could no longer be demonstrated among relapsing patients suggests that such patients might benefit from re-treatment with rituximab to maintain long-term remission.

The RAVE trial is the only clinical trial that allows the direct comparison of outcomes achieved with rituximab alone vs conventional CYC-based therapy in patients with major kidney disease. Fifty-two percent of patients in the RAVE trial (102 patients) had major kidney disease defined as the presence of red blood cell casts on urine microscopy, an increase in serum creatinine of more than 30% or a 25% decline in glomerular filtration rate, or biopsy-proven pauci-immune glomerulonephritis (45 patients). The 102 patients were equally distributed across both treatment groups (Table 1), and the treatment groups were balanced with respect to ANCA type, diagnosis of GPA vs MPA, BVAS/WG score, duration of disease, and previous exposure to CYC. However, those in the rituximab group had a lower estimated creatinine

clearance (eCrCl) (53.5 mL/minute) compared with the CYC group (70.5 mL/minute) ( $P = .01$ ) at the time of trial entry. Of the patients with major kidney disease, 75% in the rituximab group and 77% in the CYC group achieved complete remission ( $P = .82$ ). By 18 months, the eCrCl improved to 64.2 mL/minute in the rituximab group and 80.1 mL/minute in the CYC group. The degree of improvement was no different between the 2 groups ( $P = .8$ ). Patients with MPO-ANCA had a lower baseline eCrCl compared with PR3-ANCA, but there was no difference in the eCrCl change over time. These data suggest that rituximab is as effective as CYC followed by azathioprine in inducing and maintaining remission in patients with major kidney disease over 18 months.

It is notable that among patients randomized to rituximab in the RAVE trial, only 3 patients suffered relapses (only 1 severe relapse) between month 6 and 18 in the absence of B cells and ANCA. However, return of B cells or rising of ANCA titers did not predict which patient would have a relapse. Like the report by Cartin-Ceba and colleagues,<sup>92</sup> these data support that re-treatment with rituximab may not be necessary until after B-cell reconstitution and ANCA titers increase. This approach may reduce the overall immunosuppression and risks hypogammaglobulinemia and infection compared with re-treatment at fixed intervals causing continuous long-term B-cell depletion. This hypothesis is currently being investigated in a randomized controlled trial conducted by the French Vasculitis Study Group (NCT01731561).<sup>94</sup> Taken together, the above studies suggest a possible role for rituximab as a maintenance therapy agent with an effective and safe side-effect profile.

### Duration of Maintenance Therapy

Identification of patients with AAV who are at risk of relapse remains a challenge. As a result, the exact duration of maintenance therapy remains unclear, and there is significant variation amongst centers as to when to stop maintenance therapy. The duration of maintenance

**Table 1. Baseline and Follow-Up Clinical Characteristics of Patients With Severe Kidney Involvement From the RAVE Trial**

Variable	Rituximab Group (n = 51)	CYC/AZA Group (n = 51)	P
Age (y) mean (SD)	56 (15)	54 (13)	.32
Male/female (%)	47/53	57/43	.32
GPA/MPA (%)	69/31	65/35	.67
PR3/MPO (%)	59/41	55/45	.69
Newly diagnosed GN at enrollment (%)	51	63	.23
Renal BVAS/WG (0-7) mean (SD)	4.6 (1.44)	4.5 (1.42)	.68
eCrCl at entry (mL/min) mean (SD)	53.5 (4.6)	70.5 (4.7)	.01
% with eCrCl < 50 mL/min	51	41	.32
% with eCrCl < 30 mL/min	20	16	.60
eCrCl at 18 mo	64.08 ± 5.21	80.1 ± 5.1	.03

Abbreviations: AZA, azathioprine; BVAS/WG, Birmingham Vasculitis Activity Score for Wegener's Granulomatosis; CYC, cyclophosphamide; eCrCl, estimated creatinine clearance; GN, glomerulonephritis; GPA, granulomatosis polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase-3; SD, standard deviation.



therapy may be influenced by specific patient characteristics because several factors can affect the rate of relapse. There is evidence to suggest that persistent ANCA positivity is a significant risk factor for relapse. In the study by Sanders and colleagues, at the 5-year follow-up, patients with positive ANCA serology had an over 2.5 times higher rate of relapse compared with those who became ANCA negative at 6 months and remained ANCA negative.<sup>95</sup> Other predictors of relapse include a diagnosis of GPA (vs MPA), PR3-ANCA (vs MPO-ANCA), a history of relapsing disease, and lung involvement.<sup>14,26,96-98</sup> The 18-month follow-up results of the RAVE trial confirmed the association among GPA, PR3-ANCA, and history of relapsing disease as risk factors for relapse.<sup>93</sup> In contrast, newly diagnosed patients with MPA and MPO-ANCA had hardly any relapses regardless of B-cell reconstitution. Taken together, the data suggest that patients with previous relapses, diagnosis of GPA, and PR3-ANCA should be targeted for long-term remission maintenance therapy even beyond 18 months. In contrast, patients with MPO-ANCA and a new diagnosis of MPA may not need remission maintenance therapy beyond 18 months. What to do for newly diagnosed patients who have successfully achieved remission with rituximab currently remains unclear. It should be emphasized that all patients with AAV need continued close monitoring, particularly after discontinuation of immunosuppression because the highest risk of relapse is highest within months after discontinuation of maintenance therapy.

## Biologic Response Modifiers

Biologic response modifiers (BRMs) are alternative therapeutic modalities that target a specific pathway in the immune system with the goal of reducing the maladaptive responses.<sup>99</sup> Available BRMs include those that target a specific cytokine such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ; etanercept, infliximab, adalimumab), deplete B lymphocytes (rituximab), inhibit B-cell activating factor (BAFF; belimumab), or target T lymphocytes (abatacept, alemtuzumab). Of the mentioned therapies, rituximab, which was discussed in the previous section, is the only therapy that has proven to be effective when treating patients with AAV.

Of the BRMs other than rituximab, TNF- $\alpha$  inhibitors are the most extensively studied agents. TNF- $\alpha$  is the key cytokine in inducing and propagating inflammation,<sup>100</sup> and its inhibitors have been used successfully in various autoimmune diseases.<sup>101</sup> Therapies used in AAV, in particular GPA, include etanercept, infliximab, and adalimumab. Etanercept contains the extracellular portion of 2 TNF receptors that are fused to the Fc portion of immunoglobulin G1, making it soluble. Etanercept binds to the circulating TNF- $\alpha$  and renders it inactive. The use of etanercept in GPA was investigated

in a large, randomized, placebo-controlled clinical trial involving 180 patients by the Wegener's Granulomatosis Etanercept Trial research group.<sup>42</sup> Individuals were randomized to receive either etanercept or placebo in addition to standard therapy (CYC with corticosteroids or MTX). There were no differences in the rate of sustained remission or disease flares, but there was a higher rate of solid malignancy in the etanercept group ( $P = .01$ ). Given these findings, the use of etanercept in the treatment of patients with GPA is not recommended.

Infliximab and adalimumab are 2 monoclonal antibodies against TNF- $\alpha$ , neither of which have proven efficacy in the treatment of patients with AAV.<sup>102-104</sup> In a prospective randomized trial in which 17 patients with refractory GPA were randomized to receive either rituximab or infliximab, rituximab had a favorable outcome, and post hoc analysis showed that rituximab was the preferred agent in inducing and maintaining remission.<sup>103</sup> Adalimumab has only been used in 1 uncontrolled study of 14 patients.<sup>104</sup> This study suggested that the addition of adalimumab may reduce exposure to prednisone, an observation that remains to be replicated and validated in a larger cohort and a randomized trial.<sup>104</sup>

BAFF inhibitors such as belimumab play a major role in the development and survival of B lymphocytes. Belimumab is currently approved by the FDA for treatment of patients with SLE.<sup>105,106</sup> There is an ongoing large, randomized, placebo-controlled trial investigating belimumab for remission maintenance in patients with AAV when added to azathioprine. The role of anti-BAFF therapy in the management of AAV is yet to be determined.

Therapies against T lymphocytes include abatacept and alemtuzumab. Abatacept, currently approved for use in RA, is a soluble cytotoxic T-lymphocyte-associated antigen 4 that binds to CD28 and inhibits the costimulatory signal required for activation of T cells.<sup>107</sup> Abatacept has been used in an open-label trial in patients with GPA in addition to standard therapy and has been associated with disease remission and a higher rate of prednisone discontinuation.<sup>108</sup> These results are encouraging, but a randomized controlled trial is required to validate the observation. Alemtuzumab is a monoclonal antibody against CD-52 that results in T lymphocyte depletion. Alemtuzumab was used in 71 patients with AAV who had chronically relapsing disease. Sixty-five percent of patients achieved remission, but there was also a high rate of relapse, malignancy, and infections.<sup>109</sup> A prospective randomized trial is currently ongoing that is investigating the use of alemtuzumab for the treatment of patients with refractory AAV.

Despite the advancement in the use of BRMs in the treatment of AAV, rituximab is the only agent that has proven to be effective and safe. TNF- $\alpha$  inhibitors currently have no role in the treatment of patients with

AAV, and the role of BAFF inhibition and T-cell inactivation or depletion is yet to be determined.

## Conclusion

Management of AAV is directed at induction followed by maintenance of remission. The introduction of CYC in combination with glucocorticoids had changed the prognosis of severe AAV from a uniformly fatal outcome to a chronically relapsing disease process. As the toxicities associated with long-term or repeated use of CYC became apparent, treatment alternatives were sought. Evidence now supports that maintenance therapy with azathioprine or MTX can limit exposure to CYC. In more recent years, rituximab has proven to be an effective alternative to CYC altogether, and it is superior to CYC for patients with relapsing AAV. Follow-up results from the RAVE trial as well as several smaller long-term observational studies suggest that rituximab is safe. Upcoming studies should help to further define the role of rituximab for remission maintenance therapy.

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