

Pulse Versus Daily Oral Cyclophosphamide for Induction of Remission in Antineutrophil Cytoplasmic Antibody–Associated Vasculitis

A Randomized Trial

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Background: Current therapies for antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis are limited by toxicity.

Objective: To compare pulse cyclophosphamide with daily oral cyclophosphamide for induction of remission.

Design: Randomized, controlled trial. Random assignments were computer-generated; allocation was concealed by faxing centralized treatment assignment to providers at the time of enrollment. Patients, investigators, and assessors of outcomes were not blinded to assignment.

Setting: 42 centers in 12 European countries.

Patients: 149 patients who had newly diagnosed generalized ANCA-associated vasculitis with renal involvement but not immediately life-threatening disease.

Intervention: Pulse cyclophosphamide, 15 mg/kg every 2 to 3 weeks (76 patients), or daily oral cyclophosphamide, 2 mg/kg per day (73 patients), plus prednisolone.

Measurement: Time to remission (primary outcome); change in renal function, adverse events, and cumulative dose of cyclophosphamide (secondary outcomes).

Results: Groups did not differ in time to remission (hazard ratio, 1.098 [95% CI, 0.78 to 1.55]; $P = 0.59$) or proportion of patients who achieved remission at 9 months (88.1% vs. 87.7%). Thirteen patients in the pulse group and 6 in the daily oral group achieved remission by 9 months and subsequently had relapse. Absolute cumulative cyclophosphamide dose in the daily oral group was greater than that in the pulse group (15.9 g [interquartile range, 11 to 22.5 g] vs. 8.2 g [interquartile range, 5.95 to 10.55 g]; $P < 0.001$). The pulse group had a lower rate of leukopenia (hazard ratio, 0.41 [CI, 0.23 to 0.71]).

Limitations: The study was not powered to detect a difference in relapse rates between the 2 groups. Duration of follow-up was limited.

Conclusion: The pulse cyclophosphamide regimen induced remission of ANCA-associated vasculitis as well as the daily oral regimen at a reduced cumulative cyclophosphamide dose and caused fewer cases of leukopenia.

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www.annals.org

Wegener granulomatosis, microscopic polyangiitis, and the renal-limited variant of microscopic polyangiitis are all associated with antineutrophil cytoplasmic antibodies (ANCA) and are therefore referred to collectively as ANCA-associated vasculitis. The justification for grouping these diseases together as a single clinical entity goes beyond ANCA seropositivity; they cause similar histologic changes in the kidney, are associated with similar pathogenic autoantibodies, and respond similarly to induction immunosuppressive treatment. However, they also differ in important respects; for example, granuloma formation and relapse after treatment are more common in Wegener granulomatosis (1, 2).

Outcomes for these previously fatal diseases improved dramatically with the introduction of daily oral cyclophosphamide therapy (3, 4). However, cyclophosphamide has significant adverse effects that influence long-term morbidity and mortality (5, 6). Strategies to reduce these adverse effects include reducing the duration of cyclophosphamide use to 3 to 6 months (maximum, 9 months) (2) and switching to an alternative immunosuppressive regimen after induction of remission and using methotrexate instead of cyclophosphamide in patients without generalized disease and significantly impaired renal function (7). For many patients, however, cyclophosphamide remains the mainstay of therapy for inducing remission and treating relapse, so regimens that maintain efficacy while minimizing cyclophosphamide dose and maximizing safety would be welcome.

Previous studies (8) suggest that pulse cyclophosphamide regimens are safe and provide less cumulative cyclophosphamide exposure than daily oral cyclophosphamide regimens. However, small study sizes and variations in treatment regimens, including the use of treatments alongside cyclophosphamide, make the findings preliminary. We designed this trial to test the hypothesis that a regimen of

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pulsed intermittent cyclophosphamide would be as effective but less toxic than daily oral cyclophosphamide for inducing remission in patients with generalized ANCA-associated vasculitis with active glomerulonephritis.

METHODS

Trial Design and Participants

Our trial was an open-label, multicenter, randomized, controlled trial conducted over 18 months. Patients, providers, and the investigators who assessed trial outcomes were not blinded to treatment assignment.

Our inclusion criteria were newly diagnosed Wegener granulomatosis, microscopic polyangiitis, or renal-limited microscopic polyangiitis (diagnostic criteria adapted from the 1992 Chapel Hill consensus conference [9] and our group's previous studies [2, 7, 10–12]); renal involvement attributable to active vasculitis (as defined by at least 1 of the following: serum creatinine level $>150 \mu\text{mol/L}$ [$>1.7 \text{ mg/dL}$] and $\leq 500 \mu\text{mol/L}$ [$\leq 5.7 \text{ mg/dL}$], biopsy demonstrating necrotizing glomerulonephritis, erythrocyte casts, or hematuria [>30 erythrocytes per high-power field] and proteinuria [$>1 \text{ g/d}$]); and confirmatory histology or ANCA positivity.

Our exclusion criteria were coexistence of other multi-system autoimmune disease; hepatitis B or C virus or HIV infection; serum creatinine level greater than $500 \mu\text{mol/L}$ ($>5.7 \text{ mg/dL}$); previous cancer; pregnancy; or age younger than 18 or older than 80 years.

We conducted our study according to the Declaration of Helsinki. Informed consent was obtained from each participant, and each participating center reviewed the trial protocol and granted ethical approval.

Random Assignment

Random assignments were computer-generated and performed centrally by permuted blocks of 4, stratified by country and disease. Patients were enrolled by their treating physician and registered with the central trial coordinating office by fax submission of a form that contained information on center, date of birth, sex, disease, and creatinine level. We randomly assigned patients on a 1:1 basis to receive pulse or daily oral cyclophosphamide. Data were collected in record books, entered into a central computerized database, and validated against the record books before analysis. Eleven patients withdrew before random assignment; we randomly assigned 149 patients.

Interventions

We designed the pulse cyclophosphamide regimen by investigator consensus, on the basis of published experience with pulse cyclophosphamide in ANCA-associated vasculitis. Patients received 3 intravenous pulses of cyclophosphamide, 15 mg/kg , given 2 weeks apart, followed by pulses at 3-week intervals (15 mg/kg intravenously or 5 mg/kg orally on 3 consecutive days, at the physician's discretion) until remission, and then for another 3 months. The maximum

Context

Because cyclophosphamide has many adverse effects, dosing regimens that maintain efficacy but improve safety would be welcome.

Contribution

In this randomized comparison of pulse and daily oral cyclophosphamide regimens for treatment of ANCA-associated vasculitis, equal proportions of patients had remissions, but the pulse regimen seemed safer, mainly because it caused less leukopenia.

Caution

Patients and providers were not blinded to the intervention, and the study was not powered to detect differences in relapse rate.

Implication

The efficacy of pulse cyclophosphamide for treatment of ANCA-associated vasculitis seems no different from that of daily oral treatment and may be safer.

—The Editors

dose per pulse was 1.2 g . We reduced the cyclophosphamide dose by 2.5 mg/kg per pulse for persons age 60 to 70 years, 5 mg/kg per pulse for persons older than 70 years, and 2.5 mg/kg per pulse for persons with a serum creatinine level of 300 to $500 \mu\text{mol/L}$ (3.4 to 5.7 mg/dL). At minimum, blood counts were checked on day 10 and 14 after each pulse and immediately before the next pulse. We reduced the dose of the subsequent pulse by 20% for patients with a leukocyte nadir of 2 to $3 \times 10^9/\text{L}$ and 40% for those with a nadir of 1 to $2 \times 10^9/\text{L}$.

The daily oral cyclophosphamide group received cyclophosphamide, 2 mg/kg per day, until remission, followed by 1.5 mg/kg per day for another 3 months. The maximum oral dose was 200 mg , and we reduced the dose by 25% for persons older than 60 years and 50% for those older than 70 years. At minimum, blood counts were checked weekly for the first month, twice-weekly for the second month, and monthly thereafter. We withheld cyclophosphamide for persons with a leukocyte count less than $4 \times 10^9/\text{L}$, then resumed therapy at a dose reduced by 25 mg/d when their count increased to greater than $4 \times 10^9/\text{L}$.

Both groups continued the cyclophosphamide regimens for 3 months after remission, after which all patients received azathioprine, 2 mg/kg per day orally, until month 18 for remission maintenance. The maximum daily oral dose of azathioprine was 200 mg . Both groups also received prednisolone, 1 mg/kg orally, tapered to 12.5 mg at the end of month 3 and to 5 mg at the end of the study (month 18). 2-Mercaptoethanesulfonate sodium was optional in both groups. No patients received plasmapheresis.

We recommended prophylaxis for *Pneumocystis jiroveci* for all patients.

Treatment was allowed to follow local practice for patients who did not achieve remission at 9 months. We collected data on these patients but censored them for purposes of this analysis.

For more details on the protocol, see **Appendix 1** (available at www.annals.org).

Outcomes and Follow-up

We defined outcomes by using the Birmingham Vasculitis Activity Score (BVAS) index, which measures manifestations of active vasculitis during the 28 days before the date of assessment (13).

Our primary outcome was time to remission, defined as the absence of new or worse signs of disease activity on the BVAS and no more than 1 item indicating persistent disease activity (BVAS ≤ 1). Secondary outcomes included the proportion of patients who achieved remission at 6 and 9 months and the proportion with major and minor relapses. We defined major relapse as the recurrence or first appearance of at least 1 BVAS item indicating threatened vital organ function attributable to active vasculitis. We defined minor relapse as the recurrence or first appearance of at least 3 other BVAS items related to nonvital organs. An investigator classified patients as achieving remission or having relapse, and an independent observer validated these classifications retrospectively. Additional secondary outcomes were death; change in renal function; adverse events, including leukopenia and infection; and the cumulative dose of cyclophosphamide and prednisolone, which we calculated as the total cumulative drug dose at each time point in the study (3, 6, 9, 12, 15, and 18 months) divided by the number of patients in the study at that point. For each time point, we considered only the dose of drug for those patients still in the study.

Unless otherwise noted, we assessed these outcomes at baseline; at 1.5, 3, 4.5, 6, 7.5, 9, 12, 15, and 18 months after baseline; and at relapse, on the basis of standard recommendations. Clinical assessments included BVAS measures at every visit and measures of cumulative damage from any cause since disease onset, as scored by the Vasculitis Damage Index (14), at baseline and every 3 months. Laboratory assessments included measures of full blood count, C-reactive protein, alanine transaminase, serum creatinine, and glucose, as well as dipstick urine analysis. We calculated glomerular filtration rate at entry, remission, and study end by using the Modification of Diet in Renal Disease method (15).

Statistical Analysis

We determined the sample size for the trial by clinical rather than statistical considerations. We set a recruitment goal of 160 patients; we considered that number ambitious, given the rarity of these conditions (12 per 1 million persons) and the need to recruit patients and conduct the

trial within a period (5 years) that was reasonable for our resources.

We performed analyses by intention to treat. To account for censoring, we compared remission and survival by using survival methods instead of relative frequencies. We plotted Kaplan–Meier curves and made comparisons by using the log-rank test. We obtained hazard ratios by Cox regression, with treatment group as the only independent variable. We censored patients who died, were withdrawn, or were lost to follow-up at the time of death or withdrawal and censored those who did not achieve remission by 9 months at 9 months.

We used the log-rank test to compare survival; relapse; and time to relapse, which we defined as from the time patients achieved remission. We excluded patients who did not achieve remission from the analysis. We plotted Kaplan–Meier curves and made comparisons by using the log-rank test. We obtained hazard ratios by Cox regression, with treatment group as the only independent variable. We confirmed the assumption of proportional hazards by using a log-minus-log survival plot. We compared demographic and disease characteristics (adverse events, laboratory values, and BVAS [area under the curve]) between groups by using the chi-square test for categorical variables and the Mann–Whitney U test for continuous variables.

We performed exploratory subgroup analyses of the study's clinical outcomes (remission, relapse, and death) by disease subgroups; we found no apparent differences and have therefore not reported the results here.

We used SPSS for Windows, version 13 (SPSS, Chicago, Illinois), for all statistical analyses.

Role of the Funding Source

This trial was funded by the European Union (European Community Systemic Vasculitis Trial project, contract BMH1-CT93-1078 and CIPD-CT94-0307, and Associated Vasculitis European Randomised Trial project, contract BMH4-CT97-2328 and IC20-CT97-0019). The funding source had no involvement in the study's design, conduct, or reporting.

RESULTS

Patients

We recruited 160 patients from 42 hospitals in 13 European countries and Mexico (**Appendix 2**, available at www.annals.org). Eleven patients were withdrawn before random assignment: 1 declined further participation, 8 were withdrawn by their physician, and 2 did not meet the entry criteria. We randomly assigned 76 patients to the pulse group and 73 to the daily oral group (**Figure 1**). We balanced baseline characteristics between groups at entry (**Table 1**). Three patients were ANCA-negative according to immunofluorescence and enzyme-linked immunosorbent assay testing. Median follow-up in both groups was 18 months (range, 0.25 to 18 months). **Figure 1** details

Figure 1. Study flow diagram.

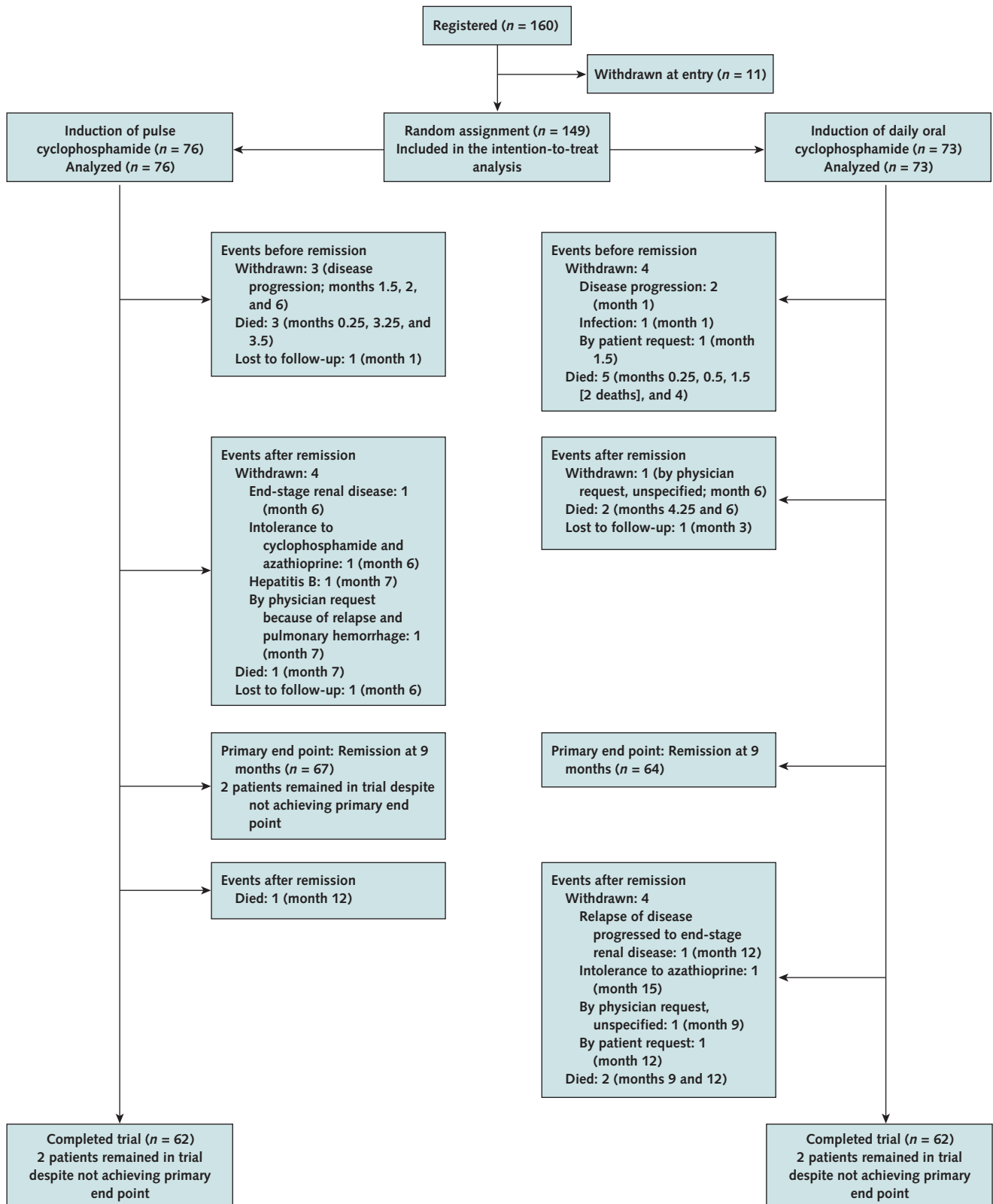


Table 1. Patient Characteristics

Characteristic	Pulse Cyclophosphamide Group (n = 76)	Daily Oral Cyclophosphamide Group (n = 73)
Mean age (SD), y	56.5 (15.3)	58.2 (13.7)
Men, n (%)	41 (54)	47 (64)
Diagnosis, n (%)		
Wegener granulomatosis	25 (33)	31 (42)
Microscopic polyangiitis	38 (50)	33 (45)
Renal limited vasculitis	13 (17)	9 (12)
Confirmatory renal biopsy, n (%)	60 (79)	57 (78)
Positive biopsy result and ANCA-positive, n (%)	60 (79)	56 (77)
Positive biopsy result and ANCA-negative, n (%)	0	1 (1)
Negative biopsy result and ANCA-positive, n (%)	16 (21)	16 (22)
PR3-ANCA-positive, n (%)	30 (39)	30 (41)
MPO-ANCA-positive, n (%)	38 (50)	37 (51)
PR3- and MPO-ANCA-positive, n (%)	4 (5)	2 (3)
PR3- and MPO-ANCA-negative, n (%)	4 (5)	4 (5)
Mean BVAS for new or worse disease (SD)*	20 (6.8)	21 (6.7)
Mean disease extension index (SD)†	4.2 (2.2)	4.5 (2.2)
Mean serum creatinine level (SD)		
μmol/L	225 (128)	222 (120)
mg/dL	2.55 (1.45)	2.51 (1.36)
Mean estimated GFR (SD), mL/min per 1.73 m ² ‡	38 (27)	35 (21)
Mean C-reactive protein level, nmol/L	523.82 (542.87)	838.11 (847.64)
Vasculitis Damage Index Score (range)	0 (0–5)	0 (0–3)
Recruiting country, n		
Belgium	1	5
Czech Republic	16	13
Finland	3	2
France	11	9
Germany	3	2
Ireland	2	5
Italy	3	4
Mexico	2	3
Netherlands	1	2
Poland	0	1
Spain	7	4
Sweden	9	7
Switzerland	1	1
United Kingdom	17	15

ANCA = antineutrophil cytoplasmic antibody; BVAS = Birmingham Vasculitis Activity Score; GFR = glomerular filtration rate; MPO = myeloperoxidase; PR3 = proteinase 3.

* A semiquantitative weighted score of disease activity attributable to active vasculitis (17).

† Number of organs affected by active vasculitis (25).

‡ Estimated by using the Modification of Diet in Renal Disease Study equation (16).

patient withdrawals (16 patients), deaths (11 patients), and losses to follow-up (3 patients).

Seven patients were withdrawn from the pulse cyclophosphamide group: 3 had uncontrolled disease, which led to end-stage renal disease in 2 patients; 1 needed dialysis despite remission; 1 developed hepatitis B; 1 could not tolerate the study medication, and 1 was withdrawn by their physician because of lung hemorrhage associated with relapse. We also lost 2 patients to follow-up.

Nine patients were withdrawn from the daily oral cyclophosphamide group: 2 by physician request after they achieved remission; 2 by patient request (1 of whom had not achieved remission); 3 for disease progression, 2 without achieving remission and 1 after relapse to end-stage renal disease; 1 for azathioprine-related hepatotoxicity; and 1 for recurrent infection without achieving remission. We also lost 1 patient to follow-up.

Median time to withdrawal did not differ between groups (pulse group, 6 months [range, 1.5 to 15 months]; daily oral group, 6 months [range, 1 to 15 months]). Seven of the 16 patients who were withdrawn and 1 of the 3 patients who were lost to follow-up had not achieved remission. Among all patients who were withdrawn or lost to follow-up, similar numbers achieved or did not achieve remission (4 patients did not achieve remission in each group, 5 achieved remission in the pulse group, and 6 achieved remission in the daily oral group).

Disease Remission

One hundred thirty-one patients (78.9%) achieved remission by 9 months. Of the 18 who did not, 5 patients in the daily oral group died, 3 in the pulse group died, and 4 patients in each group were withdrawn or lost to follow-up (Figure 1). Two patients did not achieve remission by 9

months but remained in the study; we censored them because they did not receive protocol-based treatment.

The groups did not differ in time to remission (hazard ratio [HR], 1.098 [95% CI, 0.78 to 1.55]; $P = 0.59$) (Figure 2) or proportion of patients who achieved remission at 9 months (67 [88.1%] in the pulse group vs. 64 [87.7%] in the daily oral group) (Table 2). Median time to remission was 3 months for both groups (pulse group range, 0.5 to 8 months; daily oral group range, 1 to 7.5 months).

Relapse

Nineteen (14.5%) of the 131 patients who achieved remission by 9 months subsequently had relapse; 13 (7 with major and 6 with minor relapse) in the pulse group and 6 (3 with major and 3 with minor relapse) in the daily oral group (HR, 2.01 [CI, 0.77 to 5.30]). The between-group difference in the proportion of patients who had relapse was not statistically significant, but our study was not designed or powered to test the effect of the intervention on relapse rate.

Deaths

Fourteen patients died: 5 in the pulse group and 9 in the daily oral group ($P = 0.79$) (Table 2). Four patients died within 1.5 months, 8 died before achieving remission, and 6 died after achieving remission (median months after achieving remission, 3.6 [range, 0.25 to 11 months]). Median time to death did not differ between groups (pulse group, 3.5 months [range, 0.25 to 12 months]; daily oral group, 4 months [range, 0.5 to 12 months]). In the pulse group, 1 patient died of sepsis, 1 of bowel perforation, 1 of myocardial infarction, 1 of pulmonary embolus, and 1 of pharyngeal cancer. In the daily oral group, 5 patients died of sepsis, 2 of progressive disease, 1 of pulmonary fibrosis, and 1 of gastrointestinal hemorrhage. Two patients in the pulse group and 4 in the daily oral group had achieved remission at the time of death; we thought death was associated with active disease or treatment in 3 patients in the pulse group and 7 in the daily oral group.

Renal Function

In the pulse group, the median estimated glomerular filtration rate improved from 32 mL/min per 1.73 m² (interquartile range [IQR], 15 to 52 mL/min per 1.73 m²) at study entry to 45 mL/min per 1.73 m² (IQR, 27 to 66 mL/min per 1.73 m²) at study end ($P < 0.010$). Renal function also improved among patients in the daily oral group, from 29 mL/min per 1.73 m² (IQR, 18 to 48 mL/min per 1.73 m²) to 45 mL/min per 1.73 m² (IQR, 30 to 66 mL/min per 1.73 m²) ($P < 0.010$) (Figure 3 and Table 2). Glomerular filtration rate did not differ between study groups at any time point. The between-group differences in improvement in estimated glomerular function were not statistically significant (pulse median improvement, 5 mL/min per 1.73 m² [IQR, 3 to 20 mL/min per 1.73 m²]; daily oral median improvement, 8 mL/min per 1.73 m² [IQR, 0 to 24 mL/min per 1.73 m²]; $P = 0.362$).

Six patients (5 in the pulse group and 1 in the daily oral group) developed end-stage renal disease, 3 in the context of uncontrolled disease during induction of the remission phase. The 2 groups did not differ in the development of end-stage renal disease ($P = 0.105$). Another patient in the daily oral group temporarily required dialysis but recovered independent renal function by study end.

Measures of Disease Activity

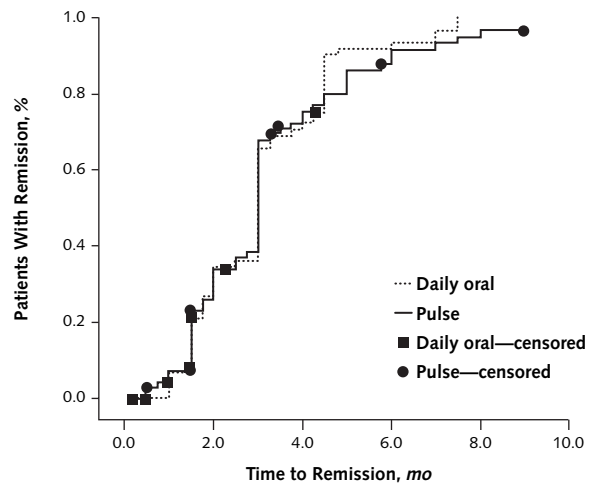
Birmingham Vasculitis Activity Scores for new or worse disease decreased promptly with treatment and were similar in both groups at all time points (Figure 4, top, and Table 2). All-cause damage, as assessed by the Vasculitis Damage Index, increased in both groups from a median of 0 at entry to 2 at months 6 and 18. Median all-cause damage did not differ between the 2 groups at any time point. Mean C-reactive protein concentrations were similar in both treatment groups at study start and decreased rapidly in both groups after initiation of induction therapy (Figure 4, bottom).

Adverse Events

Two hundred twenty-eight adverse event episodes occurred in 114 patients. Of these, 178 episodes in 85 patients were mild to moderate and 50 episodes in 29 patients were severe to life-threatening (Table 3).

Eighty-seven leukopenic episodes occurred in 53 patients. Patients were less likely to be affected by leukopenia in the pulse group than in the daily oral group (20 [26%] patients vs. 33 [45%] patients; $P = 0.016$). Four patients in the pulse group experienced multiple episodes, com-

Figure 2. Time to remission (Kaplan–Meier curves) for the pulse and daily oral cyclophosphamide groups.



Daily oral	73	43	18	4	0
Pulse	76	46	15	4	2

Sample sizes are listed for each group; missing data are from patients who were withdrawn or died.

Table 2. Main Outcome Parameters, by Treatment Group*

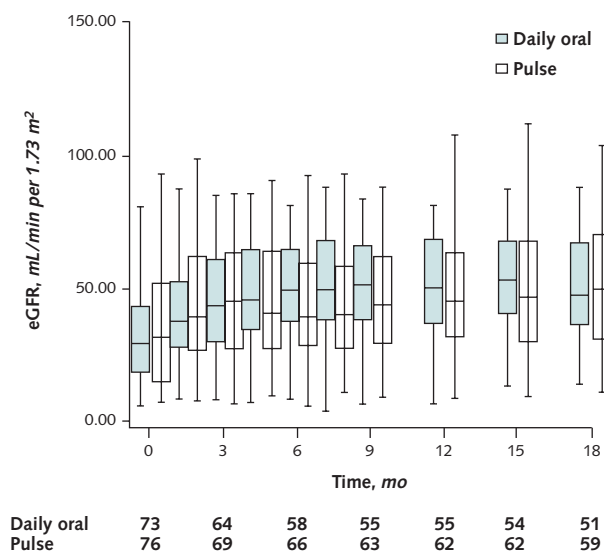
Parameter	Baseline		3 Months		6 Months	
	Pulse Group	Daily Oral Group	Pulse Group	Daily Oral Group	Pulse Group	Daily Oral Group
Total patients, <i>n</i>	76	73	72	65	66	60
Disease status, <i>n</i>						
Active disease	76	73	23	22	5	5
Achieved remission	0	0	49	43	61	55
Censored (in remission), <i>n</i>						
Died	0	0	1 (0)	4 (0)	3 (0)	7 (2)
Lost to follow-up	0	0	1 (0)	0	2 (1)	1 (1)
Withdrew	0	0	2 (0)	4 (0)	5 (2)	5 (1)
Relapse after initial remission, <i>n</i>	0	0	1	1	1	3
Renal outcomes						
End-stage renal disease, <i>n</i>	0	0	1	0	4	0
Median estimated glomerular filtration rate (IQR), mL/min per 1.73 m ² †	32 (15–52)	29 (18–48)	45 (28–64)	44 (30–63)	40 (28–60)	50 (37–64)
Cumulative cyclophosphamide dose						
Median dose for patients still in study (IQR), g	0	0	4.34 (3.5–11.3)	9.0 (7.65–11.33)	8.18 (6.5–10.0)	15.75 (11.48–19.6)

IQR = interquartile range.

* Numbers are cumulative over time. Patients who had active disease, achieved remission, died, withdrew, and were lost to follow-up always total the number of patients recruited to the study. Patients with relapse are described separately in the daily oral or pulse group. We censored those patients who did not achieve remission at 9 months because we had no treatment protocol for those who still had active disease after this time point. One patient achieved remission at 12 months and 1 had active disease until 18 months. One hundred thirty-two patients achieved remission; however, for the primary analysis, only 131 achieved remission. The pulse and daily oral groups did not differ in remission, relapse, or mortality rates at the end of study.

† Estimated by using the Modification of Diet in Renal Disease Study equation (16).

Figure 3. Sequential eGFR for the pulse and daily oral cyclophosphamide groups.



We estimated the rate by using the Modification of Diet in Renal Disease formula. Data are presented as medians plus interquartile ranges. Sample sizes are listed for each group; missing data are from patients who were withdrawn, died, were missing creatinine measurements for that visit, or developed end-stage renal failure. eGFR = estimated glomerular filtration rate.

pared with 15 in the daily oral group. The median time to the first leukopenic episode was 219 days (range, 14 to 549 days) in the pulse group and 68 days (range, 8 to 318 days) in the daily oral group (HR, 0.41 [CI, 0.23 to 0.71]). Only 10 of these episodes preceded or were simultaneous with infection. Fifty-nine of these events were very mild (leukocyte count 3 to 4 × 10⁹ cells/L) and did not progress.

We recorded 51 episodes of infection in 41 patients (22 among 20 patients in the pulse group and 29 among 21 patients in the daily oral group). The median time to the first episode of infection was 147 days (range, 12 to 472 days) for the pulse group and 68 days (range, 9 to 533 days) for the daily oral group (HR, 0.88 [CI, 0.42 to 1.83]). We identified 10 serious or life-threatening episodes of infection in the daily oral group: 1 episode each of pneumonia, bowel perforation after diverticulitis, *P. jiroveci* pneumonia with fatal outcome (the patient had not received *P. jiroveci* pneumonia prophylaxis), and herpes simplex infection and 6 episodes of infection not further characterized by the investigator, 3 of which were preceded by a leukopenic episode. We identified 7 such episodes in the pulse group: 2 episodes of pneumonia, 2 episodes of *Escherichia coli* sepsis, 1 episode each of septic shock and perirectal abscess with subsequent perforation, and 1 infection not further characterized by the investigator. One episode of *E. coli* and the episode of septic shock were simultaneously associated with leukopenia.

Table 2—Continued

9 Months		12 Months		15 Months		18 Months	
Pulse Group	Daily Oral Group	Pulse Group	Daily Oral Group	Pulse Group	Daily Oral Group	Pulse Group	Daily Oral Group
63	58	62	55	62	54	62	54
2	0	1	0	1	0	1	0
61	58	61	55	61	54	61	54
4 (1)	8 (3)	5 (2)	9 (4)	5 (2)	9 (4)	5 (2)	9 (4)
2 (1)	1 (1)	2 (1)	1 (1)	2 (1)	1 (1)	2 (1)	1 (1)
7 (4)	6 (2)	7 (4)	8 (4)	7 (4)	9 (5)	7 (4)	9 (5)
7	3	7	6	10	6	13	6
4	0	4	1	4	1	5	1
44 (29–62)	52 (38–66)	45 (32–65)	50 (37–69)	47 (29–68)	53 (41–69)	50 (30–70)	48 (36–69)
8.28 (6.55–10.68)	17.5 (13.8–24.75)	8.5 (6.74–10.93)	18 (13.49–26.31)	8.58 (6.76–11.9)	18.05 (13.48–26.77)	8.58 (6.76–11.9)	18.05 (13.5–27)

Doses of Cyclophosphamide and Prednisolone

Figure 5 and Table 2 show the median cumulative dose (IQR) of cyclophosphamide over time for each group. The median cumulative dose of cyclophosphamide differed between groups, with the daily oral group receiving nearly twice the dose administered to the pulse group (15.9 g [IQR, 11 to 22.5 g] vs. 8.2 g [IQR, 5.95 to 10.55 g]; $P < 0.001$).

The groups did not differ in prednisolone dose at any time point, with a mean cumulative dose of 7587 mg (SD, 1957) in the pulse group and 7586 mg (SD, 1460) in the daily oral group.

DISCUSSION

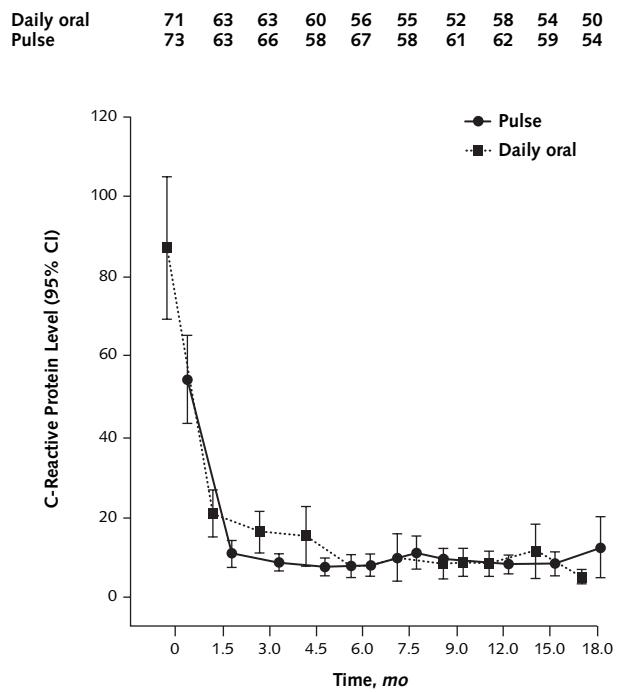
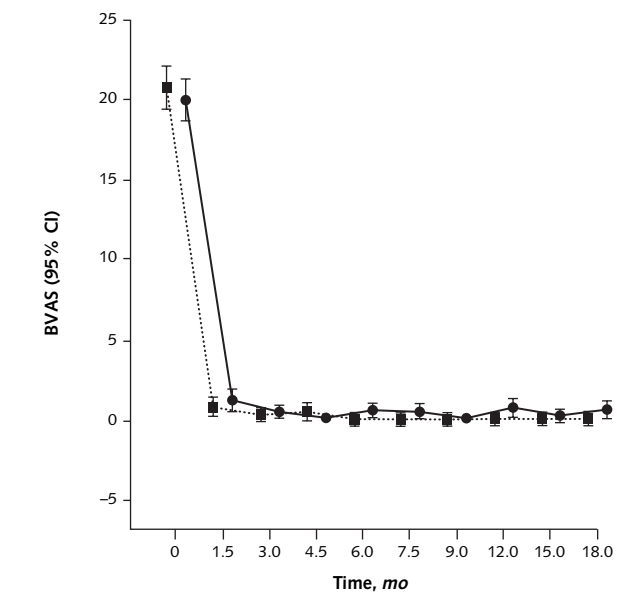
We detected no difference in time to remission and fewer episodes of leukopenia among patients with ANCA-associated vasculitis who received a consensus-designed regimen of pulse cyclophosphamide compared with those who received a regimen of daily oral cyclophosphamide. The fewer leukopenic episodes suggest that the pulse regimen might be a safer alternative for the initial treatment of patients with generalized ANCA-associated vasculitis and renal involvement.

Previous studies of pulse cyclophosphamide have been inconclusive because of small numbers. Our previously published meta-analysis (8) suggested that pulse cyclophosphamide was more likely to induce remission than daily oral cyclophosphamide and was associated with a significantly lower rate of leukopenia and infection but also with a higher relapse rate. A more recent study (16), which included patients with microscopic polyangiitis and polyar-

teritis nodosa, suggested that 12 months of treatment with pulse cyclophosphamide was associated with a lower relapse rate than 6 months of treatment. However, this study also had small numbers (only 65 patients), and patients did not receive an immunosuppressive drug after cyclophosphamide withdrawal. To our knowledge, our trial is the largest to date to compare the efficacy of pulse cyclophosphamide and daily cyclophosphamide induction regimens in a well-defined population, and our results support the findings of our previous meta-analysis.

We observed more relapses with the pulse cyclophosphamide regimen than with daily oral cyclophosphamide, but our findings are difficult to interpret. Our study was not designed to detect differences in relapse rates between the pulse and daily oral regimens and was not powered to assess this end point, and the 95% CI surrounding the estimate of treatment effect was statistically compatible with both lower and increased relapse rates, so the finding should be interpreted with caution. Several studies (17, 18) have recently shown that the risk for relapse is inversely associated with the cumulative cyclophosphamide dose received during the induction phase. The overall relapse rate in our trial was 14.4%, despite the continuation of each group's cyclophosphamide regimen for 3 months after remission. This frequency of relapse is similar to that observed in a trial that compared cyclophosphamide and azathioprine for maintenance of remission in ANCA-associated vasculitis (2), which used a similar daily oral cyclophosphamide induction regimen in a similar patient cohort but without a subsequent consolidation phase. This suggests that the 3-month consolidation phase has little effect on the risk for relapse during the first 18 months of

Figure 4. Measures of disease activity for the pulse and daily oral cyclophosphamide groups.



Data are presented as means plus 95% CIs. Sample sizes are listed for each group; missing data are from patients who were withdrawn, died, or were missing measurements for that visit. BVAS = Birmingham Vasculitis Activity Score.

follow-up, at least when cyclophosphamide is received daily and by mouth.

Of note, recent pooled data from all European Vasculitis Study Group trials completed so far suggest that re-

ceipt of prednisolone in the maintenance phase may be a decisive factor for preventing relapse over the long term (results unpublished). The results of current randomized, controlled trials show higher relapse rates in trials that discontinued corticosteroids in the first 6 months (19) or 12 months (7), as opposed to those that, like ours, continued to administer prednisolone for 18 months and longer (2).

Adverse events, particularly infection, have been reported to increase the risk for patient death (20). Patients who received pulse cyclophosphamide had fewer episodes of leukopenia and received approximately half the cyclophosphamide dose of those who received daily oral cyclophosphamide. Other adverse events did not significantly differ between groups. Although our study does not provide definitive evidence that pulse cyclophosphamide is safer than daily oral cyclophosphamide, the evidence is suggestive. Antineutrophil cytoplasmic antibody-associated vasculitis is a relapsing disease, and relapse is treated by the reintroduction of induction-of-remission regimens. Therefore, we believe that reducing total cyclophosphamide load from the start is a useful strategy. Pulse cyclophosphamide is simpler to monitor than daily oral cyclophosphamide, and the requirement to attend the clinic provides a safeguard not in place for daily oral cyclophosphamide. Pulse administration also permits bladder protection through prehydration and administration of 2-mercaptoethanesulfonate sodium.

The mortality rate was 9% at 18 months, which is slightly higher than the 5% rate reported in the CYCAZAREM (Cyclophosphamide Compared With Azathioprine for Maintenance of Remission in ANCA-Associated Vasculitis) trial (2), despite similar inclusion criteria. Early deaths before remission were similar between the 2 studies, and time to remission did not differ (results not shown). However, our study had more late deaths during the remission period. Glomerular filtration rate at entry was lower and renal recovery was poorer in our study, with a mean improvement of 13 mL/min per 1.73 m² compared with an improvement of 20 mL/min per 1.73 m² in CYCAZAREM (2). The higher proportion of patients with microscopic polyangiitis in our study (62% vs. 39%; *P* < 0.001) might account for this difference. Hauer and colleagues (21) showed that patients with microscopic polyangiitis had more chronic lesions in renal biopsies than did patients with Wegener granulomatosis, which may be less amenable to therapy. Furthermore, our study had a larger percentage of men than the CYCAZAREM trial (59% vs. 47%), which may have contributed to a worse outcome, because men have been shown to display greater disease extent and renal impairment than women at the time ANCA-associated vasculitis is diagnosed (4).

Our study has several limitations. We had too few patients to reach a conclusion with sufficient power regarding disease relapse. Longer-term, adequately powered follow-up studies are required to determine the effect of reducing the

Table 3. Adverse Events, by Treatment Group*

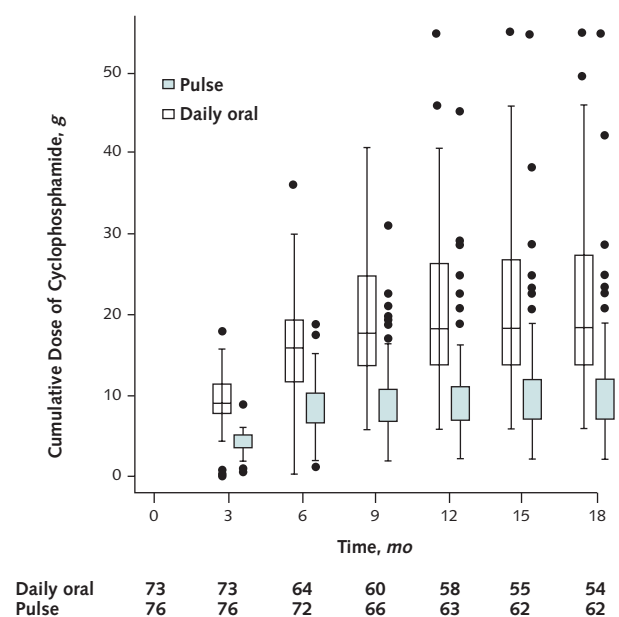
Event	Pulse Cyclophosphamide Group (n = 76)	Daily Oral Cyclophosphamide Group (n = 73)
Any adverse event		
Patients, n (%)	58 (77)	56 (77)
Episodes, n		
Mild or moderate	77	101
Severe or life-threatening	19	31
Leukopenia, n		
All patients	20	33
Patients with ≥ 2 episodes	4	15
Episodes	28	59
Infection, n		
All patients	20	21
Mild or moderate	15	19
Severe or life-threatening	7	10
New or worsening diabetes, n	8	4
Liver dysfunction, n	2	3
Alopecia, n	0	2
Hypersensitivity reaction to azathioprine, n	10	5
Osteoporosis, n	2	0
Cancer, n	1	0
Hemorrhagic cystitis, n	2	1
Amenorrhea, n	1	0
Cataracts, n	0	3
Hypertension, n	0	2
Cardiovascular events (cerebrovascular accident or myocardial infarction), n	3	2
Pulmonary embolism or deep venous thrombosis, n	2	4
Other, n	15	18

* All numbers refer to number of episodes, except where specifically noted.

cumulative dose of cyclophosphamide during induction regimens on relapse rates. A long-term follow-up of this patient cohort, which is currently under way, may detect differences in relapse rates. We know from several uncontrolled, long-term follow-up studies in single centers (22, 23) that time from induction treatment has a great influence on relapse rate. A further disadvantage of our study is that it was not placebo-controlled, which is a potential source of bias because end points were based on BVAS and were necessarily subjective. No objective biomarkers of disease remission have been validated. The addition of a 3-month consolidation phase to the daily oral cyclophosphamide regimen might bias the results against that group by increasing the risk for hematologic toxicity without improving efficacy. Unfortunately, the CYCAZAREM trial (2) had not been completed when we designed our study and it was not clear whether only 3 months of therapy with daily oral cyclophosphamide was appropriate.

In conclusion, we found no difference in remission rates for generalized but not immediately life-threatening ANCA-associated vasculitis between patients who received a consensus-designed pulse cyclophosphamide regimen and those who received a daily oral cyclophosphamide regimen. The pulse cyclophosphamide regimen allowed administration of half the cumulative cyclophosphamide dose of the daily oral regimen but used the same corticosteroid dose. Future studies are needed to explore appropriate maintenance of remission regimens for patients who receive pulse

Figure 5. Cumulative cyclophosphamide dose per person over time.



We used a box-and-whiskers plot to describe the median dose per patient and the interquartile range of sequential cumulative cyclophosphamide dose in each treatment group. Extremes and outliers are represented as circles. Sample sizes are listed for each group; missing data are from patients who were withdrawn or died.

rather than daily oral cyclophosphamide treatment to induce remission.

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Reproducible Research Statement: *Study protocol:* Available at www.vasculitis.org/protocols/CYCLOPS.pdf. *Statistical code:* Available from Dr. Harper (e-mail, L.harper@bham.ac.uk). *Data set:* Available to approved individuals through written agreements with the steering committee of the EUVAS network (contact Dr. Harper; e-mail, L.harper@bham.ac.uk).

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APPENDIX 1: DETAILS TO THE PROTOCOL

Prophylaxis against steroid-induced gastritis, fungal infection, and *Pneumocystis jiroveci* pneumonia was at the discretion of the local investigator. Patients older than 50 years received cal-

cium and vitamin D supplementation for prophylaxis against steroid-induced osteoporosis.

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