Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Stone JH, Merkel PA, Spiera R, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med 2010;363:221-32.

APPENDIX

Methods and Data Supplement[1]

This supplement contains additional information about the methods and data from the Rituximab in ANCA-Associated Vasculitis (RAVE) trial. Specific components of this supplement include:

1. Detailed Inclusion and Exclusion Criteria
2. Concomitant Medications
3. Glucocorticoid Use
    - 3.1. The Glucocorticoid Taper
    - 3.2. Glucocorticoid Use Prior to Baseline
    - 3.3. Cumulative Glucocorticoid Use at Six Months
4. Interim Analysis
5. Inferiority Trial Design and Efficacy Analysis
6. Additional Results
    - 6.1. Reasons for Primary Endpoint Failure in the Two Treatment Groups
    - 6.2. Quality-of-Life
    - 6.3 Malignancies Beyond 6 Months

[1] All citations refer to references within the manuscript.

1. Details of Inclusion and Exclusion Criteria

1.1. Inclusion Criteria:

- Diagnosis of WG or MPA according to the Chapel Hill Consensus Conference definitions[1]
- Positive serum assay for PR3-ANCA or MPO-ANCA
- Newly-diagnosed at the time of screening or presenting with a severe disease flare
- Birmingham Vasculitis Activity Scores for WG (BVAS/WG) of 3 or more
- Severe disease; i.e., one or more major BVAS/WG items or be severe enough to require treatment with cyclophosphamide to induce remission[24]. Under the current standard of care, all subjects would have been considered for treatment with cyclophosphamide and glucocorticoids because of severe disease manifestations.

1.2. Exclusion Criteria:

- Churg-Strauss syndrome or anti-glomerular basement membrane disease
- Patients with limited disease activity not normally treated with cyclophosphamide
- Alveolar hemorrhage severe enough to require mechanical ventilation
- Serum creatinine level greater than 4.0 mg/dl attributed to renal failure from the current episode of renal disease activity
- White blood cell count less than 4000/mm^3 or platelet counts less than 120,000/mm^3

- Serum hepatic transaminase levels greater than 2.5 times the upper limit of normal
- Known allergy to monoclonal antibodies or murine proteins
- Active systemic infection at time of screening
- History of deep space infection such as osteomyelitis, septic arthritis, or pneumonia complicated by empyema
- History of infection with hepatitis B or C or human immunodeficiency virus
- Acute or chronic liver disease severe enough to preclude the ability to participate in the trial.
- Malignancy: Active or history of malignancy in the last 5 years. (Individuals with squamous cell or basal cell skin carcinomas and individuals with cervical carcinoma in situ may be enrolled if they have received curative surgical treatment).
- Receipt of a live vaccine fewer than 4 weeks before potential randomization.
- Receipt of oral or intravenous cyclophosphamide within 4 months prior to enrollment
- Receipt of glucocorticoids for longer than 14 days before screening
- History of adverse effects from standard therapy (i.e., bone-marrow hypoplasia, cyclophosphamide-induced hemorrhagic cystitis or malignancy)
- Previous therapy with rituximab or alemtuzumab
- Treatment with plasma exchange within the 3 months preceding the screening visit

2. Concomitant Medications

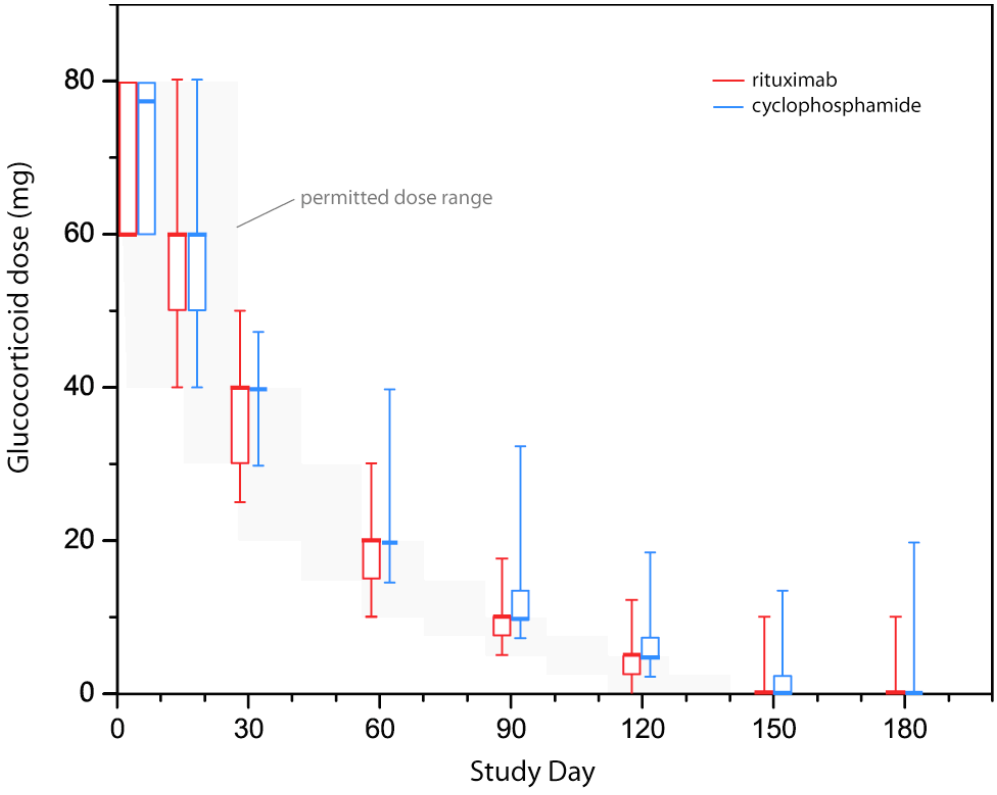
All patients received *Pneumocystis* and osteoporosis prophylaxis for the duration of the trial. The use of any immunosuppressive medication not included in the protocol was prohibited.

3. Glucocorticoid Use

3.1. The glucocorticoid taper

The use of glucocorticoids followed a strict protocol. Patients received 1-3 (1000 mg) pulses of methylprednisolone at enrollment, followed by prednisone 1 mg/kg/day (maximum 80 mg/day). The dose was tapered such that by month 5 all patients who achieved remission without flaring had completely discontinued glucocorticoids. The prednisone dose was reduced to 40 mg/day no later than by the completion of week 4 and maintained for two weeks. Stepwise dose reductions then continued every two weeks: 30 mg, 20 mg, 15 mg, 10 mg, 7.5 mg, 5 mg, 2.5 mg, and 0 mg/day. Within these parameters, the protocol allowed for investigator discretion about the oral prednisone dose between randomization and completion of week 4.

The protocol for the administration of glucocorticoids resulted in the maximal allowed and minimal required total doses of prednisone per protocol as indicated in **Supplemental Figure 1.**



Glucocorticoid Use during the Remission Induction Phase of the RAVE Trial.
The gray shaded area outlines the maximal allowed and minimally required prednisone dose per protocol. The box plots for each treatment group show the medians, upper and lower quartiles, and minimal and maximal doses (whiskers) given to patients who remained in the originally assigned treatment group through month 6. This includes patients who were treated for limited disease flares according to protocol with increases of the prednisone dose. Not included are patients who fulfilled criteria for early treatment failure, suffered a severe flare and were treated with cross-over therapy, or those who were discontinued from the planned protocol for any other reason.

3.2. Glucocorticoid Use Prior to Baseline

Several factors could have influenced the glucocorticoid use prior to the first infusion. The time intervals between referral to the site, screening, randomization or first infusion were variable. Patients who had received glucocorticoids for longer than 14 days prior to consent were excluded (see exclusion criteria). However, glucocorticoids given by referring physicians prior to the screening visit were not under the control of investigators and not governed by the protocol. The time between consent (screening) and first infusion (randomization) could be as long as 2 weeks or as short as 1 day. Because glucocorticoid use within days prior to implementation of the protocol could have affected outcomes, and particularly the number of early treatment failures, we tabulated and analyzed all glucocorticoid use within 14 days prior to consent and randomization. As shown in the manuscript's **Table 1**, no differences in the two groups were observed in the total dosage of glucocorticoids administered within 14 days prior to consent and first infusion.

3.3. Cumulative Glucocorticoid Use at Six Months

The mean (± SD) methylprednisolone doses were not different: 2165 mg (1694 mg) for the rituximab arm versus 2389 mg (3833) mg for the cyclophosphamide arm (p=0.988). However, the mean cumulative dosages of prednisone were different: 3595 mg (999 mg) and 3977 mg (1183 mg) for the rituximab and cyclophosphamide groups, respectively (p=0.025). These results include glucocorticoid usage for limited and severe disease flares that occurred during the remission induction period (6 months), as well as all glucocorticoids given between 14 days prior to consent and first infusion (randomization). However, analysis of covariance (ANCOVA) showed that the post-randomization use was conditional on the pre-randomization glucocorticoid

use. This means that the difference in cumulative glucocorticoid use cannot be clearly attributed to treatment differences.

The **Supplement Figure 1** shows the medians of the oral prednisone doses given to patients who remained in their originally assigned treatment group throughout the remission induction period including those receiving prednisone for treatment of a limited flare according to protocol.

4. Interim analysis

One planned interim analysis for futility was performed when 100 participants had completed 6 months of follow-up. We used a Lan-DeMets alpha spending function with an O'Brien-Fleming boundary, allocating 0.003 alpha to the interim analysis and 0.049 alpha to the final analysis. The trial was not stopped because the remission rate in the experimental arm was not significantly less than that in the control arm.

5. Inferiority trial design and efficacy analysis

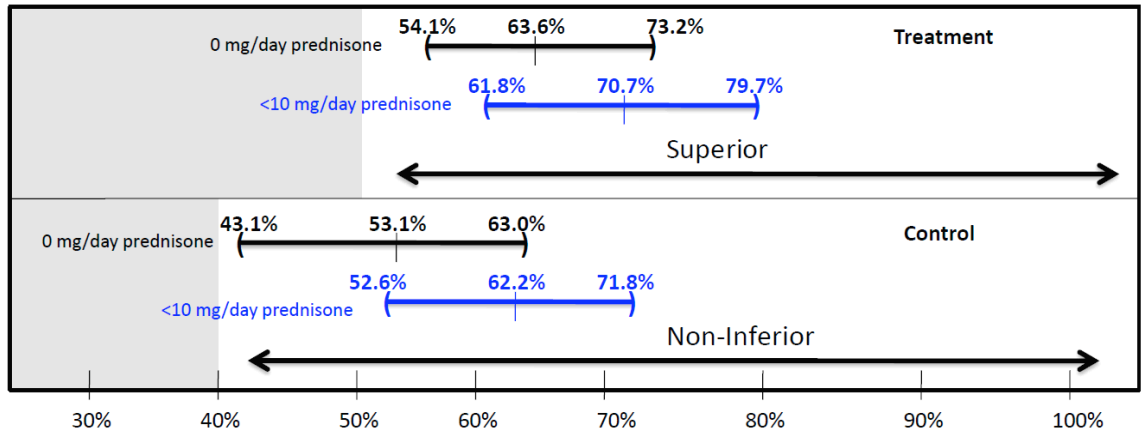
This trial was designed to test whether treatment with the investigational regimen was not inferior to treatment with conventional therapy for the induction of complete remission. Complete remission was indicated by the BVAS/WG of 0 and successful prednisone taper by 6 months after randomization (primary endpoint). Analysis of non-inferiority relied on estimating the difference in the percentage of participants who attained complete remission in the two treatment arms. The difference in the percentage of participants who attained complete remission in the rituximab arm and the control arm was analyzed using a two-sided 95%

confidence interval and a t-distribution multiplier. The lower bound of this confidence interval around the difference in proportions was used to assess non-inferiority and superiority. If the lower bound was below −20%, non-inferiority would have been rejected.

To conclude non-inferiority two conditions had to be met. First, the lower bound of the 95% confidence interval on the difference had to be above −20%. Second, if the point estimate for the complete remission rate in the rituximab arm was lower than that of the cyclophosphamide arm, the point estimate for the cyclophosphamide arm complete remission rate had to be at least 40% to meet the claim of non-inferiority for rituximab (assurance that the control was effective).

To conclude superiority the lower bound of the difference in complete remission rate at 6 months of the rituximab versus the cyclophosphamide treatment arms had to be above 0%, and the lower bound of the two-sided 95% confidence interval of the complete remission rate at 6 months in the rituximab arm had to be greater than or equal to 50%. Because the evaluation of non-inferiority and superiority was based on the same analysis, no multiplicity adjustment was made.

Supplemental Figure 2 shows the trial results for both treatment arms and how they relate to these protocol-defined non-inferiority and superiority requirements.



Supplemental Figure 2. Shown are the remission rates and 95% confidence intervals within the rituximab treated and control groups for remission (BVAS/WG=0) on 0 mg/day and < 10 mg/day of prednisone (the primary and principal secondary efficacy endpoints) with reference lines at 50% for superiority in the treated group and 40% for non-inferiority in the control group. The lower bounds of the confidence intervals in the control group were completely above 40% and the overall test for non-inferiority was significant, meaning that both criteria for non-inferiority of rituximab treatment compared to control were achieved. Even though the lower bounds of both confidence intervals in the rituximab group were above 50%, superiority was not achieved because the lower bound of the confidence interval on the difference to control was not above 0.

Non-inferiority was concluded for the primary endpoint and the secondary endpoint of remission on less than 10 mg of prednisone at 6 months, because the lower bound of the 95% confidence interval on the difference was above -20% (**see Figure 2 in the paper**). Since the point estimate of the remission rate for the rituximab arm was higher than that of the cyclophosphamide arm, the second requirement for the non-inferiority conclusion had no bearing even though it was also fulfilled (the point estimate of the remission rate of cyclophosphamide arm was 53.1% and the lower bound of its 95% confidence interval was above 40%).

Superiority was rejected because the lower bound of the two-sided 95% confidence intervals on the difference was below 0%. Since the first condition for superiority was not met, the second condition was irrelevant even though it was fulfilled (the point estimate of the remission rate of cyclophosphamide arm was 63.3% and the lower bound of its 95% confidence interval was above 50%).

6. Additional Results

6.1. Reasons for Primary Endpoint Failure in the Two Treatment Groups

The **Supplemental Table 1** indicates the reasons why some patients in the two treatment arms did not achieve the primary endpoint, disease remission (BVAS/WG = 0) at six months and successful completion of the glucocorticoid taper.

Supplemental Table 1. Reasons for Primary Endpoint Failure in the Two Treatment Groups

Reason for Primary Endpoint Failure	Rituximab (n=36)	Cyclophosphamide (n=46)	Total
"Early Treatment Failure" as defined per protocol	7	2	9
BVAS/WG not zero at month 6	19	33	52
Failed to complete prednisone taper	23	25	48
Experienced disease flare	17	24	41
Died from AAV or treatment	1	1	2*
Discontinued from trial because of adverse effects	4	10	14
Blinded crossover	6	7	13
Treated according to best medical judgment	7	6	13
Imputed to be treatment failures because 6-month data not available	1	4	5

*Two of the three deaths that occurred before 6 months are listed in this Table. The other death occurred after the patient had met other treatment failure and study termination criteria. Only safety data were collected on this patient at the time of death.

6.2. Quality-of-Life

Quality-of-life scores improved in both groups. In the rituximab group, SF-36 physical and mental health component summary scores (range 0-100) improved by 5.9 (SD=10.4) and 7.9 (12.6) points, respectively. These same summary scores improved by 6.0 (10.4) and 5.3 (12.9) points in the cyclophosphamide group. There was no significant difference between treatment groups in the change from baseline to month 6 in the quality-of-life scores or their rate of change over this period.

6.3. Malignancies Beyond Six Months

This trial was monitored rigorously by the sponsor (NIAID) and adverse events were reviewed carefully by the Data Safety Monitoring Board. As reported in the manuscript, one malignancy occurred in the rituximab group and one in the cyclophosphamide group during the first six month. Both of these were adenocarcinomas of the prostate.

Since the six-month time point, four patients randomized to rituximab and one patient randomized to cyclophosphamide have developed cancers. One patient in the rituximab group developed two cancers. This prompted a careful review of the malignancies that occurred in the trial to date. This analysis is summarized here.

The specific malignancies reported to date include prostate cancer (2), colon cancer (2), uterine cancer (1), lung cancer (1), bladder cancer (1), and papillary thyroid cancer (1). These cancers do not suggest any particular pattern to the types of malignancies observed.

Thus, the total cancers in follow-up to date are six total malignancies in five patients originally assigned to the rituximab arm, and two malignancies in two patients originally assigned to the cyclophosphamide arm.

Because of the trial design, patients have an increasing likelihood of being exposed to rituximab as follow-up continues. The protocol mandates blinded crossover treatment of severe flares that occur before 6 months and open-label rituximab treatment of all severe flares occurring between 6 months and 18. The protocol also allows treatment on an open-label basis with rituximab according to best medical judgment after a patient has been followed for 18 months, up until the common close-out date. One of the patients originally randomized to cyclophosphamide who developed a malignancy was also treated with rituximab later in the trial. When the data are analyzed "as treated" for rituximab, 6 of 124 (4.8%) of patients having received rituximab versus 1 of 73 (1.4%) of patients who never received rituximab developed one malignancy ($p=0.26$). As described in detail below, 5 of the 6 rituximab-treated patients also received other immunosuppressive agents either during or before the trial that might have contributed to their malignancy development. The malignancy in the sixth rituximab-treated patient (who did not receive other agents) was an adenocarcinoma of the prostate.

More than half of the trial participants received immunosuppressive agents (cyclophosphamide, azathioprine, methotrexate) that are associated with increased risks of cancer before enrollment in this trial. We therefore analyzed the exposure to cyclophosphamide, azathioprine, and methotrexate among the patients who developed cancer during the trial.

Supplemental Table 2 (below) shows the cancer patients' histories of exposure to these other medications before their diagnoses of cancer. The Table includes data related to the occurrence of 8 solid malignancies in seven patients. Please note that because of the importance of preserving the trial blind in the setting of ongoing patient follow-up, demographic data that might identify patients to investigators (e.g., age and sex) are not provided.

Supplemental Table 2 includes data on both exposure to the medications before trial entry (the upper part of each row) and after trial entry (lower part of each row). As an example, the first patient, diagnosed with papillary thyroid cancer, was exposed to a year-long cyclophosphamide course and a month-long methotrexate regimen before enrollment in this trial. During the trial, this patient was exposed to cyclophosphamide, azathioprine, and rituximab starting 798, 698, and 306 days, respectively, before his or her cancer diagnosis.

Supplemental Table 2. Type of Malignancy, Pre-enrollment vasculitis treatments, and Pre-Malignancy-Diagnosis Exposure to Four Medications.

Type of Cancer	Medications the patient received before cancer diagnosis	Medication Exposure Summary			
		RTX*	CYC*	AZA*	MTX*
Papillary thyroid cancer	RTX, CYC, AZA, MTX		150 mg. max; 365 days		30 mg max; 30 days
		306 days	798 days	698 days	
Uterine cancer	RTX, MTX				25 mg. max; 3102 days
		909 days			
Prostate cancer	RTX				
		71 days			
Colon cancer metastatic	RTX, CYC, MTX		150 mg. max; 240 days		25 mg. max; 1590 days
		454 days			
Prostate cancer	CYC				
			53 days		
Bladder cancer[#]	RTX, MTX				25 mg. max; 1600 days
		811 days			
Adenocarcinoma of the colon[#]	RTX, MTX				25 mg. max; 1600 days
		1180 days			
Lung neoplasm malignant	RTX, CYC		100 mg. max; 10 days		
		532 days			

*RTX = rituximab, CYC = cyclophosphamide, AZA = azathioprine, MTX = methotrexate
[#]These two malignancies occurred in the same patient.

Supplemental Table 2 demonstrates that all patients who developed malignancies in this trial had histories of exposure to at least two (and in most cases more) medications known to increase the risk of cancer. Only two patients had received just one of these medications (RTX, CYC, MTX, or AZA) before their cancer diagnosis in the trial. In both such cases, the cancer diagnosis was adenocarcinoma of the prostate.

Supplemental Table 3 shows the malignancy rates according to the exposure to RTX, CYC, AZA, and MTX.

Supplemental Table 3. Malignancy rates according to exposure to RTX, CYC, AZA, and MTX.

Actual Result				
1	2	3	4	5
	Malignancies (person-years) in <u>exposed</u> to a particular drug	Malignancies (person-years) in <u>unexposed</u> to that drug	Incidence Rate Ratio	p-value (H_0: IRR=1)
RTX	6 (211.7)	1 (152.2)	4.31	0.140
CYC	4 (270.7)	3 (93.1)	0.46	0.295
AZA	2 (180.0)	5 (182.4)	0.41	0.264
MTX	4	3		0.127*

*based on simple chi-square, not incidence-rate ratios

Columns 2 and 3 show the count of malignancies observed in each of two exposure groups along with the person-years of time of exposure to a particular medication during the trial. In Column 2, for example, 6 patients who had been exposed to rituximab experienced malignancies over

211.7 person-years of exposure. By contrast, only 1 patient never exposed to rituximab experienced a malignancy, over a period of 152.2 person-years of follow-up.

Column 4 shows the ratio of the incidence rates in the patients exposed and unexposed to a particular drug. As the first row shows, those exposed to rituximab had a 4.3 times higher incidence rate of malignancies than those not exposed. This was not statistically significant (p=0.140). Moreover, as shown in **Supplemental Table 2**, most of the RTX-treated patients who developed cancer also had histories of treatment with one or more medications known to be associated with an increased risk of cancer.

Column 5 shows statistical significance testing related to the question of whether the two incidence rates shown in columns 2 and 3 were equal. The null hypothesis tested was that the incidence rates in columns 2 and 3 were equal, i.e., had a ratio of 1. The p-values shown in column 5 indicate that there was not sufficient evidence of differing incidence rates to support rejection of the null hypothesis for any of the medications to which patients were exposed, either before or during the study.

In conclusion, the number of patients who have developed malignancies is small: n=8; 6 in one group, 2 in the other. The attribution to any single drug of "cause" for these cancers, which are common in the general population, is confounded by the fact that most patients have been exposed to multiple drugs known to be associated with an increased risk of cancer.