

Supplementary Appendix

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

Supplement to: Jones RB, Cohen Tervaert JW, Hauser T, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med* 2010;363:211-20.

Supplementary Appendix

1. Supplementary Methodology

Patients were recruited from June 2006-June 2007 from eight centres in the United Kingdom, Australia, the Czech Republic, Sweden, Switzerland, and the Netherlands.

Assessments were performed at 0, 1.5, 3, 6, 9, 12 months and at relapse, and included complete blood count, CD20 positive cell counts, erythrocyte sedimentation rate, C-reactive protein, ANCA, serum creatinine, immunoglobulin levels, and urine analysis. Glomerular filtration rate (GFR) was calculated using the four variable MDRD equation.¹ Disease activity was assessed by the Birmingham Vasculitis Activity Score 2003 (BVAS 2003).² Renal histology was analysed centrally at the Department of Pathology, Leiden University Medical Centre, by two treatment blinded pathologists. Cumulative disease damage was assessed by the Vasculitis Damage Index (VDI)³ and patient quality of life by the Short Form 36 (SF-36) questionnaire.⁴ Adverse events were classified using the European Union Clinical Trials Directive (2001/20/EC).⁵ For safety purposes additional laboratory monitoring was performed for patients receiving cyclophosphamide and azathioprine.

Supplementary Fig. 1 summarises the treatment protocols. Patients in the rituximab group received rituximab 375mg/m²/week for four consecutive weeks, and IV cyclophosphamide 15mg/kg, with the first and third rituximab infusions, and did not receive azathioprine for remission maintenance. Two doses of cyclophosphamide were permitted in view of the absence of data to support the sole use of rituximab. Withholding early potentially life or organ saving treatments was considered unethical. Control patients received IV cyclophosphamide 15mg/kg every two weeks

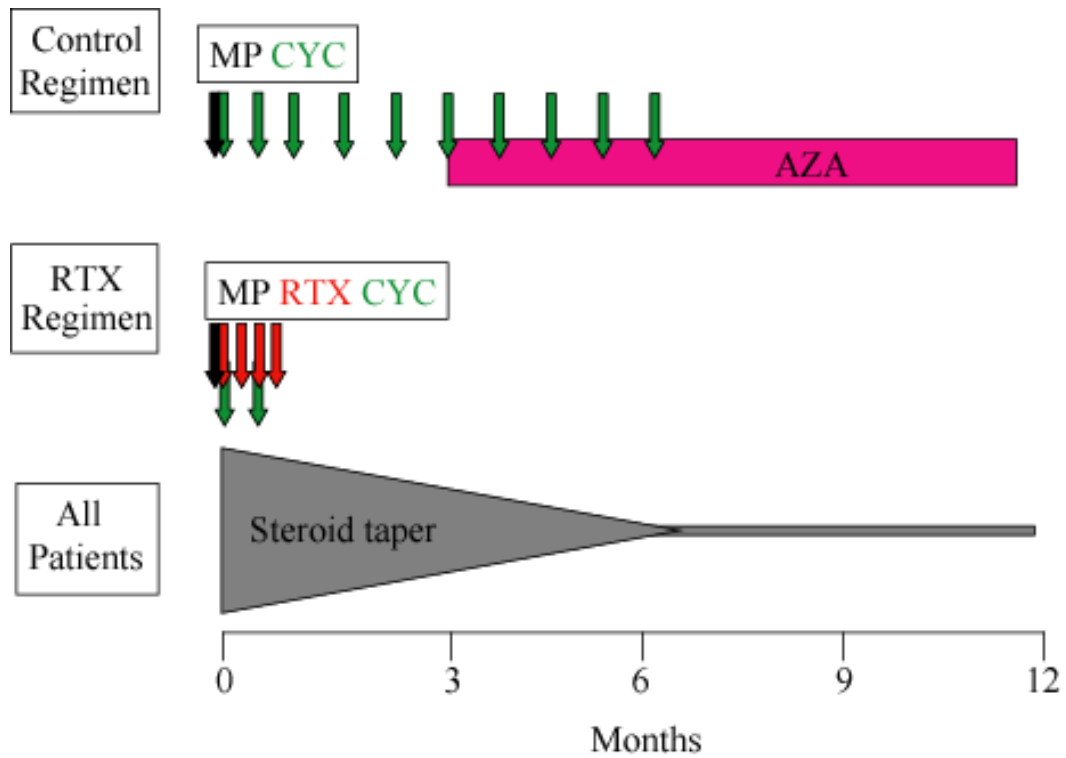
for the first three doses, then every three weeks thereafter until stable remission was achieved (minimum 6, maximum 10 doses).⁶ Oral azathioprine (2mg/kg/day) was introduced after cyclophosphamide withdrawal as remission maintenance therapy in the control group.⁷ Both groups had cyclophosphamide dose reductions for age ≥ 60 years and renal function (creatinine $>300\mu\text{mol/l}$ (3.4 mg/dl)).⁶ Prophylaxis against *Pneumocystis jiroveci* pneumonia, fungal infection, osteoporosis and gastritis was recommended.

Major relapse was defined as the recurrence or new appearance of a major BVAS 2003 item, indicative of threatened vital organ function (e.g. kidney, brain, lung, eye or gut) and attributable to vasculitis. Minor relapse required the recurrence or appearance of at least one BVAS 2003 items of less severity attributable to active vasculitis.

Randomization was performed centrally using a computer algorithm concealed from the investigators. Minimization strata were: age (≥ 60 years versus <60 years), diagnosis (Wegener's granulomatosis versus microscopic polyangiitis or renal limited vasculitis) and baseline renal function (serum creatinine $\geq 500\mu\text{mol/l}$ (5.8mg/dl) versus $<500\mu\text{mol/l}$ (5.8mg/dl)).

All analyses included 44 patients and were performed according to the intention to treat principle. The results for continuous variables that are expressed as medians and interquartile ranges (IQR) within the main text are also expressed as means \pm standard error of the mean (SEM) in Supplementary Table 1.

Supplementary Fig 1. Treatment Protocol Overview



Black arrows indicate intravenous methylprednisolone (MP), 1 gram. Red arrows indicate intravenous rituximab (RTX), 375mg/m². Green arrows indicate intravenous cyclophosphamide (CYC), 15mg/kg with dose adjustments for age and renal function. AZA=azathioprine.

Supplementary Table 1. Results with median and mean values

	RTX		CYC	
	N=33		N=11	
	Median (IQ range)	Mean ± SEM	Median (IQ range)	Mean ± SEM
Age at entry	68 (56-75)	63 ±2.90	67 (58-76)	67 ±3.20
PR3/MPO ANCA at entry	53 (14-100)	89 ±25.2	79 (28-163)	127 ±45
Total number of organs involved at entry	3 (1-3.5)	2.6 ±0.23	2 (1-4)	2.3 ±0.41
C-reactive protein at entry	28 (12-87)	60 ±12.7	25 (7-87)	53 ±17.5
Erythrocyte sedimentation rate at entry	52 (14-82)	52 ±7.1	64 (21-106)	65 ±12.6
Time to remission (days)	90 (79-112)	104.32 ±11.56	94 (91 – 100)	96.10 ±4.01
Weight adjusted prednisolone doses at 12 months (mg/kg/day)	0.071 (0.062-0.082)	0.095 ±0.018	0.082 (0.071-0.093)	0.082 ±0.019
BVAS 2003 at entry	19 (14-24)	19.65 ±1.23	18 (12-25)	19.00 ±2.14
BVAS 2003 at 3 months	0 (0-1.5)	0.68 ±0.50	0 (0-0)	0.00 ±0.00
eGFR at entry (mls/min/1.73m ²)	20 (5-44)	24 ±3.5	12 (9-33)	17 ±3.9
eGFR at 12 months (mls/min/1.73m ²)	39 (20-45)	32 ±3.4	27 (12-47)	27 ±5.9
Change in VDI from 0-12 months	2 (0-3)	2.1 ±0.40	1 (0-2)	1.4 ±0.56

Results for continuous variables expressed as medians (interquartile (IQ) range) and means (± standard error of the mean (SEM)). RTX=rituximab group, CYC =control cyclophosphamide group. BVAS 2003= Birmingham Vasculitis Activity Score 2003 version, eGFR=estimated glomerular filtration rate, VDI=Vasculitis Damage Index, PR3/MPO ANCA= proteinase 3/myeloperoxidase anti-neutrophil cytoplasm antibody.

References

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⁴ McHorney CA, Ware JJ, Rogers W, Raczek AE, Lu JF. The validity and relative precision of MOS short- and long-form health status scales and Dartmouth COOP charts. Results from the Medical Outcomes Study. *Med Care* 1992;30:MS253-65.

⁵ Directive 2001/20/EC. Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. *OJ* 2001(L121); 34-44.

⁶ de Groot K, Harper L, Jayne DRW, et al. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. *Ann Intern Med* 2009;150:670-80.

⁷ Jayne D, Rasmussen N, Andrassy K, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med* 2003;349:36-44.