

# Protocol

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Protocol for: 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.

**RITUXVAS Clinical Trial Protocol**  
**EUDRACT number: 2005-003610-15, REC reference:05/Q1604/153**

**European Vasculitis Study Group (EUVAS) Trial**

**Version 1c: 31<sup>st</sup> May 2006**

**Abbreviated trial name:** RITUXVAS

**Full Trial Name:** An international, randomised, open label trial comparing a rituximab based regimen with a standard cyclophosphamide/azathioprine regimen in the treatment of active, ‘generalised’ ANCA associated vasculitis.

**Summary**

The ANCA associated vasculitides (AASV), namely Wegener’s granulomatosis, microscopic polyangiitis, and renal limited vasculitis are autoimmune, multi-system, progressive diseases which untreated can lead to rapidly progressive renal failure and death.

Randomised, prospective, clinical trials have demonstrated the efficacy of immunosuppressive treatments for vasculitis and have defined treatment protocols at different disease points. The current ‘gold standard’ treatment for active AASV with glomerulonephritis is cyclophosphamide with steroids. However the standard treatment is associated with significant morbidity and mortality, largely due to infections and malignancy with cumulative cyclophosphamide dosing. Other effective treatments for AASV are being sought, with safer side effect profiles. Rituximab is an anti CD20 chimeric monoclonal antibody, which is used for the treatment of non-Hodgkin’s lymphoma (NHL). It is well tolerated in humans with a good safety profile. Rituximab has been shown in small series to induce remission in AASV and is now being increasingly used for other (non-ANCA) autoimmune conditions such as lupus and rheumatoid arthritis.

RITUXVAS has been designed to test the hypothesis that Rituximab leads to a higher rate of sustained remission compared to standard therapies (cyclophosphamide /azathioprine) with a lower rate of adverse events and reduced cyclophosphamide exposure as treatments for active, ‘generalised’ AASV. We plan a randomised phase II/phase III trial to compare a rituximab based regimen to standard of care. For the initial phase II part, 44 patients will be randomised 3:1 to rituximab or control. Following this analysis, the trial may be extended to a phase III stage. The trial will be conducted by 14 centres from 8 countries.

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**EUDRACT number: 2005-003610-15**

**Sponsorship provided by;** Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK, with contractual agreement with each participating centre.

**Compliance with GCP Requirements and EU Clinical Trials Directive**

RITUXVAS will be conducted in accordance with the international conference on harmonisation guidelines for Good Clinical Research Practice and the European Clinical Trials Directive 2001/20/EC.

**Participating investigators: Steering committee**

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**Trial Overview**

**Entry**  
(Eligibility criteria)



**Randomisation**  
(experimental limb 33:control limb 11)



Rituximab 375mg/m<sup>2</sup> IV x4  
Cyclophosphamide 15mg/kg IV x2  
Methylprednisolone 1g IV  
Prednisolone PO



IV Cyclophosphamide  
(minimum 3months, max  
6 months).  
Methylprednisolone 1gIV  
Prednisolone PO



Remission/maintenance  
Azathioprine 2mg/kg



**2 years  
Trial End**

**Analyses**

**6 weeks: Initial response evaluation**

**6 months: Efficacy and safety evaluation**

**24 months: Trial end and efficacy and safety evaluation**

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**List of Abbreviations**

AASV	ANCA associated vasculitis
ANCA	Anti-neutrophil cytoplasmic antibodies
BVAS	Birmingham Vasculitis Activity Score
c-ANCA	Cytoplasmic anti-neutrophil cytoplasmic antibodies
CRF	Case Report Form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CTO	Clinical Trials Office
ESR	Erythrocyte sedimentation rate
ELISA	Enzyme linked immunosorbant assay
EUVAS	European Vasculitis Study Group
GBM	Glomerular Basement Membrane
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
HACA	Human anti chimeric antibodies
HIV	Human immunodeficiency virus
IB	Investigators Brochure
IIF	Indirect immunofluorescence
IV	Intravenous
MP	Microscopic polyangitis
MPO	Myeloperoxidase
NHL	Non Hodgkin's lymphoma
PO	Oral administration
PR3	Proteinase 3
RA	Rheumatoid Arthritis
RBC	Red blood cells
RLV	Renal Limited Vasculitis
SC	Steering Committee
SAE	Serious adverse event
SF-36	Short-Form-36
SLE	Systemic Lupus Erythematosus
TMC	Trial Management Committee
VDI	Vasculitis Damage Index
WBC	White blood cell
WG	Wegener's Granulomatosis

## **Background**

### **1.1 The diseases**

Wegener's granulomatosis (WG) and microscopic polyangiitis (MP) are syndromes of primary systemic vasculitis associated with anti-neutrophil cytoplasmic antibodies (ANCA) (appendix 1)(15,16,19). WG and MP share many histological features, including a necrotising glomerulonephritis which often leads to rapidly progressive renal failure. Isolated pauci-immune necrotising, crescentic glomerulonephritis, has many features to suggest that it represents a renal-limited form of vasculitis (RLV), including the presence of circulating anti-MPO or anti-Pr3 antibodies. Common histological and serological features, and a similar response to treatment, have justified a common approach to the treatment of WG, MP and RLV.

These diseases are sub grouped according to severity. 'Generalised' vasculitis refers to systemic disease with renal involvement (creatinine up to 500) and or other imminent vital organ failure. Patients with 'generalised' vasculitis and those with more severe renal vasculitis (i.e. creatinine greater than 500) will be included in this trial.

### **1.2 Their treatment**

Untreated generalised WG and MP follow a progressive course with fatal outcome due to vital organ failure (17). With the empirical introduction of corticosteroids and cytotoxic agents, five year survival has increased from under 20% to over 60% (18).

Over the last decade the treatment of AASV has been standardised following the results of randomised trials. Cyclophosphamide (either daily oral or intravenous (IV) pulse) and prednisolone are used for remission induction (3 to 6 months) with longer therapy of azathioprine or methotrexate and low dose steroids to prevent disease relapse. Early systemic vasculitis may be treated with methotrexate in place of cyclophosphamide. The addition of plasma exchange improves renal recovery rates in severe renal vasculitis.

Adverse events with current therapy are frequent and are the major cause of mortality. The frequency of severe adverse events (10-45%) is influenced by age and the severity of renal involvement. There is a clear need for a new safer, effective therapy for AASV (1,2,3,6).

In order to address the questions of drug toxicity and frequent disease relapses, newer therapies with better safety profiles have been investigated in AASV. One such agent is rituximab, a murine/human chimeric anti CD 20 monoclonal antibody. Its use results in B cell depletion for 6-18 months (7).

B cells are key factors in autoimmune diseases with roles in autoantibody production, cytokine release and antigen presentation to T-cells. The sequence of immune events that trigger AASV are not fully understood, however evidence suggests that ANCA are pathogenic (21). Thus interruption of the cell line leading to ANCA production may be beneficial in disease suppression.

Rituximab is an established treatment in non-Hodgkin's lymphoma with a good safety track record (7). Even though the individual is rendered B cell deplete, immunoglobulin levels are maintained, and infection rates are low. Recently its use in autoimmune diseases has been realised with excellent results in rheumatoid arthritis (8). Rituximab has been shown to be effective in AASV, inducing remission in patients intolerant of cyclophosphamide (5,10-12,22,26-28). Given its good safety profile and efficacy its use a first line agent in AASV needs to be investigated.

In RITUXVAS, a rituximab based regimen will be compared with a standard cyclophosphamide/azathioprine based regimen in the treatment of AASV with glomerulonephritis. In order to achieve results applicable to clinical practice, all cases from mild to severe renal failure will be included. Standard practice includes the use of oral steroids which will be applied similarly in both treatment groups. It is recognised that necrotising crescentic glomerulonephritis progresses rapidly and immediate immune suppression is required to reverse existing and spare further organ damage. The therapeutic effect of rituximab is not immediate. Therefore two initial doses of cyclophosphamide will be given along side rituximab, thus allowing early disease control. When dialysis dependant renal failure or pulmonary haemorrhage occur, plasma exchange or intravenous steroids are standard additional therapies. These will be allowed according to local practice, and randomisation will occur after plasma exchange.

Patients in the cyclophosphamide limb will receive azathioprine as remission maintenance therapy. Patients receiving rituximab will not receive any additional maintenance therapy.

### **1.3 Rituximab**

Rituximab is an anti CD 20 chimeric mouse/human monoclonal antibody, with human IgG1 constant regions and murine light/heavy chain variable regions (7). CD 20 is a ligand which exists on developing B cells, excluding stem cells and plasma cells. The role of the CD 20 ligand in nature is not fully understood, although mediation of apoptosis has been suggested. The mechanisms by which rituximab causes B cell depletion may involve complement induced B cell lysis, Fc receptor mediated cytotoxic cell killing or the direct induction of B cell apoptosis by rituximab.

Rituximab therapy correlates positively with B cell depletion and rituximab levels. Different dosing regimens have been trialled. In patients with systemic lupus erythematosus (SLE), the efficacy of rituximab is dependant upon B cell depletion. 4 infusions of 375mg/m<sup>2</sup> rituximab infusions (one per week for four weeks) were required for this to occur (13,14). In 3 published reports 375mg/m<sup>2</sup> has resulted in B cell depletion and clinical response in patients with refractory vasculitis (10-13). On the basis of these results a dose of 4 x 375mg/m<sup>2</sup> infusions will be used in RITUXVAS.

In RITUXVAS, 2 doses cyclophosphamide will still be administered with the 1<sup>st</sup> and 3<sup>rd</sup> rituximab infusions, for two reasons. Firstly, the necrotising crescentic glomerulonephritis associated with AASV progresses rapidly and the therapeutic effect of rituximab is delayed. The use of cyclophosphamide/high dose steroid, which are standard components of induction therapy, will allow adequate immunosuppression in the early crucial treatment of AASV. Secondly, human anti-chimeric antibody (HACA) formation has been reported in NHL, SLE, and RA with varying frequencies, (4.3% of patients in RA (8)). The long term implications of these antibodies are not known. However, the possibility of anaphylactic reactions and rituximab resistance with further treatments exists. Co-administration of an immunosuppressant effective in the treatment of vasculitis is thus a logical approach to minimise HACA development.

Rituximab has now been used to treat 300,000 patients with NHL. Long-term safety regarding carcinogenicity and fertility has yet to be established. However, no major long-term adverse sequelae have been reported (1).

Up to 50% of patients receiving rituximab for an indication will develop infusion reactions with symptoms including; fevers, chills, rigors, flushing, throat irritation

rash rhinitis fatigue headache, nausea, vomiting, urticaria, angioedema, bronchospasm, myalgia, arthralgia, hypotension, hypertension and exacerbation of angina or congestive cardiac failure. Later reactions include diarrhoea, leucopenia, neutropenia, thrombocytopenia, anaemia, and infections in 30%, which may or may not be drug related (investigators brochure).

#### **1.4 Aims of RITUXVAS:**

- 1 To assess the rates of preliminary response and sustained remission of AASV following rituximab (on the basis of former studies, 86% sustained remission expected with rituximab compared to 75% in control group).
- 2 To assess safety of a rituximab regimen in terms of severe adverse events (in patients receiving standard therapies, adverse event rate is 45% at 2 years, at least 50% of which are infection related. In comparison rituximab use is only rarely associated with infections, therefore 22.5% adverse event rate expected with rituximab compared to 45% at 2 years in control group).

#### **2 Hypothesis:**

Rituximab leads to a higher rate of sustained remission compared to standard therapies (cyclophosphamide/azathioprine) with a lower rate of severe adverse events and reduced cyclophosphamide exposure.

#### **3 Trial Design**

International, randomised, controlled, prospective, open trial comparing a rituximab based regimen with a standard cyclophosphamide/azathioprine regimen in the treatment of active 'generalised' AASV. Informed consent and ethics committee approval will be obtained.

#### **3.1 Inclusion Criteria (all four must be present)**

1. **A new diagnosis of WG, MP or renal-limited vasculitis (RLV)**(appendix 1)
2. **Renal involvement attributable to active WG, MP or RLV** with at least one of the following:
  - a) Biopsy demonstrating necrotizing glomerulonephritis.
  - b) Red cell casts on urine microscopy or  $\geq ++$  haematuria
3. **ANCA positivity** ANCA positivity requires either (a) or (b)
  - (a) PR3-ANCA by ELISA or a typical cANCA pattern by indirect immunofluorescence (IIF), or both.
  - (b) MPO-ANCA by ELISA. A positive pANCA by IIF requires confirmation by MPO-ANCA ELISA.

#### **4 Written informed consent**

#### **3.2 Exclusion Criteria**

1. Previous cyclophosphamide, (greater than 2 weeks of an oral or IV pulse cyclophosphamide regimen).
2. Co-existence of another multisystem autoimmune disease, e.g. SLE, Churg Strauss Syndrome, Henoch Schonlein Purpura, rheumatoid vasculitis, essential mixed cryoglobulinaemia, anti-glomerular basement membrane antibody positivity
3. Hepatitis B e antigen positive or Hepatitis C antibody positive.
4. Known HIV positive (HIV testing will not be a requirement for this trial).
5. Previous malignancy (usually exclude unless agreed with trial co-ordinator).

6. Pregnancy, breast feeding or inadequate contraception if female.
7. Allergy to a study medication
8. Live Vaccine within last 4 weeks

### **3.3 Randomisation**

1. Variables known to influence primary end points will be balanced between groups by minimisation
  - i. Age
  - ii. Disease WG or MP/RLV
  - iii. Creatinine
2. Randomisation form (see TMF) sent by e-mail [rbjones@doctors.org.uk](mailto:rbjones@doctors.org.uk) or fax to 01223 586506 (clinical trials office).

### **3.4 Endpoints**

Primary end points will be assessed upon trial completion at 2 years.

However interim analyses will be performed when

1. 30 patients have completed 6 weeks, to assess efficacy (treatment response) and safety (severe adverse events).
2. 40 patients have completed 6 months to assess efficacy (remission rates) and safety (severe adverse events).

#### **i Primary**

1. Sustained remission (BVAS = 0 at 6 months and sustained for 6 months).
2. Severe adverse events (CTCAE grade  $\geq 3$ ) at 2 years.

#### **ii Secondary**

##### **1 Efficacy**

Response rate at 6 weeks (BVAS < 50% baseline)  
Remission at 6 months (BVAS=0 for 2 months by 6 months)  
Time to remission (BVAS=0)  
Relapses (all relapses and major/minor)  
BVAS area under the curve  
Change in GFR  
Change in SF-36  
Change in VDI

##### **2 Safety**

Severe adverse events (CTCAE grade  $\geq 3$ ) at 6 weeks and 6 months  
All adverse events  
Death  
Prednisolone cumulative dose  
Cyclophosphamide cumulative dose

#### **iii Tertiary**

Human anti-chimeric antibody testing  
Correlation of B cells with disease activity  
Change in ANCA and disease activity  
Histopathology predictors of outcome

### **3.5 Interventions**

#### **I Drug regimens**

- a) **Rituximab Regimen:** Rituximab, 375mg/m<sup>2</sup> IV once a week for 4 weeks (i.e. 4 doses total), with 2 doses of cyclophosphamide 15mg/kg, 2 weeks apart given with the 1<sup>st</sup> and 3<sup>rd</sup> rituximab dose (appendix 3).
- b) **Control (cyclophosphamide/azathioprine) Regimen;** Cyclophosphamide 15mg/kg for 3-6 months (6-10 doses total) to be given IV according to protocol (appendix 3) for remission induction. Cyclophosphamide should be converted to azathioprine for remission maintenance.
- c) **Steroids.** All patients will receive 1g IV methylprednisolone, then same daily oral corticosteroid regimen (appendix 3).
- d) **Plasma exchange or IV methylprednisolone** will be allowed according to local practice for patients with organ threatening disease. NB randomisation should not occur until completion of plasma exchange to avoid loss of rituximab during plasma exchange. The first dose of cyclophosphamide can be given prior to completion of plasma exchange.
- e) **Progressive disease** Within the first 6 months disease progression defined as a persistence of nephritic sediment or activity on a renal biopsy and a failure to improve GFR<sub>≥</sub> 10 mls/min, if GFR at diagnosis is < 50 mls/min (calculated by cockcroft gault formula, see TMF for formula) OR persistence or new occurrence of a major non-renal BVAS item at 6 weeks then additional treatment should occur:
  - i) Rituximab limb: 3<sup>rd</sup> dose of cyclophosphamide (15mg/kg)
  - ii) Cyclophosphamide limb: Plasma exchange or IV methylprednisolone (according to local practice).
- f) **Relapse Therapy.** Relapses will be categorised as major or minor.
  - i) **Rituximab limb:** Rituximab with steroid will be used for major and minor relapse. Additional cyclophosphamide may also be used for major relapse.
  - ii) **Control limb:** Increased azathioprine and steroid for minor relapse and cyclophosphamide and steroid for major relapse.

#### **II Evaluations**

Study assessments (including history and examination) will be performed at entry, 1.5, 3, 6, 9, 12, 15, 18, 21 and 24 months and at the time of relapse (see appendix 5, and TMF for assessment schedule). At each assessment the following should be performed:

- a) Blood for routine testing.
- b) 10 mls serum saved for centralised immunological analysis (including ANCA) and HACA.
- c) BVAS for each assessment and at relapse;

Every 6 months SF-36 and VDI should be performed (23-25)(see TMF).

Initial Renal histology will be reviewed by a EUVAS panel of histopathologists, based in Milan.

### **3.6 Withdrawal and treatment failure**

- 1 At patient's or physician's request. Reason for withdrawal is to be recorded in the CRF.
- 2 Patients not achieving remission within 6 months to be withdrawn from trial drug regimen and will be treated according to local practice. They will remain under trial follow up.

### **3.7 Adverse events**

- 1 Adverse effects will be actively sought and recorded in CRFs at each evaluation (see section 4.5 and TMF).
- 2 Unexpected, severe adverse events attributable to study medication must be reported within 24 hours to trial management committee. (see TMF and section 4.5 for full details).

### **3.8 Statistical analysis**

Primary end points will be assessed upon trial completion at 2 years. However interim analyses will be performed:

(a) When 30 patients have completed 6 weeks trial participation to assess

(1) treatment response (BVAS fall of > 50% baseline).

(2) safety (adverse events CTCAE grade 3 or greater)

and

(b) When 40 patients have completed 6 months trial participation to assess

(1) remission (BVAS 0 for 2 months by 6 months)

(2) safety (adverse events CTCAE grade 3 or greater)

#### **Primary end point confidence Intervals.**

A total of 30 patients will receive rituximab. The expected sustained remission rate for rituximab is 86%, based on the results of former studies. The 95% confidence interval for a sustained remission rate of 86% is 70.3%-94.7% when the sample size is 30.

The expected response rate of 86% is considered clinically significant as patients with active newly diagnosed generalised AASV without 3-6 months of cyclophosphamide are conventionally expected to experience disease progression (worsening of clinical signs and symptoms). The expected sustained remission rate in the control group is 75%.

Analyses on the 40 patients should be considered exploratory.

### **3.9 Duration:**

3 years: 6 months recruitment, two year follow-up per patient and 6 month analyses.

## **4 Trial Organisation**

### **4.1 Organisational structure and responsibilities**

#### **Principal Investigator and Research Physician**

Design and conduct of RITUXVAS  
Preparation of protocol and revisions  
Preparation of investigators brochure (IB) and CRFs  
Organising steering committee meetings  
Managing CTO  
Publication of study reports  
Members of TMC

#### **Steering committee (SC)**

(see title page for members)  
Agreement of final protocol  
All lead investigators will be steering committee members. One lead investigator per country will be nominated as national coordinator.  
Recruitment of patients and liaising with principle investigator  
Reviewing progress of study and if necessary agreeing changes to the protocol and/or investigators brochure to facilitate the smooth running of the study.

#### **Trial Management Committee (TMC)**

(Principle investigator, Research Physician, Administrator)  
Study planning  
Organisation of steering committee meetings  
Provide annual risk report MHRA and ethics committee  
SUSAR reporting to MHRA and Roche  
Responsible for trial master file  
Budget administration and contractual issues with individual centres  
Advice for lead investigators  
Audit of 6 monthly feedback forms and decide when site visit to occur.  
Assistance with international review board/independent ethics committee applications  
Data verification  
Randomisation  
Organisation of central serum sample collection

#### **Data Manager**

Maintenance of trial IT system and data entry  
Data verification

#### **Lead Investigators**

In each participating centre a lead investigator (senior nephrologist/rheumatologist/immunologist) will be identified, to be responsible for identification, recruitment, data collection and completion of CRFs, along with follow up of study patients and adherence to study protocol and investigators brochure. Investigators must maintain source documents- medical records and CRFs, signed consent forms and SF36 questionnaires. CRF data should be traceable to medical record documentation (except BVAS/VDI scores).

Lead investigators are expected to adhere to the protocol, ICH GCP guidelines and EU CT directive 2001/20/EU.

Lead investigators will be steering committee members, with one investigator per country being nominated as national coordinator.

#### **4.2 Overall study organisation**

RITUXVAS will be coordinated at the clinical trials office (CTO) in Cambridge. 14 centres in 8 different countries will take part in the study. The steering committee will meet ever 6 months to review the progress of the study. One steering committee member per country will act as national coordinator. The principle investigator, research physician, and administrator, who comprise the TMC, will be based in the CTO. The data manager will also be based in the CTO. At each centre a lead investigator will be responsible for the study conduct. The steering committee will over see the training and conduct of the trial by individual centres and staff by auditing the individual centre performance, providing information at meetings, validating data and performing site visits as needed

#### **4.3 Training and Monitoring**

RITUXVAS will be conducted in accordance with the international conference on harmonisation guidelines for good clinical research practice, the EU clinical trials directive 2001/20/EU and any local national or international regulations practice.

The lead investigators will be trained in methods of the study at investigator meetings and will be provided with an investigators brochure and trial management file (TMF) containing comprehensive study method guidelines. Advice will be available from CTO by telephone or e-mail.

The steering committee will ensure investigators receive training at meetings or by individual explanation. If inadequate reporting or problems are detected on 6 monthly feedback forms then a site visit will be performed to ensure that protocol is adhered to and local problems with the study addressed. At site visits, study records, data recording and follow up will be reviewed.

#### **4.4 Quality Control, Monitoring and safety**

(1) Monitoring of drug safety and response: End point data analysis for safety and efficacy will take place at 6 weeks, 6 months and 2 years. In view of the early analysis of data, safety issues will be addressed early on.

(2) CRF monitoring: CRF pages to be returned to CTO every 3 months with 3 monthly return form. TMC will perform quality control audit on primary end point data and other data from CRFs every 3 months. Incomplete data and inconsistencies in results will be referred to investigator for clarification. Failure to provide greater than 90% accuracy on CRFs or provide clarification of results will necessitate a site visit by TMC.

(3) Source data verification: Copies of initial ANCA results and histopathology reports (eligibility criteria) to be sent to CTO. Investigators may be required to provide copies of patient hospital records at time of study assessments for CRF data verification by TMC.

(4) Investigator audits: 6 monthly site questionnaires to be completed by each investigator and returned to TMC. Questionnaires will assess: Investigator protocol compliance, consistencies in recruitment rates, follow up, withdrawal notification, and

data return, as well as safety in terms of serious adverse events and failure to achieve disease control. Failure to return questionnaire will lead to a site visit by TMC.

(5) Regulatory body feedback: TMC to make an annual report of trial progress and safety to the MHRA and ethics committees.

#### **4.5 Monitoring of adverse events**

##### **Definitions**

###### **(1) Adverse event**

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product

###### **(2) Adverse reaction of an investigational medicinal product (AR)**

All untoward and unintended responses to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

###### **(3) Unexpected adverse reaction**

An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product).

When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

The term “severe” is often used to describe the intensity (severity) of a specific event. This is not the same as “serious,” which is based on patient/event outcome or action criteria.

###### **(4) Serious adverse event or serious adverse reaction**

Any untoward medical occurrence or effect that:

- results in death,
- is life-threatening
- requires hospitalisation or prolongation of existing inpatients' hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect.

Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.

All adverse events should be evaluated by the investigator and recorded in the CRFs. This includes the evaluation of its seriousness and the causality between the investigational medicinal products and the adverse event. Where indicated adverse events should be referred to the TMC for evaluation. The TMC office should keep

detailed records of all adverse events reported by the investigators and perform an evaluation with respect to seriousness, causality and expectedness. The TMC is responsible for the prompt notification to all investigators, research ethics committee and competent authority of each concerned member state of findings that could adversely affect the health of subjects, impact on the conduct of the trial or alter the competent authority's authorisation to continue the trial in accordance with Directive 2001/20/EC. Adverse events are defined by CTCAE (see TMF) and should be recorded in the CRF.

**Assessment of seriousness**

- Mild: The subject is aware of the event or symptom, but the event or symptom is easily tolerated
- Moderate: The subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity
- Severe: Significant impairment of functioning; the subject is unable to carry out usual activities and / or the subject's life is at risk from the event.

**Assessment of causality**

- Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product and there is a reasonable response on withdrawal.
- Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product.
- Unlikely: A causal relation is improbable and another documented cause of the AE is most plausible.
- Unrelated: A causal relationship can be definitely excluded and another documented cause of the AE is most plausible.

**(a) Serious adverse events to study treatment**

**(i) Expected events are:**

**Rituximab**

Infusion reactions (within 24hrs of infusion) occur in up to 50% of patients receiving rituximab. Symptoms include: fever chills and rash, flushing, angioedema, nausea, urticaria/rash, fatigue, headache, throat irritation, rhinitis, vomiting, puritis, hypotension and bronchospasm. Following treatment sepsis, leucopenia and neutropenia may occur.

**Cyclophosphamide**

Nausea and vomiting, neutropenia, sepsis, alopecia, haemorrhagic cystitis, infertility, bladder cancer and lymphoma.

**Prednisolone**

Sepsis, hypertension, fluid retention, hyperglycaemia and diabetes mellitus, peptic ulceration, osteoporosis, avascular necrosis, cushinoid appearance, striae, alopecia, hirsutism.

**Azathioprine**

Leucopenia, sepsis, hepatotoxicity, lymphoma, skin cancer, allergy

Please also see summary of product characteristics in TMF, for expected adverse events.

Serious expected adverse events **must** be recorded in the CRFs and if considering study drug discontinuation then TMC should be consulted.

**(ii) Unexpected (SUSARs)**

Serious unexpected suspected adverse events need to be reported within 24 hours of occurrence to the TMC. Drug should be discontinued on TMC advice. TMC will inform Roche, Basel and regulatory authorities.

**(b) Serious adverse events unrelated to study medications**

Events to be noted in CRFs.

**4.6 Patient Entry**

Participant registration form in the TMF should be e-mailed or faxed to CTO. Randomisation reply to be returned within 24hrs. Patient will receive numerical code which should be recorded at CTO and in patient record book (CRF) and TMF. . CTO will contact Roche, Basel when Rituximab required.

**4.7 Supply of study materials.**

Rituximab will be provided by national Roche affiliates and distributed after randomisation. The drug will be labelled with an individual patients study number before distribution within 48hrs of request.

**4.8 Data management.**

CRFs to be completed by local investigator. Copies of CRF records will be sent to the data manager at the CTO every 3 months. CRF data will be reviewed every 3 months by TMC. Inconsistencies, errors and incomplete data will be referred back to the investigator for clarification. Less than 90% accuracy in CRF data will necessitate a site visit. Investigators may be required to provide coded copies of patient medical notes from dates of study assessment to allow CRF data verification. The data manager is responsible for data entry into study computer.

CFRs must be retained by participating centre for 15 years from study completion. Regulatory authorities will have the right to audit such records in accordance with ICH GCP guidelines and EUCT directive 2001/20/EU.

**4.9 Laboratory measurement and sample storage.**

Standard laboratory measurements to be taken according to local laboratory practice. Normal ranges and units of measure must be included in CRFs.

10mls blood to be taken per assessment for immunological reactant testing and HACA testing. Samples will be stored frozen, on site. 3 shipments will be arranged to take samples to Addenbrooke's Hospital, Cambridge, UK for immunological reactant testing (9 10 samples per participant total). 4 out of 10 samples (taken at assessments at, 0, 3, 12, 24 months) will then be sent to Xendo laboratories, Amsterdam for HACA analysis.

**4.10 Communications**

A written trial program update will be sent to lead investigators every 6 months. Further study updates will be presented at annual EUVAS meetings and will be published on EUVAS website.

**4.11 Funding and Results Publication**

This study has received financial support from Roche Basel. It was initiated and designed by the EUVAS, RITUXVAS steering committee. Data will be collected, analysed and published independently from the source of funding by EUVAS.

**4.12 Cost implications for participants**

- 1 There will be no additional clinical costs for participating patients. There will be cost savings for the hospital as the study drug will be provided by Roche
- 2 The costs of data collection will be born by the participating centre

## **5 Ethics**

### **5.1 Ethical Considerations.**

The AASV with glomerulonephritis are rapidly progressive diseases which can be life threatening. Standard treatment regimens have significant toxicity. RITUXVAS aims to show improved sustained remission rates and safety with a rituximab based regime compared to a standard cyclophosphamide/azathioprine based regimen, for acute AASV with glomerulonephritis.

- a As rituximab may not have immediate therapeutic effect and necrotising crescentic glomerulonephritis progresses rapidly, 2 initial cyclophosphamide treatments will be given, and a third dose of cyclophosphamide if no improvement at 6 weeks. Therefore standard induction therapies will not be denied in the early, crucial, treatment phase.
- b When AASV is associated with life threatening pulmonary haemorrhage or dialysis dependent renal failure, additional therapies provide extra benefit (plasma exchange or IV steroids). These treatments will be allowed in both groups, according to local practice. Randomisation will occur after plasma exchange.
- c Patients will undergo informed consent prior to enrolment, will be provided with patient information leaflets and will be free to withdraw from the trial at any point without giving reason.
- d Approval will be sought from ethics committees.
- e Patient confidentiality will be respected according to national regulations. Data will be coded before computer entry to maintain confidentiality
- f Study follow up/blood tests will coincide with standard, disease follow-up appointments and should avoid extra hospital attendances and blood tests as much as possible.

### **5.2 Ethics Approval**

- a Central ethics approval will be obtained through the UK COREC with completion of parts A&B by the principle investigator and parts C&D by participating UK centres and standard local ethics submissions by all other sites outside the UK.
- b Sponsorship will be provided by Cambridge University Hospital NHS Foundation Trust, Cambridge, provided signature of 'site agreement to the sponsor' is obtained from all participating centres. This will take the format of a 2 page contract. The TMC may perform site inspections if necessary.

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**Appendix 1: The diseases- diagnostic criteria****Wegener's granulomatosis**

Generalised WG is characterised by granulomatous inflammation of the respiratory tract, together with necrotising vasculitis affecting small to medium-sized vessels. Necrotising glomerulonephritis is common and reflects renal involvement (16). A C-ANCA pattern by indirect immunofluorescence (IIF), with anti-proteinase 3 (PR3) reactivity by enzyme linked immunosorbant assay (ELISA), is found in over 90% of untreated patients with generalised WG. A minority of cases have anti-myeloperoxidase antibodies (MPO) instead of anti-PR3 antibodies.

For the purposes of this study, a diagnosis of generalised WG requires the presence of an active necrotising glomerulonephritis (histologically or with active urinary sediment), together with clinical, radiological or histological evidence of granulomatous inflammation in the respiratory tract, and detectable C-ANCA by IIF, or anti-Pr3 or anti-MPO ANCA by ELISA. A positive biopsy from the respiratory tract is not mandatory.

**Microscopic polyangiitis**

MP is characterised by a vasculitis predominantly affecting small vessels. Renal involvement is usual and reflected by a necrotising glomerulonephritis. Granulomata are absent. Arteritis of small or medium-sized arteries may also occur (16). MP is associated with ANCA specific for MPO or Pr3. A minority of MP patients are ANCA negative or recognise other ANCA antigens.

For the purposes of this study, a diagnosis of generalised MP requires the presence of active necrotising glomerulonephritis (histologically or with active urinary sediment), together with clinical, radiological or histological evidence of an extra-renal vasculitis, and detectable ANCA by IIF, or anti-Pr3 or anti-MPO ANCA by ELISA. A positive biopsy from an extra-renal site is not mandatory. The presence of granulomatous inflammation, or heavy immune deposition in glomeruli or vessel walls excludes this diagnosis.

**Renal-limited vasculitis or Idiopathic rapidly progressive glomerulonephritis**

Isolated pauci-immune necrotising and crescentic glomerulonephritis, typically known as idiopathic rapidly progressive glomerulonephritis has many features to suggest that it represents a renal-limited form of MP or WG, including the presence of circulating anti-MPO or anti-Pr3 antibodies.

For the purposes of this study, a diagnosis of renal-limited vasculitis, or idiopathic rapidly progressive glomerulonephritis requires renal biopsy confirmation and a positive ANCA by the criteria outlined for generalised MP. Heavy immune deposition in glomeruli excludes this diagnosis.

Renal limited vasculitis will be classified with MP for randomisation purposes, as both conditions present later than WG with greater impairment of renal function, have less response to treatment and have a lower relapse rate than WG.

**Appendix 2: Study Therapies****Rituximab- (see page 6)****Cyclophosphamide**

Cyclophosphamide is an inactive pro-drug, converted by the mixed function oxidase system in the liver to the alkylating agents 4-hydroxy-cyclophosphamide and phosphoramidate mustard, which alkylate guanine nucleotides, thus blocking cell division (20). Bioavailability after oral administration is greater than 75%, but there are large variations between individuals in the rate of production of active metabolites. A phenotypic variation in carboxylator activity affects the production of the inactive metabolite carboxyphosphoramidate from 4-hydroxy-cyclophosphamide, which may influence efficacy and toxicity. The relation of renal and hepatic failure to the production and elimination of active metabolites has not been fully determined.

Bladder toxicity is caused by renal excretion of the metabolite acrolein which can cause haemorrhagic cystitis and a markedly increased risk of bladder cancer (20). Other adverse effects include nausea and vomiting, myelosuppression with neutropenia, infections due to immunosuppression (4), alopecia and infertility. Permanent ovarian failure occurs in over 50% of women after one year's exposure and is age-related; male infertility has been less well studied. The incidence of leukaemia and /or lymphoma is increased tenfold; less common adverse effects include pulmonary fibrosis, hepatitis and the syndrome of inappropriate ADH secretion.

**Prednisolone/Methylprednisolone**

Prednisolone and methylprednisolone are synthetic derivatives of cortisone with widespread effects on metabolism and organ function (20). Desirable effects in systemic vasculitis relate to the suppression of acute and chronic inflammatory processes and immune cell function.

The major unwanted effects of corticosteroids in the short term are salt and water retention, hypertension, hyperglycaemia, central nervous system stimulation, peptic ulceration and immunosuppression. While these effects respond to reduction or withdrawal of the drug, if their use is prolonged additional effects including osteoporosis and avascular necrosis, sub capsular cataracts, skin fragility, myopathy, cushingoid facies, hirsutism, alopecia, fat re-distribution, striae and growth retardation in children may occur. IV MeP delivers a higher dose of corticosteroid, increasing the dose-related side-effects, notably hypertension and salt and water retention. Of note, in systemic vasculitis, has been the correlation of the cumulative steroid dosage with the total incidence of adverse effects, and particularly with infections (15)

**Azathioprine**

After hepatic conversion to 6-mercaptopurine, the cytotoxic effects of azathioprine are mediated by the impairment of purine synthesis, incorporation of purines into DNA, and impairment of the endonuclease repair activity of DNA polymerase (20). The drug is well-absorbed after oral administration and elimination requires hepatic metabolism by xanthine oxidase; an important drug interaction is with xanthine oxidase inhibitors, such as allopurinol. Lymphocyte function is reduced, B-cells more than T-cells, and there is suppression of the cellular component of the inflammatory response.

The major adverse effects are nausea and vomiting, dose-dependent myelosuppression and reversible, cholestatic, hepatic toxicity. An increased incidence of malignancies, particularly lymphomas and skin cancers, has been observed with prolonged administration after organ transplantation.

**Appendix 3: Drug Regimes for Remission Induction and Maintenance.****1 Steroid regimen** (same for both trial groups)

**1g IV methylprednisolone**, followed by oral prednisolone (see table below)

time from entry (weeks)	prednisolone dose (mg/kg/day)	prednisolone dose (mg/day for 60 kg patient)
0	1 (max 60mg)	60
1	0.75	45
2	0.5	30
3	0.4	25
6	0.33	20
8	0.25	15
	prednisolone dosage (mg/day)	
reduce at <b>end</b> of month 3 (16 weeks)	12.5	12.5
reduce at <b>end</b> of month 4 (20 weeks)	10	10
reduce at <b>end</b> of month 5 (24 weeks)	7.5	7.5
reduce at <b>end</b> of month 6 (28 weeks)	5	5
18- 24 months	Reduce from 5 to 0	

**Note:**

- 1mg/kg. Maximum dose in first week is 60mg/day orally.
- Round dose to nearest 5mg if dose >20mg/day, and nearest 2.5mg if dose <20mg/day.
- Single daily dose (may vary alternate day dosage by up to 5mg, i.e. 10 and 15 mg on alternate days).
- Use prednisolone, avoid enteric coated or soluble forms.
- Patients intolerant of oral medication may receive any IV steroid at an equivalent dose as a daily injection.
- Minimum dose in first three months is 10mg/day.
- Bone protection against osteoporosis recommended, e.g. use of a bisphosphonate or calcium/vitamin D supplementation.
- Stomach protection against gastric ulceration recommended, e.g. the use of a proton pump inhibitor.

**2 Induction/consolidation regime for rituximab:**

Time (weeks)	Rituximab Pulse no	IV rituximab	Cyclophosphamide pulse no	IV cyclophosphamide
<b>0</b>	<b>1</b>	<b>375mg/m<sup>2</sup></b>	<b>1</b>	<b>15mg/kg</b>
<b>1</b>	<b>2</b>	<b>375mg/m<sup>2</sup></b>		
<b>2</b>	<b>3</b>	<b>375mg/m<sup>2</sup></b>	<b>2</b>	<b>15mg/kg</b>
<b>4</b>	<b>4</b>	<b>375mg/m<sup>2</sup></b>		

**Note**

- 1 Cyclophosphamide dose reductions should be made according to age and creatinine, as with control cyclophosphamide limb (see table page 27).
- 2 Rituximab should be given according to protocol indications, irrespective of CD 19/20 count.
- 3 Rituximab should be added to 1litre of normal saline and infused at a rate of 50mg per hour increasing every half an hour by 50mg an hour up to a maximum infusion rate of 400mg per hour. For second and subsequent infusions the initial rate is 100mg per hour increasing every half an hour by 100mg per hour to a maximum of 400mg per hour. Rituximab may be infused through a peripheral line.
- 4 Premedication with 1g paracetamol orally and chlorpheniramine 10mg IV is recommended.
- 5 Patients with new disease or major disease flares will receive 15mg/kg cyclophosphamide IV directly after each dose of rituximab
- 6 Patients with minor flares will receive 1g IV of methylprednisolone just prior to first rituximab infusion
- 7 Mild infusion reactions are common, e.g. chills, rash back pain. These can be treated by stopping the infusion and restarting at a slower rate along with antihistamine/antiemetic/paracetamol administration.
- 8 Severe hypersensitivity reactions are rare but have occurred. Therefore adrenaline and hydrocortisone should always be available for administration if necessary (as per local hospital protocol), when giving IV rituximab.
- 9 Patients should be observed in hospital during infusion and following infusion for 30 minutes. Following moderate/severe infusion reactions hospital admission for observation and treatment is recommended.

**3 Induction/consolidation regimen for IV pulsed cyclophosphamide:**

<b>Time (weeks)</b>	<b>pulse no.</b>	<b>route</b>	<b>Cyclophosphamide dosage</b>
0	1	IV	15 mg/kg
2	2	IV	15 mg/kg
4	3	IV	15 mg/kg
7	4	IV	15 mg/kg
10	5	IV	15 mg/kg
13	6	IV	15 mg/kg
16	7	IV	15 mg/kg
19	8	IV	15 mg/kg.
22	9	IV	15 mg/kg
25	10	IV	15 mg/kg

**Note:**

1. The first 3 cyclophosphamide pulses are given at intervals of 2 weeks. The following doses are given at 3 weekly intervals
2. Cyclophosphamide should be continued until remission is achieved i.e. BVAS2003 score of 0 for 2 months (minimum 3 months, maximum 6 months treatment).
3. Cyclophosphamide dose reduction for age > 60 and for creatinine >300µmol/l (reductions apply for both trial and control limb patients)

<b>Pulsed CYC dose reductions for renal function and age</b>		
<b>age (years)</b>	<b>creatinine (umol/l)</b>	
	<b>&lt;300</b>	<b>&gt;300</b>
< 60	15 mg/kg/pulse	12.5 mg/kg/pulse
> 60 and < 70	12.5 “ “ “	10 “ “ “
> 70	10 “ “ “	7.5 “ “ “

- 4 Maximum cyclophosphamide pulse is 1.2g.
- 5 Dissolve cyclophosphamide in water for injection, then dilute in saline 0.9% 500 ml and administer IV over one hour.
- 6 Mesna is optional according to local practice.
- 7 The choice of antiemetic drugs to cover the cyclophosphamide pulses should follow local practice. Ondansetron 8mg is recommended.
- 8 Check FBC on day of pulse or previous day. If WBC prior to pulse < 4 x 10<sup>9</sup>/L, then postpone pulse until WBC > 4 x 10<sup>9</sup>/L, while checking WBC at least weekly. Reduce dose of pulse by 25%. With any further episodes of leucopenia, make equivalent dose reduction .
- 9 Check FBC between days 10 and 14 after a pulse. If the leucocyte nadir (i.e. the lowest leucocyte count between two cyclophosphamide pulses) is < 3 x 10<sup>9</sup>/L, even if the WBC just previous to the next pulse is > 4 x 10<sup>9</sup>/L, then reduce the dose of the next pulse by:
  - (i) leucocyte nadir 1 - 2 x 10<sup>9</sup>/L reduce cyclophosphamide dose of last pulse by 40 % of previous dose.
  - (ii) leucocyte nadir 2 - 3 x 10<sup>9</sup>/L reduce cyclophosphamide dose of last pulse by 20 % of previous dose.
- 10 **Pneumocystis carinii prophylaxis** to follow local practice but is **NOT permitted** beyond 6 months.

**Progressive disease within the first 6 months**

Disease progression defined as: a persistence of nephritic sediment and a failure to improve GFR by ≥ 10 mls/min, if GFR at diagnosis is < 50 mls/min (calculated by cockcroft gault formula, see TMF for formula) OR persistence of a major non-renal BVAS item at 6 weeks then additional treatment should occur:

- 1 Rituximab limb: 3<sup>rd</sup> dose of cyclophosphamide (15mg/kg)
- 2 Cyclophosphamide limb: Plasma exchange or IV methylprednisolone (15mg/kg x3)

**Remission maintenance regimen;**

**Control cyclophosphamide/azathioprine limb**

1. Switch cyclophosphamide to azathioprine (see below) after 3-6 months, after remission achieved (BVAS score 0 for 2 months). Azathioprine to continue until trial end.
2. Continue on oral steroids as per protocol

**Rituximab limb**

1. Continue on oral steroids as per protocol

**Azathioprine**

1. 2mg/kg. Maximum dose is 200mg.
2. Round dose down to nearest 25mg (may vary alternate day dosage, e.g. 100 and 150mg).
3. Age > 60 years, reduce dose by 25%, > 75 years by 50%.
4. Check full blood count (FBC) and ALT or AST (for hepatotoxicity):
  - a) two-weekly for one month.
  - b) two monthly for first year, then three monthly.

**Leucopenia relating to Azathioprine**

- 1 Stop oral azathioprine if white blood cells (WBC) <  $4 \times 10^9/L$ .
- 2 Restart with dose reduced by 25% when WBC >  $4 \times 10^9/L$ . Monitor weekly for four weeks.
- 3 If severe (<  $1 \times 10^9/L$ ) or prolonged (<  $4 \times 10^9/L$  for > 2 weeks), restart azathioprine at 50mg/day, increasing to target dose as weekly WBC permits. Temporary *Pneumocystis carinii* pneumonia and fungal prophylaxis are recommended until white cells >  $4 \times 10^9/L$ , G-CSF may be considered.
- 4 For falling WBC (<  $6 \times 10^9/L$  and fall of >  $2 \times 10^9/L$  over previous count), reduce dose by 25%.

**Appendix 4; Remission and relapse****Remission definition**

Full clinical remission is indicated by complete absence of clinical disease activity using BVAS 2003: BVAS score 0 for 2 months. The absence of renal disease activity is indicated by stable or falling creatinine and the absence of red cell casts. Diagnosis of complete remission is supported by a normal C-reactive protein. Sustained remission is defined by BVAS 0, maintained for at least 6 months.

**Failure to achieve remission by 6 months**

1. The trial co-ordinators should be informed.
2. Treatment should follow local practice in consultation with the trial co-ordinators.
3. Trial data collection should continue and the patient remains in the trial.

**Relapse definition**

1. Major relapse requires the recurrence or new appearance of major organ involvement such as the following, if they are attributable to active vasculitis:
  - a) an increase in serum creatinine of >30% or reduction in creatinine clearance of >25%, within a period of three months or histological evidence of active, focal, necrotizing glomerulonephritis. Biopsy is strongly recommended for recurrent haematuria or unexplained rise in creatinine.
  - b) clinical, radiological or bronchoscopic evidence of pulmonary haemorrhage or granulomata. Biopsy may be appropriate for undiagnosed opacities.
  - c) threatened vision, e.g. increasing orbital granuloma or retinal vasculitis.
  - d) significant subglottic or bronchial stenosis.
  - e) new multifocal lesions on brain MR suggestive of cerebral vasculitis.
  - f) motor mononeuritis multiplex.
  - g) gastro-intestinal haemorrhage or perforation.
2. Minor relapse requires the recurrence of disease activity of less severity, such as the following, if they are attributable to active vasculitis:
  - a) ENT: epistaxis, crusting, pain, new deafness, active nasal ulceration or proliferative mass at nasal endoscopy.
  - b) mouth ulcers.
  - c) rash.
  - d) myalgia, arthralgia, arthritis.
  - e) episcleritis or scleritis.
  - f) pulmonary symptoms without or with minor radiological changes, e.g. cough, wheeze, dyspnoea.
3. Relapse is supported by:
  - a) exacerbation of at least two constitutional symptoms (new malaise, weight loss, fever or night sweats).
  - b) rise in CRP.

If in doubt, contact a trial co-ordinator.

**Changes to drug regimens for relapse**

**(1) Rituximab Group major/minor relapse**

- 1 Rituximab 375mg/m<sup>2</sup> IV once a week, for 4 weeks. 1g Methylprednisolone IV with first dose of rituximab for **minor relapse** and IV cyclophosphamide 15mg/kg twice with each dose of rituximab for **major relapse**.
- 2 Increase oral corticosteroids to 0.5mg/kg/day, reduce dose by 5mg/week until the appropriate point on the protocol is reached.
- 3 If ineffective after 2 months, or if relapse life-threatening, follow local preference and discuss with trial management team.

**(2) Standard cyclophosphamide/azathioprine group**

**(i) Major relapse:**

- 1 Change Azathioprine to Cyclophosphamide IV pulsed (15mg/kg fortnightly for 3 doses, or until remission achieved then 3 weekly pulses). Continue cyclophosphamide for 2 months after remission achieved.
- 2 Increase oral steroids to 0.5mg/kg/day, reducing to 20mg daily by 4 weeks; reduce by 5mg daily per month until the doses of oral corticosteroids and cyclophosphamide used, approaching the 6 month point of the cyclophosphamide protocol limb are achieved. These should be maintained until the end of the study.
- 3 If ineffective after 2 months, or if relapse life-threatening, follow local preference.

**(ii) Minor relapse:**

- 1 Increase oral corticosteroids to 0.5mg/kg/day; reduce dose by 5mg/week until the appropriate point on the protocol is reached.
- 2 Increase Azathioprine to 2mg/kg if on lower dose.
- 3 If ineffective after 1 month, change cytotoxic as for major relapse.
- 4 When remission achieved return to drug regimen at 3 month point

All relapses, changes in drugs and doses are to be recorded in the record book, and patient data collected until the end of the study according to the protocol.

**Failure to recover renal function:**

In patients with renal-limited disease who remain dialysis-dependent, immunosuppressive therapy may be withdrawn after three months. A renal biopsy is strongly advised to exclude continuing disease activity before this decision is made. This action should be noted in the record book, and patient data collected until the end of the study according to the protocol.

Patients with extrarenal vasculitis should continue treatment according to the protocol

**Appendix 5: Evaluations**

These constitute the minimum information required for the study. Additional tests (eg FBC, liver function tests for cytotoxic monitoring, or disease relapse) or more frequent attendances should follow local practice (see investigator brochure for timing of evaluations).

**Entry:**

Full blood count and white cell differential (FBC)  
 C-reactive protein  
 ANCA (Indirect immunofluorescence, and proteinase 3/myeloperoxidase ELISA performed locally)  
 Creatinine  
 Rheumatoid factor, ANA, DNA binding, anti-GBM, cryoglobulins, Complement  
 Immunoglobulins  
 Hepatitis B and C serology  
 Dipstick urinalysis  
 B cell count (CD19 or CD20)  
 Renal Biopsy (not compulsory) histology sent to Milan

**Every follow up visit (i.e. at 1.5, 3, 6, 9, 12, 15, 18, 21, 24 months)**

FBC  
 Creatinine  
 CRP  
 ANCA  
 Immunoglobulins  
 CD 19/20  
 Urine dipstick  
 Urine protein/creatinine ratio or 24hour protein  
 Adverse events assessment  
 BVAS score  
 10mls serum saved for centralised immunological reactant testing (e.g. ANCA) and human anti-chimeric antibody testing.

**At 6, 12, 18, 24 month follow ups**

SF36 assessment  
 VDI assessment

**Saved Serum Samples**

10mls of serum should be taken from each patient, along with routine bloods at each study assessment (10 samples total per patient). The samples should be labelled with patient's trial identity number using the labels provided and stored on site frozen. During the study, 3 shipments will be arranged to transport the samples to Addenbrooke's Hospital Cambridge where centralised immunological tests will be performed including ANCA analysis. 4 samples per patient (0, 3, 12, 24 months follow up) will be sent from Addenbrooke's Hospital to Xendo laboratories, Amsterdam for HACA analysis and rituximab levels.

**Appendix 6: Additional whole blood samples for centralized RNA expression analysis****Whole blood sample collection at 0, 3, 6, 12 months follow up:**

2.5mls of whole blood per patient will be collected on 4 occasions for centralised RNA extraction and expression studies. Samples will be collected in PAX gene tubes (see TMF) and stored frozen, at -20°C. The samples will be labelled in coded fashion with patient trial number, date and assessment number before transfer to Addenbrooke's Hospital UK.

In Addenbrooke's Hospital the blood samples will be anonymised using a computer generated 256 bit Globally Unique Identifier (GUID) and all patient identifiable data will be encrypted using 256 bit AES (Advanced Encryption Standard) public key encryption techniques. RNA will be extracted from the peripheral blood mononuclear cells in the blood sample before being converted into cDNA. Microarray expression profiling of this cDNA will be performed by the Medical Research Council and Wellcome Trust funded CHiC TRIAD group at the University of Cambridge using an oligo-spotted array of 25,000 human genes. The aim of this work will be to identify expression profile signatures which will subsequently help to inform disease diagnosis, disease activity and likely response to treatment. Results will not have implications for individual patients. Patients who provide these additional whole blood samples will be provided with an additional patient information sheet and will be required to sign an additional consent form. It is not compulsory that all RITUXVAS trial patients provide these additional samples. At some sites additional sample collection may not be possible for logistical reasons. Therefore it is not compulsory that all investigators participate in extra sample collection.

**RITUXVAS Clinical Trial Protocol**  
**EUDRACT number: 2005-003610-15, REC reference:05/Q1604/153**

**European Vasculitis Study Group (EUVAS) Trial**

**Version 1b: 15<sup>th</sup> November 2005**

**Abbreviated trial name:** RITUXVAS

**Full Trial Name:** An international, randomised, open label trial comparing a rituximab based regimen with a standard cyclophosphamide/azathioprine regimen in the treatment of active, ‘generalised’ ANCA associated vasculitis.

**Summary**

The ANCA associated vasculitides (AASV), namely Wegener’s granulomatosis, microscopic polyangiitis, and renal limited vasculitis are autoimmune, multi-system, progressive diseases which untreated can lead to rapidly progressive renal failure and death.

Randomised, prospective, clinical trials have demonstrated the efficacy of immunosuppressive treatments for vasculitis and have defined treatment protocols at different disease points. The current ‘gold standard’ treatment for active AASV with glomerulonephritis is cyclophosphamide with steroids. However the standard treatment is associated with significant morbidity and mortality, largely due to infections and malignancy with cumulative cyclophosphamide dosing. Other effective treatments for AASV are being sought, with safer side effect profiles. Rituximab is an anti CD20 chimeric monoclonal antibody, which is used for the treatment of non-Hodgkin’s lymphoma (NHL). It is well tolerated in humans with a good safety profile. Rituximab has been shown in small series to induce remission in AASV and is now being increasingly used for other (non-ANCA) autoimmune conditions such as lupus and rheumatoid arthritis.

RITUXVAS has been designed to test the hypothesis that Rituximab leads to a higher rate of sustained remission compared to standard therapies (cyclophosphamide /azathioprine) with a lower rate of adverse events and reduced cyclophosphamide exposure as treatments for active, ‘generalised’ AASV. We plan a randomised phase II/phase III trial to compare a rituximab based regimen to standard of care. For the initial phase II part, 44 patients will be randomised 3:1 to rituximab or control. Following this analysis, the trial may be extended to a phase III stage. The trial will be conducted by 14 centres from 8 countries.

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**EUDRACT number: 2005-003610-15**

**Sponsorship provided by;** Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK, with contractual agreement with each participating centre.

**Compliance with GCP Requirements and EU Clinical Trials Directive**

RITUXVAS will be conducted in accordance with the international conference on harmonisation guidelines for Good Clinical Research Practice and the European Clinical Trials Directive 2001/20/EC.

**Participating investigators: Steering committee**

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**Trial Overview**

**Entry**  
(Eligibility criteria)



**Randomisation**  
(experimental limb 33:control limb 11)



Rituximab 375mg/m<sup>2</sup> IV x4  
Cyclophosphamide 15mg/kg IV x2  
Methylprednisolone 1g IV  
Prednisolone PO



IV Cyclophosphamide  
(minimum 3months, max  
6 months).  
Methylprednisolone 1gIV  
Prednisolone PO



Remission/maintenance  
Azathioprine 2mg/kg



**2 years  
Trial End**

**Analyses**

**6 weeks: Initial response evaluation**

**6 months: Efficacy and safety evaluation**

**24 months: Trial end and efficacy and safety evaluation**

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**List of Abbreviations**

AASV	ANCA associated vasculitis
ANCA	Anti-neutrophil cytoplasmic antibodies
BVAS	Birmingham Vasculitis Activity Score
c-ANCA	Cytoplasmic anti-neutrophil cytoplasmic antibodies
CRF	Case Report Form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CTO	Clinical Trials Office
ESR	Erythrocyte sedimentation rate
ELISA	Enzyme linked immunosorbant assay
EUVAS	European Vasculitis Study Group
GBM	Glomerular Basement Membrane
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
HACA	Human anti chimeric antibodies
HIV	Human immunodeficiency virus
IB	Investigators Brochure
IIF	Indirect immunofluorescence
IV	Intravenous
MP	Microscopic polyangitis
MPO	Myeloperoxidase
NHL	Non Hodgkin's lymphoma
PO	Oral administration
PR3	Proteinase 3
RA	Rheumatoid Arthritis
RBC	Red blood cells
RLV	Renal Limited Vasculitis
SC	Steering Committee
SAE	Serious adverse event
SF-36	Short-Form-36
SLE	Systemic Lupus Erythematosus
TMC	Trial Management Committee
VDI	Vasculitis Damage Index
WBC	White blood cell
WG	Wegener's Granulomatosis

## **Background**

### **1.1 The diseases**

Wegener's granulomatosis (WG) and microscopic polyangiitis (MP) are syndromes of primary systemic vasculitis associated with anti-neutrophil cytoplasmic antibodies (ANCA) (appendix 1)(15,16,19). WG and MP share many histological features, including a necrotising glomerulonephritis which often leads to rapidly progressive renal failure. Isolated pauci-immune necrotising, crescentic glomerulonephritis, has many features to suggest that it represents a renal-limited form of vasculitis (RLV), including the presence of circulating anti-MPO or anti-Pr3 antibodies. Common histological and serological features, and a similar response to treatment, have justified a common approach to the treatment of WG, MP and RLV.

These diseases are sub grouped according to severity. 'Generalised' vasculitis refers to systemic disease with renal involvement (creatinine up to 500) and or other imminent vital organ failure. Patients with 'generalised' vasculitis and those with more severe renal vasculitis (i.e. creatinine greater than 500) will be included in this trial.

### **1.2 Their treatment**

Untreated generalised WG and MP follow a progressive course with fatal outcome due to vital organ failure (17). With the empirical introduction of corticosteroids and cytotoxic agents, five year survival has increased from under 20% to over 60% (18).

Over the last decade the treatment of AASV has been standardised following the results of randomised trials. Cyclophosphamide (either daily oral or intravenous (IV) pulse) and prednisolone are used for remission induction (3 to 6 months) with longer therapy of azathioprine or methotrexate and low dose steroids to prevent disease relapse. Early systemic vasculitis may be treated with methotrexate in place of cyclophosphamide. The addition of plasma exchange improves renal recovery rates in severe renal vasculitis.

Adverse events with current therapy are frequent and are the major cause of mortality. The frequency of severe adverse events (10-45%) is influenced by age and the severity of renal involvement. There is a clear need for a new safer, effective therapy for AASV (1,2,3,6).

In order to address the questions of drug toxicity and frequent disease relapses, newer therapies with better safety profiles have been investigated in AASV. One such agent is rituximab, a murine/human chimeric anti CD 20 monoclonal antibody. Its use results in B cell depletion for 6-18 months (7).

B cells are key factors in autoimmune diseases with roles in autoantibody production, cytokine release and antigen presentation to T-cells. The sequence of immune events that trigger AASV are not fully understood, however evidence suggests that ANCA are pathogenic (21). Thus interruption of the cell line leading to ANCA production may be beneficial in disease suppression.

Rituximab is an established treatment in non-Hodgkin's lymphoma with a good safety track record (7). Even though the individual is rendered B cell deplete, immunoglobulin levels are maintained, and infection rates are low. Recently its use in autoimmune diseases has been realised with excellent results in rheumatoid arthritis (8). Rituximab has been shown to be effective in AASV, inducing remission in patients intolerant of cyclophosphamide (5,10-12,22,26-28). Given its good safety profile and efficacy its use a first line agent in AASV needs to be investigated.

In RITUXVAS, a rituximab based regimen will be compared with a standard cyclophosphamide/azathioprine based regimen in the treatment of AASV with glomerulonephritis. In order to achieve results applicable to clinical practice, all cases from mild to severe renal failure will be included. Standard practice includes the use of oral steroids which will be applied similarly in both treatment groups. It is recognised that necrotising crescentic glomerulonephritis progresses rapidly and immediate immune suppression is required to reverse existing and spare further organ damage. The therapeutic effect of rituximab is not immediate. Therefore two initial doses of cyclophosphamide will be given along side rituximab, thus allowing early disease control. When dialysis dependant renal failure or pulmonary haemorrhage occur, plasma exchange or intravenous steroids are standard additional therapies. These will be allowed according to local practice, and randomisation will occur after plasma exchange.

Patients in the cyclophosphamide limb will receive azathioprine as remission maintenance therapy. Patients receiving rituximab will not receive any additional maintenance therapy.

### **1.3 Rituximab**

Rituximab is an anti CD 20 chimeric mouse/human monoclonal antibody, with human IgG1 constant regions and murine light/heavy chain variable regions (7). CD 20 is a ligand which exists on developing B cells, excluding stem cells and plasma cells. The role of the CD 20 ligand in nature is not fully understood, although mediation of apoptosis has been suggested. The mechanisms by which rituximab causes B cell depletion may involve complement induced B cell lysis, Fc receptor mediated cytotoxic cell killing or the direct induction of B cell apoptosis by rituximab.

Rituximab therapy correlates positively with B cell depletion and rituximab levels. Different dosing regimens have been trialled. In patients with systemic lupus erythematosus (SLE), the efficacy of rituximab is dependant upon B cell depletion. 4 infusions of 375mg/m<sup>2</sup> rituximab infusions (one per week for four weeks) were required for this to occur (13,14). In 3 published reports 375mg/m<sup>2</sup> has resulted in B cell depletion and clinical response in patients with refractory vasculitis (10-13). On the basis of these results a dose of 4 x 375mg/m<sup>2</sup> infusions will be used in RITUXVAS.

In RITUXVAS, 2 doses cyclophosphamide will still be administered with the 1<sup>st</sup> and 3<sup>rd</sup> rituximab infusions, for two reasons. Firstly, the necrotising crescentic glomerulonephritis associated with AASV progresses rapidly and the therapeutic effect of rituximab is delayed. The use of cyclophosphamide/high dose steroid, which are standard components of induction therapy, will allow adequate immunosuppression in the early crucial treatment of AASV. Secondly, human anti-chimeric antibody (HACA) formation has been reported in NHL, SLE, and RA with varying frequencies, (4.3% of patients in RA (8)). The long term implications of these antibodies are not known. However, the possibility of anaphylactic reactions and rituximab resistance with further treatments exists. Co-administration of an immunosuppressant effective in the treatment of vasculitis is thus a logical approach to minimise HACA development.

Rituximab has now been used to treat 300,000 patients with NHL. Long-term safety regarding carcinogenicity and fertility has yet to be established. However, no major long-term adverse sequelae have been reported (1).

Up to 50% of patients receiving rituximab for an indication will develop infusion reactions with symptoms including; fevers, chills, rigors, flushing, throat irritation

rash rhinitis fatigue headache, nausea, vomiting, urticaria, angioedema, bronchospasm, myalgia, arthralgia, hypotension, hypertension and exacerbation of angina or congestive cardiac failure. Later reactions include diarrhoea, leucopenia, neutropenia, thrombocytopenia, anaemia, and infections in 30%, which may or may not be drug related (investigators brochure).

#### **1.4 Aims of RITUXVAS:**

- 1 To assess the rates of preliminary response and sustained remission of AASV following rituximab (on the basis of former studies, 86% sustained remission expected with rituximab compared to 75% in control group).
- 2 To assess safety of a rituximab regimen in terms of severe adverse events (in patients receiving standard therapies, adverse event rate is 45% at 2 years, at least 50% of which are infection related. In comparison rituximab use is only rarely associated with infections, therefore 22.5% adverse event rate expected with rituximab compared to 45% at 2 years in control group).

#### **2 Hypothesis:**

Rituximab leads to a higher rate of sustained remission compared to standard therapies (cyclophosphamide/azathioprine) with a lower rate of severe adverse events and reduced cyclophosphamide exposure.

#### **3 Trial Design**

International, randomised, controlled, prospective, open trial comparing a rituximab based regimen with a standard cyclophosphamide/azathioprine regimen in the treatment of active 'generalised' AASV. Informed consent and ethics committee approval will be obtained.

#### **3.1 Inclusion Criteria (all four must be present)**

1. **A new diagnosis of WG, MP or renal-limited vasculitis (RLV)**(appendix 1)
2. **Renal involvement attributable to active WG, MP or RLV with at least one** of the following:
  - a) Biopsy demonstrating necrotizing glomerulonephritis.
  - b) Red cell casts on urine microscopy or  $\geq$  ++ haematuria
3. **ANCA positivity** ANCA positivity requires either (a) or (b)
  - (a) PR3-ANCA by ELISA or a typical cANCA pattern by indirect immunofluorescence (IIF), or both.
  - (b) MPO-ANCA by ELISA. A positive pANCA by IIF requires confirmation by MPO-ANCA ELISA.

#### **4 Written informed consent**

#### **3.2 Exclusion Criteria**

1. Previous cyclophosphamide, (greater than 2 weeks of an oral or IV pulse cyclophosphamide regimen).
2. Co-existence of another multisystem autoimmune disease, e.g. SLE, Churg Strauss Syndrome, Henoch Schonlein Purpura, rheumatoid vasculitis, essential mixed cryoglobulinaemia, anti-glomerular basement membrane antibody positivity
3. Hepatitis B e antigen positive or Hepatitis C antibody positive.
4. Known HIV positive (HIV testing will not be a requirement for this trial).
5. Previous malignancy (usually exclude unless agreed with trial co-ordinator).

6. Pregnancy, breast feeding or inadequate contraception if female.
7. Allergy to a study medication
8. Live Vaccine within last 4 weeks

### **3.3 Randomisation**

1. Variables known to influence primary end points will be balanced between groups by minimisation
  - i. Age
  - ii. Disease WG or MP/RLV
  - iii. Creatinine
2. Randomisation form (see TMF) sent by e-mail [rbjones@doctors.org.uk](mailto:rbjones@doctors.org.uk) or fax to 01223 586506 (clinical trials office).

### **3.4 Endpoints**

Primary end points will be assessed upon trial completion at 2 years.

However interim analyses will be performed when

1. 30 patients have completed 6 weeks, to assess efficacy (treatment response) and safety (severe adverse events).
2. 40 patients have completed 6 months to assess efficacy (remission rates) and safety (severe adverse events).

#### **i Primary**

1. Sustained remission (BVAS = 0 at 6 months and sustained for 6 months).
2. Severe adverse events (CTCAE grade  $\geq 3$ ) at 2 years.

#### **ii Secondary**

##### **1 Efficacy**

Response rate at 6 weeks (BVAS < 50% baseline)  
Remission at 6 months (BVAS=0 for 2 months by 6 months)  
Time to remission (BVAS=0)  
Relapses (all relapses and major/minor)  
BVAS area under the curve  
Change in GFR  
Change in SF-36  
Change in VDI

##### **2 Safety**

Severe adverse events (CTCAE grade  $\geq 3$ ) at 6 weeks and 6 months  
All adverse events  
Death  
Prednisolone cumulative dose  
Cyclophosphamide cumulative dose

#### **iii Tertiary**

Human anti-chimeric antibody testing  
Correlation of B cells with disease activity  
Change in ANCA and disease activity  
Histopathology predictors of outcome

### **3.5 Interventions**

#### **I Drug regimens**

- a) **Rituximab Regimen:** Rituximab, 375mg/m<sup>2</sup> IV once a week for 4 weeks (i.e. 4 doses total), with 2 doses of cyclophosphamide 15mg/kg, 2 weeks apart given with the 1<sup>st</sup> and 3<sup>rd</sup> rituximab dose (appendix 3).
- b) **Control (cyclophosphamide/azathioprine) Regimen;** Cyclophosphamide 15mg/kg for 3-6 months (6-10 doses total) to be given IV according to protocol (appendix 3) for remission induction. Cyclophosphamide should be converted to azathioprine for remission maintenance.
- c) **Steroids.** All patients will receive 1g IV methylprednisolone, then same daily oral corticosteroid regimen (appendix 3).
- d) **Plasma exchange or IV methylprednisolone** will be allowed according to local practice for patients with organ threatening disease. NB randomisation should not occur until completion of plasma exchange to avoid loss of rituximab during plasma exchange. The first dose of cyclophosphamide can be given prior to completion of plasma exchange.
- e) **Progressive disease** Within the first 6 months disease progression defined as a persistence of nephritic sediment or activity on a renal biopsy and a failure to improve GFR $\geq$  10 mls/min, if GFR at diagnosis is < 50 mls/min (calculated by cockcroft gault formula, see TMF for formula) OR persistence or new occurrence of a major non-renal BVAS item at 6 weeks then additional treatment should occur:
  - i) Rituximab limb: 3<sup>rd</sup> dose of cyclophosphamide (15mg/kg)
  - ii) Cyclophosphamide limb: Plasma exchange or IV methylprednisolone (according to local practice).
- f) **Relapse Therapy.** Relapses will be categorised as major or minor.
  - i) **Rituximab limb:** Rituximab with steroid will be used for major and minor relapse. Additional cyclophosphamide may also be used for major relapse.
  - ii) **Control limb:** Increased azathioprine and steroid for minor relapse and cyclophosphamide and steroid for major relapse.

#### **II Evaluations**

Study assessments (including history and examination) will be performed at entry, 1.5, 3, 6, 9, 12, 15, 18, 21 and 24 months and at the time of relapse (see appendix 5, and TMF for assessment schedule). At each assessment the following should be performed:

- a) Blood for routine testing.
- b) 10 mls serum saved for centralised immunological analysis (including ANCA) and HACA.
- c) BVAS for each assessment and at relapse;

Every 6 months SF-36 and VDI should be performed (23-25)(see TMF).

Initial Renal histology will be reviewed by a EUVAS panel of histopathologists, based in Milan.

### **3.6 Withdrawal and treatment failure**

- 1 At patient's or physician's request. Reason for withdrawal is to be recorded in the CRF.
- 2 Patients not achieving remission within 6 months to be withdrawn from trial drug regimen and will be treated according to local practice. They will remain under trial follow up.

### **3.7 Adverse events**

- 1 Adverse effects will be actively sought and recorded in CRFs at each evaluation (see section 4.5 and TMF).
- 2 Unexpected, severe adverse events attributable to study medication must be reported within 24 hours to trial management committee. (see TMF and section 4.5 for full details).

### **3.8 Statistical analysis**

Primary end points will be assessed upon trial completion at 2 years. However interim analyses will be performed:

(a) When 30 patients have completed 6 weeks trial participation to assess

(1) treatment response (BVAS fall of > 50% baseline).

(2) safety (adverse events CTCAE grade 3 or greater)

and

(b) When 40 patients have completed 6 months trial participation to assess

(1) remission (BVAS 0 for 2 months by 6 months)

(2) safety (adverse events CTCAE grade 3 or greater)

#### **Primary end point confidence Intervals.**

A total of 30 patients will receive rituximab. The expected sustained remission rate for rituximab is 86%, based on the results of former studies. The 95% confidence interval for a sustained remission rate of 86% is 70.3%-94.7% when the sample size is 30.

The expected response rate of 86% is considered clinically significant as patients with active newly diagnosed generalised AASV without 3-6 months of cyclophosphamide are conventionally expected to experience disease progression (worsening of clinical signs and symptoms). The expected sustained remission rate in the control group is 75%.

Analyses on the 40 patients should be considered exploratory.

### **3.9 Duration:**

3 years: 6 months recruitment, two year follow-up per patient and 6 month analyses.

## **4 Trial Organisation**

### **4.1 Organisational structure and responsibilities**

#### **Principal Investigator and Research Physician**

Design and conduct of RITUXVAS  
Preparation of protocol and revisions  
Preparation of investigators brochure (IB) and CRFs  
Organising steering committee meetings  
Managing CTO  
Publication of study reports  
Members of TMC

#### **Steering committee (SC)**

(see title page for members)  
Agreement of final protocol  
All lead investigators will be steering committee members. One lead investigator per country will be nominated as national coordinator.  
Recruitment of patients and liaising with principle investigator  
Reviewing progress of study and if necessary agreeing changes to the protocol and/or investigators brochure to facilitate the smooth running of the study.

#### **Trial Management Committee (TMC)**

(Principle investigator, Research Physician, Administrator)  
Study planning  
Organisation of steering committee meetings  
Provide annual risk report MHRA and ethics committee  
SUSAR reporting to MHRA and Roche  
Responsible for trial master file  
Budget administration and contractual issues with individual centres  
Advice for lead investigators  
Audit of 6 monthly feedback forms and decide when site visit to occur.  
Assistance with international review board/independent ethics committee applications  
Data verification  
Randomisation  
Organisation of central serum sample collection

#### **Data Manager**

Maintenance of trial IT system and data entry  
Data verification

#### **Lead Investigators**

In each participating centre a lead investigator (senior nephrologist/rheumatologist/immunologist) will be identified, to be responsible for identification, recruitment, data collection and completion of CRFs, along with follow up of study patients and adherence to study protocol and investigators brochure. Investigators must maintain source documents- medical records and CRFs, signed consent forms and SF36 questionnaires. CRF data should be traceable to medical record documentation (except BVAS/VDI scores).

Lead investigators are expected to adhere to the protocol, ICH GCP guidelines and EU CT directive 2001/20/EU.

Lead investigators will be steering committee members, with one investigator per country being nominated as national coordinator.

#### **4.2 Overall study organisation**

RITUXVAS will be coordinated at the clinical trials office (CTO) in Cambridge. 14 centres in 8 different countries will take part in the study. The steering committee will meet ever 6 months to review the progress of the study. One steering committee member per country will act as national coordinator. The principle investigator, research physician, and administrator, who comprise the TMC, will be based in the CTO. The data manager will also be based in the CTO. At each centre a lead investigator will be responsible for the study conduct. The steering committee will over see the training and conduct of the trial by individual centres and staff by auditing the individual centre performance, providing information at meetings, validating data and performing site visits as needed

#### **4.3 Training and Monitoring**

RITUXVAS will be conducted in accordance with the international conference on harmonisation guidelines for good clinical research practice, the EU clinical trials directive 2001/20/EU and any local national or international regulations practice.

The lead investigators will be trained in methods of the study at investigator meetings and will be provided with an investigators brochure and trial management file (TMF) containing comprehensive study method guidelines. Advice will be available from CTO by telephone or e-mail.

The steering committee will ensure investigators receive training at meetings or by individual explanation. If inadequate reporting or problems are detected on 6 monthly feedback forms then a site visit will be performed to ensure that protocol is adhered to and local problems with the study addressed. At site visits, study records, data recording and follow up will be reviewed.

#### **4.4 Quality Control, Monitoring and safety**

(1) Monitoring of drug safety and response: End point data analysis for safety and efficacy will take place at 6 weeks, 6 months and 2 years. In view of the early analysis of data, safety issues will be addressed early on.

(2) CRF monitoring: CRF pages to be returned to CTO every 3 months with 3 monthly return form. TMC will perform quality control audit on primary end point data and other data from CRFs every 3 months. Incomplete data and inconsistencies in results will be referred to investigator for clarification. Failure to provide greater than 90% accuracy on CRFs or provide clarification of results will necessitate a site visit by TMC.

(3) Source data verification: Copies of initial ANCA results and histopathology reports (eligibility criteria) to be sent to CTO. Investigators may be required to provide copies of patient hospital records at time of study assessments for CRF data verification by TMC.

(4) Investigator audits: 6 monthly site questionnaires to be completed by each investigator and returned to TMC. Questionnaires will assess: Investigator protocol compliance, consistencies in recruitment rates, follow up, withdrawal notification, and

data return, as well as safety in terms of serious adverse events and failure to achieve disease control. Failure to return questionnaire will lead to a site visit by TMC.

(5) Regulatory body feedback: TMC to make an annual report of trial progress and safety to the MHRA and ethics committees.

#### **4.5 Monitoring of adverse events**

##### **Definitions**

###### **(1) Adverse event**

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product

###### **(2) Adverse reaction of an investigational medicinal product (AR)**

All untoward and unintended responses to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

###### **(3) Unexpected adverse reaction**

An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product).

When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

The term “severe” is often used to describe the intensity (severity) of a specific event. This is not the same as “serious,” which is based on patient/event outcome or action criteria.

###### **(4) Serious adverse event or serious adverse reaction**

Any untoward medical occurrence or effect that:

- results in death,
- is life-threatening
- requires hospitalisation or prolongation of existing inpatients' hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect.

Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.

All adverse events should be evaluated by the investigator and recorded in the CRFs. This includes the evaluation of its seriousness and the causality between the investigational medicinal products and the adverse event. Where indicated adverse events should be referred to the TMC for evaluation. The TMC office should keep

detailed records of all adverse events reported by the investigators and perform an evaluation with respect to seriousness, causality and expectedness. The TMC is responsible for the prompt notification to all investigators, research ethics committee and competent authority of each concerned member state of findings that could adversely affect the health of subjects, impact on the conduct of the trial or alter the competent authority's authorisation to continue the trial in accordance with Directive 2001/20/EC. Adverse events are defined by CTCAE (see TMF) and should be recorded in the CRF.

**Assessment of seriousness**

- Mild: The subject is aware of the event or symptom, but the event or symptom is easily tolerated
- Moderate: The subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity
- Severe: Significant impairment of functioning; the subject is unable to carry out usual activities and / or the subject's life is at risk from the event.

**Assessment of causality**

- Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product and there is a reasonable response on withdrawal.
- Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product.
- Unlikely: A causal relation is improbable and another documented cause of the AE is most plausible.
- Unrelated: A causal relationship can be definitely excluded and another documented cause of the AE is most plausible.

**(a) Serious adverse events to study treatment**

**(i) Expected events are:**

**Rituximab**

Infusion reactions (within 24hrs of infusion) occur in up to 50% of patients receiving rituximab. Symptoms include: fever chills and rash, flushing, angioedema, nausea, urticaria/rash, fatigue, headache, throat irritation, rhinitis, vomiting, puritis, hypotension and bronchospasm. Following treatment sepsis, leucopenia and neutropenia may occur.

**Cyclophosphamide**

Nausea and vomiting, neutropenia, sepsis, alopecia, haemorrhagic cystitis, infertility, bladder cancer and lymphoma.

**Prednisolone**

Sepsis, hypertension, fluid retention, hyperglycaemia and diabetes mellitus, peptic ulceration, osteoporosis, avascular necrosis, cushinoid appearance, striae, alopecia, hirsutism.

**Azathioprine**

Leucopenia, sepsis, hepatotoxicity, lymphoma, skin cancer, allergy

Please also see summary of product characteristics in TMF, for expected adverse events.

Serious expected adverse events **must** be recorded in the CRFs and if considering study drug discontinuation then TMC should be consulted.

**(ii) Unexpected (SUSARs)**

Serious unexpected suspected adverse events need to be reported within 24 hours of occurrence to the TMC. Drug should be discontinued on TMC advice. TMC will inform Roche, Basel and regulatory authorities.

**(b) Serious adverse events unrelated to study medications**

Events to be noted in CRFs.

**4.6 Patient Entry**

Participant registration form in the TMF should be e-mailed or faxed to CTO. Randomisation reply to be returned within 24hrs. Patient will receive numerical code which should be recorded at CTO and in patient record book (CRF) and TMF. . CTO will contact Roche, Basel when Rituximab required.

**4.7 Supply of study materials.**

Rituximab will be provided by national Roche affiliates and distributed after randomisation. The drug will be labelled with an individual patients study number before distribution within 48hrs of request.

**4.8 Data management.**

CRFs to be completed by local investigator. Copies of CRF records will be sent to the data manager at the CTO every 3 months. CRF data will be reviewed every 3 months by TMC. Inconsistencies, errors and incomplete data will be referred back to the investigator for clarification. Less than 90% accuracy in CRF data will necessitate a site visit. Investigators may be required to provide coded copies of patient medical notes from dates of study assessment to allow CRF data verification. The data manager is responsible for data entry into study computer.

CFRs must be retained by participating centre for 15 years from study completion. Regulatory authorities will have the right to audit such records in accordance with ICH GCP guidelines and EUCT directive 2001/20/EU.

**4.9 Laboratory measurement and sample storage.**

Standard laboratory measurements to be taken according to local laboratory practice. Normal ranges and units of measure must be included in CRFs.

10mls blood to be taken per assessment for immunological reactant testing and HACA testing. Samples will be stored frozen, on site. 3 shipments will be arranged to take samples to Addenbrooke's Hospital, Cambridge, UK for immunological reactant testing (9 10 samples per participant total). 4 out of 10 samples (taken at assessments at, 0, 3, 12, 24 months) will then be sent to Xendo laboratories, Amsterdam for HACA analysis.

**4.10 Communications**

A written trial program update will be sent to lead investigators every 6 months. Further study updates will be presented at annual EUVAS meetings and will be published on EUVAS website.

**4.11 Funding and Results Publication**

This study has received financial support from Roche Basel. It was initiated and designed by the EUVAS, RITUXVAS steering committee. Data will be collected, analysed and published independently from the source of funding by EUVAS.

**4.12 Cost implications for participants**

- 1 There will be no additional clinical costs for participating patients. There will be cost savings for the hospital as the study drug will be provided by Roche
- 2 The costs of data collection will be born by the participating centre

## **5 Ethics**

### **5.1 Ethical Considerations.**

The AASV with glomerulonephritis are rapidly progressive diseases which can be life threatening. Standard treatment regimens have significant toxicity. RITUXVAS aims to show improved sustained remission rates and safety with a rituximab based regime compared to a standard cyclophosphamide/azathioprine based regimen, for acute AASV with glomerulonephritis.

- a As rituximab may not have immediate therapeutic effect and necrotising crescentic glomerulonephritis progresses rapidly, 2 initial cyclophosphamide treatments will be given, and a third dose of cyclophosphamide if no improvement at 6 weeks. Therefore standard induction therapies will not be denied in the early, crucial, treatment phase.
- b When AASV is associated with life threatening pulmonary haemorrhage or dialysis dependent renal failure, additional therapies provide extra benefit (plasma exchange or IV steroids). These treatments will be allowed in both groups, according to local practice. Randomisation will occur after plasma exchange.
- c Patients will undergo informed consent prior to enrolment, will be provided with patient information leaflets and will be free to withdraw from the trial at any point without giving reason.
- d Approval will be sought from ethics committees.
- e Patient confidentiality will be respected according to national regulations. Data will be coded before computer entry to maintain confidentiality
- f Study follow up/blood tests will coincide with standard, disease follow-up appointments and should avoid extra hospital attendances and blood tests as much as possible.

### **5.2 Ethics Approval**

- a Central ethics approval will be obtained through the UK COREC with completion of parts A&B by the principle investigator and parts C&D by participating UK centres and standard local ethics submissions by all other sites outside the UK.
- b Sponsorship will be provided by Cambridge University Hospital NHS Foundation Trust, Cambridge, provided signature of 'site agreement to the sponsor' is obtained from all participating centres. This will take the format of a 2 page contract. The TMC may perform site inspections if necessary.

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**Appendix 1: The diseases- diagnostic criteria****Wegener's granulomatosis**

Generalised WG is characterised by granulomatous inflammation of the respiratory tract, together with necrotising vasculitis affecting small to medium-sized vessels. Necrotising glomerulonephritis is common and reflects renal involvement (16). A C-ANCA pattern by indirect immunofluorescence (IIF), with anti-proteinase 3 (PR3) reactivity by enzyme linked immunosorbant assay (ELISA), is found in over 90% of untreated patients with generalised WG. A minority of cases have anti-myeloperoxidase antibodies (MPO) instead of anti-PR3 antibodies.

For the purposes of this study, a diagnosis of generalised WG requires the presence of an active necrotising glomerulonephritis (histologically or with active urinary sediment), together with clinical, radiological or histological evidence of granulomatous inflammation in the respiratory tract, and detectable C-ANCA by IIF, or anti-Pr3 or anti-MPO ANCA by ELISA. A positive biopsy from the respiratory tract is not mandatory.

**Microscopic polyangiitis**

MP is characterised by a vasculitis predominantly affecting small vessels. Renal involvement is usual and reflected by a necrotising glomerulonephritis. Granulomata are absent. Arteritis of small or medium-sized arteries may also occur (16). MP is associated with ANCA specific for MPO or Pr3. A minority of MP patients are ANCA negative or recognise other ANCA antigens.

For the purposes of this study, a diagnosis of generalised MP requires the presence of active necrotising glomerulonephritis (histologically or with active urinary sediment), together with clinical, radiological or histological evidence of an extra-renal vasculitis, and detectable ANCA by IIF, or anti-Pr3 or anti-MPO ANCA by ELISA. A positive biopsy from an extra-renal site is not mandatory. The presence of granulomatous inflammation, or heavy immune deposition in glomeruli or vessel walls excludes this diagnosis.

**Renal-limited vasculitis or Idiopathic rapidly progressive glomerulonephritis**

Isolated pauci-immune necrotising and crescentic glomerulonephritis, typically known as idiopathic rapidly progressive glomerulonephritis has many features to suggest that it represents a renal-limited form of MP or WG, including the presence of circulating anti-MPO or anti-Pr3 antibodies.

For the purposes of this study, a diagnosis of renal-limited vasculitis, or idiopathic rapidly progressive glomerulonephritis requires renal biopsy confirmation and a positive ANCA by the criteria outlined for generalised MP. Heavy immune deposition in glomeruli excludes this diagnosis.

Renal limited vasculitis will be classified with MP for randomisation purposes, as both conditions present later than WG with greater impairment of renal function, have less response to treatment and have a lower relapse rate than WG.

**Appendix 2: Study Therapies****Rituximab- (see page 6)****Cyclophosphamide**

Cyclophosphamide is an inactive pro-drug, converted by the mixed function oxidase system in the liver to the alkylating agents 4-hydroxy-cyclophosphamide and phosphoramidate mustard, which alkylate guanine nucleotides, thus blocking cell division (20). Bioavailability after oral administration is greater than 75%, but there are large variations between individuals in the rate of production of active metabolites. A phenotypic variation in carboxylator activity affects the production of the inactive metabolite carboxyphosphoramidate from 4-hydroxy-cyclophosphamide, which may influence efficacy and toxicity. The relation of renal and hepatic failure to the production and elimination of active metabolites has not been fully determined.

Bladder toxicity is caused by renal excretion of the metabolite acrolein which can cause haemorrhagic cystitis and a markedly increased risk of bladder cancer (20). Other adverse effects include nausea and vomiting, myelosuppression with neutropenia, infections due to immunosuppression (4), alopecia and infertility. Permanent ovarian failure occurs in over 50% of women after one year's exposure and is age-related; male infertility has been less well studied. The incidence of leukaemia and /or lymphoma is increased tenfold; less common adverse effects include pulmonary fibrosis, hepatitis and the syndrome of inappropriate ADH secretion.

**Prednisolone/Methylprednisolone**

Prednisolone and methylprednisolone are synthetic derivatives of cortisone with widespread effects on metabolism and organ function (20). Desirable effects in systemic vasculitis relate to the suppression of acute and chronic inflammatory processes and immune cell function.

The major unwanted effects of corticosteroids in the short term are salt and water retention, hypertension, hyperglycaemia, central nervous system stimulation, peptic ulceration and immunosuppression. While these effects respond to reduction or withdrawal of the drug, if their use is prolonged additional effects including osteoporosis and avascular necrosis, sub capsular cataracts, skin fragility, myopathy, cushingoid facies, hirsutism, alopecia, fat re-distribution, striae and growth retardation in children may occur. IV MeP delivers a higher dose of corticosteroid, increasing the dose-related side-effects, notably hypertension and salt and water retention. Of note, in systemic vasculitis, has been the correlation of the cumulative steroid dosage with the total incidence of adverse effects, and particularly with infections (15)

**Azathioprine**

After hepatic conversion to 6-mercaptopurine, the cytotoxic effects of azathioprine are mediated by the impairment of purine synthesis, incorporation of purines into DNA, and impairment of the endonuclease repair activity of DNA polymerase (20). The drug is well-absorbed after oral administration and elimination requires hepatic metabolism by xanthine oxidase; an important drug interaction is with xanthine oxidase inhibitors, such as allopurinol. Lymphocyte function is reduced, B-cells more than T-cells, and there is suppression of the cellular component of the inflammatory response.

The major adverse effects are nausea and vomiting, dose-dependent myelosuppression and reversible, cholestatic, hepatic toxicity. An increased incidence of malignancies, particularly lymphomas and skin cancers, has been observed with prolonged administration after organ transplantation.

**Appendix 3: Drug Regimes for Remission Induction and Maintenance.****1 Steroid regimen** (same for both trial groups)**1g IV methylprednisolone**, followed by oral prednisolone (see table below)

time from entry (weeks)	prednisolone dose (mg/kg/day)	prednisolone dose (mg/day for 60 kg patient)
0	1 (max 60mg)	60
1	0.75	45
2	0.5	30
3	0.4	25
6	0.33	20
8	0.25	15
	prednisolone dosage (mg/day)	
reduce at <b>end</b> of month 3 (16 weeks)	12.5	12.5
reduce at <b>end</b> of month 4 (20 weeks)	10	10
reduce at <b>end</b> of month 5 (24 weeks)	7.5	7.5
reduce at <b>end</b> of month 6 (28 weeks)	5	5
18- 24 months	Reduce from 5 to 0	

**Note:**

- 1mg/kg. Maximum dose in first week is 60mg/day orally.
- Round dose to nearest 5mg if dose >20mg/day, and nearest 2.5mg if dose <20mg/day.
- Single daily dose (may vary alternate day dosage by up to 5mg, i.e. 10 and 15 mg on alternate days).
- Use prednisolone, avoid enteric coated or soluble forms.
- Patients intolerant of oral medication may receive any IV steroid at an equivalent dose as a daily injection.
- Minimum dose in first three months is 10mg/day.
- Bone protection against osteoporosis recommended, e.g. use of a bisphosphonate or calcium/vitamin D supplementation.
- Stomach protection against gastric ulceration recommended, e.g. the use of a proton pump inhibitor.

**2 Induction/consolidation regime for rituximab:**

Time (weeks)	Rituximab Pulse no	IV rituximab	Cyclophosphamide pulse no	IV cyclophosphamide
<b>0</b>	<b>1</b>	<b>375mg/m<sup>2</sup></b>	<b>1</b>	<b>15mg/kg</b>
<b>1</b>	<b>2</b>	<b>375mg/m<sup>2</sup></b>		
<b>2</b>	<b>3</b>	<b>375mg/m<sup>2</sup></b>	<b>2</b>	<b>15mg/kg</b>
<b>4</b>	<b>4</b>	<b>375mg/m<sup>2</sup></b>		

**Note**

- 1 Cyclophosphamide dose reductions should be made according to age and creatinine, as with control cyclophosphamide limb (see table page 27).
- 2 Rituximab should be given according to protocol indications, irrespective of CD 19/20 count.
- 3 Rituximab should be added to 1litre of normal saline and infused at a rate of 50mg per hour increasing every half an hour by 50mg an hour up to a maximum infusion rate of 400mg per hour. For second and subsequent infusions the initial rate is 100mg per hour increasing every half an hour by 100mg per hour to a maximum of 400mg per hour. Rituximab may be infused through a peripheral line.
- 4 Premedication with 1g paracetamol orally and chlorpheniramine 10mg IV is recommended.
- 5 Patients with new disease or major disease flares will receive 15mg/kg cyclophosphamide IV directly after each dose of rituximab
- 6 Patients with minor flares will receive 1g IV of methylprednisolone just prior to first rituximab infusion
- 7 Mild infusion reactions are common, e.g. chills, rash back pain. These can be treated by stopping the infusion and restarting at a slower rate along with antihistamine/antiemetic/paracetamol administration.
- 8 Severe hypersensitivity reactions are rare but have occurred. Therefore adrenaline and hydrocortisone should always be available for administration if necessary (as per local hospital protocol), when giving IV rituximab.
- 9 Patients should be observed in hospital during infusion and following infusion for 30 minutes. Following moderate/severe infusion reactions hospital admission for observation and treatment is recommended.

**3 Induction/consolidation regimen for IV pulsed cyclophosphamide:**

<b>Time (weeks)</b>	<b>pulse no.</b>	<b>route</b>	<b>Cyclophosphamide dosage</b>
0	1	IV	15 mg/kg
2	2	IV	15 mg/kg
4	3	IV	15 mg/kg
7	4	IV	15 mg/kg
10	5	IV	15 mg/kg
13	6	IV	15 mg/kg
16	7	IV	15 mg/kg
19	8	IV	15 mg/kg.
22	9	IV	15 mg/kg
25	10	IV	15 mg/kg

**Note:**

1. The first 3 cyclophosphamide pulses are given at intervals of 2 weeks. The following doses are given at 3 weekly intervals
2. Cyclophosphamide should be continued until remission is achieved i.e. BVAS2003 score of 0 for 2 months (minimum 3 months, maximum 6 months treatment).
3. Cyclophosphamide dose reduction for age > 60 and for creatinine >300µmol/l (reductions apply for both trial and control limb patients)

<b>Pulsed CYC dose reductions for renal function and age</b>		
<b>age (years)</b>	<b>creatinine (umol/l)</b>	
	<b>&lt;300</b>	<b>&gt;300</b>
< 60	15 mg/kg/pulse	12.5 mg/kg/pulse
> 60 and < 70	12.5 “ “ “	10 “ “ “
> 70	10 “ “ “	7.5 “ “ “

- 4 Maximum cyclophosphamide pulse is 1.2g.
- 5 Dissolve cyclophosphamide in water for injection, then dilute in saline 0.9% 500 ml and administer IV over one hour.
- 6 Mesna is optional according to local practice.
- 7 The choice of antiemetic drugs to cover the cyclophosphamide pulses should follow local practice. Ondansetron 8mg is recommended.
- 8 Check FBC on day of pulse or previous day. If WBC prior to pulse < 4 x 10<sup>9</sup>/L, then postpone pulse until WBC > 4 x 10<sup>9</sup>/L, while checking WBC at least weekly. Reduce dose of pulse by 25%. With any further episodes of leucopenia, make equivalent dose reduction .
- 9 Check FBC between days 10 and 14 after a pulse. If the leucocyte nadir (i.e. the lowest leucocyte count between two cyclophosphamide pulses) is < 3 x 10<sup>9</sup>/L, even if the WBC just previous to the next pulse is > 4 x 10<sup>9</sup>/L, then reduce the dose of the next pulse by:
  - (i) leucocyte nadir 1 - 2 x 10<sup>9</sup>/L reduce cyclophosphamide dose of last pulse by 40 % of previous dose.
  - (ii) leucocyte nadir 2 - 3 x 10<sup>9</sup>/L reduce cyclophosphamide dose of last pulse by 20 % of previous dose.
- 10 **Pneumocystis carinii prophylaxis** to follow local practice but is **NOT permitted** beyond 6 months.

**Progressive disease within the first 6 months**

Disease progression defined as: a persistence of nephritic sediment and a failure to improve GFR by ≥ 10 mls/min, if GFR at diagnosis is < 50 mls/min (calculated by cockcroft gault formula, see TMF for formula) OR persistence of a major non-renal BVAS item at 6 weeks then additional treatment should occur:

- 1 Rituximab limb: 3<sup>rd</sup> dose of cyclophosphamide (15mg/kg)
- 2 Cyclophosphamide limb: Plasma exchange or IV methylprednisolone (15mg/kg x3)

**Remission maintenance regimen;**

**Control cyclophosphamide/azathioprine limb**

1. Switch cyclophosphamide to azathioprine (see below) after 3-6 months, after remission achieved (BVAS score 0 for 2 months). Azathioprine to continue until trial end.
2. Continue on oral steroids as per protocol

**Rituximab limb**

1. Continue on oral steroids as per protocol

**Azathioprine**

1. 2mg/kg. Maximum dose is 200mg.
2. Round dose down to nearest 25mg (may vary alternate day dosage, e.g. 100 and 150mg).
3. Age > 60 years, reduce dose by 25%, > 75 years by 50%.
4. Check full blood count (FBC) and ALT or AST (for hepatotoxicity):
  - a) two-weekly for one month.
  - b) two monthly for first year, then three monthly.

**Leucopenia relating to Azathioprine**

- 1 Stop oral azathioprine if white blood cells (WBC) <  $4 \times 10^9/L$ .
- 2 Restart with dose reduced by 25% when WBC >  $4 \times 10^9/L$ . Monitor weekly for four weeks.
- 3 If severe (<  $1 \times 10^9/L$ ) or prolonged (<  $4 \times 10^9/L$  for > 2 weeks), restart azathioprine at 50mg/day, increasing to target dose as weekly WBC permits. Temporary *Pneumocystis carinii* pneumonia and fungal prophylaxis are recommended until white cells >  $4 \times 10^9/L$ , G-CSF may be considered.
- 4 For falling WBC (<  $6 \times 10^9/L$  and fall of >  $2 \times 10^9/L$  over previous count), reduce dose by 25%.

**Appendix 4; Remission and relapse****Remission definition**

Full clinical remission is indicated by complete absence of clinical disease activity using BVAS 2003: BVAS score 0 for 2 months. The absence of renal disease activity is indicated by stable or falling creatinine and the absence of red cell casts. Diagnosis of complete remission is supported by a normal C-reactive protein. Sustained remission is defined by BVAS 0, maintained for at least 6 months.

**Failure to achieve remission by 6 months**

1. The trial co-ordinators should be informed.
2. Treatment should follow local practice in consultation with the trial co-ordinators.
3. Trial data collection should continue and the patient remains in the trial.

**Relapse definition**

1. Major relapse requires the recurrence or new appearance of major organ involvement such as the following, if they are attributable to active vasculitis:
  - a) an increase in serum creatinine of >30% or reduction in creatinine clearance of >25%, within a period of three months or histological evidence of active, focal, necrotizing glomerulonephritis. Biopsy is strongly recommended for recurrent haematuria or unexplained rise in creatinine.
  - b) clinical, radiological or bronchoscopic evidence of pulmonary haemorrhage or granulomata. Biopsy may be appropriate for undiagnosed opacities.
  - c) threatened vision, e.g. increasing orbital granuloma or retinal vasculitis.
  - d) significant subglottic or bronchial stenosis.
  - e) new multifocal lesions on brain MR suggestive of cerebral vasculitis.
  - f) motor mononeuritis multiplex.
  - g) gastro-intestinal haemorrhage or perforation.
2. Minor relapse requires the recurrence of disease activity of less severity, such as the following, if they are attributable to active vasculitis:
  - a) ENT: epistaxis, crusting, pain, new deafness, active nasal ulceration or proliferative mass at nasal endoscopy.
  - b) mouth ulcers.
  - c) rash.
  - d) myalgia, arthralgia, arthritis.
  - e) episcleritis or scleritis.
  - f) pulmonary symptoms without or with minor radiological changes, e.g. cough, wheeze, dyspnoea.
3. Relapse is supported by:
  - a) exacerbation of at least two constitutional symptoms (new malaise, weight loss, fever or night sweats).
  - b) rise in CRP.

If in doubt, contact a trial co-ordinator.

**Changes to drug regimens for relapse**

**(1) Rituximab Group major/minor relapse**

- 1 Rituximab 375mg/m<sup>2</sup> IV once a week, for 4 weeks. 1g Methylprednisolone IV with first dose of rituximab for **minor relapse** and IV cyclophosphamide 15mg/kg twice with each dose of rituximab for **major relapse**.
- 2 Increase oral corticosteroids to 0.5mg/kg/day, reduce dose by 5mg/week until the appropriate point on the protocol is reached.
- 3 If ineffective after 2 months, or if relapse life-threatening, follow local preference and discuss with trial management team.

**(2) Standard cyclophosphamide/azathioprine group**

**(i) Major relapse:**

- 1 Change Azathioprine to Cyclophosphamide IV pulsed (15mg/kg fortnightly for 3 doses, or until remission achieved then 3 weekly pulses). Continue cyclophosphamide for 2 months after remission achieved.
- 2 Increase oral steroids to 0.5mg/kg/day, reducing to 20mg daily by 4 weeks; reduce by 5mg daily per month until the doses of oral corticosteroids and cyclophosphamide used, approaching the 6 month point of the cyclophosphamide protocol limb are achieved. These should be maintained until the end of the study.
- 3 If ineffective after 2 months, or if relapse life-threatening, follow local preference.

**(ii) Minor relapse:**

- 1 Increase oral corticosteroids to 0.5mg/kg/day; reduce dose by 5mg/week until the appropriate point on the protocol is reached.
- 2 Increase Azathioprine to 2mg/kg if on lower dose.
- 3 If ineffective after 1 month, change cytotoxic as for major relapse.
- 4 When remission achieved return to drug regimen at 3 month point

All relapses, changes in drugs and doses are to be recorded in the record book, and patient data collected until the end of the study according to the protocol.

**Failure to recover renal function:**

In patients with renal-limited disease who remain dialysis-dependent, immunosuppressive therapy may be withdrawn after three months. A renal biopsy is strongly advised to exclude continuing disease activity before this decision is made. This action should be noted in the record book, and patient data collected until the end of the study according to the protocol.

Patients with extrarenal vasculitis should continue treatment according to the protocol

**Appendix 5: Evaluations**

These constitute the minimum information required for the study. Additional tests (eg FBC, liver function tests for cytotoxic monitoring, or disease relapse) or more frequent attendances should follow local practice (see investigator brochure for timing of evaluations).

**Entry:**

Full blood count and white cell differential (FBC)  
 C-reactive protein  
 ANCA (Indirect immunofluorescence, and proteinase 3/myeloperoxidase ELISA performed locally)  
 Creatinine  
 Rheumatoid factor, ANA, DNA binding, anti-GBM, cryoglobulins, Complement  
 Immunoglobulins  
 Hepatitis B and C serology  
 Dipstick urinalysis  
 B cell count (CD19 or CD20)  
 Renal Biopsy (not compulsory) histology sent to Milan

**Every follow up visit (i.e. at 1.5, 3, 6, 9, 12, 15, 18, 21, 24 months)**

FBC  
 Creatinine  
 CRP  
 ANCA  
 Immunoglobulins  
 CD 19/20  
 Urine dipstick  
 Urine protein/creatinine ratio or 24hour protein  
 Adverse events assessment  
 BVAS score  
 10mls serum saved for centralised immunological reactant testing (e.g. ANCA) and human anti-chimeric antibody testing.

**At 6, 12, 18, 24 month follow ups**

SF36 assessment  
 VDI assessment

**Saved Serum Samples**

10mls of serum should be taken from each patient, along with routine bloods at each study assessment (10 samples total per patient). The samples should be labelled with patient's trial identity number using the labels provided and stored on site frozen. During the study, 3 shipments will be arranged to transport the samples to Addenbrooke's Hospital Cambridge where centralised immunological tests will be performed including ANCA analysis. 4 samples per patient (0, 3, 12, 24 months follow up) will be sent from Addenbrooke's Hospital to Xendo laboratories, Amsterdam for HACA analysis and rituximab levels.