

**MYCYC clinical trial protocol**  
**European Vasculitis Study Group (EUVAS) Trial**  
**EUDRACT number: 2006-001663-33, REC reference:06/Q1605/120**  
**Version 2A, 16th January 2007**

**Abbreviated trial name:** MYCYC

**Full Trial Name:** A randomised clinical trial of mycophenolate mofetil versus cyclophosphamide for remission induction in ANCA associated vasculitis.

**Summary**

There is a clear need for improved therapy in ANCA associated vasculitis where current treatments are toxic and contribute to poor outcomes. Conventional therapy combines cyclophosphamide with prednisolone but is associated with severe adverse events in 35%, early mortality, malignancy and infertility. Mycophenolate mofetil (MMF) is a newer immunosuppressive drug which has superior efficacy to azathioprine in solid organ transplantation. MMF is an effective alternative to cyclophosphamide in lupus nephritis. Open label studies and retrospective surveys point to the efficacy and low toxicity of MMF in vasculitis.

We hypothesise that MMF is not inferior in efficacy compared to cyclophosphamide for remission induction in AASV. 140 new patients will be randomised to MMF 2g/day or a European consensus intravenous cyclophosphamide regimen, with the same prednisolone dosing. Following a six month induction course all patients will receive consensus remission maintenance treatment with azathioprine and prednisolone. The primary end-point will be remission rate by six months, secondary end-points include relapse rate at 18 months and safety. The trial will be conducted in 10 countries by members of the European Vasculitis Study Group (EUVAS). The trial duration will be 42 months (24 months recruitment, 18 months follow up).

This trial has been designed according to the EULAR/EUVAS recommendations for conducting Clinical Trials in Systemic Vasculitis<sup>1</sup>.

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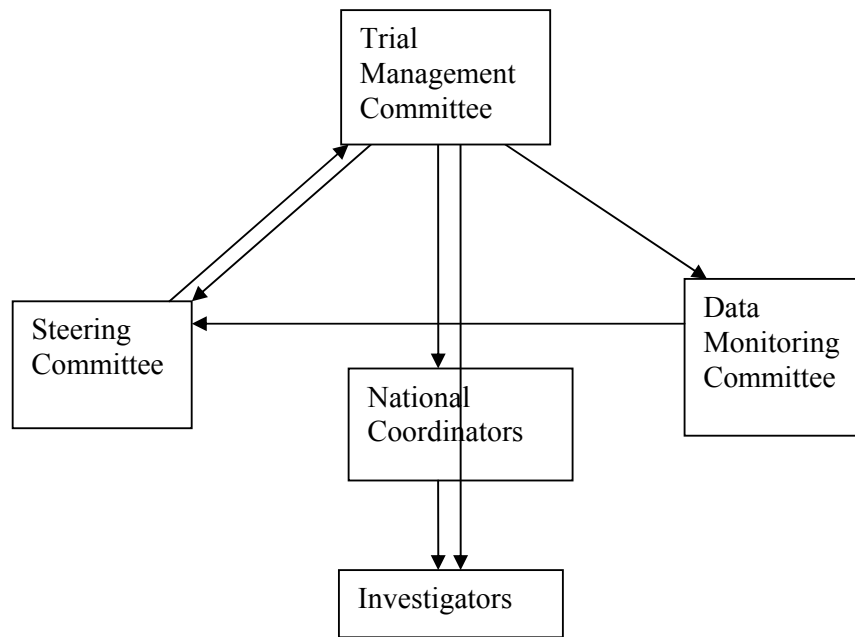
**EUDRACT number:** 2006-001663-33

**Sponsorship provided by:** Cambridge University Hospitals NHS Foundation Trust

**Compliance with GCP Requirements:**

MYCYC 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.

**Trial Organisation**



**Trial Overview**

**Entry**  
(Eligibility criteria)



**Randomisation**  
(70 patients per limb)



MMF 2g/day  
(Minimum 3  
Maximum 6 months)  
Prednisolone PO



Cyclophosphamide IV  
(minimum 3,  
maximum 6 months)  
Prednisolone PO

**Remission/Maintenance**



Azathioprine 2mg/kg/day  
Prednisolone PO



**Study end 18 months**

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List of Abbreviations

AASV	ANCA associated systemic vasculitis
ANA	Anti-neutrophil antibodies
ANCA	Anti-neutrophil cytoplasmic antibodies
ACR	American College of Rheumatology
AZA	Azathioprine
BVAS	Birmingham Vasculitis Activity Score
c-ANCA	Cytoplasmic anti-neutrophil cytoplasmic antibodies
CHiC TRIAD	Cambridge/Hinxton Centre for Translational Research in Autoimmune Disease
CMV	Cytomegalovirus
CRF	Case Report Folder/patient record book
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CTO	Clinical Trials Office
CYC	Cyclophosphamide
DMC	Data monitoring committee
ESR	Erythrocyte sedimentation rate
ELISA	Enzyme linked immunosorbant assay
EULAR	European League Against Rheumatism
EUVAS	European Vasculitis Study Group
FBC	Full Blood Count
GBM	Glomerular Basement Membrane
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GI	Gastro-intestinal
HIV	Human immunodeficiency virus
IB	Investigators Brochure
IIF	Indirect immunofluorescence
IV	Intravenous
MMF	Mycophenolate mofetil
MPA	Microscopic polyangiitis
MPO	Myeloperoxidase
p-ANCA	Perinuclear anti-neutrophil cytoplasmic antibodies
PBMC	Peripheral blood mononuclear cell
PO	Oral administration
PR3	Proteinase 3
RA	Rheumatoid Arthritis
RLV	Renal Limited Vasculitis
SC	Steering Committee
SAE	Serious adverse event
SF-36	Short Form-36
SLE	Systemic Lupus Erythematosus
SUSAR	Severe unexpected suspected adverse reaction
TPMT	Thiopurine methyl transferase
TMF	Trial management file
TMC	Trial Management Committee
UGT1A9	uridine diphosphate-glucuronosyltransferase
VDI	Vasculitis Damage Index
WBC	White blood cell
WG	Wegener's Granulomatosis

## **1. Background**

### **1.1 The diseases**

Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are primary systemic vasculitides associated with anti-neutrophil cytoplasmic antibodies (ANCA). Although WG is a granulomatous disease with systemic vasculitis, and MPA is manifest by systemic vasculitis without granuloma, these diseases are grouped together due to their histological similarities, the absence of immune deposits, the contribution of ANCA to their pathogenesis and their similar responses to immunosuppressive treatment. These diseases have an annual incidence of 20 per million and are the most common cause of rapidly progressive glomerulonephritis<sup>2</sup>. Treatment has converted these previously fatal diseases to chronic relapsing conditions with relapse rates of 50% at five years<sup>3</sup>. Data from multi-centre trials suggest that WG has an increased risk of relapse compared with MPA<sup>4</sup>.

### **1.2 Treatment of WG and MPA**

Before the advent of immunosuppressive treatment, the prognosis of WG was poor with a two year mortality of 80%. The introduction of CYC and steroids in the early 1970's has resulted in complete remission rates of 80-90% and reported five year survival rates of 60-80% depending on renal function<sup>5</sup>. The morbidity and mortality nevertheless remains high with many patients suffering severe adverse effects of treatment, often related to the use of CYC. Therapy causes severe adverse events in 25-45% of patients in the first year, irreversible damage in over 50% and is the major cause of death in the first five years. The age and severity of renal involvement influences the risk of treatment toxicity<sup>6</sup>. Toxicity of CYC includes a markedly increased risk of malignancy, which increases with cumulative dosage, contributing to the excess mortality seen in patients with ANCA associated systemic vasculitis (AASV)<sup>7</sup>. In order to address the issue of drug toxicity, newer therapies with better safety profiles but equivalent immunosuppressive potency are required to achieve disease remission. For treatment purposes the diseases have been sub grouped into: localized, early systemic, generalized, severe renal or refractory disease<sup>8</sup>. The more severe disease requires the most aggressive therapy and carries the highest mortality risk. Patient in this study will have either early systemic or generalized disease.

### **1.3 Mycophenolate mofetil as an alternative for induction therapy**

Mycophenolate mofetil (MMF) appears to be an excellent alternative to CYC for induction of remission in AASV because it has strong immunosuppressive potency combined with low toxicity profile<sup>9, 10</sup>. For prophylaxis of renal allograft rejection it is superior to azathioprine. In autoimmunity, MMF is already known to be effective in lupus<sup>11, 12</sup>, and in lupus nephritis<sup>13</sup>. Pilot studies in AASV using MMF as maintenance therapy, following induction of remission with CYC and steroids, suggested MMF was beneficial<sup>14, 15, 16</sup> resulting in development of a randomized clinical trial by the EUVAS group investigating the efficacy of MMF in maintaining disease remission. The results of this study will be available in 2008. Case studies have also suggested that MMF may be used as induction therapy<sup>17, 18</sup>, and a recent pilot study using MMF as induction therapy in patients intolerant of cyclophosphamide suggested benefit<sup>19</sup>.

MMF is a prodrug that is hydrolysed to mycophenolic acid. The active metabolite mycophenolic acid reversibly inhibits inosin-monophosphate-dehydrogenase (IMDH), a key enzyme of de novo purine synthesis<sup>20</sup>. Lymphocytes are key effector cells in AASV, whose proliferation and function

relies almost exclusively on de novo purine synthesis whereas most other cells use the salvage pathway.

Treatment with MMF is attractive due to its low toxicity profile. Patients treated with MMF to maintain remission of AASV, experienced only transient adverse events, which included gastrointestinal (GI) upset, leucopenia, respiratory infection and an episode of cytomegalovirus (CMV) – colitis. In a systematic review of transplant patients, leucopenia and CMV were significantly more common only at the very highest MMF dose (3g), compared with azathioprine. The frequency of malignancy in a three year transplant study where MMF was combined with other immunosuppressive drugs showed no difference compared to azathioprine. GI adverse effects maybe related to accumulation of the metabolite, mycophenolic acid glucuronide, which accumulates in renal failure. Dose reduction in those with a creatinine clearance of less than 25 ml/min may be required<sup>21</sup>. Paediatric MMF doses of 600mg/m<sup>2</sup> BD are equivalent to 1000mg BD in adults<sup>22</sup>

## 2. Aims of MYCYC

To investigate whether an MMF based induction regimen is as effective as a standard IV CYC regimen for the treatment of active AASV.

## 3. Hypothesis

MMF based regimen is not inferior in efficacy compared to a standard CYC/AZA based regimen in the treatment of active AASV

## 4. Trial design

International multi-centre, randomized, controlled prospective open label trial comparing an MMF based regimen with a standard intravenous CYC/AZA based regimen in the treatment of active AASV. Informed consent and ethics committee approval will be obtained. This study has the primary end point of efficacy (remission rate at 6 months). Follow-up will continue to 18 months in order to detect an influence of induction regimen on subsequent relapse rates.

### 4.1 Eligibility

- a) Inclusion (requires all)
  - i) New diagnosis of AASV (WG or MPA) (within the previous six months)
  - ii) Active disease (defined by at least one major or three minor BVAS 2003 items, see appendix 1)
  - iii) ANCA positivity (c-ANCA and PR3-ANCA or p-ANCA and MPO-ANCA) or histology confirming active vasculitis from any organ (see appendix )
  - iv) Written informed consent
  
- b) Exclusion
  - i) Previous treatment with:
    - (1) MMF: more than two weeks ever.
    - (2) Cyclophosphamide: more than two weeks daily oral or more than 1 pulse of IV CYC (15mg/kg)
    - (3) Rituximab or high dose intravenous immunoglobulin within the last twelve months
  - ii) Active infection (including hepatitis B, C, HIV and tuberculosis).
  - iii) Known hypersensitivity to MMF, AZA or CYC.
  - iv) Cancer or an individual history of cancer (other than resected basal cell skin carcinoma).



- v) Females who are pregnant, breast feeding, or at risk of pregnancy and not using a medically acceptable form of contraception.
- vi) Any condition judged by the investigator that would cause the study to be detrimental to the patient.
- vii) Any other multi-system autoimmune disease including Churg Strauss angitis, SLE, anti GBM disease and cryoglobulinaemia.
- viii) Active serious digestive system disease (e.g. inflammatory bowel disease).
- ix) Patients with imminently life threatening vasculitis (diffuse alveolar haemorrhage, intestinal perforation or major haemorrhage, cerebral vasculitis and cardiac vasculitis).
- x) Patients with rapidly progressive glomerulonephritis and declining renal function. Defined as estimated GFR fall >20% in previous two weeks.
- xi) GFR<15mls/min at entry or on dialysis.

## 4.2 Endpoints

### Primary

Proportion of patients achieving remission by 6 months. (Remission is defined as the absence of disease activity attributable to active vasculitis BVAS =0 on two occasions at least one month apart and adherence to prednisolone taper. Patients in remission require low dose immunosuppression and oral prednisolone to continue in order to maintain remission).

### Secondary

- Time to remission (months)
- Adverse events: mild/moderate/severe and infections
- Relapse (relapse rates at 18 months and relapse free survival)
- Cumulative dose of corticosteroids
- Improvement in calculated GFR at 18months
- Cumulative vasculitis damage index (VDI) scores
- Change in SF36 at 12 and 18 months
- BVAS (area under curve) between entry and 18 months
- ANCA status at 6 months

### Tertiary (optional sample collection)

- Mycophenolic acid pharmacokinetics
- Pharmacogenetics
- Gene expression profiling

## 4.3 Therapeutic regimen

### a) MMF group (appendix 3)

i) 2g per day, or maximum tolerated dose between 1 and 2g/day, for 3-6 months until remission (BVAS 0 for 2 consecutive study assessments), then switch to AZA maintenance regimen.

ii) For those <age 16 years 1200mg/m<sup>2</sup>/day, or maximum tolerated dose between 600 and 1200mg/m<sup>2</sup>/day.

iii) For persistent disease in adults at 4 weeks dose increase up to a maximum of 3g/day allowed. Dose increase only permitted if 2g/day is tolerated without moderate/severe side effects. Persistent disease is defined as persistence (NOT worsening) of major or minor BVAS items present at entry.

- b) CYC group (appendix 3)
  - i) CYC 15mg/kg at weeks 0, 2 and 4 then pulses every three weeks for 3-6 months (6-10 doses) until remission (BVAS 0 for 2 consecutive study assessments), then switch to AZA maintenance regimen.
- c) Both groups
  - i) Planned additional therapies permitted before randomization:
    - (1) Intravenous methyl prednisolone maximum 3g
    - (2) Plasma exchange is allowed according to local guidelines
 Additional therapy will be included as minimization variables.
  - ii) Prednisolone or prednisone.
    - 1mg/kg/day, tapered to 10mg/day by week 13.
  - iii) Prophylaxis
    - (1) Mandatory Sulfamethoxazole/trimethoprim 480mg (follow local protocol of either three times a week or daily) until week 26 for all patients unless allergic.
    - (2) Recommended but not mandatory
      - (a) Proton pump inhibitor, e.g. Lansoprazole 30mg/day or equivalent until prednisolone  $\leq$  10mg/day.
      - (b) Oral fungal prophylaxis, nystatin or amphotericin, until prednisolone  $<$  20mg/day according to local practice.
      - (c) Calcium and vitamin D and/or oral bisphosphonate according to local practice.
- d) Progressive disease before remission
  - i) Patients with progressive disease may receive one course (or one subsequent course if used at entry) of IV methyl prednisolone (maximum 3g) or plasma exchange (according to local practice) or intravenous immunoglobulin (2g/kg). Progressive disease is defined as:
    - (1) Persistence or worsening of a major BVAS item present at entry
    - (2) A new major BVAS item, not present at entry
    - (3) Failure to achieve an increase in GFR of  $\geq$  10 mls/min for patients with an initial GFR  $<$  50ml/min (Cockcroft-Gault). For patients  $<$  16 years of age GFR (mls/min/1.73 m<sup>2</sup>) will be calculated using the Haycock-Schwartz formula with k of 40 for creatinine expressed in  $\mu$ mol/l, or k=0.45 for creatinine expressed in mg/dl.
  - ii) Patients with progressive disease despite methylprednisolone, immunoglobulin or plasma exchange should be considered a 'treatment failure' and should be treated according to local practice but data collection should continue within the trial.

#### 4.4 Evaluations

- a) Study assessments will be performed at 0, 4 weeks and 1.5, 3, 4.5, 6, 9, 12, 18 months after entry and at the time of relapse.
- b) BVAS, bloods (FBC, CRP, creatinine, ANCA) and urine (dipstick haematuria and proteinuria), patient weight at each visit.
- c) VDI and SF-36 at 0, 6, 12 and 18 months
- d) Renal histopathology will be reviewed by a EUVAS panel.
- e) WBC, ALT, AST blood measurements will be performed and recorded in patient record books according to drug monitoring guidelines (appendix 3).

#### 4.5 Withdrawal and treatment failure

- i) At patient or physician request. Reason for withdrawal is to be recorded in the CRF.

- ii) Patients not achieving remission within six months to be withdrawn from trial drug regimen and will be treated according to local practice. They will remain under trial follow up but data collection should continue and they will be included in the intention to treat analysis.

#### **4.6 Adverse events**

1. Adverse events will be actively sought and recorded in CRF at each study evaluation.
2. All serious adverse events must be reported within 24 hours to the trial management committee. (i.e. all events irrespective of their relation to study medications, that are either life threatening, result in hospitalization or prolongation of hospital stay, result in death, result in persistent or significant disability or incapacity or congenital anomaly or birth defect.

#### **4.7 Statistical analysis**

The Primary endpoint is remission at six months defined as a BVAS = 0 on two occasions at least one month apart and adherence to the steroid protocol. Previous EUVAS studies in this patient subgroup have observed remission rates of 80-90% with a cyclophosphamide/prednisolone regimen<sup>23</sup>. In order to demonstrate non-inferiority of MMF as compared to cyclophosphamide we have calculated that 124 patients are required to detect a reduction of 12% (i.e. remission rate < 73%) or more from the expected remission rate of 85% with a power of 80%, and a significance level of 5% in a non-inferiority test (Makuch & Simon)<sup>24</sup>. Allowing for a 10% drop out rate 140 patients will be recruited.

#### **4.8 Randomization**

Minimization for variables known to influence disease outcome and end points

- 1 Cockcroft gault GFR <30mls/min in adults or calculated GFR <30 mls/min/1.73m<sup>2</sup> for patients < 16 years
- 2 Planned additional therapy at entry with Methylprednisolone >0.5g or plasma exchange
- 3 Age > 60 years

Randomization form (see Trial Management File) sent by e-mail [rbjones@doctors.org.uk](mailto:rbjones@doctors.org.uk) or fax to 01223 586506 (clinical trials office).

#### **4.9 Duration:**

42 months with 24 months recruitment and 18 months follow up

Primary end point analysis to be performed when all patients have completed 6 months induction treatment. All end point analysis to be performed when all patients have completed 18 months follow up.

## **5. Trial Organisation**

### **5.1 Organisational structure and responsibilities**

#### **Co-Principal Investigators**

Design and conduct of MYCYC  
Preparation of protocol and revisions  
Preparation of investigator brochure and patient record books  
Organising steering committee/investigator meetings  
Managing clinical trials office  
Publication of study reports  
Members of trial management committee

#### **Steering committee**

(See page 2 for members)  
Agreement of final protocol  
Recruitment of patients and liaising with principle investigator  
Reviewing progress of study and if necessary agreeing changes to the protocol and/or investigator brochure to facilitate the smooth running of the study

#### **National coordinators**

Organisation of national regulatory and ethical approval  
Organisation of national trial insurance

#### **Data Monitoring Committee**

Review annual report from Trial Management Committee in terms of risk assessment  
(Severe adverse events, treatment response rates and data return.)  
Feedback to Steering committee.

#### **Trial Management Committee**

(Co-principle investigators, Administrator)  
Study planning  
Organisation of SC meetings  
Provide annual risk report to DMC  
Responsible for trial master file  
Budget administration and contractual issues with individual centres  
Advice for lead investigators  
Audit of six monthly feedback forms and decide when site visits to occur.  
Assistance with international review board/independent ethics committee applications  
Data cleaning  
Randomisation

#### **Data Manager**

Maintenance of trial IT system and data entry  
Data cleaning

**Lead Investigators**

In each participating centre a lead investigator (senior nephrologist/rheumatologist) will be identified, to be responsible:

Protocol adherence

Recruitment of patients according to eligibility criteria

Adherence to good clinical practice and EU clinical trial directive guidelines

Adherence to local regulations

Adverse event reporting

Obtaining site approval

Data collection and return

MYCYC will be coordinated at the clinical trials office (CTO), Addenbrooke's Hospital Cambridge. 10 different countries will take part in the study. The steering committee will meet annually to review the progress of the study. Investigator meetings will be held six monthly. The co-principle investigators and administrator, who comprise the TMC, will be based in the CTO. The data manager will also be based in the CTO. The statistician is local to the CTO. At each centre a lead investigator will be responsible for the study conduct. The steering committee will oversee 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.

**5.2 Overall Study Organisation**

MYCYC will be coordinated at the clinical trials office (CTO) in Cambridge. 20 centres in 10 different countries will take part in the study. The steering committee will meet every 6 months to review the progress of the study. One investigator per country will act as national coordinator. The TMC, will be based in the CTO. The data manager will also be based in the CTO. A Data Monitoring committee will meet annually to review safety data. 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

**5.3 Training and Monitoring**

MYCYC 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 files (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.

**5.4 Quality Control, Monitoring and safety**

(1) Monitoring of drug safety and response: A data monitoring committee will meet annually to review drug safety and protocol compliance report provided by TMC. In addition an interim analysis will be performed after 70 patients have been recruited.

(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.

## **5.5 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 unfavorable 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 (SPC) 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 severity**

- 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) Adverse events to study treatment**

##### **(i) Expected events are listed in the investigator brochure documents in the trial management file.**

Adverse events **must** be recorded in the patient record books and if considering study drug discontinuation then TMC should be consulted.

##### **(ii) Unexpected (SUSARs)**

Serious unexpected suspected adverse reactions need to be reported within 24 hours of occurrence to the TMC. Drug should be discontinued on TMC advice. The TMC (on behalf of the sponsor) will then report any life threatening or fatal SUSARs to Aspreva and the regulatory authorities within 7 days of their first knowledge of the event. The TMC (on behalf of the sponsor) will then report all other non-life threatening SUSARs within 15 days of their first knowledge of the event.

**(b) Adverse events unrelated to study medications**

All adverse events to be noted in CRFs.

**5.6 Patient Entry**

Participant registration form in the trial management file should be e-mailed or faxed to the clinical trials office. Randomisation reply to be returned within 24 hours. Patient will receive numerical code which should be recorded at the clinical trials office and in the patient record book.

**5.7 Supply of study materials.**

MMF will be provided by hospital pharmacies and labeled as clinical trials supplies, using a local translation of the trial master MMF label. Any additional local regulatory requirements may be added to the label. Drug invoices should be sent to the trial management committee on a quarterly basis, for quarterly reimbursement from study funds held by the principle investigator at Addenbrooke's Hospital, Cambridge, UK.

**5.8 Data management.**

Copies of patient data should be sent to the data manager at the CTO every three months. Data cleaning to be performed by data manager and TMC. Data manager responsible for data entry into study computer.

CRFs 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.

**5.9 Laboratory measurement and sample storage.**

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

**5.10 Communications**

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

**5.11 Funding**

This study has received financial support from Aspreva. It was initiated and designed by the EUVAS MYCYC steering committee. Funding provided by Aspreva covers, fees for registration and approval at ethics committees and regulatory agencies at least at each national coordinating center according to a budget agreed on by the steering committee. Data will be collected, analysed and published independently from the source of funding.

**5.12 Cost implications for participants**

There will be no additional clinical costs for participating patients. The costs of data collection will be born by the participating centre.

**6. Ethics****6.1 Ethical Considerations**



AASV are rapidly progressive diseases which can be life threatening. Standard treatment regimes have significant toxicity. MYCYC aims to show equivalent outcome with less adverse events with a MMF based regime compared to a standard CYC/AZA based regimen for active AASV.

- i) Patients will give 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 a reason.
- ii) Approval will be sought from ethics committees.
- iii) Patient confidentiality will be respected according to national regulations. Data will be coded before computer entry to maintain confidentiality.
- iv) Study follow-up and blood tests will coincide with standard, disease follow-up appointments and should avoid extra hospital attendances and blood tests as much as possible.

## **6.2 Ethics Approval**

- a Each national coordinator will apply for their own national ethics approval as well as regulatory approval.
- b International sponsorship will be provided by Cambridge University Hospitals NHS Foundation Trust, provided signature of 'site agreement to the principle co-investigators' is obtained from all participating centres. This will take the format of a two page contract. The principle co-investigators may perform site inspections if necessary.

## **Appendix 1: The diseases - diagnostic criteria, disease classification, disease activity, remission and relapse**

### **Diagnostic Criteria**

The EUVAS group requires the following criteria for a diagnosis of AASV: history of a chronic inflammatory disease lasting at least 4 weeks with the exclusion of other causes such as infection or malignancy supported by characteristic histology on biopsy and/or a positive ELISA for either PR3 or MPO antibodies and a cANCA or pANCA on immunofluorescence.

### **Disease classification**

#### **(1) 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. A c-ANCA pattern by IIF, with specificity for PR3 (PR3-ANCA) by ELISA, is found in over 90% of untreated patients with generalised WG; some studies have found a minority of cases to have ANCA with specificity for MPO (MPO-ANCA) instead of PR3-ANCA. In WG with disease limited to the respiratory tract, ANCA positivity is less frequent.

For the purposes of this study, after a diagnosis of AASV a patient can be classified as having WG if two of the following four criteria are present (ACR classification criteria) 1) nasal or oral inflammation; 2) nodules, fixed infiltrates, or cavities on a chest radiograph; 3) microscopic hematuria or more than 5 erythrocytes per high-power field; and 4) granulomatous inflammation on biopsy.

A characteristic or compatible histology for non-renal biopsies is defined by an inflammatory infiltrate dominated by neutrophils with at least one of the following findings:

1. Necrotising vasculitis affecting small to medium-sized vessels
2. Epithelioid cell granulomas and
3. Giant cells,

After exclusion of other causes.

#### **(2) Microscopic polyangiitis**

MPA is characterised by a vasculitis predominantly affecting small vessels. Renal involvement is usual and reflected by a necrotising glomerulonephritis. Granulomata are absent. Arteritis of medium-sized arteries may also occur. MPA is associated with MPO-ANCA or PR3-ANCA; a minority of MPA is ANCA negative or has other ANCA recognizing other antigens.

For the purposes of this study, patients may be entered in the category of MPA if they have a chronic inflammatory process with non-granulomatous necrotizing vasculitis of small vessels (i.e. capillaries, venules or arterioles) that does not involve the upper respiratory tract.

### **Disease activity**

The EUVAS group subdivides AASV according to disease activity into; (1) Limited disease-Wegener's granulomatosis restricted to the upper respiratory tract, (2) early systemic disease with constitutional symptoms but no renal involvement, (3) generalized disease with renal involvement creatinine <500µ/l, (4) severe disease with creatinine >500µ/l and (5) refractory disease. Patients with early systemic and generalized disease may be entered into this trial.

### **Remission Definition**

Remission is defined as an absence of disease activity attributable to active vasculitis (BVAS 2003 = 0) for two evaluations at least a month apart with adherence to the prednisolone regimen. Dipstick haematuria must be < 2+ before renal remission is said to have occurred. Patients in remission will continue low dose immunosuppression and oral prednisolone as remission maintenance therapy.

**Sufficient disease activity for trial entry requires at least one major or 3 minor BVAS items as listed below.**

**Relapse**

Is the reoccurrence or new onset of disease attributable to active vasculitis.

**Major Relapse** is the reoccurrence or new onset of potentially organ- or life threatening disease (one or more major BVAS items).

**Minor Relapse** is the reoccurrence or new onset of disease which is neither potentially organ-threatening nor life threatening (requires the recurrence or new appearance of at least 3 minor BVAS items).

**1 Major BVAS items :**

Major entry criteria or major relapse require 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 BVAS items:**

Minor entry criteria or minor relapse require 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 Disease activity 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 coordinator.

**Appendix 2: Study Therapies****Mycophenolate mofetil (see page 6)**

MMF is a prodrug that is hydrolysed to form mycophenolic acid. Mycophenolic acid reversibly inhibits inosin-monophosphate-dehydrogenase (IMDH), a key enzyme of de novo purine synthesis. Lymphocytes are key effector cells in AASV, whose proliferation and function relies almost exclusively on de novo purine synthesis whereas most other cells use the salvage pathway.

Treatment with MMF is attractive due to its low toxicity profile. In a systematic review of transplant patients, leucopenia and CMV were significantly more common only at the very highest MMF dose (3g), compared with azathioprine. The frequency of malignancy in a three year transplant study where MMF was combined with other immunosuppressive drugs showed no difference compared to azathioprine<sup>25</sup> GI adverse effects maybe related to accumulation of the metabolite, mycophenolic acid glucuronide, which accumulates in renal failure. Dose reduction in those with a creatinine clearance of less than 25 ml/min may be required.

**Cyclophosphamide**

CYC 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<sup>26</sup>. 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. Other adverse effects include nausea and vomiting, myelosuppression with neutropenia, infections due to immunosuppression, 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 anti-diuretic hormone secretion.

**Prednisolone, prednisone**

Prednisolone is a synthetic derivative of cortisone with widespread effects on metabolism and organ function. Desirable effects in systemic vasculitis relate to the suppression of acute and chronic inflammatory processes and immune cell function. Either prednisolone or prednisone can be used in the trial at identical dosage.

The major unwanted effects of prednisolone or prednisone 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 its use is prolonged, additional effects, including osteoporosis and avascular necrosis, subcapsular cataracts, skin fragility, myopathy, Cushingoid facies, hirsutism, alopecia, fat re-distribution, striae and growth retardation in children may occur. Of note in systemic vasculitis, has been the correlation of the cumulative steroid dosage with the total incidence of adverse effects, particularly with infections<sup>27</sup>.

**Azathioprine**

After hepatic conversion to 6-mercaptopurine, the cytotoxic effects of AZA are mediated by the impairment of purine synthesis, incorporation of purines into DNA, and impairment of the endonuclease repair activity of DNA polymerase. 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. AZA reduces lymphocyte function, 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. Gene polymorphisms of the thiopurine methyl transferase (TPMT) enzyme may influence toxicity.

**Appendix 3. Study regimes****1) Pulsed Cyclophosphamide**

<b>Pulsed CYC dose reductions for renal function and age</b>		
<b>Age (years)</b>	<b>Estimated GFR using Cockcroft Gault formula or Haycock-Schwartz formula (&lt;16 years)</b>	
	<b>&gt;30</b>	<b>&lt;30</b>
< 60	15 mg/kg/pulse	12.5 mg/kg/pulse
> 60 and < 70	12.5 “ “ “	10 “ “ “
> 70	10 “ “ “	7.5 “ “ “

**Cockcroft Gault Formula**

Men: Creatinine clearance =  $\frac{(140 - \text{age}) \times \text{weight in kg}}{(72 \times \text{serum creatinine})}$

Women: Creatinine clearance =  $\frac{(140 - \text{age}) \times \text{weight in kg} \times 0.85}{(72 \times \text{serum creatinine})}$

**Haycock-Schwartz Formula**

Males and females:  $\text{pGFR} = (\text{k} \times \text{ht}) / \text{P}_{\text{Cr}}$

Where:

pGFR = predicted glomerular filtration rate (in ml/min/1.73m<sup>2</sup>);

k = an empirically derived value relating height to muscle mass;

ht = height (in cm);

P<sub>Cr</sub> = plasma concentration of creatinine (in µmol/l or mg/dl).

Most centres would use a k value of 40 (for creatinine in µmol/l) or 0.45 (for creatinine in mg/dl) for all ages.

CYC is given as intravenous pulses at weeks 0, 2, 4 and then every 3 weeks until the remission is reached at 3 - 6 months from start of therapy (max 10 doses min 6).

- CYC may be stopped from 3 months onward provided patient in remission (BVAS 0 for 2 consecutive study assessments). After completion of CYC, AZA to be commenced.
- Reductions for renal function and age according to table above.
- Maximum CYC pulse is 1.2g.
- Upon completion of CYC course AZA to be commenced.
- Dissolve CYC in water for injection, then dilute in saline 0.9% 500 ml and administer as IV infusion over one hour.
- Mesna is optional and will be administered orally in the same dose in mg as CYC in mg either from IV vials or in the form of tablets on days when CYC is administered. (If it has to be administered IV reduce mesna dose to 60% of the CYC dose).
- Prevention of emesis: the choice of antiemetic drugs to cover the CYC pulses should follow local practice. Ondansetron is suitable for this indication.

- h) Check FBC on day of pulse or previous day. If WBC prior to pulse  $< 4 \times 10^9/L$ , then postpone pulse until WBC  $> 4 \times 10^9/L$ , while checking WBC at least weekly. Reduce dose of pulse by 25%. With any further episodes of leucopenia, make equivalent dose reduction.
- i) Check FBC between days 10 and 14 after a pulse. If the leucocyte nadir (i.e. the lowest leucocyte count between two CYC pulses) is  $< 3 \times 10^9/L$ , even if the WBC just previous to the next pulse is  $> 4 \times 10^9/L$ , then reduce the dose of the next pulse by:
  - j) leucocyte nadir 1 -  $2 \times 10^9/L$  reduce CYC dose of last pulse by 40 % of previous dose.
  - k) leucocyte nadir 2 -  $3 \times 10^9/L$  reduce CYC dose of last pulse by 20 % of previous dose.
- l) If in remission by three months, or between three and six months, may switch to AZA 2mg/kg/day.

## 2) Oral Mycophenolate Mofetil

Week	MMF starting dose
0	1g/day (600 mg/m <sup>2</sup> /day for patients < 16 years)
1	2g/day (1200 mg/m <sup>2</sup> /day for patients < 16 years)

- a) MMF is given in divided doses two or three times a day as remission induction therapy for 3-6 months, followed by AZA maintenance regimen.
- b) MMF dose may be increased in adults at week 4 up to 3g/day (maximum) for persistent disease, provided MMF 2g/day is not associated with moderate or severe side effects.
- c) MMF may be stopped from 3 months onward provided patient in remission (BVAS 0 for 2 consecutive study assessments). After completion of MMF, AZA to be commenced.
- d) If the target dose is not tolerated, patients should receive the maximum tolerated dose.
- e) Age <16 years: 600mg/m<sup>2</sup>/day for one week then 1200mg/m<sup>2</sup>/day in divided doses.
- f) Total WBC testing monitoring for patients taking MMF
  - i) Weekly for 1<sup>st</sup> month
  - ii) Alternate weeks for 2<sup>nd</sup> and 3<sup>rd</sup> month
  - iii) Once a month for 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> months.
- g) Stop if WBC  $< 4 \times 10^9/l$ .  
Restart with MMF dose reduced by at least 500 mg (or approximately 25% dose reduction for patients <16 years of age) when WBC  $> 4 \times 10^9/l$ .  
Monitor weekly for one month.
- h) If severe (WBC  $< 1 \times 10^9/l$  or prolonged (WBC  $< 4 \times 10^9/l$  for  $> 2$  weeks), MMF should be withdrawn.

## 3) Corticosteroids (same for both limbs)

- a) Intravenous methylprednisolone may be given at entry (maximum 3g total); however intention to use methylprednisolone must be stated pre-randomisation.
- b) Oral prednisolone dosed according to regimen (see below). Maximum dose of oral prednisolone in first week 80mg.
- c) Oral prednisone may substitute for prednisolone at the same dose.
- d) Round dose down to nearest 5mg above 20mg and nearest 2.5mg below 20mg
- e) Single daily dose (may alternate daily dosage by up to 5 mg)
- f) Avoid enteric coated or soluble forms.
- g) A flexibility in dose of +/-12.5% from the protocol in the first 12 weeks and 25% thereafter
- h) Minimum dose in first three months 10mg/day

Time from entry (weeks)	Prednisolone (mg/kg/day)
0	1
1	0.75
2	0.5
4	0.4
6	0.3
8	0.28
10	0.25
	Prednisolone dose mg/day
At end 3 month	12.5
At end 5 month	10
At end 6 months	7.5mg
At 9 months and continue to study end	5mg

## 4) Azathioprine

- a) To replace MMF/CYC as remission maintenance therapy.
- b) Dose is 2mg/kg/day, maximum 200mg/day.
- c) Round dose down to nearest 25mg.
- d) Age > 60 years, reduce dose by 25%, age > 75 years reduce dose by 50%.
- e) Check FBC and ALT or AST (for hepatotoxicity):
  - Every two weeks for one month.
  - Every two months for the first year.
  - Then three monthly.
- f) Stop if WBC < 4 x 10<sup>9</sup>/l.  
Restart when WCC > 4x10<sup>9</sup>/l with AZA dose reduced by at least 25mg  
Monitor weekly for one month.
- g) For falling WBC (< 6 x 10<sup>9</sup>/l and fall of > 2 x 10<sup>9</sup>/l over previous count), re-check in one week and reduce dose of AZA by 25mg.
- h) TPMT activity or polymorphism assessment to be used according to local practice.

**Recommended non-mandatory treatment for relapse:****Major relapse**

1. Stop AZA
2. Treat with, cyclophosphamide or MMF as during induction therapy and with Methylprednisolone and/or plasma exchange according to local guidelines.
3. Increase prednisolone to 1mg/kg/day; reduce to 20mg/day by four weeks
4. Return again to AZA and baseline dose of prednisolone when remission is achieved defined by BVAS 0 for 2 consecutive visits 1 month apart.

**Minor relapse**

1. Continue AZA at 2mg/kg/day to maximum 200mg/day
2. Increase prednisolone to 0.5mg/kg/day then reduce to baseline steroid dose over one month



Relapsing patients will remain in the study and all changes in drugs and doses are to be recorded in the record book.

**Appendix 4: Centralised Sample Collection and Analysis**

The following samples will be taken for centralized testing:

**1) 2.5 mls of blood in PAX gene RNA tubes at 0, 3, 6, 12 months.**

RNA extraction and expression profiling of peripheral blood mononuclear cells (PBMCs) will be performed. 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.

**2) 10mls of blood at 0, 3, 6, 12 months for serum extraction:**

Immunological reactants, including ANCA.

**3) 5mls of blood at 0, 3, 6, 12 months for plasma extraction:**

Pharmacokinetic studies will be performed including activity of enzymes known to influence study drug metabolism and trough mycophenolic acid levels in patients receiving MMF.

**4) 2x 5 ml blood samples at 3 months for serum/plasma extraction (MMF limb patients only):**

At 3 months two other 5ml blood samples will be taken, one 1 hour post MMF dose and one 2 hours post MMF. Samples will be tested for MPA levels, in addition to a 12hour pre dose level (see above), in order to calculate a 3 point area under the curve estimate.

**5) 8.5mls of blood in PAX gene DNA tubes at entry for DNA extraction.**

Gene polymorphisms of the enzymes; thiopurine methyl transferase (TPMT), uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9), cytochrome p450 enzymes, known to influence the metabolism of azathioprine, MMF and cyclophosphamide respectively, will be analysed. Gene polymorphisms of FcGRII and FcGRIII receptors known to influence relapse will also be measured.

20mls of blood will be taken in sodium EDTA tubes and stored at 4°C. Blood will be shipped to UK within 24hours for DNA extraction.

All samples for centralized analysis will be frozen either as whole blood in PAX gene tubes provided or as serum (see TMF for further details of collection and storage). The samples will be labeled in coded fashion with patient trial number, date and assessment number before transfer to Addenbrooke's Hospital, UK. Centralized tests will be organized by the trial management committee at Addenbrooke's Hospital, Cambridge, UK. The majority of the analyses will be performed within Addenbrooke's Hospital and the University of Cambridge, however some of the samples will be sent to other laboratories, subcontracted by the trial management committee to perform specific sample analysis. The trial management committee will take responsibility for these samples.

In Addenbrooke's Hospital the PAX gene 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.

Results will not have implications for individual patients. Patients who provide these additional 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 MYCYC 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.

The total volume of blood drawn during the whole trial as a result of additional centralized tests is 88.5mls/patient.

**Appendix 5: Provisional costings**

Medical Input	£35 000/year for 4 years	£140 000
Secretarial support	£5 000/ year for 4 years	£20 000
Data Management and statistics		£40 000
CRFS		£1 500
Capitation	£200 per patient	£28 000
DMC (safety)		£5 000
Computer + software		£1 500
Office consumables	£1 000 per year	£4 000
Regulatory approvals	£3 000/country	£30 000
Emergency funds		£5 000
Total		£275 000

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