ACQUIVAS

Trial Title: **ACQU**ired immunodeficiency in antineutrophil cytoplasmic antibody (ANCA) associated **VAS**culitis

EudraCT Number: 2017-003563-36

Investigational Products: Pneumococcal polysaccharide conjugate vaccine
Pneumococcal polysaccharide vaccine

Statistical Analysis Plan for Final Study Report on Primary Outcome Measure and Related and Clinical Data

Version 1.0

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1 Introduction

This Statistical Analysis Plan (SAP) will be used to produce the final study report. This SAP is based on ACQUIVAS protocol version 5.0 dated 12th June 2020.

2 Trial Summary

2.1 Introduction

Antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) has been transformed from a largely fatal condition, to a chronic relapsing disorder following the introduction of cyclophosphamide and glucocorticoids 40 years ago (Flossmann, 2011). Despite refinement in the dosing regimens of these agents, and the introduction of novel agents such as rituximab, a B cell depleting therapy, the natural history of AAV is characterised by frequent relapses, necessitating repeated periods of intensified immunosuppression, and its consequent toxicity (Smith, 2012).

All-cause mortality and rates of infection remain increased in patients with AAV compared to the general population. The cumulative adverse effects of disease activity and treatments now dominate patient outcomes. These include an increased propensity for infection and acquired humoral immune deficiency as long term consequences of immunosuppression.

Vaccination against pneumococcus is recommended as a strategy to reduce infection risk in individuals with AAV. However, the effect of rituximab and B cell depletion on vaccine responses in patients with AAV is yet to be explored, and the available data from other autoimmune diseases in very limited. Poor response to vaccination has been identified as an important predictor of all-cause death in patients with AAV (Morgan, 2016). Therefore, optimisation of current vaccine strategies in autoimmune disease such as AAV is vital.

This trial will address the following hypothesis: Rituximab therapy leads to an acquired immune deficiency, as demonstrated by impaired vaccine responses, in AAV patients.

2.2 Trial Objectives

2.2.1 Primary Objective

To evaluate the humoral response to pneumococcal vaccination following rituximab therapy in AAV. The response of patients receiving rituximab will be compared to disease and healthy controls.

2.2.2 Secondary Objectives

1. To assess and compare the humoral response for each serotype in the pneumococcal vaccine following rituximab therapy in AAV

2. To assess the cellular response to pneumococcal vaccine following rituximab therapy in AAV
    a. To characterise the T cell independent (that is, response at Month 7) and T cell dependent (that is, Month 1) vaccine responses following rituximab therapy
    b. To assess whether a conjugated vaccine can overcome the postulated deficit in T independent vaccine responses following rituximab therapy (that is, Month 7))
    c. To correlate humoral responses with total CD19+ B cell counts, and CD19+ B cell subsets (memory (CD27+) and naïve (CD27-) B cells) and baseline immunoglobulin levels

3. To evaluate the incidence, type and severity of infections
4. To evaluate the incidence and severity of hypogammaglobulinaemia – IgG, IgM and IgA **(only IgG is included in the final clinical study report)**
5. To evaluate the safety of vaccination following rituximab treatment

2.2.3 Exploratory Objectives (not included in the final clinical study report)

1. To assess the functionality of antibodies produced in response to pneumococcal vaccination
2. To assess the impact of Cytomegalovirus (CMV) infection and CMV driven immunosenescence on T cell dependent and T cell independent vaccine responses in AAV patients
3. To assess the impact of CMV infection on B cell reconstitution and B cell phenotype following rituximab therapy
4. To assess whether the acute T cell response to pneumococcal vaccines predicts vaccine response itself and disease course.
5. To assess genetic factors which may influence B cell function and the development of hypogammaglobulinaemia and may therefore in turn affect vaccine responses.

2.3 Trial Outcome Measures

Note: the contents below in black are copied from the protocol. Further details are added in blue.

2.3.1 Primary Outcome Measure

The primary outcome will compare the proportions of rituximab treated patients to disease controls who respond to the Pneumococcal Polysaccharide Conjugate vaccine measured at 28 (+/- 7) days after administration of vaccine. Response is defined as at least a twofold increase in immunoglobulins in at least at least 50% of the 12 pneumococcal serotypes tested. These are the lab results on the serum blood sample collected at **Month 0 and 1** and will be recorded in an excel csv file.

2.3.2 Secondary Outcome Measures

In view of the lack of validation of any primary endpoint defining response to pneumococcal vaccination, the secondary outcome measures in this trial are extremely important.

1. Immunoglobulin (IgG) titres for each of the 12 *individual* serotype in the pneumococcal vaccine will be measured at month 0, 1, 6 and 7 in **all** participants.
   These are the lab results on the serum blood sample collected at **Month 0, 1, 6 & 7,** and will be recorded in an excel csv file. The response at Month 7 with the same definition as the primary outcome measure will also be included (for secondary objective 2)

2. PBMC extraction and immunophenotyping of circulating B cell sub-populations (CD27+ memory cells CD19+ B cells and CD27-/ CD19+ naïve B cells) and T cell sub-populations.
   These are the lab results on the PBMC blood sample collected at **Month 0, 1, 6 and 7,** and will be recorded in an excel csv file

3. Number of serious adverse events, and serious adverse events specifically related to the vaccines administered (serious adverse reactions)
   Data will be collected using the standard CCTU SAE form and recorded in the MACRO database.

4. Incidence, type, severity and treatment of infections experienced by participants after vaccinations
   Data will be collected using the INFECTION CRF and recorded in the MARCO database.

5. Changes in immunoglobulin levels will be measured over time
   Data will be collected using the Clinical LABORATORY TESTS CRF and recorded in the MARCO database on IgG.

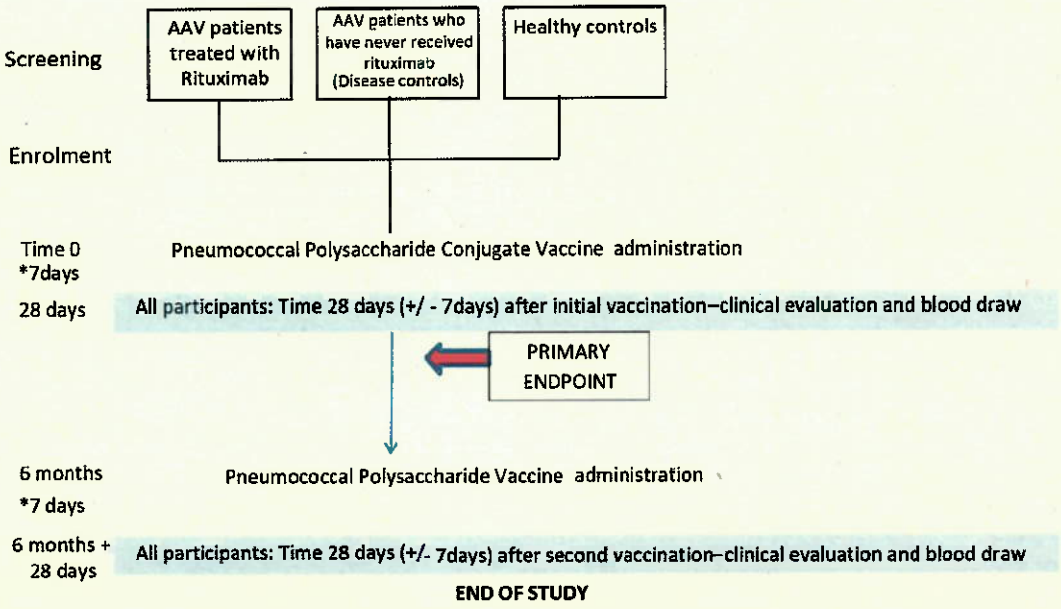
2.3.3 Exploratory Endpoints (not included in the final clinical study report)

1. Opsonophagocytosis (OPA) studies to assess functionality of antibody produced following pneumococcal vaccination (this will be performed in a proportion of patients at month 1 and month 7)
   a. OPA response will be defined as a fourfold increase in antibody titre and absolute value of >1:8 in at least 50% of antibodies tested
2. Evaluation of IFN-gamma producing CD4+ cells and T cell ELISPOT to examine functional response and the role of T cell dependent pathways in pneumococcal vaccine response
3. Relationship between CMV serostatus, anti-CMV IgG titre and markers of CMV driven immunosenescence, and vaccine response to a T-cell dependent and T-cell independent vaccine.
4. Relationship between CMV serostatus, anti-CMV IgG titre and markers of CMV driven immunosenescence, and B-cell reconstitution and phenotype following rituximab therapy.
5. Surrogate markers of T cell exhaustion will be correlated with pneumococcal vaccine response and disease course.
6. Genetic polymorphisms will be correlated with pneumococcal vaccine response

2.4 General Study Design and Plan

2.4.1 Trial Design

This is a phase IIb, open label trial to evaluate the responses of patients with ANCA associated vasculitis (AAV) to pneumococcal vaccination. It has been designed primarily to assess the immunogenicity of pneumococcal vaccines in patients with AAV treated with rituximab compared to disease controls, but also to provide mechanistic information on vaccine response by comparing AAV patients and healthy controls.



*7 days: optional study visit at 7 (+/- 2 days) for a blood draw only

Subjects who have received the Pneumococcal Polysaccharide Vaccine within 5 years of screening will not receive the Pneumococcal Polysaccharide Vaccine at month 6, and participation in the study will cease at Month 1 (primary endpoint)

2.4.2 Trial Treatment

Eligible patients will receive 1) the pneumococcal polysaccharide conjugate vaccine, a single dose of 0.5ml suspension in the pre-filled syringe, at Month 0 following the baseline assessment and enrolment; 2) the pneumococcal polysaccharide vaccine, a single dose of 0.5ml suspension, at Month 6 from the first vaccination.

2.4.3 Schedule of Assessments

Schedule of assessments for AAV patients

Trial Date M=Month	*Screening	*M0 (Baseline)	M1∞ (28 +/- 7 days after M0)	M6 (+/- 2 weeks)	M7 (28 +/- 7 days after M6)
Data Forms					
Eligibility Review	X				
Informed Consent	X				
Demographics	X	X			
Medical history	X	X			
Prior vaccination history		X			
Clinical examination	X	X			
Concomitant meds	X	X	X	X	X
BVAS/WG		X	X	X	X
VDI		X		X	
Adverse event review/ reporting	X	X	X	X	X
Clinical Labs					
Haematology bloods	X	X		X	
Immunology bloods	X	X		X	
Chemistry (electrolytes, creatinine, LFTs, CRP) bloods	X	X		X	
Serum pregnancy test (WOCBP only)		X		X	
IMP administration					
Pneumococcal Polysaccharide Conjugate Vaccine		X			
Pneumococcal Polysaccharide Vaccine				X	
Research Specimens					
Serum (for pneumococcal antigen ELISA, anti CMV-IgG and OPA# at M1 and M7)		X	X	X	X
PBMC separation and freezing		X	X	X	X
DNA		X			

*screening and baseline assessments can be conducted on the same day. If not performed on the same day, the baseline visit must occur within 14 days of screening.
Activities and blood samples listed to occur both at screening and baseline, need only be conducted once, at either visit, unless deemed otherwise necessary by the PI. Do not submit duplicate CRF pages.

#test to be performed on a subset of patients.

∞ Subjects who received the Pneumococcal Polysaccharide vaccine within 5 years of screening will cease participation in the trial at Month 1.

AAV patients recruited in Cambridge only will be invited to attend for two optional trial visits, one week after Pneumococcal Polysaccharide Conjugate vaccination, and 1 week after Pneumococcal Polysaccharide vaccine administration. See protocol Section 16.3 for details of samples to be collected at these visits. Only a blood draw will be performed on these occasions. No other assessments require completion.

Schedule of assessments for healthy controls

Trial Date M=Month	***Screening**	***M0 (Baseline)**	**M1 (28 +/- 7 days after M0)**	**M6∞ (+/- 2 weeks)**	**M7 (28 +/- 7 days after M6)**
Data Forms					
Eligibility Review	X				
Informed Consent	X				
Demographics	X	X			
Medical history	X	X			
Prior vaccination history		X			
Clinical examination	X	X			
Concomitant meds	X	X	X	X	X
Adverse event review/ reporting	X	X	X	X	X
Clinical Labs					
Haematology bloods	X	X		X	
Immunology bloods	X	X		X	
Chemistry (electrolytes, creatinine, LFTs, CRP) bloods	X	X		X	
Serum pregnancy test (WOCBP only)		X		X	
IMP administration					
Pneumococcal Polysaccharide Conjugate Vaccine		X			
Pneumococcal Polysaccharide Vaccine				X	
Research Specimens					
Serum (for pneumococcal antigen ELISA)		X	X	X	X

*screening and baseline assessments can be conducted on the same day. If not performed on the same day, the baseline visit must occur within 14 days of screening.
Activities and blood samples listed to occur both at screening and baseline, need only be conducted once, at either visit, unless deemed otherwise necessary by the PI. Do not submit duplicate CRF pages.

∞Subjects who received the Pneumococcal Polysaccharide vaccine within 5 years of screening will cease participation in the trial at Month 1.

2.5 Inclusion-Exclusion Criteria and General Study Population

2.5.1 Inclusion Criteria

To be included in the trial all participants must:

- Have given written informed consent to participate
- Be aged 40 years and over

For patients in Group 1 only (rituximab treated):

- Have a diagnosis of AAV [granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA) or eosinophilic granulomatosis with polyangiitis (eGPA)]

- Have current or historical PR3/MPO ANCA positivity by ELISA or histological confirmation of AAV
- Have ever received ≥ 2g rituximab
- Have received their last dose of rituximab at least 4 months prior to enrolment
- Be in stable remission with a prednisolone dose of ≤ 5mg/day (complete withdrawal of prednisolone ie. Dose of 0mg daily is allowed)

For patients in Group 2 only (disease controls who have never received rituximab):

- Have a diagnosis of AAV (GPA, MPA or eGPA)
- Have current or historical PR3/MPO ANCA positivity by ELISA or histological confirmation of AAV
- Have received initial induction therapy with either cyclophosphamide (oral or IV), azathioprine, methotrexate or mycophenolate mofetil
- Be on stable immunosuppression for the 6 months preceding screening including prednisolone ≤ 5mg/day (complete withdrawal of prednisolone ie. Dose of 0mg daily is allowed) AND either azathioprine, methotrexate or mycophenolate mofetil (at stable or tapering dose)

10.2 Exclusion Criteria

The presence of any of the following will preclude participant inclusion:

- Age < 40 years
- History of severe allergic or anaphylactic reactions to pneumococcal vaccinations including excipients and diphtheria toxoid
- Pneumococcal vaccination within 6 months prior to the screening visit
- Protective response to prior pneumococcal vaccination (adequate titres (>0.3 mcg/ml) in at least 50% serotypes tested) if vaccine received withing five years prior to screening visit
- Females who are pregnant, plan to become pregnant, or breast feeding
- Medical, psychiatric, cognitive or other conditions that, in the investigator's opinion, compromise the patient's ability to understand the patient information, give informed consent, comply with the trial protocol, or to complete the trial.
- History of malignancy within the past five years prior to screening or any evidence of persistent malignancy, except fully excised basal cell or squamous cell carcinomas of the skin, or cervical carcinoma in situ which has been treated or excised in a curative procedure.
- Replacement immunoglobulin (IVIg) administered intravenously or subcutaneously in the 12 weeks prior to screening visit.

For patients in Groups 1 and 2 only (AAV patients):

- Presence of another multisystem autoimmune rheumatic disease
- The prior receipt of more than 36g of cumulative cyclophosphamide ever (either IV or oral)

For patients in group 1 only (rituximab group)

- The receipt of any immune suppressing agent (azathioprine, methotrexate or mycophenolate mofetil) after rituximab

For patients in Group 2 only (disease controls):

- A relapse of AAV within the 6 months prior to screening which has necessitated an increase in prednisolone or azathioprine, methotrexate or MMF dose.
- Previous rituximab therapy at any time

For healthy controls:

- Any history of any autoimmune condition

- Any history of use of immune suppressing medication, including > 4 weeks of oral glucocorticoids, within the 5 years prior to screening.

3 Statistical Considerations

3.1 Sample Size

There is no published data on pneumococcal vaccine responses in patients with AAV treated with rituximab. Therefore, in order to estimate an effect size of rituximab administration on vaccine response, the literature on other autoimmune conditions has been reviewed and is summarised in the table below. A basic meta-analysis indicates the vaccine response following rituximab treatment is ~13%, and in disease controls ~ 38%.

By recruiting 52 patients to each of the rituximab treated and disease control groups, the trial will have an 80% power to detect a 25% difference (a conservative estimate of difference) in vaccine response rates at a 0.05 significance level.

Since data on response rates to Pneumococcal Polysaccharide Conjugate vaccination in healthy controls is limited to the very young and the very old, which is not directly applicable to the AAV patient population, 20 healthy controls will be recruited. This will generate useful data for this understudied population in the context of an established trial setting, to ensure that there is no significant difference in response between patients and controls. Only basic measures of response to vaccination will be studied in healthy controls, and if major differences are identified between disease and healthy controls, this will form the basis of a larger future trial.

Age is known to influence vaccines responses, therefore, we will attempt to age match the 20 healthy controls to the patients recruited. As 104 patients will be recruited, and only 20 healthy controls, direct age matching will not be possible. Based on historic data, we would expect an approximately even distribution of patients in each of the 4 age categories (40-49 years, 50-59 years, 60-69 years and 70 years and over). Up to 3 healthy controls will be recruited into each age category from the start of the trial, and when 50% of patients have been recruited, we will analyse the age distribution, and adjust the number of healthy controls in each group if there is an imbalance.

3.2 Analysis Populations

3.2.1 Full Analysis Population

- All subjects who were enrolled and eligible

3.2.2 Safety Population

- All enrolled subjects who were eligible and had received pneumococcal polysaccharide coagulate vaccine at Month 0

3.2.3 Efficacy Population

For outcome measures assessed at Month 0

- All enrolled subjects who 1) were eligible; 2) had received pneumococcal polysaccharide coagulate vaccine at Day 0 (Month 0); and 3) had an outcome measure available at Month 1.

For outcome measures assessed at Month 7

- All enrolled subjects who 1) were eligible; 2) had received pneumococcal polysaccharide coagulate vaccine at Day 0 (Month 0) and pneumococcal polysaccharide vaccine at Month 6 and 3) had outcome measures available at Month 7.

3.3 Statistical Analysis

Note: the contents below in black are copied from the protocol. Further details are added in blue.

3.3.1 Primary Analysis on the primary outcome measure

Summary statistics will be produced for each group of the observed response rate at month 1 (primary outcome measure), with exact binomial confidence intervals. The treatment arms: group 1(rituximab treated) vs group 2 (disease control with no rituximab) will be compared using Fisher's Exact test to provide a p-value, and the absolute differences in response rates will be estimated with confidence intervals using logistic regression on the absolute difference scale (with and without adjusting for age).

Note: recruitment to health control was stopped due to COVID-19 pandemic; the comparisons between group 1(rituximab treated) vs group 3 (health control) will be summarized by response rate and its confidence interval at Month 1.

All enrolled subjects who had pneumococcal polysaccharide coagulate vaccine will be included in the analysis.

3.4 Covariates and Subgroups

Age at registration (continuous)

3.5 Missing Data

The missing data on primary outcome measure would be rare. No imputation will be made for missing primary outcome measure.

3.6 Interim Analyses and Trial Steering Committee

The study was reviewed by the independent Trial Steering Committee (TSC) for progress of the trial and safety of participants.

Impact from the COVID-19 pandemic: one confidential interim analysis result was reviewed by the independent TSC only. The Haybittle-Peto stopping rule was a guidance for the TSC to make recommendation whether the primary objective was achieved earlier. **Conclusion**: the TSC suggested that the study was to be continued as it was.

3.7 Multi-centre Studies

There is no adjustment planned for this perspective cohort study with 2 sites.

3.8 Multiple Testing

The primary efficacy analyse on the primary outcome measure will be declared statistically significant if $p \leq 0.05$ for the comparisons between group 1 and group 2.

3.9 Estimand Strategy

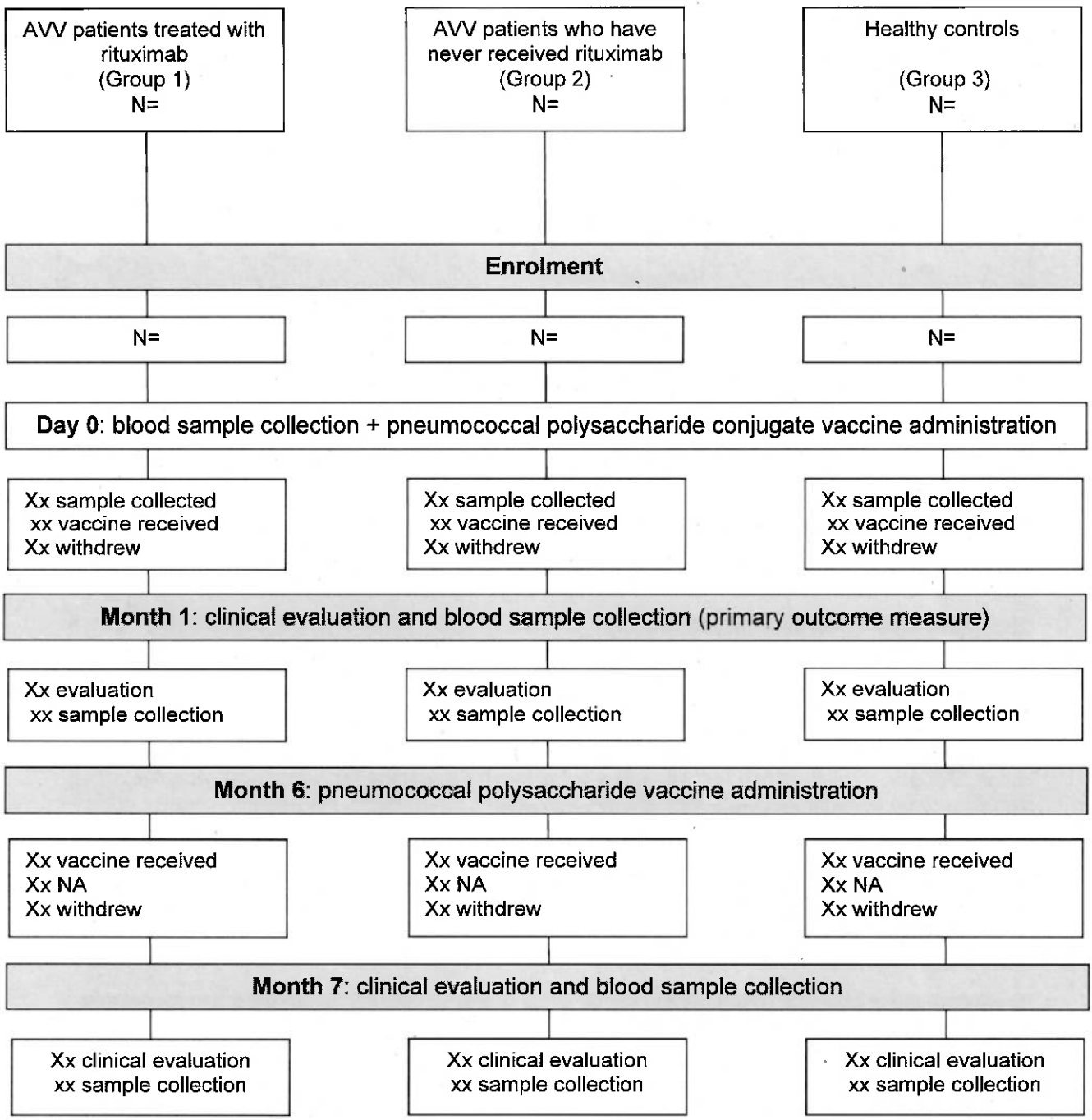
Objective	Estimand Category	Estimand attribute			
		Endpoint	Intercurrent Event Strategy	Population	Summary measure
To evaluate the humoral response to pneumococcal vaccination following rituximab therapy in AAV. The response of patients received rituximab will be compared to disease	Primary Response of patients received rituximab will be compared to disease controls at Month 1.	Response to the Pneumococcal Polysaccharide Conjugate vaccine measured at 28 days (±7) days or collected in the Month 1 visit after administration of vaccine. Response is defined as at least a twofold increase in immunoglobulins in the at least 50% of 12 pneumococcal serotypes tested.	No pneumococcal polysaccharide conjugate vaccine at Month 0 – treatment policy; data will be reported as captured, that is, as observed	Efficacy population for AVV patients treated with rituximab (group 1) and AVV patients treated without rituximab (group 2)	Rate (95%CI) Difference in rate (95% CI) between two groups at Month 1
	Supplementary 1 Response of patients received rituximab will be compared to disease controls at Month 1 and Month 7. This is also under the secondary objective 2.	Same as above at 28 days (±7) days after Month 0 vaccine or collected at Month 1 visit, at Month 6 visit, and at 28 days (±7) days after Month 6 vaccine or collected at Month 7 visit	No protocol treatment at Month 0 or Month 6 – treatment policy; data will be reported as captured, that is, as observed	Efficacy population for AVV patients treated with rituximab (group 1) and AVV patients treated without rituximab (group 2)	Rate (95%CI) Difference in rate (95% CI) between two groups at Month 1 and Month 7 Change of response from Month 1 to Month 7
	Secondary		No pneumococcal polysaccharide conjugate vaccine at Month 0 or	Efficacy population for AVV patients treated with rituximab	For each of the 12 individual serotype at Month 1 and Month 7

	Immunoglobulin (IgG) titres for each of the 12 *individual* serotype in the pneumococcal vaccine will be measured at month 0, 1, 6 and 7 in all participants.		Month 6 – treatment policy; data will be reported as captured, that is, as observed	(group 1) and AVV patients treated without rituximab (group 2)	Rate (95%CI) Difference between two groups Change of response from Month 1 to Month 7 <u>Compare the 12 individual serotype</u> Order of response rate for each group The serotype with the largest difference in response between two groups

4 Summary of Study Data

Standard descriptive summary statistics will be used in the data summary. Summary statistics will be reported to one decimal place greater than the original data. Data will be presented separately for each group and overall.

4.1 Participate disposition



4.2 Derived variables

Variables	Definition
Response is defined as at least a twofold increase in immunoglobulins in at least 50% of the 12 pneumococcal serotypes tested.	Primary outcome measure: response at month 1 Response = yes ≥6 serotypes with $\frac{\text{month 1 value}}{\text{baseline value}} \geq 2$ Response = No >6 serotypes with $\frac{\text{month 1 value}}{\text{baseline value}} < 2$ Response = missing none of them above
	Response at month 7 Response = yes ≥6 serotypes with $\frac{\text{month7 value}}{\text{month6 value}} \geq 2$ Response = No >6 serotypes with $\frac{\text{month7 value}}{\text{month6 value}} < 2$ Response = missing none of them above

4.3 Protocol Deviation

List of major protocol deviations.

This will be provided by the trial coordinator.

4.4 Demographic and Baseline Assessments

Key variables collected the Case Report Forms (CRFs) at screening/baseline will be summarised (n, mean, Std Dev, median, range for continuous variables; n percentage for categorical variables). The variables to be summarised are from the following forms.

- Enrolment form
- Clinical Labs at screening
- Vaccination history form
- BVAS/WG form at month 0
- VDI form at month 0

The following variables by cohort groups will be summarised for all patients enrolled and for patients with primary outcome measure assessed

Demographic	
Age at enrolment	Mean, median, std, range
Sex	Male Female

Race	Caucasian Black Asia Hispanic
Cohort information	
Months between last rituximab dose and enrolment (group 1)	Mean, median, std, range
Current immunosuppression treatment (group 2) Azathioprine Mycophenolate mofetil Methotrexate	 Yes No Dose
Prednisolone received (group 1 and 2)	Yes No Dose
Comorbidity	
Comorbidity check list	Yes No Unknown
Medical history (group 1 and 2)	
Years between 1st diagnosis of ANCA (MMM/YY) and enrolment Note: Day 1 of the month of 1st diagnosis of ANCA used	Mean, median, std, range
Vasculitis manifestation checklist	Yes No Unknown
Medication history (group 1 and 2)	
Medication check list	Yes No Unknown
Disease assessment (group 1 and 2)	
BVAS/WG: Disease status	Severe flare/new disease Limited flare/new disease Persistent disease Remission
Vasculitis Damage Index (VDI)	0-11
Vaccination history	
Any form of pneumococcal vaccination Polysaccharide conjugate vaccine Polysaccharide vaccine	No Yes No Yes No. of times No Yes No. of times
Years between the previous vaccination (MMM/YY) to enrolment Note: Day 1 of the month of 1st diagnosis of ANCA used	Mean, median, std, range

4.5 Treatment Received and Clinical Assessments

4.5.1 Vaccination

Month 0

- Any vaccines in the past 4 weeks prior to the vaccination administered at Month 0 (for all patients enrolled)

	Group 1	Group 2	Group3
Yes			
No			
Total			

List of details of vaccines received in the past 4 weeks

- Vaccination administered at Month 0 (for all patients enrolled)

	Group 1	Group 2	Group3
Yes			
No			
Reason for not given Xx xx			
Total			

Month 6

- Any vaccines in the past 4 weeks prior to the vaccination administered at Month 6

	Group 1	Group 2	Group3
Yes			
No			
NA			
Total			

List of details of vaccines received in the past 4 weeks

- Vaccination administered at Month 6

	Group 1	Group 2	Group3
Yes			
No			
Reason for not given Xx xx			
Total			

- Days between enrolment and pneumococcal vaccination

  N median mean std min max

4.5.2 Relapses and deaths

List of relapses or deaths

4.6 Safety (for safety population)

4.6.1 Infection

	Group 1	Group 2	Group 3
Total no. of Infections			
Site of infection xx xx …			
Worst grade of infections per site None Mild Moderate Severe			
Incidence of infection None Mild Moderate Severe			

- List of Infection events

			Date								
Trial id (group)	Site of infection	Organism	Latest vaccine administered	Latest IgG (value)	onset	resolution	outcome	Max intensity	Trt for infection	Oral prednisolone dose at the time of infection	SAE

4.6.2 AE related to pneumococcal vaccination

Reported in Month 1, 6 and 7.

4.6.3 SAE

	Group 1	Group 2	Group 3
Total no. of SAEs			
Total no. of SARs			
No. of patients experienced SAEs			
No. of patients experienced SARs			

- List of SAEs

4.7 Efficacy

4.7.1 Sample Collection and analysis

Participants with sample collected

	Group 1	Group 2	Group 3
Month 0			
Month 1			
Month 6			
Month 7			

Participants with sample analysed and response assessable

	Group 1	Group 2	Group 3
Month 0			
Month 1			
Month 7			

Eligible participants with sample analysed and response assessable

	Group 1	Group 2	Group 3
Month 0			
Month 1			
Month 7			

4.7.2 Primary outcome measure: primary analysis

Primary outcome measure: response at month 1

Method: Fisher's exact test will be performed for the comparisons between the two groups as primary analysis, together with the logistic regression adjusting for age as continuous variable; the difference in response rate and its confidence interval will be estimated using the logistic regression model without adjusting and adjusting for age.

Response at month 1	Group 1: AAV patients who have received rituximab		Group 2: AAV patients who have never received rituximab		Group 3: Healthy control	
	N	%	N	%	N	%
Yes						
No						
Total						
Comparing Group 1 vs Group 2 • P value for Fisher's exact test • P value for logistic regression adjusting for age • Response rate difference (Group2-Group1) (95%CI) without adjustment and with adjustment for age and sex						

4.7.3 Response at month 1 and month 7

Population: eligible participants with primary outcome measure available
Method: Repeated model without adjusting and adjusting for age

Response		month					
		1				7	
		N	%			N	%
Group 1: AAV patients who have received rituximab	Yes						
	No						
	Total						
Group 2: AAV patients who have never received rituximab	Yes						
	No						
	Total						
Group 3: Healthy control	Yes						
	No						
	Total						
Total	Yes						
	No						
	Total						

For all participants with both month 1 and month 7 responses available

Cochran-Mantel-Haenszel (CMH) test will be used combining all participants

- McNemar test will be used for rituximab treated group and disease control group separately

Response by cohort groups at 1 and 7 months

		Month 7		P value of McNemar's Test
Rituximab treated	**Month 1**	Responding	No responding	
	Responding			
	Not responding			
Disease control	**Month 1**	Responding	No responding	
	Responding			
	Not responding			
Healthy control	**Month 1**	Responding	No responding	
	Responding			
	Not responding			
All combined	**Month 1**	Responding	No responding	
	Responding			
	Not responding			

4.7.4 Secondary outcome measures: response in each serotype

The same analysis as section 4.7.2 and 4.7.3 for each serotype response.

4.7.5 Correlation of humoral responses with total CD19+ B cell counts, and CD19+ B cell subsets (memory (CD27+) and naïve (CD27-) B cells) and baseline immunoglobumin levels

With total CD19+8 B cell counts, and CD19+ B cell subsets (memory (CD27+) and naïve (CD27-) B cells) – on both CD19+ B cell counts and proportions

For each group, at month 1 and 7, the correlation between the number of serotype responses and CD19+ B cell, B cell subsets: scatter plot, correlation coefficient

Further, to explore the difference on groups (group 1 vs group 2), time (month 1 vs month 7), or CD19 subtype, interaction terms could be added in a regression model if appropriate depending on the distributions of CD19 and number of serotype responses. See an examples below.

Regression CD19 = number of serotype responses + group (group 1 or group 2) + assessment month (month 1 or 7) + number of serotype responses x group + number of serotype responses x assessment month + number of serotype responses x group x assessment month

Regression CD19 = number of serotype responses + type of subset + assessment month + number of serotype responses x type of subset (memory (CD27+) vs naïve (CD27-))

With baseline IgG level

For each group, at month 1 and month 7, the correlation between the number of serotype responses and IgG level at baseline: scatter plot, correlation coefficient

To explore the difference on groups, see an example below.

number of serotype responses = IgG at baseline + IgG at baseline x groups (group 1 vs group 2) + IgG at baseline * response assessment time (month 1 or month 7)

4.7.6 Change in immunoglobulin levels of IgG over time (Clinical Laboratory Tests Form)

	IgG	Group 1	Group 2	Group 3
Baseline	<3gl 3gl≤ IgG <5gl ≥5 Mean (std), median, min-max			
Month 6	IgG<3gl 3gl≤ IgG <5gl IgG≥5 Mean (std), median, min-max			
Change from Baseline at month 6	Mean (std), median, min-max			

Regression model on change from baseline at month 6 adjusting for baseline and age

4.7.7 Birmingham Vasculitis Activity Score

Disease status

	Current disease status	Group 1	Group 2 (Disease control)
Month 0	Severe flare/new disease Limited flare/new disease Persistent disease Remission Total		
Month 1	Severe flare/new disease Limited flare/new disease Persistent disease Remission Total		
Month 6	Severe flare/new disease Limited flare/new disease Persistent disease Remission Total		
Month 7	Severe flare/new disease Limited flare/new disease Persistent disease Remission Total		

4.7.8 Vasculitis Damage Index

		Group 1	Group 2 (Disease control)
Month 0	Mean (std), median, min-max		
Month 6	Mean (std), median, min-max		
Change from Baseline at Month 6	Frequency Mean(std), median, min-max		

5 Reporting Conventions

P values≥0.001 will be reported to 3 decimal places: p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

6 Technical Details

At the time of writing, it is planned to perform the final analyses using the SAS 9.4. To provide high quality code that is understandable, and allows reproduction of the analysis the following points will be followed

The population to be used in a table or figure will be explicitly set at the start of a block of code that computes the output, ideally by looking up the population from the table of tables

Any outputs will have
- The date and time included
- The name of the code file that produced the analysis
- The author
- A log capturing the version of the software and any external add-on code used.

At the start of any code file there will be a set of comments that give
- the author
- the date and time of writing
- references to inputs and outputs
- reference to any parent code file that runs the child code file

7 Summary of Changes to the Protocol

Version Number	History	Date
V1.0	Final protocol. Approved by MHRA on 2nd May 2018	16th April 2018
V2.0	Update in response to REC/HRA initial submission Approved by the REC and HRA on 6th August 2018	2nd July 2018
V3.0	Amendment to protocol 2.0 – change to primary outcome wording, update to secondary outcomes and removal of lymphocyte blood collection Approved by MHRA on 3rd September 2018 (informed of changes from V1.0 to V2.0 of the protocol and V2.0 and V3.0 of the protocol). Approved by the REC and HRA on 7th September 2018	7th August 2018

V4.0	Amendment to protocol 3.0 - Change the window period between last infusion of Rituximab and screening visit - change the window period between prior pneumococcal vaccine and screening visit - change in inclusion and exclusion criteria - duration of the trial treatment and the end of trial Approved by MHRA on 25th July 2019 (informed of changes from V3.0 and V4.0 of the protocol). Approved by the REC on 5th August 2019 and HRA on 9th August 2019	21st June 2019
V5.0	Amendment to protocol 4.0 Interim Analysis has been updated in the protocol based on recruitment prior to pause the recruitment due to COVID-19 lockdown Approved by MHRA and REC on 23rd June and 1st July 2020, HRA approval obtained on 10th August 2020	12th June 2020

8 List of Tables, Lists and Figures

These detailed specifications can have *minor* revisions during the production phase without needing to revise the SAP, this may include changing the table numbers if a reordering or deletion is appropriate, providing that the specifications in the main body of the SAP.

Table Title	Number	Population	Endpoint	Time Points or how to conglomerate	Covariates or Subgroups	Summary Statistics	Formal Analysis	Foot Notes
Disposition	1.0	NA	**Disposition**	Screening to Month 7	Participating group	count	NA	
Demographics	1.1	All eligible patients	**Age at consent**	Screening	Participating group	n, mean, SD, Median, min, max	NA	
			Age≤60 vs >60	Screening		x/n (p%)	NA	
			Sex Male Female	Screening		x/n (p%)	NA	
			Race Caucasian Black Asian Hispanic Other	Screening		x/n (p%)	NA	
Enrolment Form	1.2	All eligible patients	**Months between last rituximab dose and enrolment**	Screening	Group 1	n, mean, SD, Median, min, max	NA	Appliable for Group 1
			immunosuppression treatment at enrolment Prednisolone Azathioprine MMF	Screening + Month 0	Group 1 and 2	x/n (p%)	NA	Appliable for Group 1 and 2

Table Title	Number	Population	Endpoint	Time Points or how to conglomerate	Covariates or Subgroups	Summary Statistics	Formal Analysis	Foot Notes
			Methotrexate + associated doses					
Lab results at screening	1.3	All eligible patients	**Haematology and chemistry labs results reported at MACRO database**	Screening	Participating group	n, mean, SD, Median, min, max x/n (p%)		
Comorbidity	1.4	All eligible patients	**Hypertension** Yes vs No	Screening	Participating group	x/n (p%)		
			Ischaemic heart disease Yes vs No					
			Chronic lung disease Yes vs No					
			Cerebrovascular Yes vs No					
			Cancer Yes vs No					
			Venous thromboembolism Yes vs No					
			Diabetes Yes vs No					
			Significant history of infection requiring hospitalisation Yes vs No					
			Any of above Yes vs No					

Table Title	Number	Population	Endpoint	Time Points or how to conglomerate	Covariates or Subgroups	Summary Statistics	Formal Analysis	Foot Notes
Medical history – vasculitis manifestation	1.5	All eligible patients	**Years of 1st diagnosis of ANCA and enrolment**	Screening	Participating group	n, mean, SD, Median, min, max	NA	For group 1 and group 2
			Constitutional Yes vs No			x/n (p%)	NA	
			Joints Yes vs No			x/n (p%)	NA	
			Skin Yes vs No			x/n (p%)	NA	
			Mucous membranes/eyes Yes vs No			x/n (p%)	NA	
			Ear/nose/throat Yes vs No			x/n (p%)	NA	
			Heart Yes vs No			x/n (p%)	NA	
			Gastrointestinal Yes vs No			x/n (p%)	NA	
			Lungs Yes vs No			x/n (p%)	NA	
			Kidneys Yes vs No			x/n (p%)	NA	
			Nervous system Yes vs No			x/n (p%)	NA	
			Other Yes vs No			x/n (p%)	NA	
			Any above Yes vs No			x/n (p%)	NA	

Table Title	Number	Population	Endpoint	Time Points or how to conglomerate	Covariates or Subgroups	Summary Statistics	Formal Analysis	Foot Notes
Medical history – medication history	1.6	All eligible patients	**All items listed nder medication history at screening** Yes vs No	Screening	Participating group	x/n (p%)	NA	
Vaccination History	1.7	All eligible patients	**Any form of pneumococcal vaccination** Yes vs No Type and No. of times	Month 0	Participating group	x/n (p%)	NA	
			Years of previous last vaccination until enrolment			n, mean, SD, Median, min, max		
Disease status prior to vaccination	1.8	All eligible patients	**BVAS** Severe flare/new disease Limited flare/new disease Persistent disease Remission	Month 0	Group 1 and 2	x/n (p%)	NA	
			VDI score			x/n (p%) n, mean, SD, median, min, max		
Treatment – vaccination	2.1	All eligible patients	**Month 0: any vaccines in the past 4 weeks** Yes vs No List of details	Month 0	Participating group	x/n (p%)	NA	
			Month 0: pneumococcal polysaccharide					

Table Title	Number	Population	Endpoint	Time Points or how to conglomerate	Covariates or Subgroups	Summary Statistics	Formal Analysis	Foot Notes
			conjugate vaccine received Yes vs No Reasons for No Days between enrolment and vaccination					
	2.2	All eligible patients	**Month 6: any vaccines in the past 4 weeks** Yes No Not applicable (reasons) List of details	Month 6	Participating group	x/n (p%)	NA	
			Month 6: pneumococcal polysaccharide conjugate vaccine received. Yes No Not applicable (reasons) Reasons for No Days between enrolment and vaccination					
	2.3		**Additional treatment** Prednisolone	Month 0, 1, 6 and 7				

Table Title	Number	Population	Endpoint	Time Points or how to conglomerate	Covariates or Subgroups	Summary Statistics	Formal Analysis	Foot Notes
			Azathioprine MMF Methotrexate					
Safety	3.1	Safety population	**List of infection reported**	Trialno group vac_M0 Vac_M6 vaccinedate deathdate finfectsite MedDRA PT InfStartDate InfEndDate InfOutcome InfIntensity InfTreatment InfSAE				
			No. of infections **Site of infections** **Worst grade of infections** **Incidence of infection**	All infection forms	Participating group	x/n(p%)		
	3.2		**AE related to penumocccal vaccination** **List** **Trialno group aeterm**	Month 1, 6 and 7				
	3.3		**SAE** Total no. of SAEs Total no. of SARs No. of pts experienced SAEs No. of pts experience SARs **List of SAEs**					

Table Title	Number	Population	Endpoint	Time Points or how to conglomerate	Covariates or Subgroups	Summary Statistics	Formal Analysis	Foot Notes
	3.4		**List of relapses or deaths**					
Efficacy	4.1		**Primary outcome measure: response at Month 1**	Month1	Group 1 and 2		Fisher's exact test; Logistic regression	
			List reasons excluded in the primary analysis					
	4.2		**Response at Month 1 and 7**	Month 1 and 7	Group 1 and 2			
	4.3		**Response at month 1 for each of serotypes**					
	4.4		**Response at month 1 and 7 each of serotypes**					
	4.5		**Correlation of humoral responses with total CD19+ B cell and baseline IgG**	Month 1 and 7				
	4.6		**Change in IgG**	Month 0 and 6				
	4.7		**Disease status (BVAS)**	Month 0, 1, 6 and 7				
	4.8		**Vasculitis damage index** **Change from Month0 at Month 6**	Month 0 and 6				