Clinical Trial Protocol

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1 Protocol Signatures:

I give my approval for the attached protocol entitled "Acquired immunodeficiency in ANCA associated vasculitis (ACQUIVAS)" dated 08 October 2022.

Chief Investigator

Name:

Signature: _____

Date: _____

Site Signatures

I have read the attached protocol entitled "Acquired immunodeficiency in ANCA associated vasculitis (ACQUIVAS)" dated 08 October 2022 and agree to abide by all provisions set forth therein.

I agree to comply with the conditions and principles of Good Clinical Practice as outlined in the European Clinical Trials Directives 2001/20/EC and 2005/28/EC, the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031) and any subsequent amendments of the clinical trial regulations, the Sponsors' SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

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Amendment History

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	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	7th August 2018
V4.0	Update of inclusion and exclusion criteria	21st June 2019
V5.0	Addition of interim analysis	12th June 2020
V6.0	Amendment to protocol 5.0 – Update the inclusion criteria, clarification of the number of participants in each group.	07th December 2020
V7.0	Update the Pneumococcal Polysaccharide Vaccine pharmaceutical form in pre-filled syringe	01st February 2022
V7.1	Update to include measurement of antibodies against SARS CoV-2	08th October 2022

	vaccine on the serum samples already collected from December 2020 onwards and details of SARS Cov-2 vaccines administered.	

3 Table of Contents

1 Protocol Signatures: 2
2 Trial Management Committee(s) and Protocol Contributors 3
3 Table of Contents 4
4 Abbreviations 6
5 Trial Synopsis 7
6 Trial Flow Chart 15
7 Introduction 16
8 Rationale for Trial 17
9 Trial Design 18
9.1 Statement of Design 18
9.2 Number of Centres 18
9.3 Number of Participants 18
9.4 Participant Trial Duration 18
9.5 Trial Objectives 19
9.6 Trial Outcome Measures 20
10 Selection and withdrawal of participants 21
10.1 Inclusion Criteria 21
10.2 Exclusion Criteria 22
10.3 Participant Withdrawal Criteria 24
11 Trial Treatments 24
11.1 Treatment Summary 24
11.2 Concomitant Therapy 27
11.3 Accountability 27
12 Procedures and assessments 27
12.1 Participant identification 28
12.2 Consent 28
12.3 Screening evaluation 28
12.4 Baseline Assessments 29
12.5 Trial assessments 30
12.6 Schedule of Assessments 32
12.7 End of Trial Participation 33
12.8 Trial restrictions 33
13 Assessment of Safety 34
13.1 Definitions 35
13.2 Expected Adverse Reactions/Serious Adverse Reactions (AR /SARs) 36
13.3 Expected Adverse Events/Serious Adverse Events (SAE) 38
13.4 Evaluation of adverse events 38
13.5 Reporting serious adverse events 39
13.6 Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs) 40
13.7 Pregnancy Reporting 41
14 Toxicity – Emergency Procedures 41
15 Evaluation of Results (Definitions and response/evaluation of outcome measures) 41
15.1 Response criteria 42

16 Storage and Analysis of Samples 42
16.1 Collection and processing of serum samples (all subjects) 42
16.2 Blood collection and PBMC separation (AAV patients) 42
16.3 Additional samples 7 +/- 2 days after Pneumococcal Polysaccharide Conjugate vaccine and Pneumococcal Polysaccharide Vaccine administration (AAV patients recruited in Cambridge only) 43
17 Statistics 43
17.1 Statistical methods 43
17.2 Interim analyses 44
17.3 Number of Participants to be enrolled 44
17.4 Criteria for the premature termination of the trial 45
17.5 Procedure to account for missing or spurious data 46
17.6 Definition of the end of the trial 46
18 Data handling and record keeping 46
18.1 CRF 46
18.2 Source Data 47
18.3 Data Protection & Participant Confidentiality 47
19 Trial Management Group and Trial Steering Committee 47
20 Ethical & Regulatory considerations 48
20.1 Ethical committee review 48
20.2 Regulatory Compliance 48
20.3 Protocol Amendments 48
20.4 Peer Review 48
20.5 Declaration of Helsinki and Good Clinical Practice 48
20.6 GCP Training 49
21 Sponsorship, Financial and Insurance 49
22 Monitoring, Audit & Inspection 49
23 Protocol Compliance and Breaches of GCP 50
24 Publications policy 50
25 References 50
26 Appendices 53
26.1 Appendix 1 – Authorisation of Participating Sites 53
26.2 Appendix 2 - Safety Reporting Flow Chart 54

4 Abbreviations

AAV	ANCA associated vasculitis
AE/AR	Adverse event/Adverse Reaction
ALT	Alanine Transaminase
ANCA	Antineutrophil cytoplasmic antibody
BVAS/WG	Birmingham Vasculitis Activity Score for Wegener's Granulomatosis
CA	Competent Authority
CMV	Cytomegalovirus
CRF	Case Report Form
CTIMP	Clinical Trial of Investigational Medicinal Product
DSUR	Development Safety Update Report
EGPA	Eosinophilic Granulomatosis with polyangiitis
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-Linked ImmunoSpot
ESR	Erythrocyte Sedimentation Rate
FBC	Full Blood Count
GP	General Practitioner
GCP	Good Clinical Practice
GPA	Granulomatosis with polyangiitis
ICF	Informed Consent Form
IFN	Interferon
IgG	Immunoglobulin G
IMP	Investigational Medicinal Product
ISF	Investigator site file
IV	Intravenous
IVIg	Intravenous immunoglobulin
LFT	Liver Function Tests
MHRA	Medicines and Healthcare products Regulatory Agency
MMF	Mycophenolate mofetil
MPA	Microscopic polyangiitis
MPO	Myeloperoxidase
NIMP	Non Investigational Medicinal Product
OPA	Opsonophagocytosis assay
PBMC	Peripheral Blood Mononuclear Cells
PIS	Participant Information Sheet
PR3	Proteinase 3
R&D	Research and Development
RA	Regulatory Agency
REC	Research Ethics Committee
RNA	Ribonucleic acid
RTPCR	Reverse transcriptase polymerase chain reaction
SAE/SAR	Serious Adverse Event/Serious Adverse Reaction
SC	Subcutaneous
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
VDI	Vasculitis Damage Index

5 Trial Synopsis

Title of clinical trial	Acquired immunodeficiency in ANCA Associated vasculitis (AAV)
Sponsor name	Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge
EudraCT number	2017-003563-36
Medical condition or disease under investigation	ANCA associated vasculitis (AAV)
Purpose of clinical trial	Hypothesis: Rituximab therapy leads to an acquired immune deficiency, as demonstrated by impaired vaccine responses, in AAV patients. Aims: 1. To investigate whether rituximab leads to immune deficiency in patients with AAV when compared to both disease and healthy controls 2. To investigate whether the degree of immune deficiency is associated with the degree of B cell depletion 3. To investigate whether T-independent vaccine responses are more severely affected than T-dependent vaccine responses after rituximab and whether a conjugated vaccine will overcome this postulated deficit in T independent vaccine responses.
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.
Secondary objective (s)	1. To assess and compare the humoral response for each serotype assayed 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 and T cell dependent 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 3. 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 4. To evaluate the incidence, type and severity of infections 5. To evaluate the incidence and severity of hypogammaglobulinaemia – IgG, IgM and IgA 6. To evaluate the safety of vaccination following rituximab treatment
Trial Design	This trial will be a phase IIb, open label, multi-centre trial.
Trial Outcome Measures	Primary outcome: This will compare the proportion of patients treated with rituximab compared to disease controls who achieve at least a twofold increase in at least 50% of the 12 pneumococcal serotypes assayed, 28 (+/-7) days after Pneumococcal Polysaccharide Conjugate vaccination.
Sample Size	Three groups of subjects will be recruited to the trial. 104 patients (52 in Group 1 and 52 in Group 2 with primary endpoint data available and a maximum of 20 healthy controls will be required for analysis. The groups of patients are as follows: Group 1: AAV patients treated with rituximab (at least 4 months after last dose of rituximab) Group 2: Disease controls: AAV patients receiving azathioprine (methotrexate or MMF) maintenance therapy, who have NOT previously received rituximab Group 3: Healthy controls
Summary of eligibility criteria	Inclusion Criteria: To be included in the trial the participant

must:

1. Have given written informed consent to participate
2. Be aged 40 years and over

<u>For patients in Group 1 only (rituximab treated):</u>

1. Have a diagnosis of AAV [granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA) or eosinophilic granulomatosis with polyangiitis (eGPA)*]
2. Have current or historical PR3/MPO ANCA positivity by ELISA or histological confirmation of AAV in the case of MPA or GPA
3. Have received ≥ 2g rituximab ever
4. Have received their last dose of rituximab at least 4 months prior to screening assessment.
5. Be in stable remission with a prednisolone dose of ≤ 5mg/day (complete withdrawal of prednisolone ie. Dose of 0mg daily is allowed)

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

1. Have a diagnosis of AAV [GPA, MPA or eGPA*]
2. Have current or historical PR3/MPO ANCA positivity by ELISA or histological confirmation of AAV in the case of MPA or GPA
3. Have received initial induction therapy with cyclophosphamide (oral or IV), azathioprine, methotrexate or mycophenolate mofetil
   Be on stable immunosuppression for the 6 months preceding screening visit including prednisolone ≤ 7.5mg/day (complete withdrawal of prednisolone ie. Dose of 0mg daily is allowed) AND either azathioprine, methotrexate or

mycophenolate mofetil (at stable or tapering dose)

*diagnostic criteria for eGPA are as follows:
Asthma
AND eosinophilia (>1.0x109/Litre and/or >10% of leucocytes)
AND at least two of the following additional features of EGPA

a) a biopsy showing histopathological evidence of eosinophilic vasculitis, or perivascular eosinophilic infiltration, or eosinophil-rich granulomatous inflammation
b) neuropathy, mono or poly (motor deficit or nerve conduction abnormality)
c) pulmonary infiltrates, non-fixed
d) sino-nasal abnormality
e) cardiomyopathy (established by echocardiography or Magnetic Resonance Imaging)
f) glomerulonephritis (haematuria, red cell casts, proteinuria)
g) alveolar haemorrhage (by bronchoalveolar lavage)
h) palpable purpura
i) (ANCA) positive (MPO or PR3).

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

1. Age < 40 years
2. History of severe allergic or anaphylactic reactions to pneumococcal vaccinations
3. Pneumococcal vaccination within 6 months prior to the screening visit
4. Protective response to prior pneumococcal vaccination (adequate titres (>0.35mcg/ml) in at least 50% serotypes tested) if vaccine received within five years prior to screening visit
5. Females who are pregnant, plan to become pregnant, or breast feeding
6. 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.

7. History of malignancy within the past five years prior to screening visit 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 either intravenously or subcutaneously in the 12 weeks prior to screening.

<u>For patients in Groups 1 and 2 only (AAV patients):</u>

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

<u>For patients in group 1 only (rituximab group)</u>

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

<u>For patients in Group 2 only (disease controls):</u>

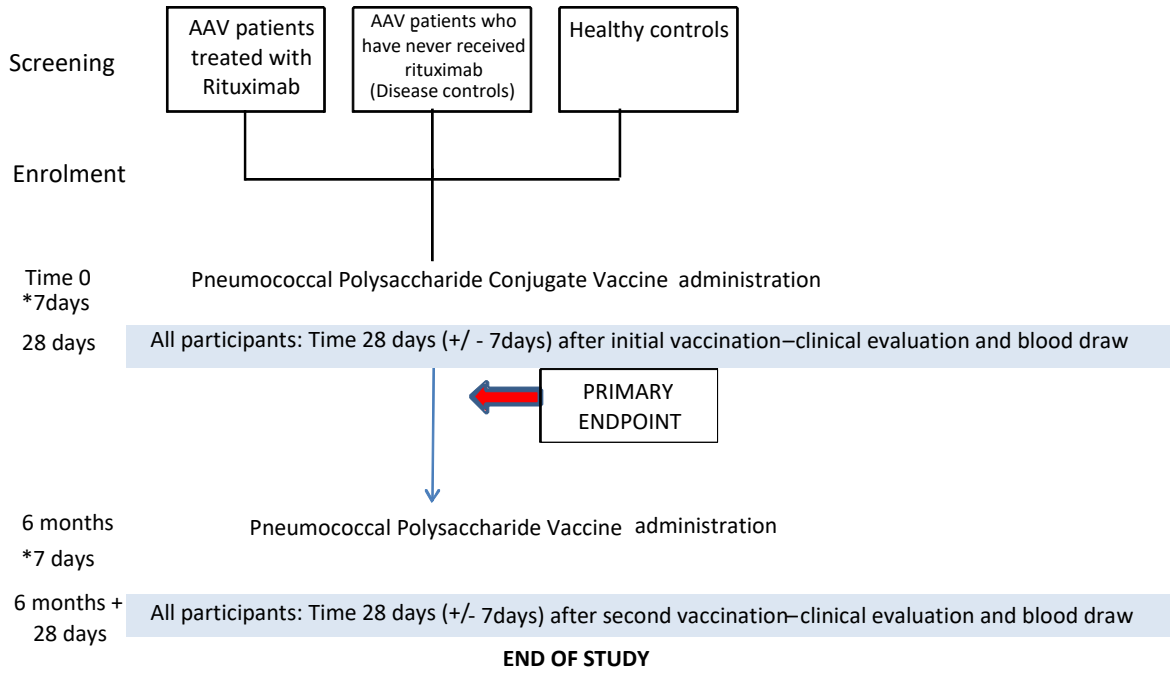
1. A relapse of AAV within the 6 months preceding screening which has necessitated an increase in prednisolone or azathioprine, methotrexate or MMF dose.
2. Previous rituximab therapy at any time

	<u>For healthy controls:</u> 1. Any history of any autoimmune condition 2. Any history of use of immune suppressing medication, including > 4 weeks of oral glucocorticoids, within the last 5 years prior to screening.
Investigational medicinal product and dosage	Pneumococcal polysaccharide conjugate vaccine– 0.5ml single dose at 0 months Pneumococcal Polysaccharide Vaccine - 0.5ml single dose at 6 months
Active comparator product(s)	N/A
NIMPs and Challenge Agents	N/A
Route(s) of administration	Pneumococcal polysaccharide conjugate vaccine– intramuscular injection Pneumococcal Polysaccharide Vaccine– intramuscular injection
Maximum duration of treatment of a participant	Each participant will receive the first vaccination at Month 0 and the second vaccination at Month 6*. *If a participant has entered the trial having received the Pneumococcal Polysaccharide Vaccine within the last 5 years, they will NOT receive the month 6 boost dose of Pneumococcal Polysaccharide Vaccine. Trial participation will end at Month 1.
Procedures: Screening & enrolment	Participants willing to take part will be asked to provide written informed consent prior to any protocol specific procedures. Participants will be screened at the screening visit to confirm eligibility.
Procedures: Baseline	For all participants (AAV patients and healthy controls): a) Age and date of birth b) Gender c) Medical history and clinical examination d) Prior vaccination history (consent will be obtained from the patient to contact their GP for further information on previous vaccinations, if required) e) Blood sample obtained for routine full blood count (including platelets and differential white cell count), biochemistry tests (including urea, electrolytes, creatinine, CRP and LFTs)

	f) Routine blood sample for Immunology profile (including ANCA andimmunoglobulin levels) g) Serum pregnancy test in women of child bearing potential h) concomitant medication review Additional assessments for AAV patients only: a) Vasculitis prior disease manifestations and drug treatments b) Assessment of vasculitis disease activity and damage at baseline c) Research blood sample for PBMC separation and PBMC freezing d) Research serum samples for anti-CMV IgG titre determination e) Blood sample for DNA extraction
Procedures: Treatment period	Participants will be seen at Month 0 for baseline assessment, and initial vaccination. A blood sample will be collected and review of adverse events will be performed in all participants at month 1 (28 days +/- 7 days). Participants (except those who have received the Pneumococcal Polysaccharide Vaccine within 5 years prior to screening) will then return at Month 6 (+/- 2 weeks) for clinical review and their second scheduled vaccine. Blood samples will be taken immediately prior to vaccination. A further blood sample and review for adverse events will be performed in all participants at Month 7 (+/- 7 days). The 0 and 6 month visits will coincide with routine clinic follow up visits for AAV patients, whenever possible. OPTIONAL visits: AAV patients recruited at Cambridge will be invited for an OPTIONAL additional visit 7 +/- 2 days after each vaccination for a blood sample.
Procedures: End of trial	The trial will end when the last participant has reached the 7 month time point
Procedures for safety monitoring during trial	At every participant visit, adverse events (AEs) will be assessed and recorded and serious adverse events (SAEs) reported, according to regulatory requirements for CTIMPs.

Criteria for withdrawal of participants	Participants will be withdrawn from the trial immediately: - if they choose to withdraw their consent to participate. - at the Investigator's decision. - if a female participant becomes pregnant or plans to become pregnant. - if AAV patients have a flare of disease which necessitates a change in maintenance immunosuppression However, any data collected prior to the point of withdrawal would be included in analyses. Otherwise, subjects will be followed until the end of the trial or until death, whichever is earlier.

6 Trial Flow Chart



*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 received the Pneumococcal Polysaccharide Vaccine at month 6, and participation in the study will cease at Month 1 (primary endpoint)

7 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).

Infection, rather than uncontrolled vasculitis, is the greatest cause of mortality in the first year of treatment, and remains a significant problem thereafter (Little, 2009). Somewhat disappointingly, rates of serious adverse events and infections following rituximab were comparable to cyclophosphamide in the RITUXVAS and RAVE trials, and were higher than in registries of patients with rheumatoid arthritis, probably as a result of prior immunosuppression burden, disease severity and major organ involvement, and the high doses of steroids initially used in AAV (Jones, 2010; Stone, 2010; Van Vollenhoven, 2010). The acquired immunodeficiency that many patients experience as a result of the disease processes and therapies used in AAV pose an additional challenge to treating physicians.

As a prophylactic measure, vaccination against pneumococcus and influenza is recommended for patients with AAV (Ntatskai, 2014; Van Assen, 2011). However, it remains unclear how effective such an approach is in the immunocompromised individual, particularly those treated with rituximab. Rituximab compromises the humoral response to influenza and pneumococcal vaccines in patients with rheumatoid arthritis. The effect on cellular responses in these patients is less clear, with some studies suggesting preservation and others compromise (Rehnberg, 2010; Oren, 2008; Van Assen 2010; Arad 2011; Adler, 2012). However, both the disease process and treatment approach to AAV is different, and the data on vaccine responses should be extrapolated from one disease to another with caution. Studies have demonstrated preservation of the cellular response and an adequate humoral response in patients with GPA receiving the influenza vaccine. However, in both studies at least half of patients had had all immunosuppression withdrawn, and none had received B cell depleting therapy (Holvast, 2009; Holvast 2010).

Although vaccination is primarily an important prophylactic intervention in patients with AAV, vaccine responses can also be used as a tool, to assess the immune competence of individuals. Consequently, immune therapy could be individually tailored, with minimisation in those with evidence of profound immune compromise, and the targeting of prophylactic interventions to those individuals to minimise infective risk. The effect of rituximab on vaccine responses in patients with AAV is yet to be explored. In particular, the association between response and the degree of B cell depletion is unknown. In addition, poor response to vaccination has been identified as an important predictor of all-cause death in patients with AAV (Morgan, 2016). Therefore, the optimisation of vaccine strategies is vital, and this trial will consider one such approach.

The ACQUIVAS trial, in addition to offering a therapeutic intervention, which aims to maximise responses to pneumococcal vaccination, also aims to understand the processes that lead to immune dysregulation in patients with AVV. The impact of sub-clinical CMV reactivation and T cell exhaustion on pneumococcal vaccine responses will be addressed as exploratory endpoints in this trial. In addition, genetic factors which influence B cell function and the development of hypogammaglobuliaemia and may therefore influence vaccine response will be studied.

Recent findings suggest that Cytomegalovirus (CMV) infection may be an important mediator of impaired immune responses in patients with AAV. Subclinical CMV reactivation amongst CMV seropositive AAV patients in remission was recently shown to promote accumulation of senescent CD4+CD28 null T-cells (Chanouzas, manuscript submitted). Accumulation of CD4+CD28null T-cells in turn has been linked to a higher risk of infection and mortality (Morgan, 2011), a reduction in the immune response to a T-cell dependent vaccine, and a relative reduction in B-cell proportions in AAV patients (Chanouzas, manuscript in preparation). This trial will therefore also assess the impact of CMV infection on vaccine responses and B-cell reconstitution and phenotype following rituximab therapy in AAV patients.

A CD8 T cell transcriptional signature has been identified that predicts, at the time of active AAV, the risk of subsequent relapse (McKinney, 2010; McKinney, 2015). This signature reflects a differentiation process known as T cell exhaustion, a dysfunctional state in which T cells progressively lose the ability to divide, kill and produce cytokines (Dolfi, 2013). T cell exhaustion is best described in viral infection where it associates with persistent infection secondary to an attenuated antiviral response. In AAV (as in other autoinflammatory diseases), exhaustion conversely associates with reduced relapse frequency. Surrogate markers of exhaustion in whole blood also predicted response to vaccination (exhaustion associating with limited response) with influenza, yellow fever and malaria (McKinney, 2015). It remains unknown whether such markers might predict either response to pneumococcal vaccinations themselves, or longer term disease course.

8 Rationale for Trial

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.

Aims:

1. To investigate whether rituximab leads to immune deficiency in patients with AAV when compared to both disease and healthy controls

2. To investigate whether the degree of immune deficiency is associated with the degree of B cell depletion

3. To investigate whether T-independent vaccine responses are more severely affected than T-dependent vaccine responses after rituximab and whether a conjugated vaccine will overcome this postulated deficit in T independent vaccine responses.

9 Trial Design

9.1 Statement of 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.

9.2 Number of Centres

We plan to include 2 UK centres within this trial, however we may increase the number of centres (UK only), if required, in order to recruit the required number of participants.

9.3 Number of Participants

Primary endpoint data will be required from 104 patients with AAV; 52 rituximab treated patients and 52 disease controls who have never received rituximab treatment. Sufficient patients will be recruited to ensure analysis can occur on primary endpoint data from 104 patients. In addition, we will require a primary end point data for a maximum of 20 healthy controls who will be age-matched to AAV participants wherever possible.

9.4 Participant Trial Duration

All participants enrolled into the trial will be required to attend for four trial visits over seven months. AAV patients recruited at Cambridge may be invited to attend for two additional visits (for a blood sample only) 7 +/- 2 days after each vaccination.

However, these are OPTIONAL, and should subjects decline this invitation, they will still be eligible to participate in the trial, provided they agree to attend all other trial visits as outlined in the protocol.

The baseline visit will be at month 0, where participants will receive their initial Pneumococcal Polysaccharide Conjugate vaccination. All participants will then return for a brief trial visit (short assessment and blood draw) after 28 +/- 7 days. The third visit will be at month 6, and participants (except those who have received the Pneumococcal Polysaccharide Vaccine within 5 years prior to screening) will receive the second vaccination (Pneumococcal Polysaccharide Vaccination) at this point. They will then return for a brief trial visit (short assessment and blood draw) after 28 +/- 7 days.

We will aim to coordinate the first and third trial visits to occur at the time of routine clinic follow up, so AAV patients will only be required to make 2 additional visits as part of the trial.

9.5 Trial Objectives

9.5.1 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.

9.5.2 Secondary objectives

1. To assess and compare the humoral response for each serotype assayed 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 and T cell dependent 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

3. 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

4. To evaluate the incidence, type and severity of infections

5. To evaluate the incidence and severity of hypogammaglobulinaemia – IgG, IgM and IgA

6. To evaluate the safety of vaccination following rituximab treatment

9.5.3 Exploratory objectives

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.

6. To correlate humoral responses to SARS CoV-2 vaccines (which became standard of care in December 2020 for this patient population) with total CD19+ B cell counts, and CD19+ B cell subsets (memory (CD27+) and naïve (CD27-) B cells) and baseline immunoglobulin levels

9.6 Trial Outcome Measures

9.6.1 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 50% of the 12 pneumococcal serotypes tested.

9.6.2 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* serotypes assayed in the pneumococcal vaccine will be measured at month 0, 1, 6 and 7 in all participants.

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.

3. Number of serious adverse events, and serious adverse events specifically related to the vaccines administered (serious adverse reactions)
4. Incidence, type, severity and treatment of infections experienced by participants after vaccinations

5. Changes in immunoglobulin levels will be measured over time

9.6.3 Exploratory endpoints

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

10 Selection and withdrawal of participants

10.1 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 in the case of MPA or GPA.
- 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 in the case of MPA or GPA.
- 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 ≤7.5mg/day (complete withdrawal of prednisolone ie. Dose of 0mg daily is allowed) AND either azathioprine, methotrexate or mycophenolate mofetil (at stable or tapering dose)

*diagnostic criteria for eGPA are as follows:
Asthma
AND eosinophilia (>1.0x109/Litre and/or >10% of leucocytes)
AND at least two of the following additional features of EGPA:

a) a biopsy showing histopathological evidence of eosinophilic vasculitis, or perivascular eosinophilic infiltration, or eosinophil-rich granulomatous inflammation
b) neuropathy, mono or poly (motor deficit or nerve conduction abnormality)
c) pulmonary infiltrates, non-fixed
d) sino-nasal abnormality
e) cardiomyopathy (established by echocardiography or Magnetic Resonance Imaging)
f) glomerulonephritis (haematuria, red cell casts, proteinuria)
g) alveolar haemorrhage (by bronchoalveolar lavage)
h) palpable purpura
i) (ANCA) positive (MPO or PR3).

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 (>0.35 mcg/ml) in at least 50% serotypes tested) if vaccine received within 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.

Subjects participating in other research studies can be recruited to this trial, provided they meet the inclusion and exclusion criteria for their group, and their participation in this trial does not contravene the protocol of the other trial. If the previous study is interventional in nature, then the participant can only be recruited to the ACQUIVAS

trial if they are in the follow up phase of the previous study, once all interventions have been completed for that study.

10.3 Participant Withdrawal Criteria

Patients will be withdrawn from the trial immediately if they choose to withdraw their consent to participate. Otherwise, subjects will be followed until the end of the trial or until death (whichever occurs first). If consent is withdrawn, data and samples that have already been collected may continue to be used in the trial, unless otherwise stated by the participant.

It is anticipated that withdrawal will be a rare event, and in particular will have minimal impact on the primary endpoint which is 28 (+/- 7) days after the administration of Pneumococcal Polysaccharide Conjugate Vaccine. Subjects who are withdrawn will not be replaced. If a female participant becomes pregnant during the trial or plans to become pregnant, then she will be withdrawn and would not receive the Pneumococcal Polysaccharide vaccine at 6 months. However, any data collected prior to the point of withdrawal would be included in analyses.

If a patient has a flare of disease activity during the trial, which necessitates a change in their maintenance immunosuppression (such as a further dose of rituximab or increase in dose of azathioprine, methotrexate or mycophenolate mofetil), and as such they no longer fulfil the eligibility criteria of Group 1 or Group 2, then they will not receive the boost Pneumococcal Polysaccharide Vaccine at 6 months and will be withdrawn from the trial. However, any data collected prior to the point of withdrawal would be included in analyses unless otherwise stated by the participant.

11 Trial Treatments

11.1 Treatment Summary

For the purpose of this trial, Pneumococcal Polysaccharide Conjugate vaccine (13-valent, adsorbed)) and Pneumococcal Polysaccharide Vaccine are both considered as Investigational Medicinal Products (IMP)s conducted with a Clinical Trial Authorisation.

11.1.1 Pneumococcal Polysaccharide Conjugate vaccine (13- valent, adsorbed)

11.1.1.1 Legal status

Pneumococcal Polysaccharide Conjugate vaccine is licensed for active immunisation for the prevention of invasive disease, pneumonia and acute otitis media caused by *Streptococcus pneumoniae* in infants, children and adolescents from 6 weeks to 17 years of age, and for the active immunisation for the prevention of invasive disease and pneumonia caused by *Streptococcus pneumoniae* in adults ≥18 years of age and the elderly.

11.1.1.2 Supply

The drug will be dispensed using standard commercial stock, according to local site supply procedures, upon receipt of a completed prescription.

11.1.1.3 Packing and Labelling

Pneumococcal Polysaccharide Conjugate vaccine is presented as a 0.5 ml suspension for injection in pre-filled syringe. Labelling will be in accordance with the provisions described in Regulation 46 of SI 2004 No 1031 (paragraph 2).

11.1.1.4 Storage conditions

Pneumococcal Polysaccharide Conjugate vaccine should be stored as per the SmPC.

11.1.1.5Maximum duration of treatment of a participant

Subjects will receive a single dose of Pneumococcal Polysaccharide Conjugate vaccine at month 0.

11.1.1.6 Dose

A single dose of 0.5ml suspension in the pre-filled syringe will be administered.

11.1.1.7Administration

Pneumococcal Polysaccharide Conjugate vaccine should be given by intramuscular injection, preferably in the deltoid muscle of the upper arm. However, in individuals with thrombocytopaenia or any coagulation disorder that would contraindicate intramuscular injection, it may be given subcutaneously. The administration of Pneumococcal Polysaccharide Conjugate vaccine should be postponed in subjects suffering from acute, severe febrile illness. However, the presence of a minor infection, such as a cold, should not result in the deferral of vaccination.

11.1.1.8Known drug reactions

Pneumococcal Polysaccharide Conjugate vaccine may be administered concomitantly with the seasonal inactivated influenza vaccine. Concomitant use with other vaccines has not been investigated, and is not permitted in this trial.

11.1.1.9Dose modifications

No dose modifications will be made.

11.1.2 Pneumococcal Polysaccharide Vaccine

11.1.2.1 Legal status

Pneumococcal Polysaccharide Vaccine is recommended for active immunisation against disease caused by the pneumococcal serotypes included in the vaccine. The vaccine is recommended for individuals 2 years of age or older in whom there is an increased risk of morbidity and mortality from pneumococcal disease. The 0.5mL dose of vaccine contains 25 micrograms of each of the following 23 pneumococcal polysaccharide serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F.

11.1.2.2 Supply

The drug will be dispensed using standard commercial stock, according to local site supply procedures, upon receipt of a completed prescription.

11.1.2.3 Packing and Labelling

Pneumococcal Polysaccharide Vaccine is presented as a 0.5 ml solution for injection in pre-filled syringe. Labelling will be in accordance with the provisions described in Regulation 46 of SI 2004 No 1031 (paragraph 2).

11.1.2.4 Storage conditions

Pneumococcal Polysaccharide Vaccine should be stored as per the SmPC.

11.1.2.5 Maximum duration of treatment of a participant

Subjects will receive a single dose of Pneumococcal Polysaccharide Vaccine at month 6.

11.1.2.6 Dose

A single dose of 0.5ml suspension will be administered.

11.1.2.7 Administration

Pneumococcal Polysaccharide Vaccine should be given by intramuscular injection. However, in individuals with thrombocytopaenia or any coagulation disorder that would contraindicate intramuscular injection, it may be given subcutaneously. The administration of Pneumococcal Polysaccharide Vaccine should be postponed in subjects suffering from acute, severe febrile illness. However, the presence of a minor infection, such as a cold, should not result in the deferral of vaccination.

11.1.2.8 Known drug reactions

Pneumococcal Polysaccharide Vaccine can be administered simultaneously with the seasonal inactivated influenza vaccine. Pneumococcal Polysaccharide Vaccine and Zostavax should not be given concurrently because of reduced immunogenicity of Zostavax with concomitant use. Therefore, administration of the two vaccines should be separated by at least 4 weeks.

11.1.2.9 Dose modifications

No dose modifications will be made.

11.2 Concomitant Therapy

AAV patients must be on a stable or tapering dose of prednisolone and/or immunosuppression (azathioprine, methotrexate or mycophenolate mofetil) for the duration of the trial. A brief increase in prednisolone dose (less than 4 weeks, and return to a baseline dose of ≤ 5mg daily for patients in GROUP 1 and return to a baseline dose of ≤ 7.5mg/day for patient in GROUP 2) will be permitted for a minor relapse of disease. Any increase in dose of azathioprine, methotrexate or mycophenolate is prohibited and any patient requiring this will be withdrawn from the trial. Administration of rituximab is not allowed during the trial. The prescription of prophylactic antibiotics is left to investigators' discretion, but will be recorded in the CRF.

11.3 Accountability

11.3.1 Drug accountability

Formal accountability records are not required. All treatments will be administered in secondary care using standard commercial stock. As part of standard vaccination procedures, batch number and expiry date of product used will be recorded at point of administration in patient records.

11.3.2 Returns and destruction

Returns are not required for this study. All used injections and packaging should be disposed of at the point of administration.

12 Procedures and assessments

All procedures and assessments will be conducted in the hospital setting by suitably trained medical professionals.

12.1 Participant identification

Potential participants will be recruited from vasculitis clinics. Clinic lists will be screened at each site for potentially eligible patients, by the patient's usual clinic care team. Potentially eligible patients could be contacted by telephone by their usual clinical care team prior to a planned clinic visit, to establish whether they may be interested in participating in the trial, and whether they would be agreeable to receive a participant information sheet via email or post. A detailed explanation will be provided at the clinic visit, and if the patient wishes, and fulfills the eligibility criteria, they could be enrolled that day. Alternatively, potential participants may be approached at a routine clinic visit by their usual clinical care team.

Posters advertising the need for healthy volunteers for this trial will be displayed in clinics, and circulated via the local and national vasculitis patient groups. Details of the site PI or research nurse will be included so that potential healthy volunteers can contact them for more information regarding the trial.

12.2 Consent

The Informed Consent form must be approved by the Research Ethics Committee (REC) and must be in compliance with GCP, local regulatory requirements and legal requirements. The investigator or designee must ensure that each trial participant, is fully informed about the nature and objectives of the trial and possible risks associated with their participation.

The investigator or designee will obtain written informed consent from each participant before any trial-specific activity is performed. The informed consent form used for this trial and any changes made during the course of this trial, must be prospectively approved by the REC. The investigator will retain the original of each participant signed informed consent form.

Should a participant require a verbal translation of the trial documentation by a locally approved interpreter/translator, it is the responsibility of the individual investigator to use locally approved translators.

Any new information which becomes available, which might affect the participant's willingness to continue participating in the trial will be communicated to the participant as soon as possible, either in writing or at the subject's next clinic visit.

AAV participants recruited at Cambridge will be asked if they would like to give consent to provide optional blood samples 1 week after each vaccination.

12.3 Screening evaluation

12.3.1 Screening Assessments (for AAV patients and healthy volunteers)

Trial specific assessments will only be conducted after participants have given written informed consent. After consent has been obtained, participants will be reviewed to ensure they meet all of the inclusion criteria and none of the exclusion criteria of the trial. In order to do this, a medical history and review of the participant's medical records will be performed.

12.3.2 Participant Enrolment (for AAV patients and healthy volunteers)

Once a participant has been confirmed eligible for the trial, participant information (date of birth and group allocation (rituximab, disease, or healthy control)) will be entered onto a CRF. A unique patient identifying number will be allocated and a target date for the 6 month administration of the Pneumococcal Polysaccharide Vaccine will be generated and entered into the participant's CRF (provided they have not received the Polysaccharide Pneumococcal vaccine within 5 years prior to the screening visit).

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12.4 Baseline Assessments

All participants will have a full medical history taken and a clinical examination (if baseline visit is separate to screening visit). The following activities will then be performed:

a) Age and date of birth
b) Gender
c) Any significant (as determined by the PI) past medical history
d) Clinical examination
e) Prior vaccination history (consent will be obtained from the patient to contact their GP for further information on previous vaccinations, if required)
f) Blood sample obtained for routine full blood count (including platelets and differential white cell count), biochemistry tests (including creatinine, CRP and LFTs)
g) Routine blood sample for Immunology profile (including ANCA and immunoglobulin levels)
h) Serum pregnancy test in women of child bearing potential

12.4.1 Additional baseline assessments (for AAV patients only)

a) Vasculitis prior disease manifestations and drug treatments
b) Assessment of vasculitis disease activity and damage at baseline
c) Research serum samples for anti-CMV IgG titre determination
d) Research blood sample for PBMC separation and PBMC freezing
e) Blood sample for DNA extraction

12.5 Trial assessments

12.5.1 Timing of assessments

The baseline visit and first treatment will be at month 0. All participants will then return for a brief trial visit 28 (+/- 7) days later. The third visit (second treatment) will be at 6 months (+/- 2 weeks), and participants will then return for a brief final trial visit 28 (+/- 7) days later (Month 7) (except those who have received the Pneumococcal Polysaccharide Vaccine within 5 years prior to screening).

We will aim to coordinate the first and third trial visits to occur at the time of routine clinic follow up, so patients will only be required to make 2 additional visits as part of the trial.

12.5.2 Assessments at each specific time point

See schedule of events, section 12.6 for full details of assessments at each time point.

Month 0 visit
The baseline visit will be at month 0, and participants will receive their Pneumococcal Polysaccharide Conjugate vaccination. See section 12.4 for details of the baseline visit assessments.

Month 1 visit
Participants will then return for a brief trial visit (to include: assessment of safety events, concomitant medications, disease activity and a blood draw) at 28 +/- 7 days.

Month 6 visit
The third visit will be at 6 months (+/- 2 weeks of target date for Pneumococcal Polysaccharide Vaccine administration generated at enrolment). Disease activity and damage, assessment of safety events and concomitant medications will be recorded. Blood samples will be taken. Participants will also receive the second vaccination (Pneumococcal Polysaccharide Vaccine) at this time point.
Subjects who received the Pneumococcal Polysaccharide vaccine within 5 year of screening, (OR it is unknown what form of the vaccine their received) will NOT receive the month 6 Pneumococcal Polysaccharide vaccine, due to concerns about tolerance and lack of response. Their participation in the trial will cease at month 1 (primary endpoint). Subjects who have received the Pneumococcal Polysaccharide conjugate vaccine within 5 years of screening, or those who received any form of the pneumococcal vaccine more than 5 years prior to screening, or those who have never been previously received the pneumococcal vaccine will receive the boost dose of Pneumococcal Polysaccharide vaccine at month 6.

Month 7 visit

Subjects who received the Pneumococcal Polysaccharide Vaccine at Month 6 will then return for a brief final trial visit (including: assessment of safety events, concomitant medications, disease activity and a blood draw) at 28 +/- 7 days.

12.5.3 Optional additional visits 1 week after Pneumococcal Polysaccharide Conjugate vaccine administration and Pneumococcal Polysaccharide Vaccine administration

Subjects recruited in Cambridge only may be invited to attend two additional visits (for a blood sample only) 7 +/- 2 days after each vaccination. However, these are **OPTIONAL**, and should subjects decline this invitation, they will still be eligible to participate in the trial, provided they agree to attend all other trial visits as outlined in the protocol. A 2.5ml RNA PAXgene sample and 30ml whole blood for PBMC separation and lymphocyte phenotyping will be taken at each additional visit. For those subjects who consent to these additional visits, an additional two 2.5ml RNA Paxgene samples will also be taken at month 0 and month 6, for comparison, which would not otherwise be taken as part of the trial protocol.

12.5.4 Assessments at the end of trial visit

Subject participation in the trial will end at the Month 7 visit. See section 12.5.2 for details of assessments to be performed at the Month 7 visit.
Subjects who received the Pneumococcal Polysaccharide vaccine within 5 years of screening will cease participation in the trial at Month 1.

12.6 Schedule of Assessments

12.6.1 (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.

12.6.2 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.

12.7 End of Trial Participation

The definition of the end of trial is the last participant's last visit at the 7 month time point. Individual participants will return to normal standard of care.

12.8 Trial restrictions

The administration of Pneumococcal Polysaccharide Conjugate vaccine and Pneumococcal Polysaccharide Vaccine should be postponed in subjects suffering from

acute, severe febrile illness. However, the presence of a minor infection, such as a cold, should not result in the deferral of vaccination.

Since there is limited data for the use of pneumococcal vaccination in pregnant women, it should be avoided during pregnancy and breast feeding. A pregnancy test will be performed on women of childbearing potential to ensure their safety before administration of either vaccine.

Women who are able to have a baby must use one of the following, reliable forms of contraception for the duration of the trial:
- Oral contraceptive (either combined or progestogen alone)
- Contraceptive implant, injections or patches
- Vaginal ring
- Intrauterine device (IUD, coil or intrauterine system)
- Condom **and** cap or diaphragm **plus** spermicide (chemical that kills sperm)
- True abstinence where this reflects theirusual and preferred lifestyle

Women do not need to use contraception if:
- they have only one partner, and the man has had an operation to cut the tubes that carry sperm (vasectomy)
- they (or their partner) are a woman who cannot become pregnant
- they practice true abstinence as part of their usual and preferred lifestyle (no sexual activity from screening until the end of the trial. If women become sexually active, they must use one of the methods listed above.

If a woman becomes pregnant during the trial they should inform their trial doctor immediately. Their trial doctor will discuss all the options available to them. With their consent, the outcome and progress of any pregnancy would be followed and they would be asked questions about the pregnancy and baby, if appropriate.

13 Assessment of Safety

The ACQUIVAS trial will use a risk-adapted safety reporting approach in view of the following:

Both Pneumococcal Polysaccharide Conjugate Vaccine and Pneumococcal Polysaccharide Vaccine, the IMPs used in the trial, are:

- licensed for the prevention of pneumococcal infection in the UK at the stated doses

- have been widely prescribed in the UK and worldwide for many years and have been subject to large, randomised controlled trials with hard endpoints and extensive safety reporting

- are in common everyday use, and are recommended for use in immunosuppressed individuals

- will be supplied from hospital stock and will be prescribed as per standard practices

13.1 Definitions

13.1.1 Adverse event (AE)

Any untoward medical occurrence in a participant or clinical trial participant administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

Please note: Recording of all serious adverse events (SAEs) and selected adverse events of interest (see section 13.3) must start from the point of Informed Consent regardless of whether a participant has yet received a medicinal product.

13.1.2 Adverse reaction to 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.

13.1.3 Unexpected adverse reaction

An adverse reaction, the nature, or severity of which is not consistent with the applicable reference safety information (RSI) as specified in section 13.1.6. When the outcome of the adverse reaction is not consistent with the applicable RSI 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 participant /event outcome or action criteria.

13.1.4 Serious adverse event or serious adverse reaction (SAE / SAR)

Any untoward medical occurrence that at any dose:

- 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
- is an important medical event - Some medical events may jeopardise the participant or may require an intervention to prevent one of the above characteristics/ consequences. Such events (hereinafter referred to as 'important medical events') should also be considered as 'serious'

Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the participant 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.

13.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature and severity of which is not consistent with the information set out in the Reference Safety Information.

13.1.6 Reference Safety Information (RSI)

A list of medical events that defines which reactions are expected for the IMP within a given trial and thus determining which Serious Adverse Reactions (SARs) require expedited reporting.

The RSI is contained in a clearly identified section of the Summary of Product Characteristics (SmPC) or the Investigator's Brochure (IB).

For this trial the Reference Safety Information is:

- Prevenar 13 suspension for injection (Pneumococcal polysaccharide conjugate vaccine (13-valent, adsorbed)): section 4.8 (undesirable effects) of SmPC approved by the MHRA for use in this trial (revised 01 January 2018).

- Pneumococcal Polysaccharide Vaccine: section 4.8 (undesirable effects) of SmPC approved by the MHRA for use in this trial (revised text 29 January 2021).

13.2 Expected Adverse Reactions/Serious Adverse Reactions (AR /SARs)

All expected Adverse Reactions are listed in the latest MHRA approved version of the RSI as specified in section 13.1.6. This must be used when making a determination as to the expectedness of the adverse reaction. If the adverse reaction meets the criteria for seriousness, this must be reported as per section 13.5.

Pneumococcal Polysaccharide Conjugate Vaccine (Prevenar 13)

Listed below are the expected adverse events associated with Pneumococcal Polysaccharide Conjugate Vaccine (Prevenar 13) administration relevant to this study population, with frequencies as follows: very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1,000); very rare (<1/10,000).

Metabolism and nutrition disorders:

Very common: Decreased appetite

Nervous system disorders:

Very common: Headaches

Gastrointestinal disorders:

Very common:	Diarrhoea; vomiting (in adults aged 18 to 49 years)
Common:	Vomiting (in adults aged 50 years and over)
Uncommon:	Nausea

Immune system disorders:

Uncommon:	Hypersensitivity reaction including face oedema, dyspnoea, bronchospasm

Skin and subcutaneous tissue disorders:

Very common:	Rash

General disorders and administration site conditions:

Very common**:**	Chills; fatigue; vaccination-site erythema; vaccination-site induration/swelling; vaccination-site pain/ tenderness; limitation of arm movement
Common:	Pyrexia
Uncommon:	Lymphadenopathy localized to the region of the vaccination Site

Musculoskeletal and connective tissue disorders:

Very common:	Arthralgia; myalgia

Pneumococcal Polysaccharide Vaccine

Listed below are the expected adverse events associated with Pneumococcal Polysaccharide Vaccine administration with frequencies as follows: very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1,000); very rare (<1/10,000).

General disorders and administration site conditions:

Very common**:**	Vaccination-site induration, fever, erythema, pain, soreness, swelling, warmth.
Rare:	Injection site cellulitis

All serious adverse events, including serious adverse drug reactions (see section 13.2) should be reported according to guidance in section 13.5. In addition, infections (any episode which requires the administration of an antimicrobial agent) will be recorded in the CRF as a selected adverse event of particular interest in the ACQUIVAS trial.

13.3 Expected Adverse Events/Serious Adverse Events (SAE)

Any pre-planned or elective surgery that necessitates hospital admission will not be considered a SAE and does not need reporting as such.

All serious adverse events, including serious adverse drug reactions (see section 13.2) should be reported according to guidance in section 13.5.

In addition to all SAEs, the following selected adverse events (AEs) are being collected as part of the ACQUIVAS trial.

1. Infections (all episodes requiring treatment with intravenous (IV) or oral antibiotics or infections that are commonly understood to be opportunistic)
2. Relapses of ANCA vasculitis
3. Adverse events directly related to vaccine administration

13.4 Evaluation of adverse events

The Sponsor expects that adverse events are recorded from the point of Informed Consent regardless of whether a participant has yet received a medicinal product. Individual adverse events should be evaluated by the investigator. This includes the evaluation of its seriousness, and any relationship between the investigational medicinal product(s) and/or concomitant therapy and the adverse event (causality).

13.4.1 Assessment of seriousness

Seriousness is assessed against the criteria in section 13.1.4. This defines whether the event is an adverse event, serious adverse event or a serious adverse reaction.

13.4.2 Assessment of causality

Definitely: A causal relationship is clinically/biologically certain. **This is therefore an Adverse Reaction**

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. **This is therefore an Adverse Reaction.**

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. **This is therefore an Adverse Reaction.**

Unlikely: A causal relation is improbable and another documented cause of the AE is most plausible. **This is therefore an Adverse Event.**

Unrelated: A causal relationship can be definitely excluded and another documented

cause of the AE is most plausible. **This is therefore an Adverse Event.**

Unlikely and Unrelated causalities are considered NOT to be trial drug related
Definitely, Probable and Possible causalities are considered to be trial drug related

A pre-existing condition must not be recorded as an AE or reported as an SAE unless the condition worsens during the trial and meets the criteria for reporting or recording in the appropriate section of the CRF.

13.4.3 Clinical assessment of severity

Mild: The participant is aware of the event or symptom, but the event or symptom is easily tolerated

Moderate: The participant 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 participant's life is at risk from the event.

13.4.4 Recording of adverse events

Selected adverse events and adverse reactions should be recorded in the medical notes and the appropriate section of the CRF and/or AE/AR log. Serious Adverse Events and Serious Adverse Reactions should be reported to the sponsor as detailed in section 13.5.

13.5 Reporting serious adverse events

Each Principal Investigator needs to record and report all serious adverse events to the Chief Investigator using the trial specific SAE form within 24 hours of their awareness of the event.

The Chief Investigator is responsible for ensuring the assessment of all SAEs for expectedness and relatedness is completed and the onward notification of all SAEs to the Sponsor immediately but not more than 24 hours of first notification. The sponsor has to keep detailed records of all SAEs reported to them by the trial team.

The Chief Investigator is also responsible for prompt reporting of all serious adverse event findings to the competent authority (e.g. MHRA) of each concerned Member State if they could:

- adversely affect the health of participants
- impact on the conduct of the trial
- alter the risk to benefit ratio of the trial
- alter the competent authority's authorisation to continue the trial in accordance with Directive 2001/20/EC

The completed SAE form can be faxed or emailed. Details of where to report the SAE's can be found on the ACQUIVAS SAE form and the front cover of the protocol.

13.6 Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

All suspected adverse reactions related to an investigational medicinal product (the tested IMP and comparators) which occur in the concerned trial, and that are both unexpected and serious (SUSARs) are subject to expedited reporting. Please see section 13.1.6 for the Reference Safety Information to be used in this trial.

13.6.1 Who should report and whom to report to?

The Sponsor delegates the responsibility of notification of SUSARs to the Chief Investigator. The Chief Investigator must report all the relevant safety information previously described, to the:

- Sponsor
- competent authorities in the concerned member states (e.g. MHRA)
- Ethics Committee in the concerned member states

The Chief Investigator shall inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

13.6.2 When to report?

13.6.2.1 Fatal or life-threatening SUSARs

All parties listed in 13.6.1 must be notified as soon as possible but no later than **7 calendar days** after the trial team and Sponsor has first knowledge of the minimum criteria for expedited reporting.
In each case relevant follow-up information should be sought and a report completed as soon as possible. It should be communicated to all parties within an additional **8 calendar days**.

13.6.2.2 Non-fatal and non-life-threatening SUSARs

All other SUSARs and safety issues must be reported to all parties listed in 13.6.1 as soon as possible but no later than **15 calendar days** after first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information should be given as soon as possible.

13.6.3 How to report?

13.6.3.1 Minimum criteria for initial expedited reporting of SUSARs

Information on the final description and evaluation of an adverse reaction report may not be available within the required time frames for reporting. For regulatory purposes, initial expedited reports should be submitted within the time limits as soon as the minimum following criteria are met:

a) a suspected investigational medicinal product
b) an identifiable participant (e.g. trial participant code number)
c) an adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship

d) an identifiable reporting source

and, when available and applicable:
- a unique clinical trial identification (EudraCT number or in case of non-European Community trials the sponsor's trial protocol code number)
- a unique case identification (i.e. sponsor's case identification number)

13.6.3.2 Follow-up reports of SUSARs

In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality should be actively sought from the reporter or other available sources. Further available relevant information should be reported as follow-up reports.
In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.

13.6.3.3 Format of the SUSAR reports

Electronic reporting is the expected method for expedited reporting of SUSARs to the competent authority. The format and content as defined by the competent authority should be adhered to.

13.7 Pregnancy Reporting

All pregnancies of a trial participant, within the trial, should be reported to the Chief Investigator and the Sponsor using the relevant Pregnancy Reporting Form within 24 hours of notification. Pregnancy reporting will stop at the point the patient completes participation in the trial (month 7). It is not necessary to report the pregnancies of trial participant partners in this trial.

Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/fetus. If the outcome meets the serious criteria, this would be considered an SAE.

14 Toxicity – Emergency Procedures

In the event of toxicity reactions, standard medical practices will be employed, including emergency care and hospitalisation if needed. All medications in this trial are licensed for use, and their uses are familiar to the physicians and staff at all participating centres.

15 Evaluation of Results (Definitions and response/evaluation of outcome measures)

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.

15.1 Response criteria

Response is defined as at least a twofold increase in immunoglobulin titres in at least 50% of serotypes tested (at least 6 of the 12 serotypes tested).

Rationale for selection of primary endpoint

An endpoint of cases of pneumococcal infection is prohibitive in a trial such as this due to the huge sample size that it would require. Therefore, a serological marker of response to pneumococcal vaccine is used as a surrogate marker. However, there is no clear agreement between experts in the field of what level of serological response confers protection in the adult population. In children, a threshold of > 0.35mcg/ml, for each serotype tested, is viewed as protective. However, such a threshold has not been validated in adults.

Endpoints used in previous studies addressing response to pneumococcal vaccine in various diseases, have included fold changes (usually twofold or fourfold) in immunoglobulin levels (titres) pre and post vaccination, or threshold values of > 0.35mcg/ml, > 0.5mcg/ml, or > 1mcg/ml post vaccination. In addition, some studies considered response to just one or two serotypes, while others considered responses to all serotypes. Serological endpoints have been widely used in vaccine studies which have directly influenced clinical practice, and recent data indicates that a poor vaccine response is associated with a higher risk of all-cause mortality, making it a highly relevant surrogate endpoint in this patient population (Morgan, 2016). In addition, data from the same cohort indicate that although antibody titres decline over time, they remain protective for 2 years, and thus a 28 day primary endpoint is appropriate.

16 Storage and Analysis of Samples

A detailed laboratory manual will be produced which will explain sample collection and storage procedures, and the practicalities of performing the assays.

16.1 Collection and processing of serum samples (all subjects)

Venous blood samples will be obtained at month 0 (baseline), month 1 after initial vaccine, month 6 (immediately prior to second vaccine) and month 7. Blood samples will be collected and centrifuged according to the laboratory manual. Following centrifugation, serum should be apportioned into clean, labelled tubes prior to storage in a -70°C to -80°C freezer. Samples will be labelled with a participant's unique trial identification number.

16.2 Blood collection and PBMC separation (AAV patients)

Whole blood will be collected into citrated tubes at months 0, 1, 6 and 7, and processed within 6 hours of sampling. Peripheral blood mononuclear cells (PBMCs) will be separated before being transferred into a cryo freezing container and placed in a -70°C to -80°C freezer.

The following assays will be centrally processed using stored serum and PBMC:

- Determination of serum IgG concentrations to 12 of the pneumococcal serotypes by LUMINEX– this will be performed on all participants (including healthy controls) at months 0, 1, 6 and 7.
- Determination of SARS CoV-2 antibody responses on samples collected after December 2020 when SARS CoV-2 vaccination became standard of care in this population.
- Immunophenotyping of B and T cells – this will be performed on all AAV patients from group 1 and 2 at month 1 and 7, and between 50% and 100% of subjects at month 0 and 6. Phenotyping will be performed on as many subjects as possible, depending on available funding.

Evaluation of the exploratory endpoints is a balance between obtaining maximal mechanistic information as part of the study, at an acceptable cost, particularly as the exploratory assays are more expensive that the pneumococcal antigen LUMINEX, on which the primary endpoint is based. As such, the exploratory assays will only be performed on a subset of samples (no additional blood samples need to be taken from subjects, as all exploratory assays can be performed on the samples taken to evaluate the primary and secondary endpoints).

The trial coordinator will liaise with sites to coordinate batch shipment of samples in secure, protective packaging to the receiving lab in Cambridge or Birmingham. On receipt, samples will be logged and stored in an approximately -70°C to -80°C freezer prior to processing. Analysis will be performed using validated analytical methods in compliance with local standard operating procedures. Unused samples will be stored for use in future approved research unless otherwise requested by the participant.

16.3 Additional samples 7 +/- 2 days after Pneumococcal Polysaccharide Conjugate vaccine and Pneumococcal Polysaccharide Vaccine administration (AAV patients recruited in Cambridge only)

For those subjects who consent to additional visits, a 2.5ml RNA PAXgene sample and 30ml whole blood for PBMC separation and lymphocyte phenotyping will be taken at each additional visit. For those subjects who consent to these additional visits, an additional two 2.5ml RNA Paxgene samples will also be taken at month 0 and month 6, for comparison, which would not otherwise be taken as part of the trial protocol. Surrogate markers of T cell exhaustion using RTPCR will be run on the PAX gene samples.

17 Statistics

17.1 Statistical methods

Summary statistics will be produced for each arm of the observed response rate, with exact binomial confidence intervals. The treatment arms 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.

Secondary analyses will include the following between group comparisons for the proportion of patients who respond to the Pneumococcal Polysaccharide Conjugate Vaccine, measured at 28 days:
a. Disease controls (Group 2) to healthy controls
b. Rituximab treated AAV patients (Group 1) to healthy controls
A detailed statistical analysis plan will be produced before the final database lock.

17.2 Interim analyses

An interim analysis will be performed as soon as serological data is available in light of the Covid-19 pandemic based on all patients recruited (approximately 50% of targeted number of participants in rituximab group and disease control group) and blood samples collected by 31 March 2020. Understanding vaccine responses, in immunosuppressed individuals is even more important at this time. Information on vaccine responses could have immediate implications on how clinicians treat their patients with regards to adjusting immunosuppressive medications, as well as informing the design of future trials of potential SARS CoV-2 vaccines in immunocompromised individuals. . The confidential interim analysis result will be reviewed by the Independent Trial Steering Committee. The Haybittle-Peto stopping rule will be a guidance for the Trial Steering Committee TSC to make a recommendation whether the primary objective is achieved earlier. If the study is to be continued as it is, the TSC will make recommendation what and how the interim results could be presented.
The Trial Management Group and Trial Steering Committee will continue to also review efficacy and safety data, and will advise on the need for any additional analyses or alterations to the conduct or even continuation of the trial if there are major safety concerns.

17.3 Number of Participants to be enrolled

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%. However, there are important considerations when reviewing this data. A number of response definitions have been used, and the timing and dosage of drugs is variable. Patients with AAV often have a history of a much higher prior burden of immunosuppression than patients with rheumatoid arthritis or inflammatory bowel disease, and may therefore not mount as good response to vaccination. Furthermore, the rituximab maintenance treatment strategy employed results in much higher cumulative doses of rituximab, which is again likely to have a negative impact on vaccine response.

Author and	Disease	Response	Rituximab (RTX)	Control

date		**definition**		**(disease)**
Bingham (2010)	Rheumatoid Arthritis	>twofold increase in at least 6 serotypes	X	MTX 61%
Kapetanovic (2006)	Rheumatoid Arthritis	>twofold increase in serotypes 23F and 6B	X	MTX ~15%
Kapetanovic (2011)	Rheumatoid Arthritis	>twofold increase in serotypes 23F and 6B	10% (90% - vaccinated within 6 months of RTX)	MTX ~20%
Mori (2013)	Rheumatoid Arthritis	>twofold increase in serotypes 23F and 6B	X	MTX ~38%
Nazi (2013)	Immune thrombocytopenic purpura	>twofold increase in antibody concentration	21% (6 months post RTX)	X

MTX – methotrexate; RTX – rituximab

Data from 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, data from 20 healthy controls will be required. 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 with primary endpoint data available will be required, and only a maximum of 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.

17.4 Criteria for the premature termination of the trial

In order to standardise assays, all samples to measure vaccine response will be processed in batch at the end of the trial, therefore, stopping rules for efficacy or futility are not applicable. All serious adverse events, including adverse reactions directly related to the vaccines will be reported, and reviewed, and any safety concerns discussed by the Trial Management Group who will also send a summary of SAEs to the Trial Steering Committee for their review.

17.5 Procedure to account for missing or spurious data

A detailed statistical analysis plan will be produced before the final database lock and this plan will include sub-group analyses. The only aspect of missing data that will affect the primary analysis is the handling of patients who drop out before 28 days. Wherever possible, reasons for participant withdrawal will be captured in the CRF. If more than 5% of data is missing, then imputation methods will be used.

17.6 Definition of the end of the trial

The competent authority and Ethics Committee must be notified of the end of a clinical trial within 90 days of its completion. The end of trial will be the date of the last participant's last visit 7 months after enrolment.

18 Data handling and record keeping

18.1 CRF

All data will be transferred into a Case Report Form (CRF) which will be pseudo-anonymised using the participant's trial identifier. All trial data in the CRF must be extracted from and be consistent with the relevant source documents. The CRFs must be completed, dated and signed by the investigator or designee in a timely manner. It remains the responsibility of the investigator for the timing, completeness, legibility and accuracy of the CRF pages. The CRF will be accessible to trial coordinators, data managers, the investigators, Clinical Trial Monitors, Auditors and Inspectors as required.

Completed originals of the CRFs should be returned to the trial coordination centre (Cambridge) within 2 weeks of the pages being completed.

The investigator will retain a copy of each completed CRF page at site. If faxed, they will retain the original. The investigator will also supply the trial coordination centre with any required, anonymised background information from the medical records as required. The investigators must ensure that the CRFs and other trial related documentation is sent to the trial coordination centre containing no participant identifiable data.

All CRF pages must be clear, legible and completed in black ink. Any errors should be crossed with a single stroke so that the original entry can still be seen. Corrections should be inserted and the change dated and initialled by the investigator or designee. If it is not clear why the change has been made, an explanation should be written next to the change. Typing correction fluid must not be used.

Trial monitors will perform ongoing source data verification according to the trial specific monitoring plan, to confirm that data entered into the CRF by authorised site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the trial is being conducted in accordance with the currently approved protocol and any other trial agreements, GCP, and all applicable regulatory requirements.

18.2 Source Data

To enable peer review, monitoring, audit and/or inspection the principal investigators must agree to keep records of all participating participants (sufficient information to link records e.g., CRFs, hospital records and samples), all original signed informed consent forms and copies of the CRF pages. Source data includes patient medical records (both paper and electronic), signed informed consent forms, and online test results. Completed BVAS/WG and VDI forms in the CRF will also constitute source data.

18.3 Data Protection & Participant Confidentiality

All investigators and trial site staff involved in this trial must comply with the requirements of the the EU General Data Protection Regulation (GDPR), Data Protection Act 2018 and and Trust Policy with regards to the collection, storage, processing, transfer and disclosure of personal information and will uphold the Act's core principles. Only link anonymised data will be shared, and the PI at each site must ensure that only anonymised data is received by the trial coordinating centres.

19 Trial Management Group and Trial Steering Committee

The role of the Trial Steering Committee (TSC) is to provide overall supervision of the trial on behalf of Arthritis Research UK. The TSC will monitor the progress of the trial and maximise the chances of completing the trial within the agreed time scale and budget. In addition, they will receive a summary of all SAEs to ensure that there are no major safety concerns. The TSC will meet regularly via teleconference.

The Trial Management Group (TMG) is responsible for the design, conduct and overall management of the trial. The members meet via regular teleconferences.

20 Ethical & Regulatory considerations

20.1 Ethical committee review

Before the start of the trial or implementation of any amendment we will obtain approval of the trial protocol, protocol amendments, informed consent forms and other relevant documents (e.g. advertisements and GP information letters, if applicable) from the REC. All relevant correspondence with the REC will be retained in the Trial Master File and/or Investigator Site File(s).

Annual reports will be submitted to the REC in accordance with national requirements. It is the Chief Investigator's responsibility to produce the annual reports as required.

20.2 Regulatory Compliance

The trial will not commence until a Clinical Trial Authorisation (CTA) is obtained from the MHRA. The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments.

Development Safety Update Reports (DSURs) will be submitted to the MHRA in accordance with national requirements. It is the Chief Investigator's responsibility to produce the annual reports as required.

20.3 Protocol Amendments

Protocol amendments must be reviewed and agreement received from the Sponsor for all proposed amendments prior to submission to the HRA, REC and/or MHRA.

The only circumstance in which an amendment may be initiated prior to HRA, REC and/or MHRA approval is where the change is necessary to eliminate apparent, immediate risks to the participants (Urgent Safety Measures). In this case, accrual of new participants will be halted until the HRA, REC and/or MHRA approval has been obtained.

20.4 Peer Review

The trial protocol has been designed by the Trial Management Group. The trial synopsis has been reviewed and approved as part of the application process for a Clinical Studies Grant from Arthritis Research UK.

20.5 Declaration of Helsinki and Good Clinical Practice

The trial will be performed in accordance with the spirit and the letter of the declaration of Helsinki, the conditions and principles of Good Clinical Practice, the protocol and applicable local regulatory requirements and laws.

20.6 GCP Training

All trial staff must hold evidence of appropriate GCP training or undergo GCP training prior to undertaking any responsibilities on this trial. This training should be updated every 2 years or in accordance with Trust policy.

21 Sponsorship, Financial and Insurance

The trial is jointly sponsored by Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge. The trial will be funded by Arthritis Research UK.

Cambridge University Hospitals NHS Foundation Trust, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to participants in the clinical trial caused through the negligence of its employees and honorary contract holders. There are no specific arrangements for compensation should a participant be harmed through participation in the trial, but no-one has acted negligently.

The University of Cambridge will arrange insurance for negligent and non-negligent harm to research subjects arising through participation in the clinical trial.

22 Monitoring, Audit & Inspection

The investigator must make all trial documentation and related records available should an MHRA Inspection occur. Should a monitoring visit or audit be requested, the investigator must make the trial documentation and source data available to the Sponsor's representative. All participant data must be handled and treated confidentially.

The Sponsor's monitoring frequency will be determined by an initial risk assessment performed prior to the start of the trial. A detailed monitoring plan will be generated detailing the frequency and scope of the monitoring for the trial. Throughout the course of the trial, the risk assessment will be reviewed and the monitoring frequency adjusted as necessary.

Remote monitoring will be conducted for all participating sites. The scope and frequency of the monitoring will be determined by the risk assessment and detailed in the Monitoring Plan for the trial.

23 Protocol Compliance and Breaches of GCP

Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on Clinical Trials and must not be used.

Protocol deviations, non-compliances, or breaches are departures from the approved protocol. They can happen at any time, but are not planned. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.

Deviations from the protocol which are found to occur constantly again and again will not be accepted and will require immediate action and could potentially be classified as a serious breach.

Any potential/suspected serious breaches of GCP must be reported immediately to the Sponsor without any delay.

24 Publications policy

Ownership of the data arising from this trial resides with the trial team. On completion of the trial the data will be analysed and tabulated and a Final Trial Report prepared. Arthritis Research UK (funder) and the Cambridge Clinical Trials Unit will be acknowledged in any publications arising from data generated in this trial.

If requested, participants will be informed of the outcome of the trial by provision of a specifically designed newsletter, once the final results have been published.

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26 Appendices

26.1 Appendix 1 – Authorisation of Participating Sites

26.1.1 Required Documentation

This documentation is required prior to initiating a participating site.

Fully signed research agreement
Local R&D confirmation of capacity and capability (where applicable)
Protocol page signed by site PI
Copy of site specific PIS/ICF
Copy of site specific GP letter
Signed copy of delegation log
CVs and GCP of all individuals on delegation log
Laboratory accreditations and reference ranges
Site initiation form
Signed minutes of initiation teleconference

26.1.2 Procedure for initiating/opening a new site

An initiation teleconference will be required before opening a new site. At an absolute minimum the site PI, a research nurse and pharmacist must be present on the call. Dr Rona Smith will conduct the initiation teleconferences.

26.1.3 Principal Investigator Responsibilities

The PI's legal responsibilities will be listed in the Participating Site Agreement. The PI's will be expected to:

- Attend the initiation meeting/teleconference
- Train new members of the trial team in the protocol and its procedures
- Ensure the ISF is accurately maintained
- Disseminate important safety or trial related information to the trial team within their site
- Perform safety reporting within the timelines
- Lead on samples management for their site

26.2 Appendix 2 - Safety Reporting Flow Chart

