Full Title: Collection of White Blood Cells [Using Leukapheresis] from Rheumatoid Arthritis Patient's for the Validation of an Advanced Treatment for Rheumatoid Arthritis (tolDC)
Short Title/Acronym: GMP Validation of tolDC
Protocol Version & Date: V3.0 27Jun2022

Statement:

This protocol has regard for the HRA guidance.

1 RESEARCH REFERENCE DETAILS

IRAS Number: 276044

NHS REC Reference: 20/YH/0203

Research Registry & References:

Sponsor Name: The Newcastle upon Tyne Hospitals NHS Foundation Trust

Sponsor Reference: 9520

Funder Name: Versus Arthritis (formally Arthritis Research UK)
Funder Reference: 21811

2 SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted. The Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the UK Policy Framework for Health and Social Care Research, the relevant Standard Operating Procedures and other regulatory requirements as applicable.

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 investigation without the prior written consent of the Sponsor.

Representative of the Research Sponsor

Name:

(print)

Position:

Signature: **Date:**

Chief Investigator

Name: Professor John Isaacs

(print)

Signature: **Date:**

Representative of Newcastle Clinical Trials Unit

Name:

(print)

Position:

Signature: **Date:**

3 STUDY SUMMARY

Study Title	GMP Validation of tolDC	
Summary of Study Design	Single centre, product/process validation study	
Summary of Participant Population	Patients over the age of 18 with ACPA-positive RA	
Planned Sample Size	Up to 9 patients (to completion)	
Intervention Duration	Up to 1 day	
Follow Up Duration	4 days	
Planned Study Period	2 months	
	Objectives	**Outcome Measures**
Primary	To collect leukocytes from ACPA+RA patients to use for GMP validation of the manufacturing process for tolDC.	Successful leukapheresis from RA patients, obtaining a product suitable for the manufacture of tolDC.
Secondary	To manufacture, from RA patient leukocytes, tolDC that meet GMP validation criteria in the Newcastle Cellular Therapies Facility.	GMP validation of the proposed manufacturing method for tolDC and the final product. The development of an Investigational Medicinal Product Dossier for use in AuToDeCRA 2 clinical trials.
Procedure	Participants to undergo one cycle of leukapheresis to obtain leukocyte samples to be used in the validation process of tolDCs.	

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4 GLOSSARY OF ABBREVIATIONS

ACR	American College of Rheumatology
ACPA	Anti-citrullinated Protein Antibody
AE	Adverse Event
ALT	Alanine Aminotrasferase
AR	Adverse Reaction
ATMP	Advanced Therapy Medicinal Product
CAPA	Corrective and Preventative Actions
CI	Chief Investigator
CMV	Cytomegalovirus
COPD	Chronic Obstructive Pulmonary Disorder
csDMARD	Conventional Synthetic DMARD
DMARD	Disease-Modifying Anti-Rheumatic Drug(s)
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
ESR	Erythrocyte Sedimentation Rate
EU	European Union
FBC	Full Blood Count
GDPR	General Data Protection Regulations
GMP	Good Manufacturing Process
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HRA	Health Research Authority
HTA	Human Tissue Act
HTLV	Human T Lymphotropic Virus
IMPD	Investigational Medicinal Product Dossier
IRAS	Integrated Research Application System
ISF	Investigator Site File
IV	Intravenous
LFTs	Liver Function Tests
MHRA	Medicines and Healthcare products Regulatory Agency
NCTF	Newcastle Cellular Therapies Facility
NCTU	Newcastle Clinical Trials Unit
NEAC	Northeast Early Arthritis Cohort
NHS	National Health Service
NSAID	Non-Steroidal Anti-Inflammatory Drug
PI	Principal Investigator
RA	Rheumatoid Arthritis
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
tolDC	Tolerogenic Dendritic Cells
tsDMARD	Targeted Synthetic DMARD
QC	Quality Control
QP	Qualified Person
U&E	Urea and Electrolytes
ULN	Upper Limit of Normal

URSE	Unexpected Related Serious Event
USM	Urgent Safety Measure

5 OBJECTIVES AND OUTCOME MEASURES

The purpose of this study is to collect samples of leukocytes from Rheumatoid Arthritis (RA) patients which can be used to validate the production and quality of an advanced therapy medicinal product (tolDC). The samples obtained will be transferred to the Newcastle Cellular Therapies Facility where the validation processes will take place.

5.1 Primary Objective/Outcomes

The Primary objective of this study is to collect a sample of leukocytes from RA patients using leukapheresis. This sample will then be used use for GMP validation of the manufacturing process of tolDC.

The primary outcome will be successful leukaphereis from RA patients, obtaining a product suitable for manufacture of tolDC.

5.2 Secondary Objectives/Outcomes

The secondary objective of this study is to manufacture, from RA patient leukocytes, tolDC that meet GMP validation criteria in the Newcastle Cellular Therapies Facility.

The secondary outcome will be GMP validation of the proposed manufacturing method for tolDC and the final product.

The validation data provided by this study will be used for development of an Investigational Medicinal Product Dossier to be submitted to MHRA for a phase IIa randomised controlled trial investigating the optimal route of delivery of the advanced therapy medicinal product tolDC (AuToDeCRA 2).

6 STUDY DESIGN

6.1 Summary of Study Design

This is a single-centre non-controlled validation study. Leukocytes will be collected from Anti-citrullinated protein antibody (ACPA) positive Rheumatoid Arthritis (RA) patients and used for the GMP process and product validation of an advanced therapy medicinal product, called tolDC.

Leukocyte samples, used to manufacture tolDC, will be collected from participants by leukapheresis. Participants will be asked to undergo a number of screening assessments including ECG and blood tests to confirm that they have no contraindications to leukapheresis. Participants meeting all the eligibility criteria will undergo a single cycle of leukapheresis to obtain the leukocyte sample.

The acquired leukocyte samples will be transferred to the Newcastle Cellular Therapies Facility, a sterile GMP (Good Manufacturing Practice) facility, where the tolDC manufacture and validation work will be carried out. Up to 9 participants will be recruited to the study to ensure the 6 adequate samples are available for the GMP validation work. Recruiting up to 9 participants allows for attrition of participant leukapheresis samples, for example due to low quality/inadequate samples for the required work.

6.2 Process and Product Validation

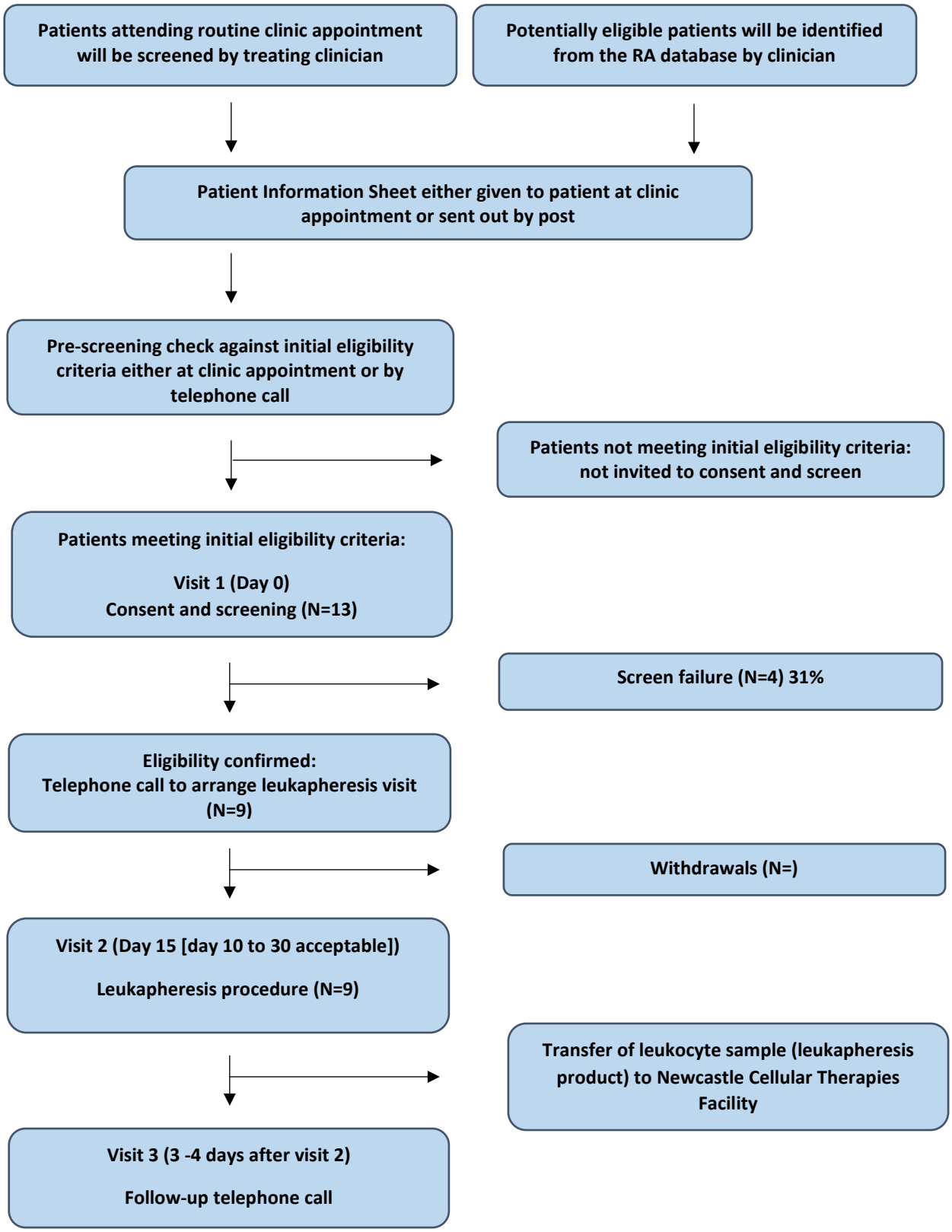
The validation process will demonstrate whether we are able to make tolDC from leukocyte samples in a sterile, GMP facility, a facility that must be used to make cells that will be given to patients. The GMP facility used in this study is the Newcastle Cellular Therapies Facility. The entire process of manufacture of tolDC will be thoroughly documented and will use only specially produced reagents (ultra-clean and sterile). As part of the validation process we will develop quality control or 'release' criteria that determine whether a particular batch of tolDC is suitable for treatment of a patient.

The validation master plan dictates that 3 process engineering runs are performed followed by 3 full validation runs to qualify a manufacturing process before clinical use. This number of full validation runs is taken from EU guidance on advanced therapy medicinal product manufacture (EudraLex Vol. 4 GMP Guidelines on GMP specific to ATMPs) which states: 'It is generally accepted that, as a minimum, three consecutive batches manufactured under routine conditions constitute a validation of the process'. In addition to the validation runs, as a new method for manufacturing tolDC will be used in this study (including peptides, labelling with ^{19}F and cryopreservation) 3 process engineering runs also need to be performed to provide material to validate the safety testing prior to use and ensure that any refinements to the manufacturing process or QC testing are incorporated before the final validation runs.

The work performed in this study will be collated and data used to produce an Investigational Medicinal Product Dossier (IMPD) which will contain detail of processes and the final 'release criteria' of the tolDC. The IMPD will ultimately be submitted to the body that regulates medicines (the Medicines and Healthcare Products Regulatory Agency or MHRA) for approval to use tolDC in future clinical trials. Once the IMPD is approved by the MHRA, the Qualified Person (QP) will use this document to release tolDC batches for use during any future clinical trials.

The IMPD and MHRA application for clinical trial authorisation are a separate study and are not covered in this protocol.

6.3 Flow Chart of study activity



6.4 STUDY SETTING

This is a single centre study set in secondary care. All clinical study activity including leukapheresis will take place at one of the Newcastle Hospitals which are part of The Newcastle upon Tyne Hospitals NHS Foundation Trust. Once collected leukocyte samples will be transferred from the Newcastle Hospitals to the Newcastle Cellular Therapies Facility where all manufacture and validation work will take place.

7 STUDY PARTICIPANTS

7.1 Justification of study population

Data generated in this study will be used to develop an IMPD for use in a future randomised controlled trial of tolDC, an Advanced Therapy Investigational Medicinal Product. To optimise the validation process it is essential that the study population recruited to this study match as closely as possible to the population that that will be participating in any future clinical trials.

TolDC are autologous dendritic cells primed *ex vivo* in a context designed to induce tolerogenic responses to citrullinated autoantigen(s) – themselves proposed to provoke autoimmunity in RA patients positive for ACPA. Therefore this is the optimal patient population in whom to deploy this particular tolDC therapy. To identify them, patients who are known to have ACPA positive RA will be screened for eligibility from clinical databases and outpatient clinics.

7.2 Inclusion Criteria

- ACPA positive RA based on local NHS laboratory cut-off. If ACPA titre is <3 times laboratory upper limit of normal, patients must also be RF positive based on local NHS laboratory cut-off
- Age 18 years or over
- Ability to provide written informed consent
- Receiving methotrexate, either as monotherapy or in combination with additional conventional synthetic disease modifying drug(s) (csDMARDs) at a stable dose for at least 4 weeks prior to screening
- At least 6 months since diagnosis of RA
- ACR Functional Class I-III
- Active disease – DAS 28 >3.2

7.3 Exclusion Criteria

- Use of investigational medicinal products within 30 days prior to screening date
- Receiving biologic DMARD or targeted synthetic DMARD (tsDMARD)
- Receiving glucocorticoids by any route accept topical/inhaled within 4 weeks of screening (nasal spray permitted)

- Receiving non-steroidal anti-inflammatory drugs (NSAID) at an unstable dose. Patients may be receiving NSAID prior to screening, provided the dose has been stable for at least 4 weeks prior to screening.
- Serious or unstable co-morbidity deemed unsuitable by PI, eg. COPD, cardiac failure
- Any known medical condition or contra-indication to leukapheresis, e.g. positive serology screen for hepatitis B or C, HIV infections
- History of hepatitis B or C, syphilis, HIV, CMV or HTLV-1/2 infections
- History of recurrent or chronic infection
- Pregnancy, or women planning to become pregnant within the study period, or women who are breast feeding
- Known hypersensitivity to local or systemic corticosteroid therapy or local anaesthetic
- Poor venous access or medical condition precluding leukapheresis
- Infection requiring hospitalization or IV antibiotics within 6 weeks of leukapheresis procedure
- Immunization with live vaccine within 6 weeks of leukapheresis procedure
- Anaemia defined as Hb<10g/dL; neutrophils< 2.00 $x10^9$/L; platelets <150$x10^9$/L
- Known active infection at screening visit or at screening (except fungal nail infection)

NB: Enrolling a patient onto the study who does not meet the inclusion/exclusion criteria is considered a protocol waiver. PROTOCOL WAIVERS ARE NOT PERMITTED.

8 STUDY PROCEDURES

8.1 Recruitment

8.1.1 Patient Identification

ACPA positive RA patients over the age of 18 will be identified by the Rheumatology outpatient department. Strategies for participant identification will be as follows:

First, to facilitate identification of RA patients already known to be ACPA+ RA, a **departmental database** of such individuals who have consented to be approached for future studies will be utilised. The Northeast Early Arthritis Cohort (NEAC) is an inception cohort of patients referred from primary care with a suspected new-onset inflammatory arthritis who consent to participate in observational research. Up to 14 individuals are enrolled every week, of whom 20% are diagnosed with RA and >250 patients with that diagnosis have already participated in this way. Importantly, the majority of these participants have given consent to be contacted to consider participation in future studies through donation of additional blood sample(s). The NEAC database captures detailed demographic, laboratory and treatment data on all cohort participants – including diagnosis and autoantibody status at inception and subsequent decision(s) regarding methotrexate treatment.

Having been identified in this way, ACPA positive RA patients will be approached during planned hospital appointments by a member of their usual care team or the rheumatology research team. Hospital appointments may be in the form of face to face visits or remote appointments by telephone, the format of the appointment will be determined by the rheumatology team in line with their standard practice. Patients will either be provided with a written information sheet at their face to face visits or by post. Alternatively, where electronically recorded evidence of current methotrexate treatment can be confirmed, information sheets may be posted to such individuals by a member of the rheumatology research team, together with contact information for the rheumatology research nurse to discuss further. If the patient is interested in participating in the study, then they can inform the rheumatology research team who will undertake a pre-screening check (see below) and, if appropriate, arrange for a screening appointment.

Second, patients with a known diagnosis of ACPA+ RA not already consented on the departmental database, will be identified during routine hospital appointments. Hospital appointments may be in the form of face to face visits or remote appointments by telephone, the format of the appointment will be determined by the rheumatology team in line with their standard practice. A member of the patients usual clinical care team will make the first approach and explain that a research study is being conducted for which they may be eligible. Patients will be provided with a written patient information sheet by the treating clinician or the rheumatology research team in person at face to face appointments or by post where remote appointments have taken place. If the patient is interested in finding out more information about the study but is not provided with a patient information sheet their clinical appointment, this will be sent out by post with a covering letter and an opt-in slip to return if the patient is interested in taking part.

8.1.2 Pre-screening check

A pre-screening checklist will be completed by a member of the clinical team to determine if a patient who has contacted the department expressing interest in study participation is likely to be eligible. The pre-screening checklist could be completed during a routine appointment for patients identified in the clinic or via telephone for those who contact the department following receipt of a written information sheet. This will ensure that patients who do not meet the initial eligibility criteria are not asked to attend the hospital for any study-specific screening procedures. The following initial eligibility criteria will be confirmed:
Patients must be:

- Receiving methotrexate, either as monotherapy or in combination with additional conventional synthetic disease modifying drug(s) (csDMARDs) at a stable dose for at least 4 weeks prior to baseline
- If patients are receiving non-steroidal anti-inflammatory drugs (NSAID) the dose must have been stable for at least 4 weeks prior to screening.

Patients must not be/have;

- Using/have used another investigational medicinal products within 30 days prior to screening date
- Receiving biologic DMARD or targeted synthetic DMARD (tsDMARD)

- Receiving glucocorticoids by any route except topical/inhaled within 4 weeks of screening (nasal spray permitted).
- Serious or unstable co-morbidity deemed unsuitable by PI, eg. COPD, cardiac failure
- History of recurrent or chronic infection
- History of hepatitis B or C, syphilis, HIV, CMV or HTLV-1/2 infections (contraindication to leukapheresis)
- Known pregnancy, or women planning to become pregnant within the study period, or women who are breast feeding
- Known hypersensitivity to local or systemic corticosteroid therapy or local anaesthetic.
- Poor venous access or medical condition precluding leukapheresis

If the patient meets the initial eligibility criteria a screening visit will be arranged.

8.2 Screening Visit

The screening visit will take place at one of the Newcastle Hospitals (part of the Newcastle upon Tyne Hospitals NHS Foundation Trust) at least 24 hours after receipt of the study information. Participants will be provided further opportunity to discuss the study and ask any questions with a study investigator who will be a member of the clinical team. If a patient decides to participate in the study then written informed consent will be obtained prior to any study-specific screening procedures**.**

8.3 Informed consent

Written informed consent will always be obtained prior to study specific procedures/investigations including screening blood draws. Participants in this study must have the capacity to consent for themselves. Proxy consent will not be allowed. Those wishing to take part will provide written informed consent by signing the study consent form, witnessed by the investigator with delegated responsibility (as recorded in the delegation log).

The original signed consent forms will be retained in the Investigator Site File, with copies in the hospital notes and a copy provided for the participant. The participant will specifically consent to inspection of their records by the Sponsor, Sponsor's representatives and representatives of regulatory and ethical authorities.

The right to refuse to participate without giving reasons will be respected.

Due to the small subject population, the information sheet and consent form for the study will be available only in English.

8.4 Study specific screening procedures

Following informed consent for the study, the participant's past medical history, full list of concomitant medication and history of blood donation will be documented in their medical records. A physical examination will be carried out consisting blood pressure, pulse, weight and height.

If any exclusions are identified at this time the patient will be deemed ineligible.

Subjects who remain potentially eligible will then go on to have the following study specific screening tests;

- Safety blood tests (FBC, U&E, LFT, ESR, clotting, electrolytes and calcium). Criteria to proceed:
    - Hb >10g/dL
    - Neutrophils >2.00x10^9/L
    - Platelets >150x10^9/L
    - eGFR >60ml/min/1.73m^2
    - Sodium and potassium within normal laboratory reference range.
    - Normal liver function; ALT and ALP <1.5xULN. Bilirubin within the normal laboratory reference range (unless there is a reasonable explanation, at the Principal Investigators discretion, i.e. Gilbert's Disease).
- DAS 28 assessment including; swollen and tender 28-joint count, Patient Global Health Questionnaire
- Blood sample for microbiological screening (HIV, syphilis, hepatitis B and C, CMV and HTLV 1 and 2; these tests are a requirement before any of the participants' cells can enter the GMP facility)
- An electrocardiogram

A pregnancy test (urine) is required at screening for any female patients of childbearing potential (post-menopausal women must be amenorrhoeic for at least 12 months to be considered of non-childbearing potential).

Once all screening results have been reported and eligibility confirmed by an investigator the participant will be contacted to make an appointment for leukapheresis.

8.4.1 Screening Failure

If the patient is deemed ineligible for study enrolment the patient will be contacted by telephone to explain this. If there is discovery of an unknown medical condition as a consequence of medical examination, ECG recording or serological screening, counselling and clinical follow up will be arranged as necessary. In such cases the patient will be contacted by telephone and either asked to come back to discuss the results with medical staff in the outpatient clinic or results will be discussed over the phone, depending on the nature and significance of the problem. A letter will be sent to the patient's GP underlining the findings and advising follow-up, with a copy sent to the patient.

8.5 Leukapheresis Procedure

This visit will take place no longer than 30 days after screening. Participants will visit the apheresis unit at the Freeman Hospital (part of The Newcastle upon Tyne Hospitals NHS Foundation Trust) for approximately half a day. All activities will be carried out by the clinical leukapheresis care team as per standard care for patients undergoing this procedure.

Before the procedure the Specialist Nurse/clinician will explain the procedure and answer any questions before obtaining written consent for the procedure using the standard Trust consent form for cell procurement.

On the day of the procedure and prior to the procedure a further safety blood test (FBC, U&E, magnesium and calcium) and blood pressure check are required, as per standard practice for leukapheresis.

The participant will be semi-supine on a bed and connected to the leukapheresis cell separator via a cannula in both antecubital fossa or forearm veins. The procedure takes up to three and a half hours and will only take the length of time needed to collect the sufficient number of cells required (approximately 100ml sample). When completed the needle and cannula are removed. After leukapheresis the patient will have their blood pressure checked and remain in the ward for a minimum of 10 minutes before being allowed to leave.

8.6 Post intervention Assessments

A telephone follow-up consultation will take place approximately 3-4 days after leukapheresis and will be performed by a delegated member of the study team. A series of questions ascertaining to any adverse events will be asked, the answers of which will be recorded in the participants medical records.

8.7 Schedule of Events

The schedule of events and study activities are summarised in the table below:

Parameters	**Pre-screening**	**Screening** **Visit 1**	**Leukapheresis** **Visit 2**	**Follow-up Phone call Visit 3**
		Day 0	**Day 15 (Day 10-30 acceptable)**	**3-4 days following visit 2**
Pre-screening Questions	x			
Patient Information Sheet	x			
Screening Visit				
Written Informed Consent		x		
Medical History		x		
Current Medication		x		
History of blood donation		x		
Physical Examination (Cardio-respiratory)		x		
Blood pressure, pulse, height and weight, venous access		x		
If no exclusion criteria identified patient will go on the following				
Safety bloods (FBC, U&E, LFT, ESR, clotting, electrolytes, calcium, magnesium)		x		
Screening bloods (HIV, syphilis, hepatitis B and C, CMV and HTLV 1 and 2)		x		
Urine Pregnancy test (for females of child bearing potential)		x		
ECG (12 lead)		x		
Swollen and Tender 28-Joint Count		x		
Patient Global Health VAS Questionnaire		x		
DAS28		x		
Confirmation of eligibility		x		
<u>Leukapheresis visit</u>				
Consent for Leukapheresis procedure			x	
Further safety bloods (FBC, U+E, magnesium, calcium)			x	
Blood pressure prior to procedure			x	
Leukapheresis procedure			x	
Blood pressure 10 minutes after procedure			x	
Adverse Events		**<u>x</u>**	x	x

8.8 Withdrawal Criteria

Given the short duration of study participation it is not foreseen that there will be a large number of withdrawals. However, the following criteria will apply in the case of withdrawals after screening or before, during and after the leukapheresis procedure.

Participants have the right to withdraw from the study at any time without having to give a reason. The Investigator site should try to ascertain the reason for withdrawal and document this reason within the Case Report Form and participant's medical notes.

The Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Participant does not have two veins suitable for the procedure
- Significant protocol deviation or non-compliance with the study procedure
- Investigator's discretion that it is in the best interest of the participant to withdraw
- An adverse event that requires discontinuation of the leukapheresis procedure
- Termination of the study by the sponsor

Participants who are withdrawn from the study will be replaced to ensure up to six suitable samples are obtained.

8.9 Storage and Analysis of Samples

It is the responsibility of the study site to ensure that samples are appropriately labelled in accordance with the study procedures to comply with the Data Protection Act. Biological samples collected from participants as part of this study will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act and 2006 Human Tissue (Scotland) Act.

The HTA consent for cell procurement will ask if the participant agrees for their cells to be stored for further research. This consent for further research will be optional.

Participant research samples will be labelled with the participant's unique study identification number only. Participant samples for tolDC manufacture will be packaged in a validated shipping container and transported by courier at ambient temperature as soon as possible after collection and on the same day as collection to the Cellular Therapies Facility at Newcastle University. The courier used for the transport of material is 1NE and should be booked using the ATOM electronic booking system. If consent for further research has not been given the samples will be destroyed at the end of the validation process.

8.10 End of Study

The end of the study will after the last follow up telephone call after the last participant has undergone leukapheresis.

9 STUDY PROCEDURE

Leukapheresis is a routine procedure used, for example, for removal of white blood cells from a donor in preparation for stem cell transplantation.

This procedure will take place at the Freeman Hospital (part of The Newcastle Upon Tyne Hospitals NHS Foundation Trust). The procedure will be carried out by the specialist nursing staff (or trained deputy), who routinely perform this procedure. Prior to the procedure routine hospital consent will be obtained by the leukapheresis team, as well as routine safety blood tests and a blood pressure check. Following the results of safety blood tests the procedure will take place.

The participant will be semi-supine on a bed and connected to the leukapheresis cell separator via a cannula in both antecubital fossa or forearm veins. The procedure takes up to three and a half hours. When completed the needle and cannula are removed and the participants are able to leave after 10 minutes, following a further blood pressure check.

9.1 Known Risks

There is a possibility of discovery of unknown chronic infection or medical condition as a consequence of serological screening and examination (counselling and clinical follow up will be arranged as necessary). Participants will be made aware of the tests that they will undertake prior to consent both verbally during screening and written in the patient information leaflet.

As with all blood sample collection there is a small risk of the following occurring, these are the known risks of leukapheresis;

- local anaesthetic, which may be used if required prior to cannulation, causing a 'stinging' sensation
- venepuncture and cannulation may cause pain, and lead to subsequent bruising
- arterial puncture with venepuncture and cannulation
- participants feeling faint during or after the procedure

A small amount of red cells will 'contaminate' the leukocyte product, leading to a very small risk of anaemia.

Citrate is used to prevent the blood clotting in the cell separator and citrate toxicity (a drop in blood calcium levels) may occur during the procedure, leading to tingling in the fingertips or around the mouth. Participants will be asked to report these symptoms if they occur, leading to a slowing of the leukapheresis procedure.

10 SAFETY REPORTING

10.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant, including occurrences which are not necessarily caused by or related to the intervention under study.
Adverse Reaction (AR)	An untoward or unintended response in a participant to which is related to the intervention under study i.e. that a causal relationship between the study intervention and an AE is at least a reasonable possibility and the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the study intervention qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • Results in death • Is life-threatening* • Requires inpatient hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect • Other important medical events that jeopardise the participant or require intervention to prevent one of the above consequences * - life-threatening refers to an event in which the participant was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to the study intervention, based upon the information provided.
Unexpected Related Serious Event (URSE)	A serious related event, the nature and severity of which is not consistent with the known information about the intervention under study.

10.2 Recording and Reporting AEs and SAEs

All AEs and SAEs occurring from the day of consent until the four day follow up telephone call is completed must be reported. Depending on the nature of the event, the reporting procedures below

will be followed. Any questions concerning adverse event reporting will be directed to the Chief Investigator or Principal Investigator in the first instance.

Adverse Event (including Adverse Reaction)

All non-serious adverse events / reactions observed at study visits, including during and after leukapheresis, will be reported on the AE study eCRF.

Severity of AEs will be graded on a three-point scale (Mild, Moderate or Severe as described in Table 10-1). Relation of the AE (i.e. causality) to the treatment will be assessed by the investigator at site.

AE Grade	Description
Mild	Discomfort is noticed but there is no disruption of normal daily activities
Moderate	Discomfort is sufficient to reduce or affect normal daily activities
Severe	Discomfort in incapacitating with inability to work or to perform normal daily activities

Table 10-1. AE Severity Grading

Expected adverse reactions

Adverse events and adverse reactions that occur in this study, whether they are serious or not, are expected treatment-related toxicities due to the study procedures:

- Citrate toxicity, with the most likely manifestations being tingling in the fingertips or a 'pins and needles' sensation.
- Venepuncture and cannulation may cause pain and lead to subsequent bruising.
- Anaemia

As this is a routine procedure for specialist haematology a low potential for unexpected adverse reactions exists.

Protocol Specifications

For purposes of this protocol:

All non-serious adverse reactions will be elicited and recorded at the study visit and telephone follow-up.

Any serious adverse events will be recorded from the day of consent and will end after the four day follow-up telephone call.

- For each SAE the following information will be collected:
    - Full details in medical terms and case description
    - Event duration (start and end dates, if applicable)
    - Action taken
    - Outcome
    - Seriousness criteria
    - Causality in the opinion of the investigator
    - Whether the event is considered expected or unexpected.

Initial SAE reports and any change of condition or other follow-up information should be sent via confidential email to the NCTU as soon as it is available or at least within 24 hours of the information becoming available. Events will be followed up until the event has resolved or a final outcome has been reached.

10.3 Recording and Reporting Unexpected Related Serious Events (URSEs)

As Leukapheresis is a routine clinical procedure to collect leukocyte samples it is not anticipated that there will URSE's. Any URSEs occurring from the intervention until 4 days post termination of the leukapheresis will be reported to the NHS REC. The NCTU, on behalf of the Sponsor, will perform this reporting.

The assessment of expectedness will be performed by the CI against the known information for the leukapheresis procedure. The reference safety information (RSI) for this study is section 9.1 – the known risks of leukapheresis. This should be used when judging expectedness.

URSEs must be reported no later than 15 calendar days after the NCTU has first knowledge of the event. Any relevant follow-up information should be sought and reported as soon as possible after the initial report.

As soon as a site suspects that a SAR may be a USRE they must contact the CI, sponsor representative and the trial manager immediately. The reporting timeframe starts at day 0 when the NCTU and/or Sponsor are in receipt of a minimum set of information:

- Sponsor study reference and study name (sponsor reference)
- Patient study number
- Date of notification of the event
- Medical description of the event
- Date and time of the onset of the event (including event end date if applicable)
- Causality assessment
- Seriousness of the event, particularly if life threatening or fatal
- An identifiable reporter (e.g., Principal Investigator)

This information must be provided by email. The site is expected to fully cooperate with the Sponsor and NCTU in order that a full and detailed report can be submitted to the NHS REC within the required timelines.

10.4 Responsibilities

Principal Investigator

- Recording any AEs and ARs which occur during Leukapheresis or during the 4 day follow-up period in line with the requirements of the protocol.
- Using medical judgement in assigning seriousness and causality and providing an opinion on expectedness of events.
- Ensuring that all SAEs and SARs, including URSEs, are recorded and reported to the Sponsor within 24 hours of becoming aware of the event and provide further follow-up information as soon as available.

Chief Investigator

- Clinical oversight of the safety of study participants, including an ongoing review of the risk.
- Using medical judgement in assigning expectedness to SARs on behalf of sponsor.
- Immediate review of all URSEs.

Sponsor

- Expedited reporting of URSEs to the REC within required timelines (may be delegated).

10.5 Notification of Deaths

Only deaths that are assessed to have a causal relationship to the intervention will be reported to the sponsor. This report will be immediate.

10.6 Reporting Urgent Safety Measures

An Urgent Safety Measure (USM) is an action that the Sponsor or an Investigator may take in order to protect the subjects of a study against any immediate hazard to their health or safety. Upon implementation of an USM by an Investigator, the NCTU must be notified immediately and details of the USM given. The NCTU must inform the NHS REC within 3 days of the USM taking place in accordance with the Sponsor's/NCTU's standard operating procedures.

11 GMP VALIDATION PROCESS

11.1 Population

Data from the GMP validation of the samples from up to nine participants recruited to the study will be presented in an IMPD.

11.2 Validation Process

The study is designed to validate the manufacturing process and no formal statistical analysis is planned. Qualitative data only will be presented. Quality, manufacturing and control data will be analysed in the Newcastle Cellular Therapies GMP facility and will be documented in an Investigational Medicinal Product Dossier.

Primary objective (GM Process engineering): The TolDC product will be made at a new GMP manufacturing site and demonstrate that it is possible to produce cit-TolDC cells of sufficient yield for clinical use. The cells collected and processed at this stage will be used to refine and assess the analytical methods used in the validation phase.

Secondary Objective (GMP Validation): The data from validation process will be used to set acceptance criteria for essential safety properties of the TolDC cells and enable future batches to be assessed prior to use in a clinical trial. Data from this and the process-engineering phase will be presented in an IMPD for regulatory assessment.

The main points in the TolDC product life-cycle where data will be collected and used to set expected acceptance limits are:

- Starting material (the donated sample): cell number, viability (live cells versus dead cells), purity (percentage of different populations of cells).

- Final TolDC product: Cell number, viability, yield (number compared to cells at the start of the process), purity (number of TolDC cells compared to non-TolDC cells) potency (ability of cells to function as intended)

The properties of the cells in the final product specification will also be measured at additional time points during the manufacture of the TolDC product for example before and after adding a label to trace the cells and before and after cryopreserving (freezing) the cells.

The analysis at each point may also be performed in duplicate or triplicate as and when required for validation of the analytical method according to current GMP regulatory expectations.

11.3 Sample Size Calculations

No formal sample size calculation has been carried out for this study. Up to nine participants will undergo leukapheresis to obtain up to 6 adequate samples to complete the GMP validation work. The reason for this is the GMP validation master plan dictates that 3 process engineering runs and then 3 full validation runs are performed to qualify a manufacturing process before clinical use. It may be possible to perform the process engineering runs using blood products from the blood bank, if this is the case then participants would not be recruited to provide leukapheresis samples to these runs. However, if the use of blood products for the process engineering runs is unsuccessful then leukapheresis samples will be obtained from participants. Leukapheresis samples from participants will be used to perform the 3 full validation runs. This number is taken from EU guidance on advanced therapy medicinal product manufacture (EudraLex Vol. 4 GMP Guidelines on GMP specific to ATMPs) which states: 'It is generally accepted that, as a minimum, three consecutive batches manufactured under routine conditions constitute a validation of the process'. As the tolDCs have not previously been manufactured using the same method (i.e. including peptides, ^{19}F labelling and cryopreservation) an additional 3 process engineering runs will be performed to provide material to validate the safety testing prior to use and ensure that any refinements to the manufacturing process or QC testing are incorporated before the final validation runs.

12 DATA HANDLING

12.1 Data Collection Tools and Source Document Identification

Data collected on screening, recruitment and follow-up will be entered by the site staff onto study specific worksheets which will then be securely sent to NCTU. Data from the worksheets will be entered by NCTU staff into a password protected data repository. Participants will be identified on study documentation and the data repository by a unique study identifier. Data management will be the responsibility of the Newcastle Clinical Trials Unit who will run validation and consistency checks on the data and will perform Quality Control (QC) checks on the transfer of data from worksheets to the data repository. Any data queries will be communicated to site and resolved as appropriate. The product validation data will be the responsibility of the Newcastle Cellular Therapies Facility and will not be held in the study data repository.

12.2 Data Handling and Record Keeping

Overall responsibility for data collection lies with the Chief Investigator. Data will be handled, computerised and stored in accordance with The Data Protection Act 2018 and General Data Protection Regulations (GDPR) 2018. The overall quality and retention of study data is the responsibility of the Chief Investigator. All study data will be retained in accordance with the applicable regulations and local policies.

12.3 Access to Data

The study data and patient medical records may be reviewed for monitoring by NCTU, or for audit purposes by the Newcastle upon Tyne Hospitals NHS Foundation Trust or NCTU.

Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee or the REC. The PI and research site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Data will be accessed only by the study team involved with the validation, there will be no research findings for wider dissemination.

12.4 Archiving

In accordance with the UK Policy Framework for Health and Social Care Research, Sponsor and NCTU SOPs and other applicable regulatory requirements all study data will be retained for 30 years. Authorisation will be obtained from the Sponsor at the end of the archiving period to destroy the study documentation.

13 MONITORING, AUDIT & INSPECTION

Study monitoring will be undertaken by Newcastle Clinical Trials Unit, on behalf of the Sponsor, to ensure the study is conducted in accordance with the Research Governance Framework. The main areas of focus will include consent, serious adverse events, and essential documents in study files. A risk assessment of the study will be carried out at the outset and the level of monitoring will be commensurate with the perceived risk. A study monitoring plan will be developed based upon the risk assessment and will be agreed by the Trial Management Group and the Sponsor. Monitoring will consist of a combination of site self-monitoring and off-site monitoring:

- Completion by site staff of a self-monitoring form, provided by the NCTU, covering recruitment status, study participant documentation, the content of the study file and a review of any SAE forms.
- A consent proforma returned to the NCTU by the site will be used to verify that the appropriate version of the consent form has been used and all relevant sections have been completed correctly.

- An eligibility checklist for all participants entered in the study will be completed which will include a signed statement of eligibility by the PI and filed in the medical notes.
- Study sample logs will be reviewed centrally.

Study oversight will be provided by the Trial Management Group.

The study may be subject to audit by representatives of the Sponsor or inspection by other regulatory bodies. The investigator site will permit study-related monitoring, audits and regulatory inspection including access to all essential and source data relating to the study if required.

14 ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Research Ethics Committee Review and Reports

The NCTU will obtain a favourable ethical opinion from an NHS Research Ethics Committee (REC) prior to the start of the study. All parties will conduct the study in accordance with this ethical opinion.

The NCTU will notify the REC of all required substantial amendments to the study and those non-substantial amendments that result in a change to study documentation (e.g. protocol or patient information sheet). Substantial amendments that require a REC favourable opinion will not be implemented until this REC favourable opinion is obtained. The NCTU will notify the REC and Sponsor of any serious breaches of GCP or the protocol, urgent safety measures or USREs that occur during the study.

An annual progress report will be submitted each year to the REC by NCTU until the end of the study. This report will be submitted within 30 days of the anniversary date on which the original favourable ethical opinion was granted.

The Sponsor will notify the REC of the early termination or end of study in accordance with the required timelines.

14.2 Peer Review

This study is funded by a grant from Arthritis Research UK and was subject to peer review of the research proposal before funding was awarded.

This protocol has been reviewed and authorised by the Sponsor, Chief Investigator and Newcastle Clinical Trials Unit.

14.3 Public and Patient Involvement

The study participant information will be reviewed by rheumatoid arthritis patient groups.

14.4 Regulatory Compliance

The study will be conducted in accordance with the UK Policy Framework for Health and Social Care Research. Before any site can enrol patients into the study, that site must have received NHS permission from the NHS Research & Development department.

14.5 Protocol Compliance

Prospective, planned deviations or waivers to the protocol are not allowed in this study and must not be used. Protocol deviations, non-compliances and breaches are departures from the approved protocol. These events should be documented on the deviation tracking log (this will be provided as part of the ISF). NCTU will ask the site to provide copies of their deviation tracking log periodically and before any monitoring visits

If the deviation constitutes a violation, the site must complete a protocol violation form (a blank template will be provided to the site as part of the ISF) and send a copy of this completed form to the Trial Manager in NCTU within 3 working days. The violation must also be entered on to the deviation tracking log.

Unintentional protocol deviations will be documented and reported to the CI and sponsor. Where necessary, Corrective and Preventative Actions (CAPA) will be implemented. Deviations that are found to frequently recur at a site are not acceptable and could be classified as a serious breach

14.6 Notification of Serious Breaches to GCP and/or the Protocol

A serious breach is a breach which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the study; or
(b) the scientific value of the study

The sponsor must be notified immediately of any incident that may be classified as a serious breach. The NCTU will notify the NHS REC within the required timelines in accordance with the NCTU and Sponsor SOP.

14.7 Data Protection and Patient Confidentiality

All investigators and study staff must comply with the requirements of the Data Protection Act 2018 and General Data Protection Regulations (GDPR) 2018, with regards to collection, storage, processing and disclosure of personal information and access to data will be limited to the minimum number of individuals necessary for quality control, audit, and analysis. Any data leaving the site will identify participants by a unique study identification code only. Security of data will be maintained by the data and linking code being stored in separate locations within password protected folders or storage media.

The study data will be stored for 30 years. The Chief Investigator will be the data custodian.

14.8 Indemnity

The sponsor will provide indemnity in the event that study participants suffer negligent harm due to the management of the study. This indemnity will be provided under the NHS indemnity arrangements for clinical negligence claims in the NHS. The substantial employers of the protocol authors will provide indemnity in the event that study participants suffer negligent harm due to the design of the study. The study site will provide indemnity in the event that study participants suffer negligent harm due to the conduct of the study at their site. For NHS Organisations this indemnity will be provided under the NHS indemnity arrangements for clinical negligence claims in the NHS. NHS Organisations must ensure

that site staff without substantive NHS contracts hold honorary contracts to ensure they can access patients and are covered under the NHS indemnity arrangements. Study staff without NHS contracts e.g. General Practitioners or Dentists will provide their own professional indemnity.

14.9 Amendments

It is the responsibility of the Research Sponsor to determine if an amendment is substantial or not and study procedures must not be changed without the mutual agreement of the CI, Sponsor and the Trial Management Group.

Substantial amendments will be submitted to the REC and will not be implemented until this approval is in place. It is the responsibility of the NCTU to submit substantial amendments.

Non-substantial amendments may be made at any time with a record of the amendment held in the Trial Master File. Any non-substantial amendment that requires an update to the study documentation will be submitted to the NHS REC for acknowledgement of the revised version of the document.

Substantial amendments and those minor amendments which may impact the site will be submitted to the relevant NHS R&D Departments for notification to determine if the amendment affects the NHS permission for that site. Amendment documentation will be provided to the study site by the NCTU.

14.10 Access to the Final Study Dataset

Access to the final study data will be restricted to the study team carrying out the validation. There will be no research findings for wider dissemination as part of this study.

15 DISSEMINATION POLICY

All data arising from the study will be the property of the Chief Investigator and Co-Investigators. We do not plan to formally publish the results of this validation study, rather it will inform the formal AuToDeCRA2 clinical trial. Results of the study will be reported to the Sponsor and Funder.

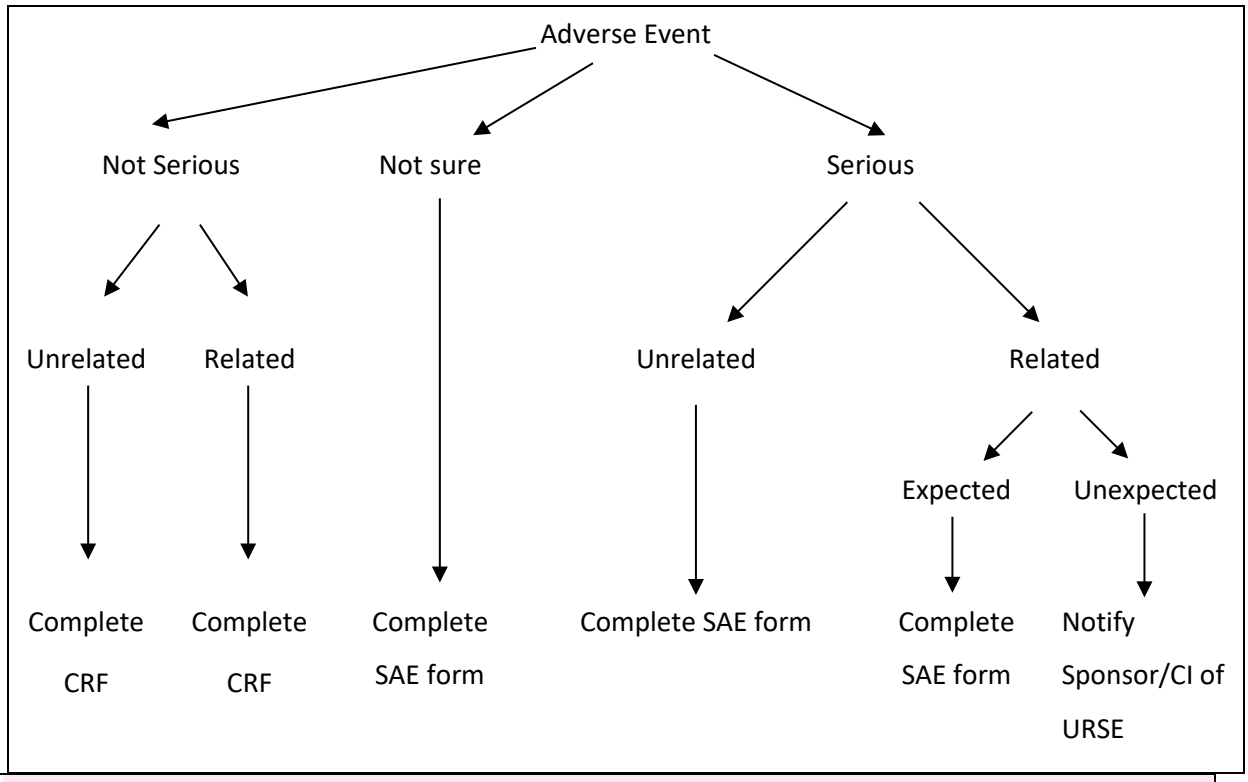
Individual participants will not be identifiable within any study report or publication. Participants will be informed about their treatment and their contribution to the study at the end of the study, including a summary of the results if they request this information.

16 REFERENCES

Bell GM, Anderson AE, Diboll J, Reece R, Eltherington O, Harry RA, Fouweather T, MacDonald C, Chadwick T, McColl EM, Dunn J, Dickinson AM, Hilkens CMU, Isaacs JD. **Autologous tolerogenic dendritic cells for rheumatoid and inflammatory arthritis**. *Annals of the Rheumatic Diseases* 2017, **76**(1), 227-234.

17 APPENDICES

17.1 Appendix 1 - Safety Reporting Diagram



Contact details for reporting SAEs and URSEs

Please send SAE form(s) via email: nctu.tolDCvalidation.sae@nhs.net

Appendix 2 – Amendment History

Amendment Number	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
Non-Substantial 01	V2.0	03Feb2021	Chrissie Butcher (Trial Manager)	Section 12.1 updated to confirm site will be collecting data on worksheets and will be entered onto the repository by NCTU staff. Other minor clarifications made.
Substantial 01	V3.0	27Jun2022	Michael White (Trial Manager)	Sections 6.1 and 11.3 updated to allow recruitment of up to 9 participants in order to get 6 adequate sample for GMP validation work. Other minor corrections made.