PROTOCOL

A phase II clinical trial of DARC

A Phase II, Open label, single centre, non-randomised phase II trial to further investigate DARC technology using ANX776 in healthy subjects and patients diagnosed with Glaucoma, Age-related Macular Degeneration, Down's Syndrome and Optic Neuritis

Protocol Version	V3.0
Date	10th November 2016
Sponsor	University College London
Sponsor Ref #	15/0959
Comprehensive Clinical Trials Unit Trial Adoption Group #	CCTU/'2015/240
Trial registration	ISRCTN: 10751859
CTA #	31894
NRES #	16/LO/1700
Public title	A Phase II Clinical Trial of DARC (DARC II)
Countries of Recruitment	UK
Health Condition(s) or Problem(s) Studied	Glaucoma, AMD, Optic Neuritis, Down's Syndrome
Intervention	Intravenous injection of a single dose of ANX776 (0.4mg) followed by assessment of retinal cells apoptosis performed using diagnostic retinal imaging
Study Type	Open label, single centre, non-randomised phase II trial to further investigate DARC technology using ANX776 in healthy subjects and patients diagnosed with Glaucoma, Age-related Macular Degeneration, Down's Syndrome and Optic Neuritis
Date of First Enrolment	1st January 2017
Target Sample Size	120 participants: - Healthy volunteers: 40 subjects - Glaucoma: 20 subjects - AMD: 20 subjects - Down's syndrome: 20 subjects - Optic Neuritis: 20 subjects

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1. Trial design

This Phase II trial will be a single-centre, open-label, cross-sectional study in patients diagnosed with Glaucoma, Age-related Macular Degeneration, Optic Neuritis, individuals with Down's syndrome, and healthy volunteers.

A sample size of 120 subjects is planned in 5 study groups:

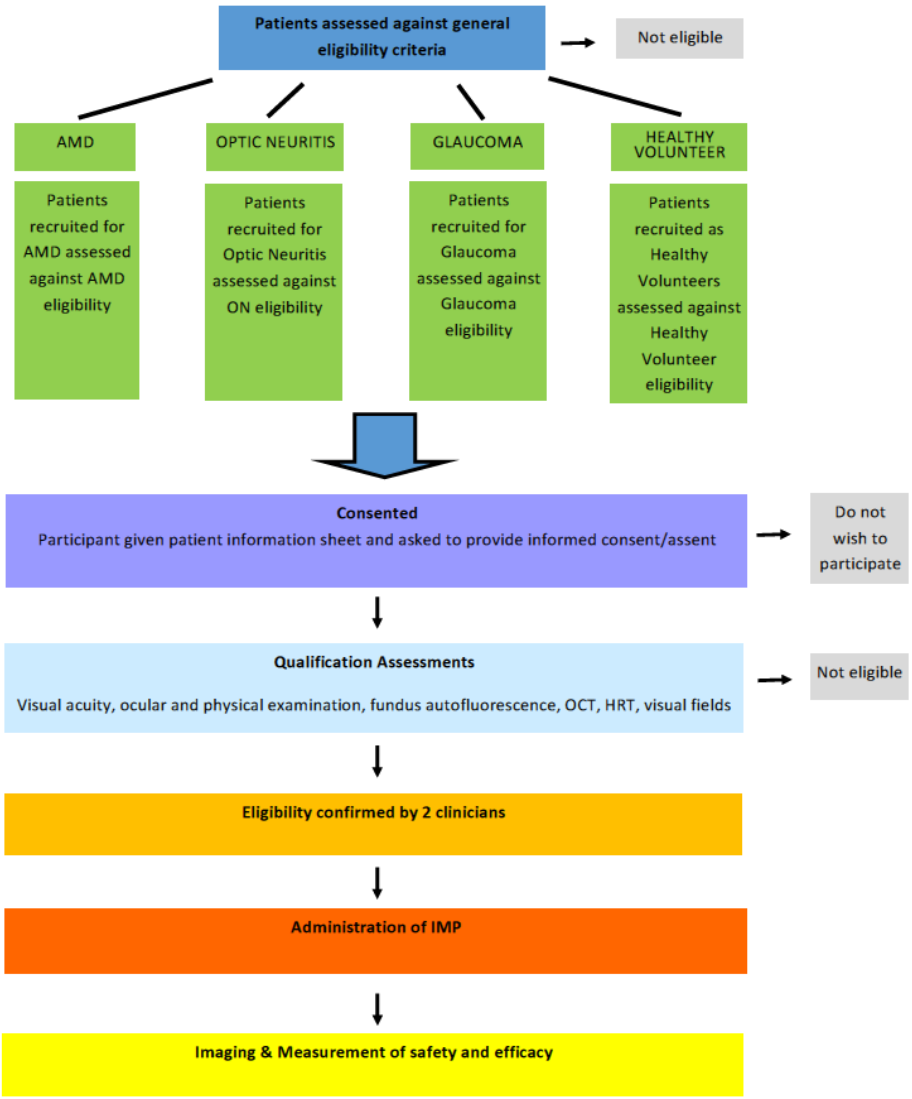
- Healthy volunteers: 40 subjects
- Glaucoma: 20 subjects
- Age-related Macular Degeneration: 20 subjects
- Optic Neuritis: 20 subjects
- Down's Syndrome: 20 subjects

The Down's syndrome participants will be recruited and receive the intervention last.

Subjects in each study group will receive the same amount of ANX776 and each individual subject will receive 1 single intravenous injection. All subjects will be enrolled in accordance with the inclusion and exclusion criteria outlined in Section xxx and via the recruitment strategy in Section xxx.

DARC imaging will be performed in all participants to assess retinal cell apoptosis. However, in a subset of participants (as described in section xxx), fNIR spectroscopy will be performed simultaneously as an optional procedure.

2. Trial Diagram



3. Abbreviations

AE	Adverse Event
Anx-A5	Annexin A5
AR	Adverse Reaction
AMD	Age-related Macular Degeneration
CI	Chief Investigator
CRF	Case Report Form
cSLO	Confocal Scanning Laser Ophthalmoscope
CTA	Clinical Trial Authorisation
CCTU	Comprehensive Clinical Trials Unit
DARC	Detection of Apoptosing Retinal Cells
DSUR	Development Safety Update Report
EU	European Union
FDA	(US) Food and Drug Administration
fNIRS	Functional Near-Infrared Spectroscopy
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GS	Glaucoma Suspect
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
IOP	Intraocular pressure
IRB	Institutional Review Board
ITT	Intention to Treat
MHRA	Medicines and Healthcare products Regulatory Agency
MS	Multiple Sclerosis
NIRS	Near-Infrared Spectroscopy
OCT	Optical Coherence Tomography
OHT	Ocular Hypertension
PED	Pigment Epithelial Detachment
PI	Principal Investigator
PIN	Participant Identification Number
PIS	Participant Information Sheet
PS	Phosphatidylserine
QA	Quality Assurance
QC	Quality Control
QMMP	Quality Management and Monitoring Plan
R&D	Research and Development
REC	Research Ethics Committee
RGC	Retinal Ganglion Cell
RNFL	Retinal Nerve Fibre Layer
RPE	Retinal Pigmented Epithelium
RRMS	Relapsing-Remitting Multiple Sclerosis
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SPC	Summary of Product Characteristics
SPMS	Secondary Progressive Multiple Sclerosis
SSA	Site Specific Approval
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMG	Trial Management Group
TMT	Trial Management Team
ToR	Terms of Reference
TSC	Trial Steering Committee
UCL	University College London

4. Methods

4.1. Site Selection

The trial sponsor has overall responsibility for site and investigator selection and has delegated this role to CCTU.

4.1.1. Study Setting

The Trial will be conducted at 1 site in the UK, all patients will be recruited from the NHS clinics at the Western Eye Hospital, Imperial College Healthcare NHS Trust. Down's Dyndrome (DS) participants will be identified by the research team at Intellectual and Developmental Disabilities Research Group (CIDDRG) from the cohorts of people with DS who have previously consented to be contacted about future studies.

4.1.2. 6.1.2 Site/Investigator Eligibility Criteria

Once the site has been assessed as being suitable to participate in the trial, the trial team will provide them with a copy of this protocol and relevant Summary of Product Characteristics (SPC) or Investigator Brochures (where appropriate).
To participate in the DARC II trial, investigators and trial sites must fulfil a set of criteria that have been agreed by the DARC II Trial Management Group (TMG) and that are defined below.
Eligibility criteria:

- A named clinician is willing and appropriate to take Principal Investigator responsibility
- Suitably trained staff are available to recruit participants, enter data and collect samples
- For IMP trials the site should have a pharmacy that is able to store, prepare and dispense IMP appropriately

4.1.3. Principal Investigator's (PI) Qualifications and Agreements

The investigator(s) must be willing to sign a CCTU Clinical Trial Agreement or an Investigator Agreement to comply with the trial protocol (confirming their specific roles and responsibilities relating to the trial, and that their site is willing and able to comply with the requirements of the trial). This includes confirmation of appropriate qualifications, familiarity with the appropriate use of any investigational products, agreement to comply with the principles of GCP, to permit monitoring and audit as necessary at the site, and to maintain documented evidence of all staff at the site who have been delegated significant trial related duties.

4.1.4. Resourcing at Site

The investigator(s) should be able to demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period (i.e. the investigator(s) regularly treat(s) the target population). They should also have an adequate number of qualified staff and facilities available for the foreseen duration of the trial to enable them to conduct the trial properly and safely.
Sites will be expected to complete a delegation of responsibilities log and provide staff contact details.
The site should have sufficient data management resources to allow prompt data return to CCTU.

4.2. Site approval and activation

The Clinical Trial Authorisation (CTA) for the trial requires that the Medicines and Healthcare products Regulatory Agency (MHRA) is supplied with the names and addresses of all participating sites and named Principal Investigators. Trial staff at CCTU will perform this task.

On receipt of the signed Clinical Trial Agreement or Investigator Agreement, approved delegation of responsibilities log and staff contact details, written confirmation will be sent to the site PI. The CCTU will notify the PI in writing of the plans for site initiation. Sites will not be permitted to recruit any patients until a letter for activation has been issued. The Trial Manager or delegate will be responsible for issuing this after a green light to recruit process has been completed.
The site must conduct the trial in compliance with the protocol as agreed by the Sponsor and, by the regulatory authority(ies) (as appropriate), and which was given favourable opinion by the Research Ethics Committee (REC) and/or Institutional Review Board (IRB). The PI or delegate must document and explain any deviation from the approved protocol, and communicate this to the trial team at CCTU.

5. Participants

5.1. Eligibility Criteria

5.1.1. Participant selection

There will be **NO EXCEPTIONS** (waivers) to eligibility requirements for the DARC II trial. Questions about eligibility criteria should be addressed PRIOR to attempting to enrol a participant.
The eligibility criteria for this trial have been carefully considered and are the standards used to ensure that only medically appropriate participants are entered. Participants not meeting the criteria should not be entered into the trial for their safety and to ensure that the trial results can be appropriately used to make future treatment decisions for other people with similar diseases or conditions. It is therefore vital that exceptions are not made to these eligibility criteria.
Participants will be considered eligible for enrolment in this trial if they fulfil all of the **general** inclusion criteria and none of the **general** exclusion criteria as defined below.
Patients should then be assessed against the inclusion and exclusion criteria of the specific study group that they are being enrolled into the trial for.
Eligibility criteria should be confirmed by 2 clinicians ensuring the PI signs to confirm eligibility before the patient is enrolled. Prior to enrolment subject eligibility status will be confirmed against eligibility criteria by study investigators and signed off by one of the Principal Investigators.

5.2. Inclusion Criteria

5.2.1. General Participant Inclusion Criteria

- Age ≥ 18 years.
- Clear optical media in the studied eye.
- Refractive error not higher than spherical equivalent of 10 D and best corrected visual acuity equal to 6/24 or better at qualification.
- Women of childbearing potential identified as not pregnant and have consented to complete a pregnancy test.
- Subjects who have capacity to consent, have personally signed and dated the informed consent document indicating that they have been informed of all pertinent aspects of the study.

5.2.2. Group Specific Participant Inclusion Criteria

- No table of contents entries found.Glaucoma group subjects will show progression in one or more of the parameters measured and will have at least one eye with a diagnosis of glaucoma (abnormal optic disc and/or visual field defect or both); be diagnosed as a glaucoma suspect or ocular hypertensive (elevated 10P).

- Subjects proven to be able to perform reliable visual field testing using the HFA 640, central 24-2 program, to yield full thresholds, and have had good fundoscopy with assessment of their optic disc.

5.2.2.1. Down's Syndrome Participant Inclusion Criteria

- Down's Syndrome.

5.2.2.2. AMD Participant Inclusion Criteria

- Patients with AMD as defined by:
    - Early AMD mainly characterised by drusen, retinal pigment epithelium (RPE) pigment changes.
    - Late AMD mainly characterised as: geographic atrophy of the RPE (dry AMD).
- Neovascular AMD (wet AMD).

5.2.2.3. Optic Neuritis Participant Inclusion Criteria

- Clinical diagnosis of optic neuritis affecting one eye within two years.
- Visual acuity in affected eye ≤ 6/12 at worst point.
- Corrected vision in unaffected eye ≥ 6/6.
- No history of optic neuritis or other ocular disease in either eye prior to the episode of optic neuritis.
- Subjects proven to be able to perform reliable visual field testing using the HFA 640, central 24-2 program, to yield full thresholds, and have had good fundoscopy with assessment of their optic disc.

5.2.2.4. Healthy Volunteers Participant Inclusion Criteria

- Confirmation of medical history as confirmed by General Practitioner.
- No evidence of any eye disease.

5.2.2.5. Exploratory Optional fNIRS Inclusion Criteria

- Participants who are bald, or with short (less than 3cm), fair (unpigmented) hair

5.2.3. Exclusion Criteria

5.2.3.1. General Participant Exclusion Criteria

- Presence of severe, unstable or uncontrolled systemic disease.
- Known intolerance to IMP.
- Body weight <40kg or >150kg.
- Inability to comply with the study or follow-up procedures.
- Any subjects with a known history of clotting diseases (including DVTs), and subjects taking anticoagulants.
- Ocular surgery within the past 3 months or planned surgery in the study eye, during the course of the trial.
- Pregnant or lactating, or not using adequate contraception* for the duration of the trial (and 30 days post injection of study drug).
- Currently being treated for cancer or any other disease likely to adversely affect participation in this study.
- AIDS / HIV.
- History of alcoholism or drug addiction.
- History or active uveitis.
- History of systemic vasculitis, collagenosis or ongoing treatment of cancer.
- Evidence of previous retinal vascular disease.

- Individuals with terminal illness, or mental illness affecting their compliance with the study
- Any other disease, condition or laboratory abnormality that in the opinion of the CI may increase the risk for the participation or may interfere with the interpretation of study results and in the judgement of the Investigator would make the subject inappropriate for entry into the study.
- Central corneal thickness <450 pm or >650pm.
- Currently, or within the last 3 months, enrolled in a clinical trial of an investigational medicinal product.
- History of retinal laser photocoagulation.
- Media opacities or retinal pathology or amblyopia significantly limiting visual acuity, visual field test or retinal imaging.
- Any other condition or pathological process that in the opinion of the investigator would not make the patient suitable for the trial.

* For the purposes of this study, females will be considered of childbearing potential unless they are naturally postmenopausal or permanently sterilised (i.e. hysterectomy). For women of childbearing potential who may participate in the study, the following reliable form of contraception are acceptable (e.g. oral contraceptive and condom, intra--uterine device (IUD) and condom, diaphragm with spermicide and condom).

5.2.3.2. Group Specific Participant Exclusion Criteria

5.2.3.3. Glaucoma Participant Exclusion Criteria

- Uncontrolled I0P >24mmHg.
- Angle closure/narrow glaucoma. Mean deviation at HVF >12dB.

5.2.3.4. Down's Syndrome Participant Exclusion Criteria

- Lack of capacity to consent.
- Significant learning disability
- Dementia

5.2.3.5. AMD Participant Exclusion Criteria

- Presence of ocular conditions with increased risk of choroidal neovascularisation (CNVM).
- Current or past use for more than 30 days of chloroquine, hydroxychloroquine, chlorpromazine, thioridazine, quinine sulfate, clofazimine, cisplatin, carmustine, (BCNU), deferoxamine, amiodarone, isoretinoin, or gold.

5.2.3.6. Optic Neuritis Participant Exclusion Criteria

- Corticosteroid use in the past 2 months.

5.2.3.7. Healthy Volunteers Participant Exclusion Criteria

- Evidence of any historical retinal eye disease.

5.2.3.8. Exploratory Optional fNIRS Exclusion Criteria

- There are no specific exclusion criteria for the exploratory optional fNIRS.

5.3. Eligibility Criteria for Individuals Performing the Interventions

The IMP should be administered by a study investigator in adherence with the trial administration SOP and also the hospital's policies and procedures for intravenous injections.

5.4. Co-enrolment Guidance

The Principal investigator or Co-Investigator(s) at trial sites will be responsible for ascertaining whether the patient is currently taking part in any clinical trial. All patients will only be enrolled once into the DARC II trial. The investigator will be responsible for checking the patient notes against the screening/enrolment log at site prior to screening to ensure that the patient is not already enrolled in the trial.
Patients may not be enrolled if currently taking part in any other trial of an IMP without the permission of the Chief Investigator. Co-enrolment in observational studies is acceptable.

5.5. Screening Procedures

Written informed consent to enter into the trial must be obtained from participants, after explanation of the aims, methods, benefits and potential hazards of the trial and **BEFORE** any trial-specific procedures are performed or any blood is taken for the trial. The only procedures that may be performed in advance of written informed consent being obtained are those that would be performed on all patients in the same situation as usual standard of care.

5.6. Informed Consent Procedure

Written informed consent will be taken from each subject by an investigator or delegate following appropriate explanation of the aims, methods, possible benefits and risks of the study. The investigator or designee will explain that the participants are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason, and without their clinical care being affected. No clinical trial procedure will be conducted prior to taking consent from the participant.
If new safety information results in significant changes in the risk/benefit assessment, the participant information sheet and consent form will be reviewed and updated if necessary, and participants will be re-consented as appropriate.

5.6.1. Informed Consent for Optic Neuritis, AMD & Glaucoma Patients

Eligible patients for the trial in these affected groups will be approached by members of their usual care team who will present the study to them and provide them with the patient information sheet (PIS) to take home.
Individuals will be given sufficient time to allow them to decide whether or not they would like to participate. Patients will have it explained to them that they are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason. Prior to documenting consent in writing, Investigators will ensure all aspects of the study have been fully understood and that all questions have been answered satisfactorily.
No clinical trial procedures will be conducted prior to having obtained fully informed consent in writing.
A copy of the signed Informed Consent form will be given to the participant. The original signed form will be retained at the study site in the site file and a copy placed in the medical notes.

5.6.2. Informed Consent for Down's Syndrome Participants

For participants in this affected group the consent process will be managed as follows:
A domiciliary visit will be arranged for each potential Down's syndrome participant. The trial will be discussed with the DS participant and this will be facilitated by the use of easy read information sheets. The trial team fully expect that all DS participants who will take part in the DARC II trial will be capable of forming an opinion, explicitly expressing their wishes in regards to participation, and signing an easy read consent form. The opinion of the DS participant will be fully considered and respected. If a DS participant is not considered to have capacity to consent they will be excluded from the DARC II trial. If the potential participant is found to lack the capacity to form an opinion and to consent or withdraw from this study they will not be included in this research.

To be considered to have capacity to consent the DS participant will need to meet the following 5 criteria:

1. The subject has had an interview with the Principal Investigator for Down's syndrome (xxxxxxx) in which they have been given the opportunity to understand the objectives, risks and inconveniences of the trial and the conditions under which it is to be conducted.
2. The subject has been informed of their right to withdraw from the trial at any time.
3. The subject has given their informed consent to taking part in the trial.
4. The subject may, without being subject to any resulting detriment, withdraw from the clinical trial at any time by revoking their informed consent.
5. The subject has been provided with a contact point where they may obtain further information about the trial.

Down's syndrome patients that are considered to have a significant learning disability or dementia will be excluded from participating in the DARC II trial. All potential Down's syndrome participants will previously have completed Cognitive Ability Assessments and Dementia Diagnosis Interviews. Down's syndrome participants will need to demonstrate an IQ of at least XX at their last Cognitive Ability Assessment (which must have been in the previous 3 months) if order to be considered to have sufficient cognitive ability to have capacity to consent. And Down's syndrome participants with an QI of below XX will be considered to have a significant learning disability and be excluded from the trial. The parent/carer of Down's syndrome participants will also need to have completed a Dementia Diagnosis Interview (within the last 3 months) and there must be no indication or diagnosis of dementia for the potential Down's syndrome participant. If the Down's syndrome participant has a diagnosis of dementia or in the experience of the Down's syndrome Principal Investigator has experienced significant cognitive decline they will be excluded.

Down's syndrome participants will have all the trial procedures explained to them again on the day of the intervention and before and trial specific procedures take place. If the Down's syndrome participant gives any indication that they do not wish to proceed with the trial, then they will be withdrawn immediately.

The inclusion of DS participants has been carefully considered by the trial team. The trial interventions have been designed to minimise pain, discomfort and fear for the all participants. The consent process will be conducted by xxx (the PI for the DS cohort) who is already known to all of the potential DS participants.

We will begin the recruitment of the DS participants last. The first Down's syndrome participant will not receive the intervention until a minimum of 20 participants from the other study groups (Glaucoma, AMD, Optic Neuritis and healthy volunteers) have received the intervention and completed the 30 day follow up for adverse events. At this point the IDMC will review the available safety data and make a recommendation to the TSC regarding whether recruitment of Down's syndrome participants can commence. The TSC will ultimately make the decision regarding the commencement of recruitment of Down's syndrome participants (section xxx).
Despite the study drug being an investigational medicinal product, the intervention is considered low risk. The trial requires a home visit, a hospital visit, and a follow up telephone call so the burden for potential participants is minimal. No serious adverse events were observed in the phase I clinical trial of DARC. It is unlikely there will be any direct benefit to participants in the DARC II trial. However it is entirely possible that the onset of dementia may be detected earlier through their participation in the DARC II trial. DS patients will be required to complete Cognitive Ability Assessments, and their parent/carer will complete a Dementia Diagnosis Interview. If onset of dementia is detected this will be fed back to the DS participant and their parent/carer appropriately. The potential for the DARC in

the DS population is huge, and the trial team strongly believe that DS participants should be given the opportunity to participate in the trial.

5.6.3. Informed Consent for Healthy Volunteers

Healthy Volunteers will be recruited by way of advertisements and so may contact the trial team directly expressing interest in being recruited. Healthy volunteers will be provided with the Patient Information Sheet for healthy volunteers prior to asking for their consent.

5.6.4. Informed Consent for Optional Exploratory fNIRS

Functional near-infrared spectroscopy will be offered as an optional procedure to participants who are bald, or with short (less than 3cm), fair (unpigmented) hair. The informed consent form will specifically collect consent for the optional fNIRS assessment.

6. Interventions

6.1. Description of intervention

The DARC compound or ANX776 consists of Annexin V (AnxV), covalently bound to an infrared fluorescent dye molecule called Dye-776 (DY-776). The AnxV variant used in this project is commonly known as Anx-128 and differs from the wild type by two single amino acid mutations. The addition of an exposed thiol group at the N-terminus affords increased conjugation efficiency for the molecular tag. DY-776 is a proprietary dye produced by the company Dyomics and is available for conjugation in its maleimide form, providing 1:1 dye:protein ratio. ANX776 is a diagnostic agent and therefore a subject will only receive a single dose of the IMP.

The retinal imaging procedure will be conducted in both eyes after pharmacological mydriasis (1% tropicamide and 2.5% phenylephrine). In preparation for the administration of the IMP the participant will be cannulated, and seated comfortably in chair while resting their chin on a chin-rest and facing the retinal camera. When the individual is ready, a member of clinical staff qualified to administer intravenous injections will inject ANX776. Following the injection retinal images will be taken at predetermined time intervals, as per protocol. During this time, participants will be closely monitored for safety by the study doctor. All aspects of the intervention will be identical for all study groups.
fNIR spectroscopy will be performed as an optional procedure using an optical cap with NIRS optodes in patients defined in section xxx. This ‘cap’ will be placed on the participant’s head and secured beneath the chin using a soft Velcro strap. During the time a participant is having their retinal imaging done (chin in chinrest, and focusing on the camera), the signal reading generated by the fNIRS will be recorded by the device. Hence there will be no extra burden to the participant.

6.2. Treatment Schedule

The ANX776 IMP will be given as a single intravenous bolus dose (0.4mg) to the trial participants. This is the optimal dose as deemed by the results of the phase I trial of DARC. The injection will be given through a cannula inserted into the participants arm. The cannula will be removed immediately after the injection.

6.3. Dispensing

The ANX776 IMP will be prescribed by the study Investigator with suitable prescribing qualifications. It can be administered by any member of clinical staff qualified to administer intra venous infusions.

6.4. Accountability

ANX776 is classed as an IMP and therefore will be accounted for in line with MHRA regulations. The amount of ANX776 administered to each patient will be recorded in the CRF, including recording the

batch number. The unused IMP will be disposed of as per local standard procedures. IMP shipping instructions for the site will be in the Summary of Drug Arrangements.
Biotec Services International will inform the site contact listed on the drug order form by e- mail when a shipment is sent. On receipt pharmacy will segregate and store the drug appropriately and protected from light at -20°C immediately, and log receipt of each batch of ANX776 on the Accountability Log in a timely manner. Following delivery of ANX776 pharmacy will inspect and verify the contents and conditions of the shipment. An acknowledgement of receipt of ANX776 will be included with the received vials. Usual procedures for monitoring of temperature and transport conditions of the IMP will apply and will be documented on the IMP shipping form. Upon receipt of the IMP, the site pharmacy will confirm receipt of the IMPs by posting/faxing back the accompanying shipping form to the supplier and copies retained at trial sites file. Pharmacy must file a copy of the acknowledgement receipt in the Pharmacy Site File.
If the vials containing ANX776 arrive damaged, pharmacy must quarantine the product, and contact the Sponsor Representative. The Sponsor will arrange for replacement stock to be sent if required. All correspondence relating to delivery problems or any other problem with respect to ANX776 must also be documented and filed in the Pharmacy Site File. If the ANX776 has not arrived by the expected date and time, pharmacy must contact the Trial Management Team in the first instance.
In cases where the IMP is damaged or not stored correctly, this will warrant an urgent notification to the manufacturer who will advise the site and sponsor as to whether a replacement needs to be arranged. The sponsor will be responsible for liaising with Dompé Farmaceutici S.p.A and Biotec Services International (where vials will be labelled and released to the Clinic) for dispatching replacement IMPs to sites. Site pharmacy will be responsible for logging receipt of the IMPs on the site accountability log within the site pharmacy file. Site pharmacy will be responsible for storing the IMP in line with storage requirements as set out in the IMPD (briefly, at -20°C and protected from light, prior to dispensing each vial, thawing at 2-8°C, in the dark, without agitation will be required). Site pharmacy will monitor temperature of IMP storage and report to the sponsor any temperature excursions that have occurred. Details of reporting temperature excursion are in the Summary of drug arrangements. Full IMP accountability will be conducted during the trial. All IMP dispensed by pharmacy will be logged on the site accountability log within the site pharmacy file.
Once the IMP is dispensed, it will be stored at site pharmacy at 2-8°C, protected from light. The storage temperature will be monitored by a thermograph, with a Temperature log kept as standard procedure. The IMP will be warmed to room temperature in the IMP administration room where it will be protected from light prior to IV administration. The IMP will be administered to subjects within 24 hours of dispensing and the administration of the IMP will be documented in the source data and CRF. All used/unused IMP vials will be returned to site pharmacy, to be then updated in the drug accountability log in the pharmacy site file. Drug destruction will be conducted, once agreed with the sponsor and in accordance to local practice, and this will be documented in the drug destruction log in the hospital pharmacy file.

6.5. Source of IMPs including placebo

The IMP will be packaged, labelled and QP released for clinical trial use by an MIA(IMP) holder.

The IMP is being manufactured by:
Dompé Farmaceutici spa - Via Campo di Pile, 67100 l'Aquila, Italy.

Drug substance release tests:
Dompé Farmaceutici spa - Via Campo di Pile, 67100 l'Aquila, Italy.

Drug product characterisation release tests:
Dompé Farmaceutici spa - Via Campo di Pile, 67100 L'Aquila, Italy.

Drug Product manufacture and release tests (Sterility, Bioburden, Subvisible Particles):
Patheon UK Limited - Kingfisher Drive, Covingham, Swindon, Wiltshire, SN3 5BZ, UK.

The IMP is being packaged, labelled and released to the clinic by:
Biotec Services International – Biotec House, Central Park, Western Avenue, Bridgend Industrial Estate, Bridgend, CF31 3RT, UK.

The sourcing of IMPs is discussed in the summary of drug arrangements.
IMP storage, dispensing and relevant administrative activities will be based at the Western Eye Hospital's pharmacy that has compliant freezer and fridge facilities as well as a clinical trials dedicated technician.

6.6. Compliance and Adherence

It is not expected that there will be any problems with compliance as ANX776 will be administered by trial clinicians in charge of the patients care.
Any interruptions or dose modifications will be expected to be recorded in the patient's hospital notes and on the trial CRFs.

6.7. Concomitant Care

All medication and treatment will be continued as normal and will be recorded in the CRF.
Proxymetacaine hydrochloride 0.5% and Fluorescein Sodium 1% eye drops will be used for intraocular pressure assessment. Tropicamide 1% and phenylephrine 2.5% eye drops will be used to induce pharmacological mydriasis during visit days. Any concomitant glaucoma medication that the subject is receiving will be permitted. There are no comparator products or placebos.

6.8. Overdose of Trial Medication

All study groups will receive the same intervention, consisting of a single intravenous injection of 0.4mg of ANX776; there will be no dose escalation. Injection will be followed by assessment of retinal cell apoptosis performed using diagnostic retinal imaging.
The DARC Phase I evaluation of safety and tolerability has shown ANX776 to be well tolerated at all trialled doses (0.1mg, 0.2mg, 0.4mg and 0.5mg) with only minor and unrelated adverse events having been observed. In adherence with the protocol, all participants will be closely monitored for continued evaluation of safety and tolerability.

6.9. Protocol Treatment Discontinuation

In consenting to the trial, participants are consenting to trial treatments, trial follow-up and data collection. However, an individual participant may stop treatment early or be stopped early for any of the following reasons:

- Unacceptable treatment toxicity or adverse event
- Inter-current illness that prevents further treatment
- Any change in the participant's condition that in the clinician's opinion justifies the discontinuation of treatment
- Withdrawal of consent for treatment by the participant

As participation in the trial is entirely voluntary, the participant may choose to discontinue trial treatment at any time without penalty or loss of benefits to which they would otherwise be entitled. Although not obliged to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason, whilst remaining fully respectful of the participant's rights.
Participants who discontinue protocol treatment, for any of the above reasons, should remain in the trial for the purpose of follow up and data analysis.

6.10. Accountability procedures for investigational products

ANX776 will only be used for trial subjects.
All intravenous injections will be prepared under GMP regulations and identical dosages of the tracer will be given to all subjects according to the study group.
The setting up of protocol procedures and IMP pathway logistics will be done by the dedicated Pharmacy Clinical Trials Team at St Mary's Hospital. The on-site pharmacy based at the Western Eye Hospital will be in charge of storage, dispensing and keeping related records.

6.11. Contingency planning for abnormal findings

If the DARC II trial procedures result in any abnormal findings, these findings will be fed back to the participant (and their parent/carer for Down's syndrome participants) in a timely and appropriate manner by the study doctor. The trial team will ensure that the patients GP is informed, and that the participant is directed to appropriate care services.

7. Outcomes

7.1. Primary Outcomes

The primary outcome is the efficacy of the intervention ascertained by the DARC Count, which is the number of apoptosing retinal cells visualised 4 hours after the ANX776 injection.

7.2. Secondary Outcomes

Safety is defined as absence of an adverse event of grade 3 or above in the following study groups:

- 20 participants with AMD,
- 20 participants presenting with acute vision loss from optic neuritis,
- 20 participants with Down's syndrome,
- 20 participants with glaucoma,
- 40 healthy volunteers.

Safety will be measured by adverse events following the Common Terminology Criteria for Adverse Events (CTCAE) scale.
In addition, in a subset of participants, an exploratory secondary objective is to assess if the ANX776 signal is detected in the brain using fNIR spectroscopy.

8. Participant Timeline

Figure 1: Participant Timeline

Type of procedure	Procedure	Study Visit
Qualification Procedures	Informed Consent	X
	Medical and Ophthalmic Histories	X
	Prior & Concomitant Medications/Procedures	X
	Demographic data	X
	Cognitive Ability Assessments	X[8]
	Dementia Diagnosis Interview	X[9]
	Physical Examination	X
	Vital Signs	X

	Visual Acuity	**X[4]**
	Slit-Lamp Bio-microscopy (incl. Corneal Pachymetry and IOP)	**X[4]**
	Visual Field (HVF)	**X, X, X[1,3,4]**
	Fundus Exam (Post-Pupil Dilation)	**X[4]**
	Baseline fundus autofluorescence (IR)*	**X[4]**
	Optical Coherence Tomography (OCT)	**X, X, X[1,3,5]**
	Heidelberg Retinal Tomography (HRT)	**X, X, X[1,3,4]**
	Childbearing potential / pregnancy test	**X**
	Eligibility Confirmed	**X**
Intervention Procedures	**Intravenous Administration of Study Drug - ANX776**	**X[6]**
	DARC assessment by cSLO Retinal Imaging*	**X, X, X, X[2,6]**
	Adverse Medical Event Query	**X[6]**
Post intervention	**Safety follow up telephone call**	**X[7]**

1. Up to 2 reliable historical tests, obtained no more than 24 months prior to the qualification / pre-intervention visit, can be used as part of this qualification assessment of Glaucoma, healthy volunteers, AMD, Downs syndrome and Optic Neuritis subjects.
2. During injection, 15 min, 2 hours and 4 hours post injection.
3. Confirmation of historical findings for Glaucoma, Optic neuritis and AMD participants.
4. Down's participants are exempted from this test.
5. For Down's participants, historical data will be used.
6. Can be conducted up to 12 weeks from confirmation of eligibility.
7. 30 days after DARC Intervention +/- 1 week
8. Down's participants only
9. Down's participants' parent /carer only

* In a subset of participants this will be done in conjunction with fNIR spectroscopy (as described in section xxx)

9. Procedures

9.1. Qualification/ pre-intervention assessment

Procedures performed before the intervention:

- Serious and Adverse Medical Event Query, Medical and Ophthalmic Histories, Prior and concomitant Medication/Procedures will be documented.
- Inclusion/Exclusion criteria will be evaluated.
- Demographic data, Ocular History, Physical Examination, Vital Signs, Visual Acuity, Slit-Lamp Bio-microscopy, Corneal Pachymetry, Intraocular Pressure, Visual Field, Fundus Exam (Post-Pupil Dilation), Cup/Disc Ratio (Post-Pupil Dilation), , Baseline fundus autofluorescence (488 and IR) will be performed.
- Glaucoma and healthy volunteers will require a minimum of 3 Visual field tests, 3 OCT and 3 HRT's to assess baseline characteristics. However, up to 2 reliable historical tests, obtained no more than 9 months prior to qualification, can be used for these tests in the Glaucoma group.
- AMD and Optic Neuritis subjects will have an OCT assessment confirming the status of the disease.

- For Down's syndrome participants, due to their characteristics, historical data will be used.

9.2. Treatment procedures

ANX776 is a diagnostic agent and therefore a subject will only receive a single dose (0.4mg) of the IMP. As this is a safety study, no dosage modifications are permitted.
After qualification, subjects who have signed the informed consent, have fulfilled all inclusion criteria, and have not met any exclusion criteria will be enrolled in the study. The participant details and study group will be recorded on the trial enrolment log and patient identification log kept within the site file at site.

9.3. Intervention (within 12 weeks of Qualification)

Procedures performed:

- Adverse Medical Event Query, Concomitant medications/Procedures will be documented.
- Physical Examination, Vital Signs, Visual Acuity, Slit-Lamp Bio-microscopy, Intraocular Pressure, Fundus Exam (Post-Pupil Dilation), Cup/Disc Ratio (Post-Pupil Dilation) will be performed.
- Cognitive Ability & Dementia Assessments (only for Down's Syndrome participants)
- Administration of intravenous injection of 0.4mg of ANX776.
- DARC imaging at following intervals:
    - during the injection*
    - at 15 min post-injection*
    - at 2 hours post-injection*
    - at 4 hours post-injection*

  *In a subset of participants this will be done in conjunction with fNIR spectroscopy (as described in 5.1.2).

9.4. Safety follow up telephone call (30 days after intervention)

The site will conduct a safety follow up via telephone call to the subject/carer 30 days after IMP administration, to check for any symptoms and/or adverse events.

9.5. Early Stopping of Follow-up

If a participant chooses to withdraw from the trial after the intervention, they should be asked if they are willing to receive the safety follow up telephone call 30 days after the intervention. If, however, the participant exercises the view that they no longer wish to be followed up either, this view must be respected and the participant withdrawn entirely from the trial. CCTU should be informed of the withdrawal in writing using the appropriate DARC II trial documentation. Data already collected will be kept and included in analyses according to the intention-to-treat principle.

9.6. Loss to Follow-up

Due to the design and short duration of the trial i.e. single visit, loss to follow-up is not anticipated to be an issue. Following completion of study visit, patients from Glaucoma, AMD, Optic Neuritis and Down's syndrome groups will continue with care in their respective clinics.

9.7. Trial Closure

The end of the trial will be defined as resolution of all data queries following the last visit by the last participant.
The REC and MHRA will be notified within 90 days of the end of the trial. A summary report of the research will be sent to the REC and MHRA within 12 months of the end of the trial.
A site may be deemed ''closed'' once all trial-related activities at that site are reconciled and/or complete and a letter confirming that close down is complete has been sent to the site PI from UCL

CCTU. The letter should include instructions to archive all trial related material according to any archiving SOPs. This includes ensuring that:

- Investigator/institution files are reviewed and all essential documentation for a particular site are confirmed as being present providing a clear audit trail of trial conduct at that site.
- All site data are collected, entered and validated, and all queries resolved where feasible. This includes queries resulting from reconciliation of the clinical database.
- All findings from previous trial monitoring visits are addressed or reasons documented regarding why they cannot be addressed.
- Investigators are made aware of the trial publication policy, as documented in the trial protocol and/or trial contracts/agreements.
- Investigators are aware of and have implemented relevant ongoing requirements such as site archiving, subsequent audit/inspection procedures and any ongoing reporting requirements.

9.8. Data queries

The site may only be closed once all outstanding data queries have been answered and resolved and documentation returned to UCL CCTU as necessary. The Trial Manager will ensure that all queries have been resolved and that data is complete and accurate as far as possible. If local research staff are unavailable towards the end of the end of the trial and cannot complete data queries, a member of the trial team at UCL CCTU will go to the site to collect the missing data.

10. Ethics and Dissemination

10.1. Research Ethics Approval

Before initiation of the trial at any clinical site, the protocol, all informed consent forms and any material to be given to the prospective participant will be submitted to the relevant REC for approval. Any subsequent amendments to these documents will be submitted for further approval. Before initiation of the trial at each additional clinical site, the same/amended documents will be submitted for local Research and Development (R&D) approval.

The rights of the participant to refuse to participate in the trial without giving a reason must be respected. After the participant has entered the trial, the clinician remains free to give alternative treatment to that specified in the protocol, at any stage, if s/he feels it to be in the best interest of the participant. The reasons for doing so must be recorded. After randomisation the participant must remain within the trial for the purpose of follow up and data analysis according to the treatment option to which they have been allocated. However, the participant remains free to change their mind at any time about the protocol treatment and follow-up without giving a reason and without prejudicing their further treatment.

10.2. Competent Authority Approvals

This protocol will be submitted to the UK Regulatory Authority (MHRA).

This is a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC. Therefore, a CTA is required in the UK.

The progress of the trial, safety issues and reports, including expedited reporting of SUSARs, will be reported to the MHRA in accordance with relevant and local requirements and practices.

10.3. Other Approvals

The protocol will be submitted by those delegated to do so to the relevant R&D department of each participating site or to other local departments for approval as required in each country. A copy of the local R&D approval (or other relevant approval as above) and of the Participant Information Sheet (PIS) and consent form on local headed paper must be forwarded to the co-ordinating centre before participants are randomised to the trial.

The protocol has received formal approval and methodological, statistical, clinical and operational input from the CCTU Protocol Review Committee.

10.4. Protocol Amendments

The CCTU will ensure that the trial protocol, patient information sheet, consent form, GP letter, patient safety card and submitted supporting documents have been approved by the research ethics committee and site Research & Development department prior to any patient recruitment. The protocol and all agreed substantial protocol amendments, will be documented and submitted for ethical approval prior to implementation.

Plans for communicating substantial protocol amendments (e.g. changes to eligibility criteria or outcomes) to relevant parties (e.g. investigators, REC, trial participants, trial registries, journals, regulators) will be developed. The TSC will be responsible for the decision to amend the protocol and how substantive changes will be communicated to relevant stakeholders.

10.5. Consent or Assent

Patients will be provided with a Patient Information Sheet (PIS) and given time to read it fully. Following a discussion with a medical qualified investigator or suitable trained and authorised delegate, any questions will be satisfactorily answered and if the participant is willing to participate, written informed consent will be obtained. During the consent process it will be made completely and unambiguously clear that the participant is free to refuse to participate in all or any aspect of the trial, at any time and for any reason, without incurring any penalty or affecting their treatment.
Consent will be re-sought if new information becomes available that affects the participant's consent in any way. This will be documented in a revision to the patient information sheet and the participant will be asked to sign an updated consent form. These will be approved by the ethics committee prior to their use.
A copy of the approved consent form is available from the CCTU trial team.

10.6. Confidentiality

Data protection and information governance principles will be followed throughout the study, which will be overseen by the Trial Manager and Clinical Project Manager based at CCTU. Any confidentiality concerns expressed by potential patients will be addressed prior to providing informed consent.
Patients will be assigned a trial number upon enrolment. This number will be used on all trial-related documentation in place of personal identifiable data and used to identify patients on the CRFs. Person identifiable information will be held securely at the sites and will be removed from documents and replaced with the trial number in the event of being sent off-site. Patient names will not be passed to anyone outside the research team who is not involved in the trial.
The records obtained during the trial, as well as related health records, will remain strictly confidential at all times. The information will be held securely on paper and electronically at the treating hospital under the provisions of the 1998 Data Protection Act. Information will be transferred from hospital sites to UCL CCTU on CRFs to enable analysis of the trial results to be undertaken. Patient names will only appear on their consent form, which will be kept at the hospital site in the medical notes, a copy will not be sent to the CCTU.
Patient records will be available to people authorised to work on the trial within NHS Trusts but may also need to be made available to people authorised by the Sponsor for monitoring and audit purposes. By signing the consent form patients agree to this access for the DARC II trial and any further research that may be conducted in relation to it, even if they withdraw from the trial. When a patient withdraws consent from the trial, unless they object, their data will remain on file and will be included in the final trial analysis.
All trial staff will have a duty of confidentiality to participants in the DARC II trial.

The Case Report Forms (CRFs) will not bear the subject's name or other personal identifiable data. The subject's initials, date of birth and trial identification number, will be used for identification.
For other information regarding Data Management please see section xxx.

10.7. Declaration of Interests

The Chief Investigator for this trial is a named Inventor on the granted DARC patent which is owned by UCLB, and is unlicensed. We have therefore aimed to mitigate any risks arising from conflict of interest by using Principal Investigators for each affected group. The Chief investigator will not personally be involved in the recruitment, IMP administration nor follow up assessment for all trial patients. Data resulting from the trial will be monitored by an Independent Data Monitoring Committee.

10.8. Indemnity

UCL holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant in the clinical trial. UCL does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or not. This does not affect the participant's right to seek compensation via the non-negligence route.
Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of UCL or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to UCL's insurers, via the Sponsor's office.
Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to UCL, upon request.

10.9. Finance

DARC II is fully funded by Wellcome Trust translational award grant number WT099729. It is not expected that any further external funding will be sought.

10.10. Archiving

The investigators agree to archive and/or arrange for secure storage of DARC II trial materials and records for a minimum of 10 years after the close of the trial unless otherwise advised by the CCTU.
Essential documents are those which enable both the conduct of the trial and the quality of the data produced to be evaluated and show whether the site complied with the principles of GCP and all applicable regulatory requirements.
UCL CCTU will notify the site when trial documentation can be archived and which documents must be archived for the 10 year period. All archived documents must continue to be available for inspection by appropriate authorities upon request.

10.11. Access to Data

Requests for access to trial data will be considered, and approved in writing where appropriate, after formal application to the TMG/TSC. Considerations for approving access are documented in the TMG/TSC Terms of Reference.

10.12. Ancillary and Post-trial Care

Once having completed the IMP administration visit patients will not be invited back for any further follow up visits. Safety will be assessed before the trial leaves the site and advised who to contact should problems arise once they are discharged.

10.13. Publication Policy

10.13.1. Trial Results

The results of the trial will be disseminated regardless of the direction of effect.
The trial will be performed and reported in accordance with the CONSORT guidance.
Trial findings will be disseminated to all potential beneficiaries of the research including patients, carers and relatives, and also doctors, advisory bodies and health care Commissioners. This will take the form of papers in high impact open access (included in the budget) medical journals and also presentations at national and international medical conferences. We will seek publication of the trial protocol once finalised. Trial results will also be disseminated to the trial patients in a one-page summary written in lay language.

10.13.2. Authorship

Publications generated from the trial will be attributed to the DARC II Trial Management Group, which will consist of all those who have wholeheartedly collaborated in the trial. The main report will be drafted by the TMG, and the final version will be reviewed by the TSC before submission for publication. TMG members will be named and their affiliations listed in the main report. All publications will be in compliance with the CCTU Publication Policy.

10.13.3. Reproducible Research

There are no plans for granting public access to the full protocol, participant-level dataset and statistical code.