Nicotin**AM**ide **in G**laucoma (NAMinG): A randomised, placebo-controlled, multi-centre, Phase III trial

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Date	05-Sep-2023
Sponsor	University College London (UCL)
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IRAS #	1006433

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Table of Contents

General information

This document was constructed using the Comprehensive Clinical Trials Unit (CCTU) at UCL Protocol template Version 6. It describes the NAMinG trial, sponsored by UCL and co-ordinated by CCTU.

It provides information about procedures for entering participants into the trial, and provides sufficient detail to enable: an understanding of the background, rationale, objectives, trial population, intervention, methods, statistical analyses, cost-effectiveness analyses, ethical considerations, dissemination plans and administration of the trial; replication of key aspects of trial methods and conduct; and appraisal of the trial's scientific and ethical rigour from the time of ethics approval through to dissemination of the results. The protocol should not be used as an aide-memoire or guide for the treatment of other patients. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to registered investigators in the trial. Sites entering participants for the first time should confirm they have the correct version through a member of the trial team at CCTU.

CCTU supports the commitment that its trials adhere to the SPIRIT guidelines. As such, the protocol template is based on an adaptation of the Medical Research Council CTU protocol template and the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 Statement for protocols of clinical trials [1]. The SPIRIT Statement Explanation and Elaboration document [2] can be referred to, or a member of CCTU Protocol Review Committee can be contacted for further detail about specific items.

Sponsor

University College London (UCL) is the trial sponsor and has delegated responsibility for the overall management of the NAMinG trial to CCTU. Queries relating to UCL sponsorship of this trial should be addressed to the CCTU Director, CCTU at UCL, Institute of Clinical Trials & Methodology, 90 High Holborn 2nd Floor, London, WC1V 6LJ or via the Trial Team.

Funding

NAMinG is fully funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme, grant number 132758; a Medical Research Council (MRC) and National Institute of Health Research (NIHR) partnership. The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NIHR or the Department of Health and Social Care.

It is not expected that any further external funding will be sought.

Trial Registration

This trial has been registered with the ClinicalTrials.gov Register, where it is identified as NCT05405868.

Trial Administration

Please direct all queries to the NAMinG Trial Manager at UCL CCTU in the first instance; clinical queries will be passed to the Chief Investigator by the Trial Manager.

Coordinating Site:

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Structured trial summary

Acronym or short title	**N**icotin**AM**ide **in G**laucoma (NAMinG): A randomised, placebo-controlled, multi-centre, Phase III trial
Scientific Title	A Phase III, double-masked, randomised, placebo-controlled trial investigating the safety and efficacy of nicotinamide (NAM) to slow visual field loss in adults with open-angle glaucoma
CCTU Trial Adoption Group #	CCTU/2020/365
Sponsor R&D ID #	135050
CTA #	20363/0457/001-0001
REC #	23/EM/0175
IRAS #	1006433
Primary Registry and Trial Identifying Number	ClinicalTrials.gov Identifier: NCT05405868
Date of Registration in Primary Registry	3rd June 2022
Source of Monetary or Material Support	National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation programme Grant Award: 132758
Sponsor	University College London with sponsor responsibilities delegated to CCTU.
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Countries of Recruitment	UK
Health Condition(s) or Problem(s) Studied	Adults with recently diagnosed (within 12 months) early to moderate open-angle glaucoma (OAG)
Intervention(s)	All enrolled participants will receive Standard of Care Intraocular Pressure (IOP) lowering treatment* prior to randomisation to either one of the following arms: **Arm A:** Nicotinamide 750mg tablets; two tablets a day (1.5g/day) for the first 6 weeks, thereafter four tablets a day (3.0g/day) for the remainder of the treatment period (total 27 months). **Arm B:** Matching placebo tablets; two tablets a day for the first 6 weeks, thereafter four tablets a day for the remainder of the treatment period (total 27 months).

	<u>*Standard of Care Intraocular Pressure (IOP) lowering treatment:</u> A single initial therapy (to be initiated prior to the baseline/randomisation visit), determined by patient choice of either preservative-free latanoprost (eye drops) or selective laser trabeculoplasty (SLT), will be permitted as standard of care. Treatment escalation is permitted, as detailed in the protocol section 5.3.4
Key Inclusion and Exclusion Criteria	**Inclusion Criteria:** 1. Patients who have been recently diagnosed (within the last 12 months) with early to moderate open-angle glaucoma (OAG) in at least one eye* (including primary OAG, normal tension glaucoma (NTG) and pseudoexfoliation glaucoma) 2. Open angle on gonioscopy 3. Adults aged 18 years or over 4. Snellen visual acuity 6/12 or better in at least one eye meeting the Visual Field (VF) criteria** 5. Visual Field (VF) mean deviation (MD) no worse than -12dB in either eye 6. A negative pregnancy test result at the screening and baseline visit prior to randomisation for women of childbearing potential 7. Ability to provide informed consent to participate 8. Able and willing to attend trial visits and comply with trial procedures for the duration of the trial **Exclusion Criteria:** 1. Pigment dispersion glaucoma 2. Pregnancy (or planned pregnancy during the trial) and/or breastfeeding 3. Women of childbearing potential and male participants with a partner of childbearing potential not willing to use highly effective contraception as described in section 3.2.1.1 for the duration of the trial treatment and for the time period specified following last trial treatment administration. 4. Current treatment with either isoniazid, pyrazinamide, carbamazepine, phenobarbital or primidone 5. Current liver disease or laboratory results with elevated levels of liver transaminases (AST or ALT >3 x ULN) at screening visit. 6. Renal failure (eGFR <30mL/min/1.73m^2) at screening visit 7. Conditions affecting both eyes *** which may affect the Visual Field test result:

	a. Diabetic retinopathy or any other retinal disease, causing VF loss b. Clinically relevant cataract (likely to require cataract surgery within the next 2 years) c. Dementia or other non-glaucomatous neurological disease causing VF loss d. Adnexal conditions causing VF loss (including, but not limited to blepharochalasis) 8. Diagnosed with cancer in the last 5 years (with exception of non-melanoma skin cancer) 9. Any clinical condition that, in the investigator's opinion, would make the participant unsuitable for the trial 10. Concurrently enrolled in any other interventional trial or participation in previous clinical trial of glaucoma 11. Current use of, and unwilling to abstain from, over-the counter additional vitamin B3/NAM oral supplements (including skin preparations such as ointments/emulsions), Ginkgo Biloba and/or Coenzyme Q10 supplements, throughout the duration of their participation in the trial **** Data to be collected from both eyes; the eye with the worst visual field loss at baseline will be used in the primary analysis.*** ***** As described in section 3*** ****** If only one eye is affected and the other is eligible, participant can still enter the trial.***
Study Type	A UK multi-centre, randomised, masked, placebo-controlled, phase III trial with an internal 6-month pilot. The internal 6-month pilot will aim to recruit 108 participants within the first 6 months from 4 sites, adopting a stop-go criterion. Participants, Investigators, site teams and the analysing statistician will be masked to the treatment allocation.
Study setting	NHS Outpatient Clinics
Date of First Enrolment	September 2023
Target Sample Size	496 in total, 248 participants in each arm
Trial Duration	60 months
Primary Outcome(s)	The difference between the treatment arms in VF MD at 27 months.
Key Secondary Outcomes	**Clinical safety and efficacy outcomes:** 1. The difference in VF MD at 3 months (0-3 months – neuro-recovery) between the NAM group and the placebo group, measured using the HFA Mark II (or

	II-i) or HFA3 with the SITA Standard 24-2 programme. 2. Safety profile of high dose NAM measured by liver function tests (LFTs), estimated glomerular filtration rate (eGFR) and glycosylated haemoglobin (HbA1c) at screening, month 3 and month 18. 3. Adverse events during the study period for each participant from baseline to month 27. 4. Quality-of-Life outcome differences between the two treatment groups at baseline, month 3 and month 27, measured by the EQ-5D-5L (with vision bolt-on) and GQL-15. **Mechanistic outcomes:** The following outcomes will be measured from the blood samples and VFs collected over the course of the trial. <u>At Moorfields Eye Hospital (MEH), King's College Hospital, and up to 2 further sites ONLY (Biomarker Sub-Study):</u> 1. Impact of NAM treatment on mitochondrial function (ATP-linked oxygen consumption rate (OCR)) in the NAM and placebo groups between baseline and month 27. 2. The association between a) ATP-linked OCR and b) NAD levels and rate of VF loss in the placebo group between baseline and month 27. 3. The association between lymphocyte mitochondrial function (ATP-linked OCR) and serum NAM levels, lymphocyte NAD+ and oxidised glutathione levels in the placebo and NAM groups at baseline. 4. Association of NAM, NAD and OCR levels with the EPIC Food Frequency Questionnaire responses in the placebo and NAM groups at baseline. 5. The effect of NAM on a) rate of VF loss (between month 3 and month 27), and b) level of mitochondrial function overall and in participants with low vis high baseline mitochondrial function and low vs high baseline NAM levels. 6. The association between lymphocyte mitochondrial function (ATP-Linked OCR) and quality of life measures in the placebo and NAM groups between baseline and month 27. <u>At all sites:</u> 7. The association between baseline NAM levels and rate of VF loss (between baseline and month 27) in the placebo group at all sites.

	8. Association between NAM dosing and IOP (between baseline and month 27) in the active and placebo groups at all sites.
Surrogate Outcome(s)	The primary analysis will be repeated with imaging/structural measures as the outcome variable. 1. The difference in OCT peripapillary RNFL thickness/OCT macular GCL thickness between treatment arms at month 27. 2. The difference in OCT peripapillary RNFL thickness/ OCT macular GCL thickness between treatment arms at month 3. 3. The difference in the rate of OCT peripapillary RNFL/macular GCL loss between treatment arms between month 3 and month 27. 4. The effect of high dose NAM on the rate of VF progression between month 3 and month 27, with the rate of OCT peripapillary RNFL/macular GCL loss as a Bayesian prior. 5. The correlation between the rate of VF progression and OCT peripapillary RNFL/macular GCL loss between month 3 and month 27.
Exploratory Outcome(s)	The differences between treatment groups in the rate of VF loss at predefined subsets of VF locations. If there is no neuro-recovery (significant difference in VF MD between months 0 and 3) the rates of progression will be measured between months 0 and 27 months. If there is a statistically significant VF improvement between month 0 and 3 months (neuro-recovery), the rate of progression will be measured between month 3 and 27 months (neuroprotection evaluation).
Ancillary studies	Biomarker Sub-Study (refer to Appendix 1) Future Genetic and Gene Expression Sub-Study (refer to Appendix 2)

Roles and responsibilities

These membership lists are correct at the time of writing; please see terms of reference documentation in the TMF for current lists.

Protocol contributors

Name	Affiliation	Role
Professor David Garway-Heath	UCL Institute of Ophthalmology	Chief Investigator
Mr Gerassimos Lascaratos	Kings College Hospital NHS Foundation Trust	Co-CI
Professor Gus Gazzard	UCL Institute of Ophthalmology	Co-Applicant
Professor Anthony Schapira	UCL Institute of Neurology	Co-Applicant
Professor Timothy Jackson	Kings College London	Co-Applicant
Dr David Chau	UCL Institute of Neurology	Co-Applicant
Professor James Morgan	Cardiff University	Co-Applicant
Mrs Kate Lees	Patient and Public Involvement and Engagement (PPIE)	Co-Applicant
Gemma Jones	UCL CCTU	Head of Clinical Trials Operations & Co-Applicant
Dr Hakim-Moulay Dehbi	UCL CCTU	Head of Statistics & Co-Applicant
Kashfia Chowdhury	UCL CCTU	Trial Statistician
Felicia Ikeji	UCL CCTU	Clinical Project Manager
Nazma Begum-Ali	UCL CCTU	Trial Manager
Zainab Mohamed	UCL CCTU	Data Manager

Role of trial sponsor and funders

Name	Affiliation	Role
University College London (UCL)	N/A	Regulatory sponsor
UCL Comprehensive Clinical Trials Unit (CCTU)	UCL	UCL as the trial sponsor has delegated all sponsor duties to UCL CCTU. A Clinical Project Manager (CPM) will oversee the Trial Manager (TM) who will be responsible for the day-to-day management of the trial, identifying and providing support to the trial teams at all participating sites and coordinating centres. Responsibilities include securing arrangements to initiate, manage and finance the trial. CCTU staff will be involved in site set up including trial protocol and participant information development, ethics and regulatory submissions, case report form development and

		database construction, in collaboration with the NAMinG Trial Management Group.
National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation programme	Part of the UK Government health research organisations, funded by the Department of Health and Social Care (DHSC)	Peer review of study proposal Study funder

Trial Team

Name	Affiliation	Role and responsibilities
Professor David Garway-Heath	UCL Institute of Ophthalmology	Chief Investigator, primarily responsible for the concept, design and the conduct of the trial.
Mr Gerassimos Lascaratos	Kings College Hospital NHS Foundation Trust	Co- CI, responsible for the design, supervision, and interpretation of the trial
Gemma Jones	UCL CCTU	Head of Clinical Trials Operations, providing contracting and oversight of trial delivery
Dr Hakim-Moulay Dehbi	UCL CCTU	Head of Statistics providing statistical advice and oversight of the study statistician
Felicia Ikeji	UCL CCTU	Clinical Project Manager, providing oversight of governance, trial conduct, Quality Management in line with CCTU SOPs and prevailing legislation and budget management
Kashfia Chowdhury	UCL CCTU	Statistician, providing statistical analysis at all stages of the trial; CRF development, writing of Statistical Analysis Plan, analyses for IDMC reports, data cleaning and final analysis.
Nazma Begum-Ali	UCL CCTU	Trial Manager, providing the day-to-day management of the trial including the development of trial protocol and supporting patient documents, ethics and regulatory submissions, sites set up and support to all participating sites.
Zainab Mohamed	UCL CCTU	Data Manager, responsibilities include the development of the CRF and Metadata design, Database testing, Data Management Plan, Data queries, Preparation of reports, data cleaning.

Trial Management Group

Name	Affiliation	Role and responsibilities
Professor David Garway-Heath	UCL Institute of Ophthalmology	Chair, Professor of Ophthalmology for Glaucoma and Allied Studies, Chief Investigator. Overall responsibility for the conduct of the trial, ensuring deliverables and expectations of the oversight group are met.
Mr Gerassimos Lascaratos	Kings College Hospital NHS Foundation Trust	Deputy Chair, Consultant Ophthalmic Surgeon. Assist the CI in the trial set-up and trial

		management, and lead on the mechanistic aspects of the trial.
Professor Gus Gazzard	UCL Institute of Ophthalmology	Professor of Ophthalmology. Support recruitment and retention at MEH and other sites, advise on logistics and data analysis.
Professor Anthony Schapira	UCL Institute of Neurology	Professor of Neurological Science in Clinical and Movement Neurosciences. Expertise in mitochondrial function, support the development of laboratory manuals and conduct of trial.
Professor Timothy Jackson	Kings College London	Professor of Ophthalmology Support recruitment and retention at Kings and other sites and advise on logistics.
Dr David Chau	UCL Institute of Neurology	Senior Scientist in Clinical and Movement Neurosciences. Experience in laboratory techniques to evaluate mitochondrial function and related assays.
Professor James Morgan	Cardiff University	Professor of Ophthalmology in School of Optometry and Vision Sciences. Expertise in neuronal damage in glaucoma models, advise on laboratory investigations and data interpretation
Carol Bronze	Glaucoma patient	Patient & Public Involvement and Engagement Representative. Oversee the PPIE activities and steer the Participant Advisory Group (PAG)
Gemma Jones	UCL CCTU	Head of Clinical Trials Operations at UCL CCTU. Oversee implementation of CCTU policies and SOPs for trial delivery.
Dr Hakim-Moulay Dehbi	UCL CCTU	Senior oversight statistician. Senior oversight and supervision of trial statistician in designing the statistical analysis plan (SAP), statistical analyses and statistical reports.
Felicia Ikeji	UCL CCTU	Clinical Project Manager. Oversight of the trial conduct as per protocol, regulations and trial budget.
Kashfia Chowdhury	UCL CCTU	Trial Statistician. Produce statistical reports and provide statistical input on trial analyses.
Nazma Begum-Ali	UCL CCTU	Trial Manager & Trial Management Group Facilitator. Manage the day-to-day running of the trial according to the protocol and regulations.
Zainab Mohamed	UCL CCTU	Data Manager. Support the TM and manage data collection as per protocol across participating sites.

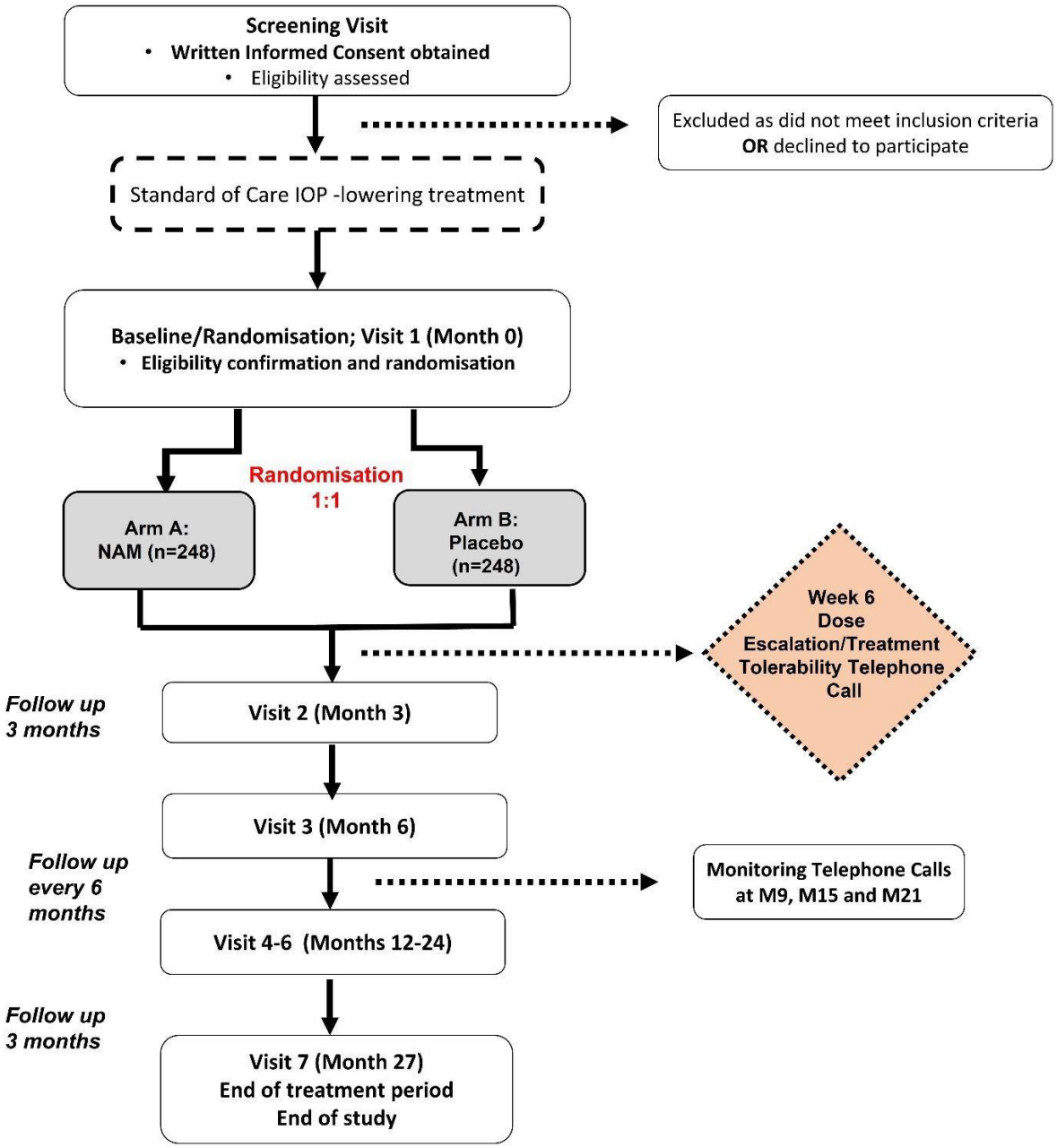
Trial Steering Committee

Name	Affiliation	Role
Professor Andrew McNaught	Gloucestershire Hospitals NHS Foundation Trust	Independent Clinician Committee Chair
Dr Catey Bunce	The Royal Marsden NHS Foundation Trust	Independent Statistician
Professor Jennifer Burr	University of St Andrews	Independent Clinician
Jacqueline Mitton	Glaucoma patient	PPIE representative

Independent Data Monitoring Committee

Name	Affiliation	Role
Professor Colm O'Brien	The Mater Hospital, Dublin	Independent Clinician Committee Chair
Professor David Crabb	City University of London	Independent Statistician
Professor Alastair Denniston	University of Birmingham	Independent Clinician

Trial Diagram



Abbreviations

AE	Adverse Event
ALT	Alanine Aminotransferase
AMD	Age-Related Macular Degeneration
AR	Adverse Reaction
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BCP	Business Continuity Plan
CA	Competent Authority
CI	Chief Investigator
CNS	Central Nervous System
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CCTU	Comprehensive Clinical Trials Unit
DMP	Data Management Plan
DNA	Deoxyribonucleic Acid
DR	Disaster Recovery
DSUR	Development Safety Update Report
EC	Ethics Committee
eGFR	Estimated Glomerular Filtration Rate
EHR	Electronic Health Records
EMGT	Early Manifest Glaucoma Trial
EU	European Union
FDA	(US) Food and Drug Administration
FWA	Federal Wide Assurance
GAT	Goldmann Applanation Tonometry
GCL	Ganglion Cell Layer
GCP	Good Clinical Practice
GDPR	General Data Protection Regulations
GP	General Practitioner
HFA	Humphrey Field Analyzer
HRA	Health Research Authority
HTG	High Tension Glaucoma
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
IOP	Intraocular Pressure
ISF	Investigator Site File
ITT	Intention to Treat
LFT	Liver Function Test
MD	Mean Deviation
MEH	Moorfields Eye Hospital
MHRA	Medicines and Healthcare products Regulatory Agency
NAD	Nicotinamide Adenine Dinucleotide
NAE	Notifiable Adverse Event
NAM	Nicotinamide
NHS	National Health Service
NICE	The National Institute for Health and Care Excellence
NIHR	National Institute for Health and Care Research
NMNAT1	Nicotinamide Mononucleotide Adenylyltransferase 1
NMNAT2	Nicotinamide Mononucleotide Adenylyltransferase 2
NTG	Normal Tension Glaucoma
OAG	Open Angle Glaucoma
OCR	Oxygen Consumption Rate
OCT	Optical Coherence Tomography
OHT	Ocular Hypertension
ON	Optic Nerve
ONH	Optic Nerve Head
ORA	Ocular Response Analyzer
PAG	Participant Advisory Group
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
PIN	Participant Identification Number
PIS	Participant Information Sheet
POAG	Primary Open-Angle Glaucoma
PPIE	Patient & Public Involvement and Engagement
PSF	Pharmacy Site File
QA	Quality Assurance
QC	Quality Control
QMG	Quality Management Group

QMMP	Quality Management and Monitoring Plan
QMS	Quality Management System
R&D	Research and Development
RGC	Retinal Ganglion Cell
RNA	Ribonucleic Acid
RNFL	Retinal Nerve Fibre Layer
RPE	Retinal Pigment Epithelium
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SE	Sealed Envelope
SITA	Swedish Interactive Thresholding Algorithm
SLT	Selective Laser Trabeculoplasty
SPC	Summary of Product Characteristics
SSA	Site Specific Approval
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMG	Trial Management Group
TT	Trial Team
ToR	Terms of Reference
TSC	Trial Steering Committee
UCL	University College London
ULN	Upper Limit of Normal
UK	United Kingdom
UKGTS	United Kingdom Glaucoma Treatment Study
VF	Visual Field
WOCBP	Women of Child Bearing Potential

Glossary

Aqueous Humour	The clear fluid produced by the eye that fills and helps form the anterior and posterior chambers of the eye. It provides nutrition to the eye as well as maintains pressure within the eye.
Adenosine Triphosphate (ATP)	The principal molecule for storing and transferring energy in cells.
COVID-19	Coronavirus disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2)
DBA/2J mouse glaucoma model	A model for congenital experimental glaucoma. It develops anterior segment anomalies with synechiae and pigment dispersion leading to raised IOP and glaucomatous damage.
EMGT Criteria	Early Manifest Glaucoma Trial Criteria to define visual field progression.
European Quality of Life -5 Dimension – Visual Scale (EQ-5D-5L)	Quality of Life questionnaire to assess health status using 5 dimensions each with 5 response levels of severity.
Glaucoma	A common eye condition where the optic nerve (ON), which connects the eye to the brain, becomes damaged. It's often associated with increased pressure inside the eye.
GQL-15 Questionnaire	15-item Glaucoma Quality of Life Questionnaire to measure severity of visual disability.
HbA1c	Glycated haemoglobin (or HbA1c) is when haemoglobin (a protein within red blood cells that carries oxygen throughout the body), joins with glucose in the blood becoming 'glycated'. The measurement of HbA1c provides the average blood sugar levels of a person over the last 2 or 3 months.
Highly effective contraception	Defined as contraceptive methods that can achieve a failure rate of < 1% per year when used consistently and correctly.
Humphrey Field Analyzer	A diagnostic tool commonly used by optometrists to assess the retina's ability to detect a light stimulus at specific points within the visual field. This is called retinal sensitivity and is recorded in 'decibel's (dB).
Intraocular Pressure (IOP)	The fluid pressure of the eye.
Latanoprost	Eye drop medication used to lower pressure inside the eye. It is one of the current methods of treatment for glaucoma.
Mitochondria	Membrane bound cell organelles that generate most of the chemical energy (adenosine triphosphate (ATP)) needed to power the cell's biochemical reactions. They are known as the "energy factory" of our body as their job is to process oxygen and convert substances from the food we eat into energy.
Mitochondrial dysfunction	Occurs when the mitochondria do not work as well as they should due to a disease or another condition. The mitochondria fail to produce enough energy for the body to function properly. This disease can affect almost any part of the body.

Nicotinamide Adenine Dinucleotide (NAD)	A coenzyme central to metabolism.
Nicotinamide (NAM)	A water-soluble form of Vitamin B3, which is naturally present in niacin rich foods such as fish, poultry, nuts, legumes, eggs and cereal grains. It can also be taken as a supplement. Vitamin B3 is essential for good health and deficiency can lead to serious illness. It is a: - Cellular energy precursor - Modulator of inflammatory cytokines - Inhibitor of the nuclear enzyme poly polymerase, which plays a significant role in DNA repair, maintenance of genomic stability and cellular response to injury
Normal Tension Glaucoma (NTG)	The optic nerve (ON) becomes damaged even though eye pressure stays within the normal levels.
Open Angle Glaucoma (OAG)	The most common form of glaucoma. The drainage angle formed by the cornea and iris remains open. This causes pressure in the eye to gradually increase. This pressure is associated with damage to the ONH.
Optical Coherence Tomography (OCT)	A non-invasive imaging test that uses light waves to take cross-section pictures of the retina
Primary OAG	Defined as a syndrome of 'characteristic' optic nerve damage associated with an open anterior chamber angle and no identifiable cause for elevated intraocular pressure.
Pseudoexfoliation glaucoma	Glaucoma develops in some patients with a condition called exfoliation syndrome (also known as pseudoexfoliation). It is caused by the abnormal accumulation of protein in the drainage system and other structures of the eye.
Retinal Nerve Fibre Layer (RNFL)	Formed by the retinal ganglion cell (RGC) axons as they pass from the RGC body to the ONH. It is the thickest near the ONH.
Selective Laser Trabeculoplasty (SLT)	A form of laser treatment used to treat intraocular pressure in patients with glaucoma.
Sirtuins	Sirtuins are a family of signalling proteins involved in metabolic regulation.
Trabecular Meshwork	An area of tissue located in the anterior chamber angle of the eye, near the cornea. It is the drainage structure through which the aqueous humour flows.
Visual Field	The total area in which objects can be seen in the side (peripheral) vision as you focus your eyes on a central point.
Visual Field Progression	A significant change from baseline or a significant rate of decline in VF sensitivity.
Women of Child Bearing Potential (WOCBP)	A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A post-menopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may

	be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

1 Background

1.1 Rationale

Glaucoma is the leading cause of irreversible blindness worldwide, the second cause in the UK and a leading cause of certifications for sight impairment[3]. Prevalence rises from about 2% in the over 50s to 5% in the over 80s in white European populations and is more common in people of West-African descent; socioeconomic factors influence prevalence and care outcomes[4]. About 300,000 people are diagnosed with glaucoma in the UK[5]. As the population ages, prevalence is predicted to rise by 44% between 2015 to 2035[6]. Hospital Eye Services are responsible for 10% of all NHS outpatient activity and glaucoma accounts for 25% of eye appointments (over a million each year). The average individual lifetime cost of glaucoma was estimated at £40,000 in 2003[7]. The UK population lifetime costs are at least £12bn and set to rise. Aside from financial costs, glaucoma causes a psychological burden with a fear of blindness, social withdrawal, depression and a higher risk of falls.

Open-angle glaucoma (OAG) is a common chronic optic neuropathy causing progressive deterioration in vision. OAG is the world's commonest cause of irreversible blindness[8]. Median age (interquartile range) at diagnosis is 67 (55-76) years[9]. The major risk factors for OAG are raised eye pressure (intraocular pressure; IOP) and greater age.

NICE Guidelines recommend that patients with OAG should be offered IOP lowering treatment (Selective Laser Trabeculoplasty (SLT) as first line treatment or eye drops if the patient declines SLT, or is not suitable for SLT, or as an interim measure while SLT is being arranged). Typically, treatment is escalated if IOP-lowering is insufficient or the glaucoma progresses, so that up to 75% of patients require more than one type of IOP-lowering medication after two years of therapy[10]. Combination therapy usually requires taking drops several times a day, causing inconvenience and frequent ocular and systemic side effects[11][12]. Many patients have difficulty instilling drops[13]. The only oral agent for IOP-lowering, acetazolamide, is a potent diuretic and unsuitable for long term use. Current treatments have shown to slow vision loss however, many patients continue to lose vision despite treatment. Although most treated patients do not experience noticeable vision loss, an unacceptably high proportion lose vision and become vision-impaired or blind despite our best current treatments: 24% of glaucoma patients are blind in one eye, and 11% in both eyes, at the end of life[14].

There is wide variation in susceptibility to IOP, with some patients retaining a healthy optic nerve (ON) and good vision despite years of high IOP and other patients progressing rapidly to vision impairment with IOP within the statistical normal range. All current treatments for glaucoma are licenced on the basis of their ability to lower IOP. There are no licenced treatments to reduce ON susceptibility ('neuroprotective' treatments). An oral neuroprotective treatment would be beneficial for patients progressing despite IOP-lowering, significantly reducing sight loss, and, potentially, for all patients by reducing dependence on eye drop therapy for disease control. Patients themselves want to know the best treatments for glaucoma and whether dietary supplements can slow progression[15].

This trial proposes a neuroprotective treatment to make the ON more resistant to IOP. There is strong evidence that mitochondrial function is associated with OAG susceptibility, and that nicotinamide (NAM) improves mitochondrial function.

Whilst it is well established that IOP is the only currently modifiable risk factor for glaucoma progression, it is widely accepted that other risk factors modulate the susceptibility of an eye to IOP. This has led to a considerable body of research into neurodegenerative mechanisms and potential neuroprotective approaches[16]. Various pathways contributing to the neurodegeneration have been implicated[17], and many have focussed on the role of mitochondria[18]. The knowledge gap addressed here is the relative contribution that mitochondrial function makes to glaucomatous neurodegeneration and whether boosting mitochondrial function with NAM slows progression in humans in the same way that it does in animal models. Clinical and experimental evidence is summarised below. Biomarkers for mitochondrial-related susceptibility have not yet been established in humans.

Retinal ganglion cells (RGCs) probably have more mitochondria than any other CNS neurone[18]. Oxygen consumption per tissue weight is even higher in the retina than the brain[19]. RGCs, in particular, have very high energy consumption attributed largely to demands to support axon potential generation in the unmyelinated portion of RGC axons and to maintain axonal transport across the high pressure gradient as axons leave the eye at the optic nerve head (ONH). The former is evidenced by the trafficking and distribution of mitochondria within RGC axons[20] and the latter by the giant mitochondria in the ONH astrocytes which support RGC axonal metabolism at an anatomical site of particular vulnerability[21]. These energy demands make the portion of the ON in the eye particularly susceptible to mitochondrial dysfunction[22]. Mitochondrial dysfunction has emerged as an important susceptibility factor for glaucoma[23–26] as evidenced below:

a) Genetic evidence: the role of mitochondria in glaucoma susceptibility is further supported by genetic studies. Khawaja and colleagues, using gene set analyses of a large cohort of patients, identified mitochondrial enzyme pathways critical to the pathogenesis of primary open angle glaucoma (POAG)[27]. Polymorphisms in the optineurin gene (which mediates mitophagy) are associated with OAG[28], including OAG with normal IOP (normal tension glaucoma; NTG) and younger age of onset of high-tension glaucoma (HTG). Inherited mitochondrial defects are associated with characteristic optic neuropathies: Leber's hereditary optic neuropathy (mitochondrial genome mutations) and dominant optic atrophy (mutations in a nuclear gene encoding inner mitochondrial membrane proteins)[29]. Phenotypically, dominant optic atrophy may mimic NTG[30].

b) Ex vivo and animal model evidence: degeneration of RGC mitochondria is an early feature of human glaucoma[31] and mitochondrial dysfunction has also been identified in lamina cribrosa[32] and trabecular meshwork cells[33] of glaucomatous eyes. The effects of raised IOP on mitochondrial function and mitophagy have been well described in animal glaucoma models[34–36]. Differential expression of genes in the mitochondrial dysfunction and oxidative phosphorylation pathways is an early feature in the DBA/2J mouse glaucoma model[37]. Data support a model where age-dependent declines of NAD+ and glutathione in the retina render RGCs vulnerable to damage from elevated IOP[37].

c) Clinical evidence: initial reports of mitochondrial abnormalities in lymphocytes of glaucoma patients[38] have been replicated by others[39]. There is evidence that susceptibility to IOP is related to mitochondrial function. Lascaratos and Garway-Heath and colleagues demonstrated that patients who do not develop glaucoma despite raised IOP have better lymphocyte mitochondrial function compared to glaucoma patients with normal IOP and age similar controls[40]. Lascaratos and Garway-Heath and colleagues have also shown that NTG patients have lower lymphocyte mitochondrial function compared to those with HTG (Figure 1)[41]. Mitochondrial function of patients with severe glaucoma was worse than in those with mild/moderate disease.

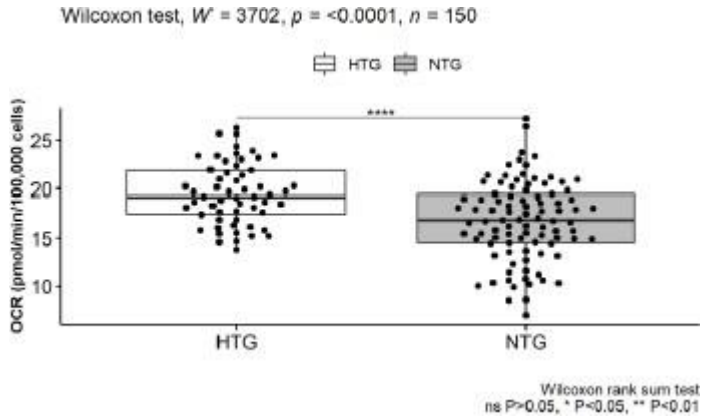


Figure 1. ATP-linked oxygen consumption rate (OCR) for 57 HTG and 93 NTG patients[41].

One publication of a trial of NAM in OAG was identified[42]. This evaluated oral NAM (1.5-3g/day) versus placebo over 3 months in patients with OAG. Significantly improved retinal function (measured electrophysiologically with the photopic negative response) was observed with the higher dose (3g/day) in 23% compared to 9% of those on placebo[42]. A similar positive effect was seen on the VFs and NAM was well tolerated. This demonstrates a neuro-enhancement effect over the short-term. However, the rate of OAG progression was not evaluated, so disease modification has not been shown. A trial is needed to assess long-term neuro-protection.

NCT03797469 (clinicaltrials.gov trial registry) is a small (n=36) interventional study assessing the effect of NAM and pyruvate to increase concentration and improve performance during VF testing. NCT04784234 (clinicaltrials.gov trial registry) is a 6-month trial of GlaucoCetin (a medical food that includes more than 20 ingredients including very low dose NAM) versus placebo that aims to evaluate improvement in vision and visual function in patients with OAG. ChiCTR1900021998 (WHO ICRTP trial registry) is a small (n=125) study evaluating the effect of nicotinamide riboside on the rate of OCT RNFL thinning over 24 months. None of the above trials assesses the effect of NAM on progressive visual function loss in OAG.

Untreated, glaucoma results in permanent vision loss. Current IOP-lowering therapies slow the rate of loss, so that most patients retain useful vision. However, visual disability and blindness rates remain unacceptably high amongst patients receiving treatment[14]. The only modifiable risk factor for worsening (progression) is raised IOP, yet there is a wide variation in susceptibility to IOP, with 40% of OAG presenting with IOP within the normal range, visual field (VF) deterioration occurring at all IOP levels (including normal) and progression occurring despite IOP lowering. Clinical studies underline the inadequacy of current IOP-lowering approaches, with over 40% of patients progressing after 7 years in one study[43] and 60% with statistically significant deterioration after an average 8 years follow-up in another study[44]. Models for predicting rates of progression have poor to moderate accuracy[45,46]. The identification of biomarkers for rapid deterioration, indicating patients most at risk, would considerably improve glaucoma care efficiency and enable personalised medicine. Mitochondrial function is a promising biomarker for glaucoma susceptibility and risk stratification. The same biomarker may predict treatment response.

Nicotinamide adenine dinucleotide (NAD) is an essential cofactor in oxidative phosphorylation and adenosine triphosphate (ATP) production and a potent mediator of axon neuroprotection. NAM is the major precursor of NAD in neurones, synthesised through the salvage pathway[47]. Older age is a major risk factor for OAG and ageing is associated with mitochondrial functional decline and a reduction in tissue NAD and key NAD salvage pathway transcripts[37,48]. The accelerated ageing seen in Werner syndrome is associated with impaired mitophagy and NAD+ depletion. The mutation is for a gene (WRN) that regulates a key enzyme for NAD+ synthesis, NMNAT1. NAD+ repletion restores NAD+ metabolic profiles and improves mitochondrial quality through DCT-1 and ULK-1-dependent mitophagy[49].

NAD and ATP levels are crucial for linking cellular energy status to molecular processes that regulate cell survival. NAD+ may act directly or through modulation of the activity of the enzymes PARP and SIRT1, implicated in DNA repair and control of cell metabolism[50]. The importance of the NAD+-sirtuin axis has been emphasised in systemic conditions in which NAD depletion plays a role (cardiac and renal disease) and NAD+ supplementation has been proposed as a promising therapy[51]. The diverse mechanisms by which NAM has its therapeutic effect has been reviewed recently and include direct effects of NAM and NAD, and effects through sirtuin activation[52].

In addition to glaucoma, mitochondrial dysfunction has been implicated in other ocular disease, notably age-related macular degeneration (AMD) affecting retinal pigment epithelial (RPE) cells; improved function (ATP synthesis) has been demonstrated in cultured RPE cells when treated with nicotinamide mononucleotide[53].

NAM plasma levels are lower in glaucoma patients than in age-matched controls in one report[54] and preliminary data from Lascaratos and Garway-Heath and colleagues show that fibroblast NAD levels in NTG patients (n=5) is significantly ($p<0.01$) lower than in age-similar controls (n=5).

There is clinical evidence that better than average mitochondrial function, measured in lymphocytes, provides resistance to OAG in the context of high IOP[40] and worse than average mitochondrial function, and lower plasma NAM[54], confers susceptibility to OAG. The laboratory evidence shows

that high IOP leads to mitochondrial metabolic dysfunction, and NAD+ depletion, in the retina and ON.

The safety of high-dose NAM has been reviewed[55]. Conclusions of the review were "The therapeutic index of nicotinamide is wide but at very high doses reversible hepatotoxicity has been reported in animals and humans" and "High-dose nicotinamide should still, however be considered a drug with toxic potential at adult doses in excess of 3 gm/day." There is potential for effects on mitochondrial function and/or drug interactions with drugs such as isoniazid, pyrazinamide, carbamazepine, phenobarbital and primidone. In a survey of 6000 schizophrenic patients on 'megadoses' (up to 6g/day) of nicotinamide or nicotinic acid, 3 cases of jaundice were reported; one cleared spontaneously, and another cleared when concomitant phenothiazine was stopped[56]. In the European Nicotinamide Diabetes Intervention Trial (ENDIT), where doses of between 0.5 and 3.0g/day for 5 years were administered in a placebo-controlled trial to 522 patients, there was no difference in adverse events in general between treatment groups; nine participants had elevated liver transaminases, 5 from the NAM group and 4 from the placebo group[57]. Nausea, vomiting, reversible hepatic toxicity and hyperglycaemia have been reported with very high doses (>3g/day)[55].

There are limited (human and animal) data available on the effects of NAM in pregnancy and fertility. No adverse events have been reported[58,59] but, the half-life of Nicotinamide is dose-dependent and reported to be 4 hours for the doses used in the proposed study.[60]

In this trial a NAM dose of 3.0g/day has been selected based on preclinical glaucoma models; trials of neurorecovery in patients with glaucoma; and the literature on the tolerability and safety. A step wise dose introduction will be incorporated to improve tolerability of escalation to the higher dose of 3.0g/day[42].

1.1.1 Explanation for choice of comparators

The potential of NAM treatment for neurodegenerative diseases has gained widespread attention, with several current trials in Alzheimer's disease (AD), Parkinson's disease, peripheral small-fibre neuropathy and Friedreich's ataxia. Whereas there are common features between glaucoma and other age-related neurodegenerations, the very high energy requirements of RGCs and their dependency on mitochondrial function suggest glaucoma may be particularly responsive to NAM. Given the role of mitochondria in glaucoma susceptibility, NAM has been proposed as a strong therapeutic candidate[61]. High dose oral NAM raises tissue NAD+ levels through synthesis in the salvage pathway and reverses age-dependent declines in key NAD-salvage pathway transcripts[37]. Higher tissue NAD+ leads to higher ATP levels and mitochondrial membrane potential, and axonal protection[48].

Animal models: high-dose NAM provided profound protection from glaucoma in a mouse model, associated with an increase in retina NAD levels and up-regulation of NMNAT2, a key NAD producing enzyme in RGCs (and which has been shown to protect neurones)[37]. Further evidence for the neuroprotective effects of NAM comes from a series of experiments using a variety of neuronal insults (rat ocular hypertension, axotomy and intravitreal rotenone)[62]. There was an IOP-dependent

decline in NAD in the retina and ON of ocular hypertensive rats and an associated loss of RGCs which was prevented by oral NAM treatment in a dose-dependent manner (Figure 2).

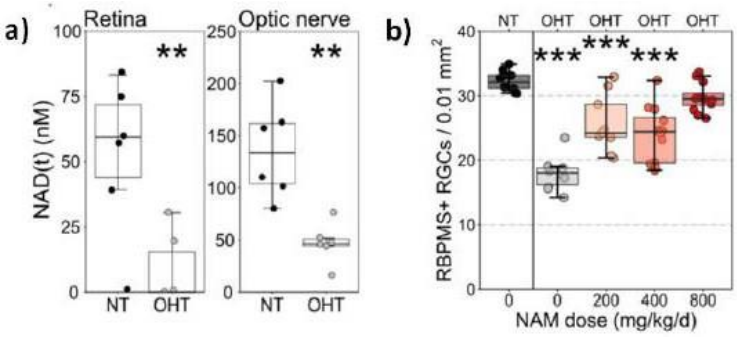


Figure 2. a) Retina and optic nerve NAD levels in normotension (NT) control and ocular hypertensive (OHT) rats; **b)** Retinal ganglion cell (RGC) counts in NT and OHT rats at 3 NAM doses (from Tribble et al[62]).

Other sequelae illustrating neuronal metabolic dysfunction induced by ocular hypertension were prevented by NAM. NAM's neuroprotective effects in the RGC were achieved by increasing oxidative phosphorylation, buffering and preventing metabolic stress, and increasing mitochondrial size and motility.

Human data: oral NAM (1.5-3g/day) for 3 months significantly improved retinal function (measured by the photopic negative response) at the higher dose (3g/day) in 23% of 57 glaucoma patients compared to 9% of those randomised to placebo[42]. A similar positive effect was seen on the VFs and NAM was well tolerated. This demonstrates short-term benefit on visual function neuroenhancement).

A trial is needed to assess long-term neuro-protection. In a pilot study, we showed mitochondrial function in skin fibroblasts from a normal-tension glaucoma patient significantly improved with NAM treatment[63].

There is clinical evidence that high-dose NAM supplementation leads to better retinal function and laboratory evidence that high-dose NAM supplementation reverses mitochondrial metabolic dysfunction and provides profound neuronal protection in the high-IOP eye.

Therefore, it is anticipated that high dose NAM supplementation will provide protection from OAG progression in patients with poor mitochondrial function both resulting from systemic metabolic dysfunction (possibly related to low serum NAM levels) and local metabolic dysfunction resulting from high IOP.

A treatment protecting the ON from glaucoma damage would have a large impact on patient well-being and costs of care. Neuroprotection would significantly reduce sight loss and the burden of IOP-lowering eyedrop treatment needed for disease control. This anticipates a reduction in NHS costs resulting from a more stable clinical population (fewer outpatient appointments and fewer IOP-

lowering treatment escalations, including surgery) and a reduction in social care costs of visual impairment and blindness. At a proposed dosage of 3.0g/day, NAM costs about £13 a month.

Matching placebo will be used as a comparator (control group) to NAM.

The proposal for this trial is a placebo-controlled trial to evaluate the long-term safety and efficacy of NAM to preserve vision, to elucidate its mechanism of action and identify biomarkers for patients who may benefit most. Whilst the primary outcome of this trial will be a conventional VF outcome, data will be collected to validate potential imaging surrogate outcomes.

1.1.2 Hypothesis

Clinical Hypothesis:

Treatment with high-dose NAM, compared to placebo, reduces the amount of VF loss (VF mean deviation (MD)) by 0.158dB on average over 27 months in recently diagnosed patients on standard IOP-lowering treatment.

Mechanistic Hypothesis:

Lymphocyte mitochondrial function (ATP-linked OCR) is associated with the rate of VF progression; NAM improves lymphocyte mitochondrial function.

This trial has two components:
1) overall benefit – assessment of change in VF sensitivity (months 0-27; primary outcome) and;
2) neuro-recovery – assessment to assess potential VF improvement (months 0-3; key secondary outcome).

1.2 Objectives

1.2.1 Primary Objectives

To evaluate the effect of high-dose NAM on VF loss (change from baseline in MD) in recently diagnosed glaucoma patients over 27 months.

1.2.2 Secondary Objectives

Clinical safety and efficacy:

1. Evaluate the effect of high dose NAM on VF sensitivity over the initial 3 months (0-3 months – neuro-recovery).
2. Evaluate NAM safety.
3. Evaluate quality of life outcome (EQ-5D-5L (with vision bolt-on) and GQL-15) differences between treatment arms.

Mechanistic:

1. Quantify the association between mitochondrial function and rate of VF loss (placebo group).
2. Assess whether any benefit of NAM on a) rate of VF loss and b) level of mitochondrial function is greater when baseline mitochondrial function is poor or when baseline serum NAM (vitamin B3) levels are low.
3. Test hypothesis that lower lymphocyte mitochondrial function correlates with lower serum NAM levels and lower lymphocyte NAD+ levels; assess each as a biomarker for VF progression.
4. Evaluate association of NAM, NAD and OCR levels with the EPIC Food Frequency Questionnaire outcomes between treatment arms.
5. Assess whether NAM lowers IOP.
6. Quantify the association between mitochondrial function and quality of life measures.

1.2.3 Surrogate Objectives

1. Establish whether any effect of NAM on structural (OCT) outcomes (peripapillary RNFL loss and macular GCL loss) is similar to the effect on VFs (VF loss).
2. Evaluate the effect of high-dose NAM on the rate of VF progression, with the rate of OCT peripapillary RNFL/macular GCL thinning as a Bayesian prior.

1.2.4 Exploratory Objectives

To evaluate the difference in the rate of VF progression between treatment arms. The rate of VF loss is widely recognized as a clinically relevant outcome. NAM treatment has been reported to result in a step improvement in the VF ('neuro-recovery'); this is not necessarily related to disease modification (preservation of RGCs over an extended period). Inclusion of a step improvement in calculations of the rate of VF change would result in a spurious estimation of the rate of change (likely over-estimating the potential disease modification effect). Therefore, we will test for a step improvement at 3 months. We will calculate rates of VF loss between 0-27 months if there is no evidence for neuro-recovery (statistically significant difference at month 3 VF between treatment arms) or 3-27 months if there is evidence for neuro-recovery at 3 months. We will also evaluate differences between treatment groups in the rate of VF loss at predefined subsets of VF locations.

1.3 Trial Design

This is a UK multi-centre, randomised, masked, placebo-controlled, phase III trial with an internal 6-month pilot (See Section 6.7), designed to evaluate the effect of high-dose NAM on VF loss (change from baseline in Mean Deviation) in recently diagnosed glaucoma patients over a 27-month period.

The visit schedule includes 8 visits: Screening (-2 weeks up to -3 months before baseline), Visit 1 (Baseline, Month 0), Visit 2 (Month 3), Visit 3, (Month 6), Visit 4 (Month 12), Visit 5 (Month 18), Visit 6 (Month 24), and Visit 7 (Month 27); the primary endpoint of the trial. The total duration of the trial for each participant will be up to 30 months from the screening visit.

For a summary of the participant schedule of assessments, please refer to Section 6.2.

1.4 Benefit Risk Assessment

NAMinG is a low-risk trial investigating vitamin B3, which can be found in food or used as a dietary supplement. Animal models[37,62] and human trials[42] have shown that NAM can improve retinal function in glaucoma. The dose used in this trial (up to 3.0g/day) has been used in previous clinical trials and has shown to have a stable safety profile and is well tolerable[55,56]. To ensure tolerability a low dose of 1.5g/day will be introduced for the first 6 weeks before increasing to 3.0g/day for the rest of the trial duration[42]. If a dose is not tolerated than there are steps to reduce the dose to the maximal tolerated dose as specified in section 5.3.4. The IMP is a tablet, which is easier to administer than the current standard of care treatment, which is eye drops[13]. Adverse events will be assessed throughout the trial duration, participants will be able to record side effects experienced in their dosing diaries which will be reviewed at each clinic visit. In between the clinic visits contact will continue to be made via telephone calls to monitor progress. The known side effects[55–57] related to the IMP are listed in section 7.1.3. Participants are also excluded from taking certain medication whilst participating in the trial, which are listed in section 5.4 and this will be explained to participants by the investigators and research teams during the informed consent process.

The assessments that are above standard of care are the blood samples that are to be collected and the completion of questionnaires and dosing diaries.

The benefits of this trial would outweigh the risks involved.

Table 1 below summarises the risks, frequencies and mitigations of the IMP Nicotinamide:

Potential Risk	Risk Frequency	Risk Management
Liver enzyme abnormalities	≤1.6%	If elevated levels of liver aminotransferase enzymes >3 times the upper limit of normal then IMP will be discontinued as detailed in section 5.7. These are exclusion criterions and will be tested at Screening and then monitored during the trial. The IMP used for this trial should not cause flushing. However, if any side effects experienced cannot be tolerated then the current IMP dose that the participant is taking can be reduced to the previous tolerated dose level (refer to section 5.3.4).
Gastrointestinal symptoms	≤ 1.6%	
Fatigue	≤ 0.4%	
Headache	≤ 0.5%	
Eye symptoms	≤ 0.4%	
Flushing	≤ 1.5%	

		All adverse events will be recorded, and serious adverse events reported.

Table 2 below summarises the risks and mitigations of all tests and/or procedures above standard care that are being performed:

Intervention	Potential Risk	Risk Management
Blood sample collection	Bruising, dizziness	Blood samples are only collected at certain time points and the amount of blood drawn will be within the daily limit. The different blood sample types can all be taken from one blood draw. Some blood samples are optional, and consent will be obtained.
Questionnaire completion	Duration and length	Only 3 short questionnaires to complete at 3 visits in the trial. Research teams can help participants in completing these.
Dosing Diary completion	Inconsistent completion	Research teams will provide instructions on how to complete the diary and inform participants these need to be brought to each clinic visit for review. Telephone calls in between clinic visits will review IMP adherence and remind participants to complete the diaries.
Visual Field (VF) assessment	Participant may get tired	Visual Field assessments will occur during clinic visits and these are conducted as standard of care. Additional VF tests will be carried out during the additional clinic visits that fall outside of routine and at some clinic visits the test will be repeated twice. Participants will be given a break at those visits where the VF assessment needs repeating twice.
Urine pregnancy test for women of child bearing potential (WOCBP)	Participant may refuse/Unknown risk to	Pregnant or breastfeeding females will be excluded from the trial. Participants (male

	pregnant women or an unborn baby	and WOCBP) not willing to use highly effective methods of contraception will also not be included in the trial. Participants will be explained the importance of a pregnancy test for WOCBP and the use of highly adequate methods of contraception throughout the trial. A urine pregnancy test, which participant can easily self-administer, will be conducted at screening, baseline and all subsequent clinic visits.

2 Selection of Sites/Investigators

2.1 Site Selection

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

2.1.1 Trial Setting

This trial will take place at up to 7-10 NHS hospitals across the UK (additional sites will be added if required), all of which were selected based on their expertise with glaucoma trials and geographical location to represent a diverse group of patients. Five of the proposed sites are in the top 20% of areas of greatest social deprivation in England, thus, the trial participant make-up will reflect the intended target populations and is skewed toward those at higher risk.

A list of participating sites can be obtained upon request from the NAMinG Trial Manager at UCL CCTU.

2.1.2 Site/Investigator Eligibility Criteria

Once a 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 (SmPC(s)) or Investigator Brochure(s) (where appropriate).

To participate in the NAMinG trial, investigators and trial sites must fulfil a set of criteria that have been agreed by the Sponsor and NAMinG Trial Management Group (TMG) and that are defined below.

Eligibility criteria:

1. A named clinician is willing and appropriate to take Principal Investigator responsibility
2. Suitably trained staff are available to recruit participants, enter data and collect samples
3. The site should be able to store, prepare and dispense IMP appropriately

2.1.2.1 Principal Investigator's (PI) Qualifications and Agreements

The Principal Investigator(s) must be willing to sign a Principal Investigator Declaration (Schedule 5 of the Clinical Trial Site 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 (provide an up-to-date CV), familiarity with the appropriate use of any investigational products and agreement to comply with the principles of GCP. The PI must agree to permit monitoring and audit as necessary at the site, and to maintain documented evidence of staff who have been delegated significant trial related duties.

2.1.2.2 Resourcing at site

- The investigator should demonstrate potential for recruiting the required number of suitable participants within the agreed recruitment period.
- The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.
- The investigator should have available an adequate number of qualified staff and suitable facilities for the anticipated duration of the trial in order to conduct the trial properly and safely.
- The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational medicinal product, and their trial-related duties and functions.
- The site should have sufficient data management resources to allow prompt data return to the CCTU (refer to the Data Management Plan for timelines).
- Sites will be expected to complete a delegation of responsibilities log and provide staff contact details.

In the event of any UK Government restrictions (i.e. exceptional circumstances), the trial design has been adjusted to align most of the trial visits with NHS standard of care visits. As all recruited participants will have early-to-moderate disease, they should be suitable for follow-up in virtual/data acquisition glaucoma clinics, however sites should follow their local policy if restrictions are implemented. Further changes to the design of the trial will be applied if necessary.

2.2 Site approval and activation

Site training will be performed prior to the activation of each site and will include all processes for the trial including but not limited to protocol training, data management procedures, procedures for handling of investigational medicinal product, adverse event reporting procedures, procedures for laboratory samples and frequency and expectations for monitoring visits. A log of Site Initiation Visit

attendees will be kept in the TMF as a record of participants present. The Visit may occur in person or via Videoconference as outlined in the QMMP.

The trial manager or delegate will notify the PI in writing of the plans for site activation. Sites will not be permitted to recruit any patients until a letter for activation has been issued. On receipt of the signed Clinical Trial Site Agreement (including the signed PI Declaration), completed delegation of responsibilities log and staff contact details, the Trial Manager or delegate will complete the green light process and issue written confirmation of site activation to the site PI.

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 Ethics Committee (EC). The PI or delegate must document and explain any deviation from the approved protocol and communicate this to the trial team at CCTU.

A list of activated sites may be obtained from the Trial Manager at UCL CCTU.

3 Selection of Participants

There will be **NO EXCEPTIONS** (waivers) to eligibility requirements at the time of trial entry. Questions about eligibility criteria should be addressed PRIOR to attempting to randomise 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 the inclusion criteria and none of the exclusion criteria as defined below.

Open Angle Glaucoma (OAG) is defined as a reproducible glaucomatous VF defect in at least one eye in reliable VFs with corresponding damage to the Optic Nerve Head (ONH), with an open angle on gonioscopy and in the absence of a retinal, neurological or adnexal condition that could account for the VF loss.

Definition of a glaucomatous VF defect: a Glaucoma Hemifield Test outside normal limits (ONL) AND a cluster of 3 or more locations, 2 of which are depressed on the pattern deviation plot at a P value of <5% and 1 of which is depressed at a P value of <1%, on at least 2 consecutive fields. At least 1 common location in the cluster should be depressed at <5% in the 2 consecutive VFs.

Definition of a reliable VF: false positive response rate ≤15%.

Definition of a cluster: each depressed location in the clusters should be adjacent to at least one other depressed location.

3.1 Participant Inclusion Criteria

1. Patients who have been recently diagnosed (within the last 12 months) with early to moderate open-angle glaucoma (OAG) in at least one eye* (including primary OAG, NTG and pseudoexfoliation glaucoma)
2. Open angle on gonioscopy
3. Adults aged 18 years or over
4. Snellen visual acuity 6/12 or better in at least one eye meeting the visual field (VF) criteria**
5. Visual Field (VF) mean deviation (MD) no worse than -12dB in either eye
6. A negative pregnancy test result at the screening and baseline visit prior to randomisation for women of childbearing potential
7. Ability to provide informed consent to participate
8. Able and willing to attend trial visits and comply with trial procedures for the duration of the trial

** Data to be collected from both eyes, the eye with the worst visual field loss at baseline will be used in the primary analysis.*

*** As described in section 3*

3.2 Participant Exclusion Criteria

1. Pigment dispersion glaucoma
2. Pregnancy (or planned pregnancy during the trial) and/or breastfeeding
3. Women of childbearing potential and male participants with a partner of childbearing potential not willing to use highly effective contraception as described in section 3.2.1.1 for the duration of the trial treatment and for the time period specified following last trial treatment administration.
4. Current treatment with either isoniazid, pyrazinamide, carbamazepine, phenobarbital or primidone
5. Current liver disease or laboratory results with elevated levels of transaminases (AST or ALT >3 x ULN) at screening visit.
6. Renal failure (eGFR <30mL/min/1.73m^2) at screening visit
7. Conditions affecting both eyes*** which may affect the VF test result:
    a. Diabetic retinopathy or any other retinal disease causing VF loss
    b. Clinically relevant cataract (likely to require cataract surgery within the next 2 years)
    c. Dementia or other non-glaucomatous neurological disease causing VF loss
    d. Adnexal conditions causing VF loss (including, but not limited to blepharochalasis)
8. Diagnosed with cancer in the last 5 years (with exception of non-melanoma skin cancer)
9. Any clinical condition that, in the investigator's opinion would make the participant unsuitable for the trial
10. Concurrently enrolled in any other interventional trial or participation in previous clinical trial of glaucoma

11. Current use of, and unwilling to abstain from, over-the-counter additional vitamin B3/NAM oral supplements (including skin preparations such as ointments/emulsions), Ginkgo Biloba and/or Coenzyme Q10 supplements, throughout the duration of their participation in the trial

**** If only one eye is affected and the other is eligible, participant can still enter the trial.*

3.2.1 Women of Childbearing Potential

A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming postmenopausal unless permanently sterilised. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

Female participants of childbearing potential must not become pregnant and agree to use a highly effective method of birth control whilst administering the IMP and for up to 30 days after stopping trial treatment. A highly effective method of birth control is defined as contraceptive methods that can achieve a failure rate of < 1% per year when used consistently and correctly.

Birth control measures must be employed, beginning at Screening and during the time of participation in this trial.

The method of contraception should be clearly documented in the participants medical notes during the Screening visit (prior to baseline/randomisation).

3.2.1.1 Birth control methods considered as highly effective

Highly effective contraceptive methods[64] include:

- Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - Oral
  - intravaginal
  - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - Oral
  - injectable
  - Implantable (*low user dependency)*
- Intrauterine device (IUD) – (*low user dependency)*
- Intrauterine hormone-releasing system (IUS) – (*low user dependency)*

- Bilateral tubal occlusion – (*low user dependency)*
- Vasectomised male partner – (*low user dependency).* This is a highly effective birth control method provided that the partner is the sole sexual partner of the WOCBP participant and that the vasectomised partner has received medical assessment of the surgical success. Investigators at sites must document azoospermia prior to the female participants' entry into the trial.
- Sexual abstinence, defined as refraining from heterosexual intercourse (i.e., with male partner) when this is in line with the preferred and usual lifestyle of the participant, during the entire period of administering the trial treatment and for up to 30 days after stopping trial treatment.

3.2.1.2 Unacceptable birth control methods

Periodic abstinence (calendar, ovulation, symptothermal and post-ovulation methods) withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) **are not considered acceptable methods of contraception in clinical trials.** Female condom and male condom **should not be used together**.

Progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action, double barrier methods such a combination of condom with cap, diaphragm, or sponge with spermicide **are not considered highly effective** (i.e., have a failure rate of more than 1% per year).

3.2.1.3 Pregnancy testing

Following written consent, all women of childbearing potential will undergo a urine pregnancy test **prior** to randomisation at Screening (-2 weeks up to -3 months before baseline), Visit 1 (Baseline Month 0), Visit 2 (Month 3), Visit 3 (Month 6), Visit 4 (Month 12), Visit 5 (Month 18), Visit 6 (Month 24) and Visit 7 (Month 27). The results of this pregnancy test must be available prior to dispensing the IMP and should be clearly documented in the participants medical notes and in the trial database.

A urine pregnancy test must be obtained from all WOCBP **30 days after stopping last treatment**. Research teams at sites will be required to post a pregnancy test kit to the participant and then follow-up with a phone call to obtain the result.

3.2.1.4 Male Participants

A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy. Male participants with medical confirmation of azoospermia and/or infertility will be considered permanently sterile.

It is recommended as precautionary measure **and** advised that sexually active male participants or their female partners of childbearing potential use highly effective contraception methods during

treatment and for at least 30 days after cessation of the IMP. Research teams at sites must follow-up with a phone call 30 days after cessation of the IMP to obtain pregnancy information regarding their female partners.

Investigators at participating sites should inform all male participants of reproductive potential, including those planning to have children, of the potential risks and document this in the participants medical notes.

3.3 Recruitment

Participants will be recruited from 7-10 UK NHS sites (additional sites will be added if required) with established research infrastructure and a proven research track record in glaucoma trials; most of the proposed hospital sites have previously recruited for UKGTS, LiGHT and TAGS, with good deliverability. The selected sites (additional sites will be added if required) also have good geographic location to represent a diverse group of patients. Five out of the 7 sites are in the top 20% of areas of greatest social deprivation in England; West African heritage is a risk factor for OAG.

The trial will be advertised at each site (using a REC/HRA approved trial poster) as well as public domain websites (such as Sponsor webpage, NIHR Be Part of Research) and potential participants provided with the trial team's contact details. UCL has Beacon of Public Engagement status and we will use the IoO 'News section' to update participants on the study progress. The Chief Investigator is the Glaucoma UK Professor of Ophthalmology at UCL (Glaucoma UK is the national patient support organisation for people with glaucoma) and he will present updates at the Glaucoma UK AGM and in the Glaucoma UK newsletter.

The 'new patient' numbers at each site have been reviewed and an estimated (on average) participation rate of <10% of eligible patients is required to meet the trial recruitment target. A total target of 496 participants will be recruited to this trial, 248 in each arm, over a period of 16 months. The initial 4 sites will recruit over 16 months, which will include a 6-month internal pilot. The other 3-6 sites will recruit over 10 months.

Recruitment for this trial does not compete with other studies at the same time for recently diagnosed glaucoma patients in any of the sites. Recruitment will be monitored regularly to identify any barriers to recruitment, which will include an internal pilot with a specific stop/go criteria. Please refer to section 6.7 below.

MEH will recruit from various MEH satellite sites throughout London where Hindi and Urdu is predominately spoken. The PPI/E will lead a Participant Advisory Group (PAG) which will ensure patient information is culturally sensitive, developed with reference to the BAME toolkit and the INCLUDE Ethnicity Framework. For these participants, where English may not be their first language, a translated version of the PIS and ICF and questionnaires (where necessary) will be provided in Hindi and Urdu and will be made available.

Information will be collated on the number of eligible participants identified and approached for participation against those randomised and who declined participation (with reasons for declining sought), including drop-out rates and reasons for drop-out (which will be monitored with simple questionnaires). This information will be collated by the Participant Advisory Group (PAG) chaired by the lead PPIE, who will report to the Trial Management Group (TMG). The TMG will evaluate data and PAG reports at regular intervals.

Reporting over the trial set-up and recruitment period will be conducted on a regular basis to the NAMinG Trial Management Group (TMG), Independent trial oversight committees and the trial funder. Remedial actions will be put in place if any concerns arise.

3.4 Retention

To enhance recruitment and maintain retention of participants, trial visits have been aligned with NHS standard routine clinic visits (5 out of the 8 visits are routine), and evening/Saturday clinics will be made available where feasible.

The retention of participants in the trial for the primary outcome is fundamental to the assessment of effectiveness. The importance of attending scheduled protocol visits until trial completion will be explained to all participants at the start of the trial to ensure that only those able to commit to the trial protocol are recruited.

Participants who discontinue trial IMP will be encouraged to return for the scheduled follow-up visits. Additional blood samples that are collected and which are not related to safety (PBMCs and DNA/RNA) will be made optional.

Travel costs will be offered to all participants (at an agreed amount) for study visits.
Quarterly Participant Newsletters provided by UCL CCTU/PAG will keep participants informed on trial progress and developments in the field.

3.5 Co-enrolment Guidance

This trial will use screening and enrolment logs to track currently enrolled participants to ensure that each participant can only be enrolled once. The online randomisation service used for this trial, (www.sealedenvelope.com) will also prohibit the same participant from being randomised twice.

Investigators will be responsible for maintaining logs and ascertaining whether potential participants are currently taking part in another clinical trial.

Participants cannot be concurrently enrolled in any other interventional trial or have participated in a previous glaucoma trial. Co-enrolment in observational trials is acceptable.

3.6 Screening Procedures and Informed Consent

Patients will be provided with a Participant Information Sheet (PIS) and given time to read it fully. Following a discussion with a medically qualified investigator or suitable trained and authorised delegate, any questions will be satisfactorily answered and if the participant is willing (and has the capacity to consent and wishes) 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.

If a patient has capacity and is willing to provide verbal consent, but is physically unable to sign the consent form, a witness independent of the trial team will be identified and asked to sign the witness signature field in the consent form, to attest to the patient's verbal consent to participate.

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.

Signed consent forms must be kept by the investigator and a copy given to the participant. The consent process should be documented in the participants medical records. With the consent of the participant, a letter should be sent to the general practitioner informing him/her of the trial and the participant's involvement in it.

Potential participants will be identified principally at 'New Patient' clinics (referred by their GP or optometrist), if the site has such clinics. Otherwise, potential participants will be identified at their first visit to the Glaucoma Clinic. Standard of care assessments will be performed as usual and, when a diagnosis of OAG is confirmed, this will be followed by a discussion of the standard of care treatment options available.

Potentially eligible participants should be provided with the Patient Information Sheet (PIS) during a routine visit or sent a copy in the post with routine appointment letters prior to attending the Screening Visit.

Written informed consent to enter and be randomised into the trial must be obtained from participants after explanation of the aims, methods, benefits and potential hazards of the trial, including the optional sub-studies (see section 21), and **BEFORE** any trial-specific procedures or blood samples for the optional sub-studies are performed.

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.

The following assessments will be performed as standard of care, some of which will be used to assess eligibility:

- Snellen Visual Acuity (both eyes)
- Visual Field assessment - Humphrey Visual Field Analyzer Mark II (or II-i) or HFA3 with the SITA Standard, SITA FAST or SITA Faster programme (both eyes)
- Slit lamp biomicroscopy
- IOP measurement (Goldmann Applanation Tonometry (GAT))
- Spectral Domain Optical Coherence Tomography (SD-OCT) Imaging (Spectralis preferred, when available) (both eyes)
- Gonioscopy and central corneal thickness measurement (any available method) if not previously done

Standard of care IOP-lowering treatment will have been initiated (single initial therapy of preservative-free latanoprost or SLT).

Screening Visit (-2 weeks up to -3 months before baseline):
A preliminary evaluation of eligibility for the trial, based on VF testing and clinical evaluation/imaging of the optic nerve and retina, should be made by the site investigators before informed consent is obtained.

Once written informed consent is obtained, the following additional assessments must be performed:

- Medical History
- Review of concomitant medication (including dietary supplements)
- Review of adverse events
- Discussion about IOP-lowering treatment to ensure treatment aligns with the trial algorithm
- Blood sample collection: Glycosylated haemoglobin (Hb1Ac), LFTs, eGFR, serum NAM
- Urine Pregnancy test (for WOCBP)

Potential trial participants should be asked to bring with them to the Screening Visit all prescribed and over-the-counter medicines used AND food supplements and vitamins.

It is the site investigators responsibility to review what IOP-lowering treatment participants are currently receiving and whether this is in line with the treatment algorithm detailed in section 5.3.4. Any changes to IOP-lowering treatment should be considered and discussed prior to obtaining consent at this visit and then aligned with the trial treatment algorithm and commenced only after consent is obtained and prior to randomisation at the baseline visit (Month 0).

The Visual Field results from the screening visit in addition to the last Visual Field results performed at the routine clinic visit, will be transferred using secure software and in an anonymised manner (refer to section 4 for details) to the Moorfields Reading Centre (an image grading centre for clinical

trials in ophthalmology, located at Moorfields Eye Hospital). These tests will be assessed against the VF component of the participant **eligibility criteria.**

Results from the review of the visual field tests by Moorfields Reading Centre and the results from the serum NAM from Central Labs will be provided to sites within 10 working days of this screening visit.

At the end of the screening visit research teams should arrange with the participant the date of the baseline visit (Visit 1), with the view of participant returning 2 weeks after being screened (minimum, preferred timeframe), or within 3 months at the latest (maximum timeframe).

3.7 Trial Visits

Participants will be asked to attend 8 clinic visits: Screening (-2 weeks up to -3 months before baseline), Visit 1 (Baseline, Month 0), Visit 2 (Month 3), Visit 3 (Month 6), Visit 4 (Month 12), Visit 5 (Month 18), Visit 6 (Month 24), and Visit 7 (Month 27, which is end of IMP treatment and end of trial).

In between the clinic visits, telephone calls will also need to take place as detailed below.

Visit dates are referenced to the Baseline Visit (Visit 1). A window of –1 week/+4 weeks will be permitted for each visit from Visit 2 (Month 3) to Visit 7 (Month 27).

Additional visits (unscheduled visits) will be permitted as deemed clinically necessary (i.e. uncontrolled IOP, visual field progression or standard of care IOP-lowering treatment change occurring in between scheduled visits). Sites are encouraged to undertake VF testing, IOP measurements and assessments of AEs at all additional clinic visits. These unscheduled visits must be clearly documented in the participants medical notes.

Pre-Randomisation Assessments:

Please refer to section 3.6 for pre-randomisation assessments.

Visit 1 (Month 0 – Baseline/Randomisation):

Investigators (or delegated individual) should review all results received from the screening visit assessments to re-confirm eligibility, i.e., the participant **meets ALL the inclusion criteria and NONE of the exclusion criteria.**

To complete the randomisation process, investigators MUST be in receipt of the Moorfields Reading Centre VF eligibility assessment and the results of the serum NAM from central labs.

Participants will then be randomised to one of the treatment arms.

An Initial 6-month supply of IMP will be dispensed at this visit.

All participants will be provided with instructions for administering the IMP in addition to a dosing diary for the daily recording of IMP intake and dosing regimen.

The IMP dosing diary will be used to monitor medication compliance/adherence in addition to pill counts and blood tests for NAM levels at screening and months 3 and 27.

The participant should be instructed to bring their IMP dosing diary and IMP bottles (used/unused) to each clinic visit.

At the Baseline visit (Visit 1), the following assessments will take place:

- Review of any changes to medical history
- Review of concomitant medication
- Review of adverse events
- EPIC-Norfolk FFQ (Food Frequency Questionnaire)
- Quality of Life questionnaires (EQ-5D-5L with vision bolt-on and GQL-15)
- Blood pressure
- Weight and Height
- Visual acuity
- Clinical exam (slit lamp biomicroscopy)
- IOP measurement (GAT) and ORA or Corvis ST (if available)
- Two Visual Field tests (24-2 SITA Standard) per eye
- SD-OCT imaging
- Blood sample collection: PBMC* and DNA/RNA
- Urine Pregnancy test (for WOCBP)

**PBMC to be collected for participants taking part in the Biomarker Sub-Study (Moorfields Eye Hospital, King's College Hospital and up to 2 further sites ONLY).*

Dose Escalation/Tolerability Reminder Telephone call (telephone call 1):

During Week 6 after starting trial IMP, a phone call must be made to the participant to remind them to increase their dose to the next dose level of 3.0g/day, which should commence from the start of Week 7 until the remainder of the treatment period (Month 27). Direct questions should be asked during the phone call to check on treatment compliance, adverse events and concomitant medication. The outcome of this assessment should be documented in the participants medical notes.

Visit 2 (Month 3):

This visit should take place 3 months after the participant starts their trial IMP at the Baseline/Randomisation visit. The following assessments will be undertaken:

- Review of any changes to medical history
- EPIC-Norfolk FFQ (Food Frequency Questionnaire)
- Quality of Life questionnaires (EQ-5D-5L with vision bolt-on and GQL-15)
- Review of concomitant medication
- Review of adverse events
- Review of IMP Dosing diary and pill count
- Blood pressure
- Visual acuity
- Clinical exam (slit lamp biomicroscopy)
- IOP measurement (GAT) and ORA or Corvis ST (if available)
- Two Visual Field tests (24-2 SITA Standard) per eye
- SD-OCT imaging
- Blood sample collection: Glycosylated haemoglobin (HbA1c), LFTs, eGFR, serum NAM, PBMC*****
- Urine Pregnancy test (for WOCBP)

**PBMC to be collected for participants taking part in the Biomarker Sub-Study (Moorfields Eye Hospital, King's College Hospital and up to 2 further sites ONLY).*

The research team should record any missed intake of IMP, collect the dosing diaries and provide the participants with a new diary.

No further IMP will be dispensed at this visit (initial 6-month provided during Baseline).

Visit 3 (Month 6):

This visit should take place 3 months after Visit 2. The following assessments will be undertaken:

- Review of any changes to medical history
- Review of concomitant medication
- Review of adverse events
- Review of IMP Dosing diary and pill count
- Blood pressure
- Visual acuity
- Clinical exam (slit lamp biomicroscopy)
- IOP measurement (GAT) and ORA or Corvis ST (if available)
- Two Visual Field tests (24-2 SITA Standard) per eye
- SD-OCT imaging
- Urine Pregnancy test (for WOCBP)

The research team should record any missed intake of IMP, collect the dosing diaries and provide the participants with a new diary.

Any unused or any empty bottles of IMP should be returned to site pharmacy at this visit for accountability and disposal. Sponsor approval will be required prior to destruction of IMP.

Next 6-month supply of IMP will be dispensed at this visit.

Monitoring Telephone Call 2 (Month 9):

This is the second telephone call in the trial, but the first of 3 monitoring telephone calls that will be made to the participant by a member of the research team at site. Direct questions should be asked during the phone call to check on treatment compliance, adverse events and concomitant medication. The outcome of this assessment should be documented in the participants medical notes.

Visit 4 (Month 12):

This visit will take place 6 months after Visit 3. The following assessments will be undertaken:

- Review of any changes to medical history
- Review of concomitant medication
- Review of adverse events
- Review of IMP Dosing diary and pill count
- Visual acuity
- Clinical exam (slit lamp biomicroscopy)
- IOP measurement (GAT) and ORA or Corvis ST (if available)
- Visual Field (24-2 SITA Standard)
- SD-OCT imaging
- Urine Pregnancy test (for WOCBP)

The research team should record any missed intake of IMP, collect the dosing diaries and provide the participants with a new diary.

Any unused or any empty bottles of IMP should be returned to site pharmacy at this visit for accountability and disposal. Sponsor approval will be required prior to destruction of IMP.

Next 6-month supply of IMP will be dispensed at this visit.

Monitoring Telephone Call 3 (Month 15):

This is the third telephone call in the trial, but the second of 3 monitoring telephone calls that will be made to the participant by a member of the research team at site. Direct questions should be asked during the phone call to check on treatment compliance, adverse events and concomitant medication. The outcome of this assessment should be documented in the participants medical notes.

Visit 5 (Month 18):

This visit will take place 6 months after Visit 4. The following assessments will be undertaken:

- Review of any changes to medical history
- Review of concomitant medication
- Review of adverse events
- Review of IMP Dosing diary and pill count
- Blood pressure
- Visual acuity
- Clinical exam (slit lamp biomicroscopy)
- IOP measurement (GAT) and ORA or Corvis ST (if available)
- Visual Field (24-2 SITA Standard)
- SD-OCT imaging
- Blood samples collection: Glycosylated haemoglobin (HbA1c), LFTs, and eGFR
- Urine Pregnancy test (for WOCBP)

The research team should record any missed intake of IMP, collect the dosing diaries and provide the participants with a new diary.

Any unused or any empty bottles of IMP should be returned to site pharmacy at this visit for accountability and disposal. Sponsor approval will be required prior to destruction of IMP.

Next 6-month supply of IMP will be dispensed at this visit.

Monitoring Telephone Call 4 (Month 21):

This will be the last monitoring telephone call (in the trial) made to the participant by a member of the research team at site. Direct questions should be asked during the phone call to check on treatment compliance, adverse events and concomitant medication. The outcome of this assessment should be documented in the participants medical notes.

Visit 6 (Month 24):

This visit will take place 6 months after Visit 5. The following assessments will be undertaken:

- Review of any changes to medical history
- Review of concomitant medication
- Review of adverse events
- Review of IMP Dosing diary and pill count
- Visual acuity
- Clinical exam (slit lamp biomicroscopy)
- IOP measurement (GAT) and ORA or Corvis ST (if available)
- Two Visual Field tests (24-2 SITA Standard) per eye

- SD-OCT imaging
- Urine Pregnancy test (for WOCBP)

The research team should record any missed intake of IMP, collect the dosing diaries and provide the participants with a new diary**.**

Any unused or any empty bottles of IMP should be returned to site pharmacy at this visit for accountability and disposal. Sponsor approval will be required prior to destruction of IMP.

Final 3-month supply of IMP will be dispensed at this visit.

Visit 7 (Month 27):

This will be the end of treatment visit and the final visit of the trial and will take place 3 months after Visit 6. The following assessments will be undertaken:

- Review of any changes to medical history
- EPIC-Norfolk FFQ (Food Frequency Questionnaire)
- Quality of Life questionnaires (EQ-5D-5L with vision bolt-on and GQL-15)
- Review of concomitant medication
- Review of adverse events
- Review of IMP Dosing diary and pill count
- Blood pressure
- Visual acuity
- Clinical exam (slit lamp biomicroscopy)
- IOP measurement (GAT) and ORA or Corvis ST (if available)
- Two Visual Field tests (24-2 SITA Standard) per eye
- SD-OCT imaging
- Blood samples collection: serum NAM, DNA/RNA and PBMC*****
- Urine Pregnancy test (for WOCBP)

*******PBMC to be collected for participants taking part in the Biomarker Sub-Study (Moorfields Eye Hospital, King's College Hospital and up to 2 further sites ONLY).*

Any unused or any empty bottles of IMP should be returned to site pharmacy at this visit for accountability and disposal. Sponsor approval will be required prior to destruction of IMP.

For details on all assessments performed at each visit, please refer to Section 6.2 for Participant Timeline.

4 Examination Tests, Equipment and Techniques

4.1 IOP Measuring Methods

Goldmann applanation tonometry (GAT) AND, either Corvis ST or ORA tonometry (depending on availability at each site) should be used to take IOP measurements. If neither the Corvis ST nor the ORA tonometer's are available, then GAT alone should be performed.

4.2 Visual Field Analyser

The Humphrey Visual Field Analyzer Mark II (or II-i) or HFA3, with the 24-2 SITA Standard programme will be used. The test is to be performed on both eyes (as per SoC) throughout the trial duration. On Visit 1 (Baseline), Visit 2, Visit 3, Visit 6, and Visit 7 (last visit) two sets of VF tests should be obtained, with a break of at least 30 minutes between each test. Study visits have shorter intervals at the beginning and end of the follow-up period with additional VF tests to add precision to the estimates of rates for VF change.

Please Note: the two VFs done at the trial Baseline Visit (Visit 1) must be set as the baseline 'reference' tests in the progression analysis software.

4.3 SD-OCT Imaging

The Heidelberg Spectralis (Glaucoma Module Premium Edition, when available) will be the preferred choice to be used for this trial. However, sites will be permitted to use devices from another manufacturer if Spectralis is not available. The other devices that can be used are listed below:

- CZM Cirrus or Plex Elite
- Topcon 3D, Maestro or Triton
- OptoVue RTVue, iScan, iFusion80 or iVue80

As a minimum, peripapillary retinal nerve fibre layer (RNFL) and macular ganglion cell layer (GCL) scans are essential.

The test is to be performed on both eyes (as per SoC) throughout the trial duration. The same device used to scan a participant at Baseline should be used throughout the duration of the trial.

Sites will be provided with trial specific manuals for performing IOP, Visual Fields and SD-OCT imaging, including instructions on data export and transfer.

5 Trial treatments / Intervention

5.1 Introduction

Following randomisation, NAM or matching placebo will be administered by the participant (to be taken with food) at the dose 1.5g/day (1 x 750mg tablet twice a day: one in the morning and one in the evening) for the first 6 weeks followed by an increase to 3.0g/day (2 x 750mg tablet twice a day:

two in the morning and two in the evening) until the remainder of the treatment period at Month 27.

Please refer to dosing regimen in Table 3.

Arm A (Active Treatment)
Nicotinamide (NAM) 750mg tablets; two tablets a day (1.5g/day) for the first 6 weeks from Baseline (Visit 1), increased to four tablets a day (3.0g/day) until end of trial treatment at Month 27.

Arm B (Comparator)
Matching placebo tablets; two tablets a day (1.5g/day) for the first 6 weeks from Baseline (Visit 1), increased to four tablets a day (3.0g/day) until end of trial treatment at Month 27.

Table 3. Dose Regimen for oral NAM or Matching Placebo tablets

Timepoint	Total daily dose	Regimen	Total daily tablets	Duration
From Baseline, Month 0	1.5g	1x750mg tablet twice a day, morning and evening	2	up to end Week 6
Dose Escalation/Tolerability Reminder Telephone Call During Week 6				
From Week 7	3.0g	2x750mg tablets twice a day, morning and evening	4	until end Month 27

Between clinic visits, at Month 9, Month 15 and Month 21, monitoring telephone calls will be made to the participants from the research team at participating sites to check on treatment compliance, adverse events and concomitant medication.

For any dose modifications or dose intolerability, please refer to section 5.3.4.

5.1.1 Non-Investigational Medicinal Products (NIMPs)

All participants will undergo standard of care IOP-lowering treatment (initiated prior to randomisation at baseline; Visit 1, Month 0); selective laser trabeculoplasty (SLT) initial therapy or, if contra-indicated or by patient choice, a single therapy of preservative-free latanoprost (eye drops).

Patients already prescribed alternative IOP-lowering treatment should either be offered SLT or be changed to preservative-free latanoprost at the Screening Visit, following discussion and informed consent. For patients who have already had a treatment escalation (SLT plus drops or preservative-free latanoprost plus another medication) since treatment initiation, the treatment escalation should be reviewed against the trial treatment escalation algorithm (section 5.3.4). If the treatment intensity is greater than recommended, then de-escalation should be considered. If the treatment intensity is in line with the recommendations, then the compounds should be switched to be in line with the algorithm.

During the trial, it may be necessary to provide further standard of care IOP-lowering treatment to participants. Refer to Section 5.3.4.

5.2 Arm A

5.2.1 Nicotinamide (NAM) 750mg Tablets

Oral Nicotinamide (NAM) 750mg tablets will be manufactured and supplied by Blackmores Institute (Warriewood New South Wales, AU) as packed product (bulk tablets, bottles, labelling and packaging components) in bottles containing 140 tablets per bottle.

Nicotinamide (NAM) bottles will be shipped to Eramol (UK) Limited (Kent, UK), who will be responsible for secondary labelling (in compliance with GMP, Annex 13), including masking of all IMP into kit cartons (using masked numbered codes provided by the unmasked statistician at UCL CCTU), QP release and distribution to all participating sites.

5.2.2 Regulatory Status

Nicotinamide 750mg tablet is an unlicensed product in the UK and will be used outside of its current indication for the NAMinG trial.

5.3 Arm B

5.3.1 Matching Placebo Tablets

Matching placebo tablets will be manufactured and supplied by Blackmores Institute (Warriewood New South Wales, AU) as packed product (bulk tablets, bottles, labelling and packaging components) in bottles containing 140 tablets per bottle; identical in size, appearance and taste of Nicotinamide to maintain masking.

Matching placebo bottles will be shipped to Eramol (UK) Limited (Kent, UK), who will be responsible for secondary labelling (in compliance with GMP, Annex 13), including masking of all IMP into kit cartons (using masked numbered codes provided by the unmasked statistician at UCL CCTU), QP release and distribution to all participating sites.

5.3.2 Regulatory Status

The matching placebo tablet is unlicensed and currently not approved for use in any indication in the UK.

5.3.3 Dispensing & Storage

Prescribing of trial medication should only be performed by members of the research team who have the necessary qualifications, training and experience to do so and by those who have been delegated this task by the Principal Investigator (PI) as documented on the delegation log.

Once eligible participants are randomised (Visit 1, Month 0), the investigator or delegated member of the research team should complete the trial prescription and provide this to a member of the site pharmacy team.

Oral NAM or matching placebo will be dispensed by the site pharmacy in masked packaging i.e. pre-labelled with the inclusion of the treatment allocation code. Handling and management of the IMPs, including the additional dispensing labelling (as per GMP annex 13) will be subject to each site's local pharmacy policy.

Participants will be provided with IMP supplies at Visit 1 (Baseline/Randomisation), and every 6 months thereafter, at Visit 3 (M6); Visit 4 (M12); Visit 5 (M18); and Visit 6 (M24). Sites will be provided with an initial supply of IMP based upon either the confirmed recruitment number or the storage capacity, which will be agreed prior to site activation. Site re-supplies will be the responsibility of the investigator/Pharmacy personnel and re-ordered as they reach critical stock levels.

Re-ordering and expiry of medication will be managed by the NAMinG Trial Manager or delegate at UCL CCTU via Eramol (UK) Limited and using the Sealed Envelope Ltd, www.sealedenvelope.com

Kit codes will be managed by the unmasked trial statistician at UCL CCTU.

Detailed information regarding the labelling, administration and storage of the IMP will be provided in the NAMinG IMP Management Plan.

5.3.4 Dose Modifications, Interruptions and Discontinuations

IMP

Dose modifications will be discouraged, however, if intolerable side effects are experienced, dose reductions may be considered at the discretion of the treating clinician, following the guidelines below.

If a participant does not tolerate a particular IMP dose, that dose may be halved and then increased by 750mg (one tablet) every 2 weeks until the desired dose is achieved (1.5g/day (2 tablets) in the first 6 weeks or 3.0g/day (4 tablets) thereafter, or until the maximum tolerated dose (if lower) is achieved. A re-challenge to 3.0g/day should only be attempted once. If the dose is still not tolerated it should be reduced to the previous tolerated dose level, and this will become the participant's maximum tolerated dose for the remainder of the treatment period. Please refer to **Table 4** below.

Table 4. Dose Modification Strategy

TIMEPOINT	Week 1 to Week 6	From Week 7 onwards (up to Month 27 - End of treatment)
DOSAGE	**1.5g/day**; One 750mg tablet twice a day (1 in the morning; 1 in the evening)	**3.0g/day**; Two 750mg tablets twice a day (2 in the morning; 2 in the evening)
DOSE DECISION	During the Week 6 Dose Escalation/Tolerability Phone Call: If **NOT TOLERATED – reduce dosage to half; 750mg/day (750mg tablet daily)** for 2 weeks and then increase by 750mg (one tablet) every 2 weeks until 3.0g/day (4 tablets) or the maximum tolerated dose (if lower) is achieved. **750mg/day**; **1 tablet** a day → **+ 2 WEEKS** **1.5g/day**; **2 tablets** a day → **+ 2 WEEKS** **2.25g/day**; **3 tablets** a day → **+ 2 WEEKS** **3.0g/day**; **4 tablets** a day	If **NOT TOLERATED and the participant has not previously been re-challenged – reduce dosage to half; 1.5g/day (750mg twice a day)** for 2 weeks and then increase by 750mg (one tablet) every 2 weeks until 3.0g/day (4 tablets) or the maximum tolerated dose (if lower) is achieved. **1.5g/day**; **2 tablets** a day → **+ 2 WEEKS** **2.25g/day**; **3 tablets** a day → **+ 2 WEEKS** **3.0g/day**; **4 tablets** a day If **NOT TOLERATED and the participant has previously been re-challenged – the dosage may be decreased to the previous tolerated dose level** (reduce by one tablet).

If the participant cannot tolerate adverse side effects, the participant and investigator should make a decision about whether or not to stop the trial medication. If trial medication is stopped, then UCL CCTU should be informed at the earliest opportunity and the participant should continue to be followed up for the remainder of the trial duration unless they explicitly withdraw their consent for follow-up.

Standard of care IOP-lowering

Additional Standard of Care IOP-Lowering treatment will be permitted if either:

1. IOP rises above the level of 26mmHg or,
2. IOP-lowering is <20% compared to the pre-treatment level or,
3. Visual field progression occurs (as per EMGT criteria; three black triangles on the Humphrey Visual Field Analyser Guided Progression Analysis; GPA)

The algorithm to pursue clinical treatment escalation is as follows:

1. Repeat SLT (if previous therapy was SLT and SLT was effective), or preservative-free latanoprost (if initial therapy was SLT and SLT was ineffective), or SLT (if initial therapy was preservative-free latanoprost), then
2. Timolol 0.1% (β-blocker) once in the morning (if no contra-indication to β-blockers), or Brinzolamide drops twice a day (if β-blockers contra-indicated), then
3. Brinzolamide drops twice a day (as an add-on to Timolol), then
4. Treatment as deemed clinically necessary at the discretion of the Investigator

Brimonidine should be avoided unless deemed necessary because it may confound the effect of NAM.

Participants being considered for trial eligibility who have been diagnosed within the last 12 months may have had treatment escalation which does not conform to the preferred schema. Prior to obtaining participant consent (at the screening visit), the investigator should discuss with the participant alteration of IOP-lowering therapy to adhere to the preferred schema. Following consent, treatment changes may be made as deemed appropriate by the investigator.

Tetracyclines

Participants who receive medications in the Tetracyclines drug class whilst participating in the trial should interrupt their trial medication (stop taking the IMP) until the prescribed course of Tetracyclines is complete. Once complete, participants are advised to resume trial medication at a dose of 1.5g/day for one week and then increase to 3.0g/day (or the maximum tolerated dose) for the remainder of the treatment period until Month 27.

(see also Section 5.7 for treatment discontinuation)

5.4 Concomitant Care

All medications (prescribed/non-prescribed) taken by the participant upon enrolment and throughout the duration of the trial must be recorded. Exclusion criteria include concomitant use of the following medications:

Carbamazepine, Phenobarbital, Primidone (anti-epileptic drugs)

A possible interaction between nicotinamide and carbamazepine or phenobarbital or primidone (anticonvulsants) has been reported (leading to an increase of blood levels of carbamazepine, phenobarbital and primidone), occurring probably by inhibition of cytochrome p-450 by nicotinamide.

Isoniazid

This medication is used to treat tuberculosis (TB) and may lower levels of niacin in the body and cause a deficiency.

Pyrazinamide

Pyrazinamide, the pyrazine analogue of nicotinamide, is an anti-tuberculous agent. Given the structural similarity with nicotinamide, interactions are likely.

Participants MUST NOT take additional Vitamin B3 supplements during the course of the trial. This includes skin preparations (ointments/emulsions). Higher doses of NAM can lead to toxicity.

Additionally, participants **MUST NOT** take the supplements Ginkgo biloba and/or Coenzyme Q10 (CoQ10). Either may confound the effect of NAM.

Participant's trial medication should be interrupted for the duration of treatment with:

Tetracyclines
This group of medications are antibiotics used to treat various infections. Please refer to section 5.3.4 for more detail on trial dose interruptions.

5.4.1 Concomitant Covid-19 Vaccine use with NAM

No data are currently available to demonstrate any interactions between the UK Government approved COVID-19 vaccines and the IMP being used in this trial.

Any planned vaccines can continue to be deployed to participants who have enrolled or choose to participate in the trial.

5.5 Overdose of Trial Medication

Doses higher than prescribed in the trial can lead to nausea, vomiting and reversible hepatotoxicity.

This trial prohibits additional use of vitamin B3 supplements and skin preparations from being taken during participation in the trial.

Accidental or deliberate overdose of trial medication will be treated accordingly. The re-introduction of trial medication dosing should be determined by the clinical investigator. Any participant taking a deliberate overdose of trial medication should discontinue and be withdrawn from the trial medication but should continue to be followed-up for the remainder of the trial duration unless they explicitly withdraw their consent for follow-up.

The event should be documented in the patient's medical notes and UCL CCTU informed.

5.6 Unmasking

Unmasking of the trial treatment allocation will be performed after database lock and once primary analysis has been completed. Participants will receive information on the results of the trial at the same time as being unmasked to give context and aid interpretation.

Further details of unmasking can be found in the NAMinG Randomisation and Unmasking Plan.

5.6.1 Emergency Unmasking

All recruited participants will be given an emergency contact card with details of the local clinical trial team, including emergency telephone number available 24 hours a day, 7 days per week.

In the event of a medical emergency, the treating physician should assume the patient is on the active arm of the clinical trial and treat accordingly. It is to the treating physician's discretion whether or not trial treatment is withdrawn or if the treatment allocation requires unmasking.

The unmasking feature will be available in the Sealed Envelope randomisation service, which can be accessed 24 hours a day. Unmasking can be performed by the delegated user at the site, and this will not affect remaining participants in the trial, nor will it reveal the treatment allocation to the masked CCTU trial team. Where possible, other members of the local site team not involved in the clinical management, should also remain masked.

On receipt of the unmasked treatment allocation details, the PI or treating healthcare professional will treat the participant's medical emergency as appropriate. It will also be documented at the end of the trial in any final trial report and/or statistical report.

Where unmasking is not possible using the procedure described above, due to extenuating circumstances, the trial site team must contact the UCL CCTU Trial Team via the trial mailbox cctu.Naming@ucl.ac.uk immediately.

Detailed information regarding unmasking is provided in the NAMinG Randomisation and Unmasking Plan.

5.6.2 Unmasking for the Submission of SUSAR reports

All SAEs that are related to the trial medication (i.e., SARs) and are suspected to be unexpected i.e. SUSARs, need to be submitted to the regulatory agencies within pre-specified timelines (refer to section 7.1.3 for expected SARs). When SAE reports are received at the CCTU, if the event is recorded as being a SUSAR then the following procedure will be used to unmask the SUSAR to determine if the participant was receiving active trial medication, and therefore, that the SUSAR needs onward reporting to the regulatory agencies:

- A member of the CCTU trial SUSAR Reporting Team (SRT) will unmask the participant's trial treatment allocation using the Sealed Envelope randomisation service.

- If the participant is revealed to the CCTU SRT to be receiving active treatment, the CCTU trial SRT member will report the SUSAR on the ICSR (Individual Case Safety Report) Submissions portal available through the MHRA website and to the MHRA and REC as required.

- This information will not be forwarded to the trial team at the CCTU or at the sites. It will be kept in a separate file by the CCTU SUSAR reporting team to maintain the mask.

5.7 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
- Pregnancy
- Death
- Elevated liver transaminases >3 times the upper limit of normal (ULN)
- eGFR <30mL/min/1.73m²
- New cancer diagnosis (excluding non-melanoma skin cancer)

If treatment is discontinued, participants should be encouraged to attend follow-up visits as per protocol.

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.
It should be clear to the patient and recorded in the patient notes what aspect(s) of the trial the participant is discontinuing their participation. These could include:

- Early cessation of **further treatment**
- Early cessation **from further trial follow-up**

Patients should remain in the trial for the purpose of follow-up and data analysis (unless the patient withdraws their consent from all stages of the trial). If a patient ceases follow-up early, refer to Section 6.4.

Data on patients who stop follow-up early will be kept and included in analysis.

5.8 Accountability & Unused Trial Medication

Accountability logs will be maintained by the pharmacy teams at participating sites to record details of all IMP received, dispensed, returned, expired, and destroyed. The Accountability Log should

contain at a minimum, the quantity of IMP received from the sponsor, participant trial ID, date of dispensing, and date and quantity of any IMP returned.

Accountability logs will be provided by UCL CCTU to use for this trial, however where Trust local policy permits the use of local versions only, or if the site wishes to use local versions, this will be granted upon review of local logs and approval from UCL CCTU.

If an electronic accountability log is used, it must be printable and have a clear audit trail. Any local training undertaken by pharmacy teams on the use of an electronic accountability system should be documented in the Site Pharmacy File (e.g., file notes, training logs).

Any unused or empty bottles returned by the participant will be destroyed by the site pharmacy **upon receiving confirmation from the Sponsor** and in accordance with the site local policy for destruction.

Any expired medication will be destroyed by the local site pharmacy upon receiving confirmation from UCL CCTU.

Copies of accountability logs and destruction logs must be provided to UCL CCTU upon request.

5.9 Compliance and Adherence

Investigators and research team members should ensure participants are made aware of the importance of following and complying with the dosing regimen, at the baseline visit and at subsequent visits throughout the trial duration.

5.9.1 Participant Dosing Diary

Participants will be provided with a dosing diary to take home and record information about their daily trial medication intake (including missed doses and reasons). Any adverse events that are experienced should also be recorded in this diary. The diary should be maintained throughout the duration of the trial as they will be reviewed at each clinic visit. All participants will be asked to bring their diaries and all trial medication including any unused and empty bottles to each clinic visit.

Instructions on diary completion and dosing will be provided to the research teams at sites.

5.9.2 IMP Pill Count

Treatment adherence and compliance will be assessed by the site research teams during the trial scheduled clinic visits upon review of the dosing diary and via the conduct of a pill count. Any discrepancies should be discussed with the participant. Pill counts and any missed doses will be recorded in the trial specific CRFs with reasons for non-compliance documented.

5.9.3 Telephone Calls

Additional treatment compliance will be monitored at the Dose Escalation/Tolerability Reminder Telephone Call during week 6, and the 3 monitoring telephone calls in between visits 3-6 (M6-M24).

Pharmacy teams at sites will be responsible for maintaining adequate IMP dispensing logs and return records.

Compliance will be additionally assessed at the end of the trial by reviewing serum NAM levels collected at Screening, Month 3, and Month 27.

5.10 Eligibility Criteria for Individuals Performing the Interventions

Nursing staff, medical staff and any other delegated members of the clinical trial team at participating sites should have the appropriate qualifications to manage patients with glaucoma. Protocol-specific training including IMP dosing regimen will be provided to participating sites by the NAMinG Trial Manager or delegate at UCL CCTU prior to site activation or during a Site Initiation Visit (SIV). New site staff who start work on the trial after site activation occurs, will be provided with protocol specific training prior to performing trial related procedures.

Each member of the trial team at each participating site will have their roles within the trial, as delegated by the PI, documented on the NAMinG site delegation log. Current CVs and GCP certificates of all individuals working on the trial will be collected by the NAMinG trial team at UCL CCTU to document their qualifications and relevant experience.

The IMP used for this trial will be administered by the participant. All participants enrolled in this trial must be available and willing to attend the visit time points during the 30 months duration of the trial. The participant must be instructed by a member of the research team at sites about how to administer the IMP. All participants will be provided instructions for administering the IMP in addition to home dosing diaries (specifying the dose to be taken each day).

6 Assessments & Follow-Up

6.1 Outcomes

6.1.1 Primary Outcome(s)

The primary outcome is the difference between the treatment arms in Visual Field (VF) mean deviation (MD) at 27 months.

6.1.2 Secondary Outcomes

Clinical safety and efficacy outcomes:

1. The difference in VF MD at 3 months (0-3 months – neuro-recovery) between the NAM group and the placebo group, measured using the HFA Mark II (or II-i) or HFA3 with the SITA Standard 24-2 programme.
2. Safety profile of high dose NAM measured by liver function tests (LFTs), estimated glomerular filtration rate (eGFR) and glycosylated haemoglobin (HbA1c) at screening, month 3 and month 18.
3. Adverse events during the study period for each participant from baseline to month 27.

4. Quality-of-Life outcome differences between the two treatment groups at baseline, month 3 and month 27, measured by the EQ-5D-5L (with vision bolt-on) and GQL-15.

Mechanistic outcomes:

The following outcomes will be measured from the blood samples and VFs collected over the course of the trial.

<u>At Moorfields Eye Hospital (MEH), King's College Hospital, and up to 2 further sites ONLY (Biomarker Sub-Study):</u>

1. Impact of NAM treatment on mitochondrial function (ATP-linked oxygen consumption rate (OCR)) in the NAM and placebo groups between baseline and month 27.
2. The association between a) ATP-linked OCR and b) NAD levels and rate of VF loss in the placebo group between baseline and month 27.
3. The association between lymphocyte mitochondrial function (ATP-linked OCR), and serum NAM levels, lymphocyte NAD+ and oxidised glutathione levels in the placebo and NAM groups at baseline.
4. Association of NAM, NAD, and OCR levels with the EPIC Food Frequency Questionnaire responses in the placebo and NAM groups at baseline.
5. The effect of NAM on a) rate of VF loss (between month 3 and month 27) and b) level of mitochondrial function overall and in participants with low vs high baseline mitochondrial function and low vs high baseline NAM levels.
6. The association between lymphocyte mitochondrial function (ATP-linked OCR) and quality of life measures in the placebo and NAM groups between baseline and month 27.

<u>At all sites:</u>

7. The association between baseline NAM levels and rate of VF loss (between baseline and month 27) in the placebo group at all sites.
8. Association between NAM dosing and IOP (between baseline and month 27) in the active and placebo groups at all sites.

6.1.3 Surrogate Outcomes

The primary analysis will be repeated with imaging/structural measures as the outcome variable.

1. The difference in OCT peripapillary RNFL thickness/OCT macular GCL thickness between treatment arms at month 27.
2. The difference in OCT peripapillary RNFL thickness/OCT macular GCL thickness between treatment arms at month 3.
3. The difference in the rate of OCT peripapillary RNFL/macular GCL loss between treatment arms between month 3 and month 27.
4. The effect of high dose NAM on the rate of VF progression between month 3 and month 27, with the rate of OCT peripapillary RNFL/macular GCL loss as a Bayesian prior.
5. The correlation between the rate of VF progression and OCT peripapillary RNFL/macular GCL loss between month 3 and month 27.

6.1.4 Exploratory Outcomes

The differences between treatment groups in the rate of VF loss at predefined subsets of VF locations. If there is no neuro-recovery (significant difference in VF MD between months 0 and 3), the rates of progression will be measured between months 0 and 27 months. If there is statistically significant VF improvement between month 0 and 3 months (neuro-recovery), the rate of progression will be measured between month 3 and 27 months (neuroprotection evaluation).

6.1.5 Future genetic and gene expression sub-study

This sub-study will include an optional blood sample collection for DNA and RNA (gene expression) which will be analysed to address questions beyond the immediate scope of the main trial and used to identify the genetic and gene expression profiles of those susceptible, or resistant, to glaucoma progression and genetic profiles associated with more favourable clinical outcomes in response to NAM.

6.1.6 Outcome Taxonomy

Below is a table presenting the classifications (domains) of the trial outcomes based on taxonomy.

Table 5. Trial Outcome Taxonomy[65]

	Domains		
Outcome	**Physiological/clinical**	**Functioning**	**Resource use**
Difference in VF	Eye outcomes	Physical functioning	
Safety of NAM	Metabolism and nutrition		
Adverse events			Adverse events/effects
EQ5D		Global quality of life	
GQL-15		Perceived health status	
Lymphocyte mitochondrial function	Metabolism and nutrition		
Lymphocyte NAD levels	Metabolism and nutrition		
Lymphocyte oxidised glutathione	Metabolism and nutrition		
Retinal nerve fibre layer/macular OCT parameters	Eye outcomes	Physical functioning	
Serum NAM, pill counts, dosing diary		Delivery of care (adherence/ compliance)	

6.2 Participant Timeline

Schedule of Assessments:

Visit Number	SCREENING	VISIT 1[1] BASELINE / RANDOMISATION	Dose Escalation / Tolerability Reminder Telephone Call (1)	VISIT 2	VISIT 3	Monitoring Telephone Call 2	VISIT 4	Monitoring Telephone Call 3	VISIT 5	Monitoring Telephone Call 4	VISIT 6	VISIT 7 (End of Treatment)	Unscheduled Visit[2]
Month		**Month 0 (2 weeks or up to 3 months after Screening)**	**During Week 6**	**Month 3**	**Month 6**	**Month 9**	**Month 12**	**Month 15**	**Month 18**	**Month 21**	**Month 24**	**Month 27**	
Protocol Window				**(+4 weeks)**	**(-1 week / +4 weeks)**		**(-1 week / +4 weeks)**		**(-1 week / +4 weeks)**		**(-1 week / +4 weeks)**	**(-1 week / +4 weeks)**	
Inclusion/exclusion criteria (Eligibility review)	X	X											
Informed Consent	X												
Demographic data	X												
Weight and Height		X											
Review of Medical History and any changes	X	X		X	X		X		X		X	X	
Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood pressure [3]	X	X		X	X				X			X	
Visual Acuity [4]	X	X		X			X		X			X	

Visit Number	SCREENING	VISIT 1[1] BASELINE / RANDOMISATION	Dose Escalation / Tolerability Reminder Telephone Call (1)	VISIT 2	VISIT 3	Monitoring Telephone Call 2	VISIT 4	Monitoring Telephone Call 3	VISIT 5	Monitoring Telephone Call 4	VISIT 6	VISIT 7 (End of Treatment)	Unscheduled Visit[2]
Month		**Month 0 (2 weeks or up to 3 months after Screening)**	**During Week 6**	**Month 3**	**Month 6**	**Month 9**	**Month 12**	**Month 15**	**Month 18**	**Month 21**	**Month 24**	**Month 27**	
Protocol Window				**(+4 weeks)**	**(-1 week / +4 weeks)**		**(-1 week / +4 weeks)**		**(-1 week / +4 weeks)**		**(-1 week / +4 weeks)**	**(-1 week / +4 weeks)**	
Clinical Examination [5], slit lamp biomicroscopy	X	X		X	X		X		X		X	X	
IOP measurement [6]	X	X		X	X		X		X		X	X	X
Visual Field [7]	X	X (2x)		X (2x)	X (2x)		X		X		X (2x)	X (2x)	X
SD-OCT Imaging [8]		X		X	X		X		X		X	X	
Urine Pregnancy Test [9]	X	X		X	X		X		X		X	X	
Liver Function Test (LFTs) [10]	X			X					X				
eGFR [11]	X			X					X				
Glycosylated haemoglobin (HbA1c) [12]	X			X					X				
PBMCs [13]		X		X								X	
Serum NAM levels [14]	X			X								X	
DNA & RNA blood sample [15]		X										X	

Visit Number	SCREENING	VISIT 1[1] BASELINE / RANDOMISATION	Dose Escalation / Tolerability Reminder Telephone Call (1)	VISIT 2	VISIT 3	Monitoring Telephone Call 2	VISIT 4	Monitoring Telephone Call 3	VISIT 5	Monitoring Telephone Call 4	VISIT 6	VISIT 7 (End of Treatment)	Unscheduled Visit[2]
Month		**Month 0 (2 weeks or up to 3 months after Screening)**	**During Week 6**	**Month 3**	**Month 6**	**Month 9**	**Month 12**	**Month 15**	**Month 18**	**Month 21**	**Month 24**	**Month 27**	
Protocol Window				**(+4 weeks)**	**(-1 week / +4 weeks)**		**(-1 week / +4 weeks)**		**(-1 week / +4 weeks)**		**(-1 week / +4 weeks)**	**(-1 week / +4 weeks)**	
Randomisation		X											
Dispense study medication		X			X		X		X		X		
IMP administration		X	X	X	X	X	X	X	X	X	X	X	
Week 6 telephone call [16]			X										
IMP treatment adherence / compliance (review of IMP Dosing diary and pill count)			X	X	X	X	X	X	X	X	X	X	
AE/SAE review [17]	X	X	X	X	X	X	X	X	X	X	X	X	X
Quality of Life questionnaires: EQ-5D-5L with vision bolt-on and GQL-15		X		X								X	
EPIC-Norfolk FFQ (Food Frequency Questionnaire)		X		X								X	

1. *Baseline visit for all participants should ideally occur 2 weeks after the screening visit (minimum preferred timeframe). Maximum time between screening and baseline should be no longer than 3 months.*
2. *Participants can attend unscheduled visits for instances of uncontrolled IOP, visual field progression or standard of care IOP-lowering treatment change, that occurs in between the trial scheduled visits.*
3. *Only blood pressure assessment to be performed.*
4. *Snellen Visual Acuity Chart. Test to be performed on both eyes (as per SoC) throughout the trial duration.*
5. *Clinical exam includes slit lamp biomicroscopy. Biomicroscopy data collected should include information on disc haemorrhages and any (new) retinal cause which might impact the VF (i.e. vein occlusion).*
6. *IOP measurement using Goldmann applanation tonometry AND either Corvis ST or ORA (Ocular Response Analyser). Whichever is chosen at baseline (Corvis or ORA), the same method must be used throughout the trial duration.*
7. *Visual Field measured by the Humphrey Field Analyser (HFA) Mark II (or II-i) or HFA3 with 24-2 programme (SITA Standard). The test is to be performed on both eyes (as per SoC) throughout the trial duration. The test is to be repeated twice at Baseline, month 3, month 6, month 24 and month 27 (final visit), with at least a 30-minute break in between each test.* ***The VF tests done at the Baseline visit must be set as the baseline 'reference' tests in the progression analysis software.***
8. *SD-OCT imaging of the RNFL. Preferred instrument is the Heidelberg Spectralis: Glaucoma Module Premium Edition (when available) with peripapillary RNFL and macular GCL scans. However sites can use other devices if this is not available. The same method must be used throughout the trial duration. The test is to be performed on both eyes (as per SoC) throughout the trial duration.*
9. *Pregnancy test required for WOCBP. A pregnancy test MUST be posted to participant home 30 days after last treatment visit and results obtained.*
10. *Liver function tests (LFTs)*
11. *Renal function (eGFR).*
12. *Glycosylated haemoglobin will be assessed by measuring HbA1c.*
13. *Peripheral Blood Mononuclear Cells (PBMCs) to be collected by the Moorfields Eye Hospital, King's College Hospital, and up to 2 further sites ONLY (Biomarker Sub-Study). Samples will be processed at the UCL Institute of Ophthalmology. Please refer to the Laboratory Sample Management Plan for more details on sample collection, processing, storage and shipment.*
14. *Serum NAM to be collected by ALL participating sites and transferred to The Doctors Laboratory (TDL). Screening serum NAM sample must be transferred to TDL on the same day of collection to allow completion of the randomisation process at baseline. Please refer to the Laboratory Sample Management Plan for more details on sample collection, processing, storage, and shipment.*
15. *DNA/RNA blood sample is optional and at baseline and M27 ONLY.*
16. *Research teams at participating sites MUST make a telephone call to participants during Week 6 to check on treatment compliance, adverse effects, concomitant medication, and to remind them to increase their dose to the next level of 3.0g/day starting from Week 7 until the remainder of the treatment period (Month 27).*
17. *AEs and SAEs should be reported from the time of consent.*

6.3 Participant Transfers

If a participant moves away from the area of the consenting centre making continued follow up difficult, every effort should be made for them to be followed at another participating trial centre. Written consent should be taken at the new centre and then a copy of the participant's source documents should be provided to the new centre. Responsibility for the participant remains with the original consenting centre until the new consent process is complete.

6.4 Early Stopping of Follow-up

If a participant chooses to discontinue their trial treatment, they should continue to be followed up as closely as possible to the follow-up schedule defined in the protocol, providing they are willing. They should be encouraged and facilitated not to leave the trial, even though they no longer take the trial treatment. 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. The CCTU should be informed of the withdrawal in writing using the appropriate NAMinG trial documentation. Data already collected will be kept and included in analyses according to the intention-to-treat principle for all participants who stop follow up early, unless specifically requested by the participant.

Any optional consent e.g. future use of stored samples already collected can be refused when leaving the trial early (but this should be discouraged and should follow a discussion).

Patients may change their minds about stopping trial follow-up at any time and re-consent to participation in the trial.

Participants who stop trial follow-up early will not be replaced.

(See also Section 5.7 Protocol discontinuation)

6.5 Loss to Follow-up

A 'loss to follow-up' is a participant who is no longer contactable and has missed at least one follow-up visit within the protocol defined visit window of -1 week / +4 weeks. Research teams at sites should attempt to contact the participant at least three times via two different methods of contact (i.e., telephone and letter) before they are declared as a loss to follow-up.

Efforts will be made to minimise loss to follow-up, such as tracing participants via their NHS number using NHS Digital records (Spine) (where they might have changed their GP) which supports the IT infrastructure for health and social care in England once approvals for access have been granted. Consent for this will be sought prior to the participant entering the trial.

6.6 Completion of Protocol Follow-up

Participants will complete protocol follow-up upon completion of the final visit CRF.

6.7 Internal Pilot: Stop-Go Criteria

108 patients will be recruited during the first 6-month pilot trial across the initial 4 sites. Recruitment and loss to follow-up will be closely monitored with interim assessments at 2 months and 4 months. The following stop-go criteria will be adopted at 6 months:

1. **GREEN**: trial allowed to continue if recruitment at 100% of pre-specified target;
2. **AMBER**: measures taken to enhance recruitment if trial recruits at less than 100%, but better than 60% of its target;
3. **RED**: trial will end if recruitment falls to less than 60% of the target.

7 Safety reporting

The principles of GCP require that both investigators and Sponsors follow specific procedures when notifying and reporting adverse events or reactions in clinical trials. These procedures are described in this section of the protocol. **Section 7.1** lists definitions, **Section 7.3** gives details of the investigator responsibilities and **Section 7.4** provides information on CCTU responsibilities.

7.1 Definitions

Definitions of harm of the EU Directive 2001/20/EC Article 2 based on the principles of GCP apply to this trial.

Table 6: Definitions

Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial subject to whom a medicinal product has been administered including occurrences that are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the medicinal product in question set out in the Summary of Product Characteristics (SPC) or Investigator Brochure (IB) for that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: • results in death • is life threatening* • requires hospitalisation or prolongation existing hospitalisation** • results in persistent or significant disability or incapacity • consists of a congenital anomaly or birth defect • is another important medical condition***

* The term life threatening here refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that might hypothetically cause death if it was more severe (e.g., a silent myocardial infarction)

** Hospitalisation is defined as an in-patient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisation for pre-existing conditions (including elective procedures that have not worsened) do not constitute an SAE

*** Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. The following should also be considered serious: important AEs or ARs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above (e.g., a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not result in hospitalisation, or development of drug dependency).

7.1.1 Medicinal Products

An investigational medicinal product is defined as the tested investigational medicinal product and the comparators used in the study (see section 5 of this protocol).

Adverse reactions include any untoward or unintended response to drugs. Reactions to an IMP or comparator should be reported appropriately.

7.1.2 Adverse Events

Adverse events include:

- an exacerbation (i.e., increase in the frequency or intensity) of a pre-existing illness, episodic event or symptom (initially recorded at the screening/baseline visit), that is detected after trial drug administration/intervention
- occurrence of a new illness, episodic event or symptom, that is detected after trial drug administration/intervention
- Elevated levels of liver aminotransferase enzymes >3 times the upper limit of normal (ULN)
- eGFR <30mL/min/1.73m²

Adverse events do **NOT** include:

- Medical or surgical procedures: the condition that leads to the procedure is the adverse event
- Pre-existing disease or a condition present before treatment that does not worsen
- Hospitalisation where no untoward or unintended response has occurred e.g., elective cosmetic surgery
- Overdose of medication without signs or symptoms

7.1.3 Adverse Reactions (ARs)

The following adverse reactions are potentially related to NAM:

- Flushing, redness, itching and rash of the skin
- Gastro-intestinal symptoms such as sore mouth, heartburn, vomiting, nausea, diarrhoea, flatulence
- Headache, dizziness
- Liver enzyme abnormalities, jaundice
- Eye symptoms such as blurred or disturbances in vision, hyphema, periorbital oedema
- Fatigue
- Alopecia
- Heart palpitations
- Decreased insulin sensitivity

7.2 Notifiable Adverse Events

7.2.1 Pregnancy

Female participants with a positive pregnancy test at screening and baseline will not be eligible for inclusion in this trial. WOCBP and male participants will be advised to use highly effective methods of contraception throughout the duration of the trial (see Section 3.2.1).

From the time of consent, all pregnancies and suspected pregnancies that occur in female participants must be reported immediately of the site becoming aware to UCL CCTU using the notification and follow-up of pregnancy form.

All confirmed pregnancies in the female partners of male participants should be reported to UCL CCTU **within 24 hours** of the site becoming aware. The pregnant participant or the male participant of the pregnant partner will be advised to discontinue their trial medication immediately.

Before any additional outcome of pregnancy or pregnancy complications can be reported to UCL CCTU, both pregnant female participants and the female partners of male participants must be given a copy of the trial pregnancy monitoring information sheet and asked to complete a pregnancy monitoring informed consent form for follow-up in pregnancy. The process should follow GCP, and the processes used to obtain consent for the clinical trial.

Outcomes should be reported to UCL CCTU no later than 10 months after the initial report. Any complications of pregnancy including miscarriage, congenital abnormality or birth defect resulting from the pregnancy must be reported as an SAE to UCL CCTU, within the timelines outlined in section 7.3.2 below.

7.3 Investigator responsibilities

All non-serious AEs and ARs, whether expected or not, should be recorded in the patient's medical notes and in the trial specific database. SAEs and SARs should be reported to UCL CCTU immediately and **no later than 24 hours** after the investigator becoming aware of the event, via remote data

entry into the trial specific database. Upon site completion of the SAE/SAR form, the trial team at UCL CCTU should be notified.

7.3.1 Investigator Assessment

7.3.1.1 Seriousness

When an AE or AR occurs, the investigator responsible for the care of the participant must first assess whether or not the event is serious using the definition given in Table 6. If the event is classified as 'serious' then an SAE form must be completed and CCTU (or delegated body) notified immediately (**within 24 hours**) after the investigator becoming aware of the event.

7.3.1.2 Severity or Grading of Adverse Events

The severity of all AEs and/or ARs (serious and non-serious) in this trial should be graded using the toxicity gradings in the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (2017).

Grades for AEs and ARs according to the CTCAE are:

Grade 1: Mild; asymptomatic or mild symptoms; clinical diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.

Grade 3: Severe or medically significant but not life threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL**.

Grade 4: Life threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE or AR.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money etc.

** Self-care AD refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

Where no specific grading criteria exist for an event, the event should be graded according to the CTCAE general guidelines outlined above.

7.3.1.3 Causality

The investigator must assess the causality of all serious events or reactions in relation to the trial therapy using the definitions in Table 7. There are five categories: unrelated, unlikely, possibly, probably, and definitely related. If the causality assessment is unrelated or unlikely to be related, the event is classified as an SAE. If the causality is assessed as possibly, probably or definitely related, then the event is classified as an SAR.

Table 7: Assigning Type of SAE Through Causality

Relationship	Description	Event type
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out	SAR
Probably	There is evidence to suggest a causal relationship and the influence of other factors is unlikely	SAR
Possibly	There is some evidence to suggest a causal relationship (e.g., because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant treatment)	SAR
Unlikely	There is little evidence to suggest that there is a causal relationship (e.g., the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g., the participant's clinical condition, other concomitant treatment)	Unrelated SAE
Unrelated	There is no evidence of any causal relationship	Unrelated SAE

If an SAE is considered to be related to trial treatment, and treatment is discontinued, interrupted or the dose modified, refer to the relevant Dose Modifications, Interruptions and Discontinuations sections of the protocol.

7.3.1.4 Expectedness

If there is at least a possible involvement of the trial treatment (or comparator), the sponsor will assess the expectedness of the event. If information of expectedness is provided by the investigator, this should be taken into consideration by the sponsor. An unexpected adverse reaction is one not previously reported in the current and approved version of the Reference Safety Information (the IB) for the trial, or one that is more frequently reported or more severe than previously reported.

7.3.1.5 Reference Safety Information (RSI)

The Reference Safety Information (RSI) for the NAMinG trial is section 6.9 of the current approved IB and for a list of expected toxicities associated with the drugs being used in this trial refer to section 5.3 of the IB. If a SAR is assessed as being unexpected it becomes a SUSAR (suspected, unexpected, serious adverse reaction) and MHRA/REC reporting guidelines apply (see Notifications sections of the protocol).

7.3.2 Notifications

7.3.2.1 Notifications by the Investigator to CCTU

CCTU must be notified of all SAEs immediately after site staff become aware of the event (in no circumstances should this notification take longer than 24 hours).

Investigators should notify CCTU of any SAEs and other Notifiable Adverse Events (NAEs) occurring from the time of consent until 30 days after the last protocol treatment administration, including SARs and SUSARs. From this point forward the site will not actively monitor SAEs or NAEs but will notify the CCTU of any SARs and SUSARs if they become aware of them until trial closure.

Any subsequent events that may be attributed to treatment should be reported to the MHRA using the yellow card system (https://yellowcard.mhra.gov.uk/the-yellow-card-scheme/).

- The trial specific SAE eform must be completed by the site staff named on the delegation log with this responsibility assigned by the PI. The form should be reviewed and signed off by the investigator (listed on the delegation log) who is responsible for the participant's care with attention paid to the grading and causality of the event. . The responsible investigator should check the SAE eform at the earliest opportunity, make any changes necessary and sign. Systems should be in place at the site to enable the investigator to check the form for clinical accuracy as soon as possible.

The minimum criteria required for reporting an SAE are the primary event term, trial number and partial date of birth (month and year), name of reporting investigator and sufficient information on the event to confirm seriousness. Any further information regarding the event that is unavailable at the time of the first report should be sent as soon as it becomes available.

- Follow-up: Participants must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline values (if applicable), or until the event has stabilised. Follow-up should continue after completion of protocol treatment and/or trial follow-up if necessary. If there are ongoing SAEs at the trial end this should be discussed with TMG. Follow-up SAE eforms (clearly marked as follow-up) should be completed and entered on the trial specific database as further information becomes available. Additional information and/or copies of test results etc may be provided separately. The participant must be identified by trial number and partial date of birth (month and year) only. The participant's name should not be used on any correspondence and should be blacked out and replaced with trial identifiers on any test results.
- Staff should follow their institution's procedure for local notification requirements.

SAE REPORTING

Within 24 hours of investigator becoming aware of an SAE:

The SAE must be entered on to the sponsor's central database and the trial team at CCTU notified at: **cctu.naming@ucl.ac.uk**

- **Where the central database is not available** (e.g., system updates, system failure), a paper version of the SAE form (pCRF) will be provided to sites. The pCRF SAE must then be scanned and sent by encrypted email to the trial team at CCTU at: **cctu.naming@ucl.ac.uk**

For further details on safety reporting please refer to the NAMinG Safety Management Plan for Sites.

7.3.2.2 CCTU responsibilities

The Clinical Reviewer (Chief Investigator or a medically qualified delegate) will review all SAE reports received. The causality assessment given by the local investigator at the hospital cannot be overruled; in the case of disagreement, both opinions will be provided in any subsequent reports.

The clinical reviewer will complete the assessment of expectedness in light of the Reference Safety Information (RSI)for the active treatment or comparator.

CCTU is undertaking the duties of trial sponsor and is responsible for the reporting of SUSARs and other SARs to the regulatory authorities (MHRA and competent authorities of other European member states and any other countries in which the trial is taking place) and the ECs as appropriate. Fatal and life threatening SUSARs must be reported to the competent authorities within 7 days of CCTU becoming aware of the event; other SUSARs must be reported within 15 days.

CCTU will keep investigators informed of any safety issues that arise during the course of the trial.

In the UK an Annual Progress Report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended.

A Development Safety Update Report (DSUR) will be submitted to the MHRA within 60 days of the international birth date of the trial and annually until the trial is declared ended.

Any drug companies involved (Blackmores Institute) will also be notified of all reportable (serious and unexpected and drug-related/unknown relationship) events.

7.3.2.3 Urgent Safety Measures

The CCTU or investigator may take appropriate urgent safety measures in order to protect research participants against any immediate hazard to their health or safety.

In the UK if any urgent safety measures are taken the CI/PI/CCTU shall immediately (no later than 3 days from the date the measures are taken), give written notice to the MHRA and the REC of the measures taken and the circumstances giving rise to those measures, according to the relevant CCTU SOP.

8 Quality Assurance & Control

8.1 Risk Assessment

The Quality Assurance (QA) and Quality Control (QC) considerations for the NAMinG trial are based on the standard CCTU Quality Management Policy that includes a formal Risk Assessment, and that acknowledges the risks associated with the conduct of the trial and proposals of how to mitigate them through appropriate QA and QC processes. Risks are defined in terms of their impact on the rights and safety of participants; project concept including trial design, reliability of results and institutional risk; project management; benefit risk of the trial (see section 1.4); and other considerations.

The trial proposal from the CI was reviewed and developed in line with the processes required by the CCTU Trial Adoption Group (TAG) and this protocol has been reviewed, updated and finalised passing through the Protocol Review Committee process.

According to the MHRA Trial Risk Categorisation, this trial is defined as risk Category B (i.e. somewhat higher than that of standard medical care).

QA is defined as all the planned and systematic actions established to ensure the trial is performed and data generated, documented and/or recorded and reported in compliance with the principles of GCP and applicable regulatory requirements. QC includes the operational techniques and activities performed within the QA system to verify that the requirements for quality of the trial related activities are fulfilled.

The NAMinG Risk Assessment has been reviewed by the CCTU's Quality Management Group (QMG).

8.2 Central Monitoring at CCTU

CCTU staff will review data and other information provided by investigators to identify trends, outliers, anomalies, protocol deviations and inconsistencies. The trial database will also be programmed to generate reports on errors and error rates. The frequency and type of central monitoring will be detailed in the NAMinG trial DMP and Quality Management and Monitoring Plan (QMMP).

8.3 Monitoring

The frequency, type and intensity of routine on-site monitoring and the requirements for triggered on-site monitoring will be detailed in the NAMinG QMMP, including any provision for remote or self-monitoring.

The QMMP will detail the procedures for review and sign-off of monitoring reports. In the event of a request for a trial site inspection by any regulatory authority UCL CCTU must be notified as soon as possible.

8.3.1 Direct access to Participant Records

Participating investigators must agree to allow trial-related monitoring, including audits, REC review and regulatory inspections, by providing access to source data and other documents as required. Participant consent for this must be obtained as part of the informed consent process for the trial.

8.3.2 Confidentiality

CCTU plan to follow the principles of the UK DPA regardless of the countries where the trial is being conducted.

Participant's data will be collected and kept securely. Confidentiality of participant's personal data is ensured by not collecting participant names and other personally identifiable information on CRFs and receiving only pseudonymised data. At trial enrolment, participants will be allocated a Participant Identification Number (PIN), which will be used on all trial related paperwork sent to UCL

CCTU and in the trial database. The trial database and any trial related paperwork will collect data in a pseudonymised format referring to the participant's trial PIN alongside their partial date of birth (month and year). This additional identifier will allow for verification that the correct trial PIN has been provided.

Any documents (e.g. screening and enrolment logs) linking PIN to participant's personally identifiable information will be kept securely at site; only redacted copies will be sent to Sponsor if requested. Only the site research teams will hold identifiable personal data such as the participant's name and/or hospital number on the informed consent forms and enrolment logs, which will be kept in a locked secure location with restricted access to only the site research team.

Copies of participant's trial data will be kept at the participating site in a secure location with restricted access. Unless working at a site, CCTU staff will only have access to the pseudonymised data collected on the trial CRFs (i.e. they will not have access to any other personal data) and applicable source data, moreover only staff working on the trial will have access to these data. Data stored electronically are held on secure servers, that have restricted access.

The informed consent form will carry the participant's name and an appropriate signature; these will be retained at the trial site (participant's hospital). The consent forms will only be accessed by UCL CCTU staff for purposes of monitoring the consent procedure at the site.

Trial-specific samples will be labelled 'NAMinG', with the trial PIN and partial date of birth (month and year) only.

8.4 Source Data

The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial participants. Source data are contained in source documents and are defined by EU guidelines as all information in original records that are used for the reconstruction and evaluation of the clinical trial. Source documents are the first place where the source information is recorded. In most instances these can include hospital records, clinical and office charts, laboratory notes, X-rays, source data worksheets and pharmacy dispensing records.

Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., via an audit trail). Each data element should only have one source.

For this trial the CRFs/eCRFs will not be the source document for any data elements.

The following data should all be verifiable form source documents, which may include paper notes and electronic health records:

- Signed consent forms
- dates of visits including dates any trial specimens were taken and processed in the laboratory

- eligibility and baseline values
- adverse events of any grade
- serious adverse events
- dates IMP was dispensed and (if necessary) IMP returned
- pharmacy or clinic IMP accountability and prescription logs
- concomitant medications
- medical history
- local blood results
- pregnancy test results
- BP, height and weight
- Participant dosing diaries
- Visual field test results
- SD-OCT imaging
- Visual acuity results
- Clinical examination, including slit-lamp biomicroscopy results
- IOP measurement results
- EPIC Norfolk FFQ (Food Frequency Questionnaire)
- EQ-5D-5L with vision bolt on Questionnaire
- GQL-15 Quality of Life Questionnaire

Paper CRFs (pCRFs) will be provided to the site to be used as a back-up for instances when the EDC is unavailable (e.g., system updates, build maintenance, system failures). pCRFs will be provided to sites and should only be used as a temporary measure until the EDC is restored.

A Source Data Agreement will be put in place as part of the site activation process with each site. This will define the source documents and the data therein, together with location of these source documents and any applicable plans for transmission of source data between the site and CCTU.

8.5 Data Collection and Transfer Methods

Each site will be allocated a unique site code and enrolled participants will be given a unique randomisation Participant Identification Number (PIN), generated by Sealed Envelope, which will be used for all data collection forms (minus the screening related CRFs). At the initial screening visit, the site will generate a unique Screening ID number in sequential order following consent. Data will be collected at the time-points indicated in the Schedule of Assessments (section 6.2, Participant Timeline).

The preferred method of data collection is direct online entry of data onto the Electronic Data capture (EDC) cloud-based system sponsor central database OpenClinica. pCRFs can be used as an intermediary (i.e. as CRF worksheets, if sites wish to) between the source data and the EDC, however, ultimately all data are to be entered into the EDC where formal data sign off will be required by the investigators.

Standard Operating Procedures (SOP) for completing the eCRF will be implemented prior to the start of the trial. The site investigator will be responsible for confirming the data in the eCRF prior to data lock.

Research teams at participating sites will receive training on data collection, data entry, secure data transfer and storage at the site initiation meeting(s) and when necessary.

8.6 Data Management

Data will be collected at the time-points indicated in the Participant Timeline (Section 6.2).

Data will be entered remotely in the approved NAMinG database by delegated members of the research teams at participating sites and protected using established CCTU procedures.

Participants will be given a unique randomisation Participant Identification Number (PIN), which will be referred to throughout the trial. Data will be entered under this identification number onto the central database stored on the servers based at CCTU. The database software provides a number of features to help maintain data quality, including maintaining an audit trail, allowing custom validations on data, allowing users to raise data query requests, and search facilities to identify validation failure and missing data.

Data collection, data entry and data queries (raised by a member of the NAMinG trial team), data coding and database lock will be conducted in line with the CCTU Standard Operating Procedures (SOPs) and trial specific Data Management Plan (DMP).

The database will be password protected and only accessible to members of the NAMinG trial team at CCTU, delegated site staff and external regulators if requested. Database users will only be granted permissions to use the database functionality appropriate to their role in the clinical trial. The servers are protected by firewalls and are patched and maintained according to best practice. The physical location of the servers is protected by CCTV and security door access.

Identification logs, screening logs and enrolment logs will be completed and held at the trial site in a locked cabinet within a secured room.

After completion of the trial, the database will be retained on the servers of UCL for on-going analysis of secondary outcomes. All data storage will adhere to UK Data Protection Act 2018 and the EU General Data Protection Regulation (GDPR) 2016 (and subsequent updates and amendments).

8.7 Data Storage

Trial data will be stored in a database created specifically for the NAMinG trial.

The database is hosted by OpenClinica. The data are stored on secure, GDPR-compliant, cloud-based servers held within UK and EU: https://www.openclinica.com/privacy-policy/

The randomisation service is hosted by Sealed Envelope LTD. The data are stored on a secure, GDPR-compliant, cloud-based servers held within the EU: https://www.sealedenvelope.com/security/

The Moorfields Grading Platform is hosted by the Moorfields Reading Centre. The data are stored on secure, GDPR compliant, cloud-based servers held within the UK.

The identification, screening and enrolment logs, linking participant identifiable data to the pseudo-anonymised PIN, will be held locally by the trial site. This will either be held in written form in a locked filing cabinet or electronically in password protected form on hospital computers. After completion of the trial, the identification, screening and enrolment logs will be stored securely by the sites for 5 years after trial closure unless otherwise advised by CCTU.

8.8 Data Archiving

Once all primary and secondary analysis has been completed the trial data will be archived. Once the trial data has been archived the trial database will be decommissioned and will no longer be available. Any subsequent/ further analysis will be performed using the archived data.

8.9 Quality Issues

Quality Issues are issues that can have an impact on patient safety, rights, and well-being; data integrity and/or scientific rigor; and compliance with regulatory requirements; these can be classified as protocol deviations, potential serious breaches, near misses etc.

A protocol deviation is any departure from procedures documented in this protocol, this includes deviations that cannot be predicted. If a protocol deviation is identified the Trials team should be contacted and CCTU's protocol deviation reporting process will be followed.

A 'serious breach' is a deviation from procedures documented in this protocol, GCP or other clinical trial regulations that is likely to affect to a significant degree:

1. The safety or physical or mental integrity of the participants in the trial, or
2. The scientific value of the trial.

If a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee and/or Regulatory authority (MHRA) within 7 days.

9 Statistical Considerations

9.1 Sample Size

We aim to detect a difference at 27 months between treatment arms of 0.158dB, which is 57% of the effect size of IOP lowering, observed in the UKGTS study.

A sample size of 420 evaluable participants is required (210 /arm) to detect a difference between treatment groups of 0.158dB in change in VF MD from baseline to month 27, with 90% power and at the 5% level of significance (two-sided).

The SD of change from baseline from the UKGTS is estimated to be 0.5dB. Assuming a 15% attrition rate, the total sample size is 496 participants (248/arm).

9.2 Assignment of Intervention

9.2.1 Randomisation Procedures

Following the Screening visit, the PI or delegate at site will enter the participant's screening number, site details, initial standard of care IOP-lowering treatment type (eye drops or SLT) and baseline serum NAM levels to re-confirm participant eligibility on the SealedEnvelope.com secure online system, which will then allocate a unique randomisation participant identification number (PIN) to the participant, which will be referred to throughout the trial.

Delegated staff at participating sites will be provided with a secure login to the SealedEnvelope.com Website, according to their role in the trial. The randomisation result will be shown directly online as a unique IMP kit code, with an email confirmation sent to the user, research team and to the CCTU trial team. The investigator will provide details of the allocated unique kit code assigned to the participant on the trial prescription.

9.2.2 Randomisation Method

Participants will be randomised on a 1:1 ratio to either NAM or Placebo, which will be supplied in a masked manner. The dynamic randomisation method, minimisation, will be used to allocate trial participants to one of the groups, balanced by trial site, initial IOP-lowering treatment type (eye drops or SLT); and baseline serum NAM levels. The minimisation algorithm will incorporate a random element to maximise balance in the minimisation factors between the randomised groups. Randomisation will be performed via www.sealedenvelope.com (online web-based system).

9.2.3 Sequence generation

A sequence of unique kit codes for every trial medication kit (active/placebo), will be generated by Sealed Envelope and will be entered into their web-based, secure randomisation system. The IMP kit code list will be provided to a qualified person at Eramol (UK) Limited, who is responsible for ensuring that trial medication and placebo kits are labelled appropriately, and that the trial team and participants remain masked to treatment allocation. A copy of the sequence will be held securely at CCTU.

Treatment for each participant will be prescribed in accordance with their allocated IMP kit code generated by Sealed Envelope. The trial staff at CCTU along with the site pharmacies will ensure that appropriate amounts of the labelled active/placebo drug kits will be delivered to site pharmacies to allow adequate supplies (of the relevant numbered kits) for pharmacy dispensing.

9.2.4 Allocation concealment mechanism

On the day of randomisation, delegated staff at site will enter the participant's screening number, site details, initial standard of care IOP-lowering treatment type (eye drops or SLT) and baseline serum NAM levels into Sealed Envelope, which will then allocate a unique randomisation participant identification number (PIN) to the participant, which will be referred to throughout the trial.

Sealed Envelope will also allocate a kit code to the participant at each of the IMP dispensing visits (refer to section 5.3.3). The kit code will be shown directly online, with an email confirmation to the user, the site PI and members of the NAMinG research team UCL CCTU.

9.2.5 Allocation Implementation

Following confirmation of participant eligibility, eligible participants will be randomised at the baseline visit (Visit 1; Month 0) by a clinical investigator or delegated member of the research team using the Sealed Envelope web-based service. The responsibility for enrolling and prescribing trial medication for the participant lies with the PI at each recruiting participating site, however this can also be undertaken by other delegated clinicians who have been delegated the task and which has been recorded on the NAMinG Delegation Log. Secure login usernames and passwords for Sealed Envelope will be provided to delegated staff prior to green light site activation and/or as required. A complete list of users can be obtained from UCL CCTU.

The users will be required to log in and answer eligibility questions before entering stratification data and being permitted to randomise a participant. The randomisation result will be shown directly online as a unique kit code, with an email confirmation to the user and the CCTU trial team. The investigator will provide details of the allocated unique kit code assigned to the participant on prescription forms which are then sent to the site pharmacist to enable the dispensing of trial medication. A full accountability trail will be maintained from receipt of trial medication at the site pharmacy, prescribing by the local investigator, distribution of the trial medication to the participant, to destruction of the undispensed trial medication at local site pharmacies.

9.2.6 Masking

This will be a double-masked designed trial. Investigators, pharmacy staff, research teams at sites, participants, the research team at UCL CCTU and the analysing trial statistician will be masked to treatment allocation. The only exception being the delegated unmasked trial statistician at UCL CCTU who will be responsible for providing the masked trial kit codes to Sealed Envelope and Eramol (UK) Limited.

Masking will be maintained by using matching medicinal preparation for NAM and placebo (matched size/appearance, taste of tablets and identical containers (bottles)). The masked treatment identity will be maintained in the online Sealed Envelope randomisation service except in the case of medical emergency (see section 5.6.1).

Detailed information regarding randomisation and un-masking will be provided in the NAMinG Randomisation and Unmasking Plan.

9.3 Statistical Considerations

9.3.1 Statistical Analysis Plan

All trial analyses will be according to the Statistical Analysis Plan (SAP), which will be prepared before the first substantive unblinded analysis and agreed in advance by the Trial Steering Committee (TSC)

and Independent Data Monitoring Committee (IDMC). The rest of this section provides the general statistical principles.

9.3.2 Interim Analyses

No interim analyses will be performed apart from those required by the IDMC.

9.3.3 Statistical Methods – Outcomes

A CONSORT diagram will be used to describe the course of participants through the trial.

Summary measures for the baseline characteristics of each group will be presented as mean and standard deviation for continuous (approximate) normally distributed variables (or medians and interquartile ranges for non-normally distributed variables) and frequencies and percentages for categorical variables.

The primary endpoint will be analysed using a linear mixed model to estimate the difference between treatment groups in VF MD at 27 months. The model will include month 27 VF MD as the outcome and baseline VF MD, IOP-lowering treatment type (eye drops vs laser) and baseline serum NAM level as covariates. Site will be included as a random effect to account for variability in outcomes between sites. This will be modelled with an identity covariance structure and fitted using restricted maximum likelihood (REML). Primary outcome analysis will be at the level of the participant using data on the eye with the worse baseline VF MD (study eye). A sensitivity analysis will be conducted, with the primary model adjusted for additional covariates: age, sex, glaucoma bilaterality, IOP, smoking history, presence of disc haemorrhages and corneal thickness.

<u>Analysis of secondary outcomes:</u>

The key secondary outcome, improvement in VF MD in the first 3 months, will be analysed using a similar model to the primary outcome. Similar models will also be used to evaluate differences in quality-of-life measures, EQ-5D-5L and GQL-15 at 27 months between the two treatment arms.

<u>Analysis of mechanistic outcomes:</u>

To quantify the association between mitochondrial function and VF MD longitudinally, a repeated measures model will be used. Mitochondrial function will be regarded as time-varying covariate. The model will be adjusted for IOP-lowering treatment type (eye drops vs laser) and baseline serum NAM level.

To evaluate whether NAM improves mitochondrial function, the change in the ATP-linked OCR in the PBMCs of patients from the time of randomisation (month 0) to the end of the trial (27 months) will be compared between the NAM group and the placebo group (the Biomarker Sub-Study cohort) using the primary outcome model.

Using an interaction term in two separate models with the two outcomes a) VF MD and b) ATP-linked OCR, we will explore whether a differential treatment effect is observed according to participants' baseline mitochondrial function level (low vs high) and baseline NAM levels (low vs high) (the Biomarker Sub-Study cohort).

To assess the relationship between ATP-linked OCR (as an outcome variable) and:

- Baseline serum NAM
- Baseline lymphocyte NAD+ and,
- Baseline oxidised glutathione levels,

separate regression analyses will be used (placebo group and the Biomarker Sub-Study cohort; n = 100).

To quantify the association between baseline NAM levels and rate of VF loss, a linear mixed model will be used with VF MD as the outcome and baseline NAM measure (continuous variable) as a covariate.

To evaluate whether NAM influences IOP, the mean IOP change from month 3 to the end of the trial will be compared between the NAM group and the placebo group. We will also use the primary outcome model to test the effect of treatment with IOP as the outcome.

Analysis of surrogate outcomes:

The primary outcome mixed models will be repeated using the surrogate outcome OCT peripapillary RNFL/macular GCL to estimate treatment difference at 3 and 27 months.

Analysis of exploratory outcomes:

To compare between treatment groups of the rate of VF loss at predetermined VF locations, a linear mixed effects model will be used with time as a continuous variable. The appropriate model will be based on whether there is neuro-recovery between 0 and 3 months:

i) If there is VF MD improvement between 0 and 3 months the rate of progression will be measured between 3 and 27 months.
ii) If there is no VF MD improvement between 0 and 3 months the rate of progression will be measured between 0 and 27 months.

We will also consider fitting a non-linear model using a restricted cubic spline or other appropriate method, and compare model fit with the linear model, using AIC.

Other tests will be regarded as exploratory and will include the evaluation of any interactions between NAM and topical latanoprost treatment on VF progression and IOP. These will be analysed without multiplicity adjustment, but significant results would be clearly labelled as exploratory and would require confirmation in further studies.

9.3.4 Additional Analyses - Subgroup

Using an interaction term in the primary outcome model, we will explore whether a differential treatment effect is observed according to the following factors: participants with lower vs higher baseline IOP, IOP treatment (SLT vs latanoprost), lower vs higher baseline mitochondrial function, younger (<68yrs) vs older (≥68yrs) participants, stage of glaucoma and in participants with lower vs higher baseline NAM (vit B3 levels).

9.3.5 Additional Analyses – Adjusted

The primary and secondary outcome models will be adjusted for baseline VF (MD), IOP-lowering treatment type (eye drops vs laser) and baseline serum NAM level. A supportive analysis of the primary outcome will include additional adjustment for age, sex, stage of glaucoma, glaucoma bilaterality, IOP, smoking history, presence of disc haemorrhages and corneal thickness.

9.3.6 Analysis Population and Missing Data

The primary analysis will be in the intention-to-treat (ITT) population. Analysis will include data up to the point of drop-out for patients no longer attending appointments or withdrawing consent. Participants who leave the trial procedures, but who are still followed up for data collection, will otherwise still contribute data and be included in the ITT analysis. There will be no imputation for missing data for any of the trial outcomes.

A modified ITT analysis will be performed on the primary outcome that will exclude participants who have non-glaucomatous VF change (i.e. VF loss with a retinal or neurological cause) prior to or at the 27 month visit.
An additional sensitivity analysis will be carried out on the mechanistic outcome, effect of NAM on rate of VF loss, which will exclude VF measures of participants following non-glaucomatous VF change.

10 Economic Evaluations

No Health Economic Evaluations are planned for this trial.

11 Regulatory & Ethical Issues

11.1 Compliance

11.1.1 Regulatory Compliance

This trial will adhere to the conditions and principles of GCP as outlined in the Medicines for Human Use (Clinical Trials) Regulation 2004 (SI 2004/1031), as amended.

In conducting the trial, the Sponsor, UCL CCTU and sites shall comply with the protocol and with all relevant guidance, laws and statutes, as amended, applicable to the performance of clinical trials and research including, but not limited to:

- UK Policy Framework for Health and Social Care Research, issued by the Health Research Authority
- Declaration of Helsinki 1996

- Data Protection Act 2018 (DPA number: Z6364106),
- General Data Protection Regulation (EU) 2016/679 (GDPR)
- Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031) (IMP trials only)
- The Human Medicines (Amendment etc.) (EU Exit) Regulations 2019 (SI 2019/775)
- Human Tissue (Quality and Safety for Human Application) Regulations 2007, and any other national and local applicable regulations

11.1.2 Site Compliance

Agreements that include detailed roles and responsibilities will be in place between participating sites and CCTU.

Participating sites will inform CCTU as soon as they are aware of a possible serious breach of compliance, so that CCTU can fulfil its requirement to report the breach if necessary (see section 8.9).

11.1.3 Data Collection & Retention

Clinical notes and administrative documentation should be kept in a secure location (for example, locked filing cabinets in a room with restricted access) and held for a minimum of 5 years after the end of the trial. During this period, all data should be accessible, with suitable notice, to the competent authorities, the Sponsor, and other relevant parties in accordance with the applicable regulations. The data may be subject to an audit by the competent authorities. Medical files of trial participants should be retained in accordance with the maximum period of time permitted by the hospital, institution or private practice.

11.2 Ethical Approvals

11.2.1 Ethical Considerations

The following ethical considerations relating to the trial have been considered and steps have been taken to minimise the issues and their impact on participants and they have been mentioned in the Participant Information Sheet:

- Any additional visits required by the trial
- Any additional tests required by the trial, for example: blood, urine pregnancy test, additional VF tests, etc)
- Use of placebo and when and/or whether its use would be revealed
- Any additional short-term or long-term risks of participating in the trial
- Effects on life insurance (sometimes known as assurance) policies
- Participants will not be able to choose their own treatment if this is a randomised controlled trial (RCT)
- Availability of trial treatment after the trial if the participant is responding
- Reimbursement of time and expenses (if payment is even available)
- The collection of sensitive or personal samples or data

- Publication of data and feedback of overall results (not individual results) to participants
- Blood and tissue samples as donations – not sharing in the profit in the unlikely instance that something is developed
- Coincidental findings: extra tests may uncover some other previously unknown condition

11.2.2 Ethics Committee 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 EC for HRA approval.

Following main REC approval and Health Research Authority (in England) approvals and before initiation of the trial at each clinical site, the local information pack will be submitted to each Trust's Research and Development (R&D) office by UCL CCTU. The local information pack will contain the protocol, all informed consent forms, and information materials to be given to the prospective participant, the Clinial Trial Site Agreement, the Organisation Information Document (OID), and the validated Schedule of Events Cost Attribution Template (SoECAT). In Wales, Scotland and Northern Ireland, the R&D office will be asked to give approval. In England, the R&D office will be asked to confirm capacity and capability. UCL CCTU must receive confirmation of capacity and capability from each participating site before recruitment can begin.

Any further substantial amendments will be submitted and approved by the main REC and HRA.

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.

11.3 Competent Authority Approvals

This protocol will be reviewed by/submitted to the national competent authority (MHRA in the UK), where the trial will be conducted.

This is a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by The Medicines for Human Use (Clinical Trials) Regulations 2004/1031. Therefore, a Clinical Trial Authorisation (CTA) is required in the UK.

The progress of the trial and safety issues will be reported to the competent authority, regulatory agency or equivalent in accordance with relevant national and local requirements and practices in a timely manner.

Safety reports, including expedited reporting and SUSARS will be submitted to the competent authority in accordance with each authority's requirements in a timely manner.

11.4 Other Approvals

This protocol, along with other relevant trial documentation will be submitted to the Health Research Authority (HRA) at the same time as it is submitted to the relevant EC for national approval.

The protocol will also be submitted by those delegated to do so to the relevant R&D department of each participating site for local approval (confirmation of capacity and capability). A copy of the local permissions (or other relevant approvals 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 review and methodological, statistical, clinical and operational input from the CCTU Protocol Review Committee.

11.5 Trial Closure

Trial closure is defined as the date when all data have been received, cleaned and all data queries resolved at all sites and the database locked for final analysis.

The MHRA and the REC/HRA will be notified within 90 days of trial completion. Within one year of the end of the trial, the CCTU will submit a final trial report with the results of the trial, including any publications/abstracts of the trial, to the HRA and the MHRA. In case the trial is ended prematurely, the CCTU will notify the HRA and the MHRA within 15 days, including the reasons for the premature termination.

12 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.

13 Finance

NAMinG is fully funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme, grant number 132758; a Medical Research Council (MRC) and National Institute of Health Research (NIHR) partnership. The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NIHR or the Department of Health and Social Care.

It is not expected that any further external funding will be sought.

14 Oversight & Trial Committees

Trial oversight is intended to preserve the integrity of the trial by independently verifying a variety of processes and prompting corrective action where necessary.

There are a number of committees involved with the oversight of the trial and these committees are detailed below. In multi-centre trials this oversight is considered and described both overall and for each recruiting centre by exploring the trial dataset or performing site visits as described in the NAMinG QMMP.

14.1 Trial Team

The Trial Team (TT) will be set up to assist with developing the design, co-ordination and day to day operational issues in the management of the trial, including budget management. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TT terms of reference (ToR).

14.2 Trial Management Group

A Trial Management Group (TMG) will be formed comprising the Chief Investigator, other lead investigators (clinical and non-clinical) and CCTU staff and PPI contributors. A Trial Management Group (TMG) will be set up to assist with developing the design, co-ordination and strategic management of the trial. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TMG ToR.

14.3 Trial Steering Committee

The Independent Trial Steering Committee (TSC) is the independent group responsible for oversight of the trial in order to safeguard the interests of trial participants. The TSC provides advice to the CI, CCTU, the funder and sponsor on all aspects of the trial through its independent Chair. The ultimate decision for the continuation of the trial lies with the TSC. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TSC ToR.

14.4 Independent Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) will be appointed for the trial. The Independent Data Monitoring Committee (IDMC) is the only oversight body that has access to unblinded accumulating comparative data. The IDMC is responsible for safeguarding the interests of trial participants, monitoring the accumulating data and making recommendations to the TSC on whether the trial should continue as planned. The membership, frequency of meetings, activity (including review of trial conduct and data) and authority will be covered in the IDMC ToR. The IDMC will consider data in accordance with the statistical analysis plan and will advise the TSC through its Chair.

14.5 Trial Sponsor

The role of the sponsor is to take on responsibility for securing the arrangements to initiate, manage and finance the trial. UCL is the trial sponsor and has delegated the duties as sponsor to CCTU via a signed letter of delegation.

15 Patient & Public Involvement

Patient and Public Involvement (PPI) in research is defined by INVOLVE (an advisory group established by the NIHR) as research being carried out 'with' or 'by' members of the public rather than 'to', 'about' or 'for' them. INVOLVE intends 'public' to include patients, potential patients, carers and other users of health and social care services, as well as people from organisations that represent people who use services. In some cases, this may include involvement of a trial's participants in guidance or oversight of a trial.

15.1 Potential Impact of PPI

PPI will help with recruitment strategies to improve recruitment where required. PPI will help with the trial design and be able to provide patient perspectives and promote the trial. PPI will review the participant information sheet to ensure it has been written in lay terms for the understanding of a participant enrolling in this trial. PPI will help with the dissemination of trial results and feedback to participants and the public. PPI will be able to engage with stakeholders and monitor changes in response to PPI activities and comments.

15.2 Identifying PPI Contributors

A Participant Advisory Group (PAG) will be set-up for the trial and will include a PPI lead. PPI members will be recruited from a diverse group of people with different backgrounds, and diversity is important in this trial as certain BAME groups are high risk from Glaucoma. Terms of Reference for the PAG will be created and a recruitment flyer will be used as an advertisement to recruit PPI members. Training for PPI members will be provided where required and courses identified as helpful. PPI members will be reasonably reimbursed for their time and travel as a PPI member.

15.3 Protocol Design & Trial Set Up

PPI has been considered from the start of the trial, with the CI taking part in PPI activities to gain insight into patient's perspective on the trial design. The PPI lead has already been appointed and was a co-applicant on the trial funding application. The PPI lead has been involved in the trial set-up

and design and has had input in reviewing the trial protocol, participant information sheet and participant dosing diaries.

15.4 PPI in the Ongoing Running of the Trial

The PPI lead will chair the PAG, and this group will meet at regular intervals throughout the trial duration. The PAG will evaluate feedback on recruitment and retention of participants onto the NAMinG trial and will support the development of trial newsletters. The PPI lead will be a member of the TMG and will report back to the TMG any outcomes of the PAG meetings. The PPI lead will also be a member of the TSC. A monetary budget is available for any necessary training that PPI members require to fulfil their roles.

16 Publication & Dissemination of Results

16.1 Publication Policy

16.1.1 Trial Results

The results of the trial will be disseminated regardless of the direction of effect. The publication of the results will comply with the UCL CCTU Publication Policies.

A lay summary of the results will also be produced to be disseminated to the participants who took part and who express an interest in the findings. The results will also be communicated to the public and service users, NIHR, NICE and the wider scientific community. The Participant Advisory Group will guide communication with participants and maintain contact with regular Newsletter updates.

A summary of results will be submitted to the REC via the HRA (https://www.hra.nhs.uk/approvals-amendments/managing-your-approval/ending-your-project/final-report-form/) and published through an open-access mechanism in a peer-reviewed journal within 12 months of the trial closure.

A summary of results will be published within one year of the end of study, in the registry where the clinical trial is registered.

16.1.2 Authorship

All individuals who have made substantial intellectual, scientific and practical contributions to the trial and the manuscript should, where possible, should be credited as authors; all individuals credited as authors should deserve that designation. It is the responsibility of the CI and, ultimately, the Sponsor to ensure that these principles are upheld.

16.1.3 Reproducible Research

The latest version of the protocol will be made available as supplementary material upon publication of the final clinical investigation report.

Applications for access to the trial dataset at the end of the trial, should be submitted formally in writing to UCL CCTU and will be considered, and approved in writing after formal consideration by the trial oversight committees and the CI.

17 Data and/or Sample Sharing

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.

Data will be shared accordingly based on the following principles:

- No data should be released that would compromise an ongoing trial or study.
- There must be a strong scientific or other legitimate rationale for the data to be used for the requested purpose.
- Investigators who have invested time and effort into developing a trial or study should have a period of exclusivity in which to pursue their aims with the data before key trial data are made available to other researchers.
- The resources required to process requests should not be under-estimated, particularly successful requests which lead to preparing data for release. Therefore, adequate resources must be available in order to comply in a timely manner or at all, and the scientific aims of the study must justify the use of such resources.
- Data exchange complies with Information Governance and Data Security Policies in all of the relevant countries.

In order to reflect the NIHR's position on open access to research materials, where research materials recording the outcome of the Research or details of the progress of the Research are submitted for publication, UCL shall either: 1) subject to confidentiality requirements and to applicable data protection considerations, make all information and data on which the research materials are based available on an open access basis; or 2) include a statement with the research materials detailing how such information and data can be accessed.

UCL shall ensure that the outcome of the Research is prepared for publication in a suitable peer-reviewed journal and shall ensure that it, and any other publication, including patent applications, of or resulting from research caried out by the grant shall acknowledge the NIHR's financial support and carry a disclaimer relevant to the programmes set out in the NIHR's research outputs and publications guidance as amended from time to time.

Data will be available for sharing after the trial results have been published (within 12 months of the end of trial). Researchers wishing to access NAMinG trial data should contact the Trial Management Group in the first instance.

18 Ancillary Studies

There are two optional sub-studies included in the NAMinG trial, which eligible participants will be able to take part in should they wish to do so. A separate participant information sheet and informed consent form will be available for these sub-studies.

The Future Genetic and Gene Expression Sub-Study is open to all eligible NAMinG participants. The Biomarker Sub-Study is open to eligible NAMinG participants at the selected sites that will be taking part in the Biomarker Sub-Study (Moorfields Eye Hospital, Kings College Hospital, and up to 2 further sites ONLY).

For further details about the sub-studies please refer to section 21: Appendices.

18.1 Consent or Assent in Ancillary Studies

Consent will be sought from all eligible NAMinG participants to participate in the optional future genetic and gene expression sub-study to address questions beyond the immediate scope of the main trial and to identify the genetic and gene expression profiles of those susceptible, or resistant, to glaucoma progression and genetic profiles associated with more favourable clinical outcomes in response to NAM (see appendix 2 for details).

Consent will include the option for use of routinely collected clinical data after the end of the trial (beyond the outcomes to be collected during the trial), including when a participant may have transferred to another centre, for long-term assessment of the impact of standard of care with or without NAM on visual function. Patients will be prospectively recruited by experienced members of the clinical team at each site and patient information will not be used from an existing database without pre-existing patient consent.

Consent will be sought from eligible NAMinG participants at the selected sites taking part in the Biomarker Sub-Study to participate in the analysis of peripheral blood mononuclear cells (PBMCs) to investigate mitochondrial function and any association between mitochondrial function and quantitative phenotypic traits (see appendix 1 for details).

They will be asked to consent to samples being collected and stored at the UCL Institute of Ophthalmology for purposes of this trial as well as for use in future research.

All samples will be retained under the participant's trial ID.

18.2 Ancillary and Post-trial Care

No arrangements are in place to provide trial drug to trial participants' post completion of trial participation. This will be made clear in the Patient Information Sheet.

19 Protocol Amendments

Any amendments to the protocol will be determined by the CI, TMG, TSC and IDMC. The sponsor will ensure amendments to essential documentation i.e. protocol, participant information sheets/informed consent forms and other supporting documents are submitted and approved by the EC/HRA/MHRA prior to implementation. The ethical and regulatory approved amended documentation will then be disseminated to local R&Ds at participating sites for their approval and implementation.

Table 8. Summary of Protocol Amendments

Protocol version	Protocol date	Summary of changes
1.0	31-May-2023	N/A – new document
2.0	05-Sep-2023	Update to section 3.6, 3.7, 6.2 and section 7.3.2 that reporting of AEs/SAEs will be from the time of consent

20 References

1 Chan AW, Tetzlaff JM, Altman DG et Al. SPIRIT 2013 Statement: Defining Protocol Items for Clinical Trials. *Ann Intern Med* 2013; 158:200-207.

2 Chan AW, Tetzlaff, Gotzsche et Al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013; 346: e7586.

3 Quartilho A, Simkiss P, Zekite A, Xing W, Wormald R, Bunce C. Leading causes of certifiable visual loss in England and Wales during the year ending 31 March 2013. Eye 2016;30:602-7.

4 Higginbotham EJ. How much influence do socioeconomic factors have on glaucoma prevalence and therapeutic outcomes? Arch Ophthalmol. 2006;124:1185-6.

5 Minassian D, Reidy A. Future sight loss UK: An epidemiological and economic model for sight loss in the decade 2010-2020. RNIB Report July 2009.

6 Royal College of Ophthalmologists (RCOphth): The Way Forward Glaucoma Report. Options to help meet demand for the current and future care of patients with eye disease. RCOphth; 2016.

7 Guide Dogs for the Blind Association study: The costs of blindness: An analysis of the costs of visual impairment and blindness in the United Kingdom. Ethical Strategies Limited; 2003.

8 Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, et al; Vision Loss Expert Group of the Global Burden of Disease Study. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. Lancet Glob Health. 2017;5:e1221-e1234.

9 Boodhna T, Crabb DP. Disease severity in newly diagnosed glaucoma patients with visual field loss: trends from more than a decade of data. Ophthalmic Physiol Opt. 2015;35:225-30.

10 Kass MA, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. Archives of Ophthalmology. 2002;120:701-13.

11 Saade CE, Lari HB, Berezina TL, Fechtner RD, Khouri AS. Topical glaucoma therapy and ocular surface disease: a prospective, controlled cohort study. Can J Ophthalmol. 2015;50:132-6.

12 Roughead EE, Kalisch LM, Pratt NL, Killer G, Barnard A, Gilbert AL. Managing glaucoma in those with comorbidity: not as easy as it seems. Ophthalmic Epidemiol. 2012;19:74-82.

13 Tatham AJ, Sarodia U, Gatrad F, Awan A. Eye drop instillation technique in patients with glaucoma. Eye (Lond). 2013;27:1293-1298.

14 Mokhles P, Schouten J, Beckers H, Azuara-Blanco A, Tuulonen A, Webers C. Glaucoma blindness at the end of life. Acta Ophthalmol. 2017;95:10-11.

15 Glaucoma Top 10 James Lind Alliance Priority Setting Partnerships. [cited 2021 April 24]. Available from: https://www.jla.nihr.ac.uk/priority-setting-partnerships/sight-loss-and-vision/top-10-priorities/glaucoma-top-10.htm

16 Chang EE, Goldberg JL. Glaucoma 2.0: neuroprotection, neuroregeneration, neuroenhancement. Ophthalmology. 2012;119:979-86.

17 He S, Stankowska DL, Ellis DZ, Krishnamoorthy RR, Yorio T. Targets of Neuroprotection in glaucoma. J Ocul Pharmacol Ther. 2018;34:85-106.

18 Osborne NN, del Olmo-Aguado S. Maintenance of retinal ganglion cell mitochondrial functions as a neuroprotective strategy in glaucoma. Curr Opin Pharmacol. 2013;13:16-22.

19 Yu DY, Cringle SJ, Balaratnasingam C, Morgan WH, Yu PK, Su EN. Retinal ganglion cells: Energetics, compartmentation, axonal transport, cytoskeletons and vulnerability. Progress in Retinal and Eye Research. 2013;36:217-246.

20 Casson RJ, Chidlow G, Crowston JG, Williams PA, Wood JPM. Retinal energy metabolism in health and glaucoma. Prog Retin Eye Res. 2021;81:100881.

21 Li Y, Li D, Ying X, Khaw PT, Raisman G. An energy theory of glaucoma. Glia 2015;63:1537-1552.

22 Chrysostomou V, Trounce I, Crowston J. Mechanisms of retinal ganglion cell injury in aging and glaucoma. Ophthalmic Res. 2010;44:173-8.

23 Kong GY, Van Bergen NJ, Trounce IA, Crowston JG. Mitochondrial dysfunction and glaucoma. J Glaucoma. 2009;18:93–100.

24 Yu-Wai-Man P, Griffiths PG, Chinnery PF. Mitochondrial optic neuropathies – disease mechanisms and therapeutic strategies. Prog Retin Eye Res. 2011;30:81-114.

25 Kamel K, Farrell M, O'Brien C. Mitochondrial dysfunction in ocular disease: Focus on glaucoma. Mitochondrion. 2017;35:44-53.

26 Lascaratos G, Garway-Heath DF, Willoughby CE, Chau KY, Schapira AH. Mitochondrial dysfunction in glaucoma; understanding genetic influences. Mitochondrion. 2012;12(2):202-12.

27 Khawaja AP, Cooke Bailey JN, Kang JH, Allingham R, Hauser M, Brilliant M, et al. Assessing the Association of Mitochondrial Genetic Variation With Primary Open-Angle Glaucoma Using Gene-Set Analyses. Invest Ophthalmol Vis Sci. 2016;57:5046-5052.

28 Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, et al. Adult-onset primary open-angle glaucoma caused by mutations in optineurin. Science. 2002;295:1077-9.

29 Yu-Wai-Man P, Griffiths PG, Hudson G, Chinnery PF. Inherited mitochondrial optic neuropathies. Journal of Medical Genetics. 2009;46:145-158.

30 O'Neill EC, Danesh-Meyer HV, Kong GX, Hewitt AW, Coote MA, Mackey DA, et al; Optic Nerve Study Group. Optic disc evaluation in optic neuropathies: the optic disc assessment project. Ophthalmology. 2011;118:964-70.

31 Tribble J, Vasalauskaite A, Redmond T, Young R, Hassan S, Fautsch M, et al. Midget retinal ganglion cell dendritic and mitochondrial degeneration is an early feature of human glaucoma. Brain Communications. 2019;1:fcz035.

32 McElnea EM, Quill B, Docherty NG, Irnaten M, Siah WF, Clark AF, et al. Oxidative stress, mitochondrial dysfunction and calcium overload in human lamina cribrosa cells from glaucoma donors. Mol Vis. 2011;17:1182-91.

33 Izzotti A, Longobardi M, Cartiglia C, Saccà SC. Mitochondrial damage in the trabecular meshwork occurs only in primary open-angle glaucoma and in pseudoexfoliative glaucoma. PLoS One. 2011;6:e14567.

34 Kimball EC, Jefferys JL, Pease ME, Oglesby EN, Nguyen C, Schaub J, et al. The effects of age on mitochondria, axonal transport, and axonal degeneration after chronic IOP elevation using a murine ocular explant model. Exp Eye Res. 2018;172:78-85.

35 Kimball EC, Pease ME, Steinhart MR, Oglesby EN, Pitha I, Nguyen C. A mouse ocular explant model that enables the study of living optic nerve head events after acute and chronic intraocular pressure elevation: Focusing on retinal ganglion cell axons and mitochondria. Exp Eye Res. 2017;160:106-115.

36 Munemasa Y, Kitaoka Y, Kuribayashi J, Ueno S. Modulation of mitochondria in the axon and soma of retinal ganglion cells in a rat glaucoma model. J Neurochem. 2010;115:1508-19.

37 Williams PA, Harder JM, Foxworth NE, Cochran KE, Philip VM, Porciatti V, et al. Vitamin B3 modulates mitochondrial vulnerability and prevents glaucoma in aged mice. Science. 2017;355:756-760.

38 Abu-Amero KK, Morales J, Bosley TM. Mitochondrial abnormalities in patients with primary open-angle glaucoma. Invest Ophthalmol Vis Sci. 2006;47:2533-41.

39 Lee S, Sheck L, Crowston JG, Van Bergen NJ, O'Neill EC, O'Hare F, et al. Impaired complex-I-linked respiration and ATP synthesis in primary open-angle glaucoma patient lymphoblasts. Invest Ophthalmol Vis Sci. 2012;53:2431-7.

40 Lascaratos G, Chau KY, Zhu H, Gkotsi D, King R, Gout I, et al. Resistance to the most common optic neuropathy is associated with systemic mitochondrial efficiency. Neurobiol Dis. 2015;82:78-85.

41 Petriti B, Chau D, Lascaratos G, Campbell P, Lazaridis G, Garway-Heath DF. Normal Tension Glaucoma patients have reduced systemic mitochondrial function compared to High Tension Glaucoma patients. Invest Ophthalmol Vis Sci. 2020;61:1009.

42 Hui F, Tang J, Williams PA, McGuinness MB, Hadoux X, Casson RJ, et al. Improvement in inner retinal function in glaucoma with nicotinamide (vitamin B3) supplementation: A crossover randomized clinical trial. Clin Exp Ophthalmol. 2020;48:903-914.

43 Canadian Glaucoma Study Group. Canadian Glaucoma Study: 1. Study design, baseline characteristics, and preliminary analyses. Can J Ophthalmol. 2006;41:566-75.

44 Heijl A, Buchholz P, Norrgren G, Bengtsson B. Rates of visual field progression in clinical glaucoma care. Actac Ophthalmol. 2013;91:406-12.

45 De Moraes CG, Sehi M, Greenfield DS, Chung YS, Ritch R, Liebmann JM. A validated risk calculator to assess risk and rate of visual field progression in treated glaucoma patients. Invest Ophthalmol Vis Sci. 2012;53:2702-2707.

46 Nitta K, Wajima R, Tachibana G, Inoue S, Ohigashi T, Otsuka N, et al. Prediction of visual field progression in patients with primary open-angle glaucoma, mainly including normal tension glaucoma. Sci Rep. 2017;7:15048.

47 Liu L, Su X, Quinn WJ, Hui S, Krukenberg K, Frederick DW, Redpath P, et al. Quantitative Analysis of NAD Synthesis-Breakdown Fluxes. Cell Metab. 2018;27:1067-1080.

48 Braidy N, Liu Y. NAD+ therapy in age-related degenerative disorders: A benefit/risk analysis. Exp Gerontol. 2020;132:110831.

49 Fang EF, Hou Y, Lautrup S, Jensen MB, Yang B, SenGupta T, et al. NAD+ augmentation restores mitophagy and limits accelerated aging in Werner syndrome. Nat Commun. 2019;10:5284.

50 Fricker RA, Green EL, Jenkins SI, Griffin SM. The Influence of Nicotinamide on Health and Disease in the Central Nervous System. Int J Tryptophan Res. 2018;11:1178646918776658.

51 Hershberger KA, Martin AS, Hirschey MD. Role of NAD+ and mitochondrial sirtuins in cardiac and renal diseases. Nat Rev Nephrol. 2017;13:213-225.

52 Song SB, Park JS, Chung GJ, Lee IH, Hwang ES. Diverse therapeutic efficacies and more diverse mechanisms of nicotinamide. Metabolomics. 2019;15:137.

53 Ebeling MC, Polanco JR, Qu J, Tu C, Montezuma SR, Ferrington DA. Improving retinal mitochondrial function as a treatment for age-related macular degeneration. Redox Biol. 2020;34:101552.

54 Nzoughet J, Chao de la Barca JM, Guehlouz K, Leruez S, Coulbault L, Allouche S, et al. Nicotinamide Deficiency in Primary Open-Angle Glaucoma. Invest Ophthalmol Vis Sci. 2019;60:2509-2514.

55 Knip M, Douek IF, Moore WP, Gillmor HA, McLean AE, Bingley PJ, et al; European Nicotinamide Diabetes Intervention Trial Group. Safety of high-dose nicotinamide: a review. Diabetologia. 2000;43:1337-45.

56 Hoffer A. Megavitamin B-3 therapy for schizophrenia. Canadian Psychiatric Association Journal. 1971;16:499-504.

57 Gale EA, Bingley PJ, Emmett CL, Collier T; European Nicotinamide Diabetes Intervention Trial (ENDIT) Group. European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. Lancet. 2004;363:925-31.

58 https://pubmed.ncbi.nlm.nih.gov/11126400/

59 https://pubmed.ncbi.nlm.nih.gov/24507733/

60 She J, Sheng R, Qin ZH. Pharmacology and Potential Implications of Nicotinamide Dinucleotide Precursors. Aging and Disease. 2021;12;1879-1897.

61 Williams PA, Harder JM, John SWM. Glaucoma as a Metabolic Optic Neuropathy: Making the Case for Nicotinamide Treatment in Glaucoma. J Glaucoma. 2017;26:1161-1168.

62 Tribble J, Otmani A, Sun S, Ellis SA, Cimaglia G, Vohra R, et al. Nicotinamide provides neuroprotection in glaucoma by protecting against mitochondrial and metabolic dysfunction [pre-print] bioRxiv 2020.10.21.348250.

63 Durani Z, Gkotsi D, Chau D, Garway-Heath DF. The effects of Vitamin B3 on fibroblast mitochondrial function in glaucoma patients. Invest Ophthalmol Vis Sci. 2018;59:6036.

64 https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf

65 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5854263/

21 Appendices

21.1 Appendix 1 Biomarker Sub-Study

21.1.1 Introduction

In this trial, we propose to measure systemic mitochondrial function in the peripheral blood to understand whether the potential protective effect of NAM is mediated by measurable improvement in mitochondrial function and whether this effect is maintained throughout the observation period.

For the first time in the context of a glaucoma clinical trial, we use mitochondrial function (a known biological marker of ageing) to evaluate the 'metabolic signature' of each patient and determine whether a subgroup of glaucoma patients (perhaps those with lower mitochondrial function at baseline or those with other clinical features yet to be determined) may be more responsive to treatment with NAM. Using mitochondrial function as a novel, clinically useful, biomarker for glaucoma opens the door to 'personalised treatment plans' and enforces compliance and confidence to treatment by introducing a measurable biological treatment outcome measure. In a neurodegenerative disease like glaucoma, mitochondrial function has the potential to reveal the underlying systemic susceptibility of patients and play an important role in risk stratification in clinic, indicating which individuals need closer monitoring and perhaps more intensive treatment. The same biomarker may predict treatment response.

We will measure the mitochondrial oxygen consumption rate (OCR) on live peripheral blood mononuclear cells (PBMCs) of our patients using the Extracellular Flux (XFe24) analyser (Seahorse Bioscience) at the UCL Institute of Ophthalmology. Due to practical considerations and equipment availability, this will only be performed on participants from our London based recruitment sites (Moorfields Eye Hospital, King's College Hospital, and up to 2 further sites ONLY). This experimental method is well established in our group at UCL and enables the measurement of basal respiration, ATP-linked respiration, proton leak, maximal respiration, spare respiratory capacity and non-mitochondrial respiration by the sequential addition of pharmaceutical modulators of mitochondrial oxidative phosphorylation. This approach has been successful in identifying mitochondrial impairment in PBMCs from patients with Alzheimer's disease (Maynard et al 2015). Mitochondrial respiration is considered the single most useful test of mitochondrial function in intact cells (Brand et al 2011).

PBMC samples will be shipped to the UCL Institute of Ophthalmology for storage for research purposes of the main trial. Instructions for sample collection, packing and shipping will be included in the NAMinG Laboratory Sample Management Plan.

21.1.2 Eligibility criteria

The eligibility criteria for participants who consent to participant in the *optional* Biomarker sub-study are identical to those in the inclusion and exclusion criteria of the main NAMinG trial, with the addition that the participant is randomised at one of the hospitals that is taking part in this sub-study.

We intend to recruit at least 200 participants to the Biomarker Sub-Study.

A participant information sheet (separate to the main trial) will be provided, and it will include information on the optional biomarker sub-study (including the amount of blood sample being collected). Blood samples will be collected at Visits 1, 2 and 7. Participants will be given an

opportunity to discuss the biomarker sub-study with the local trial team and to have any questions answered. Participants who do not wish to participate in the biomarker sub-study will not be excluded from the main trial.

A separate informed consent form will also be provided for the biomarker sub-study.

21.1.3 Aims of the sub-study

The aims of this sub-study are to investigate differences in mitochondrial function between the placebo and NAM arms, and any association between mitochondrial function and quantitative phenotypic traits, such as the rate of glaucoma progression. We will also evaluate whether the benefit of NAM treatment is greatest in those with poor baseline mitochondrial function. These aims are identical to the secondary mechanistic objectives that are listed in the main NAMinG trial (see section 1.2.2). The outcomes are also identical to the secondary mechanistic outcomes that are listed in the main NAMinG trial (see section 6.1.2).

21.1.4 UCL Institute of Ophthalmology

Samples will be collected as whole blood in a BD Vacutainer CPT Heparin tube, packaged and sent by courier at ambient temperature from individual participating sites to the Institute of Ophthalmology within the Faculty of Brain Sciences of University College London, Bath St, London, EC1V 9EL. CPT™ is an evacuated, sterile blood collection tube containing buffered sodium citrate or sodium heparin anticoagulant, liquid density medium and inert gel barrier. The PBMC layer (and separate serum sample where possible) will be extracted from the whole blood sample in accordance with the analytical plan agreed by the NAMinG biomarker sub-study investigators (Professor David Garway-Heath and Mr Gerassimos Lascaratos) and their teams. The Institute of Ophthalmology will store blood samples in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act, 2004, and any amendments thereto. UCL will be the custodian of the samples and any accompanying data.

21.1.5 Confidentiality

Blood samples for the biomarker sub-study will be labelled with the unique NAMinG participant identification number ensuring the pseudonymity of the participants who have provided the samples. At UCL Institute of Ophthalmology, brief clinical details will be stored with the sample data. PBMC analysis results will be stored on a web-based, secure confidential database, including after completion of the NAMinG trial. Participants may ask for their information to be removed from this database at any time, in accordance with the GDPR.

Biomarker sub-study samples will be linked to the main trial data held on the secure trial database at UCL CCTU via the unique NAMinG participant identification number to integrate with the main trial data analyses. Participants will be informed of this in the information sheet. Any information collected during the trial will be kept confidential.

21.1.6 Withdrawal

Participants can choose to withdraw from the biomarker sub-study at any time, without it affecting their participation in the main trial. If participants withdraw from the main trial (withdrawal from treatment and/or withdrawal from follow-up), their data and samples will be retained for further use as described in the participant information sheet. If participants request that their samples and

data be withdrawn from the biomarker sub-study, every effort will be made to destroy samples and data that have been provided, but in some cases, this may not be possible, e.g. when further analyses have been carried out by collaborators.

21.2 Appendix 2 Future Genetic and Gene Expression Sub-Study

21.2.1 Introduction

Blood samples will be collected for future DNA/RNA analysis to look for known and new glaucoma genes and, if these are present, whether they are associated with any particular quantitative traits (such as disc size, intraocular pressure, and location or rate of visual field loss). These DNA samples will contribute to, and may be analysed together with, the 1500 samples of the UKGTS, LIGHT and TAGS trials. DNA methylation and RNA analyses will give insight into gene expression.

DNA/RNA bloods will be shipped to the UCL Institute of Ophthalmology for storage for future analysis. Instructions for sample collection, packing and shipping will be included in the NAMinG Laboratory Sample Management Plan.

21.2.2 Eligibility criteria

The eligibility criteria for participants who consent to participant in the *optional* Future Genetic and Gene Expression sub-study are identical to those in the inclusion and exclusion criteria of the main NAMinG trial.

A participant information sheet (separate to the main trial) will be provided, and it will include information on the optional genetic sub-study including the amount of blood sample being collected. Blood samples will be collected at Baseline and Month 27 ONLY. Participants will be given an opportunity to discuss the genetic sub-study with the local trial team and to have any questions answered. Participants who do not wish to participate in the genetic sub-study will not be excluded from the main trial.

A separate informed consent form will also be provided for the genetic sub-study.

21.2.3 Aims of the sub-study

The aim of this sampling is to address questions beyond the immediate scope of the main trial and to identify the genetic and gene expression profiles of those susceptible, or resistant, to glaucoma progression and genetic profiles associated with more favourable clinical outcomes in response to NAM.

21.2.4 UCL Institute of Ophthalmology

Samples will be collected as whole blood in a Tempus Blood RNA tube (Thermo Fisher), packaged and sent by courier in dry ice from individual participating sites to the Institute of Ophthalmology within the Faculty of Brain Sciences of University College London, Bath St, London, EC1V 9EL. The inherited material (DNA and RNA genes) will be extracted from the whole blood sample in accordance with the analytical plan agreed by the NAMinG future genetic and gene expression sub-study investigators and their teams. The Institute of Ophthalmology will store blood samples in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act,

2004, and any amendments thereto. UCL will be the custodian of the samples and any accompanying data.

21.2.5 Sample and data sharing

The DNA/RNA samples at the UCL Institute of Ophthalmology will be stored for ongoing and future projects by the NAMinG genetic sub-study investigators and their teams. A committee including core investigators, scientific, lay and charity representatives will review requests for data and sample access. These samples will be made available to responsible investigators in the UK and around the world for use in future ethically approved research.

21.2.6 Confidentiality

Blood samples for the genetic sub-study will be labelled with the unique NAMinG participant identification number ensuring the pseudonymity of the participants who have provided the samples. At UCL Institute of Ophthalmology, brief clinical details will be stored with the sample data. DNA/RNA analysis results will be stored on a web-based, secure confidential database, including after completion of the NAMinG trial. Participants may ask for their information to be removed from this database at any time, in accordance with the GDPR.

Genetic sub-study samples will be linked to the main trial data held by Professor Garway-Heath's team at the end of the trial via the unique NAMinG participant identification number to integrate with routinely collected clinical data to support further analysis for future research. Participants will be informed of this in the information sheet. Any information collected during the trial will be kept confidential.

Pseudonymised (de-identified) information and DNA/RNA collected during the trial may be transferred both within and outside the European Economic Area as part of ongoing collaboration with other researchers. This may include combining data from participants' samples with those of other patients in order to determine important factors related to glaucoma. This information may be made available to other researchers to enable large-scale analysis and new discoveries. Pseudonymised (de-identified) data will be hosted centrally through a secure web-based database holding research data without personal details. This will meet high security standards and safety measures, including ISO27001 certification, and will enable sharing of data to approved groups. Participants are informed of this in the NAMinG patient information sheet and will consent to these specific aspects. Personal data will be held separately from research data on a separate, secure web-based database meeting the same security standards.

21.2.7 Withdrawal

Participation in the genetic sub-study is voluntary and participants can choose to withdraw at any time. If participants decide not to take part or to withdraw from the genetic sub-study, participation and treatment in the main trial will not be affected. If participants withdraw from the main trial (withdrawal from treatment and/or withdrawal from follow-up), their data and samples will be retained for further use as described in the participant information sheet. If participants request that their samples and data be withdrawn from the genetic sub-study, every effort will be made to destroy samples and data that have been provided, but in some cases, this may not be possible, e.g. when further analyses have been carried out by collaborators.