Official Title: A Phase 1, Open-label, Positron Emission Tomography Study in Healthy Subjects to Determine the Relationship Between Plasma Concentration and Target Occupancy of ASN51 Following Repeated Oral Doses

NCT ID: NCT05725005

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Asceneuron S.A.

Trial Protocol

Confidential

Trial title	A phase 1, open-label, positron emission tomography study in healthy subjects to determine the relationship between plasma concentration and target occupancy of ASN51 following repeated oral doses.
Short title	PET study of repeated ASN51 in healthy volunteers
Version and date of protocol	Version 2, dated 23 January 2023
HMR code	22-012
Sponsor code	ASN51-103
Invicro code	COM221594
IRAS number	1006677
Trial medication	ASN51 (O-linked-β-N-acetylglucosaminidase inhibitor)
Phase of trial	Phase 1
Place of trial	Hammersmith Medicines Research (HMR) Cumberland Avenue London NW10 7EW Tel: +44(0)20 8961 4130 Fax: +44(0)20 8961 8665 Invicro Burlington Danes Building, Imperial College London Hammersmith Hospital, Du Cane Road London W12 0NN
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Sponsor	Asceneuron S.A. EPFL Innovation Park, Bâtiment B 1015 Lausanne, Switzerland, CH-1015 Tel: +41 (0)21 353 8242 Fax: +41 (0)21 693 8247
Planned dates of trial	January 2023 to April 2023

Version control history

Version	Reason for change
2, dated 23 January 2023	Response to GNA
1, dated 07 November 2022	N/A

1 Signatures

The investigator and the sponsor have discussed this protocol. The investigator agrees to perform the investigation and to abide by this protocol and any future agreed amendments, except in case of medical emergency.

Chief investigator

[redacted]
HMR

[redacted] 24-Jan-2023
Signature Date

Principal investigator

[redacted]
Invicro

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Signature Date

Statistician

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Signature Date

Sponsor

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Asceneuron S.A.

[redacted] 23-Jan-2023
Signature Date

2 Summary

2.1 Trial medication

ASN51 is a reversible, substrate-competitive inhibitor of O-linked-*N*-acetylglucosaminidase (O-GlcNAcase). It is being developed as an orally-administered treatment for neurodegenerative diseases such as Alzheimer's Disease (AD), Progressive Supranuclear Palsy (PSP), and other tauopathies.

2.2 Objectives

2.2.1 Primary objectives

- To assess the pharmacodynamic (PD) response in peripheral blood mononuclear cells (PBMCs) following single and repeated oral doses of ASN51 in healthy subjects.
- To assess brain O-GlcNAcase occupancy using [^{18}F]-IMA601 positron emission tomography (PET), following repeated oral doses of ASN51 in healthy subjects.

2.2.2 Secondary objectives

- To assess the safety and tolerability of repeated oral doses of ASN51 in healthy subjects
- To assess the relationship between the plasma concentration of ASN51 and the time-course of brain O-GlcNAcase occupancy using [^{18}F]-IMA601 PET, following repeated oral doses of ASN51 in healthy subjects
- To assess the pharmacokinetics (PK) of repeated doses of ASN51 in healthy subjects
- To assess the trough O-GlcNAcase occupancy of ASN51 after repeated doses in healthy subjects
- To determine the effect of food on the PD response in PBMCs following repeated ASN51 dosing in healthy subjects

2.3 Endpoints

2.3.1 Primary endpoints

PD:

- O-GlcNAcylation of PBMCs
- [^{18}F]-IMA601 regional total volume of distribution (V_T) at each brain scan

2.3.2 Secondary endpoints

Safety and tolerability:

- vital signs (blood pressure, pulse rate, tympanic temperature, and respiratory rate), 12-lead safety electrocardiogram (ECG), physical and neurological examination, laboratory safety tests (haematology, clinical chemistry, coagulation, and urinalysis), Columbia-Suicide Severity Rating Scale (C-SSRS), and adverse events (AEs)

PK:

- plasma concentration of ASN51 at the time of each postdose PET scan
- PK parameters including C_{max}, C_{max}/Dose, t_{max}, $t½$, λ_Z, AUC_{tau}, AUC_{last}, AUC_{inf}, AUC_{inf} /Dose, $\%AUC_{extrap}$, CL_{SS}/F, V_Z/F, C_{trough}, $R_{ac(Cmax)}$, $R_{ac(AUC)}$.

PD:

- O-GlcNAcylation of PBMCs with or without prior feeding
- Trough [^{18}F]-IMA601 V_T

PK/PD:

- relationship between ASN51 plasma concentration, PBMC target engagement, and brain receptor occupancy (RO) over time

2.4 Type of trial

This is a phase 1, open-label, dose escalation, PET study to investigate the brain occupancy of O-GlcNAcase, and the PD response in PBMCs, after repeated doses of ASN51 in healthy subjects.

2.5 Trial population

a **Total** Up to 12 healthy volunteers, excluding replacements

b **Age** 25–55 years

c **Main inclusion criteria**

Normotensive male volunteer (PET subjects), or male or female volunteer of non-childbearing potential (PBMC-only subjects), deemed healthy on the basis of a clinical history, physical and neurological examination, ECG, vital signs, and laboratory tests of blood and urine; agree to follow the contraception requirements of the trial; able to give fully informed written consent.

d **Main exclusion criteria**

Significant (> 10%) recent weight change; positive tests for hepatitis B & C, HIV; severe adverse reaction to any drug; sensitivity to trial medication; drug or alcohol

abuse; regular consumption of xanthine-containing products; frequent use of nicotine-containing products; severe adverse reaction to any drug; sensitivity to trial medication (all subjects) or PET imaging radioligand (PET subjects); use of over-the-counter medication (with the exception of paracetamol [acetaminophen]) during the 7 days before the first dose of radioligand (PET subjects) or trial medication (PBMC subjects) (or longer if the medicine is a potential enzyme inducer), or prescribed medication during the 28 days before first dose of radioligand (PET subjects) or trial medication (PBMC subjects); received vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) within 2 weeks of screening; participation in other clinical trials of unlicensed medicines, or loss of more than 400 mL blood, within the 3 months before the first dose of tracer (PET subjects) or trial medication (PBMC subjects); clinically relevant abnormal findings at the screening assessment, including ECG abnormalities (all subjects) or those identified by MRI scan (PET subjects only); acute or chronic illness; clinically relevant abnormal history of or concurrent medical (including neurological or psychiatric) condition; positive C-SSRS result; vegan; possibility that volunteer will not cooperate; unsatisfactory venous access; objection by General Practitioner (GP);

PET subjects only: significant exposure to research related radiation (more than 10 mSv) within the previous 12 months; contraindications to arterial cannulation (eg Allen's test indicates risk) or MRI scanning (eg presence of a cardiac pacemaker or other implanted electronic device or a history of claustrophobia).

2.6 Trial design and methods

Enrolment of up to 12 healthy subjects is planned, in up to 2 groups (Groups 1 and 2). Each group will consist of up to 6 subjects.

All subjects will receive once-daily (QD) doses of ASN51, by mouth, for 14 days: Group 1 will receive [redacted] ASN51 QD and Group 2 will receive [redacted] ASN51 QD. Group 2 will proceed only if the safety and tolerability of the previous dose level are acceptable, and the plasma concentrations of ASN51 are predicted to remain below the toxicokinetic limit, as determined by the Safety Review Group (SRG).

On Day 11, to evaluate the effect of food on PBMC response, subjects will receive ASN51 in the fed state following a United States Food and Drug Administration (FDA) high-fat breakfast. Subject fasting requirements are further detailed in section 11.

In each group, 2 subjects will have a PBMC-only study design (section 8.1.2) and 4 will have a PBMC and PET scanning study design (section 8.1.3).

Schematic diagrams of each study design are in section 8.2.

PBMC-only subjects

2 subjects in each of Groups 1 and 2 will not have PET scans during the study. They will:

- be screened within 28 days before their first dose of trial medication.
- be resident on ward from 1 day before their first dose (Day –1) until 6 days after their final dose (Day 20).
- return for a follow-up visit 9–11 days after their final dose of trial medication (Days 23–25).

PET subjects

4 subjects in each of Groups 1 and 2 will have PET scans during the study. PET subjects will:

- be screened within 21 days before imaging session 1 (see below).
- be resident on ward from 1 day before their first dose (Day –1) until 6 days after their final dose (Day 20); and have imaging sessions as described below.
- return for a follow-up visit 9–11 days after their final dose of trial medication (Day 23 ± 2 days), or 2–4 days after imaging session 3 (see below), whichever is later.

PET subjects will have up to 3 imaging sessions, as follows.

- *Imaging session 1*. Subjects will have a baseline PET scan between Day –7 and Day –3. Subjects will be admitted to the ward the day before their baseline PET scan, and will be discharged after their scan.
- *Imaging session 2*. Subjects will have an on-treatment PET scan at about 5 h postdose on Day 1.
- *Imaging session 3*. Subjects will have an on-treatment PET scan at 3–9 days after their final dose of ASN51:
  - 3 subjects per group on Day 17
  - 1 subject on Day 22 (± 1 day) (Group 1 only)
  - 1 subject on Day 23 (± 1 day) (Group 2 only)

The timing of on-treatment PET scans (imaging sessions 2 and 3) may be altered based on emerging data or study logistical requirements.

Subjects will receive an intravenous dose of the radiolabelled tracer, [^{18}F]-IMA601, at the start of each PET scan.

Arterial blood sampling will be done during each PET scan to quantify the parent tracer-related radioactivity over the course of the PET scan, and to establish a tracer metabolite-corrected plasma input function. The total arterial blood volume required for each tracer injection will not exceed 120 mL. Arterial cannulation and arterial

blood sampling may be reduced or removed if analysis of PET data from previous subjects indicates that non-invasive analysis of the PET scan data can be done. If a non-invasive analysis is not possible, the use of an arterial cannula with each PET scan will continue through the study.

In the case of a technical failure (such as unsuccessful tracer synthesis), subjects may be asked to attend an additional on-treatment imaging session as described in section 12.2. However, no subject will have more than 3 PET scans and 3 doses of [^{18}F]-IMA601 during the study.

2.7 Assessments

The following assessments will be made.

2.7.1 Pharmacodynamics

Blood samples for PBMC assays will be taken before and frequently during dosing (up to 144 h [6 days] after the last dose). Trough samples will be taken before study drug administration.

Occupancy of O-GlcNAcase by ASN51 will be assessed by PET imaging using the radioligand [^{18}F]-IMA601 (PET subjects only).

Blood samples will be taken during each PET scan for arterial input function and radio metabolite analysis (PET subjects only).

2.7.2 Safety and tolerability

Laboratory assessments (routine haematology, clinical chemistry, coagulation, and urinalysis), physical and neurological examinations, 12-lead ECGs, and vital signs (blood pressure, pulse rate, respiratory rate, and tympanic temperature) and C-SSRS will be done frequently until the subject's last visit. AEs will be recorded from screening until the subject's last visit.

Laboratory safety variables to be assessed during the study are in Table 4.

From screening until the follow-up visit, AEs and concomitant medication will be documented as they are reported by the subjects. Subjects will be questioned about AEs on admission to the ward, when procedures are done, and when they return to the ward for outpatient visits/at follow-up.

2.7.3 Pharmacokinetics

Blood samples for assay of ASN51 will be taken before and frequently during dosing (up to 144 h [6 days] after the last dose), and at follow-up. Trough samples will be taken before study drug administration.

PET subjects only: for subjects having imaging session 3 on Day 22 or 23, additional PK samples will be taken approximately 2 h before and after the PET scan.

2.7.4 Other

Structural MRI scans will be done during the screening period (PET subjects only).

3 Contents

3.1 Table of Figures

3.2 Table of Tables

4 List of abbreviations

AChE	acetylcholinesterase
AD	Alzheimer's disease
AE	adverse event
ALT	alanine aminotransferase
ANOVA	analysis of variance
ARSAC	Administration of Radioactive Substances Advisory Committee
AST	aspartate aminotransferase
$\%AUC_{extrap}$	percentage of AUC that was extrapolated
AUC	area under the concentration–time curve
AUC_{24}	AUC during the first 24 h after dosing
AUC_{inf}	AUC extrapolated to infinite time
AUC_{last}	AUC to last measurable concentration
AUC_{tau}	AUC during the dosing interval
BLQ	below the limit of quantification
BMI	Body Mass Index
BSA-CF	body surface area conversion factor
CI	confidence interval
C_{last}	last measurable plasma concentration
CL_{ss}/F	clearance/fraction of dose absorbed at steady state
C_{max}	maximum plasma concentration
C_{min}	minimum measured concentration
CNS	central nervous system
COVID-19	Coronavirus disease 2019
CRF	case report form
C-SSRS	Columbia-Suicide Severity Rating Scale
CT	computed tomography
CTA	clinical trial authorisation
CYP	cytochrome P450
CV	coefficient of variation
D	decreased by more than predetermined amount
DRF	dose-range finding (studies)
DDS	Drug Development Solutions
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
FDA	United States Food and Drug Administration
FIH	first-in-human
FSH	follicle-stimulating hormone

g	gram(s)
g	G force
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GP	General Practitioner
h	hour(s)
Hb	haemoglobin
hERG	human Ether-à-go-go-Related Gene
HIV	human immunodeficiency virus
HMR	Hammersmith Medicines Research
IB	investigator's brochure
ICF	information and consent form
ICH	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICRP	International Committee for Radiation Protection
IMP	investigational medicinal product
INR	international normalised ratio
IUD	intrauterine device
IV	intravenous
kg	kilogram(s)
λ_z	terminal rate constant
LIMS	laboratory information management system
LSM	least square means
MBq	megabecquerel
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
MHRA	Medicines and Healthcare Products Regulatory Agency
MIA(IMP)	manufacturing authorisation for investigational medicinal products
min	minute(s)
mm Hg	millimetres of mercury
MRI	magnetic resonance imaging
mSv	millisievert
MTD	maximum tolerated dose
NFT	neurofibrillary tangle

NMDA	N-methyl-D-aspartate
NOAEL	no observed adverse effect level
O-GlcNAc	O-linked-*N*-acetylglucosamine
O-GlcNAcase	O-linked-*N*-acetylglucosaminidase or OGA
PBMC	peripheral blood mononuclear cell
PD	pharmacodynamic(s)
PET	positron emission tomography
PK	pharmacokinetic(s)
PSP	Progressive Supranuclear Palsy
QA	quality assurance
QD	once-daily
QTc	QT interval corrected for pulse rate
QTcF	QT interval corrected according to Fridericia's formula
RBC	red blood cells
REC	research ethics committee
RES	Research Ethics Service
RO	receptor occupancy
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome-related coronavirus 2
SD	standard deviation
SOP(s)	standard operating procedure(s)
SRG	Safety Review Group
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{½}$	terminal elimination half-life
TEAE	treatment-emergent adverse event
t_{last}	time of last measurable concentration
t_{max}	time of maximum plasma concentration
TOPS	The Overvolunteering Prevention System
UK	United Kingdom
ULN	upper limit of normal
US	United States of America
WBC	white blood cells
WHO	World Health Organization
V_T	volume of distribution

5 Trial personnel

Chief investigator	[redacted] MBBS JCPTGP MPhil HMR Tel: [redacted] Email: [redacted]
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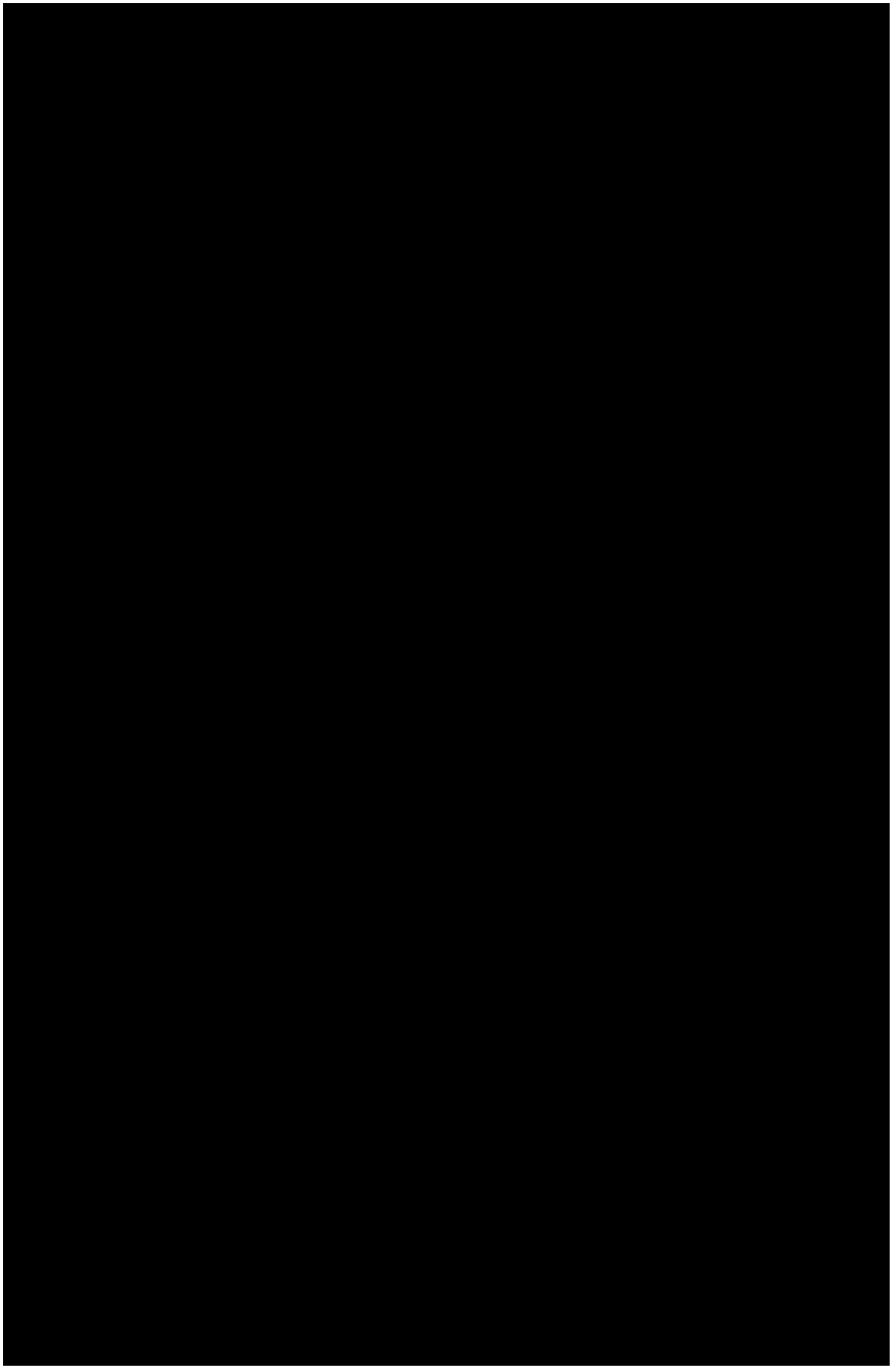
Statistician	[redacted] BSc MSc [redacted] BSc MSc HMR
Sponsor's representative	[redacted] MD Asceneuron S.A Tel: [redacted] Email: [redacted]
Sponsor's primary medical officer (European Union)	[redacted] MD MSc PhD Asceneuron S.A. Tel: [redacted] Email: [redacted]
Sponsor's secondary medical officer (United States)	[redacted] MD Asceneuron S.A. Tel: [redacted] Email: [redacted]
Monitor	Niche Science & Technology Ltd Unit 26, Falstaff House Bardolph Road Richmond-upon-Thames London, TW9 2LH Tel: [redacted] Email: [redacted]
Laboratory safety tests	The HMR Analytical Laboratory HMR
Assays of IMP concentration	DDS Newmarket Road, Fordham Cambridgeshire CB7 5WW Tel: [redacted] Email: [redacted]

Derivation of pharmacokinetic (PK) parameters	[redacted] BSc HMR
Pharmacovigilance	[redacted] MD MSc PhD Asceneuron S.A. EPFL Innovation Park, Bâtiment B 1015 Lausanne Switzerland, CH-1015 Tel: [redacted] Email: [redacted] **SAE reporting:** Email: [redacted] Mobile: [redacted]

6 Introduction

6.1 Background



6.2 Review of investigational medicinal product

Asceneuron SA is developing ASN51, a new chemical entity [redacted]

6.2.1 *Non-clinical studies*

Several non-clinical studies of ASN51 have been conducted to date, as described below.

Non-clinical pharmacology

[redacted]

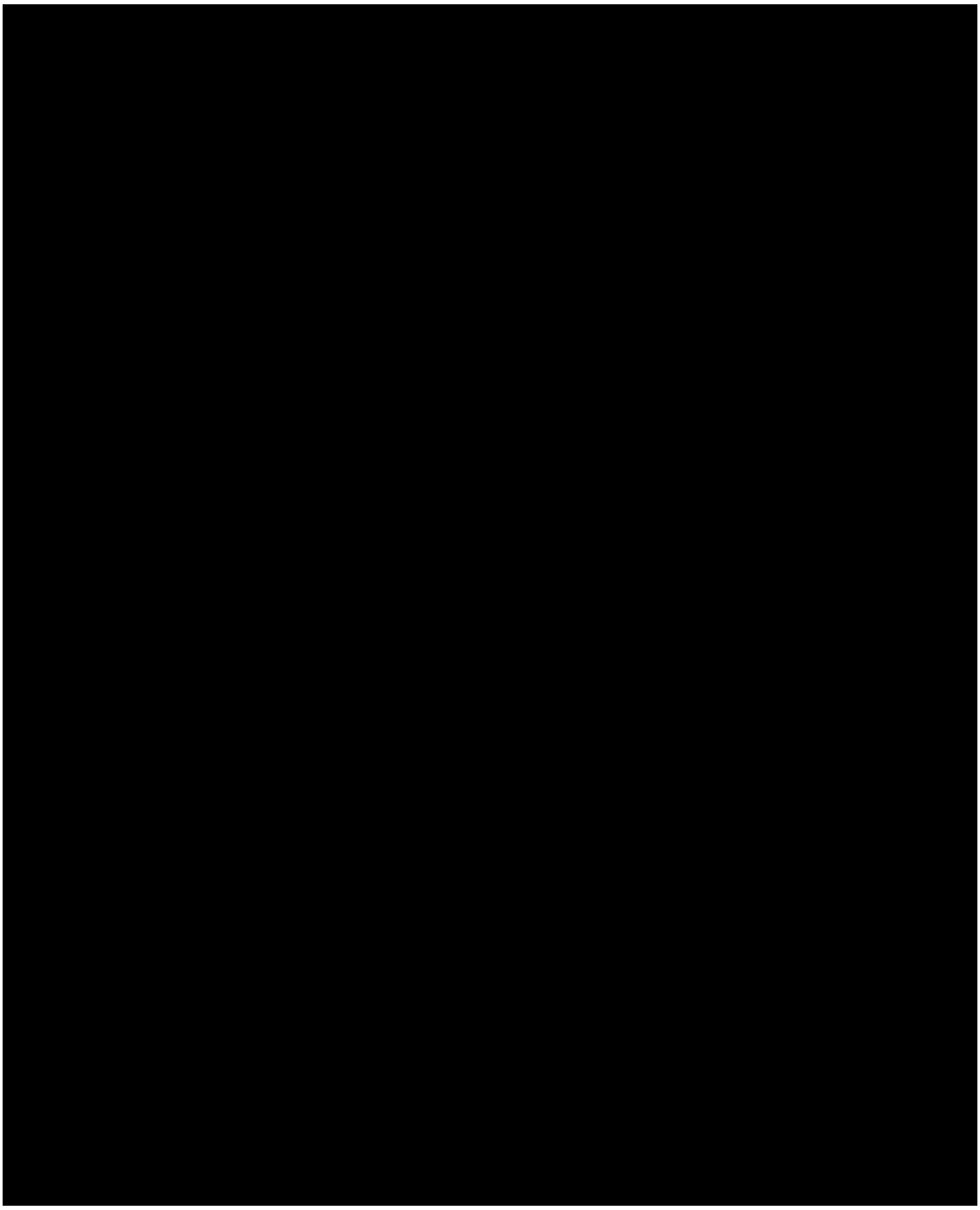
Further information about non-clinical pharmacology studies of ASN51 is available in the IB[9].

Pharmacokinetics

PK studies of ASN51 were done in rats, dogs, and monkeys. Additional information is available from *in vitro* studies on plasma protein binding and metabolism in liver microsomes and hepatocytes of various species, cytochrome P450 (CYP)-profiling (inhibition and induction) and from toxicokinetic studies in rats and dogs.

Further information about the non-clinical PK of ASN51 is available in the IB[9].

Toxicology



Further information about non-clinical studies of ASN51 is available in the IB[9].

6.2.2 Clinical studies

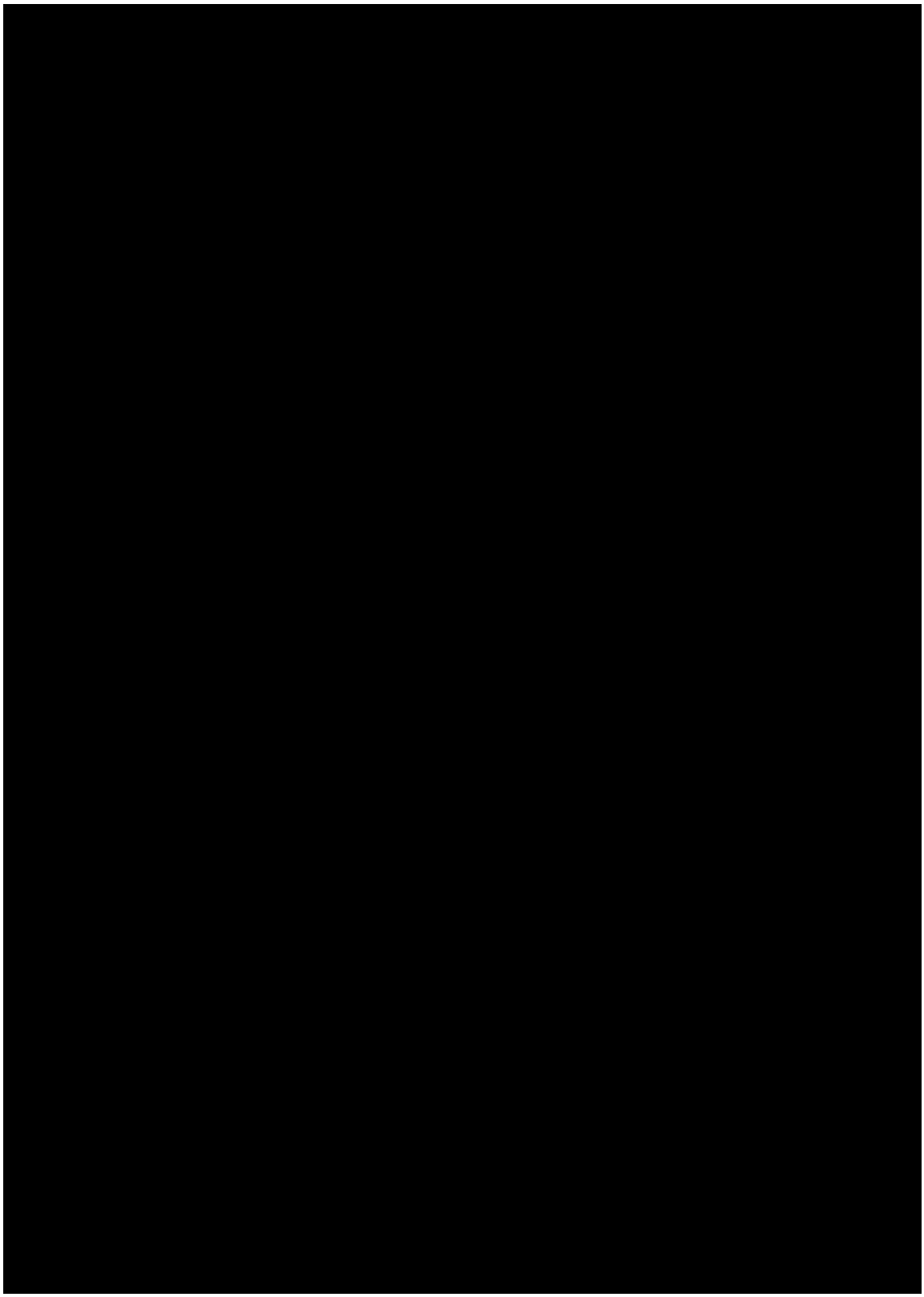
To date, ASN51 has been assessed 2 clinical studies in healthy subjects: a first-in-human (FIH) single- and multiple-ascending dose study (ASN51-101), and an adaptive-design positron emission tomography (PET) study (ASN51-102).

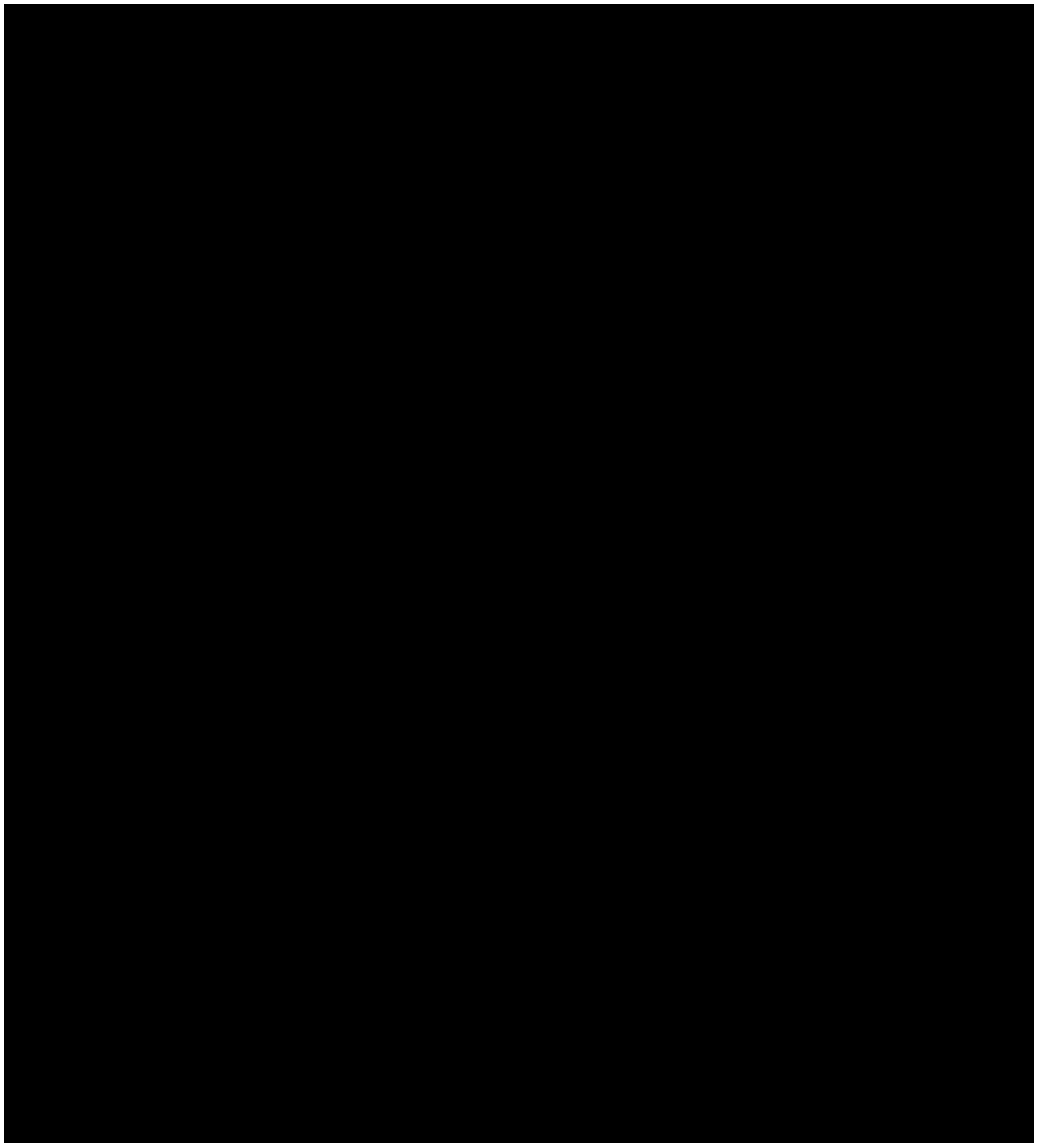
There were no deaths or serious adverse events (SAEs) in either study. Overall, ASN51 was safe and well tolerated. All treatment-emergent adverse events (TEAEs) were mild or moderate in severity. All AEs were reversible. There were no

clinically significant laboratory, vital sign, electrocardiogram (ECG), cardiac telemetry, or physical or neurological examination findings in either study, and no positive Columbia-Suicide Severity Rating Scale (C-SSRS) results.

Further information about clinical studies of ASN51 is available in the IB[9].

6.2.2.1 Study ASN51-101





Further information is available in the IB[9].

6.2.2.2 Study ASN51-102

Further information is available in the IB[9].

6.3 Review of non-investigational medicinal product

Further details are included in the [^{18}F]-IMA601 non-IMP dossier (non-IMPD)[28].

6.4 Rationale for the trial

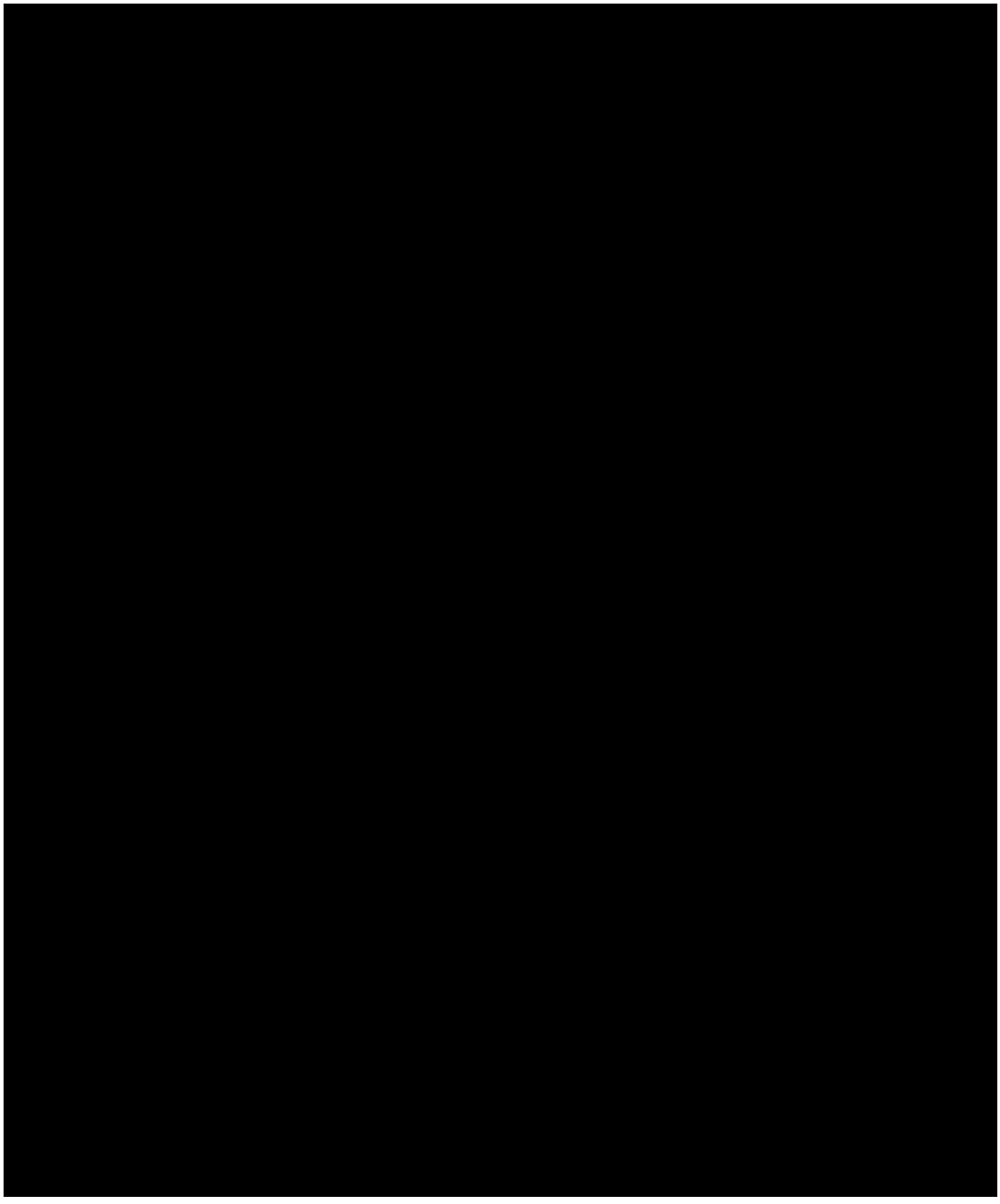
[redacted]

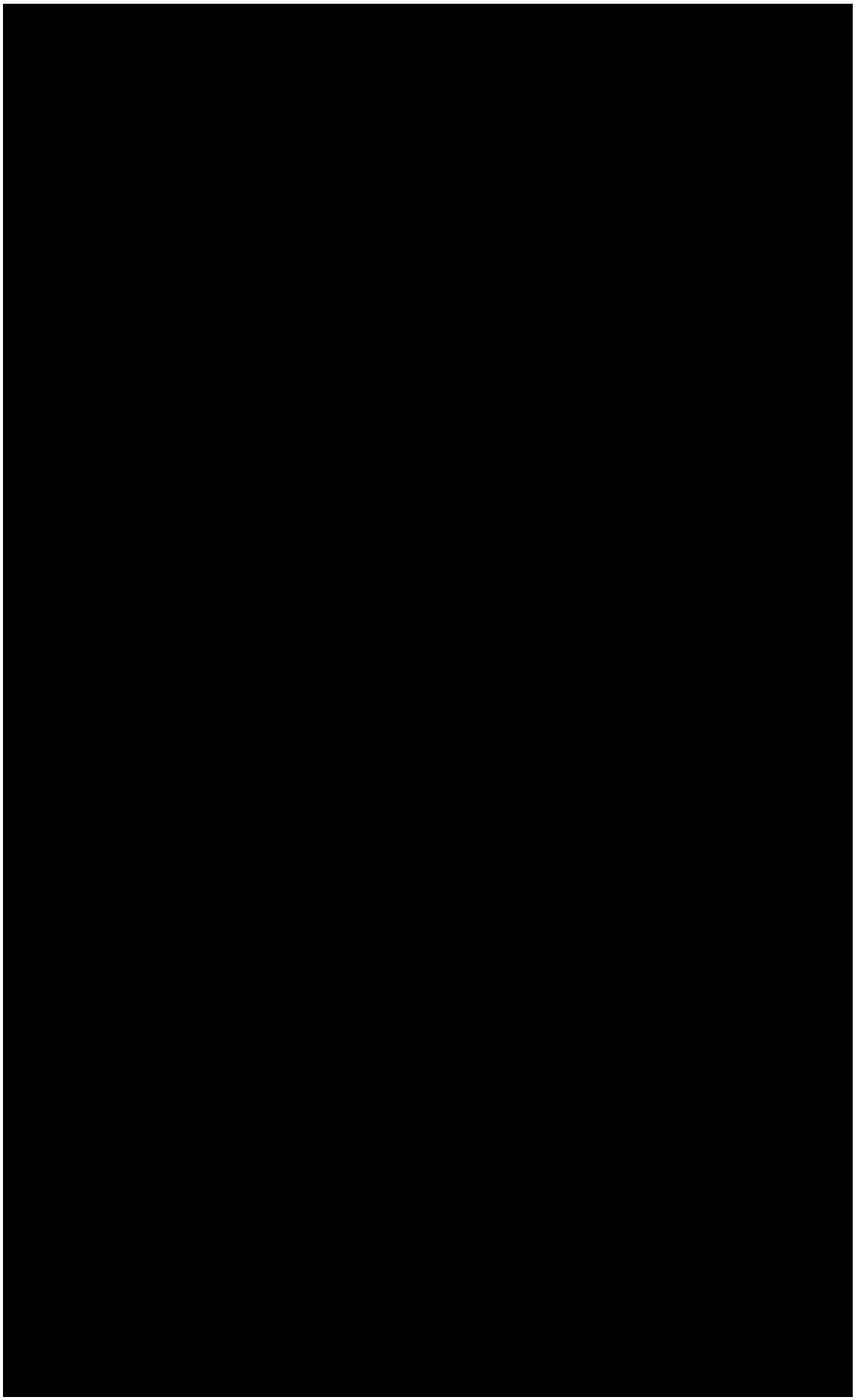
The results of this study will be used to select doses for subsequent studies in patients.

6.5 Participant input into trial design

This is a phase 1, exploratory study in healthy volunteers, with no anticipated therapeutic benefit to the participants; involvement of patients, service users or members of the public in the design of the trial is not appropriate.

6.6 Rationale for choice of doses





6.7 Assessment and management of risk

Risk associated with ASN51 (all subjects)

Other safety considerations (all subjects)

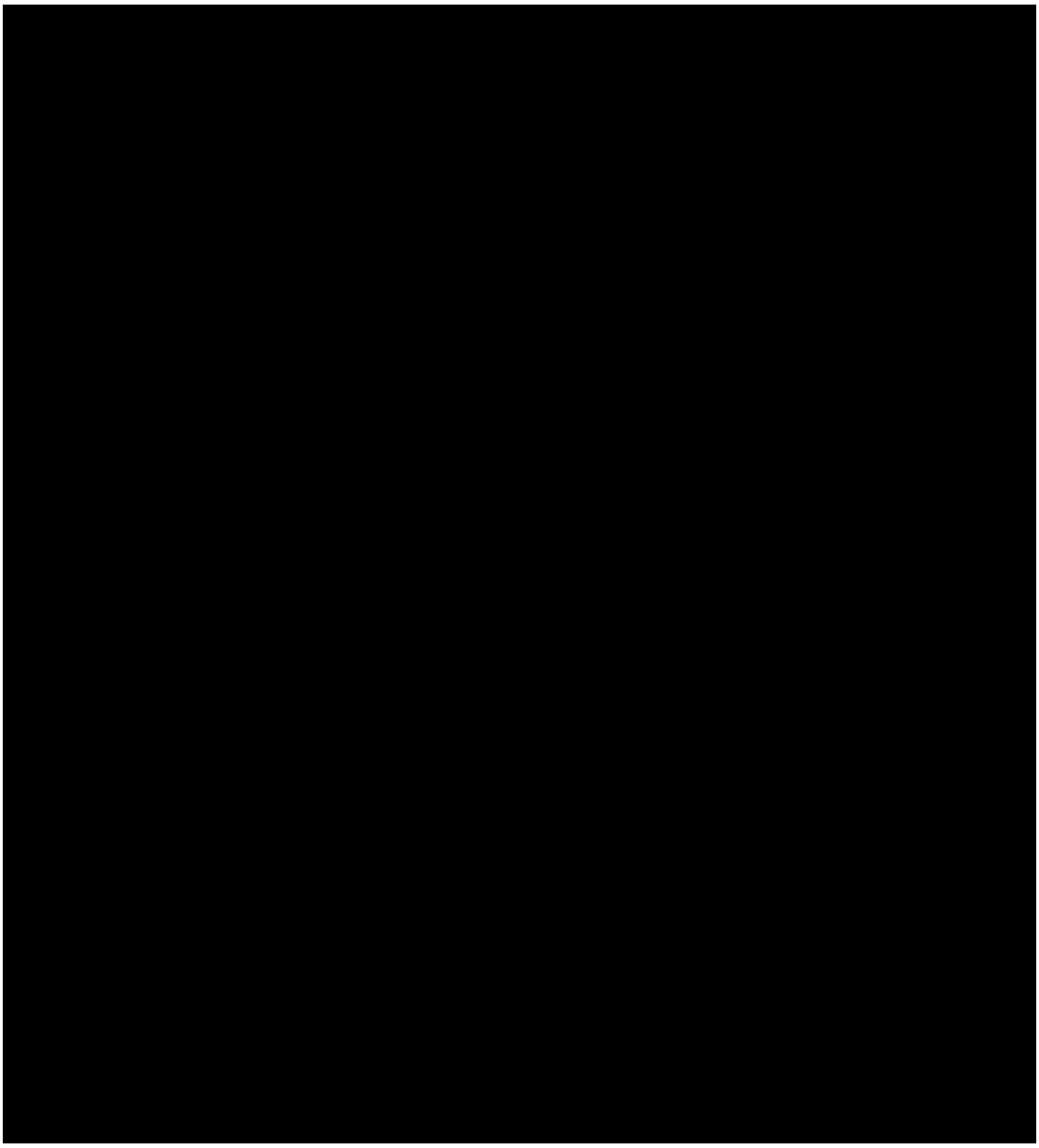
Any other safety risk to study participants is mitigated by the following considerations.

- Subjects will be monitored frequently throughout the study for safety and tolerability, including vital signs, 12-lead safety ECG, physical and neurological examination, laboratory safety tests, and C-SSRS. The safety monitoring practices employed by this protocol are adequate to protect the subjects' safety and should detect all expected TEAEs.
- HMR maintains its own resuscitation team (SS329) to deal with medical emergencies, including anaphylaxis and cardiac arrest. The team will be on duty continuously, until at least 24 h after each dose.
- Any risks are adequately mitigated by safety assessments, and by the medical cover provided by the investigator site, and by appropriate investigational medicinal product (IMP) manufacture, according to Good Manufacturing Practice (GMP).
- Subjects will stay on the ward from the day before first study drug administration until 6 days (ie 3 half-lives) after the last study drug administration.

There will be no direct health benefit for study participants from receipt of study medication. An indirect health benefit to the healthy subjects enrolled in this trial is the free medical examination received at screening and during the study.

Overall, the risk benefit balance of the present study is considered to be acceptable.

Risk of testicular toxicity (all subjects)



Risk associated with the imaging techniques (PET subjects only)

In this study, each subject will be exposed to radiation from PET and computed tomography (CT) scans (for attenuation correction). To mitigate this risk, the study will exclude subjects who have had previous exposure to ionising radiation such that, their exposure would be > 10mSv for the previous year (see exclusion criterion 35).

The maximal radiation exposure for each subject over the whole study is presented in Table 2.

Table 2: Expected maximal radioactive exposure of each subject

Assessment	Number of scans	Activity from PET tracer (MBq)	Effective dose per scan (mSv)	Total exposure (mSv)
PET emission scan	3	100	2.05	6.15
Low-dose CT scan	3	–	0.28	0.84
Total				**6.99**

A total exposure of 6.99 mSv falls into category IIb (upper limit 10 mSv) of the guidance published by the International Committee for Radiation Protection (ICRP). There is no dose limit for research purposes in the UK. However, as in many member states in the EU, the UK follows the ICRP 62 guidance that provides a guideline of 10 mSv (or less) per study where research subjects are not expected to benefit personally[12].

Magnetic resonance imaging (MRI) to delineate brain anatomy will be done in this study to aid PET image analysis. There are no known risks to subjects associated with MRI scanning, provided that they have no contraindications to MRI as listed in the exclusion criteria (section 9.3, exclusion criterion 36). Potential risks associated with metallic implants will be mitigated by administration of a questionnaire and careful screening.

Risk associated with arterial cannulation (PET subjects only)

Before the PET-CT scan, subjects will have a cannula inserted into the radial artery by qualified staff using aseptic technique under local anaesthesia. That insertion may be uncomfortable, cause minor local bleeding and bruising, and can rarely lead to complications (eg, a blood clot could form around the cannula). Most people have no after-effects of cannulation. However, very occasionally, it may cause a small scar. Very rarely, more serious complications can occur, although these usually occur when the radial artery is cannulated in sick people for therapeutic purposes, rather than in healthy subjects for research purposes.

Assessment of the patency of collateral arterial circulation will be assessed at screening and at each imaging session using Allen's test, to minimise the risk of complications.

Use of healthy young male volunteers (PET subjects only)

Despite the potential risk of testicular toxicity, radiation exposure, and repeated arterial blood sampling described above, healthy young volunteers are the most suitable population for the present study, because they:

- have no confounding pathology (eg vascular disease), or concomitant medicine use (eg aspirin), that would contraindicate arterial cannulation, and are better able to tolerate the procedure.
- are better suited to tolerate repeated blood draws for PK and PBMC analyses (to a maximum total of 537 mL; see section 12.5).
- are better able to tolerate repeated PET scans.

The intended blood volume to be taken from PET subjects in this study (537 mL, plus a maximum intended overage of 30 mL unless medically indicated that further samples are needed for safety reasons) is slightly higher than that of a standard blood donation visit. However, the total volume of blood will be taken over approximately 4 weeks. To ensure the safety of the subjects, volunteers will be screened to confirm that they have no haematological abnormalities, and will be excluded if they lose or donate more than 400 mL blood during the 3 months before the trial. Male volunteers are at lower risk of anaemia, and must agree not to donate blood or blood products for up to 3 months after their final visit. Volunteers will be closely monitored and if the investigator (or delegate) has concerns about the results of regular safety blood tests, no further samples will be taken until it is considered clinically safe to do so.

6.8 Conducting the trial during the COVID-19 pandemic

The investigator and sponsor have reviewed the risks of conducting the trial during the current Coronavirus disease 2019 (COVID-19) pandemic. Our priority is the safety of trial subjects and staff, but we also have an ethical duty to preserve the scientific integrity of the trial as far as possible.

ASN51 is unlikely to increase the risk of contracting COVID-19 infection, or to worsen the severity of an infection. ASN51 has no immunosuppressive effect, and is not associated with cough, pyrexia, or anosmia, which would require isolation of subjects.

ASN51 is unlikely to affect the efficacy of a vaccine against severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2). However, to delineate AEs caused by ASN51 or a vaccine, vaccines will be prohibited from 7 days before screening until the follow-up visit. That's ethically acceptable owing to the short duration of participation for each subject.

This clinical trial will be done in accordance with HMR's COVID-19 risk mitigation policy (RMP), which documents HMR's COVID-19 virus testing strategy for volunteers and staff, social distancing measures, and management of COVID-19-like symptoms. HMR's RMP was first notified to the MHRA and HRA on 22 May 2020 and applies across all HMR's trials. The mitigation measures specified in the HMR COVID-19 RMP are deemed adequate for this trial. Any deviations from the RMP will be documented in a separate COVID-19 trial-specific risk assessment, prepared

by the investigator. Any deviations from the protocol that result from the COVID-19 pandemic, and COVID-19 related AEs or SAEs, will be documented.

Taking the above factors into account, and the proposed mitigation, we believe that it is medically and ethically acceptable to proceed with the trial during the current COVID-19 pandemic.

7 Objectives and endpoints

7.1 Objectives

7.1.1 Primary objectives:

- To assess the pharmacodynamic (PD) response in peripheral blood mononuclear cells (PBMCs) following single and repeated oral doses of ASN51 in healthy subjects
- To assess brain O-GlcNAcase occupancy using [^{18}F]-IMA601 positron emission tomography (PET), following repeated oral doses of ASN51 in healthy subjects

7.1.2 *Secondary objectives:*

- To assess the safety and tolerability of repeated oral doses of ASN51 in healthy subjects
- To assess the relationship between the plasma concentration of ASN51 and the time-course of brain O-GlcNAcase occupancy using [^{18}F]-IMA601 PET, following repeated oral doses of ASN51 in healthy subjects
- To assess the PK of repeated doses of ASN51 in healthy subjects
- To assess the trough O-GlcNAcase occupancy of ASN51 after repeated doses in healthy subjects
- To determine the effect of food on the PD response in PBMCs following repeated ASN51 dosing in healthy subjects

7.2 Endpoints

7.2.1 Primary endpoints:

PD:

- O-GlcNAcylation of PBMCs
- [^{18}F]-IMA601 regional total volume of distribution (V_T) at each brain scan

7.2.2 Secondary endpoints:

Safety and tolerability:

- vital signs (blood pressure, pulse rate, tympanic temperature, and respiratory rate), 12-lead safety ECG, physical and neurological examination, laboratory safety tests (haematology, clinical chemistry, coagulation, and urinalysis), C-SSRS, and adverse events (AEs)

PK:

- plasma concentration of ASN51 at the time of each postdose PET scan
- PK parameters including C_{max}, C_{max}/Dose, t_{max}, $t½$, λ_Z, AUC_{tau}, AUC_{last}, AUC_{inf}, AUC_{inf} /Dose, $\%AUC_{extrap}$, CL_{SS}/F, V_Z/F, C_{trough}, $R_{ac(Cmax)}$, $R_{ac(AUC)}$

PD:

- O-GlcNAcylation of PBMCs with or without prior feeding
- Trough [^{18}F]-IMA601 V_T

PK/PD:

- relationship between ASN51 plasma concentration, PBMC target engagement, and brain RO over time

8 Overall trial design

8.1 Trial design

8.1.1 Trial design

This is a phase 1, open-label, dose escalation, PET study to investigate the brain occupancy of O-GlcNAcase, and the PD response in PBMCs, after repeated doses of ASN51 in healthy subjects.

Enrolment of up to 12 healthy subjects is planned, in up to 2 groups (Groups 1 and 2). Each group will consist of up to 6 subjects.

All subjects will receive once-daily (QD) doses of ASN51, by mouth, for 14 days: Group 1 will receive [redacted] ASN51 QD and Group 2 will receive [redacted] ASN51 QD. Group 2 will proceed only if the safety and tolerability of the previous dose level are acceptable, and the plasma concentrations of ASN51 are predicted to remain below the toxicokinetic limit, as determined by the Safety Review Group (SRG; see section 8.5).

On Day 11, to evaluate the effect of food on PBMC response, subjects will receive ASN51 in the fed state following a United States Food and Drug Administration (FDA) high-fat breakfast[29]. Subject fasting requirements are further detailed in section 11.

In each group, 2 subjects will have a PBMC-only study design (section 8.1.2) and 4 will have a PBMC and PET scanning study design (section 8.1.3).

Schematic diagrams of each study design are in section 8.2.

8.1.2 PBMC-only subjects

2 subjects in each of Groups 1 and 2 will not have PET scans during the study. They will:

- be screened within 28 days before their first dose of trial medication.
- be resident on ward from 1 day before their first dose (Day –1) until 6 days after their final dose (Day 20).
- return for a follow-up visit 9–11 days after their final dose of trial medication (Days 23–25).

8.1.3 PET subjects

4 subjects in each of Groups 1 and 2 will have PET scans during the study. PET subjects will:

- be screened within 21 days before imaging session 1 (see below).
- be resident on ward from 1 day before their first dose (Day –1) until 6 days after their final dose (Day 20); and have imaging sessions as described below.
- return for a follow-up visit 9–11 days after their final dose of trial medication (Day 23 ± 2 days), or 2–4 days after imaging session 3 (see below), whichever is later.

PET subjects will have up to 3 imaging sessions, as follows.

- *Imaging session 1*. Subjects will have a baseline PET scan between Day –7 and Day –3. Subjects will be admitted to the ward the day before their baseline PET scan, and be discharged the following day after their scan.
- *Imaging session 2*. Subjects will have an on-treatment PET scan at about 5 h postdose on Day 1.
- *Imaging session 3*. Subjects will have an on-treatment PET scan at 3–9 days after their final dose of ASN51:
  - 3 subjects per group on Day 17
  - 1 subject on Day 22 (± 1 day) (Group 1 only)
  - 1 subject on Day 23 (± 1 day) (Group 2 only)

The timing of on-treatment PET scans (imaging sessions 2 and 3) may be altered based on emerging data or study logistical requirements.

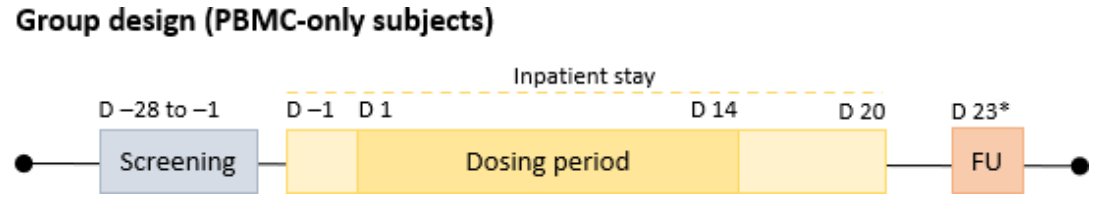
Subjects will receive an intravenous (IV) dose of the radiolabelled tracer, [^{18}F]-IMA601, at the start of each PET scan.

Arterial blood sampling will be done during each PET scan to quantify the parent tracer-related radioactivity over the course of the PET scan, and to establish a tracer metabolite-corrected plasma input function. The total arterial blood volume required for each tracer injection will not exceed 120 mL. Arterial cannulation and arterial blood sampling may be reduced or removed if analysis of PET data from previous subjects indicates that non-invasive analysis of the PET scan data can be done. If a non-invasive analysis is not possible, the use of an arterial cannula with each PET scan will continue through the study.

In the case of a technical failure (such as unsuccessful tracer synthesis), subjects may be asked to attend an additional on-treatment imaging session as described in section 12.2. However, no subject will have more than 3 PET scans and 3 doses of [^{18}F]-IMA601 during the study.

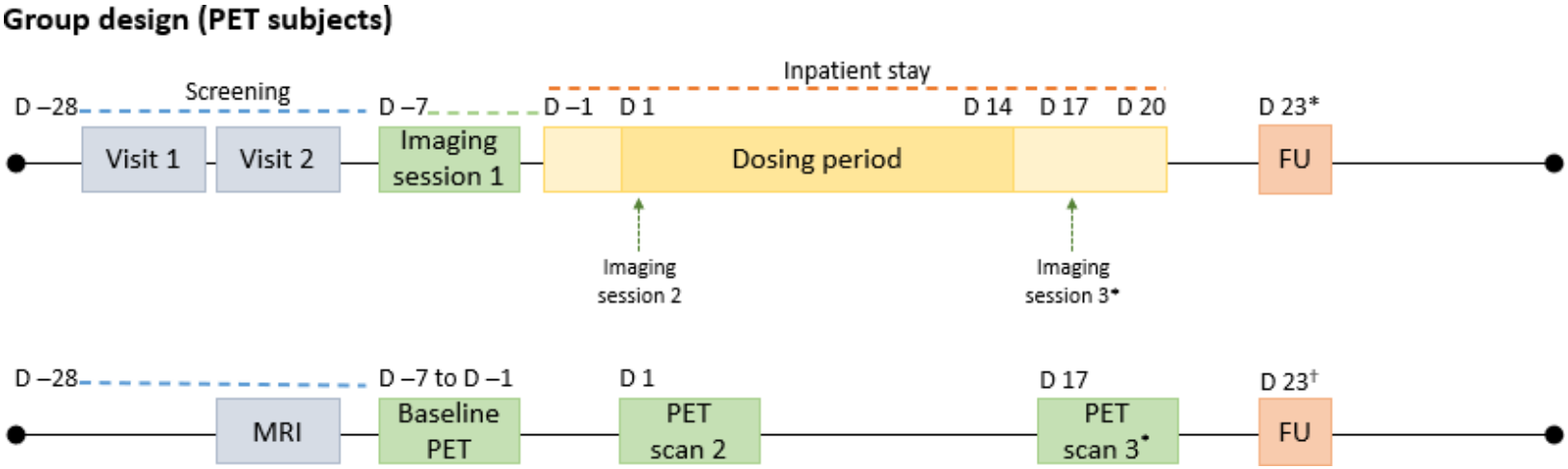
8.2 Study flow chart

Figure 1 Schematic of the study design (PBMC-only subjects)



** The follow-up visit will be 9–11 days after a subject's last dose (Days 23–25).*
Subject fasting requirements are detailed in section 11

Figure 2 Schematic of the study design (PET subjects)



** 1 subject in Group 1 will have imaging session 3 on Day 22, and 1 subject in Group 2 will have imaging session 3 on Day 23.*
† The follow-up visit will be 9–11 days after a subject's last dose (Days 23–25) or 2–4 days after imaging session 3, whichever is later.
Subject fasting requirements are detailed in section 11

8.3 Definition of the end of the trial

The end of the trial is defined as the final follow-up visit by the last subject. If the trial is terminated prematurely, the trial ends when the sponsor notifies the investigator in writing that the trial has finished, or when the last subject attends the final follow-up visit, whichever is later.

8.4 Stopping criteria

8.4.1 Trial stopping criteria

The trial will be stopped if either of the following occurs:

- 1 or more SAEs considered to be at least possibly related to study treatment; or
- 2 or more severe or clinically significant AEs considered to be at least possibly related to study treatment at any dose level.

If, after an internal safety review, it is appropriate to restart the trial, a substantial amendment will be submitted to the MHRA and research ethics committee (REC). The trial will not restart until the amendment has been approved by the MHRA and REC.

8.5 Criteria for proceeding to Group 2

8.5.1 Review of data by the Safety Review Group

Before proceeding to Group 2, the SRG will review the safety, tolerability and PK data from Group 1. The SRG will include (as a minimum) the principal investigator (or medically qualified delegate) and the sponsor's medically qualified representatives (Primary Medical Officer and the Head of Translational Medicine [or delegate]). Each dose decision will be made and documented in line with HMR SOPs. All data used to support dose selection will be quality checked.

The SRG will review safety, tolerability, and PK data up to 6 days after the final dose (Day 20) from all subjects dosed in Group 1. In the event of a subject withdrawal prior to Day 20, all available safety, tolerability, and PK data from that subject will be included in the SRG review. The review will include (as a minimum) data from at least 4 evaluable subjects. An evaluable subject is one who completes dosing and has undergone procedures until 6 days after final dosing (Day 20), and had no major protocol deviations.

The data reviewed will include, as a minimum:

- AEs and SAEs, including description, frequency, intensity, onset, duration, and relationship to treatment
- vital signs

- 12-lead safety ECG
- laboratory safety tests
- PK parameters

Group 2 will proceed only if the following are true.

- The safety and tolerability of [redacted] ASN51 QD are deemed acceptable
- In any individual subject, C_{max} and AUC_{24} after [redacted] ASN51 QD are predicted not to exceed the following limits, which are based on the NOAEL in 4-week toxicology in dogs (as detailed in sections 6.2.1).

C_{max}: 3,000 ng/mL	AUC_{24}: 25,000 ng·h/mL

The trial will be stopped if any of the criteria in section 8.4.1 are met.

9 Trial population

9.1 Planned number of subjects

Up to 12 healthy volunteers, excluding replacements

9.2 Inclusion criteria

The inclusion criteria detailed below should be used to determine eligibility at screening only (unless otherwise specified).

1. Male healthy volunteers (PET subjects); or healthy males or females of non-childbearing potential (PBMC-only subjects). A woman is considered to be of non-childbearing potential if she meets one of the following criteria:
   - post-menopausal (amenorrhea for at least 12 months, and follicle-stimulating hormone (FSH) at screening confirms post-menopausal status)
   - has no uterus, ovaries or fallopian tubes
2. Aged 25–55 years.
3. Deemed healthy based on medical history, physical examination, ECG, vital signs, neurological examination, and laboratory tests of blood and urine.
4. Body weight ≥ 50.0 kg (men) or ≥ 45.0 kg (women).
5. Body mass index (BMI; Quetelet index) in the range 18.0–30.9 kg/m^2 (inclusive).

$$\text{Body Mass Index} = \frac{\text{weight [kg]}}{(\text{height [m]})^2}$$

6. Sufficient intelligence to understand the nature of the trial and any hazards of participating in it. Ability to communicate satisfactorily with the investigator

and to participate in, and comply with the requirements of, the entire trial (ie be fluent in the local language).

7. Willingness to give written consent to participate after reading the ICF, and after having the opportunity to discuss the trial with the investigator or their delegate.
8. Agree to follow the contraception requirements of the trial as described in section 11.
9. Agree not to donate blood or blood products during the study and for up to 3 months after the final visit.
10. Willingness to give written consent to have data entered into The Overvolunteering Prevention System (TOPS).
11. Agree not to post any personal medical data or information related to the study on any website or social media site (eg Facebook, Twitter, etc) until the trial has ended.

9.3 Exclusion criteria

The exclusion criteria detailed below should be used to determine eligibility at screening only (unless otherwise specified).

1. Clinically relevant abnormal medical history, physical or neurological findings, ECG, or laboratory values at screening, or before the baseline PET scan (PET subjects only), or before the first dose of trial medication, that could interfere with the objectives of the trial or the safety of the volunteer.
2. History or presence of acute or chronic illness, or clinically-significant medical abnormality, sufficient to invalidate the volunteer's participation in the trial or make it unnecessarily hazardous.
3. History or presence of any disease, medical condition, or surgery (eg stomach bypass), likely to affect the absorption, distribution, metabolism, or excretion of medicines. Subjects with a history of cholecystectomy should be excluded.
4. Impaired endocrine, thyroid, hepatic, respiratory or renal function, diabetes mellitus, coronary heart disease, or history of any psychotic mental illness.
5. Presence or history of severe or clinically significant adverse reaction to any drug; or a history of sensitivity to ASN51 (all subjects) or the PET radioligand, [^{18}F]-IMA601 (PET subjects only), or any components of those medications.
6. History of psychiatric disorders, including substance use disorders, according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) criteria.
7. Any diagnosis of intellectual disability (intellectual developmental disorder) or mental retardation.

8. History of epilepsy or seizures, other than a single instance of benign febrile convulsion in childhood.
9. History of clinically significant head trauma, including closed head injury with loss of consciousness.
10. History of symptomatic orthostatic hypotension (ie postural syncope).
11. History of neuroleptic malignant syndrome.
12. History of chronic urinary tract infections.
13. Significant suicide risk as assessed by C-SSRS.
14. Frequent use (≥ 3 days a week) of any nicotine-containing product (eg cigar, cigarette, or snuff; or any product used for smoking cessation [eg nicotine gum or plasters]) within 3 months before the baseline PET scan (PET subjects) or first dose of trial medication (PBMC subjects).

    Use of any nicotine-containing product is prohibited from within 1 week of the baseline PET scan (PET subjects) or the first dose of trial medication (PBMC subjects).
15. Presence or history of drug or alcohol abuse, or intake of more than 14 units of alcohol weekly, within the 12 months before the baseline PET scan (PET subjects) or the first dose of trial medication (PBMC subjects).
16. Evidence of drug abuse on urine testing.
17. Regular consumption (eg ≥ 4 days/week) of excessive quantities of xanthine-containing beverages (eg ≥ 5 cups of coffee or the equivalent per day) within 30 days before screening, or between screening and the baseline PET scan (PET subjects) or the first dose of trial medication (PBMC-only subjects).
18. Receipt of an investigational product (including prescription medicines) or device within 3 months* before the baseline PET scan (PET subjects) or the first dose of trial medication (PBMC subjects); in the follow-up period of another clinical trial at the time of screening for this study.

    *(or 5 half-lives, or twice the duration of the biological effect, of the investigational product – whichever is longer).
19. Use of the following:
    - a prescription medicine during the 28 days* before the baseline PET scan (PET subjects) or the first dose of trial medication (PBMC subjects);
    - an over-the-counter medicine, including vitamins, herbal, or dietary supplements (including St John's Wort), with the exception of acetaminophen (paracetamol), during the 7 days* (or 28 days if the medicine is a potential hepatic enzyme inducer) before the baseline PET scan (PET subjects) or the first dose of trial medication (PBMC subjects).

*(or 5 half-lives of the medicine – whichever is longer).

20. Loss of more than 400 mL blood during the 3 months before the trial, eg as a blood donor.
21. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels ≥ 1.5 × upper limit of normal (ULN) at screening, or before the baseline PET scan (PET subjects), or before the first dose of trial medication. A repeat is allowed on one occasion for determination of eligibility.
22. QT value, measured at screening, or before the baseline PET scan (PET subjects) or first dose of trial medication (PBMC subjects), greater than 450 msec (men) or 470 msec (women), using Fridericia's formula (QTcF) for correction.

    Triplicate measurements will be made, and a mean QTcF value higher than those stated will lead to exclusion. A repeat (in triplicate) is allowed on one occasion for determination of eligibility.
23. Family history of long QT syndrome or sudden death.
24. Clinically significant ECG abnormalities, as determined by the investigator or medical monitor, including (but not limited to):
    - abnormal ST-T-wave morphology or left ventricular hypertrophy.
    - PR (PQ) interval shortening < 120 msec (PR < 120 msec but > 110 msec is acceptable if there is no evidence of ventricular pre-excitation).
    - PR (PQ) interval prolongation (> 220 msec), intermittent second- (Wenckebach block while asleep or in deep rest is not exclusionary) or third-degree atrioventricular (AV) block.
    - Persistent or intermittent complete bundle branch block, incomplete bundle branch block, or intraventricular conduction delay with QRS > 120 msec.
25. Blood pressure and pulse rate in seated position at the screening examination outside the ranges: blood pressure 90–140 mm Hg systolic, 40–90 mm Hg diastolic; pulse rate 40–100 beats/min. The average of triplicate measurements (each 5 min apart) will be used to assess eligibility.

    Repeat measurements are permitted if values are borderline (ie values that are within 5 mm Hg for blood pressure or 5 beats/min for pulse rate) or if requested by the investigator. Subjects can be included if the repeat value is within range or still borderline, but deemed not clinically significant by the investigator.
26. Significant (> 10%) weight loss or gain within 30 days before the (first) screening visit or before the baseline PET scan (PET subjects) or first dose of trial medication (PBMC subjects).
27. Unsatisfactory venous access
28. Subjects with a COVID-19 vaccination within 2 weeks of screening, or who are due to receive a dose of a COVID-19 vaccine while participating in the study.

29. Positive test for hepatitis B, hepatitis C or human immunodeficiency virus (HIV).
30. Vegan, or unwilling to eat a high-fat breakfast including eggs and dairy (eg for personal or religious reasons).
31. Employed by, or a first-degree relative of an employee of, Asceneuron S.A. or a clinical trial site participating in this study.
32. Possibility that the volunteer will not cooperate with the requirements of the protocol.
33. Objection by general practitioner (GP) to volunteer entering trial.

The following exclusion criteria also apply to subjects who will be given PET scans during the study.

34. Significant structural brain abnormality, as determined by MRI.
35. Significant exposure to ionising radiation as part of research (defined as International Commission on Radiological Protection [ICRP][11] category IIb or above: no more than 10 mSv in addition to natural background radiation including this trial), within the 12 months before the first dose of tracer.
36. Unsuitable or unwilling to undergo the imaging procedures, as determined by an MRI safety questionnaire. Reasons for exclusion include but are not limited to: presence of a cardiac pacemaker or other implanted electronic device; ferromagnetic metal foreign bodies, intracranial aneurysm clips or other metallic objects; non-MRI compatible heart valves; inner ear implants; or a history of claustrophobia.
37. Contraindication for arterial cannulation: Allen's test indicating potential risk in placement of the arterial cannula.

9.4 Withdrawal of subjects from the trial

9.4.1 Subject withdrawal

Subjects are free to withdraw from the trial at any time without giving reasons. Furthermore, the investigator may withdraw a subject for reasons such as intolerance to trial medication, intercurrent illness, need for medication which is contraindicated, significant non-compliance with the requirements of the trial, or withdrawal of consent. The investigator will assess the reasons for withdrawal as far as possible and will fully record the circumstances and medical details.

Subjects will be informed before they agree to take part in the trial that, if they withdraw or are withdrawn:

- the investigator will stop collecting information about them; and
- they can ask the investigator to destroy any identifiable samples taken from them.

The investigator will ask withdrawn subjects to consent to a follow-up examination, to check that they have come to no harm as a result of taking part in the trial. Provided that the subject agrees, they will undergo, at withdrawal from the trial (or as soon as possible afterwards), the standard medical examination and laboratory tests which they would have undergone had they completed it. The investigator will record in the case report form (CRF) the results of the follow-up examination of withdrawn subjects, if they give their consent for that.

9.4.2 Individual subject stopping criteria

A subject who meets any of the following criteria will receive no further doses.

- SAE considered related to study treatment.
- ALT ≥ 3 × ULN.
- Any condition that, in the judgment of the investigator or sponsor, might place the subject at risk or invalidate the trial.

Subjects who meet the above criteria will be considered discontinued from dosing. However, the investigator may ask the subject to continue their participation in the study, so they can be monitored for safety, PK and PD, or the investigator may withdraw the subject from the trial.

9.4.3 Replacement of withdrawn subjects

Withdrawn subjects will be replaced at the discretion of the sponsor and investigator. Replacements for withdrawn subjects will receive the treatment intended for the withdrawn subject, from the start of the trial.

For subjects withdrawn because of AEs considered possibly related to study treatment (see section 14.2), possible replacement will be discussed by mutual agreement between sponsor and investigator.

10 Treatments

10.1 Treatments administered

All subjects

All subjects will receive once-daily doses of ASN51 for 14 days (see section 8.1).

All doses will be given orally, in the fasted or fed state, with about 240 mL of water. Extra water can be given if a subject has trouble swallowing the tablets; that will be recorded in source documents.

Fasting requirements are described in section 11.

PET subjects

PET subjects will also receive IV doses of the radiolabelled tracer [^{18}F]-IMA601 (non-IMP) on up to 3 occasions (1 at each imaging session).

If one or both on-treatment PET scans need to be rescheduled, eg owing to a technical failure, subjects may have additional imaging sessions. However, subjects will receive no more than 3 doses of [^{18}F]-IMA601 during the study.

10.2 Overdose

Symptoms of overdose of ASN51 are not yet known. In the case of accidental overdose, subjects should be treated symptomatically as no specific antidote is available.

10.3 Blinding

This is an open-label study.

10.4 Method of assigning subjects to treatment groups

Subjects will be assigned a screening number at the screening visit.

After passing all of the screening assessments, subjects will be allocated to a group according to their availability and the scheduled trial dates.

Subjects will be numbered consecutively, from 1001–1006 (Group 1) or 2001–2006 (Group 2), in the order in which they arrive on the ward and are entered into the trial (ie subjects who will have PET scans during the study will be allocated subject numbers on the day of imaging session 1; all other subjects will be numbered on Day –1).

Replacements for withdrawn subjects will be given a number equal to that of the subject that they replaced plus 100. So, Subject 1001 would be replaced by Subject 1101, and Subject 2004 would be replaced by Subject 2104, and so on.

10.5 Selection and timing of dose for each subject

The treatments to be administered are described in sections 8.1 and 10.1. Subjects will be assigned to treatments as described in section 10.4.

Doses will be given at the same time each day (± 15 mins from the dosing time on Day 1).

10.6 Previous and concomitant treatment

Previous treatment restrictions are described in section 9.3.

During the trial, concomitant medication may be given if the subject's GP believes it to be necessary. In addition, up to 2 g paracetamol (acetaminophen) will be allowed

per day for mild analgesia (up to 1 g per dose). Any other concomitant treatment will be given only if deemed strictly necessary by the investigator or co-investigator. In any case, all concomitant treatments will be reported in the CRF along with their daily dosage, duration and reasons for administration. Subjects who have received any concomitant treatment may be withdrawn from the trial at the discretion of an investigator.

10.7 Assessment of compliance

All subjects will be dosed with ASN51 on the research ward under the supervision of 2 suitably trained members of HMR staff. Mouth checks will be done.

Subjects who will have PET scans will be dosed with radiolabelled tracer, [^{18}F]-IMA601 at Invicro, under the supervision of suitably trained members of staff, at least one of whom will be a registered physician or nurse.

11 Dietary and lifestyle restrictions

Subjects will abide by HMR house rules while on the ward or at Invicro.

Subjects will receive their doses of ASN51 in the fed and fasted states, as follows.

- On Days 1 and 14, subjects will fast (no food or drink other than water) from midnight on the evening before dosing, until 4 h after dosing.
- On Days 2–10, 12, and 13, the study medicine will be given before a standard breakfast.
- On Day 11, subjects will take the study medicine after a high-fat breakfast consisting of 2 fried eggs, 2 strips of bacon, 2 slices of toast and butter, 4 ounces of hash brown potatoes, and a glass of whole milk. Subjects will start the meal approximately 30 min before dosing, and must eat all of it by at least 10 min before dosing.

Other standard meals and drinks will be provided at appropriate times. Because the timings of on-treatment PET scans may change, meals for PET subjects may be provided at unusual times.

Other than the water required to take the study medicine, water will not be allowed for 2 h before and after dosing (Days 1 and 14 only).

No food or drink containing Seville oranges or grapefruit, and no smoking or use of nicotine-containing products, will be allowed from 7 days before the baseline PET scan (PET subjects) or the first dose of trial medication (PBMC subjects) until the end of the study. No alcoholic drinks will be allowed 48 h before the baseline PET scan (PET subjects) or first dose of trial medication (PBMC subjects), until the end of the study. No caffeine- or xanthine-containing products (eg coffee, tea, cola drinks and chocolate) will be allowed from 24 h before: the baseline PET scan (PET subjects) or the first dose of trial medication (PBMC subjects) until discharge from

the ward; imaging session 3 (if applicable, PET subjects); and the follow-up visit (all subjects). Subjects should be advised to avoid eating food containing poppy seeds from screening until the end of the study, because that can cause a positive drugs of abuse test result.

No strenuous exercise will be allowed for 7 days before the baseline PET scan (PET subjects) or the first dose of trial medication (PBMC subjects) until the end of the study.

Subjects must not sunbathe or use a sunbed during the study.

Subjects must use a reliable method of contraception, as follows.

11.1 Contraception requirements

Men

Male subjects must not plan to father a child, or donate sperm, during the trial (from 1 day before the baseline PET scan [PET subjects] or first dose of trial medication [PBMC subjects] until 3 months after their follow-up visit).

Male subjects must not have sex without using a condom, if their partner is a woman of childbearing potential, from 1 day before the baseline PET scan (PET subjects) or first dose of trial medication (PBMC subjects) until 90 days after their last dose of trial medication. Also, their female partners of childbearing potential must use an additional method of contraception (such as an oral contraceptive, cap, or diaphragm [used with spermicide], intrauterine device [IUD], or tubal ligation). Subjects do not need to use any contraception if: they've had a vasectomy, and surgical success has been confirmed by medical assessment, or if their partner is not of childbearing potential. Partners who are not of childbearing potential are defined as: men; post-menopausal women (no menstrual periods for at least 12 months, and FSH at screening confirms post-menopausal status); or women who have no uterus, ovaries, or fallopian tubes.

Subjects who practise true abstinence or who only have same-sex relationships need not use contraception, provided it is in line with their preferred and usual lifestyle (note: periodic abstinence (eg calendar, ovulation, symptothermal, and post-ovulation methods) and withdrawal are not acceptable methods of contraception). Should any such subject stop practising true abstinence, they must use contraception as described above.

Women

Women of non-childbearing potential may take part in the trial as PBMC-only subjects. A woman is considered to be of non-childbearing potential if she meets one of the criteria in inclusion criterion 1.

12 Procedures and observations

Only subjects who meet all the inclusion and no exclusion criteria will be eligible for enrolment into the trial (see section 9). Each subject will be allocated a unique trial number (see section 10.4).

The schedule of procedures is in section 12.1.

Additional timepoints may be introduced, and changes to timepoints may be made, in accordance with section 12.2.

The following assessments will be made.

Pharmacodynamics

Blood samples for PBMC assays will be taken before and frequently during dosing (up to 144 h [6 days] after the last dose). Trough samples will be taken before study drug administration.

Occupancy of O-GlcNAcase by ASN51 will be assessed by PET imaging using the radioligand [^{18}F]-IMA601 (PET subjects only).

Blood samples will be taken during each PET scan for arterial input function and radio metabolite analysis (PET subjects only).

Safety and tolerability:

Laboratory assessments (routine haematology, clinical chemistry, coagulation, and urinalysis), physical and neurological examinations, 12-lead ECGs, and vital signs (blood pressure, pulse rate, respiratory rate, and tympanic temperature) and C-SSRS will be done frequently until the subject's last visit. AEs will be recorded from screening until the subject's last visit.

Laboratory safety variables to be assessed during the study are in Table 4.

From screening until the follow-up visit, AEs and concomitant medication will be documented as they are reported by the subjects. Subjects will be questioned about AEs on admission to the ward, when procedures are done, and when they return to the ward for outpatient visits/at follow-up.

Pharmacokinetics

Blood samples for assay of ASN51 will be taken before and frequently during dosing (up to 144 h [5 days] after the last dose), and at follow-up. Trough samples will be taken before study drug administration.

PET subjects only: for subjects having imaging session 3 on Day 22 or 23, additional PK samples will be taken approximately 2 h before and after the PET scan.

Other

Structural MRI scans will be done during the screening period (PET subjects only).

12.1 Schedule of procedures

	Screening[1]		Imaging session 1[2]	Treatment period (including imaging session 2 [Day 1], and imaging session 3 [Day 17])								Imaging session 3[4]	Follow-up[5]
	Visit 1	Visit 2 (MRI)		Day –1	Day 1[3]	Days 2–10	Day 11	Days 12–13	Day 14	Day 17[4]	Day 20		
Informed consent	X												
Inclusion/exclusion criteria	X			X									
Demographics	X												
Medical/surgical history	X												
Allen's test[6]	X		X		X					X		X	
MRI questionnaire[7]	X	X											
MRI scan[7]		X											
Admission				X									
Inpatient stay				←	---	---	---	---	---	---	→		
Venous cannulation[8]			X		X				X	X		X	
Arterial cannulation[9]			X		X					X		X	
Administration of [^{18}F]-IMA601[10]			X		X					X		X	
PET scan[6]			X		X					X		X	
Dose of ASN51[11]					←	---	---	---	→				
High-fat breakfast[12]							X						
Discharge											X		
Outpatient visit												X[6]	X
Safety assessments													
Physical and neurological examination[13]	X			X									X
FSH test[14]	X												X
Height, weight, and BMI[15]	X	X											
Vital signs[16]	X		X	X	X	X	X	X	X	X	X	X	X
12-lead ECG[17]	X		X	X	X	X	X	X	X	X	X	X	X
Serology	X												
Laboratory safety tests[18]	X		X[6]	X		X[18]			X		X		X
Urine drug screen and cotinine, and alcohol urine test	X			X									

	Screening[1]		Imaging session 1[2]	Treatment period (including imaging session 2 [Day 1], and imaging session 3 [Day 17])								Imaging session 3[4]	Follow-up[5]
	Visit 1	Visit 2 (MRI)		Day –1	Day 1[3]	Days 2–10	Day 11	Days 12–13	Day 14	Day 17[4]	Day 20		
C-SSRS	X												X
Blood samples													
PBMC samples[19]					X	X	X	X	X	X	X		
PK plasma samples[20]					X	X	X	X	X	X	X	X	X
Arterial blood samples[9]			X		X					X		X	
Ongoing subject review													
Concomitant medicines	←		→										
Adverse events	←		→										

Abbreviations: BMI: body mass index; C-SSRS: Columbia-Suicide Severity Rating Scale; ECG: electrocardiogram; FSH: follicle stimulating hormone; MRI: magnetic resonance imaging; PBMC: peripheral blood mononuclear cell; PD: pharmacodynamics; PET: positron emission tomography; PK: pharmacokinetics.

Notes:

1. Screening will be within:
   - 28 days before the first dose of trial medication (PBMC-only subjects; see section 8.1.2)
   - 21 days before imaging session 1 (PET subjects only; see section 8.1.3)
2. *PET subjects only* – imaging session 1 (baseline PET scan) will be within 3–7 days before the first dose of trial medication. Subjects will be admitted to the ward the day before their baseline PET scan, and have laboratory safety tests done for inclusion/exclusion criteria before their PET scan.
3. *PET subjects only* – imaging session 2 (on-treatment PET scan) will be at about 5 h postdose on Day 1. Water will not be allowed from 2 h before until 2 h after dosing.
4. *PET subjects only* – imaging session 3 will take place on:
   - Day 17 (3 out of 4 PET subjects per group)
   - Day 22 (1 subject in Group 1)
   - Day 23 (1 subject in Group 2).

   NB. In the event of technical failures, a PET scan may need to be repeated or rescheduled – detailed in section 12.2. Subjects will have no more than 3 PET scans in total during the study.
5. Follow-up will be:
   - on Day 23 (+ 2 days) (PBMC-only subjects)
   - On Day 23 (+ 2 days) or 2–4 days after imaging session 3, whichever is later (PET subjects).

   Additional follow-up owing to SAEs is described in section 12.3.
6. PET subjects only. A sample will be taken for clinical chemistry only.

7. *PET subjects only* – subjects will complete an MRI questionnaire before the MRI scan, to exclude unsuitable subjects. Questionnaires will be done at Screening Visit 1 with the screening physician, and at Screening Visit 2 at Invicro with the radiographer. Screening MRI will be done once all other screening results are available. If a subject is being re-screened (ie missed cohort) and there is already a recent MRI scan available within 3 months from their first screening period (ie if subjects were a reserve subject for a previous group but were not dosed and are no longer within the screening window) they do not need a repeat MRI scan.
8. Subjects will have several cannulas, as below.
   - *All subjects* – on Days 1 and 14, subjects will have a cannula inserted for PK sampling.
   - *PET subjects only* – at each imaging session, subjects will have an additional cannula inserted for administration of the radioligand [^{18}F]-IMA601.
9. *PET subjects only* – once per imaging session
10. *PET subjects only* – [^{18}F]-IMA601 administration at the start of each PET scan.
11. Subjects will receive once-daily oral doses of ASN51 on Days 1–14 in the fasted and fed state (fasting requirements and water restrictions are in section 11). Doses will be given at approximately the same time each day (± 15 min from dosing time on Day 1).
12. All subjects will be given an FDA high-fat breakfast before dosing on Day 11; see section 11.
13. Full physical and neurological examinations will be done at screening and at the follow-up visit. Brief (symptom-directed) physical examinations will be done at all other timepoints.
14. FSH tests in post-menopausal women (screening only).
15. Weight only at screening visit 2 (MRI; PET subjects only).
16. Vital signs consist of seated systolic and diastolic blood pressure, pulse rate, tympanic temperature, and respiratory rate. Vital signs will be done at the following timepoints.
    - *PBMC-only subjects:* screening; Day –1; predose and at 2, 4, 8, and 12 h postdose on Day 1; before dosing on Days 2–13; before, and at 2, 4, 8, and 12 h after dosing on Day 14; at 24 h (Day 15), 48 h (Day 16), 72 h (Day 17), 96 h (Day 18), 120 h (Day 19), and 144 h (Day 20) after the last dose of ASN51; and at follow-up
    - *PET subjects* will have the same timepoints as PBMC-only subjects, plus additional assessments at: imaging session 1 (baseline) and imaging session 3 (if held on Day 22 or Day 23)

    Triplicate measurements (obtained ≥ 5 min apart) will be made at screening. Single measurements will be made at all other timepoints. Measurements should begin with subjects in a seated position, after resting for at least 5 min. Additional unscheduled assessments may be done at the discretion of the investigator. Morning vital signs should always be taken before study drug administration.
17. During the period of residence, ECGs will be recorded at the following timepoints.
    - *PBMC-only subjects:* screening; Day –1; predose and at 2, 4, 8, and 12 h postdose on Day 1; before dosing on Days 2–13; before, and at 2, 4, 8, and 12 h after dosing on Day 14; at 24 h (Day 15), 48 h (Day 16), 72 h (Day 17), 96 h (Day 18), 120 h (Day 19), and 144 h (Day 20) after the last dose of ASN51; and at follow-up
    - *PET subjects* will have the same timepoints as PBMC-only subjects, plus additional assessments at: imaging session 1 (baseline) and imaging session 3 (if held on Day 22 or Day 23)

    Triplicate recordings (obtained ≥ 5 min apart) will be made at screening. Single recordings will be done at all other time points. Recordings should be made with subjects in a supine position, after resting for 5 min.

18. Laboratory safety tests consist of haematology, clinical chemistry, coagulation, and urinalysis. Samples are to be taken at screening, Day –1, before dosing on Days 8 and 14, on Day 20, and at follow-up. Samples for clinical chemistry only are to be taken at Imaging session 1.
19. Plasma samples for the PBMC assay will be collected as follows.

    PBMC-only subjects

    - before and at 4, 8, and 12 h after dosing on Day 1
    - before dosing on Day 2
    - before and at 4 h after dosing on Day 11
    - before and at 4 h, 8 h, and 12 h after dosing on Day 14
    - at 24 h (Day 15), 72 h (Day 17), and 144 h (Day 20) after the last dose of ASN51

    PET subjects

    - before and at 4, and 8 h after dosing on Day 1
    - before dosing on Day 2
    - 4 h after dosing on Day 11
    - before and at 4 h, and 8 h after dosing on Day 14
    - at 24 h (Day 15), 72 h (Day 17), and 144 h (Day 20) after the last dose of ASN51
20. Plasma samples for PK assay of ASN51 will be collected at as follows.

    PBMC-only subjects

    - before and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 16 h after dosing on Day 1
    - before dosing on Days 2, 4, and 7
    - before and at 8 h after dosing on Day 11
    - before and at 0.25, 0.5, 1, 2, 4, 6, and 12 h after dosing on Day 14
    - at 24 h (Day 15), 48 h (Day 16), 96 h (Day 18), and 144 h (Day 20) after the last dose of ASN51; and at follow-up

    PET subjects will have the same timepoints as PBMC-only subjects, plus 2 additional samples at imaging session 3 at about 2 h before and 2 h after the PET scan.

    Samples scheduled immediately before the PET scan should be taken as close as possible to the planned start of the PET scan. Samples scheduled for the end of the PET scan should be taken as soon as possible after the PET scan is finished. Samples scheduled during the PET scan should be taken either before or after the PET scan, as close to the scheduled timepoint as possible.

When more than one assessment is scheduled at a specific timepoint, PET procedures should be prioritised (if applicable), followed by PK, PBMC, and scheduled safety assessments. Unscheduled safety assessments due to an emergency should always be prioritised. Blood sampling should be done on time. Other procedures should be done as close as possible to the scheduled timepoint.

12.2 Sampling timepoints and additional tests

With the sponsor's approval, additional timepoints may be introduced, and changes to timepoints may be made, if we have reason to believe that the change might improve the quality of the data (for example, if we believe that an important effect of the IMP is occurring at a time when no measurements are scheduled), or if extra procedures are needed in the interest of subject safety. However, the total volume of blood taken in the trial will not exceed the value given in section 12.5.

An additional 48 hours' residence in the ward, and additional outpatient or imaging visits, will be permitted, in the event of a technical failure, to accommodate a later PET scan (detailed further below), and/or if extra observations or samples of blood or urine are needed.

Extra procedures and changes to timepoints which have a significant impact on the scientific value and/or safety of the trial participants will be implemented only after approval of a substantial amendment from the Regulatory Authority (MHRA), unless the changes constitute an urgent safety measure.

With the sponsor's approval, the interval between inpatient and outpatient visits may be changed, if data collected during the trial support the change. The following will **not** be regarded as protocol deviations.

Table 3: Acceptable deviation times

Procedure	Study day	Timepoint	Acceptable deviation
PK and PBMC blood sampling*	Days 1 and 14	Predose	Up to 30 min before dosing
		Up to and including 1 h post dose	± 5 min of the scheduled time
		After 1 h to 4 h post dose	± 10 min of the scheduled time
		After 4 h to 24 h post dose	± 15 min of the scheduled time
	Days 2–13	Predose	Up to 30 min before dosing
	Day 15–20	> 24 h post dose	± 1 h of the scheduled time
	Day 21 onward	Outpatient visits	± 1 day
All other procedures	Days 1 and 14	Predose	Up to 90 min before dosing**
		Up to and including 4 h post dose	± 10 min of the scheduled time
		After 4 h to 24 h post dose	± 15 min of the scheduled time
	Days 2–13	Predose	Before dosing
	Day 15–20	> 24 h post dose	± 1 h of the scheduled time
	Day 21 onward	Outpatient visits	± 1 day

* For subjects having PET scans, samples scheduled immediately before the scan should be taken as close as possible to the planned start of the scan. Samples scheduled for the end of the scan should be taken as soon as possible after the scan is finished. Samples

Procedure	Study day	Timepoint	Acceptable deviation

scheduled during the scan should be taken either before or after the scan, as close to the scheduled timepoint as possible.
** Samples for urinalysis may be collected anytime from when the subject awakes until the scheduled time.

PET scans

Timings of on-treatment PET scans may change based on emerging data.

Occasionally, a technical failure, such as unsuccessful radiolabelled tracer synthesis or a PET scanner malfunction, may require a PET scan to be repeated or rescheduled. However, subjects will have no more than 3 PET scans and 3 doses of [^{18}F]-IMA601 during the study. Therefore, if the failure affects an imaging session before the administration of radioactivity, a scan may be repeated (ideally within about 24 h for on-treatment scans). If the scan is scheduled after a subject has been discharged from the ward, the subjects may be asked to stay at the research unit for up to 24 h longer. Otherwise, the subject may be discharged and later readmitted to the research unit. If a subject can receive no further doses of [^{18}F]-IMA601, then they may be replaced, on a case-by-case basis, at the discretion of the sponsor.

12.3 Follow-up

Subjects will return to the ward 9–11 days after their final dose of trial medication, or 2–4 days after imaging session 3 (whichever is later; PET subjects only) for a follow-up visit. Withdrawn subjects who consent to a follow-up visit will undergo the same procedures (see section 9.4).

The follow-up period may be extended if:

1. a subject has an unresolved AE at the follow-up visit, which, in the opinion of the investigator, merits further follow-up;
2. plasma concentrations of IMP were higher than predicted, and/or the half-life of the IMP was very long, and the investigator considers that additional follow-up is necessary; or
3. new information becomes available that supports an extended follow-up period.

The investigator will decide on the nature of the follow-up. For example, subjects may have a telephone follow-up at which they are asked about AEs, or subjects may be asked to attend extra outpatient visits for additional monitoring of blood levels or effects of IMP, and for extra safety tests. The extra safety tests might include tests that are not described in this protocol. The investigator reserves the right, during or after the study, to repeat safety tests or to do any extra safety tests that are in the best interest of the subjects.

If a subject has an SAE during the study, they will also have an additional follow-up by telephone at 28 days (± 3) days after their final dose of trial medication.

12.4 Methods

Blood collection

Blood will be taken by venepuncture or via a cannula. At HMR, cannulae will be inserted under local anaesthesia with lidocaine 0.5%, for withdrawal of venous blood.

After each blood sample, the cannula will be flushed with 3–5 mL normal saline, to keep it patent. In order to minimise dilution of each subsequent blood sample with normal saline, the following procedure will be used: about 2 mL will be drawn via the cannula into the sampling syringe, and immediately re-injected gently via the cannula. The definitive blood sample will be taken after a 10-second delay.

Additional cannulation (PET subjects only)

On the day of each imaging session, before each PET scan, an additional cannula will be inserted into a forearm vein, as per site standard operating procedures (SOPs), for administration of the radiolabelled tracer [^{18}F]-IMA601.

An arterial cannula will be inserted to take blood for analysis of the radioligand and metabolites. The details of sampling times and analysis of the arterial samples (done at the imaging centre) will be provided in a PET blood sampling protocol.

Blood volumes of specific types of samples may vary from those described below, but any change to the sample volumes will not cause the total volume of blood taken during the study to exceed that given in section 12.5.

Samples for laboratory safety tests

Blood will be taken for:

- haematology (2 mL in EDTA)
- clinical chemistry (3.5 mL in tubes with a gelatin plug)
- serology and serum FSH (3.5 mL in tubes with a gelatin plug)
- coagulation (3 mL in sodium citrate)

Blood samples will be collected into 13 × 75 mm tubes. Urine will be collected in Universal containers. Samples will then be transferred to the laboratory. For logistical reasons, blood and/or urine samples may be collected in tubes other than those stated above.

Processing and analysis of samples for laboratory safety tests

Processing of samples will be done by the HMR Analytical Laboratory in accordance with the laboratory's SOPs.

The HMR Analytical Laboratory will do safety tests on blood and urine samples using instruments interfaced to a validated laboratory information management system (LIMS). Data from analysers that are not interfaced will be entered manually into the LIMS.

Table 4: Laboratory safety tests

Haematology:

- haemoglobin (Hb)
- red blood cells (RBC)
- mean corpuscular volume (MCV)
- mean corpuscular haemoglobin (MCH)
- mean corpuscular haemoglobin concentration (MCHC)
- haematocrit
- white blood cells (WBC) and differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
- platelets

Coagulation:

- prothrombin time (PT)
- activated partial thromboplastin time (aPTT)
- international normalized ratio (INR)

Urinalysis:

- dipstick: protein, blood, ketones, glucose, bilirubin, urobilinogen, leukocyte esterase, specific gravity, nitrites, pH
- microscopy: only if dipstick test for protein, blood, leukocyte esterase or nitrites is abnormal

Serology:

- hepatitis B (hepatitis B surface antigen)
- hepatitis C antibody
- HIV screen (HIV 1 and 2)

Clinical chemistry:

- urea
- creatinine
- uric acid
- total bilirubin
- total protein
- albumin
- globulin
- alkaline phosphatase (AP)
- aspartate aminotransferase (AST)
- alanine aminotransferase (ALT)
- gamma-glutamyl transpeptidase (GGT)
- glucose*
- phosphate
- cholesterol
- triglycerides
- potassium
- sodium
- calcium
- chloride

* fasting [at screening and follow-up]

FSH tests

Serum FSH tests will be done using a chemiluminescent immunoassay method.

Drugs of abuse, alcohol, and cotinine tests

Urine will be tested for drugs of abuse, alcohol, and cotinine according to the laboratory's SOP. Tests will include: amphetamines, cocaine, opiates, cannabis, barbiturates, benzodiazepines, alcohol, and cotinine.

Processing of blood samples for pharmacodynamic analysis

Blood samples (5 mL) will be taken.

Details will be provided in a PBMC sampling manual.

Processing of blood samples for pharmacokinetic analysis

Blood samples (2 mL) will be taken into K_2EDTA tubes, and immediately placed on ice. Samples will be centrifuged at 1500 *G* for 10 min at 4°C and the plasma divided into 2 aliquots of approximately equal volume (minimum of 250 µL in each aliquot) in screw-capped polypropylene cryotubes. Plasma samples will be frozen at about –80°C or below within 1.5 h after collection, and stored until dispatch to DDS (Cambridgeshire, UK) for analysis.

Physical and neurological examination

Physical and neurological examination will be done by a physician. Complete physical examinations will examine: general appearance; head, ears, eyes, nose and throat; thyroid; lymph nodes; back and neck; heart; chest; lungs; abdomen; skin; and extremities. The following systems will be assessed: cardiovascular, dermatologic, respiratory, gastrointestinal, musculoskeletal and neurological.

Brief physical examinations will be symptom-directed, and include neurological assessment of cranial nerves, motor, sensation, coordination, reflexes, and gait.

Complete neurological examinations will include the following.

Motor system: upper and lower limbs will be inspected for tremor and fasciculation. Tone will be recorded for all limbs as 'normal', 'increased' or 'decreased'. Tendon reflexes will be elicited bilaterally (biceps, triceps, brachioradialis, knee, ankle) and graded 0 (absent even with reinforcement), + or ++ (normal) or +++ (increased). Plantar responses will be recorded as downgoing or equivocal (normal), or upgoing (abnormal).

Romberg test: this test is designed to identify loss of proprioceptive sensation, resulting in a sensory ataxia. The sensory ataxia is accentuated when the subject stands still with both feet together, arms at each side and eyes closed (Romberg Test). If the subject falls or staggers after 1 min, they are considered to have a sensory ataxia (a positive Romberg sign). If the sign is absent, the test will be repeated with the subject standing on the balls of the feet.

Coordination: will be examined using the 'finger–nose' test bilaterally, whereby the subject touches the examiner's finger at a distance of about 50 cm, with his index finger and then touches his nose, as fast as they can.

Direct pupillary reflexes: elicited by shining a torch into the pupil of the eye being tested and noting pupillary constriction (normal). Consensual pupillary reflexes will be elicited by shining the torch in the pupil of one eye and noting pupillary constriction in the contralateral eye (normal).

Height and weight

Height and weight will be measured by trained staff at HMR.

Vital signs

Blood pressure and pulse rate will be measured using SpaceLabs oscillometric equipment. Measurements will be made with subjects in a seated position; subjects will remain seated for at least 5 min before vital signs are measured.

Tympanic temperature will be measured using digital thermometers.

Respiratory rate will be measured by observation of the chest.

Repeat vital signs measurements

During the trial, if vital signs fall outside the ranges in Table 5, a physician will review and decide on an appropriate course of action. The procedure will be repeated only if instructed by a physician.

Table 5: Vital signs ranges

Vital sign	Range
Seated systolic blood pressure	90–140 mm Hg
Seated diastolic blood pressure	40–90 mm Hg
Seated pulse rate	40–100 beats/min
Temperature	35.5–37.8°C
Respiration rate	10–16 breaths/min

If the result of the repeat measurement is still out of range, the investigator will decide on an appropriate course of action.

Standard 12-lead ECGs

12-lead ECGs will be recorded using Mortara ELI250c and ELI280 cardiographs. Each recording will be printed on a single A4 page at paper speed 25 mm/sec and calibrated to 10 mm/mV. Recordings will be made with subjects in a supine position; subjects will remain supine for at least 5 min before the ECG is recorded. PR, RR, QRS and QT intervals will be captured on source documents. QT interval will be corrected using Fridericia's formula (QTcF).

During the trial, if ECG values fall outside the ranges in Table 6, a physician will review and decide on an appropriate course of action. The procedure will be repeated only if instructed by a physician.

Table 6: ECG ranges

ECG parameter	Range
Ventricular rate	35–100 beats/min
QRS	≤ 120 msec
PR interval	110–220 msec
QTcF (men)	≤ 450 msec
QTcF (women)	≤ 470 msec

If the result of the repeat measurement is still out of range, the investigator will decide on an appropriate course of action.

Columbia-Suicide Severity Rating Scale (C-SSRS)

The C-SSRS is a suicidal ideation rating scale used to evaluate suicidality[30]. The questionnaire will be administered by the investigator or his delegate. The 'baseline' questionnaire will be used at screening, and the 'since last visit' questionnaire will be used at follow-up.

Structural MRI

Structural MRI acquisition will be performed at Invicro using either a 3 Tesla Siemens (Siemens Healthcare, Erlangen, Germany) or 3 Tesla GE (GE Healthcare, UK) clinical MRI system, and will consist of a structural scanning protocol. MRI scans will be evaluated by a radiologist to exclude any subjects with major pathology or abnormalities. A radiologist will provide a report of each MRI scan to the investigator at HMR.

PET scan

Dynamic PET scans will be performed at Invicro using a Siemens Horizon PET/CT scanner (Siemens Healthcare, Erlangen, Germany). The subjects will be placed in the PET scanner with head padding to minimise movement during the scan. A low dose CT scan will be performed to correct for the attenuation of emitted radiation. Subjects will then receive an IV bolus injection of up to 100 MBq of the [^{18}F]-IMA601 radioligand. Dynamic emission data will be recorded for up to about 90 min after injection of the radioligand.

Allen's test

If arterial cannulation will be used, the patency of subjects' ulnar and radial arteries in both wrists will be checked, at screening and before insertion of the arterial cannula on scanning days.

12.5 Total volume of blood removed

The total volume of blood taken from each volunteer in the trial will be about 184.5 mL (Table 7; PBMC-only subjects) or 537 mL (Table 8; PET subjects). Additional blood samples for assay of ASN51, PBMCs, or for laboratory safety tests may be taken as described in section 12.2. No more than an extra 80 mL of blood will be taken from any PBMC-only subject, and no more than an extra 30 mL of blood from any PET subject, unless medically indicated for safety reasons.

Table 7: Planned blood volume (PBMC-only subjects)

Test	Planned number of tests	Volume (mL)	Total planned blood volume (mL)
Haematology	6	2	12
Clinical chemistry	6	3.5	21
Coagulation	6	3	18
Serology (and FSH)	1	3.5	3.5
Pharmacokinetics	30	2	60
PBMC sampling	14	5	70
		Total	**184.5 mL**

Table 8: Planned blood volume (PET subjects)

Test	Planned number of tests	Volume (mL)	Total planned blood volume (mL)
Haematology	6	2	12
Clinical chemistry	7	3.5	24.5
Coagulation	6	3	18
Serology	1	3.5	3.5
Arterial	3	120	360
Pharmacokinetics	32	2	64
PBMC sampling	11	5	55
		Total	**537 mL**

13 Trial materials

13.1 Identity of test product(s)

Immediate-release [redacted] containing [redacted] ASN51 will be provided to the HMR pharmacy by Asceneuron S.A, and will be packed and dispatched in containers.

Full details of ASN51 are included in the IB[1] and IMPD[27].

The sponsor will provide to HMR a certificate of analysis, and a Certificate of GMP Compliance for the test product for HMR's Qualified Person to release batches of IMP.

13.2 Packaging and labelling

The trial medication will be packaged and labelled by the HMR Pharmacy, in accordance with The Rules Governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practice (GMP), and with HMR's Manufacturing Authorisation for IMPs (MIA(IMP)). The IMP labels will include all the information required by Annex 13 to GMP[13].

13.3 Storage and accountability of IMP

The IMP will be stored and accounted for according to GMP and HMR SOPs.

At the end of the trial, all unused IMP supplies will be returned to the sponsor or destroyed in accordance with the sponsor's instructions.

13.4 Non-investigational medicinal product

The radioligand [^{18}F]-IMA601 (non-IMP) will be prepared by Invicro.

Product name: [^{18}F]-IMA601 (also known as [^{18}F]OGA1)

Dosage form: sterile solution for injection

Effective dose: maximum 2.33 mSv for each PET scan (including components from both the PET [2.05 mSv] and CT scans [0.28 mSv])

Specific activity: will not exceed 100 MBq for each PET scan

Route of administration: IV bolus

Manufacturer/source of procurement: Invicro*

* cGMP grade precursor will be procured from an external commercial source

Full details are included in the [^{18}F]-IMA601 non-IMPD[28].

13.5 Other trial supplies

Supplies of containers to be used to package IMP (eg bottles, vials, syringes), components to be used in the assembly of a dose (eg labels), and excipients to be used in the preparation of the IMP (eg sterile saline solution) will be placed in quarantine on receipt by HMR, pending assessment and release by Pharmacy Quality Control.

14 Adverse events

14.1 Definitions of adverse events

Adverse event (AE)

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with that treatment.

Adverse drug reaction (AR)

All untoward and unintended responses to an IMP related to any dose administered. Note that, according to the ICH Guideline for Good Clinical Practice (ICH GCP), the phrase 'response to an IMP' means that a causal relationship between the medicinal product and an AE is at least a reasonable possibility, ie a relationship cannot be ruled out.

Unexpected AR

An AR, the nature or severity of which is not consistent with the applicable product information (eg IB for an unauthorised investigational product, or summary of product characteristics for an authorised product).

Serious adverse event (SAE) or serious adverse drug reaction (SAR)

An AE or AR that:

- is fatal;
- is life-threatening;
- requires or prolongs inpatient treatment;
- results in persistent or significant disability or incapacity; or
- is a congenital anomaly or birth defect.

Note that*:*

- the term 'life-threatening' in the definition of 'serious' refers to an event or reaction in which the patient was at risk of death at the time of the event; it does not refer to an event or reaction which hypothetically might have caused death had it been more severe; and
- in accordance with the ICH Guideline on Clinical Safety Data Management: Definitions and Standards of Expedited Reporting, events or reactions that are not immediately life-threatening or may not result in death or hospitalisation, but might jeopardise the subject or require intervention to prevent one of the other outcomes listed above, should usually be considered serious.

Significant AE or AR

Any SAE or SAR, or an AE or AR which is not serious but is otherwise significant. The following should normally be considered significant:

- a marked haematological or other laboratory abnormality;
- an AE or AR that leads to an intervention, including withdrawal of drug treatment, dose reduction or significant additional concomitant therapy; or
- any AE or AR that the investigator considers to be significant.

14.2 Procedures for recording adverse events

Subjects will be carefully monitored for AEs. The investigator or delegate will question the subjects about AEs using a non-leading question, such as 'How are you feeling?'. The investigator will also record AEs reported spontaneously by the subjects. Clinically significant changes in the findings of physical examination, and clinically significant abnormalities in the results of objective tests (eg laboratory variables, x-ray, ECG) may also be recorded as AEs. The investigator will use the following criteria when deciding whether to report an abnormal result as an AE.

1. The test result is associated with accompanying symptoms.
2. Results of additional diagnostic tests cause concern or necessitate medical intervention.
3. As a consequence of the test result, the dose administered to the subject is changed, the subject is withdrawn, or the subject is given concomitant treatment.
4. The investigator considers the result to constitute an AE.

If any of the above criteria are met, the investigator will report the result as an AE.

A record will be kept in the source documents of all AEs as reported, whether believed to be related or unrelated to the treatment. The record will include the following.

- **Clinical symptoms**: a simple, brief description.
- **Date and time of onset and end** of clinical symptoms.
- **Frequency**: constant or intermittent.
- **Severity**. The following categories will be used:

  Mild: the AE does not interfere with the volunteer's daily routine, and does not require intervention; it causes slight discomfort.

  Moderate: the AE interferes with some aspects of the volunteer's routine, or requires intervention, but is not damaging to health; it causes moderate discomfort.

  Severe: the AE results in alteration, discomfort or disability which is clearly damaging to health.

- **Relationship to treatment**: The assessment of relationship of AEs to the administration of IMP is a clinical decision based on all available information at

the time of the completion of the case report form. The following categories will be used.

Unrelated

- The AE is clearly not related to the study drug/procedure, beyond a reasonable doubt.

Unlikely related

- The AE is doubtfully related to the study drug/procedure.

Possibly related

- The AE may be related to the study drug/procedure.

Probably related

- The AE is likely related to the study drug/procedure.

Definitely related

- The AE is clearly related to the study drug/procedure.

For the purposes of reporting to regulatory agencies, AEs deemed 'definitely related', 'probably related' or 'possibly related' will be considered related to study treatment; and those deemed 'unrelated' or 'unlikely related' are considered unrelated to study treatment.

AEs listed as related are considered to have a suspected 'reasonable causal relationship' to the study drug/intervention (ICH E2A). The expression 'reasonable causal relationship' is meant to convey in general that there are facts (evidence) or arguments to suggest a causal relationship.

- **Action taken**: none, drug treatment, subject withdrawn, other (specified).
- **Outcome**: recovered/resolved, recovering/resolving, not recovered/not resolved, or unknown.

14.3 Procedures for dealing with serious adverse events

In the event of any SAE which, in the investigator's opinion, justifies termination or modification of the trial (see section 8.4), dosing will be stopped and the sponsor's medical monitor will be informed immediately (within 24 h of the investigator becoming aware of the event) by telephone or email, as follows.

Medical monitor:	[redacted] MD
Tel:	[redacted]
Mobile:	[redacted]
Email:	[redacted]

All SAEs occurring after the signing of the ICF until the follow up visit, regardless of study drug relationship, must be reported to the sponsor's pharmacovigilance

department, with as much information as possible, within 24 h of the investigator becoming aware of the event. The investigator will complete an SAE form and provide it to the sponsor's pharmacovigilance department, via the following contact details.

Monitor:	[redacted] MD
Tel:	[redacted]
Mobile:	[redacted]
Email:	[redacted]

The sponsor will also be notified by email [redacted]

The investigator will notify the REC of SAEs that occur during this trial, if applicable, in accordance with the SOPs issued by the Research Ethics Service (RES)[23].

The sponsor is responsible for determining the expectedness of the event, using the reference safety information in the IB[1]. The sponsor will notify the MHRA of all suspected unexpected serious adverse reactions (SUSARs), and will be responsible for ensuring that the REC is notified of SUSARs, if applicable. SUSARs that are fatal or life-threatening must be notified to the MHRA and REC within 7 days after the sponsor has learned of them. Other SUSARs must be reported to the REC and MHRA within 15 days after the sponsor has learned of them.

If a subject has an SAE during the study, they will have an additional telephone follow-up as described in section 12.3.

14.4 Procedures for handling withdrawals due to adverse events

The investigator will assess the reason for withdrawal as far as possible and will fully record the circumstances and medical details. Provided that subjects give written informed consent, they will undergo the standard medical examination and laboratory tests at withdrawal from the trial which they would have undergone had they completed it (see also section 9.4).

14.5 Procedures for reporting pregnancies

Subjects will be asked to follow the contraception guidance in section 11.

If, during the study, the investigator becomes aware of a pregnancy in a subject or their partner, they will inform the sponsor's pharmacovigilance department immediately (within 24 h of the investigator becoming aware of the event), as follows.

Monitor:	[redacted] MD
Tel:	[redacted]
Mobile:	[redacted]
Email:	[redacted]

The investigator will follow-up the pregnancy according to HMR SOPs, provided the subject (or their partner) consents to that. A pregnancy will not constitute an SAE unless it meets one of the criteria in section 14.1.

15 Data management and quality assurance

Data will be securely stored within HMR and Invicro. Data collected in paper source documents at HMR (see section 18.2) will be transcribed into an electronic CRF (eCRF).

The investigator is responsible for ensuring the accuracy and completeness of the data entered into the eCRF, and the timeliness of data entry. Clinical data (including AEs, concomitant medication, etc) will be entered into a 21 CFR Part 11-compliant Medrio M-1 database. The Medrio M-1 system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Data reported in the eCRF, derived from source documents, should be consistent with the source documents or the discrepancies should be explained. Where the data violate data validation checks, queries will be generated for resolution by clinical staff. All edits made to the database upon resolution of queries will be recorded in an electronic audit trail.

The database will be locked after the following have been completed: all expected eCRF data have been entered and accounted for; all discrepancies have been resolved; data have been coded as appropriate; SAEs have been reconciled; all site audit findings impacting the database have been closed; and QC inspection has been completed.

Data in source documents will be checked by the HMR Quality Assurance (QA) Department. In addition, the HMR QA Department will audit the trial report; that audit will include checks to ensure that statistical output is correctly reproduced in the report. If requested, the investigator will provide the sponsor, MHRA, and REC with direct access to the original source documents.

16 Statistical methods

16.1 Pharmacokinetic methods

The PK analysis will be done by the Statistics and Data Management Department at HMR, using WinNonlin 8.3 or higher.

Actual times will be used to derive PK parameters. Missing data will not be imputed.

For calculation of all PK parameters, and for individual concentration–time plots, plasma concentrations below the limit of quantification of the assay (BLQ) will be treated as follows: values that occur before t_{max} will be taken as zero; all other values will be taken as missing.

For calculation of plasma concentration summary statistics, BLQ values will be taken as zero, unless they fall between two quantifiable concentrations, in which case they will be treated as missing.

The pharmacokinetic parameters listed in section 16.1.1 will be derived.

16.1.1 Plasma Parameters

Text symbol	Definition	Calculation
	Concentrations during and after multiple dosing	
C_{trough}	Trough plasma concentration	Trough plasma concentration (measured concentration at the end of a dosing interval at steady state [taken directly before next administration]) obtained directly from the concentration–time data.
	Concentrations and times (Day 1 and after final dose)	
C_{max}	Maximum (peak) plasma concentration	Obtained directly from the concentration–time data.
C_{max}/Dose	Dose-normalised C_{max}	Calculated as C_{max} /Dose administered
t_{max}	Time to reach maximum (peak) plasma concentration	Obtained directly from the concentration–time data.
	Half-life (after final dose)	
λ_z	Terminal rate constant	Estimated by linear regression of logarithmically transformed concentration versus time data.
$t_{½}$	Terminal half-life	Calculated from the terminal slope of the log concentration–time curve, as follows: $t_{½} = \frac{\log_e 2}{\lambda_z}$
	Areas under the curve	
AUC_{tau}	Area under the plasma concentration–time curve during a dosing interval (tau)	Calculated using the trapezoidal method.
AUC_{last} (after final dose)	Area under the plasma concentration–time curve from time zero to time of last measurable concentration	Calculated using the trapezoidal method.

Text symbol	Definition	Calculation
AUC_{inf} (after final dose)	Area under the plasma concentration–time curve from time zero to infinity	Calculated using the trapezoidal method for the interval 0 to t_{last} (time t_{last} is the time at which the last non-zero level was recorded), plus the area under the exponential curve from t_{last} to infinity, calculated as follows: $AUC_{t-\inf} = \frac{\hat{C}_t}{\lambda_z}$ where $\hat{C}_t$ is the predicted value of the concentration at t_{last}.
AUC_{inf}/Dose (after final dose)	Dose-normalised AUC to infinity	Calculated as AUC_{inf} /Dose administered
$\%AUC_{extrap}$ (after final dose)	Percentage of AUC_∞ extrapolated from t_{last} to infinity	$\%AUC_{extrap} = \frac{100\times AUC_{t-\inf}}{AUC_{\inf}}$
Clearance, volume of distribution and mean residence time (after final dose)		
CL_{SS}/F	Apparent total clearance from plasma after non-intravenous administration calculated at steady state	Calculated using the following formula: $CL_{SS}/F = \frac{Dose}{AUC_{tau}}$
V_z/F	apparent volume of distribution after non-intravenous administration calculated at steady state	Calculated using the following formula: $V_z/F = \frac{Dose}{\lambda_z \bullet AUC_{tau}}$
Accumulation and time invariance ratios		
$R_{ac(AUC)}$	Accumulation ratio for AUC	Calculated from AUC_{tau} at steady state and AUC_{tau} after a single dose
$R_{ac(Cmax)}$	Accumulation ratio for C_{max}	Calculated from C_{max} at steady state and C_{max} after a single dose

16.2 Statistical methods

16.2.1 Planned analyses

Final statistical analysis, including analysis of PK parameters, will be done by HMR. A statistical analysis plan will be prepared by the HMR Statistics and Data Management Department after completion of the final protocol and before database lock.

All statistical analysis and reporting will be done using SAS 9.4 or higher.

16.2.2 Statistical hypotheses

The trial is an exploratory one, and there are no null hypotheses to be tested.

16.2.3 Analysis populations

The following populations will be identified:

Safety population:	All subjects who received at least one dose of study drug.
PET population:	All subjects in the safety population who had a baseline PET scan, at least 1 post-baseline PET scan, and a PK result immediately preceding a PET scan.
PD analysis population:	All subjects in the safety population for who a PBMC measure is available.
PK analysis population:	The PK analysis population will consist of the subjects who provide evaluable data for the comparisons of interest. These subjects should have at least one quantifiable plasma concentration, should not have violated any major entry criterion likely to confound the PK analysis, and should not have deviated significantly from the protocol between enrolment and successful study completion.

The primary endpoint will be analysed using the PET and PD populations.

16.2.4 General considerations for data analyses

The minimum set of summary statistics for numeric variables will be: n, mean, standard deviation (or standard error), median, minimum, and maximum. 95% confidence intervals (CI) will be presented where appropriate for data interpretation.

Categorical data will be summarised in frequency tables with n and percentage. Summaries of a categorical variable will include all recorded values.

The minimum and maximum values will be presented to the same number of decimal places as the raw data collected on the CRF (or to 3 significant figures for derived parameters). The mean, median and percentiles (eg Q1, and Q3) will be presented to one additional decimal place. The standard deviation (SD) and standard error will be presented to 2 additional decimal places.

'Baseline' will be the latest value obtained before IMP administration (predose on Day 1, or Day –1 if not recorded at predose, or screening if not recorded at predose or on Day –1). Out-of-range laboratory tests may be repeated. If a test is out of-range at baseline and repeated before dosing, the latest repeat value before dosing will be used as baseline. However, if a test is out-of-range and repeated at any other time during the study, the out-of-range value (not the repeat value) will be included in statistical summaries.

16.2.5 Study population analyses

16.2.5.1 Disposition of subjects

The disposition of all subjects in all analysis populations will be summarised including: number completing the study, by treatment; and number discontinued from the study.

All subjects who withdraw or are withdrawn from the study will be listed, by treatment, with the reason for withdrawal.

16.2.5.2 Demographic and baseline characteristics

Demographic and baseline characteristics (eg physical examination, vital signs and ECGs) will be summarised.

Subjects who take concomitant medication will be listed. All non-trial medication will be coded using the World Health Organisation (WHO) ATC index (version 2021 or higher).

Medical and surgical history data will also be listed. Medical and surgical history will be coded using the version of the Medical Dictionary for Regulatory Activities (MedDRA) current at the time of database lock.

16.2.5.3 Treatment compliance

Dates and times of dosing will be listed.

16.2.6 Safety data analyses

Summaries and listings of safety data will use the safety population.

16.2.6.1 Adverse events

AEs will be coded using the version of the Medical Dictionary for Regulatory Activities (MedDRA) current at the time of database lock.

All AEs will be listed.

A treatment-emergent adverse event (TEAE) is an AE that emerges during treatment (having been absent before treatment) or that worsens after treatment[14].

The number of subjects with at least one TEAE will be tabulated by actual treatment and MedDRA system organ class and preferred term.

For each of the following, the number of TEAEs and the number of subjects with TEAEs will be summarised by actual treatment as follows:

- TEAEs, by system organ class and preferred term
- drug-related TEAEs, by system organ class and preferred term

Subjects with more than one TEAE will be counted only once, at the maximum causality, for each system organ class and preferred term. AEs with missing severity and/or causality will be treated as severe and possibly related, respectively.

AEs leading to withdrawal, deaths and other SAEs will be listed separately (fatal events will be listed separately from non-fatal events).

16.2.6.2 Clinical laboratory evaluations

Data from haematology, coagulation, clinical chemistry, and urinalysis will be summarised by treatment.

Data from haematology, coagulation and clinical chemistry outside the normal range will be listed separately and summarised.

16.2.6.3 Other safety measures

Vital signs at each planned assessment, and change in vital signs from baseline at each planned post-baseline assessment, will be summarised by actual treatment.

Vital signs data outside of the normal range will be listed and summarised.

A separate listing of vital sign findings, classified as clinically significant by the investigator will also be provided.

QT interval will be corrected using Fridericia's (QTcF) formulae.

ECG variables will be summarised by treatment and timepoint. Differences from baseline will be summarised by treatment and timepoint.

QTcF values > 450 msec, PR interval shortening < 110 msec, and PR interval prolongation >220 msec, and increases of QTcF from baseline of > 30 msec and > 60 msec, will be listed by treatment and timepoint and summarised. A separate listing of ECG findings classified as abnormal by the investigator will also be provided.

Abnormal physical and neurological examination findings will be listed.

Positive C-SSRS data will be listed.

16.2.7 PET data analyses

Start and end date of each PET scan will be listed together with the scan start/stop time and the Dose drawn up, Dose administered, Residual 1, and Residual 2.

[^{18}F]-IMA601 regional total volume of distribution (V_T) at each brain scan will be listed for each session and for change from baseline.

The RO values will be listed for imaging sessions 2 and 3, respectively, together with the plasma concentration of ASN51 at the time of the start of each postdose PET scan, which will be calculated by log-linear interpolation from the two closest PK data points by subject and treatment (to be provided by the sponsor).

These concentration data will be transferred to Invicro for the PK-RO analysis. The PK-RO analysis will be provided by Invicro and sent to HMR for the inclusion in the CSR.

16.2.8 Pharmacokinetic data analyses

PK data will be summarised using the PK analysis population.

For log-transformed parameters, the primary measure of central tendency will be the geometric mean[15]; for untransformed parameters, it will be the arithmetic mean or median.

For all variables, N (number of subjects in receiving the treatment in the population), n (number of observations), arithmetic mean, median, minimum, maximum, SD, %CV, and the 95% CI of the arithmetic mean will be derived. For log-transformed variables, all of the above plus the geometric mean, its 95% CI, and the SD of the log-transformed variables, will be provided.

Plasma concentrations and PK parameters will be listed and summarised, by treatment, using descriptive statistics. Individual and mean plasma concentration–time profiles will be presented graphically. All available data will be used to derive PK parameters in individual subjects.

16.2.9 Pharmacodynamic data analyses

O-GlcNAcylation of PBMCs will be summarised using the PD population.

Time invariance of target engagement will be investigated by comparing the PD and PK/PD relationship over time.

Food effect on the PBMC assay will be investigated by comparing Day 11 and 14 PBMC data.

In addition to the descriptive statistics of the PBMC data (ie N, mean, median, SD, min, max, CV%), time-matched PBMC parameters (Day 1 vs Day 14, and Day 11 vs Day 14) will be assessed using an Analyses of variance (ANOVA) approach. ANOVA will be performed on the natural log(ln)-transformed PBMC parameters. Each ANOVA will include calculation of least square means (LSM), the difference between LSM of the two conditions, and the standard error and 90% confidence interval (CI) associated with this difference. The LSM, difference in LSMs, and associated 90% CI will be back transformed to present geometric LSM, the ratio of geometric means, and associated 90% CI. The 90% CI of geometric mean ratio will be calculated to check whether it lies within the interval 0.70 to 1.43.

Furthermore, PBMC PK/PD of Day 1 and Day 14 will be investigated by a regression analysis, where slope and intercept will be compared with appropriate statistical methods descriptively.

16.3 Determination of sample size

Since this trial is hypothesis generating, no formal calculation of sample size is appropriate.

The sample size chosen is considered adequate to allow modelling of the relationship between ASN51 plasma concentrations and pharmacodynamic target engagement using a PBMC O-GlcNAcylation assay.

PET analyses of 8 subjects is sufficient to define the occupancy of O-GlcNAcase and is within the range generally accepted for PET studies.

17 Ethical and regulatory requirements

The trial proposal will be reviewed by a recognised REC, and by the MHRA. The trial will not proceed unless the sponsor obtains from the MHRA a clinical trial authorisation (CTA), and the REC approves the trial. The trial will not proceed at any site until that site has obtained site-specific approval. No subject will have a PET scan without approval from the Administration of Radioactive Substances Advisory Committee (ARSAC).

The trial will be done at HMR, in compliance with The Medicines for Human Use (Clinical Trials) Regulations 2004 and current amendments[19], The Medicines for Human Use (Clinical Trials) (Amendment) (EU Exit) Regulations 2019[20], The Human Medicines (Amendment etc) (EU Exit) Regulations of 2019 and 2020[21,22], GMP[13], the SOPs issued by RES for RECs in the UK[23], and Good Clinical Practice, which has its origins in the Declaration of Helsinki.

All subjects must give written consent to participate in this trial. Consent for screening evaluations may be obtained using the information and consent form for the HMR healthy volunteer panel, which has been approved by the Health Research Authority's Generic Review Committee. The trial-specific information and consent form will be signed by the subject either before any screening evaluation or after the investigator confirms the eligibility of the subject for the trial and before the subject is randomised to receive the first administration of IMP. Before giving consent, subjects must read the information sheet about the trial. They must also read the consent form. They will then discuss the trial with the investigator or his deputy and be given the opportunity to ask questions. The trial-specific information sheet and the consent form must be approved by the REC.

Each subject is free to withdraw from the trial at any time, without giving a reason. If a subject withdraws, the investigator will ask the subject to consent to a follow-up examination. For withdrawn subjects, the investigator will use a special information and consent form which has been ethically approved. If the subject consents to the follow-up examination but asks the investigator to destroy all identifiable samples taken from the subject and/or not enter into the eCRF results of the follow-up examination, the investigator will comply with the subject's requests.

The sponsor will ensure that the MHRA and the REC, are informed promptly of SUSARs (see section 14.3), and that any new reports of SUSARs from other ongoing trials of the IMPs under investigation in this trial are notified to the MHRA, and to the REC, if applicable. The sponsor will provide the investigator, the REC and the MHRA with annual safety reports of each IMP under investigation, and listings of all suspected serious adverse reaction (SSAR) reports. The sponsor will also inform the investigator promptly of any new safety or toxicology data that might affect the safety of the subjects in this study.

The investigator will promptly inform the sponsor and, if applicable, the REC of any SAE that occurs during this trial (see section 14.3). The investigator will provide the REC with annual progress reports of the trial, if the trial lasts longer than a year.

The investigator will report to the REC any protocol deviation that is, in his opinion, of clinical significance. The investigator will also inform the REC in the event of several deviations which, although of no clinical significance, cause inconvenience and/or discomfort to the volunteers. The sponsor will notify the MHRA and REC of any serious breach of GCP (for example, the investigator puts subjects' safety at risk, falsifies data, or persistently fails to comply with this protocol or good clinical practice).

Within 90 days after the end of the trial, the sponsor will ensure that the REC and the MHRA are notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial.

The sponsor will supply a summary of the clinical trial report to the MHRA and REC within 1 year after the end of the trial.

Trial procedures at HMR will be subject to audits by the HMR QA Department, to ensure compliance with the protocol and applicable regulatory requirements.

18 Trial documentation

18.1 Protocol amendments

After the protocol has been approved by the REC and the MHRA, no changes may be made without the agreement of both the investigator and the sponsor.

The MHRA and REC do not need to approve any substantial change to the protocol that needs to be implemented urgently to avoid an immediate hazard to trial subjects. The sponsor will ensure that the MHRA and REC are informed of urgent amendments or a temporary halt to the trial in accordance with The Medicines for Human Use (Clinical Trials) Regulations 2004 (and current amendments)[19] and the SOPs issued by RES for NHS RECs[23].

All agreed protocol amendments will be recorded on a written agreement which will be signed and dated by the investigator and sponsor, and attached to the original

protocol. The REC and/or MHRA must approve substantial amendments before they are implemented.

18.2 Case report forms

The eCRF will be designed and produced by HMR.

To preserve confidentiality, the CRF will not bear the subject's name. The subject number and/or HMR volunteer number will be used for identification.

The investigator is responsible for ensuring the accuracy and completeness of the data entered into the eCRF, and the timeliness of data entry. Clinical data (including AEs, concomitant medication, etc) will be entered into a 21 CFR Part 11-compliant Medrio database. The Medrio system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Data reported in the eCRF, derived from source documents, should be consistent with the source documents or the discrepancies should be explained. Where the data violate validation checks, queries will be generated for resolution by clinical staff. All edits made to the database upon resolution of queries will be recorded in an electronic audit trail.

Source documents

Before the start of the study, the sponsor and investigator will sign an agreement listing the source documents to be used in this trial.

18.3 Reporting of results

HMR will prepare a draft report for discussion with the sponsor. The report will contain results and discussion of the trial, to which will be attached tables, figures and listings in compliance with ICH E3[24].

Completed eCRFs will be supplied separately to the sponsor by HMR.

19 Obligations of the sponsor and investigator

19.1 Monitoring, auditing, and inspection

The trial will be monitored by the sponsor.

A sample of documents generated by HMR which form part of this trial, and the ensuing data, will be audited by the HMR Quality Assurance Department to assess compliance with the quality management system of HMR. That system incorporates the requirements of The Medicines for Human Use (Clinical Trials) Regulations 2004 (and current amendments)[19], ICH GCP, GMP[13], and the SOPs issued by RES for RECs in the UK[23], and is based on ISO 9001.

The sponsor may do a quality assurance audit, and regulatory authorities may inspect this study, at any time during or after the study. The sponsor and investigator agree

to allow auditors and inspectors direct access to all relevant documents, and to allocate time to discuss findings with the auditors or inspectors.

19.2 Compensation of volunteers

The sponsor agrees to abide by the Association of the British Pharmaceutical Industry Guidelines for medical experiments in non-patient human volunteers (2018 edition)[25], and undertakes to compensate the subjects for injuries which are considered, on the balance of probabilities, to have arisen as a result of their participation in the trial.

19.3 Confidentiality

All personal details of the participating subjects and the results of the trial will be kept strictly confidential. Each subject's GP (or equivalent physician) will be informed of the nature and timing of the trial.

All unpublished documents including the protocol, the CRF, and the IB are confidential. Those documents cannot be disclosed to a third party without the written consent of the sponsor. However, submission of those documents to a REC is expressly permitted.

The investigator agrees that the sponsor maintains the right to use the results of this trial, in their original form and/or in a global report, for submission to governmental and regulatory authorities of any country.

19.4 Publication

If the data merit, the investigator and the sponsor will discuss the preparation of a manuscript for publication in a peer-reviewed professional journal or an abstract for presentation, oral or written, to a learned society or symposium. Either party may undertake the task but both must agree to the strategy before the work is started. Each party will allow the other 30 days to comment before any results are submitted for publication or presentation. Authorship should reflect work done by the investigators and personnel of the sponsor, in accordance with generally recognised principles of scientific collaboration.

19.5 Archiving

The sponsor and HMR will keep in a trial master file all the essential documents required by GCP. HMR will ensure that the investigator's master file, and all data generated during the trial, will be archived in a secure place for at least 25 years. Documents will be stored such that they are readily available for inspection at the request of the sponsor or a regulatory authority. Any transfer of ownership of the investigator's data or documents will be documented, and the sponsor will be informed.

20 Premature termination of the trial

The sponsor and investigator reserve the right to terminate this trial should severe AEs, SAEs or any other safety issue occur during the trial. If the trial is terminated prematurely, and the sponsor or investigator, as appropriate, will provide a written statement of the reasons for termination. The sponsor will ensure that the MHRA and REC are notified, as described in section 17.

21 References

1. World Health Organisation. https://www.who.int/news-room/fact-sheets/detail/dementia