Manchester
Academic Health Science Centre

Imaging cerebral neuro-inflammation in acute and chronic cerebrovascular disease: a predictor of outcome and biomarker for guiding treatment (IN-CVD)

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EudraCT Number: **2014-000591-26**

ISRCTN No **13797354**

Protocol Version: **VERSION 4.0 (15/03/2016)**

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SIGNATURES/PROTOCOL APPROVAL

This document describes the IN-CVD trial and provides information about procedures for entering patients into it.

The protocol should not be used as a guide for the treatment of patients outside the trial.

Every care was taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to Investigators in the trial, but centres entering patients for the first time are advised to contact the Trial Manager at the MAHSC-CTU to confirm they have the most recent and approved version.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) regulations 2004 and ICH Good Clinical Practice guidelines. The trial will be conducted in compliance with the protocol, the Data Protection Act 1998, the Declaration of Helsinki, Human Tissue Act (2004), the Research Governance Framework (2005) and other regulatory requirements as appropriate.

Protocol Authorised by:

Name & Role	Site	Signature	Date
Professor. Karl Herholz (Chief Investigator)	Wolfson Molecular Imaging Centre, University of Manchester		
Dr Gillian Heap (Sponsor Representative)	The Christie NHS Foundation Trust		

Principal Investigator

I, as Principal Investigator for the **IN-CVD** trial confirm that I will be responsible to ensure that all members of the local clinical trial team are appropriately trained on the trial protocol and have the relevant qualifications and experience to carry out their role in accordance with the trial protocol.

Name	Site	Signature	Date

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LIST OF ABBREVIATIONS

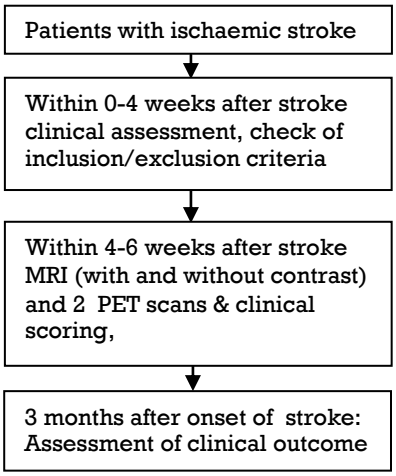
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRP	C-Reactive Protein
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CTPM	Clinical Trial Project Manager
CVD	Cerebral Vascular Disease
EC	European Commission
EU	European Union
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
IB	Investigator Brochure
ICF	Informed Consent Form
IDMC	Independent Data Monitoring Committee
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
MA	Marketing Authorisation
MAHSC – CTU	Manchester Academic Health Science Centre – Clinical Trials Unit
MCA	Middle Cerebral Artery
MHRA	Medicines and Healthcare products Regulatory Agency
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Score
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NHS R&D	National Health Service Research & Development
NIHSS	National Institutes of Health Stroke Scale
PET	Positron Emission Tomography
PI	Principal Investigator
PIS	Participant Information Sheet
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
RCT	Randomised Control Trial
REC	Research Ethics Committee
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SmPC	Summary of Product Characteristics
SOP(s)	Standard Operating Procedure(s)

SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
TSPO	Translocator protein
VCI	Vascular Cognitive Impairment
WMIC	Wolfson Molecular Imaging Centre

TRIAL SUMMARY

Title:	Imaging cerebral neuroinflammation in acute and chronic cerebrovascular disease: a predictor of outcome and biomarker for guiding treatment.
Design:	Observational, cross-over study, two phase study.
Objectives:	*Phase 1 (months 1-12):* 1) Evaluating the tolerability of scanning using a questionnaire given to the participants, asking them to grade the discomfort associated with the [^{18}F]GE-180 procedure on a 5 point scale, the average score must be neutral (score 3) or better. 2) The technical feasibility will be assessed by evaluating the Translocator Protein TSPO on imaging using the [^{18}F]GE-180 as the radiotracer. In particular, we will examine whether patients with the heterozygote genotype (identified by blood tests but analysed retrospectively), who are expected to present as medium binders, will provide quantifiable scans. 3) A Correlation coefficient of 0.5 or higher between the focal abnormalities observed with the two different tracers in ischemic stroke patients, indicates that measurements are comparable. *Phase 2 (months 13-39):* *1)* Examine whether the extent and intensity of microglial activation is related to functional outcome in stroke patients (disability scores, such as the NIHSS and the Modified Rankin Score, relative to initial assessment) *2)* Determine the location of microglial activation including infarct border zones and functionally connected brain regions in comparison with structural lesions as delineated on MRI and neurological deficits in patients after stroke *3)* Examine whether the intensity of microglial activation is related to systemic inflammation
Endpoints	*1)* Data acquisition in at least 10 patients and feasibility analysis completed within 12 months. *2)* Data acquisition on 50 patients (shown to not have homozygote genotype) and collection of functional outcome data (as assessed at 3 month follow-up). *3)* Analysis and statistical comparison of MRI/PET scans compared to the functional outcome.
Patient Group:	Ischaemic stroke patients (n=50 excluding patients with homozygote genotype)
Eligibility	Ischaemic stroke in MCA territory (I63.0 to I63.5), mild to moderate severity (Modified Rankin Scale score1-3)

Trial Treatment and methods:



Trial duration per participant: 12 weeks (± 2 weeks)

Estimated total trial duration: 48 months

Total number of participants planned:
Phase 1 = 10 patients
Phase 2 = 40 patients
TOTAL number = 50
(Recruited from the research hospital sites where initial clinical assessment is carried out and 3 month follow-up; All other procedures will be performed at WMIC and Central Manchester University Hospital NHS Foundation Trust).

1.0 BACKGROUND

Reduced blood supply to the brain causes ischaemia and can result in severe tissue damage. An acute and severe regional blood flow reduction would typically cause a stroke, while a less severe but chronic and widespread reduction may cause impairment of brain function, including an impairment of cognitive processes e.g. memory and action planning. Although the severity and duration of the blood flow impairment is an important factor to predict the severity of symptoms and functionality, basic and clinical research has demonstrated that inflammation also contributes to the outcome.

Inflammation of brain tissue and blood vessels can increase the severity of stroke, and there are also indications that it may increase the severity of cognitive impairment. Microglial cells are an important type of glial cell. These non-neuronal cells have several functions in supporting nerve cells, including regulating immune and inflammatory responses in the brain. Microglia may damage tissue as a result of secreting inflammatory proteins (cytokines), but will also ultimately contribute to the removal of irreversibly damaged brain cells [Ransohoff and Cardona, (2010)]. Activated microglia are also present along neuronal pathways in areas remote from the initial damage, and probably contribute to the impairment of neuronal function and limit recovery in stroke patients [Dirnagl *et al.*, (1999)].

Acute stroke and vascular cognitive impairment (VCI) are part of a spectrum of cerebrovascular disease. In acute cerebral infarcts, the penumbra accumulates an abundance of activated microglia [Ransohoff and Cardona, (2010)]. The presence of microglia-derived brain macrophages is an important contributor to the early and late stages of the tissue pathology and therefore therapeutic strategies aimed at modulating microglial activation are key [Dirnagl *et al.*, (1999)]. Experimental and clinical studies have demonstrated that the neuroinflammatory response contributes to the extent of brain tissue damage and can be primed by systemic inflammation [Okun *et al.* (2011), Emsley *et al.* (2007), Drake *et al* (2011)]. However, neuroinflammation cannot satisfactorily be detected by current clinical brain imaging methods. Using better imaging techniques in anticipation of identifying patients with increased microglial activation, will permit us to more accurately correlate clinical outcomes in practice and in clinical studies using anti-inflammatory drugs [Emsley *et al.* (2005)].

1.1 Rationale for the proposed trial

There are two main objectives for the study; initially to evaluate whether a technique for microglial imaging is feasible and secondly to evaluate whether imaging of microglial activation predicts outcome and clinical progression in patients with stroke.

PET and the radiotracer [^{11}C]-(R)-PK11195 can provide a quantitative assessment of microglial activation in human subjects. [^{11}C]-(R)-PK11195 (1-[2-chlorophenyl]-N-methyl-N-[1-methyl-propyl]-3-isoquinoline carboxamide) is a specific ligand for the mitochondrial 18kDa translocator protein (TSPO), which is particularly abundant in activated microglia. Labelled with carbon-11 (^{11}C), the radiotracer has been used to image activated microglia in several neurological disorders including completed ischaemic stroke [Pappata *et al.* (2000), Garhard *et al.* (2005), Price *et al.* (2006), Thiel *et al.* (2010)]. Clinical and preclinical animal model data suggest that it is a very sensitive tool to monitor the evolution of microglial activation after a stroke demonstrating the early recruitment of microglia in the ischemic border zone and later involvement of the neocortex and thalamus [Myers *et al.* (1991)]. Unfortunately, the physical half-life time of ^{11}C (~20 min) is too short to allow routine clinical use of [^{11}C]-(R)-PK11195. This study however, will measure the extent of the inflammation in patients with stroke using positron emission tomography (PET) and a new radiotracer ([^{18}F]GE-180) that binds specifically to activated microglia.

In the past few years, a new generation of tracer has been developed [Winkeler *et al.* (2010)]. (S)-N,N-diethyl-9-(2-[18F]fluoroethyl)-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide (^{18}F-GEH120714, [^{18}F]GE-180) is a novel PET ligand with high affinity (0.87 nM) and selectivity for TSPO. Additionally, it has an *in vivo* performance superior to that of [^{11}C]-(R)-PK11195. Labelling with fluorine-18 (^{18}F) that has a physical half-life of 110 minutes, makes it much more amenable for routine clinical use. [^{18}F]GE-180 is being developed by GE Healthcare (patent WO2011117421) as a clinical PET biomarker of microglial activation.

All second-generation tracers provide high-affinity binding with low background, but signal intensity is influenced by a genetic polymorphism (Ala147Thr) that has recently been identified on the TSPO gene [Owen *et al.* (2012)]. For each individual, the genetic polymorphism and the associated binding status can be determined using blood samples.

Previous PET studies of microglial activation in similar, but smaller patient groups using [^{11}C]-(R)-PK11195 have been conducted. In a study of volunteers at high risk for ischaemic stroke, a significant relationship of systemic inflammation, as indicated by increased plasma C-reactive protein (CRP) levels was found [Drake *et al* (2011)]. In another study in patients with mild cognitive impairment and Alzheimer's disease, increased microglial binding was found [Edison *et al.* (2008)]. A recently completed study in patients with gliomas compared to normal controls found an increased signal in malignant tumours and, to a lesser degree but still significant, also in normal brain tissue. Methods for TSPO receptor quantification using supervised reference tissue extraction techniques have been developed and validated [Turkheimer *et al.* (2007)].

Preclinical studies using [^{18}F]GE-180 in WMIC laboratory demonstrated excellent TSPO binding [Owen *et al.* (2011)]. [^{18}F]GE-180 uptake was significantly higher in the core of ischaemic lesions (+24%) and lower in the healthy contralateral tissue (-18%) than [^{11}C]-(R)-PK11195 and thus gave a significantly better contrast between the lesion and the healthy tissue. Full displacement of [^{18}F]GE-180 was obtained within 5-10min post-injection of the cold ligand (Figure 1). Pilot studies in human patients are currently being conducted by GE Healthcare in Canada. These data also indicate that the biological properties of [^{18}F]GE-180 are similar to 18F-DPA-714, suggesting high specific binding with moderate dependency on receptor activation status [Owen *et al.* (2011)].

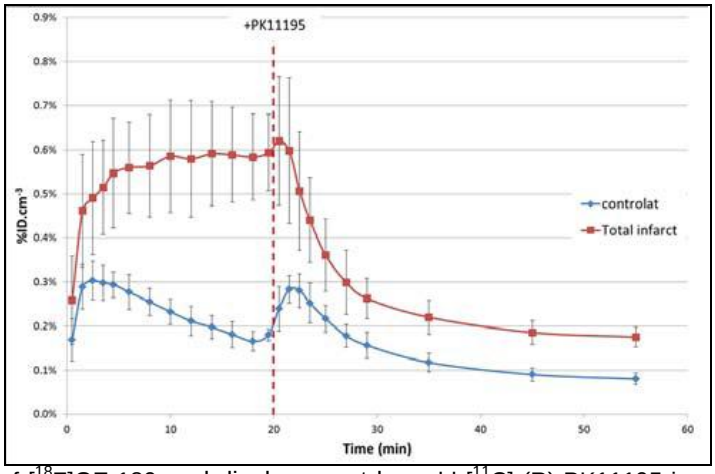


Figure 1. Accumulation of [^{18}F]GE-180 and displacement by cold [^{11}C]-(R)-PK11195 in an experimental ischemic infract [Dickens *et al.* (2014)].

By evaluating the relationship of the inflammatory changes with the clinical symptoms and their change (progression or recovery) after 3 months, it will be determined whether neuroinflammation does have an impact on the severity and prognosis of clinical impairment. This will answer the important issue of whether microglial activation is actually associated with more severe symptoms and poor clinical outcome and will potentially open new avenues for effective drug development to target this pathophysiological mechanism. This is highly relevant for treatment since drugs that aim to suppress neuroinflammation are currently being tested, and the study will indicate whether these drugs could also be beneficial to patients with stroke.

2.0 TRIAL OBJECTIVES AND ENDPOINTS

The study aims to evaluate the association between microglial activation and the outcome in stroke patients. The data produced by the study will assess whether neuroinflammation has an impact on the severity and prognosis of clinical impairment in stroke patients. The first year of the trial will assess the technical feasibility, whilst ensuring that the patients can tolerate the

scanning procedures. If phase 1 of the study is successful, the trial will move onto phase 2. During phase 2 of the study, data will be collected on a further 40 patients, ensuring that by the end of the trial there will be data on 50 stroke patients. The data from the entire study will be used to identify the location and quantifying the degree of microglial activation after stroke and determine if a correlation between clinical outcome and inflammation can be demonstrated.

2.1 Phase 1 Objective

The initial objective is to evaluate whether the proposed technique for microglial imaging is feasible. This evaluation will be performed over the first twelve months, (or as soon as the first 10 patients have received both [^{11}C]-(R)-PK11195 and [^{18}F]GE-180 scans.) and will reviewed by the IDMC. Criteria of feasibility will be assessed by:

A) Evaluate the tolerability of scanning using a questionnaire given to the participants, asking them to grade the discomfort associated with the [^{18}F]GE-180 procedure on a 5 point scale, the average score from the tolerability questionnaire must be neutral (score 3) or better.

B) Evaluate the technical feasibility of the imaging using the [^{18}F]GE-180 as the radiotracer. In particular, we will examine whether patients with the heterozygote genotype (as identified by the blood test which will be tested retrospectively), who are expected to present as medium binders, will provide quantifiable scans.

C) A Correlation coefficient of 0.5 or higher between the focal abnormalities observed with the two different tracers in ischemic stroke patients, indicates that measurements are comparable.

Analysis of data from the 3 month follow-up assessments are not necessary for the phase 1 data analysis but will be included in the phase 2 analysis.

2.2 Phase 2 Objective

The objective for phase 2 of the study is to determine whether the extent of neuroinflammation has an impact on the severity and prognosis of clinical impairment.

To assess whether imaging of microglial activation predicts outcome and clinical progression in patients with stroke, the clinical status will be compared to the degree of neuroinflammation.
The clinical status will be determined using:

A) The Modified Rankin Scale (mRS), which is being used in most stroke trials as a clinical indicator of the severity of impairment will be assessed.

B) The National Institutes of Health Stroke Scale (NIHSS), which provides a finer grained indicator of functional and neurological impairment will be assessed.

The neuro-inflammation will be assessed using:

A) A comparison between [^{18}F]GE-180 PET findings with the extent of changes in proton diffusion and late structural damage by MRI will be determined.

2.3 Endpoints

Phase 1:

1) Data acquisition in 10 patients.

2) Technical feasibility analysis completed within 12 months. Assessing if it is possible to obtain quantifiable scans from patients with heterozygote genotype and that the frequency of the low-binding genotype (Thr147/Thr147) in this population is rare.

3) Tolerability levels recorded for 10 patients. The threshold for tolerability is the average score of neutral (score 3 out of 5) or better, as assessed by the tolerability questionnaire.

Phase 2:

1) Data acquisition on 50 patients (replacing the patients that are shown to have a low binders, genotype Thr147/Thr147, as assessed by the blood test)

2) Completion and analysis of the clinical assessments (performed 3 months apart) on 50 patients (replacing the patients with a low binders, genotype Thr147/Thr147, as assessed by the blood test).

It is expected that a few patients, even though they might not indicate that at the time of inclusion/consent, will leave the study before undertaking the PET scan. MRI scanning is usually perceived as the most stressful investigation because it involves the patient being scanned in a confined space and it also involves a significant level of noise, compared to PET scanning. Patients leaving the study at this stage before receiving the first PET scan, will be replaced by additional recruits. Patients, who leave the study at a later stage would not be replaced and counted as follow-up failures.

3.0 TRIAL DESIGN

3.1 Overall design

This study aims to recruit 50 patients in total receiving standard of care stroke assessments. Patients in phase 1 (n=10) will be asked to have two PET scans (using [^{11}C]-(R)-PK11195 and

[^{18}F]GE-180) and two MRI scans (one with and one without contrast). If the phase 1 analysis is favourable, the project will progress to phase 2. Patients in the second phase will be asked to have one PET scan (using [^{18}F]GE-180) and two MRI scans (one with and one without contrast). All patients will remain on the study for 3 months after the onset of the stroke , at which point they will have outcome assessments (mRS and NIHSS). Following this, their participation in the study will cease and no further follow-up data will be captured. Patients will receive supportive care as per standard practice.

The study aims to recruit 10 patients in the first year, estimating a rate of 1 per month per NHS site, enabling 2 months for phase 1 review. Phase 2 estimated accrual for this study estimated at 2 patients per month. Thus, patient accrual is expected to be completed within 24 months. The total duration for phase 1 recruitment and analysis (12 months) analysis and phase 2 (24 months recruitment + 3 months for follow-up) recruitment is 39 months. The following 9 months will be dedicated to analysing the scans and completing coefficient correlation analysis on the outcome measure and the neuroinflammation. The total duration of the study is 48 months.

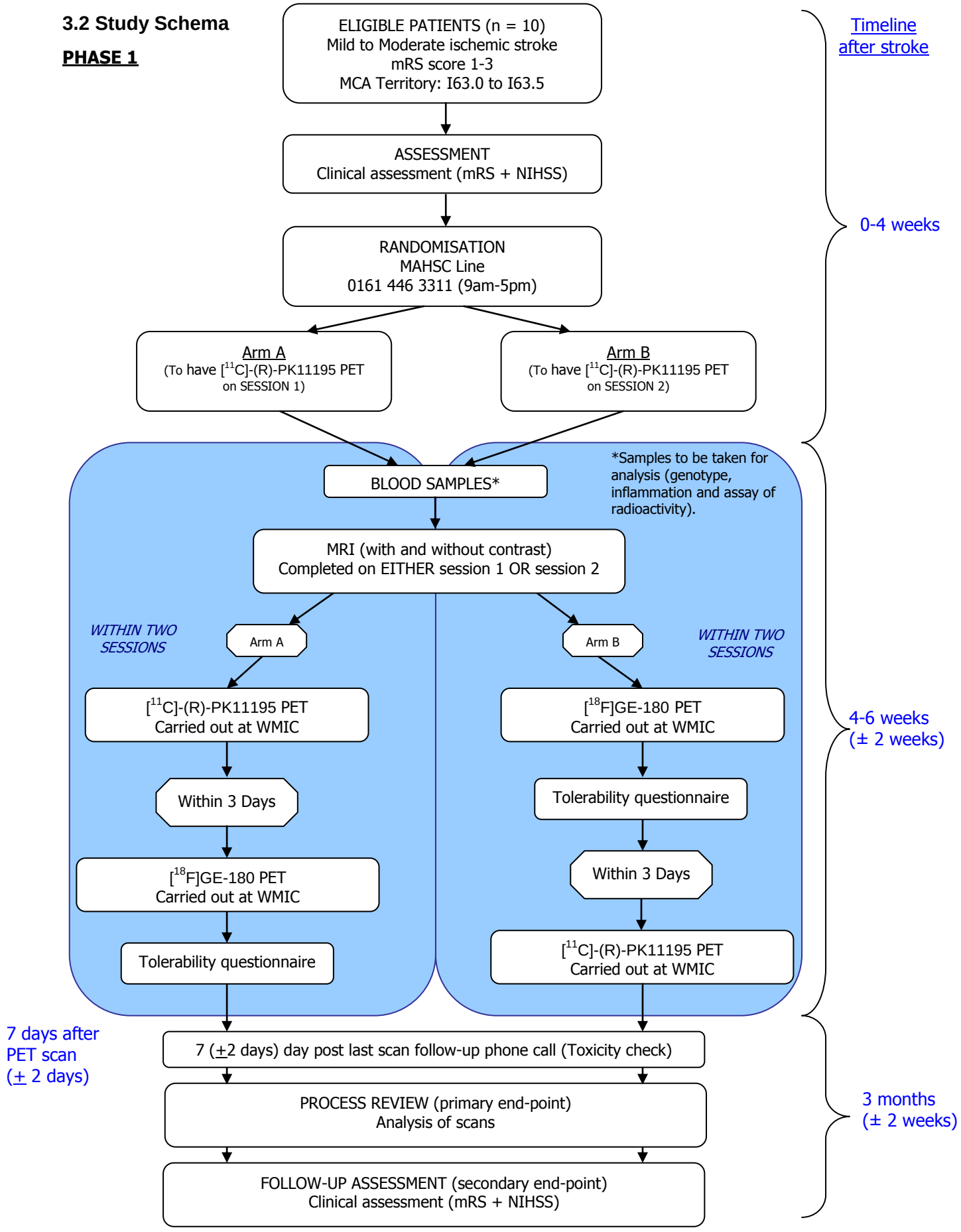
The study is designed to determine whether ^{18}F-GE-180 PET as a biomarker can predict the clinical outcome in patients with cerebrovascular disease (CVD). Patients with sub-acute stroke of mild to moderate severity will be studied using standard research techniques and following the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) criteria for diagnostic studies. In particular, the study protocol will ensure:

- patient selection based on standard diagnostic criteria
- short time interval between initial clinical assessment and PET
- verification of diagnosis at the time of follow-up
- independence of clinical diagnosis and clinical assessment from the result of the index test (PET) by blinding of clinical staff to PET results
- inclusion of failed and uninterpretable test results or withdrawal of consented patients into the final report

The selection of clinical instruments and the timing of assessments in the study are being guided by the design of major stroke trials and by diagnostic studies in vascular cognitive impairment.

3.2 Study Schema

PHASE 1



PHASE 2

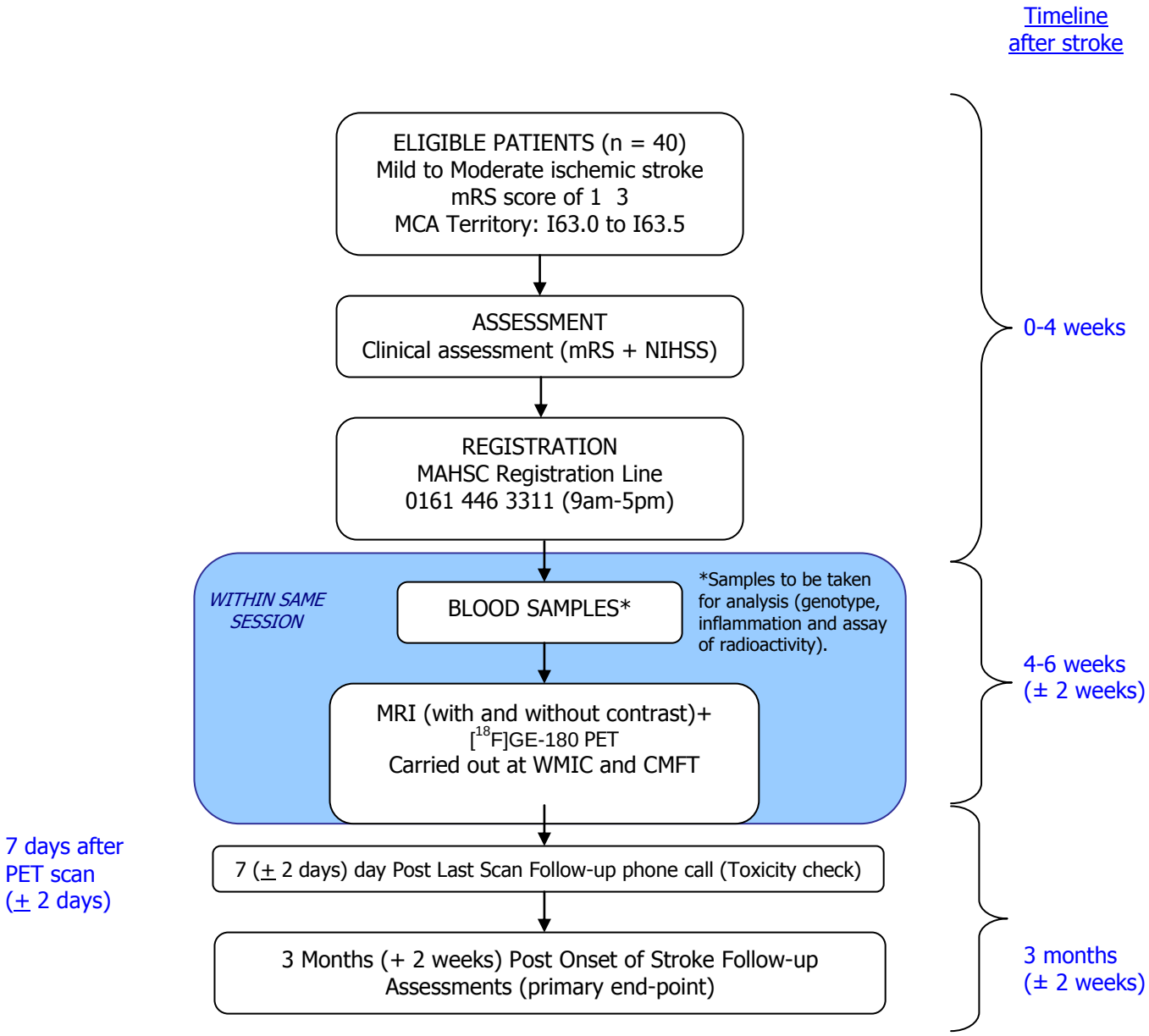


Figure 2. Schematic of the study procedure (Phase 1 and Phase 2)

3.3 Outcome measures

The primary outcome measure will be the functional score on the NIHSS at clinical follow-up after 3 months. The baseline value will be taken into account as a covariate. The Modified Rankin Scale and its change during follow-up will be analysed as a secondary outcome parameter.

- Imaging and outcome parameters will also be correlated with CRP as a marker of systemic inflammation. Regional image analysis will be performed to relate cognitive and motor symptoms with the brain location of structural abnormalities on MR and microglial

activation on PET. Technical feasibility of the [^{18}F]GE-180 scans and the possibility of reducing the scan time to 20 minutes are also among the outcome measures.

4.0 SELECTION OF TRIAL PARTICIPANTS

4.1 Inclusion criteria

Stroke patients (n=50):

1. Age: 18+
2. Ischemic stroke in MCA territory
3. Mild to moderate severity (Modified Rankin Scale score 1-3)

Patients must be able to give Informed consent or patients that wish to consent but are unable to sign or mark the consent form due to mobility issues may give their consent orally in the presence of at least one witness. The witness/es must sign the consent form as evidence that the information was accurately explained to and understood by the participant and that consent was freely given.

4.2 Exclusion criteria

1. Neurological diagnosis of neurodegenerative disease
2. Inability to understand study information and/ or express willingness to consent to the study due to communication difficulties (.
3. History of brain surgery, brain tumour, neuroinflammatory or neurodegenerative disease
4. Severe uncontrolled systemic illness
5. Patients in whom carotid endarterectomy/carotid stenting is due to be carried out within three months of recruitment to the study.
6. Treatment with other drugs known to influence microglial activation, e.g. minocycline , Corticosteroids or Benzodiazepines (2 weeks prior to date of the scan).
7. Pregnancy / Breastfeeding women
8. Contraindications to MRI scanning
9. Patients receiving treatment with Antabuse (see appendix 1 for details)

4.3 Discontinuation / withdrawal of participants and 'stopping rules'

The study would be stopped if any previously unknown serious safety concerns were identified or if new information comes to light that makes the aims or objectives of the study futile or if requested by the study sponsor or funder.

4.3.1 Withdrawal of patients

When consenting to the trial, patients are consenting to trial procedure, trial follow-up and data collection. Patients will be withdrawn from scanning prior to completion for the following reasons:

- Adverse event or an intercurrent illness
- Unable to complete the visits or study documentation (such as questionnaires etc) as required
- Deterioration in health
- Patient decision
- Investigator decision
- For safety reasons e.g. recommendation of IDMC that trial should not continue.
- Benzodiazepines and other drugs known to influence microglial activation, e.g. minocycline or corticosteroids in the last three days prior to the PET scan
- If a patient is withdrawn from the PET scanning prior to having a tracer there is no need to complete the follow-up.

Patients will only be withdrawn from further follow-up in the case of:

- Patient explicitly stating their wish not to contribute further data to the study

4.3.2 Early discontinuation of trial

The early stopping rules for efficacy will be based on the phase 1 analysis. Depending on the outcome of this stage, we will either continue without modification, make appropriate modifications or, in case of insurmountable problems or poor tolerability of the scanning protocol will stop the study. This critical 'Go/No-Go' decision will also be based on advice from the trial steering committee, the sponsor and the funder, who will be informed about study progress, the results of the tracer comparison and patient feedback prior to the second milestone meeting.
Efficacy will be based on:

- The comparison of the new tracer ([^{18}F]GE-180) with the already established [^{11}C]-(R)-PK11195 in 10 patients.
- Patient tolerability questionnaire about the feasibility in terms of experience and comfort.
- Technical feasibility of the scans, whether the participants with the heterozygote genotype will provide quantifiable scans.

An Independent Data Monitoring Committee (IDMC) will be appointed who will oversee patient safety and any recommendations from the IDMC (which may include stopping the trial after phase 1) will be considered by the Trial Steering Group. The IDMC will be presented with the

phase 1 data for consideration and a decision whether to continue onto phase 2 will be reached before the end of month 12.

4.3.3 Withdrawal from trial participation by sites

Should a site choose to close to recruitment the CI or PI must inform MAHSC-CTU in writing. Follow up as per protocol must continue for all patients recruited into the trial at that site and other responsibilities continue as per the written agreement between the trial sponsor and research site.

5.0 RECRUITMENT OF TRIAL PARTICIPANTS

5.1 Identifying Participants

Potentially eligible participants will be identified and approached whilst in hospital. A member of the patient's existing care team will first approach the patient about the trial and if they are interested then the Chief Investigator, Clinical Fellow or other appropriately trained staff (as assigned in the delegation log) will discuss the study with the patient. The participants must have been diagnosed with cerebrovascular disease and meet the inclusion criteria to be considered for this clinical trial.
Patients suffering ischaemic stroke will be eligible for screening. Initial screening will be for modified Rankin Scale 1-3 at time of screening. Patients otherwise satisfying inclusion / exclusion criteria will be recruited and an NIHSS and updated mRS assessment will be performed.

5.2 Consenting Participants

Patients deemed eligible for entry into the trial (after review of diagnosis, treatment history and key eligibility criteria) will be provided with a verbal and written explanation of the trial. The PI, or, where delegated by the PI, other appropriately trained site staff (GCP trained, suitably qualified and experienced), are required to provide a full explanation of the trial and all relevant treatment options to each patient prior to trial entry. During these discussions the information described in the Patient Information Sheet should be discussed with the patient. A contact number should be given to the patient, should they wish to discuss any aspect of the trial with site staff. This will be given to the patients on a patient card, that can be kept on their person at all times.

After adequate time has been given (a minimum of 24 hours), all queries have been addressed and the clinical team is confident that the patient understands the trial and all requirements, patients will be consented onto the trial signing a current approved written informed consent form. Written informed consent on the current approved version of the consent form for the trial must be obtained before any trial–specific procedures are conducted.

Four copies of the consent forms will be completed:

1. An **original, signed copy** of the patient consent form should be retained in the Investigator Site File.
2. A **copy** of the signed patient consent form should be retained in the patient notes.
3. A **copy** of the signed patient consent form should be sent to the Wolfson Molecular Imaging Centre.
4. A **copy** of the patient consent form should be given to the patient.

The right of the patient to refuse to participate in the trial without giving reasons must be respected. All patients are free to withdraw at any time from the protocol procedures without giving reasons and without prejudicing further treatment. Patient withdrawal of consent from the trial must be explicitly documented in the source documents.

With the patient's permission, their GP will be informed of their patient's trial participation. If new safety information results in significant changes in the risk/benefit assessment, the patient information sheet and associated consent form would be reviewed and updated if necessary. If the patient information sheet and consent form is updated, at the discretion of the CI all patients (including those currently participating on the trial), would be informed of the new information, given a copy of the revised documents and if appropriate asked to re-consent to continue in the trial.

5.3 Screening for Eligibility

Pre-trial evaluations should be carried out as detailed below before registration. Clinical assessments should be performed within 4 weeks of initial CVD (cerebrovascular disease) diagnosis.

1. Diagnosis of CVD
2. Medical history, patients demographics and baseline symptoms
3. Concomitant medication (see details of prohibited medications in Appendix 1)
4. Vital signs: blood pressure, heart rate and rhythm, respiratory rate, temperature
5. Physical exam
6. MRI compatibility assessed by following standard practices as defined by local SOPs*

7. Pregnancy status assessed by following standard practices as defined by local SOPs*

* Not required for patients who fail the other screening points, so should be completed following successful completion of all other screening assessments.

5.4 Ineligible and non-recruited participants

Ineligible and non-recruited participants will be treated as per standard care at the discretion of the treating clinician. Best supportive care is recommended.

6.0 RANDOMISATION AND REGISTRATION PROCEDURES

Trial site(s) will be required to keep a record of all patients approached to participate in the study, in order to monitor recruitment. Details of patients approached and the number who declined participation should be provided to MAHSC CTU (summary details only, no identifiers will be collected). Details of patients who were consented to the trial but were found ineligible/ did not proceed for any other reason will also be collected by MAHSC CTU (in anonymised form using the screening log provided by MAHSC). Sites will be provided with instructions on how to provide this information to the CTU.

Any patient who consents to the trial and has been deemed eligible will be centrally randomised/registered by the MAHSC CTU. The clinical team at the research site will contact the CTU by a telephone call to a dedicated telephone number:

Randomisation/Registration line at MAHSC CTU:
0161 446 3311 (9am-5pm, Mon-Fri)

Sites should ensure that the following have been completed before randomisation/registration:

- Patient consent form must be signed
- Pre-registration evaluations must have been carried out at site as detailed in the previous section and the patient's eligibility for the trial must have been confirmed.

Randomisation and registration is completed within a bespoke computer system which is maintained by a MAHSC CTU statistician. A member of MAHSC CTU staff will access the system and check with the member of the clinical team calling that the patient's eligibility for the trial has been confirmed. The MAHSC CTU staff member will then provide the caller with verbal confirmation of the unique Participant ID assigned.

An e-mail confirmation of the participant entering into the trial will be sent to the designated circulation list by MAHSC CTU. The Participant ID will be used for the purpose of participant identification and data collection during the study.

Upon registration, participants will be given a trial specific participant card, which will have the trial title, participant ID number, contact details of the Principal Investigator and out of hours contact details in cases of emergency.

Phase 1 of the trial will require randomisation of the patients. Patients will be randomised 5:5 Arm A to Arm B. It is acknowledged that this randomisation process will not be completely random thus ensuring equal grouping. The caller will be given a unique ID for the patient as well as study ARM allocation, which will determine if the patient is having [^{11}C]-(R)-PK11195 on Day 1 or Day 2.
Phase 2 of the trial will only require registration of the patients. Therefore, the caller will be given a unique ID for the patient only.

7.0 RADIOTRACER & SCANNING DETAILS

7.1 Radiotracer summary

This study uses PET imaging with a new radiotracer ([^{18}F]GE-180) that binds specifically to areas of inflammation. This will be used in combination with standard outcome assessments to see if there is a relationship between the degree and extent of inflammation with symptoms/functional impairment.

The new radiotracer [^{18}F]GE-180, will be compared to an existing radiotracer known as [^{11}C]-(R)-PK11195. This also binds to sites of inflammation, and is widely used in other PET studies. [^{18}F]GE-180 has a longer half-life and therefore has the potential to be used in routine clinics.

7.2 Radiotracer details

7.2.1 Details of trial treatment

- **Name of radiotracers**
    - **[^{18}F]GE-180 / [^{18}F]GEH120714**
      (S)-N,N-diethyl-9-(2-[18F]fluoroethyl)-5-methoxy-2,3,4,9-tetrahydro-1H-carbazole-4-carboxamide

- **[^{11}C]-(R)-PK11195**
  (1-[2-chlorophenyl]-N-methyl-N-[1-methyl-propyl]-3-isoquinoline carboxamide)

- **Description of the products**

[^{18}F]GE-180
Each vial contains ~10mL of an endotoxin and particle free solution of [^{18}F]GE-180 in Formulation Buffer (GE Healthcare), with a radioactivity concentration of ≤1100MBq / mL at end of synthesis. The maximum concentration of [^{18}F]GE-180 will be 20µg per injectable volume. The total concentration of [^{18}F]GE-180, known and unknown chemical impurities will not exceed 60µg per injectable volume.

[^{11}C]-(R)-PK11195
Each vial contains ~10mL of an endotoxin and particle free solution of [^{11}C]-(R)-PK11195 in 0.9% saline. The concentration of [^{11}C]-(R)-PK11195 will not exceed 10µg per injectable volume. The concentration of desmethyl [^{11}C]-(R)-PK11195 (precursor) and total unknown impurities will each not exceed 3µg per injectable volume.

- **Drug manufacturer**

[^{18}F]GE-180 and [^{11}C]-(R)-PK11195 are manufactured in accordance with EU Good Manufacturing Practice at the following facility:

Wolfson Molecular Imaging Centre (WMIC)
The University of Manchester
27 Palatine Rd
Manchester
M20 3LJ
UK

MIA(IMP) 35030
MS 35030

- **How the drug will be supplied and packaged**

The products are aseptically filtered into a type I glass vial sealed with a type I synthetic rubber closure and aluminium overseal. The product vial will be stored within a vial shield (lead or tungsten) in the absence of light at ≤25^{o}C.

The primary and secondary product packaging will be labelled by the manufacturer in accordance with Eudralex Volume 4, Annex 13 (Manufacture of Investigational Medicinal Products).

- **Disposal of unused tracer material**

Unused tracer material is monitored to determine the radioactivity content and the volume of material. A radioactive waste disposal document is completed that determines when the activity level will have decayed sufficiently for the material to be disposed of as non-radioactive chemical waste. The sample is held in a lead safe until it has decayed and is then double-checked with a radiation monitor prior to disposal. The procedure is outlined in the WMIC standard operating procedure: SOP_QC_00025 Disposal of Radioactive Samples Solid Chemical and Solvent Waste

7.3 Scanning summary

7.3.1 Magnetic Resonance Imaging (MRI)

In phase 1, MR imaging will be conducted using the Philips 1.5 Tesla Achieva MR Scanner at the Wolfson Molecular Imaging Centre (WMIC). It will consist of the following standard anatomical sequences (an approximately 30 min acquisition time slot required):

- T1 inversion recovery (IR) sequence
- T2 fluid-attenuated inversion recovery (FLAIR) sequence
- post-contrast enhanced T1 sequence

7.3.2 Positron Emission Tomography (PET) with [^{11}C]-(R)-PK11195

In phase 1, PET imaging will be conducted on the High Resolution Research Tomograph (HRRT) at the Wolfson Molecular Imaging Centre (WMIC).

Patient preparation: Patients may eat and drink normally on the day of the scan. As [^{11}C]-(R)-PK11195 is a ligand for the peripheral benzodiazepine receptor, patients must not be taking Benzodiazepines with a long half-life that would interfere with the [^{11}C]-(R)-PK11195 binding. A venous blood sample will be collected for the analysis of peripheral inflammatory markers.

A PET scanning session with [^{11}C]-(R)-PK11195 constitutes of:

- a short transmission scan of approximately 10 min to enable subsequent correction for photon attenuation in the head,
- followed by an emission scan with [11C]-(R)-PK11195 (approximately 60 min)

Tracer injection: A target dose of 740MBq (370MBq minimum) of [^{11}C]-(R)-PK11195 will be injected intravenously into the patient shortly after the emission scan starts;
Data acquisition: Dynamic PET data will be acquired over 60 min in list mode.

7.3.3 Positron Emission Tomography (PET) with [^{18}F]GE-180

In phase 1 and phase 2, PET imaging will be conducted on the High Resolution Research Tomograph (HRRT) at the Wolfson Molecular Imaging Centre (WMIC). In phase 2 only 20

patients will have PET imaging with low dose head CT scan (1 minute) at Central Manchester University Hospitals NHS Foundation Trust instead of HRRT .

Patient preparation: Patients may eat and drink normally on the day of the scan. As [^{18}F]GE-180 is a ligand for the peripheral benzodiazepine receptor, patients must not be taking benzodiazepines with a long half-life that would interfere with the [^{18}F]GE-180 binding. A venous blood sample will be collected for the analysis of peripheral inflammatory markers and for the classification of the affinity of the binding to [^{18}F]GE-180.

A PET scanning session with [^{18}F]GE-180 constitutes of

- tracer injection outside the PET camera, a target dose of 200 MBq (120 MBq minimum) to be administered intravenously,
- followed by an uptake period of approximately half an hour during which a discrete venous blood sample may be taken for an assay of radioactivity
- participant to be placed in the camera for an emission scan of approximately half an hour (exact length still to be determined) acquired in list mode
- immediately followed by a short transmission scan of approximately 10 min to enable subsequent correction for photon attenuation in the head at the end of which another discrete venous blood sample may be taken for an assay of radioactivity

The total length of a PET scanning session with [^{18}F]GE-180 is not to exceed two hours. The decision on the exact length of the tracer uptake waiting time and the emission scan is to be based on the experience provided by GE Healthcare from the evaluation of their first in man investigations with [^{18}F]GE-180 and on the tolerability of the procedure by the study participants.

8.0 DETAILS OF RISK

8.1 Risks from radiotracers

Ionising radiation dose to the patients coming from the injection of radiotracers or exposure to external radiation source (CT or Transmission scans) will be kept following ALARA (“As Low as Reasonably Achievable” principle). [^{18}F]GE-180 is a developmental unregistered Investigational Medicinal Product whose safety profile is still under investigation; therefore undocumented side effects may occur. The effective dose for the full research study to each patient is around 9 mSv, which is for Phase I study which includes both radiotracer injections (C-11) Pk11195 and (F-18) GE-180. For Phase II study, there will be only one radiotracer injection (F-18) GE-180, and the effective dose corresponding is 5.5 mSv. These effective doses have been calculated using target injection activities. The equivalent radiation background received in UK per year is 2.7 mSv; therefore the equivalent UK background equivalent is 3.3 years for full study or Phase I

and 2 years for Phase II. In both cases the patient will received less than 4 times the normal dose of radiation that individuals are normally exposed to when living for one year in the United Kingdom in a normal environment.

For a healthy adult of 30-40 year old the estimated lifetime risk of fatal cancer associated with the total Phase I part of the study (9 mSv) is 1 in 2000 and as for Phase II the risk is 1 in 3400 (using UK specific detriment of 5.3*10-5 per mSv NRPB R260).

Study team must remain vigilant for at least 1 hour after the injection of GE-180 to observe subjects for signs of possible anaphylactoid reactions and other emergencies after dosing and access to appropriate clinical supplies must be immediately available

8.2 Risks from scans

Exposure to magnetic resonance scans poses a very small risk related to possible interaction of metal bodies with the strong magnetic field. These will be minimised by following the standard operating procedures applied to all studies held at WMIC that involve a MRI and/or PET scans.

9.0 TRIAL ASSESSMENTS

PHASE 1 Table 1. Schedule of assessment for phases 1 and 2.

		Trial Procedure (ARM A)		Trial Procedure (ARM B)		3 months after stroke**
	Screening Assessment*	**Visit 1**	**Visit 2**	**Visit 1**	**Visit 2**	
Assessments						
National Institutes of Health Stroke Scale (NIHSS)	X					X
Modified Rankin Scale (mRS)	X					X
Tolerability questionnaire			X	X		
Adverse Event Log		X	X	X	X	X
Concomitant/Prohibited Medications	X	X	X	X	X	
Tests						
Blood tests***		X		X		
MRI scan (with and without contrast)$^{+}$		X^{+}	X^{+}	X^{+}	X^{+}	
PET scan ([^{11}C]-(R)-PK11195)		X			X	
PET scan ([^{18}F]GE-180) ****			X	X		

* Carried out whilst patient is still in hospital
** Carried out at the hospital site *** Whole blood sample (3ml EDTA tube) and plasma sample (2ml cryovial)
**** Two blood samples of 10ml each may be taken per scan for the measurement of radioactivity.
$^{+}$ To be carried out on either Day 1 or Day 2.

PHASE 2

	Screening Assessment*	Trial Procedure	3 months after stroke**
Assessments			
National Institutes of Health Stroke Scale (NIHSS)	X		X
Modified Rankin Scale (mRS)	X		X
Adverse Event Log		X	X
Concomitant/Prohibited Medications	X	X	
Tests			
Blood tests***		X	
MRI scan (with and without contrast)		X	
PET scan ([^{18}F]GE-180)****		X	

* Carried out whilst patient is still in hospital
** Carried out at the hospital site
*** Whole blood sample (3ml EDTA tube) and plasma sample (2ml cryovial)
**** Two blood samples of 10ml each may be taken per scan for the measurement of radioactivity.

Two weeks after initial stroke, most patients would expect to switch tablets from aspirin to clopidogrel as a national recommendation. At each scan visit, a vein will be cannulated for the scanning procedure. Venous access is needed for the administration of the contrast agent in Magnetic Resonance Imaging (MRI) and of the radiopharmaceutical in Positron Emission Tomography (PET). The cannula can be removed on completion of the visit that day. If possible, blood will be taken from the administration cannula at the time of insertion. This can be sometimes difficult and may necessitate a further venepuncture for another blood sampling cannula.

Three types of blood analysis may be performed.

1. The concentration of peripheral inflammatory markers such as C-Reactive Protein (CRP) and Interleukin 6 (IL6) in plasma will be measured. This is to study the relationship between markers of peripheral inflammation and markers of inflammation in the central nervous system.
2. Genomic DNA will be extracted from whole blood (3ml EDTA tube). The affinity of the binding to [^{18}F]GE-180 will be classified as high affinity binder (HAB), medium affinity binder (MAB) or low affinity binder (LAB) by analysing the rs6971 genotype of the participant (Owen et al., 2012).
3. Measurements of [^{18}F]GE-180 activity in the venous plasma samples taken before and after the PET emission scan will be performed. These are used to assess whether the transient equilibrium analysis (Carson et al., 1993) is a valid approach and to optimise the time window past injection when the [^{18}F]GE-180 emission scan is acquired.

During the study period, the treating clinician may have organised additional tests such as heart tests or other blood tests. Patients should be strongly encouraged to keep these appointments. It is routine for a patient to be offered a follow up appointment in the stroke clinic at approximately 6-8 weeks after their stroke. If the patient is an inpatient then we will liaise with the medical and therapy services in order to minimise any impact of travel to WMIC on the clinical care.

Three months (± 2 weeks) after the initial stroke patients will return to the hospital site for follow up. Patients will be assessed by the clinical team to assess if they have had any other symptoms that would indicate a recurrent stroke. The NIHSS and mRS will be assessed for second time as outcome measures.

10.0 TRIAL CLOSURE

All patients will be followed up 3 months (± 2 weeks) after their initial stroke.

The first possible endpoint is at the end of year 1. If the trial does not satisfy the 'Go/NoGo' criteria (defined in section 4) the trial will be closed. All patient data gathered in the first year will be kept by WMIC and used for analysis.
The data acquisition endpoint is at the end of 39 months. Data will have been collected on 50 patients (excluding any low -binding patients as described in previous sections).
The final endpoint of the trial is at the end of 4 years (39 months of data collection plus 9 months of scanning analysis and statistical analysis of the correlation between microglial activation and the functional outcome of the patients). All data collected from phases 1 and 2 will have been analysed.

11.0 STATISTICS AND DATA ANALYSIS

11.1 Sample size calculation

The project is primarily a feasibility study aimed at obtaining provisional evidence that baseline PET scan data are useful predictors of clinical outcomes. In the context of clinical trial development, it is recommended that not too much emphasis should be placed on formal power calculations for the evaluation of statistical significance at this stage [Lancaster *et al.* (2004)], but that investigators should concentrate, instead, on confidence interval estimation. The main analytical question to be answered by the present project is whether baseline PET score measurements can predict (i.e. are associated or correlated with) clinical outcome in stroke survivors. We assume that to be of any utility the correlation would have to be at least 0.40 (explaining about 16% of the variation in outcome). Taking this to be the true value and assuming bivariate normality, we obtain the following result: when the sample size is 37, a one-sided 90.0% confidence interval for rho=0.400 will have limit 0.200 (*nQuery Advisor*). It is expected that the analysis will be based on at least 40 participants with non-missing data. The allowance for loss to follow-up using probability weights should have little impact on the precision of the estimates (but probably making them marginally less precise), but the inclusion in the prediction model of baseline covariates with known prognostic value should lead to increased precision.

11.2 Interim analysis

The interim analysis will be carried out at the end of phase 1. It will be based on the feasibility of obtaining scans on patients who heterozygous for the TSPO rs6971 polymorphism. The analysis will also include information from the tolerability questionnaires that patients will be asked to complete at the end of the 2 day scanning process.
Main objective is to obtain information on:

- Technical feasibility of the [^{18}F]GE-180 scans and the possibility of reducing the scan time to 20 minutes.
- Use tolerability information gathered from patients about the comfort and duration of the scans.
- Ensure that the correlation between [^{11}C]-(R)-PK11195 and [^{18}F]GE-180 images has a coefficient correlation of 0.5 or higher.

11.3 Image analysis

The MR images will be used to assess the integrity of the blood-brain barrier and to provide anatomical information for a region-of-interest analysis of the PET images.
Dynamic PET data from the [^{11}C]-(R)-PK11195 scans will be processed as previously described (Su et al., 2013). Principally, characterisation of the radiotracer binding in the lesion target areas (infarct core and periphery, affected white matter tracts) will be based on the comparison with an appropriate reference region(s) in the contralateral hemisphere. Thus, each study participant serves as its own control avoiding the scanning of a separate control cohort in this clinical trial.
Summed PET images will be calculated from the [^{18}F]GE-180 data, and for comparison by correlation analysis also of [^{11}C]-(R)-PK11195, enabling visual assessment and semi-quantitative analyses based on target-to-reference region ratios. Of particular interest is the quality of the [^{18}F]GE-180 static images governed by the length of the image acquisition and the injected activity, two adaptable scanning parameters.

11.4 Primary and secondary analysis

<u>Factors associated with three-month mortality</u>
Although death of the participant precludes measurement and analysis of other clinical outcomes such as the NHSS and mRS this is not a missing data problem in the conventional sense: it is itself a particularly important outcome. We expect the three-month mortality to be less than 10%. The initial analysis will explore the relationship between baseline characteristics (including the imaging biomarker measurements). This analysis will be primarily descriptive and informal – there will be insufficient data to enable the development of a robust risk prediction model.
Subsequent analyses (described below) will be carried out to infer patterns of clinical outcome in those who have not died. That is, the target population will be the stroke survivors.

<u>Factors associated with loss to follow-up (other than death) – construction of inverse probability weights</u>

In total, it is anticipated that about 10-15% of the surviving stroke patients will have missing clinical outcome data. As long as the attrition is kept lower than 15%, it would be expected to have only a small effect on statistical precision and, similarly, only a small impact on bias in the estimates of the regression coefficients in the prediction models (see below). However, associations between baseline characteristics and loss to follow-up will be examined both descriptively and through the use of logistic regression modelling. Predicted probabilities of follow-up will be used to compute inverse probability weights to ensure that the observed outcomes fairly represent the full cohort of stroke survivors [Dunn G (2000)]. There are likely to be three sources of missing data: (a) initial diagnosis not validated at the time of follow-up - presumably, there will be very few of these; (b) discontinuation and drop-out; and (c) machine failure (although there will be opportunities for a second appointment to rectify this problem). Group (b) will be the main focus of the analyses, although the possibility of (a) and (c) not occurring completely at random will be investigated.

Development and validation of clinical prediction models

All analyses of clinical outcomes in the stroke survivors will be carried out using the *regress* command of *Stata*, incorporating inverse probability weights to allow for attrition using Stata's *pweight* option. The statistical significance of any predictive effects will be evaluated using robust standard errors, p-values and associated confidence intervals (using the Huber-White 'sandwich' estimator). Prediction models include the relevant PET scan variable, TSPO Ala147Thr polymorphisms (high versus medium) and the possibility of an interaction between the two. Other baseline demographic and clinical covariates will be added to the model so that the 'unique' contribution of the PET scan data can be assessed (i.e. how much information does it provide over and above the routinely collected demographic and clinical variables?). We will be giving considerable attention to confidence interval estimation rather just relying on statistical significance.

12.0 PHARMACOVIGILANCE

12.1 Definitions

Adverse Event (AE) - An adverse event is any untoward medical occurrence in a patient from trial-specific procedures. An AE may therefore be any unfavourable and unintended sign (including abnormal findings), symptom or disease concurrently associated with the use of the trial procedures.

Adverse Events of Medical Significance:

1.Adverse Reaction (AR) - An adverse reaction is any untoward and unintended response in a patient to a trial procedure which is related to any dose (e.g. radiotracers) administered to that patient. Note: Any adverse event judged by either the reporting investigator or the sponsor as having reasonable causal relationship to the trial procedures qualifies as an AR if there is evidence or argument to suggest a causal relationship.

2.Serious Adverse Event / Reaction (SAE / SAR) - A serious adverse event or reaction in a trial subject that:

- results in death,
- is life-threatening,
- requires in-patient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability / incapacity
- is a congenital anomaly / birth defect, or
- other important medical condition (medical judgement should be exercised in deciding whether an adverse event / reaction should be classified as serious in other situations. Important adverse events / reactions that are not immediately life-threatening or do not result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious).

Note: For the purpose of this definition, life-threatening refers to an event in which the trial patient was at risk at the time of an event; not an event that might have caused death if it were more severe.

Events not to be treated as SAEs: The following events do not require reporting as an SAE, but must be recorded in the relevant section(s) of the CRF

- Disease related illness / events
- Elective hospitalisation
- Disease related deaths

12.2 Detecting and reporting AEs

All adverse events relating to the study procedures that occur between informed consent and end of trial procedures must be recorded in the patient notes. If the patient is at the WMIC then the clinical fellow or another appropriately trained member of the team must ensure that the event is recorded using the protocol at WMIC and the information is also recorded in the patient

notes (which will be held at the research hospital). Every effort should be made to maintain a complete record of adverse events.

Where required, adverse events will be reported to MAHSC CTU via completion on the trial CRF. Serious adverse events should also be reported immediately on an SAE report form, as detailed in section 12.2.1 below.

12.2.1 SAE Processing and Reporting at the MAHSC-CTU

AEs meeting the definition of a Serious Adverse Event (SAE) must be reported to the MAHSC-CTU using the trial specific SAE Report Form **immediately, and within 24 hours** of observing or learning about the event.

Centres should respond as soon as possible to requests from the CI or designated representative (via the MAHSC-CTU) for further information that may be required for final assessment of the SAE.

All SAEs must be reported by faxing a completed SAE Report Form **immediately, and within 24 hours** of becoming aware of the event to the IN_CVD Trial Manager at the MAHSC-CTU

Fax: 0161 446 8148

On receipt of the SAE Report Form, the CTPM will send an acknowledgement of the SAE to the relevant members of the trial team at the participating site. This acknowledgement will include an SAE reference number which should be included on all future correspondence regarding the SAE. The CTPM then passes the SAE form to the CI. The CI reviews the site PI's assessments of seriousness and causality.

12.2.2 Detecting and reporting a SUSAR

If an SAE is suspected to be a SUSAR, regulatory time constraints apply for expedited reporting. The CTPM will then liaise with the CI to evaluate the event for seriousness, causality and expectedness to determine whether or not the case qualifies for expedited reporting.

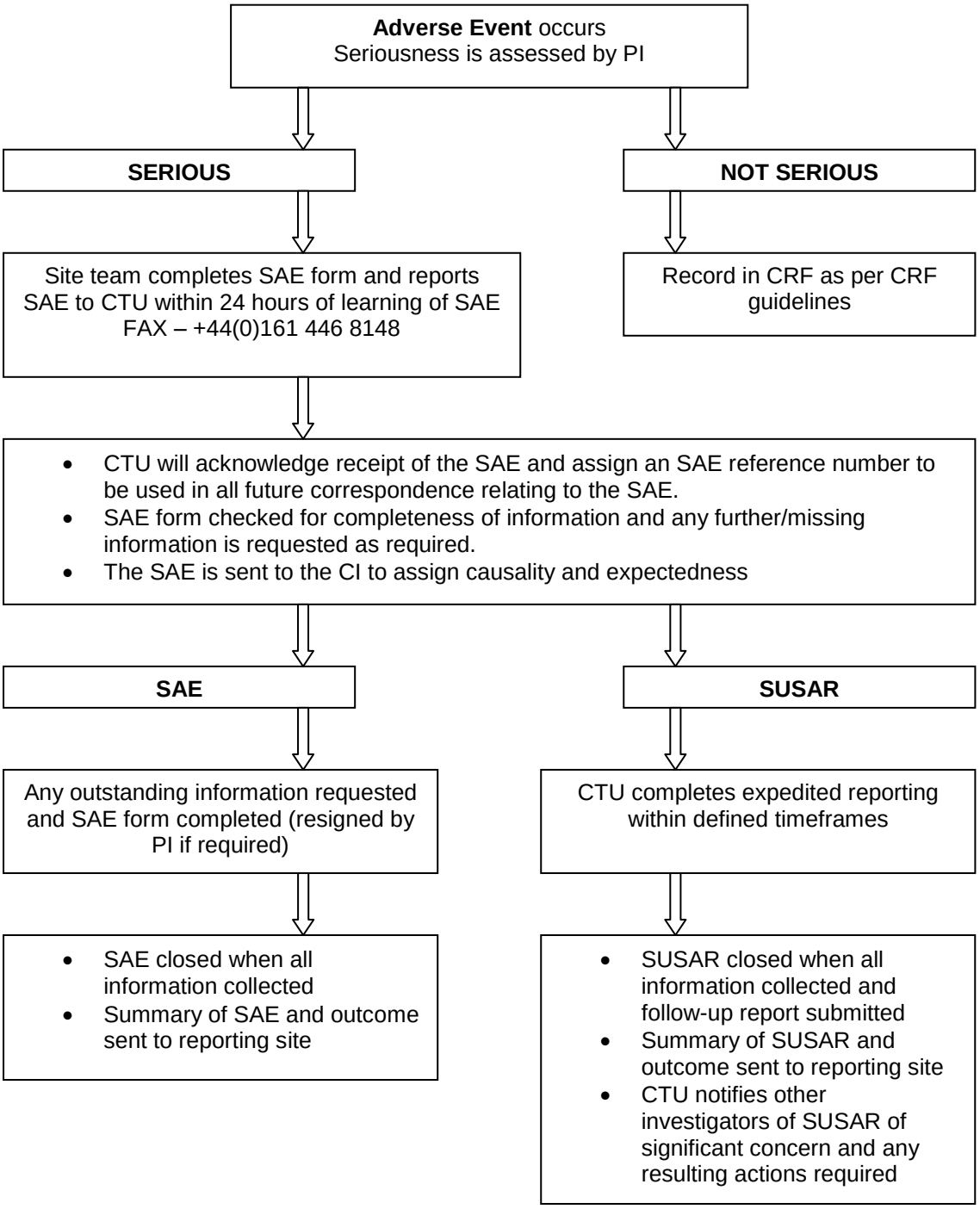


Figure 3. Flow diagram to describe the reporting procedure for adverse events/reactions.

SAEs assessed as having a causal relationship to the radiotracer and as being unexpected (SUSARs) will undergo expedited reporting to the relevant authorities and GE Healthcare by the MAHSC-CTU.

12.3 Pregnancy Reporting

All patients will be asked to complete a pregnancy questionnaire prior to scanning, as per standard local procedures (following SOPs at WMIC). However, if a patient becomes pregnant during the trial, the patient must be withdrawn from study immediately. The pregnancy of a patient must be reported to the MAHSC-CTU using the Pregnancy Notification and Outcome Form immediately, and within 24 hours of the participating site becoming aware of the pregnancy. The PI must obtain consent to follow the pregnancy and liaises with the obstetrician until delivery/outcome and for the postpartum period. Parental and neonatal outcomes must be recorded (by updating the Pregnancy Notification and Outcome form) even if they are completely normal and without AEs. Abnormal outcomes for the foetus or child observed at birth or during the follow-up period (e.g. spontaneous or therapeutic abortion, stillbirth, neonatal death, congenital abnormality) are considered SAEs and are reported to the MAHSC-CTU on the trial specific SAE form.

The MAHSC-CTU will fax all Pregnancy Reports to the sponsor immediately, but within 24 hours of the MAHSC-CTU becoming aware.

12.4 New Safety Findings

If a new safety finding emerges (from sources such as IMP manufacturers, data analysis, IDMC findings), the CI will review the finding for its impact on the patients participating in the relevant trial(s). If there is a potential impact on patient's safety, the sponsor will take appropriate action in conjunction with the CTPM, CI and research team. Appropriate reporting mechanisms will be followed in the event of actions being taken.

12.5 Urgent Safety Measures

Where an urgent safety measure (to prevent immediate hazard to patient's health and safety) is necessary, prior authorisations from the MHRA and ethics are not required.

If the PI takes urgent action that is not consistent with the protocol to prevent harm to a patient on a trial, the PI must immediately inform the CI and CTPM and give full details of the measures taken and the decision making process surrounding the action(s) taken. The CTPM will inform the CI, sponsor, ethics and the MHRA of these measures immediately, but no later than 3 days of the actions being taken.

An amendment will be formally submitted as soon as possible by the CI and CTPM to the relevant bodies in conjunction with the sponsor.

Examples of issues requiring urgent safety measures may be:

- A single report of a SAR with an unexpected outcome (e.g. death)
- An increase in rate of occurrence of SAR which is judged to be clinically important
- A post-trial SUSAR occurring after the patient has left the trial
- A new event relating to the use or development of the IMP likely to affect the safety of patients e.g.:
    - an SAE that could be associated with the procedures and which could lead to modification of the trial conduct

12.6 Periodic Safety Reports

Annual progress reports will be submitted to the REC, in accordance with their procedures.
A Development Safety Update Report (DSUR) will be submitted annually to the MHRA and REC in accordance with requirements.
These periodic reports will be prepared by MAHSC CTU with the input and review of the chief investigator and will be circulated to the sponsor and participating sites.

13.0 TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

13.1 Oversight Committees

13.1.1 Trial Management Group (TMG)

A Trial Management Group (TMG) will be established and will include those individuals responsible for the day-to-day management of the trial.

Notwithstanding the legal obligations of the Sponsor and Chief Investigator, the TMG have operational responsibility for the conduct of the trial including monitoring overall progress to ensure the protocol is adhered to and to take appropriate action to safeguard the patients and the quality of the trial.

The TMG will meet at least quarterly once the trial is actively recruiting. Minutes will be taken at TMG meetings and copies of the minutes will be filed in the Trial Master File. The CTPM and CI will ensure that all relevant issues and actions discussed during the meeting are followed up and resolved and details of significant issues will be made available to participating sites. Minutes of all TMG meetings are available on request.

13.1.2 Independent Data Monitoring Committee (IDMC)

An IDMC will be instigated to review accruing trial data and to assess whether there are any safety issues that should be brought to the patient's attention, whether any safety amendments should be made or if there are any reasons the trial should not continue. The IDMC will conduct an early safety review after the first 10 patients. The IDMC will provide a recommendation for the trial by month 12 (end of phase 1).

The IDMC will be independent of the investigators, funder and sponsor. The CI and TMG with the support of the MAHSC-CTU will be responsible for nominating IDMC members. The Committee's terms of reference, roles and responsibilities will be defined in a charter agreed by all members of the IDMC. This charter will outline any stopping rules and the frequency of analysis and IDMC meetings during the trial. The IDMC will meet in confidence at regular intervals, as appropriate to the progress of the trial and as per the schedule agreed with IDMC members.

Reports to the IDMC will be prepared and presented by the trial team and CTPM prior to the IDMC meeting. The IDMC Chairman will then report their recommendations to the Chairman of the TMG and may request additional reports or information if required. This report will be submitted to the TMG, and if required, the REC and the MHRA and the CI and CTPM will ensure that all actions and recommendations are followed up.

13.2 Trial management: MAHSC-CTU

13.2.1 Data Collection

Each research site is responsible for maintaining source data for their trial patients in their medical notes, and for transcribing this data onto a trial specific case report form (CRF). All entries on the CRF, including corrections, must be made by an authorised member of trial staff. Research site staff will also provide trial patients with copies of the tolerability questionnaire at the time indicated on the schedule of assessments.

Research sites will submit original completed copies of the CRF and patient completed questionnaires to MAHSC CTU, keeping a copy at site with the Investigator Site File. The hospital sites and WMIC will each have site files, there will be a CRFs which must be completed and filed at the hospital sites.

13.2.2 Data Handling at MAHSC-CTU

Data provided to the MAHSC-CTU will be checked for errors, inconsistencies and omissions. If missing or questionable data are identified, the MAHSC-CTU will request that the data be

clarified. All aspects of data collection and handling throughout the life cycle of the trial will be described in trial specific documents held in the trial master file at MAHSC CTU.

Upon completion of relevant data management processes prior to any interim/ final analysis the trial data will be passed to the trial statistician for analysis.
All data handling and analysis will be conducted in line with Good Clinical Practice guidelines and the Data Protection Act 1998.

13.2.3 Trial monitoring

On-site monitoring will be based on a risk-based strategy and a risk assessment will be completed by the MAHSC-CTU as part of the trial set-up process to ascertain the frequency and intensity of monitoring visits required (although additional monitoring may be conducted if necessary). This risk assessment and associated monitoring plan will be stored at the MAHSC-CTU.

The purpose of these visits are:

- To verify that the rights and well-being of patients are protected.
- To verify accuracy, completion and validity of reported trial data from the source documents.
- To evaluate the conduct of the trial within the institution with regard to compliance with the currently approved protocol, GCP and with the applicable regulatory requirements.

14.0 ETHICAL AND REGULATORY REQUIREMENTS

The trial will be conducted in accordance with the principles of good clinical practice (GCP).

The sponsor and MAHSC-CTU will ensure that the trial protocol, patient information sheet, consent form, GP letter and submitted supporting documents have been approved by the appropriate regulatory body and research ethics committee prior to any patient recruitment.

Any agreed substantial amendments will also be submitted for ethical and regulatory approval prior to implementation. The study is being conducted under the ethical framework of a Clinical Trials Authorisation (CTA) from the MHRA. Under the CTA scheme the responsibility for the management and well being of a patient is no different from everyday clinical practice, namely the attending physician and the hospital Trust. Drug companies are, liable on a no fault basis for the quality and fitness for use of their product.

It is the responsibility of the PI at each site to ensure that the trial has local R&D approval and the sponsor and MAHSC-CTU will verify this, plus the presence of all other essential documentation (and potentially an initiation meeting) before giving the site the “green light” to open the trial to recruitment. The PI is also responsible for ensuring that any subsequent amendments gain the necessary approvals.

The CI, MAHSC-CTU and sponsor will ensure that the ethics committee(s) and regulatory body(ies) are notified that the trial has finished (either as expected or prematurely) within required timeframes with summary reports to be provided as required.
Contracts will be established with all relevant organisations and stored at the MAHSC-CTU.

14.1 Sponsorship and indemnity

The Christie NHS Foundation Trust will act as the sponsor for this study. Delegated responsibilities will be assigned to the MAHSC-CTU to manage the trial on behalf of the sponsor and to the participating sites recruiting patients into this trial.

As the sponsor is an NHS organisation the NHS indemnity scheme will apply to meet the potential legal liability for harm to participants arising from the management of the research. The University of Manchester have written the protocol and are therefore responsible for providing insurance or making indemnity arrangements for harm to participants arising from the design of the protocol.

Participating sites will be liable for clinical negligence and other negligent harm to participants taking part in the study and covered by the duty of care owed to them by the sites concerned. For participating sites which are part of the NHS, the NHS indemnity scheme will also apply; and for participating sites which are part of the University, University insurance will apply.

The manufacturer supplying IMP has accepted limited liability related to the manufacturing and original packaging of the study drug and to the losses, damages, claims or liabilities incurred by study participants based on known or unknown adverse events which arise out of the manufacturing and original packaging of the study drug, but not where there is any modification to the study drug (including without limitation re-packaging and blinding).

14.2 Participant Confidentiality and Data Protection

Patients will be assigned a unique trial ID via the MAHSC-CTU trials line which will be used throughout their participation in the trial. Any personal data recorded will be regarded as

confidential, and any information which would allow individual patients to be identified will not be released into the public domain.

Each investigator should keep a separate Trial ID and screening log of all participants consented and screen status. The investigator must maintain this screening log and all other trial documents (including participant's written consent forms) which are to be held at the participating centres, in strictest confidence. The investigator must ensure the patients' confidentiality is maintained.

The MAHSC-CTU will maintain the confidentiality of all patients and will not reproduce or disclose any information by which patients could be identified. The Investigator and trial site staff involved with this trial may not disclose or use for any purpose other than performance of the trial, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the trial.

Representatives of the MAHSC-CTU and the regulatory authorities will be required to have access to patients notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any confidential information to other parties.

All Investigators and trial site staff involved with the trial must comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

Patient notes and trial files at site must be kept in a secure storage area with limited access. Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual patients.

15.0 TRIAL CONDUCT

15.1 Protocol Amendments

Any changes in research activity will be reviewed and approved by the Chief Investigator and submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to enrolment into an amended protocol.

Deviations from the protocol may be taken by an investigator without prior approval from the sponsor or regulatory bodies in order to eliminate an immediate hazard to a patient. The rationale must be submitted to the MAHSC-CTU and the appropriate regulatory bodies as soon as possible after the deviation.

15.2 Protocol Deviations and Serious Breaches

The Regulations and guidance state that no deviation must be made from an approved trial protocol, unless it is an urgent safety measure taken to protect a patient from immediate harm, therefore the Christie does not allow protocol waivers for any other reason. Participating sites must contact the MAHSC-CTU if a potential protocol deviation has occurred (or if an event has occurred and it is unclear whether it should be classified as a deviation) and the MAHSC-CTU will advise the site what information and actions are required.

For Clinical Trials of Investigational Medicinal Products (CTIMPs), there is a legal requirement to report serious breaches of GCP or the trial protocol to the MHRA and REC within a defined timeframe. If a major deviation on a CTIMP meets the criteria for a serious breach, it is notified immediately to the Sponsor and reported to the REC and the MHRA within 7 days of confirmation. Complete investigations of breaches are fully documented, filed in the TMF and a copy sent to the sponsor.

16.0 END OF TRIAL

The end of the trial is defined as the point at which the analysis is completed on all the participants.

The CI and/or TSC have the right at any time to terminate the trial for clinical or administrative reasons.

The end of the trial will be reported to the REC and Regulatory Authority within the required timeframe if the trial is terminated prematurely. Investigators will inform patients of any premature termination of the trial and ensure that the appropriate follow up is arranged for all involved.

Following the end of the trial a summary report of the trial will be provided to the REC and Regulatory Authority within the required timeframe.

17.0 PEER REVIEW

Peer review of the IN_CVD trial has been completed during the consideration of the trial for funding by the Medical Research Council Mechanism Evaluation programme (EME).

18.0 PUBLICATION POLICY

The main trial results will be published in the name of the trial in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, appointed from amongst the Trial Management Group, and high accruing clinicians. All participating centres and clinicians will be acknowledged in this publication together with staff from the MAHSC-CTU. All presentations and publications relating to the trial must be authorised by the TMG and sponsor, on whose behalf publications should usually be made. Authorship of any secondary publications e.g. relating to the various biological studies will reflect the intellectual and time input into these studies, and will not be the same as on the primary publication. No investigator may present or attempt to publish data relating to the IN_CVD without prior permission from the TMG and sponsor.

19.0 TRIAL RECORD RETENTION AND ARCHIVING

Essential documents are documents that individually and collectively permit evaluation of the conduct of the trial and substantiate the quality of the data collected. Essential documents will be maintained at the MAHSC-CTU and at the Investigator Sites in a way that will facilitate the management of the trial, audit and inspection. They should be retained for a sufficient period (at least 5 years) for possible audit or inspection. Documents should be securely stored and access restricted to authorised personnel.

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APPENDICES

Appendix 1: List of Prohibited Medications

No drug interactions involving GE-180 are known at this stage.

The formulation contains NMT 10.0% ethanol. At the maximum injection of 10mL, the maximum blood concentration of ethanol will be approximately 2 mg/100ml (0.02%), assuming a human blood volume of 4L. this is below the concentration that will cause sensitization by most drugs (e.g. metronidazole). However, there is the potential for alcohol hypersensitisation to occur in the presence of disulfiram (Antabuse), used for the treatment of addiction to alcohol. As this can lead to serious adverse reactions, patients taking Antabuse should be excluded from receiving GE-180.

Prohibited Medication
ANTABUSE