Clinical Trial Protocol

Trial Title:	A phase 1 clinical trial evaluating the safety and efficacy of up to two administrations of the adrenal PET tracer [^{18}F]CETO in healthy volunteers and patients with primary aldosteronism
Protocol Number:	CCTU0224
EudraCT Number:	2018-004851-18
Investigational Product:	2-[^{18}F]fluoroethyl 1-[(1R)-1-(4-chlorophenyl)ethyl]-1H-imidazole-5-carboxylate (abbreviated to [^{18}F]CETO)
Protocol Version:	1.2

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1 Protocol Signatures:

I give my approval for the attached protocol entitled “A phase 1 clinical trial evaluating the safety and efficacy of up to two administrations of the adrenal PET tracer [^{18}F]CETO in healthy volunteers and patients with primary aldosteronism” version 1.2, dated 19 December 2019.

Chief Investigator

Name: Professor Mark Gurnell

Signature:

Date:

2 Protocol and amendment history

Amendment No.	Protocol version no.	Date	Details of changes made
1	1.1	20 November 2019	Modified sections related to follow up of participants with abnormal SST results, eligibility criteria, IMP specifications, trial stopping criteria and reference safety information following initial MHRA review.
2	1.2	19 December 2019	Additional update to the trial stopping criteria following subsequent MHRA review prior to approval. Addition of Protocol amendment table.

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4 Abbreviations

[^{18}F]CETO	2-[^{18}F]fluoroethyl 1-[(1R)-1-(4-chlorophenyl)ethyl]-1H-imidazole-5-carboxylate
[^{18}F]FDG	[^{18}F]fluorodeoxyglucose
ACTH	Adrenocorticotrophic Hormone
AE/AR	Adverse event/Adverse Reaction
AVS	Adrenal Vein Sampling
CA	Competent Authority
CLRF	Clinical Research Facility
CRF	Case Report Form
CT	Computed Tomography
CTIMP	Clinical Trial of Investigational Medicinal Product
CUH	Cambridge University Hospitals NHS Foundation Trust
DRC	Direct Renin Concentration
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
FIH	First In Human
GP	General Practitioner
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
MBq	Mega-Becquerel
MHRA	Medicines and Healthcare products Regulatory Agency
NIMP	Non-Investigational Medicinal Product
PA	Primary aldosteronism
PAC	Plasma Aldosterone Concentration
PET	Positron Emission Tomography
PIS	Participant Information Sheet
R&D	Research and Development
RA	Regulatory Agency
REC	Research Ethics Committee
SAE/SAR	Serious Adverse Event/Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
WBIC	Wolfson Brain Imaging Centre

5 Trial Synopsis

Title of clinical trial	A phase 1 clinical trial evaluating the safety and efficacy of up to two administrations of the adrenal PET tracer [^{18}F]CETO in healthy volunteers and patients with primary aldosteronism
Sponsor name	Cambridge University Hospitals NHS

	Foundation Trust and the University of Cambridge
EudraCT number	2018-004851-18
Medical condition or disease under investigation	Primary Aldosteronism (PA)
Purpose of clinical trial	First In-Human (FIH) trial evaluating the safety and adrenal uptake of the PET radiotracer [^{18}F]CETO.
Primary objective	To evaluate the safety of up to two administrations of [^{18}F]CETO in up to 6 patients with primary aldosteronism and 5 healthy volunteers.
Secondary objective (s)	To: • Assess [^{18}F]CETO uptake by the adrenal glands • Evaluate uptake in bilateral vs unilateral cases of PA following [^{18}F]CETO administration in up to 6 patients.
Trial Design	Phase 1 single centre, open label, micro-dosing study
Trial Outcome Measures	<u>Primary Outcome Measure</u> • The primary outcome measure is the overall safety of [^{18}F]CETO. This will be assessed according to the frequency of adverse events, serious adverse events, clinically significant changes in vital signs, ECG and laboratory parameters. <u>Secondary Outcome Measures</u> • [^{18}F]CETO uptake by the adrenal glands will be assessed by measurement of Standardized Uptake Values (SUV) over the left and right adrenal glands: all assessments will be performed by a dedicated blinded reviewer. • Evaluation of adrenal uptake of [^{18}F]CETO in bilateral versus unilateral cases of PA will be performed by comparing SUV values of both adrenal glands in three patients with each

	subtype of PA (using a dedicated blinded reviewer).
Sample Size	Up to 11 participants: 5 healthy controls and 6 patients with PA
Summary of eligibility criteria	Inclusion Criteria: *Healthy Volunteers* To be included in the trial the participant must: • give written informed consent • be aged 50 years or over • have no underlying medical conditions • be able to lie down for at least 2 hours and not be claustrophobic In addition, all female participants must be: • post-menopausal (no menses for 12 months, without an alternative medical cause) *Patients* To be included in the trial the patient must: • give written informed consent • be aged 40 years or over • be able to lie down for at least 2 hours and not be claustrophobic • fulfil the following criteria: • have a confirmed diagnosis of PA as per Endocrine Society guidelines • have undergone successful lateralisation of the cause of PA to one or both adrenal glands by adrenal vein sampling (AVS) • be willing to have two PET-CT scans. In addition, all female patients must have a negative (blood) pregnancy test after the screening visit. Exclusion Criteria: **All participants**: • allergy to radiographic contrast agents • allergy or contraindication to synacthen injection

	• pregnancy, breastfeeding, or the intention to become pregnant during the 6 months following trial participation • assessed by the investigator as being unable or unwilling to comply with the requirements of the study protocol. • receipt of another IMP as part of a CTIMP • prior radiation exposure as part of previous research studies • recreational drug use, or substance/alcohol dependency • clinically abnormal screening blood tests Additional exclusion criteria for **healthy volunteers:** • women of child-bearing potential (i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile) • exposure to radiation during their work • received more than 10 mSv of radioactivity in the past 12 months • any subject with a history of adrenal disease or who, at the screening visit, reports symptoms, or exhibits physical signs, that could be consistent with previously unsuspected adrenal disease Additional exclusion criteria for **patients**: • allergy or contraindication to dexamethasone treatment (or lactose intolerant)
Investigational medicinal product and dosage	[^{18}F]CETO, not exceeding 10 micrograms per injection
Active comparator product(s)	None
NIMPs and Challenge Agents	Dexamethasone 500micrograms four times daily will be self-administered by patients in the three days prior to their repeat PET-CT scan as part of standard clinical care. Healthy volunteers will not receive Dexamethasone pre-treatment as they will only undergo a single scan.
Route(s) of administration	Intravenous
Maximum duration of treatment	Single dose repeated on up to one occasion

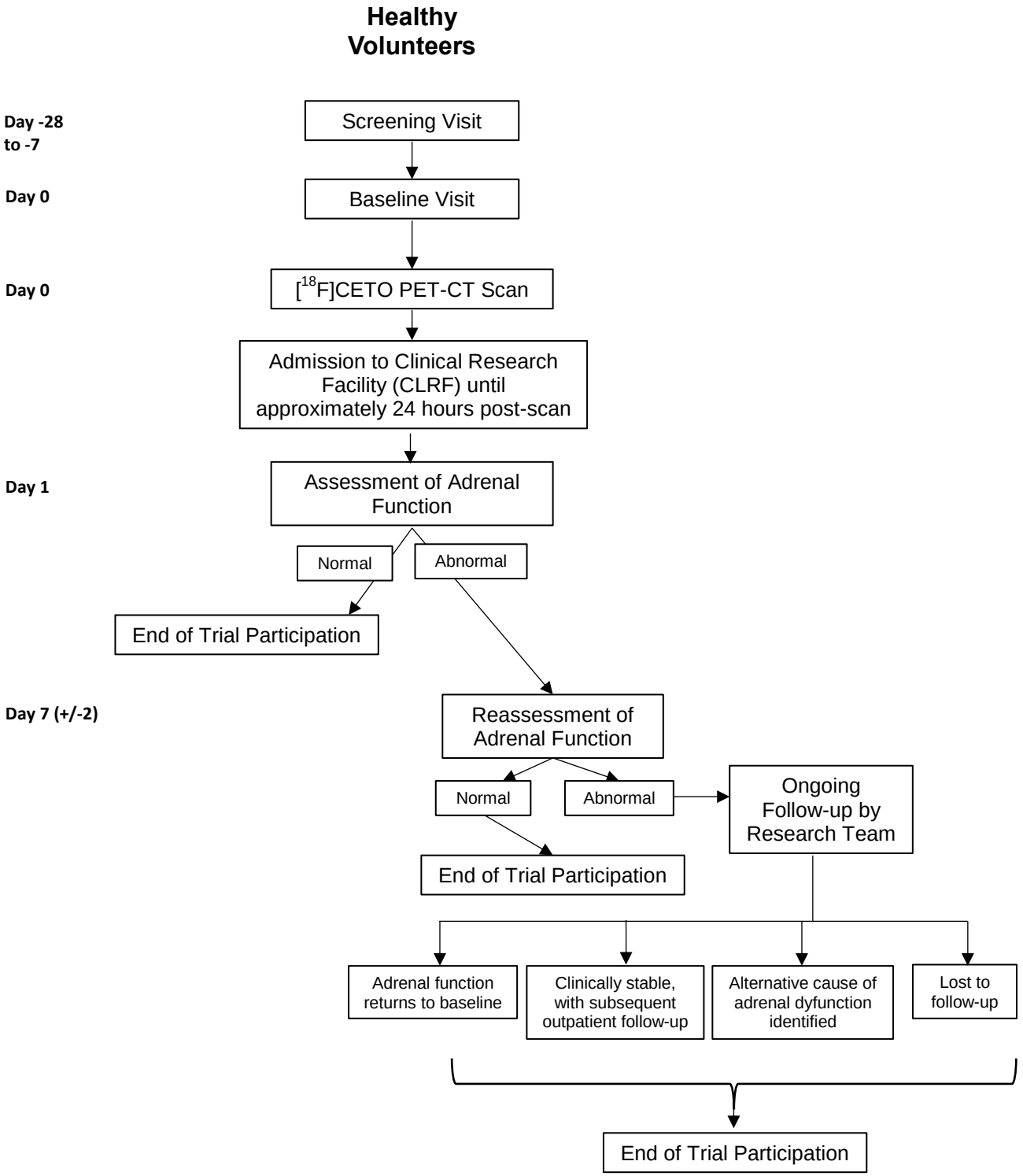
of a participant	in patients but not healthy volunteers
Procedures: Screening & enrolment	• Medical history (including medications) and physical examination • 12-Lead ECG • Pregnancy test (serum hCG) in females of child-bearing potential • Blood tests: - full blood count (FBC) (haemoglobin, white cell count, platelet count) - urea & electrolytes including creatinine (U&Es), bicarbonate - liver function (LFTs) [bilirubin, alanine transaminase (ALT), alkaline phosphatase (ALP)] -short synacthen test (SST) • Urine tests: - drugs of abuse screen (amphetamine, cocaine, marijuana, benzodiazepine, tricyclic antidepressant, barbiturates, metamphetamine, morphine, methadone, MDMA) • Inclusion/exclusion criteria review
Procedures: Baseline	• Confirmation of continued willingness and eligibility to participate in the study • Update to medical history (including current medication) and physical examination • Recording of participant demographics • Repeat 12-lead ECG • Pregnancy test (urine hCG) in females of child-bearing potential • Blood tests: - FBC - U&Es, bicarbonate - LFTs - Direct Renin Concentration (DRC) - Plasma Aldosterone Concentration (PAC) - Adrenocorticotropic Hormone (ACTH)
Procedures: Treatment period	Each participant will receive 100-200 MBq [^{18}F]CETO by intravenous injection. Following diagnostic and attenuation correction CT, dynamic PET image acquisition will be performed for 90 minutes. Following completion of the PET-CT scan, both patients and healthy volunteers will be

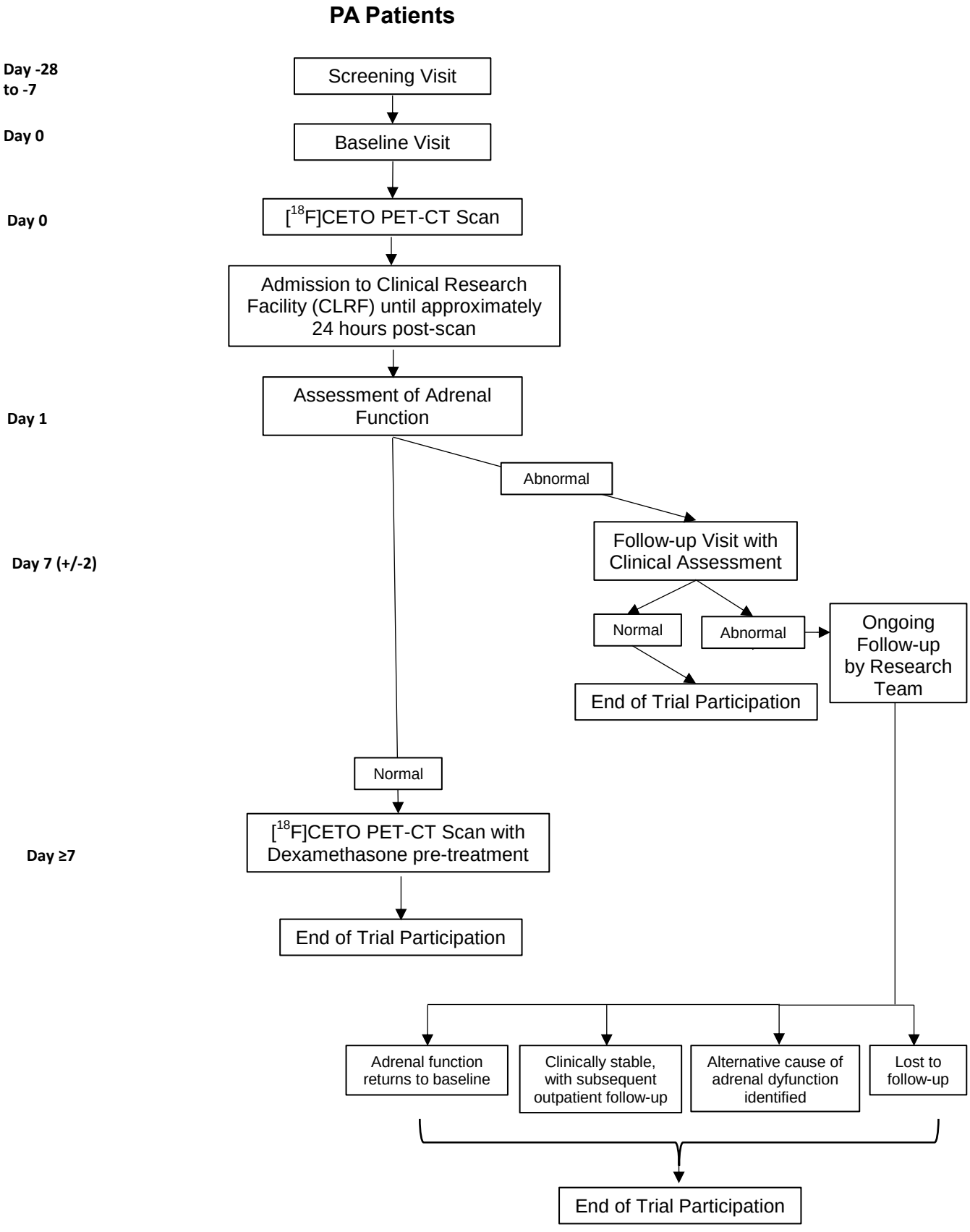
	admitted for an approximately 24-hour observation period on the Clinical Research Facility (CLRF). On the morning following the scan, blood samples will be taken which will include the following: • FBC • U&Es • LFTs • Short Synacthen Test *Patient-specific Activities (for patients who returned normal SST results)* Patients will be provided with a prescription for dexamethasone 500 micrograms to be taken four times daily for three days (total 12 doses). Dexamethasone will be self-administered for three days prior to the repeat PET-CT scan. Patients will be provided with contact details for the research team if they have any questions while taking dexamethasone. Patients will undergo a repeat scan ≥7 days after the first PET-CT scan following dexamethasone pre-treatment as part of standard clinical care. Blood samples (FBC, U&Es, LFTs, ACTH, DRC, PAC) will be taken prior to the second PET-CT scan. In the event that no abnormal findings were recorded after the first scan, patients will be observed for up to four hours following the second scan, and then discharged with no requirement for admission to the CLRF or an additional SST.
Procedures: End of trial	Healthy volunteers will conclude their involvement in the trial after their final assessment has demonstrated normal adrenal function. In the extremely unlikely event that an abnormality persists, the participant will continue to be monitored within the trial until one of the following conditions is met: • Adrenal function returns to baseline, as determined by follow-up SST. • Participant remains clinically stable,

	and where further follow-up can be managed safely in the outpatient setting. • An alternative cause for the adrenal dysfunction is identified. • Participant is lost to follow-up. Upon which their involvement in the trial will end. Patients will conclude their involvement in the trial after the second [^{18}F]CETO PET-CT scan. However, any patient with abnormal adrenal function test results following the first scan will not undergo a second PET-CT scan. Instead, they will continue to be monitored within the trial until one of the following conditions is met: • Adrenal function returns to baseline, as determined by follow-up SST. • Patient remains clinically stable, and where further follow-up can be managed safely in the outpatient setting. • An alternative cause for the adrenal dysfunction is identified. • Patient is lost to follow-up Upon which their involvement in the trial will end.
Procedures for safety monitoring during trial	Automatic trial stopping criteria will be met if a single participant experiences any serious adverse reaction (i.e. a serious adverse event considered at least possibly related to the IMP administration). Stopping criteria will additionally apply if any severe non-serious adverse reactions (ARs) develop in two participants, independent of whether or not these are within the same system-organ-class. The following will then occur: • The trial will be halted via a substantial amendment to the MHRA. • No further dosing or recruitment will be undertaken • A safety review by the Data Monitoring Committee (DMC) will be conducted to determine how to proceed with the trial • Any patients who have their dosing

	discontinued as a result of a SAR or a severe non-serious AR will be withdrawn from the trial • Re-initiation of the trial can only be considered after MHRA approval via a substantial amendment. Any concurrently active participants will be re-consented before further IMP doses are administered, and all completed participants will be informed of the SAR or severe non-serious ARs. Finally, if there is no demonstrable [^{18}F]CETO uptake into human adrenal tissue in the first 4 participants (either healthy volunteers and/or patients), the trial will be halted and the DMC will be asked to consider if the trial should be stopped.
Criteria for withdrawal of participants	• A participant can decide to withdraw from the trial at any point without further explanation • A participant can be withdrawn from the trial at the Investigator's discretion, as a result of safety considerations (e.g. an adverse event regardless of relation to the IMP) or protocol violation (e.g. failure to return for visits). • Withdrawal from the trial will also occur in the event of participant pregnancy. • Healthy volunteers will be withdrawn from the trial in the event that they are taking medication at the point of [^{18}F]CETO administration.

6 Trial Flow Chart




PA Patients
Day -28 to -7
Screening Visit
Day 0
Baseline Visit
Day 0
[18F]CETO PET-CT Scan
Admission to Clinical Research Facility (CLRF) until approximately 24 hours post-scan
Day 1
Assessment of Adrenal Function
Abnormal
Day 7 (+/-2)
Follow-up Visit with Clinical Assessment
Normal
Abnormal
Ongoing Follow-up by Research Team
End of Trial Participation
Normal
Day ≥7
[18F]CETO PET-CT Scan with Dexamethasone pre-treatment
End of Trial Participation
Adrenal function returns to baseline
Clinically stable, with subsequent outpatient follow-up
Alternative cause of adrenal dyfunction identified
Lost to follow-up
End of Trial Participation

7 Introduction

7.1 Background

At least one quarter of the UK adult population has hypertension, which is a major risk factor for heart attacks and stroke. Primary aldosteronism (PA), a treatable form of hypertension, accounts for 5-10% of all cases, and 20-25% of those with difficult to control ('resistant') hypertension[1]. Determining whether one (potentially curable with surgery) or both (requiring long-term drug treatment) adrenal glands are the source of PA in any given patient remains challenging. Existing lateralising procedures (i.e. investigations to distinguish one from two gland involvement) include computed tomography (CT) or magnetic resonance imaging (MRI). However, these often lack appropriate sensitivity and specificity, while the highly invasive adrenal vein sampling (AVS), in which small catheters are placed in to each adrenal vein, is time-consuming, technically demanding, and fails in 20-50% of cases[2]. Such diagnostic challenges have traditionally resulted in less than 300 adrenalectomies per year in the UK for PA (HES data). Therefore, there is an urgent need for an accurate and more widely available method for reliably diagnosing the cause of PA.

To address this, we have adopted a novel diagnostic approach using positron emission tomography – computed tomography (PET-CT) as an alternative to AVS. Currently this uses metomidate labelled with carbon-11 ([^{11}C]MTO) as a radiotracer, which is taken up preferentially by the adrenal gland, and in particular by adrenal tumours causing PA. However, its utility is limited by a short tracer half-life, which means the scan can only be performed in centres with an on-site cyclotron facility (currently less than 10 NHS sites in the UK). We therefore wish to investigate an innovative new tracer with a longer half-life, [^{18}F]CETO, for the lateralisation of PA.

<u>Primary Aldosteronism</u>

PA is characterised by excessive, unregulated production of aldosterone by the adrenal cortex, which is independent of the normal renin-angiotensin-aldosterone (RAS) pathway. Aldosterone excess may arise either from an aldosterone-producing adenoma (APA) or bilateral adrenal hyperplasia (BAH). APAs have been demonstrated to harbour somatic mutations in various genes, including *KCNJ5, ATP1A1* and *CACNA1D*[3].

Elevated aldosterone levels result in increased salt and water reabsorption through the kidney, leading to a rise in blood pressure (BP). It may also be associated with hypokalaemia (low serum potassium levels) due to renal potassium loss. The increased circulating blood volume results in suppression of renin production by the kidney's juxtaglomerular apparatus (JGA). Initial biochemical evaluation of a patient for possible PA requires calculation of the aldosterone to renin ratio (ARR), with an elevated ratio suggestive of PA. Confirmatory testing (e.g. saline infusion test, captopril challenge test or fludrocortisone suppression test), to demonstrate autonomous aldosterone production, may be required following initial screening.

Once a diagnosis of PA has been confirmed, patients move to the second phase of investigation, in which a lateralisation procedure is used to determine whether the cause of PA is unilateral or bilateral. Unilateral disease can be managed with adrenalectomy

and therefore represents a potentially reversible cause of PA. Current guidelines advise that cross-sectional imaging (e.g. adrenal CT or MRI) alone is insufficient to establish a diagnosis of unilateral PA, unless the patient is young (<35 years old) with clear evidence of a normal adrenal gland on the side opposite to a suspected lesion. For all other cases, AVS is considered the gold standard for lateralisation. However, this procedure has several limitations, in particular it is technically challenging with failure to adequately cannulate both adrenal veins in 25 to 50% of cases[4]. It additionally requires avoidance of certain antihypertensive medications, which can confound interpretation of the findings, and this can prove to be a major stumbling block because of the resistant nature of hypertension requiring multiple antihypertensive agents. Patients may therefore fail to proceed to surgery even in the context of unilateral PA due to an inability to successfully perform AVS or because of inconclusive results. This highlights the need to identify an alternative lateralisation technique in PA.

Positron Emission Tomography (PET)

Etomidate is an anaesthetic agent that binds to aldosterone synthase and 11β-hydroxylase (the final enzyme in cortisol synthesis) encoded for by the genes *CYP11B2* and *CYP11B1*, respectively. It can be chemically modified by changing the ethanol ester to a methyl ester group, to produce the compound metomidate ([^{11}C]MTO). Due to its high specificity for the adrenal cortex, [^{11}C]MTO has been used in Uppsala since 2000[5] and Cambridge since 2008[6] in the diagnostic work up of adrenal tumours. Previous studies in patients with PA have demonstrated elevated Standardized Uptake Values (SUV, used in nuclear medicine for quantitative analysis of PET- imaging) in aldosterone-producing adenomas in comparison to non-functional and cortisol-producing adrenal adenomas, presumably due to a higher expression of aldosterone synthase[5,6]. We have previously shown [^{11}C]MTO PET can be a sensitive and specific alternative to AVS for the investigation of PA[5]. However, [^{11}C]MTO is labelled with the short-lived radioisotope carbon-11 ($t_{1/2}$ =20 minutes), which limits this technique to the few PET centres with on-site radiopharmacies with cyclotrons for its manufacture. Therefore, a key objective is to overcome this barrier to wider expansion of this technique by developing a radiopharmaceutical labelled with the longer-lived radioisotope fluorine-18 ($t_{1/2}$ =110 minutes), which can be supplied to external centres.

To address this, we have developed [^{18}F]CETO, a fluorine-18 analogue of metomidate, as a potential alternative for PET imaging in patients with PA. Subsequent roll out of this radiopharmaceutical would facilitate the creation of a national network for molecular imaging in PA. There are several properties of [^{18}F]CETO which indicate it would bring substantial additional advantages for imaging the adrenal glands by PET. It is radiolabelled with the fluorine-18 radioisotope with a $t_{1/2}$ of 110 minutes, enabling wider dissemination, and in particular use by centres without a cyclotron facility. Fluorine-18 is the primary radioisotope used for commercial PET radiopharmaceuticals (e.g. as used for [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) the most widely produced and used PET radiopharmaceutical). Fluorine-18 radiopharmaceuticals can be transported to PET centres 100-150 miles away from the radiopharmacy (up to 4-6 hours from radiosynthesis to use) for application in clinical PET studies (as exemplified by [^{18}F]FDG). We believe molecular imaging with [^{18}F]CETO could provide a ready solution for distinguishing unilateral and bilateral disease in patients with hypertension secondary to PA. In this way, many more patients than is currently the case could be

considered for surgical intervention/ablative strategies, with the prospect of achieving cure and thereby avoiding the requirement for long-term anti-hypertensive medication and its attendant side effects.

We will use dexamethasone pre-treatment prior to the repeat (i.e. second) PET scan in the patient subgroup. Such pre-treatment (given as dexamethasone 500 micrograms four times daily for three days) is used routinely when performing [^{11}C]MTO PET-CT scans, and is considered a core component of standard clinical care [we and others have shown that MTO also binds to the enzyme 11-β-hydroxylase, which is structurally very similar to aldosterone synthase[7]; by lowering adrenocorticotrophic hormone (ACTH) levels in the body (which occurs following dexamethasone treatment), expression of the enzyme 11-β-hydroxylase is concomitantly lowered]. Technological advancements in PET-CT imaging, combined with dexamethasone pre-treatment, have been shown to improve discrimination between abnormal and normal adrenal tissue[5].

Manufacture of [^{18}F]CETO

The product, [^{18}F]CETO (2-^{18}Fluoroethyl 1-[(1R)-1-(4-chlorophenyl)ethyl]-1H-imidazole-5-carboxylate), is a radiopharmaceutical radiolabelled with the short-lived positron-emitting radioisotope fluorine-18 ($t_{1/2}$ = 110 min) for application in human imaging studies using PET. Its manufacture begins with a stable chemical precursor. Using this precursor, a one-step radiochemical method has been developed for incorporating the fluorine-18 radioisotope through a [^{18}F]nucleophilic reaction with cyclotron-generated [^{18}F]fluoride to produce [^{18}F]CETO in radiochemical yields of up to 38% at end-of-synthesis (Figure 1). This method has been successfully implemented onto commercial derived radiosynthesis modules for high radioactivity manufacture under GMP. The [^{18}F]CETO is then obtained in yields of 1–5 GBq at end-of- synthesis with molar activities of >200 GBq/micromol and radiochemical purity of >95%.

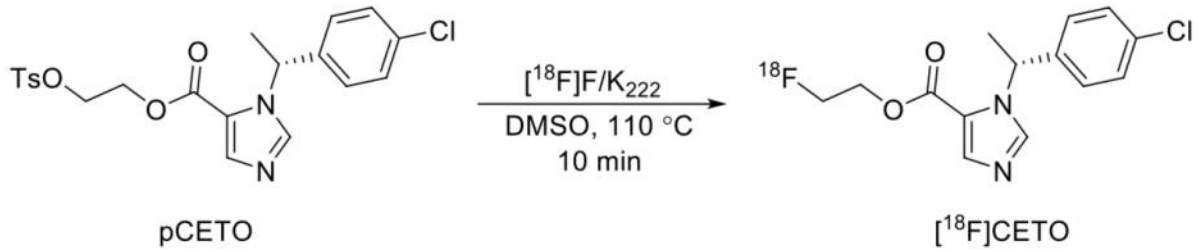


Figure 1: Synthesis pathway for [^{18}F]CETO.

Starting with 20-30 GBq of [^{18}F] fluoride gives approximately 1-5 GBq of [^{18}F]CETO (radiochemical purity >95%) with a synthesis time of around 90 minutes including quality control. The molar activity is greater than 150 GBq/micromol at time for injection. [^{18}F]CETO is produced in close proximity to the PET-CT unit and administered within 60 minutes from release of [^{18}F]CETO. The shelf-life at room temperature does not exceed 8 hours.

As described in the following section, we have established, through a series of *in vitro* studies on human adrenal tissue (including resected adrenal adenomas), and by *in vivo*

PET imaging studies on rats and non-human primates, that [^{18}F]CETO demonstrates high, specific uptake by the adrenal glands.

7.2 Data from non-clinical studies (for early phase trials)

[^{18}F]CETO has undergone a series of preclinical studies to evaluate its selectivity for the adrenal glands and future potential application in the investigation of PA. This has been achieved through a combination of *in vitro* and *in vivo* (rat and non-human primate) studies.

In Vitro PET Studies – using human adrenal tissue, a human adrenal cell line, and adrenal gland from Cynomolgus Monkeys

Autoradiography of human tissue was performed on normal adrenal gland and adrenocortical adenomas with [^{18}F]CETO. Human liver, kidney, small intestine and spleen, as well as normal adrenal gland, liver and spleen from Cynomolgus monkey, were used as control tissues. In each case, samples were exposed to [^{18}F]CETO and its specific binding and displacement by metomidate, or cold (i.e. unlabelled) CETO, were investigated. We were able to demonstrate that while uptake of [^{18}F]CETO in adrenocortical adenomas and adrenocortical carcinomas showed some variation between different tumours, in general it exceeded the uptake observed in non-adrenocortical tumours (phaeochromocytoma) and in organs close to the human adrenal glands (e.g. liver, kidney). Furthermore, the uptake of [^{18}F]CETO in human and Cynomolgus monkey normal adrenal gland, human and Cynomolgus monkey spleen, and in human adrenocortical adenomas (Figure 2) and carcinomas was specific, whilst the uptake of [^{18}F]CETO in human liver, kidney, small intestine and Cynomolgus monkey liver was mostly non-specific.

Secondly, human adrenal adenomas with differing expression levels of aldosterone synthase [as determined by immunohistochemistry (IHC)] were studied to test for a possible correlation between IHC expression level and SUV values. However, no clear correlation was observed.

Thirdly, human adrenal cancer cells (from the cell line HAC15) were used in preliminary pharmacodynamic studies together with tissue from normal Cynomolgus monkey adrenal gland. Initial results indicated that [^{18}F]CETO shows fast binding kinetics and a high affinity for adrenocortical tissue and cells. The specific uptake of [^{18}F]CETO in normal Cynomolgus monkey adrenal gland exceeded the uptake in HAC15 cells. The study was repeated using normal Cynomolgus monkey adrenal gland and the results were used to calculate the equilibrium dissociation constant of [^{18}F]CETO and its target; a K_d-value of 90 picomol confirmed [^{18}F]CETO as a high affinity ligand.

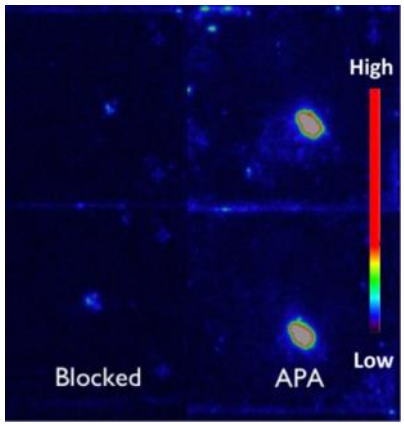


Figure 2. Frozen sections from two human aldosterone-producing adenomas (APA) showing uptake of [^{18}F]CETO, which is blocked by unlabelled ('cold') metomidate), confirming specific binding of [^{18}F]CETO.

In Vivo PET Studies – Rat

Rats were injected with [^{18}F]CETO and dynamic PET-MR performed. Specificity of binding was assessed following blockade with metomidate. The rat model demonstrated minimal liver uptake [SUV at 60 minutes of 0.5, which is considerably less than [^{11}C]MTO (3.0)], resulting in an adrenal:liver SUV ratio of 44 [which is substantially greater than for [^{11}C]MTO (ratio of 4)]. This property resulted in improved visualisation of the right adrenal, with less confounding background tracer uptake in the liver (Figure 3). *In vivo* studies of rats demonstrated that the stability of [^{18}F]CETO was comparable to [^{11}C]MTO. [^{18}F]CETO was identified to have a biphasic metabolic profile. There was a decrease to 20% of parent compound within 10 minutes, followed by slower metabolism, decreasing to 5% at 90 min. When combined, these features should yield substantially better "signal-to-noise" PET images for [^{18}F]CETO.

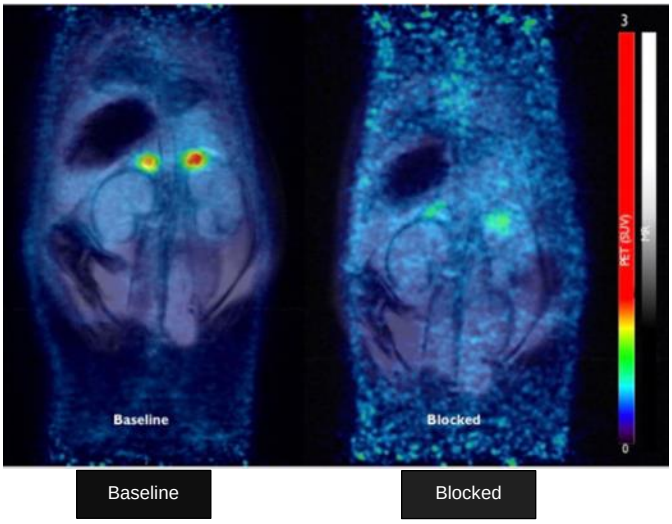


Figure 3: Dynamic PET, combined with Magnetic Resonance Imaging, demonstrates high uptake of [^{18}F]CETO by rat adrenal tissue, which is specific (blocked by cold competing metomidate).

In Vivo PET Studies – Non-human primate (Cynomolgus Monkey - *Macaca fascicularis*)

In vivo PET studies in *Macaca fascicularis* were performed to compare the biodistribution of [^{18}F]CETO with [^{11}C]MTO. The studies demonstrated high specific binding to the adrenal glands combined with low non-specific uptake in the liver and the kidneys at 90 minutes (Figure 4). No adverse effects were observed following [^{18}F]CETO administration.

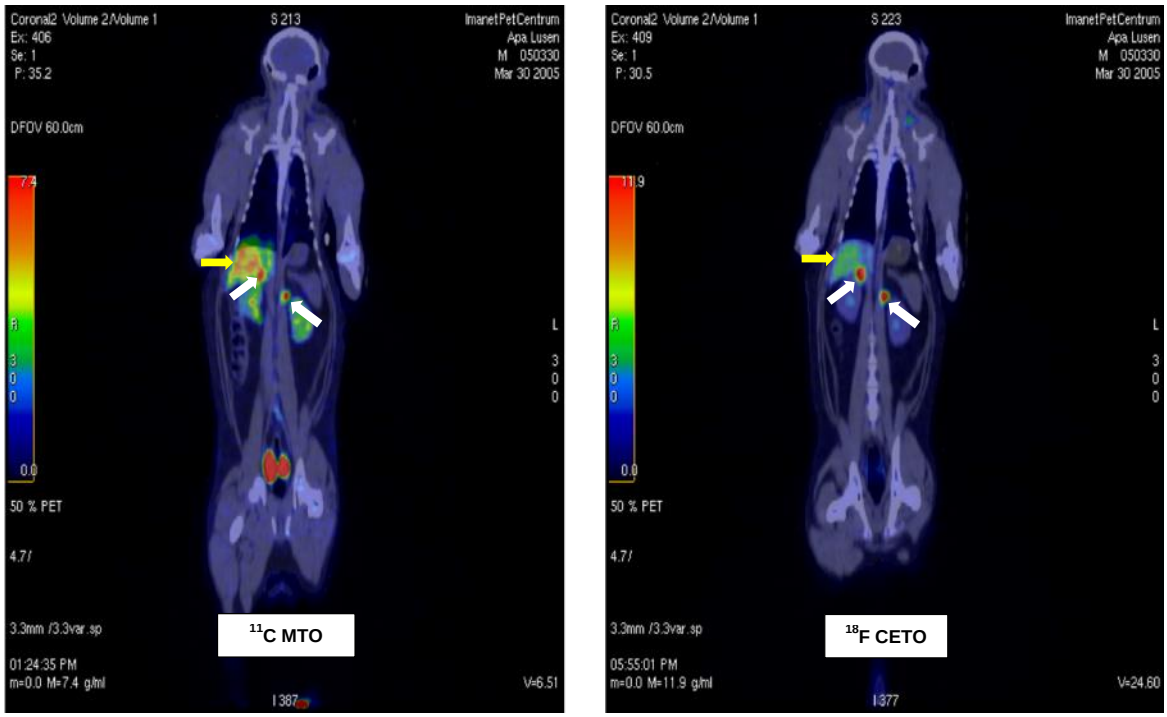


Figure 4: Comparison of [^{11}C]MTO and [^{18}F]CETO uptake in non-human primates (*Macaca fascicularis*). Following administration of [^{18}F]CETO, there is increased adrenal gland (white arrows) and reduced liver (yellow arrow) uptake when compared to [^{11}C]MTO.

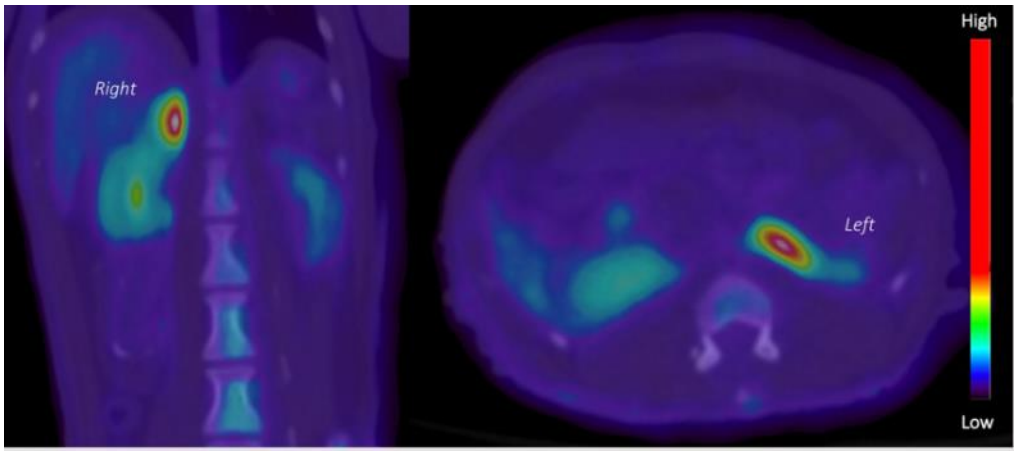


Figure 5: At 90 minutes post-injection with [^{18}F]CETO in *Macaca fascicularis*, uptake of [^{18}F]CETO in the right- and left adrenal glands, respectively, is clearly visible and distinguishable from any other uptake of [^{18}F]CETO in surrounding organs.

Results from metabolite analyses of venous blood samples from *Macaca fascicularis* show [^{18}F]CETO has a bi-phasic metabolism with a fast first phase with an approximate plasma half-life of 10 minutes followed by a second slower phase. At the end of a typical dynamic PET-scan of 90 minutes [^{18}F]CETO represents about 10% of radioactivity in plasma. The kinetics of metabolism are compatible with the kinetics of tissue uptake, and [^{18}F]CETO shows sufficient stability to give high uptake in the adrenals as well as elimination of the tracer from blood.

Pharmacology Studies – Rats

Toxicology assessment for [^{18}F]CETO, based on the micro-dosing guidelines, was performed in rats, using 1000-fold and 100-fold the expected human dose of 10 micrograms/per subject (details provided in the investigator brochure and Appendix 1). Administration of [^{18}F]CETO by a single intravenous injection was well tolerated in male and female rats at dose levels of 0.0143 and 0.143 mg/kg. Based on these outcomes, we consider [^{18}F]CETO has appropriate properties for phase 1 human PET studies.

As anticipated at these high doses of 0.143mg/kg (1000-fold dosing), [^{18}F]CETO suppressed plasma corticosterone levels in keeping with its known pharmacological properties. While this is unlikely to be relevant to human studies with [^{18}F]CETO given the much lower dose that will be administered, we will undertake an assessment of adrenal function 24 hours after [^{18}F]CETO administration to confirm this is not a clinically relevant concern.

7.3 Clinical data

To date, no clinical trials of [^{18}F]CETO have been performed in humans. We are therefore seeking the necessary approvals to allow us to undertake a First-in-Human (FiH) trial. We propose to study up to five healthy volunteers and six patients with confirmed PA to assess the safety and tolerability of [^{18}F]CETO. These studies will also provide an early indication of the extent of uptake of [^{18}F]CETO by both normal and abnormal human adrenal glands. Healthy volunteers will receive a single dose of the tracer followed by a PET-CT scan. Six patients with PA (three with unilateral PA and three with bilateral PA) will each receive [^{18}F]CETO followed by a PET-CT scan on up to two occasions to allow us to determine whether pre-treatment with a short (72 hour) course of dexamethasone can improve the signal to background uptake of [^{18}F]CETO, as we have previously demonstrated for 11C-Metomidate[1].

8 Rationale for Trial

AVS is currently the gold standard for lateralisation in PA. However, it has several limitations, including technical failures and a need to discontinue confounding medications that may interfere with interpretation. These severely limit its availability and utility in routine clinical practice. Our solution to address this has been to use PET coupled with the radiopharmaceutical [11C]metomidate ([^{11}C]MTO) for molecular adrenal imaging. However, [^{11}C]MTO is limited by a short half-life ($t_{1/2}$ = 20 minutes) therefore restricting this imaging modality to facilities with an on-site cyclotron. [^{18}F]CETO has a longer half-life ($t_{1/2}$= 110 minutes). It can be distributed to centres 100-150 miles from the primary radiopharmacy site. Preclinical studies have suggested a higher adrenal selectivity with reduced uptake by adjacent organs (e.g. liver, spleen) in comparison to [^{11}C]MTO. Therefore, we anticipate [^{18}F]CETO will offer increased

efficacy in PA lateralisation in addition to promoting the creation of a national network for molecular imaging in PA.

The expected dose of [^{18}F]CETO is in the range of 0.2μg to 2μg which is based on data from a validated production method as described in the investigational medicinal product (IMP) dossier. The selected dose is close to the maximum allowed, estimated from dosimetry calculations and taking into account animal *in vivo* studies. Based on clinical data derived from this study, we will be able to predict what dose should be sufficient to give the required signal to noise ratio, and to be used in future studies. The administration of up to 10μg of the study drug is regarded as safe due to [^{18}F]CETO being a very close analogue to the well-known registered drug Etomidate. The total radiation dose to patients participating in two [^{18}F]CETO PET-CT scans is below 10mSv which is the current standard limit for healthy controls in clinical PET research studies[8]. There is no anticipated clinical benefit for participants in this study. This is a Proof-of-Concept study to investigate the safety and efficacy of [^{18}F]CETO.

9 Trial Design

9.1 Statement of Design

This is a Phase 1, single-centre, open label, micro-dosing study.

9.2 Number of Centres

This is a single-centre study conducted at Cambridge University Hospitals NHS Foundation Trust.

9.3 Number of Participants

We intend to recruit 11 participants: 5 of these subjects will be healthy controls, and 6 will be patients with PA (three with unilateral disease and three with bilateral disease as lateralised on prior AVS). Recruitment of healthy volunteers and patients will occur concurrently (i.e. it will not be necessary for all healthy volunteers to be recruited before patients will be recruited).

9.4 Participants Trial Duration

The trial duration will be approximately 1 month and will consist of:

- A screening visit (1-2 hours duration) conducted up to 28 days prior to the scan
- A baseline visit (lasting up to 2 hours) prior to the PET-CT scan
- Injection of [^{18}F]CETO followed by a 90 minute PET-CT scan for both healthy volunteers and patients
- All participants will remain in the PET-CT unit for up to 4 hours, following which they will be admitted to the clinical research facility (CLRF) for approximately 24 hours
- Where required, a 7 (+/- 2) day follow up visit (lasting up to 2 hours) for repeat synacthen testing
- Patient self-administration of Dexamethasone for 3 days
- Patients (but not healthy volunteers) will attend for a repeat administration of [^{18}F]CETO followed by a 90 minute PET-CT scan ≥7 days following the initial scan. On this occasion, they will be discharged following a 4 hour observation period on the PET-CT unit. There will be no requirement for patients to have an overnight

observation period on the CLRF following the repeat PET-CT scan due to tolerance of [^{18}F]CETO administration and normal blood results following the initial PET-CT scan.

9.5 Trial Objectives

9.5.1 Primary objective

- To evaluate the safety of up to two administrations of [^{18}F]CETO in up to 6 patients with primary aldosteronism and 5 healthy volunteers.

9.5.2 Secondary objectives

- To assess [^{18}F]CETO uptake by the adrenal glands
- To evaluate uptake in bilateral vs unilateral cases of PA following [^{18}F]CETO administration in up to 6 patients

9.6 Trial Outcome Measures

9.6.1 Primary outcome Measure

- The primary outcome measure is the overall safety of [^{18}F]CETO. This will be assessed according to the frequency of adverse events, serious adverse events, clinically significant changes in vital signs, ECG and laboratory parameters.

9.6.2 Secondary outcome measures

- [^{18}F]CETO uptake by the adrenal glands will be assessed by measurement of Standardized Uptake Values (SUV) over the left and right adrenal glands: all assessments will be performed by a dedicated blinded reviewer.
- Evaluation of adrenal uptake of [^{18}F]CETO in bilateral versus unilateral cases of PA will be performed by comparing SUV values of both adrenal glands in three patients with each subtype of PA (using a dedicated blinded reviewer).

10 Selection and withdrawal of participants

10.1 Inclusion Criteria

Inclusion Criteria:

Healthy Volunteers

To be included in the trial the participant must:

- give written informed consent
- be aged 50 years or over
- have no underlying medical conditions
- be able to lie down for at least 2 hours and not be claustrophobic

In addition, all female participants must be:

- post-menopausal (no menses for 12 months, without an alternative medical cause)

Patients

To be included in the trial the patient must:

- give written informed consent
- be aged 40 years or over

- be able to lie down for at least 2 hours and not be claustrophobic
- fulfil the following criteria:
  - have a confirmed diagnosis of PA as per Endocrine Society guidelines
    - At least one paired measurement of plasma renin and aldosterone, measured off Spironolactone/Eplerenone within past 12 months, showing ARR above local threshold value.
    - One of the following two criteria:
      - Plasma aldosterone>190pmol/L following saline infusion.
      - Spontaneous hypokalaemia, suppressed plasma renin and plasma aldosterone>550pmol/L.
  - have undergone successful lateralisation of the cause of PA to one or both adrenal glands by adrenal vein sampling (AVS).
  - be willing to have two scans

In addition, all female patients must have a negative (blood) pregnancy test at the screening visit.

10.2 Exclusion Criteria

All participants:

- allergy to radiographic contrast agents
- allergy or contraindication to synacthen
- pregnancy, breastfeeding, or the intention to become pregnant during the 6 months following trial participation
- positive pregnancy test at the screening or baseline visits
- assessed by the investigator as being unable or unwilling to comply with the requirements of the study protocol.
- receipt of another IMP as part of a CTIMP
- prior radiation exposure as part of previous research studies
- recreational drug use, or substance/alcohol dependency
- clinically abnormal screening blood tests [including abnormal short synacthen test (SST)].

Additional exclusion criteria for **healthy volunteers**:

- women of child-bearing potential (i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy)
- exposure to radiation during their work
- received more than 10 mSv of radioactivity in the past 12 months
- any subject with a history of adrenal disease or who, at the screening visit, reports symptoms, or exhibits physical signs, that could be consistent with previously unsuspected adrenal disease

Additional exclusion criteria for **patients**:

- allergy or contraindication to dexamethasone treatment (or lactose intolerant)

10.3 Participant Withdrawal Criteria

A participant can decide to withdraw from the trial at any time without further explanation. Participants may be removed from the trial at their choice or at Investigator's discretion if it is felt to be clinically appropriate. Reasons for participant withdrawal will be recorded in the Case report form (CRF). The participant will be reassured that their future care will not be affected by their withdrawal, and that their relationship with their responsible physician remains unaltered. The participant will be informed that any of their study data already stored will be retained, and will be used, if necessary, in the evaluation of the study drugs and for adherence to the relevant regulatory requirements.

Any participants who have their dosing discontinued will be withdrawn from the trial (as described in Section 11.5 Ongoing Safety Review). Withdrawal from the trial will also occur in the event of participant pregnancy. Healthy volunteers will be withdrawn from the trial in the event that they are taking medication at the point of [^{18}F]CETO administration.

11 Trial Treatments

For the purpose of this trial, the radiopharmaceutical [^{18}F]CETO, administered intravenously, is considered a Investigational Medicinal Product (IMP).

Oral dexamethasone is considered as a Non- Investigational Medicinal Product (nIMP) within this trial and will be supplied by local site's own stock, using standard labels and packaging.

The investigator is responsible for ensuring that deliveries of [^{18}F]CETO and other study materials are safely and properly received, recorded, handled, and stored in accordance with all applicable regulatory guidelines, and used in accordance with this protocol. This responsibility may be delegated to appropriately trained personnel.

11.1 Treatment Summary

[^{18}F]CETO injection is a radiopharmaceutical, and will be handled in accordance with all applicable regulations. Radiopharmaceuticals will be used only by personnel (e.g radiopharmacists, radiographers) who are qualified by specific training in safe use and handling of radioactive drugs and materials. Also, care will be taken to ensure minimum radiation exposure to the patient and all personnel involved in the procedure.

The IMP consists of the radiopharmaceutical [^{18}F]CETO. The manufacture of [^{18}F]CETO will be performed at the Wolfson Brain Imaging Centre (WBIC) Radiopharmaceutical Unit in Cambridge, UK. Details of manufacture of [^{18}F]CETO for injection are outlined in the IMPD. Due to being radiolabelled with a short-lived radioisotope (fluorine-18 $t_{1/2}$ = 110 minutes) the IMP is produced "on demand" for which stability studies have established a shelf life of 8 hours. No long-term storage is required.

A batch release certificate of the product is provided by the Qualified Person (QP) of the manufacturing site to the investigational centres to confirm that the product is suitable for administration, and that it meets the drug product specifications stated in the Investigational Medicinal Product Dossier (IMPD), including the filter integrity test requirements.

A summary of the IMP components is given below

Chemical name: 2-[^{18}F]fluoroethyl1-[(1R)-1-(4-chlorophenyl)ethyl]-1H-imidazole-5-carboxylate

Formulation: 15.2 mL phosphate buffer pH 7.4, 0.8 mL EtOH, 0.9% Sodium Chloride Injection

Manufacturer: Radiopharmaceutical Unit, Wolfson Brain Imaging Centre, Cambridge Biomedical Campus, CB2 0QQ

Storage: At room temperature, Shelf life of 8 hours

11.1.1 Name and description of IMP [^{18}F]CETO

11.1.1.1 *Legal status*

The trial is being carried out under a Clinical Trial Authorisation. The drug is therefore only to be used by the named investigators, for the participants specified in this protocol, and within the trial.

11.1.1.2 *Supply*

For the trial studies at Cambridge University Hospitals NHS Foundation Trust (CUH) PET-CT Unit, the [^{18}F]CETO will be supplied as a sterile solution in multi-dose vials by the nearby WBIC Radiopharmaceutical Unit (University of Cambridge), where it will be manufactured, packaged and labelled at a qualified and GMP-validated facility. The product will be delivered using “Type A” radioactivity containers to reduce any radioactivity exposure to the operators. Supply will be initiated on receipt of a suitably signed radiopharmaceutical prescription form as part of the manufacturing standard operating procedures.

Before administration, the suitability of each preparation will be assessed for radioactivity, as well as by high-performance liquid chromatography (HPLC) and pH measurement. Suitable preparations will be certified by a QP according to approved standard operating procedures (SOPs) of the GMP manufacturing site.

11.1.1.3 *Packing and Labelling*

Labels will be prepared in accordance with Good Manufacturing Practice Annex 13 requirements and local regulatory guidelines.

[^{18}F]CETO will only be dispatched to site after receipt of confirmation that the regulatory checklist is complete. Due to the short half-life of [^{18}F]CETO orders will be made on a per-patient basis. For each injection, a release certificate will be provided by email which will include the following information: batch number, date of preparation, radioactive concentration of injection (MBq/ml) at stated time and shelf life.

11.1.1.4 *Storage conditions*

[^{18}F]CETO must be stored in a secure area with access limited to authorised site staff only. [^{18}F]CETO Injection should be stored at room temperature in a lead-shielded container and in accordance with national regulations for radioactive materials. The precautions normally taken when handling radioactive materials will be observed. Maintenance of a temperature log (manual or automated) is required. For further information investigators should refer to the investigator brochure and IMP Handling Manual.

11.1.1.5 *Maximum duration of treatment of a participant*

Healthy volunteers will be given a single dose of [^{18}F]CETO only.

Patients will have administration of up to two [^{18}F]CETO doses across two separate visits.

11.1.1.6 *Radiation Exposure*

The administered activity of [^{18}F]CETO will be at a dose of 2 Mega-Becquerel (MBq)/kg, up to a maximum of 200MBq (based on a 100kg patient). The activity concentration of 2MBq/kg has been identified on pre-clinical animal data to show good adrenal uptake. The administered radioactivity of [^{18}F]CETO will be recorded on the PET treatment record, and should be within +/-10% of the prescribed activity based on weight. No dose escalation will be performed in the trial; however, dose optimisation will be performed on conclusion of the study for guiding optimal dose in future studies.

		Maximum Effective dose (mSv)	
		Healthy Volunteers	Patients
Initial PET-CT Scan	PET	4.10	4.10
	Low dose CT	0.81	0.81
Repeat PET-CT Scan (Patients only)	Second PET	n/a	4.10
	Second low dose CT	n/a	0.81
	Total dose	**4.91**	**9.82**

Abbreviations: n/a not applicable

11.1.1.7 *Dose*

The total dose of CETO (comprising both [^{18}F]-labelled and naturally occurring unlabeled CETO) in a single bolus will not exceed 10 micrograms. This takes into account the microdosing toxicology study performed in rats, where a dose 1000 times the dose of the planned participant dose was investigated. The dose showed no toxicologic findings in rats. The expectation is that a dose of 1/1000 of this can therefore safely be given to humans without any expected toxicologic effect and the safety margin should be sufficient to account for potential species differences between rats and humans.

11.1.1.8 *Administration*

Since the [^{18}F]CETO vials could contain more radioactivity than is required for 1 subject dose at the time of administration, the correct volume will be calculated prior to administration. The injectable volume of [^{18}F]CETO will be determined by the PET-CT radiographer, based on the concentration (MBq/ml) provided by WBIC Radioipharmaceutical Unit. Where multiple participants will be dosed on a given day, calculation and preparation of each consecutive dose can only be done once the previous dose has been administered. [^{18}F]CETO calculated volume will be injected intravenously immediately prior to scan commencement by a trained radiographer. This will be followed by administration of 0.9% Saline flush, up to a volume of 10ml using a syringe. The administration site will be evaluated pre- and post administration for any reaction (e.g. bleeding, hematoma, redness, or infection.

Aseptic conditions must be observed during withdrawal of a dose from the vial, including microbial decontamination of the rubber stopper with a suitable disinfectant before removal of a dose.

11.1.1.9 *Known drug reactions*

There are no expected drug reactions to [^{18}F]CETO administration.

11.1.1.10 *Known drug interactions and concomitant medications*

There are no expected drug interactions for [^{18}F]CETO. Therefore, continuation of pre-existing concomitant medications for patients is permitted. Concomitant medications at the point of [^{18}F]CETO administration are not permitted for healthy volunteers. Administration of medication to treat adverse events following [^{18}F]CETO administration is permitted.

11.1.1.11 *Dose modifications*

No dose modifications will be made to the dose of [^{18}F]CETO.

11.2 Accountability and dispensing

11.2.1 PET-CT responsibilities

IMP for the trial will be ordered in accordance with the usual clinical process for the CUH PET-CT centre. Documentation of the IMP administered to a subject will be recorded on the appropriate CRF, including date, batch number, total volume, total radioactivity, start/stop time of administration, and injection site. This will be undertaken by a suitably qualified member of the PET-CT unit. Drug accountability IMP accountability will be maintained by the WBIC Radiopharmaceutical Unit in accordance with their SOP(s).

Treatment compliance:
Subjects will receive[^{18}F]CETO Injection under direct supervision of study personnel. Each administration volume and total radioactivity injected should be checked. The label on the IMP container should include the batch number, expiry date/time activity in MBq, time of activity measurement, and volume. The volume per administration should be recorded in each participant's source document and CRF.

11.2.2 Returns and destruction

After decay, the IMP will be returned to the WBIC for disposal. IMP destruction will be performed by the WBIC Radiopharmaceutical Unit in accordance with existing unit SOP(s).Waste must be disposed of according to national regulations for radioactive material.

12 Procedures and assessments

All procedures and assessments will be carried out at CUH.

12.1 Participant identification

Healthy volunteers will be recruited using the approved CETO Healthy Volunteer Poster or through word of mouth. Patients will be identified from the endocrine clinic at CUH and from endocrine referrals received by neighbouring trusts as per routine standard clinical practice.

12.2 Consent

The Informed Consent Form must be approved by the Research Ethics Committee (REC) and must be in compliance with GCP, local regulatory requirements and legal requirements. The investigator or designee must ensure that each trial participant is fully informed about the nature and objectives of the trial and possible risks associated with their participation.

The investigator or designee will obtain written informed consent from each participant before any trial-specific activity is performed. The informed consent form used for this trial and any change made during the course of this trial, must be prospectively approved by the REC. The investigator will retain the original of each participant-signed informed consent form. Written informed consent will be sought at least 24 hours following the participant being in receipt of the Participant Information Sheet.

Should a participant require a verbal translation of the trial documentation by a locally approved interpreter/translator, it is the responsibility of the individual investigator to use locally approved translators.

Any new information which becomes available, which might affect the participant's willingness to continue participating in the trial, will be communicated to the participant as soon as possible.

12.3 Screening evaluation

12.3.1 Screening Assessment

Trial-specific assessments will only be conducted after participants have had all of their questions answered and have provided written informed consent.

The following will be undertaken at the screening assessment:

- Photo ID check
- Medical history (including medications) and
- Physical examination

- 12-Lead ECG
- Pregnancy test (serum hCG) in females of child-bearing potential
- Blood tests:
  - full blood count (FBC) (haemoglobin, white cell count, platelet count)
  - urea & electrolytes including creatinine (U&Es), bicarbonate
  - liver function tests (LFTs) [bilirubin, alanine transaminase (ALT), alkaline phosphatase (ALP)]
  - short synacthen test (SST)
- Urine tests:
  - drugs of abuse point of care screen (amphetamine, barbiturates, benzodiazepine cocaine, marijuana, MDMA, metamphetamine, methadone, morphine, tricyclic antidepressants),
- Inclusion/exclusion criteria review

Data from the screening visit, in conjunction with review of the inclusion/exclusion criteria, will be medically reviewed before confirmation of participant eligibility to proceed to a baseline visit and PET-CT scan.

Time will be dedicated to answer any further participant queries regarding the trial. Information regarding what to expect on the scan day will be provided to the participant. All participants will be advised of the need to fast for a minimum of 4 hours before the [^{18}F]CETO injection on the PET-CT scan date.

Following the screening visit, a member of the trial team will contact the participant's GP to verify their eligibility to be enrolled in the trial. Confirmation of the participant's relevant past medical and surgical history, current medications, recreational drug use, alcohol consumption, previous clinical trial involvement and previous radiation exposure will be verified with the GP. Written communication verifying participant eligibility will be sought from the participant's GP prior to proceeding to the baseline visit and PET-CT scan..

All healthy volunteers will be registered on The Over-Volunteering Prevention System (TOPS) database (which is available via the CLRF), and any existing record of participants on the database will be reviewed prior to confirming their eligibility for this trial.

On verification of eligibility, participants will be informed of their successful recruitment onto the trial by a member of the research team. A baseline visit will then be booked with the participant.

12.3.2 Participant Registration/Randomisation

All consenting participants will be provided with a unique participant ID number at their screening visit which will be used on all trial documentation and will be added to the CRF following consent. Participants who subsequently pass screening will retain their participant ID throughout the trial which will be included on the CRF.

12.3.3 Intensive Care Unit (ICU)

The ICU team will be provided with details of the study prior to its commencement. In the days preceding admission of trial participants to the CLRF, the ICU team will be provided with details of the impending admission. Evidence of notification will be retained.

12.4 Baseline Assessments

Baseline assessments are scheduled to occur on the day of the PET-CT scan and will occur a minimum of 1 week after the screening visit. Participants will require a minimum 4 hour fast before the PET-CT scan, however water is permitted during this fasting period. On completion of the PET-CT scan, participants will be permitted to eat and drink as normal, with no restrictions.
Photo ID will be reviewed by a member of the clinical research team to confirm participant identity prior to dosing.

Baseline activities will include:
- Confirmation of continued willingness and eligibility to participate in the study
- Update to medical history (including current medication) and physical examination
- Recording of participant demographics
- Repeat 12-lead ECG
- Pregnancy test (to confirm the negative result from the screening visit, a rapid turnaround urine hCG assay will be used) in females of child-bearing potential
- Insertion of intravenous cannula
- Blood tests:
  - FBC
  - U&Es, bicarbonate
  - LFTs
  - Direct Renin Concentration (DRC)
  - Plasma Aldosterone Concentration (PAC)
  - Adrenocorticotropic Hormone (ACTH)

12.5 Trial assessments

IMP Administration & PET-CT

Healthy volunteers and patients will attend the PET-CT unit at CUH. Recruitment of healthy volunteers and patients will occur concurrently (i.e. it will not be necessary for all healthy volunteers to be recruited before patients will be recruited). All scans will be performed on a GE Discovery PET-CT scanner. During the [^{18}F]CETO injection and the PET-CT scan, a clinical research fellow and research nurse will supervise the participant. We will perform sentinel dosing for the first overall IMP dose on the trial. Specifically, only a single participant will receive the IMP and proceed through the trial before any subsequent participants are administered with the IMP. The Principal Investigator will be present during the first injection of [^{18}F]CETO in the first participant. For subsequent injections, the Principal Investigator will be contactable by the unit in the unlikely event of an emergency. Details of the emergency equipment and processes for the PET-CT Unit can be found in section 13 Toxicity - Emergency Procedures.

A detailed description of the IMP administration and PET-CT scan process is described below:

- Participants will be asked to void their bladder before the PET-CT scan
- A positioning attenuation correction (CTAC) scan will be performed.
- Participants will then receive a 2MBq/kg IV injection of [^{18}F]CETO, up to a maximum of 200 MBq. The radiotracer will be injected over approximately 30 seconds.
- A dynamic PET scan will occur immediately following the injection of [^{18}F]CETO and will last for 90 minutes from the point of injection. Dynamic scanning will be performed to provide information on the optimum image acquisition window.
- List-mode PET data acquisition will last for the duration of imaging and facilitate the creation of retrospective static and dynamic images needed for optimisation.
- Pulse oximetry will be acquired and recorded throughout imaging.
- At the end of the scan participants will be asked to void their bladder and drink water *ad libitum* as this promotes the removal of radioactive material from the body
- Vital signs will be measured, and a 12-lead ECG will be performed after the scan

In the unlikely event of a failure of tracer production (with no ability to rectify on the day in question), participants will be rescheduled for a PET-CT scan on a separate date.

Period of Observation

Following the IMP administration and PET-CT scan, the following will occur:

After completion of the scan, participants will remain in the PET-CT unit for up to 4 hours following injection of the radiotracer. They will be accompanied throughout this time by a trial research nurse and members of the PET-CT team, and will be reviewed on a regular basis by a clinical research fellow or the Principal Investigator. The unit is a fully accredited NHS PET facility and is fully equipped to deal with any adverse event following [^{18}F]CETO administration.

- Vital signs will be checked at approximately 30 minute intervals throughout this time.
- Participants will subsequently be accompanied to the CLRF (which lies on the same corridor as the PET-CT unit) by the clinical research fellow and research nurse, where they will be observed in the Early Phase Unit of the Clinical Research Facility (CLRF) until approximately 24 hours following injection of [^{18}F]CETO.
- On arrival at the CLRF, vital signs will be checked and repeated when clinically indicated thereafter. A clinical member of the research team will be available for advice from the baseline visit up to the point of participant discharge from the CLRF. They will be able to attend the CLRF to review the participant within approximately 10 minutes of a request for clinical review.
- In the unlikely event that the participant acutely deteriorates, a CUH rapid response team will attend the CLRF and make a decision regarding transfer to the main hospital. The Early Phase unit is on Level 3 of the CLRF, with a link

corridor to allow direct access to the main hospital. It will take approximately 2 minutes for participants to be transferred to the intensive care unit in the rare instance that this situation was to arise.

The following morning, participants will have repeat blood tests including a short synacthen test (SST) to evaluate adrenal function. Although the doses of [^{18}F]CETO administered in humans will be significantly lower than used in the rat microdosing studies, and it is therefore very unlikely that clinically relevant short-term adrenal suppression will occur, we have included a SST following injection of the radiotracer to provide reassurance that adrenal function remains normal in participants. Following review of the results by a clinical member of the research team, participants will be discharged from the CLRF. In the unlikely event that a participant returns an abnormal SST result, they will be reviewed by a member of the clinical research team and the decision will be taken as to whether they temporarily need low dose hydrocortisone treatment until the test is repeated one week later.

Dexamethasone Prescription (Patients only)

Prior to discharge from the CLRF and where SST results were confirmed to be acceptable, patients will be provided with a prescription for dexamethasone 500 micrograms four times daily for three days (total 12 doses). Dexamethasone will be self-administered for three days (total 12 doses) prior to the repeat PET-CT scan. Patients will be provided with contact details for the research team if they have any questions while taking dexamethasone. The purpose of this pre-treatment with dexamethasone will be to improve the ability to distinguish between abnormal and normal adrenal tissue in the subsequent scan. Therefore, a second scan with dexamethasone pre-treatment will only be required for patients and not healthy volunteers.

Follow up visit for those with abnormal synacthen test result

Any individuals with an abnormal SST approximately 24 hours post injection of [^{18}F]CETO will be invited to a follow-up visit at 7 (+/-2 days) for a repeat test. At that visit, the participant will also be assessed by a clinical member of the research team. A repeat SST will be conducted during this visit, and the results assessed by the clinical research fellow and, if clinically indicated (for example if a further abnormal result is returned), by the Principal Investigator before the participant goes home.

In the extremely unlikely event that the abnormality persists, the participant will continue to be monitored within the trial until one of the following conditions is met:

- Adrenal function returns to baseline, as determined by follow-up SST.
- Participant/patient remains clinically stable, and where further follow-up can be managed safely in the outpatient setting.
- An alternative cause for the adrenal dysfunction is identified.
- Participant/patient is lost to follow-up.

Upon which their involvement in the trial will end.

Repeat PET-CT (Patients only)

Any patient who had an abnormal short synacthen test following the first scan will not be eligible to undergo the second scan. Eligible patients will undergo a second PET-CT scan (with dexamethasone pre-treatment) at ≥7 days after the initial scan

Prior to undertaking the second scan, the following assessments will be performed:

- Photo ID check
- Review of Dexamethasone medication diary
- Repeat urine pregnancy test for women of childbearing potential
- Blood tests:
  - FBC
  - U&Es, bicarbonate
  - LFTs
  - Direct Renin Concentration (DRC)
  - Plasma Aldosterone Concentration (PAC)
  - Adrenocorticotropic Hormone

Patients who fail to demonstrate 100% self-administration of dexamethasone in accordance with instructions provided by the trial team, assessed by review of the Dexamethasone medication diary, will not be able to proceed to a repeat PET-CT scan.

As no abnormal findings were recorded for these patients following the first scan, patients will only be observed for up to 4 hours in the PET-CT Unit after completion of the second scan. They will then be discharged by a clinical member of the research team with no requirement for admission to the CLRF or an additional SST.

Ongoing Safety Review

The safety data and PET-CT data will be reviewed by the Data Monitoring Committee (DMC) following injections of [^{18}F]CETO in the first 3 participants to provide an initial evaluation of [^{18}F]CETO safety and confirm the trial is able to proceed.

Furthermore, automatic trial stopping criteria will be met if a single participant experiences any serious adverse reaction (SAR, i.e. a serious adverse event considered at least possibly related to the IMP administration). Stopping criteria will additionally apply if any severe non-serious adverse reactions (ARs) develop in two participants, independent of whether or not these are within the same system-organ-class. The following will then occur:

- The trial will be halted via a substantial amendment to the MHRA.
- No further dosing or recruitment will be undertaken
- A safety review by the Data Monitoring Committee (DMC) will be conducted to determine how to proceed with the trial
- Any patients who have their dosing discontinued as a result of a SAR or a severe non-serious AR will be withdrawn from the trial
- Re-initiation of the trial can only be considered after MHRA approval via a substantial amendment. Any concurrently active participants will be re-consented before further IMP doses are administered, and all completed participants will be informed of the SAR or severe non-serious ARs.

12.6 Schedule of Assessments

Assessment	Screening	Baseline	[^{18}F]CETO PET-CT Scan Day (Volunteers)	Morning following Scan (Volunteers)	[^{18}F]CETO PET-CT Scan Day (Patients)	Morning following Scan (Patients)	SST Follow-up Visit (Volunteers & Patients) – if necessary	Repeat [^{18}F]CETO PET-CT Scan Day (Patients)
Visit	1	2[b]	3[b]	3[b]	3[b]	3[b]	4	5
Informed consent	X							
Eligibility assessments	X							
Medical history	X	X					X	X
Physical	X	X					X	
Vital signs only			X	X	X	X		X
Demographics		X						
Bloods & Urine								
FBC	X	X		X		X	X	X
U&Es	X	X		X		X	X	X
LFTs	X	X		X		X	X	X
Serum HCG[a]	X							
Urine HCG[a]		X						X
DRC		X						X
PAC		X						X
ACTH		X						X
Bicarbonate	X	X						X
SST	X			X		X	X	
Drugs of abuse	X							
ECG	X	X	X		X			X
Adverse event assessments			X		X		X	X
Photo ID Check	X		X		X			X

Assessment	Screening	Baseline	[^{18}F]CETO PET-CT Scan Day (Volunteers)	Morning following Scan (Volunteers)	[^{18}F]CETO PET-CT Scan Day (Patients)	Morning following Scan (Patients)	SST Follow-up Visit (Volunteers & Patients) – if necessary	Repeat [^{18}F]CETO PET-CT Scan Day (Patients)
IMP administration			X		X			X
Dexamethasone pre-treatment								X

Key:

[a]*Females of child-bearing potential.*

[b]*Visits 2 and 3 will occur over two consecutive days*

Abbreviations: ACTH Adrenocorticotrophic Hormone; DRC, Direct Renin Concentration; ECG, Electrocardiogram; FBC, Full Blood Count; HCG, Human Chorionic Gonadotrophin; IMP, Investigational Medicinal Product; LFTs, Liver Function Tests; PAC, Plasma Aldosterone Concentration; SST Short Synacthen Test; U&Es, Urea & Electrolytes.

12.7 End of Trial Participation

Healthy volunteers will conclude their involvement in the trial after their final adrenal function assessment has confirmed normal adrenal function or they are transitioned to clinical care as a patient following a confirmed abnormal result.

Patients will conclude their involvement in the trial after the second [^{18}F]CETO PET-CT scan or, in the event of an abnormal result following the first scan, after their final adrenal function assessment has confirmed normal adrenal function. Participants who continue to be monitored within the trial until one of the following conditions is met:

- Adrenal function returns to baseline, as determined by follow-up SST.
- Participant/patient remains clinically stable, and where further follow-up can be managed safely in the outpatient setting.
- An alternative cause for the adrenal dysfunction is identified.
- Participant/patient is lost to follow-up.

Upon which their involvement in the trial will end.

Healthy volunteers will not receive routine follow up after the trial. Patients will be returned to their normal clinical care.

12.8 Trial restrictions

Women of childbearing potential will be required to use highly effective contraception for the duration of the trial and for 7 days after the completion of the last scan. This includes:

- Intrauterine Device (IUD)
- Oral contraceptive (progestogen alone – note combined oral contraceptives containing oestrogen and progesterone are not permitted). Progesterone-only hormonal contraception preparations are only considered acceptable if the main mechanism of action is inhibition of ovulation.
- True abstinence (where this is in accordance with the participants preferred and usual lifestyle)

Men will be required to use adequate contraception for the entire duration of the trial and for 7 days after completion of the last scan.

- Barrier contraception (condom and occlusive cap e.g. diaphragm or cervical cap with spermicide)
- True abstinence (where this is in accordance with the participants preferred and usual lifestyle)

13 Assessment of Safety

13.1 Definitions

13.1.1 Adverse event (AE)

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

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

Please note: Recording of all adverse events must start from the point of Informed Consent regardless of whether a participant has yet received a medicinal product.

13.1.2 Adverse reaction to an investigational medicinal product (AR)

All untoward and unintended responses to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

13.1.3 Unexpected adverse reaction

An adverse reaction, the nature, or severity of which is not consistent with the applicable reference safety information (RSI) (e.g. investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product). When the outcome of the adverse reaction is not consistent with the applicable RSI this adverse reaction should be considered as unexpected.

The term “severe” is often used to describe the intensity (severity) of a specific event. This is not the same as “serious,” which is based on participant /event outcome or action criteria.

13.1.4 Serious adverse event or serious adverse reaction (SAE / SAR)

Any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect.
- is an important medical event - Some medical events may jeopardise the participant or may require an intervention to prevent one of the above characteristics/ consequences. Such events (hereinafter referred to as ‘important medical events’) should also be considered as ‘serious’

Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the participant was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.

13.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature and severity of which is not consistent with the information set out in the Reference Safety Information.

13.1.6 Reference Safety Information (RSI)

A list of medical events that defines which reactions are expected for the IMP within a given trial and thus determining which Serious Adverse Reactions (SARs) require expedited reporting.

For this trial the Reference Safety Information is: located in the most recent version of the Investigator's Brochure for the PET Radiopharmaceutical [^{18}F]CETO approved by the MHRA for use in this trial.

Due to the absence of any previous human data for [^{18}F]CETO, all events will be considered unexpected. Therefore, all SARs in the trial will meet the unexpected criteria and will be reported as SUSARs.

13.2 Expected Adverse Reactions/Serious Adverse Reactions (AR /SARs)

All expected Adverse Reactions are listed in the latest MHRA approved version of the RSI as specified in section 12.1.6. This will be used when making a determination as to the expectedness of the adverse reaction. If the adverse reaction meets the criteria for seriousness, this will be reported as per section 12.5

13.3 Expected Adverse Events/Serious Adverse Events (AE/SAE)

We are not aware of any adverse effects associated with [^{18}F]CETO from previous studies.

Before the PET/CT scan, a cannula will be sited, and this will be used for injecting the tracer. There may be local bruising following insertion of the cannula. Localised bruising associated with cannula insertion will not be recorded as an AE.

13.4 Evaluation of adverse events

The Sponsor expects that adverse events will be recorded from the point of Informed Consent regardless of whether a participant has yet received a medicinal product. Individual adverse events will be evaluated by the investigator. This includes the evaluation of its seriousness, and any relationship between the investigational medicinal product(s) and/or concomitant therapy and the adverse event (causality).

13.4.1 Assessment of seriousness

Seriousness will be assessed against the criteria in section 12.1.4.This defines whether the event is an adverse event, serious adverse event or a serious adverse reaction

13.4.2 Assessment of causality

Definitely: A causal relationship is clinically/biologically certain. **This is therefore an Adverse Reaction**

Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product and there is a reasonable response on withdrawal. **This is therefore an Adverse Reaction.**

Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the AE and administration of the investigational medicinal product. **This is therefore an Adverse Reaction.**

Unlikely: A causal relation is improbable and another documented cause of the AE is

most plausible. **This is therefore an Adverse Event.**

Unrelated: A causal relationship can be definitely excluded and another documented cause of the AE is most plausible. **This is therefore an Adverse Event.**

Unlikely and Unrelated causalities are considered NOT to be trial drug related
Definitely, Probable and Possible causalities are considered to be trial drug related

A pre-existing condition will not be recorded as an AE or reported as an SAE unless the condition worsens during the trial and meets the criteria for reporting or recording in the appropriate section of the CRF.

13.4.3 Clinical assessment of severity

Mild: The participant is aware of the event or symptom, but the event or symptom is easily tolerated

Moderate: The participant experiences sufficient discomfort to interfere with or reduce his or her usual level of activity

Severe: Significant impairment of functioning; the subject is unable to carry out usual activities and / or the participant's life is at risk from the event.

13.4.4 Recording of adverse events

Adverse events and adverse reactions will be recorded in the medical notes and the appropriate section of the CRF and/or AE/AR log. Serious Adverse Events and Serious Adverse Reactions will be reported to the sponsor as detailed in section 12.5.

13.5 Reporting serious adverse events

Each Principal Investigator will record all adverse events and report serious adverse events to the Chief Investigator and Sponsor using the trial specific SAE form within 24 hours of their awareness of the event.

The Principal Investigator will be responsible for ensuring the assessment of all SAEs for expectedness and relatedness is completed and the onward notification of all SAEs to the Sponsor immediately but not more than 24 hours of first notification. The sponsor will keep detailed records of all SAEs reported to them by the trial team.

The Chief Investigator will be responsible for prompt reporting of all serious adverse event findings to the competent authority (e.g. MHRA) of each concerned Member State if they could:

- adversely affect the health of participants
- impact on the conduct of the trial
- alter the risk to benefit ratio of the trial
- alter the competent authority's authorisation to continue the trial in accordance with Directive 2001/20/EC

The completed SAE form must be emailed to the CTC. Details of where to report the SAE's can be found on the SAE form and the front cover of the protocol.

13.6 Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

All suspected adverse reactions related to an investigational medicinal product (the

tested IMP and comparators) which occur in the concerned trial, and that are both unexpected and serious (SUSARs) are subject to expedited reporting. Please see section 12.1.6 for the Reference Safety Information to be used in this trial.

13.6.1 Who should report and whom to report to?

The Sponsor will delegate the responsibility of notification of SUSARs to the Chief Investigator. The Chief Investigator must report all the relevant safety information previously described, to the:

- Sponsor
- competent authorities in the concerned member states (eg MHRA)
- Ethics Committee in the concerned member states

The Chief Investigator shall inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

13.6.2 When to report?

13.6.2.1 Fatal or life-threatening SUSARs

All parties listed in 12.6.1 will be notified as soon as possible but no later than **7 calendar days** after the trial team and Sponsor has first knowledge of the minimum criteria for expedited reporting.
In each case relevant follow-up information will be sought and a report completed as soon as possible. It will be communicated to all parties within an additional **8 calendar days**.

13.6.2.2 Non-fatal and non-life-threatening SUSARs

All other SUSARs and safety issues will be reported to all parties listed in 12.6.1 as soon as possible but no later than **15 calendar days** after first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information will be given as soon as possible.

13.6.3 How to report?

13.6.3.1 Minimum criteria for initial expedited reporting of SUSARs

Information on the final description and evaluation of an adverse reaction report may not be available within the required time frames for reporting. For regulatory purposes, initial expedited reports will be submitted within the time limits as soon as the minimum following criteria are met:
a) a suspected investigational medicinal product
b) an identifiable participant (e.g. trial participant code number)
c) an adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship
d) an identifiable reporting source

and, when available and applicable:

- a unique clinical trial identification (EudraCT number or in case of non-European Community trials the sponsor's trial protocol code number)
- a unique case identification (i.e. sponsor's case identification number)

13.6.3.2 Follow-up reports of SUSARs

In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality will be actively sought from the reporter or other available sources. Further available relevant information should be reported as follow-up reports.
In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.

13.6.3.3 Format of the SUSARs reports

Electronic reporting is the expected method for expedited reporting of SUSARs to the competent authority. The format and content as defined by the competent authority will be adhered to.

13.7 Pregnancy Reporting

Pregnancies within the trial will be reported to the Chief Investigator and the Sponsor using the relevant Pregnancy Reporting Form within 24 hours of notification . Participants will be advised to notify the trial team where they become aware of a pregnancy following the conclusion of their participation in the trial as follows:

- Pregnant participants: conception occurring within 1 month of the last IMP dose
- Pregnant partners of male participants: conception occurring between the date of the first IMP dose and 7 days following the last IMP dose

Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/fetus. If the outcome meets the serious criteria, this would be considered an SAE.

14 Toxicity – Emergency Procedures

Administration of [^{18}F]CETO will take place in a NHS PET-CT unit which is fully equipped to deal with any adverse reaction following tracer administration. The unit contains a resuscitation trolley which contains equipment for use in the event of a clinical emergency, such as airway support and for vascular access. The equipment contained in the resuscitation trolley is compliant with UK Resuscitation Council recommendations. At a minimum, all clinical members of the research team, in addition to PET/CT clinical staff members, are trained in providing basic life support. Therefore, they will be suitably qualified to use the available resuscitation equipment. A clinical member of the research team will also be present during and following tracer administration. The unit is fully supported by CUH's rapid response team in the unlikely event of an acute deterioration following [^{18}F]CETO administration. Any participant who experiences a SAR will not receive any further doses of [^{18}F]CETO.

Similarly, the CLRF is embedded within the main hospital and is fully supported by the CUH clinical teams should a participant require urgent/high dependency care.

15 Evaluation of Results (Definitions and response/evaluation of outcome measures)

The study is primarily a safety study. Although adrenal uptake of [^{18}F]CETO will be assessed as a secondary objective, the study is not powered to allow statistical analysis of the findings.

List-mode PET data acquisition during the dynamic scanning phase will enable retrieval of static images at any point for the duration of the PET-CT scan. Analysis of static images across different timepoints will allow us to determine when adrenal background uptake is most favourable and, in patients with primary aldosteronism, when the signal (tumour) to background (normal adrenal uptake) is maximally separated. Assessment of adrenal uptake of [18F]CETO will be undertaken by two nuclear medicine physicians.

Confirmation of adrenal uptake will be assessed in the first 4 participants to ensure that the study can continue. This will necessarily be performed in a non-blinded manner and will depend on a joint assessment by a nuclear medicine physician and the PI. In contrast, all SUV measurements performed as part of the secondary outcome measures will be determined at the end of the study by two nuclear medicine physicians who will independently assess each study in a fully blinded manner [i.e. with no knowledge of participant status (healthy volunteer versus patient) nor the date/timing of the scan – all scans will be fully anonymised].

We will be comparing the adrenal uptake (as measured by SUV_{max} over the adrenal gland) between healthy volunteers and patients on [^{18}F]CETO PET-CT. In detail, a region of interest (ROI) will be drawn over the adrenal glands on each axial slice of the co-registered PET-CT scan, and the maximum standardised uptake value (SUV_{max}) recorded. The SUV_{max} is a better indicator of true uptake in small lesions when compared to mean SUV due to partial volume affects caused by the limited spatial resolution. Each participant will have SUV_{max} generated at 3 timepoints over the 90 minute duration of the dynamic PET-CT. In patients, there will be a comparison of the SUV_{max} between the adrenal glands, to determine the ability of [^{18}F]CETO distinguish between unilateral and bilateral adrenal disease.

Additional data, including formal pharmacokinetic studies, will be obtained in a parallel study undertaken by our trial collaborators in Uppsala, Sweden.

16 Statistics

16.1 Statistical methods

The trial has not been undertaken with a power calculation as the primary objective is to assess the safety and tolerability of [^{18}F]CETO and not to evaluate tracer efficacy. Summary statistics of safety data will be prepared for review and evaluation by the independent DMC

16.2 Interim analyses

Due to the short duration of the trial, no interim analysis is planned. Safety signals will be actively monitored by the independent DMC.

16.3 Number of Participants to be enrolled

Up to 11 participants will be recruited consisting of up to 5 healthy volunteers and 6 patients with a diagnosis of PA. The recruitment of 5 healthy volunteers is a sufficient number to evaluate the safety and tolerability of a single [^{18}F]CETO administration. The recruitment of up to 6 PA patients will provide additional safety data and also provide preliminary information regarding [^{18}F]CETO uptake in normal and abnormal adrenal tissue.

16.4 Criteria for the premature termination of the trial

The safety data and PET-CT data will be reviewed by the DMC following injections of [^{18}F]CETO in the first 3 participants to provide an initial evaluation of [^{18}F]CETO safety, and confirm the trial is able to proceed.

Furthermore, automatic trial stopping criteria will be met if a single participant experiences any serious adverse reaction (SAR, i.e. a serious adverse event considered at least possibly related to the IMP administration). Stopping criteria will additionally apply if any severe non-serious adverse reactions (ARs) develop in two participants, independent of whether or not these are within the same system-organ-class. The following will then occur:

- The trial will be halted via a substantial amendment to the MHRA.
- No further dosing or recruitment will be undertaken
- A safety review by the DMC will be conducted to determine how to proceed with the trial
- Any patients who have their dosing discontinued as a result of a SAR or a severe non-serious AR will be withdrawn from the trial
- Re-initiation of the trial can only be considered after MHRA approval via a substantial amendment. Any concurrently active participants will be re-consented before further IMP doses are administered, and all completed participants will be informed of the SAR or severe non-serious ARs.

Finally, if there is no demonstrable [^{18}F]CETO uptake into human adrenal tissue in the first 4 participants (either healthy volunteers and/or patients), the trial will be halted and the DMC will be asked to consider if the trial should be stopped.

16.5 Procedure to account for missing or spurious data

For participants who wish to withdraw entirely from the study, existing data acquired to the point of withdrawal will be included in the final study analysis, with missing data handled according to standard missing data methods, including missing-at-random methods.

16.6 Economic evaluation

No economic evaluation is planned.

16.7 Definition of the end of the trial

The end of trial will be the date of the last patient's last follow up visit.

17 Data handling and record keeping

17.1 Case Report Form (CRF)

All data will be transferred into a CRF which will be link-anonymised. All trial data in the CRF will be extracted from and be consistent with the relevant source documents. The CRFs will be completed, dated and signed by the investigator or designee in a timely manner. It remains the responsibility of the investigator for the timing, completeness, legibility and accuracy of the CRF pages. The CRF will be accessible to trial coordinators, data managers, the investigators, Clinical Trial Monitors, Auditors and Inspectors as required.

All CRF pages will be clear, legible and completed in black ink. Any errors will be crossed with a single stroke so that the original entry can still be seen. Corrections will be inserted and the change dated and initialled by the investigator or designee. If it is not clear why the change has been made, an explanation will be written next to the change. Typing correction fluid will not be used.

17.2 Source Data

To enable peer review, monitoring, audit and/or inspection the investigator agrees to keep records of all participating participants (sufficient information to link records e.g., CRF, hospital records and samples), all original signed informed consent forms and copies of the CRF pages.

17.3 Data Protection & Participant Confidentiality

All investigators and trial site staff involved in this trial will comply with the requirements of the General Data Protection Regulation (GDPR), the Data Protection Act 2018 and Trust Policy with regards to the collection, storage, processing, transfer and disclosure of personal information and will uphold the Act's core principles.

18 Independent Data Monitoring Committee

A single independent Data Monitoring Committee will oversee the trial and will be formed as follows:

- 1st DMC Member (Endocrinologist)
- 2nd DMC Member (Endocrinologist)
- 3rd DMC Member (Nuclear Medicine Physician)
- 4th DMC Member (Early Phase Clinical Trialist)
- Sponsor Representative (non-voting)

19 Ethical & Regulatory considerations

19.1 Ethical committee review

Before the start of the trial or implementation of any amendment we will obtain approval of the trial protocol, protocol amendments, informed consent forms and other relevant documents e.g., advertisements and GP information letters if applicable from the REC. All correspondence with the REC will be retained in the Trial Master File/Investigator Site File.

Annual reports will be submitted to the REC in accordance with national requirements. It will be the Chief Investigator's responsibility to produce the annual reports as required.

19.2 Regulatory Compliance

The trial will not commence until a Clinical Trial Authorisation (CTA) is obtained from the MHRA. The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments.

Development Safety Update Reports (DSURs) will be submitted to the MHRA in accordance with national requirements. It is the Chief Investigators responsibility to produce the annual reports as required.

19.3 Protocol Amendments

Protocol amendments will be reviewed and agreement received from the Sponsor for all proposed amendments prior to submission to the HRA, REC and/or MHRA.

The only circumstance in which an amendment may be initiated prior to HRA, REC and/or MHRA approval is where the change is necessary to eliminate apparent, immediate risks to the participants (Urgent Safety Measures). In this case, accrual of new participants will be halted until the HRA, REC and/or MHRA approval has been obtained.

19.4 Peer Review

The review was performed by the Research Advisory Committee at Cambridge University Hospitals NHS Foundation Trust. The Committee provide independent peer review for proposed studies that will be sponsored solely by the Trust or jointly with the University of Cambridge. To maintain the independence of the committee all reviews are undertaken in a confidential manner.

19.5 Declaration of Helsinki and Good Clinical Practice

The trial will be performed in accordance with the spirit and the letter of the declaration of Helsinki, the conditions and principles of Good Clinical Practice, the protocol and applicable local regulatory requirements and laws.

19.6 GCP Training

All trial staff will hold evidence of appropriate GCP training or undergo GCP training prior to undertaking any responsibilities on this trial. This training should be updated every 2 years or in accordance with the host Trust's policy.

20 Sponsorship, Financial and Insurance

The trial is sponsored by Cambridge University Hospitals NHS Foundation Trust and University of Cambridge. The trial will be funded by a UK Medical Research Council grant award to F Aigbirhio, J Aston, M Brown, F Gilbert and M Gurnell (Reference MR/P01710X/1).

Cambridge University Hospitals NHS Foundation Trust, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to participants in the clinical trial caused through the negligence of its employees and honorary contract holders. The University of Cambridge will arrange insurance for

negligent harm caused as a result of protocol design and for non-negligent harm arising through participation in the clinical trial.

Participants will be reimbursed for their time on the trial. They will be paid £250 upon completion of all trial visits. Participants who fail screening will be paid £50 in recognition of their time. Participants will also be reimbursed for reasonable travel costs.

21 Monitoring, Audit & Inspection

The investigator will make all trial documentation and related records available should an MHRA Inspection occur. Should a monitoring visit or audit be requested, the investigator will make the trial documentation and source data available to the Sponsor's representative. All participant data will be handled and treated confidentially.

The Sponsor's monitoring frequency will be determined by an initial risk assessment performed prior to the start of the trial. A detailed monitoring plan will be generated detailing the frequency and scope of the monitoring for the trial. Throughout the course of the trial, the risk assessment will be reviewed and the monitoring frequency adjusted as necessary.

22 Protocol Compliance and Breaches of GCP

Prospective, planned deviations or waivers to the protocol will not be allowed under the UK regulations on Clinical Trials and will not be used.

Protocol deviations, non-compliances, or breaches are departures from the approved protocol. They can happen at any time, but are not planned. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.

Deviations from the protocol which are found to occur constantly again and again will not be accepted and will require immediate action and could potentially be classified as a serious breach.

Any potential/suspected serious breaches of GCP must be reported immediately to the Sponsor without any delay.

23 Publications policy

Ownership of the data arising from this trial resides with the trial team. On completion of the trial, the data will be analysed and tabulated and a Final Trial Report prepared. All data arising from the trial will reside with the CI. Publication of any research findings by collaborators will not be permitted prior to the initial trial publication by the CI or without express written permission.

24 References

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25 Appendices

A Single Dose Study of pCI-Fluoroetomidate by Intravenous Injection in Wistar Rats with a 14-Day Recovery Period (Summary)

The objective of this study was to determine the potential toxicity of pCI-Fluoroetomidate, when given intravenously as a single dose to Wistar rats and to evaluate the potential reversibility of any findings.

The study design was as follows:

Text Table 1 - Experimental Design

Group No.	No. of Animals Main (Recovery) Male	Female	Test Material	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)
1	10 (5)	10 (5)	Vehicle	0	0	1
2	10 (5)	10 (5)	Test Item	0.0143	0.0143	1
3	10	10	Test Item	0.143	0.143	1
4[a]	-	3	Test Item	0.143	0.143	1

[a] Group was only used for Corticosterone determination.

The following parameters and end points were evaluated in this study:

- clinical signs
- body weights
- food consumption
- clinical pathology parameters (hematology, coagulation, and clinical chemistry), corticosterone determination (non-GLP),
- gross necropsy findings
- organ weights
- and histopathologic examinations.

No mortality occurred during the study. There were no test item-related clinical signs or effects on body weight, food consumption, clinical pathology and organ weights or any macro- and microscopic alterations observed in males and females up to 0.143 mg/kg.

A clear decrease in corticosterone levels were seen in all three females at 24 hours postdose when compared to predose.

In conclusion, administration of pCI-Fluoroetomidate by a single intravenous injection was well tolerated in male and female rats at dose levels of 0.0143 and 0.143 mg/kg.

Further details regarding the study can be obtained from the CETO trial team. Please quote the CETO Toxicology-Charles River report, *'A Single Dose Study of pCI-Fluoroetomidate by Intravenous Injection in Wistar Rats with a 14-Day Recovery Period'*, Test facility study number 20158118.