

Clinical Study Protocol

***HD-CSF: Studying cerebrospinal fluid
to understand key CNS pathobiological targets in Huntington's disease***

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Synopsis

<p>Study Title: HD-CSF: Studying cerebrospinal fluid to understand key CNS pathobiological targets in Huntington's disease</p>
<p>Short Study Title: HD-CSF</p>
<p>Funding Source: Medical Research Council Clinician Scientist Fellowship MR/M008592/1</p>
<p>Study Location: University College London Institute of Neurology / National Hospital for Neurology & Neurosurgery</p>
<p>Number of Participants planned: 80</p>
<p>Principal Investigator: Dr. Edward Wild MRC Clinician Scientist, UCL Institute of Neurology; Honorary Consultant Neurologist, National Hospital for Neurology & Neurosurgery, Queen Square London WC1N 3BG, UK</p>
<p>Study period: Estimated date first subject enrolled: Q4 2015 Estimated date last subject completed: April 30th, 2019</p>
<p>Objectives: Primary: The primary objective of this study is to generate a high quality cerebrospinal fluid (CSF) sample collection and evaluate biomarkers and pathways that contribute to the development of Huntington's disease (HD). Secondary:</p> <ul style="list-style-type: none"> • To generate a high quality plasma sample collection matching the CSF collections, which will also be used to evaluate biomarkers and pathways of relevance to HD research and development. • To collect phenotypic and clinical data for each participant.
<p>Study Design: HD-CSF is a longitudinal observational study. At baseline participants will attend a Screening Visit and Sampling Visit (collectively referred to as Core Baseline activities) and may attend an optional third visit (optional Repeat Sampling Visit). At follow-up 24 months later the screening Visit, sampling (collectively referred to as Core Follow-up activities) and optional Repeat Sampling Visit will be repeated. During the Screening Visit, medical history, and clinical and phenotypic data including an optional MRI scan will be obtained. Participants who meet the eligibility requirements of the study and are willing to continue in the study, will return for a Sampling Visit. During that visit, biosamples will be collected following an overnight fast: blood will be obtained via venepuncture and CSF will be obtained via lumbar puncture. Some participants may be</p>

invited to return for a Repeat Sampling Visit approximately 4-8 weeks later. Participant cohorts are as follows:

1. Healthy controls, n= 20
2. Pre-manifest HD, n=20
3. Early to moderate manifest HD, n = 40

Diagnosis and main criteria for inclusion:

Healthy controls as well as Huntington's disease gene expansion carriers (HDGECs) will be enrolled. The latter will include two groups: pre-manifest HD and early to moderate HD.

Inclusion Criteria:

1. All eligible participants
 - a. Are 18-75 years of age, inclusive; and
 - b. Are capable of providing informed consent. A legal representative will be used only in the event of communication difficulties to verify that the person has understood and consented; and
 - c. Are capable of complying with study procedures, including fasting, blood sampling and lumbar puncture; and
 - d. Are participating in the Enroll-HD study
2. For the **Healthy Control** group, subjects eligible are persons who meet the following criteria:
 - a. Have no known family history of HD; or
 - b. Have known family history of HD but have been tested for the huntingtin gene glutamine codon (CAG) expansion and are not at genetic risk for HD (CAG < 36).
3. For the **Pre-manifest HD** group, participants eligible are persons who meet the following criteria:
 - a. Do not have clinical diagnostic motor features of HD, defined as Unified Huntington's Disease Rating Scale (UHDRS) Diagnostic Confidence Score < 4; and
 - b. Have CAG expansion ≥ 40 ; and
4. For the **Early to moderate HD** group, participants eligible are persons who meet the following criteria:
 - a. Have clinical diagnostic motor features of HD, defined as UHDRS Diagnostic Confidence Score = 4; and
 - b. Have CAG expansion ≥ 36 ; and
 - c. Have Stage I, II or III HD, defined as UHDRS Total Functional Capacity (TFC) scores between 4 and 13 inclusive.

Exclusion Criteria:

1. For all groups, participants are ineligible if they meet any of the following exclusion criteria:
 - a. Current use of investigational drugs or participation in a clinical drug trial within 30 days prior to Sampling Visit; or
 - b. Current intoxication, drug or alcohol abuse or dependence; or
 - c. If using any antidepressant, psychoactive, psychotropic or other medications or nutraceuticals used to treat HD, the use of inappropriate (e.g., non-therapeutically

- high) or unstable dose within 30 days prior to Sampling Visit; or
- d. Significant medical, neurological or psychiatric co-morbidity likely, in the judgment of the Principal Investigator, to impair participant's ability to complete essential study procedures; or
 - e. Needle phobia, frequent headache, significant lower spinal deformity or major surgery; or
 - f. Antiplatelet or anticoagulant therapy within the 14 days prior to sampling visit, including but not limited to: aspirin, clopidogrel, dipyridamole, warfarin, dabigatran, rivaroxaban and apixaban; or
 - g. Clotting or bruising disorder; or
 - h. Screening blood test results outside the clinical laboratory's normal range for the following: white cell count, neutrophil count, lymphocyte count, hemoglobin (Hb), platelets, prothrombin time (PT) or activated partial thromboplastin time (APTT); or
 - i. Screening blood test results for C-reactive protein (CRP) > 2× upper limit of normal; or
 - j. Predictable non-compliance as assessed by the Principal Investigator; or
 - k. Inability or unwillingness to undertake any of the essential study procedures; or
 - l. Exclusion during history or physical examination, final decision to be made by the Principal Investigator; including but not limited to:
 - i any reason to suspect abnormal bleeding tendency, e.g. easy bruising, petechial rash; or
 - ii any reason to suspect new focal neurological lesion, e.g. new headache, optic disc swelling, asymmetric focal long tract signs; or
 - iii any other reason that, in the clinical judgment of the operator or the Principal Investigator, it is felt that lumbar puncture is unsafe.

Participants are ineligible for the optional MRI component if they meet any of the following criteria:

- a. Contraindication to MRI, including, but not limited to, MR-incompatible pacemakers, recent metallic implants, foreign body in the eye or other indications, as assessed by a standard pre-MRI questionnaire; or
- b. Pregnant (as confirmed by urine pregnancy test); or
- c. Claustrophobia, or any other condition that would make the subject incapable of undergoing an MRI.

Sample Size:

Power calculations were based on a 12-subject CSF analysis of mutant huntingtin using a novel single molecule counting immunoassay¹. Detecting cross-sectional differences between control and HD requires very small numbers (<5 per group for >90% power at 5% significance). 20 subjects per group gives >90% power to detect predicted longitudinal change in mutant huntingtin over two years.

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List of Abbreviations and Definitions of Terms

Abbreviation	Definition
AE	Adverse Event
APTT	Activated partial thromboplastin time
CAG	Cytosine-arginine-glutamine codon whose count in the HTT gene determines the genetic diagnosis of HD
CRP	C-reactive protein
CSF	Cerebrospinal fluid
eCRF	electronic Case Report Form
GCP	Good Clinical Practice
Hb	Hemoglobin
HD	Huntington's disease
HDGEC	Huntington's disease gene expansion carrier
HTT	huntingtin protein
ICH Guidelines	International Conference on Harmonisation Guidance for Industry
IRB	Institutional Review Board
KMO	kynurenine mono-oxygenase
KP	kynurenine pathway
PT	Prothrombin time
REC	Regional Ethics Committee
SAE	Serious Adverse Event
TFC	Total Functional Capacity
TMS	Total Motor Score
UHDRS	Unified Huntington's Disease Rating Scale

1. Introduction

1.1 Background and Rationale

Huntington's disease (HD) is an autosomal dominant genetic disease, which typically manifests beginning in adulthood in the form of movement symptoms, cognitive decline, and psychiatric changes.² Currently the only approved treatment for HD is tetrabenazine, but several clinical trials are expected to launch shortly to explore novel therapeutic approaches to treating this disease. In preparation for such trials, biomarkers are needed to evaluate: (1) how well these novel therapeutics reach their intended target and have a biological effect (pharmacodynamic markers); (2) the effectiveness of these novel therapeutics at improving clinical signs and symptoms (efficacy biomarkers); and (3) the state of disease patients are in throughout the trial (disease progression biomarkers). Cerebrospinal fluid (CSF) is an ideal fluid compartment for assessing HD biomarkers, particularly pharmacodynamics markers, due to its proximity to the brain.

Evidence from preclinical animal studies as well as post-mortem human brain studies suggests that the kynurenine pathway (KP) may be abnormally regulated in HD.³ Thus, this enzymatic pathway may be a target for therapeutic intervention. However, the KP has not been extensively investigated in HD patients and premanifest HD gene expansion carriers (HDGECs). To further investigate the potential dysregulation of the pathway, and inter-participant variability of the dysregulation, we propose to measure levels of some of the key KP metabolites in CSF and plasma from HD patients, premanifest HDGECs and healthy controls. The results of this study will serve not only to support the biological rationale for pursuing this line of treatment for HD, but will also set the ground work for the use of particular metabolites as pharmacodynamic biomarkers in future clinical trials of therapeutics modulating the KP, such as inhibitors of kynurenine mono-oxygenase (KMO).

Several therapeutic approaches focused on lowering huntingtin protein (HTT) in the brain are currently pursued, and studies in animals suggest this is a promising approach.⁴ However, one of the key tools needed to pursue such approaches in humans is the ability to demonstrate that the intervention did lower HTT levels in the brain. Fortunately, assays have been developed that can detect HTT in CSF.¹ We propose to further the development and validation of CSF HTT assays by measuring HTT levels in CSF and plasma from HD patients, premanifest HDGECs and healthy controls. This will also help to understand to what extent CSF mHTT level predicts disease progression in HD and could be used to guide future treatment decisions. The results of these studies will lead to the establishment of the best practices for measuring HTT in CSF from patients before and after HTT lowering therapies.

Several CSF and plasma HD biomarker discovery programs have resulted in the generation of many substances potentially differentially expressed in HD.^{2,5} While promising, these need to be replicated in a new sample set and with more quantitative assays. The samples and data generate by HD-CSF will be used to conduct biochemical analyses to understand the pathobiology of HD and possible biomarkers in CSF and plasma.

1.2 Rationale for Current Study

With promising new therapeutic trials expected to begin in the next few years, exploration of potential biomarkers needs to be accelerated now. There is currently no high quality repository of CSF from well-characterised HDGECs spanning the disease spectrum. The

current study will provide such a repository in order to expedite the research into biomarkers for HD. We also need to understand to what extent proposed CSF and plasma biomarkers can predict the progression of HD to help design future trials and guide clinical decision-making in HD.

2. Study Objectives

The overall objective of this study is to generate a high quality CSF sample collection and use it to identify and validate biomarkers for HD clinical development. In one usage, the sample collection will be assayed to determine if the KP is dysregulated in premanifest and early HD in comparison to healthy controls, and to evaluate the variability in KP metabolite levels within each participant group. This information will help assess the potential for KMO inhibitors as therapies for HD and guide the use of such assays as pharmacodynamic biomarkers in clinical trials. The sample collection will also enable the further development and validation of assays to measure HTT in CSF, which may be an attractive pharmacodynamic biomarker for HTT lowering clinical trials. Last, the sample collection will enable the continued evaluation of a number of potential novel biomarkers of disease progression and, potentially, efficacy in HD.

2.1 Primary Objective

The primary objective of this study is to generate a high quality CSF sample collection and use it to evaluate biomarkers and pathways that will enable the development of novel treatments for HD.

2.2 Secondary Objective(s)

The secondary objectives of this study are:

- To generate a high quality plasma sample collection matching the CSF collections, which will also be used to evaluate biomarkers and pathways of relevance to HD research and development.
- To collect phenotypic and clinical data for each participant.

3. Study Design

3.1 Overall Study Design

This is a longitudinal observational study.

Recruitment: Participants will be recruited from the Huntington's Disease Multidisciplinary Clinic at the UCLH National Hospital for Neurology and Neurosurgery, from the database of participants in the Enroll-HD study.

Study Visits: There are two time points, core baseline and core follow-up activities. At baseline, participants will attend two study visits: a **Screening Visit** and a **Sampling Visit**. During the **Screening Visit**, which may coincide with an Enroll-HD visit, medical history, clinical and phenotypic data (including a screening blood sample and an optional MRI scan) will be obtained. These data will determine participant eligibility for participation in the study and will be used in the analysis of biomarker data. Participants meeting the eligibility requirements of the study and willing to continue in the study, will return for a **Sampling Visit** within 30 days of the Screening Visit. During that visit, biosamples will be collected following an overnight fast: blood will be obtained via venepuncture and CSF will be obtained via lumbar puncture. Participants will be contacted by telephone approximately 24-72 hours after the Sampling Visit for safety and

adverse event monitoring. The Screening Visit, Sampling Visit and Telephone Call are all part of the Core Baseline activities. Some participants may be invited to return for an optional **Repeat Sampling Visit** 4-8 weeks following the Sampling visit.

Core Follow-up visits will occur 24 months (+/- 3 months) after Core Baseline visits and will take exactly the same format as the Core Baseline visits.

HD-CSF is designed to use the standardised phenotypic data from Enroll-HD. Where possible, routine, planned Enroll-HD visits will be used to plan recruitment into HD-CSF. However, where such scheduling may jeopardise a potential participant's inclusion in HD-CSF, assessments equivalent to an Enroll-HD Core visit may be performed at the HD-CSF screening visit.

Biosample Preparation: Samples will be processed and stored as described in Sections 10.1, 10.2 and 10.3 until ready for analysis.

Laboratory analyses: Samples will either be analysed locally or be shipped to collaborators authorised by the Principal Investigator, for investigations into biomarkers and pathogenic mechanisms in HD including, but not limited to, evaluation of the kynurenine pathway, measurement of huntingtin protein and other HD pathobiology, biomarker discovery or validation studies.

Statistical analysis: Statistical advice has been provided by the UCL/UCLH Biostatistics Unit. For each set of laboratory analyses conducted, a statistical analysis plan will be finalised before samples are sent to the laboratory conducting the studies. In brief, a two-stage approach will be used with linear regression models comparing change in molecular markers between clinical groups and for primary and secondary outcomes, with those found to be associated with disease progression then being examined for associations with measures of phenotypic change using a similar model.

3.2 Safety

The procedures for performing lumbar punctures and venous blood draws have been designed to maximize participant safety.

Study-related risks are explained in the informed consent document. In particular, the following risks may be associated with lumbar puncture: pain; headache (approximately 5%), infection, bleeding and nerve root damage. Most headaches resolve spontaneously but occasionally a headache may be persistent; in rare cases this may necessitate treatment, which may include a second procedure (a blood patch), carried out in a clinical setting.

See Appendix A –Principal Investigator Obligations for additional information.

4. Study Population

Three participant cohorts will be included in the study:

1. Healthy controls, n= 20
2. Pre-manifest HD, n=20
3. Early to moderate HD, n=40

4.1 Diagnosis and Main Selection Criteria

A total of 80 participants, aged between 18 and 75 years, inclusive, will be enrolled in the study. Eligible participants include healthy controls, people who are in the pre-manifest stage of HD, and people diagnosed with early or moderate HD.

4.1.1 Inclusion Criteria

1. All eligible participants:

- a. Are male or female, 18-75 years of age, inclusive; and
- b. Are capable of providing informed consent. A legal representative will be used only in the event of communication difficulties to verify that the person has understood and consented; and
- c. Are capable of complying with study procedures, including fasting, blood sampling and lumbar puncture; and
- d. Are participating in the Enroll-HD study;
- e. No contraindication to MRI scan

2. For the **Healthy Control** group, subjects eligible are persons who meet the following criteria:

- a. Have no known family history of HD; or
- b. Have a known family history of HD but have been tested for the huntingtin gene glutamine codon (CAG) expansion and are **not** at genetic risk for HD (CAG < 36)*.

3. For the **Pre-manifest HD** group, participants eligible are persons who meet the following criteria:

- a. Do not have clinical diagnostic motor features of HD, defined as Unified Huntington's Disease Rating Scale (UHDRS) Diagnostic Confidence Score < 4; and
- b. Have CAG expansion $\geq 40^*$; and

4. For the Early to moderate HD group, participants eligible are persons who meet the following criteria:

- d. Have clinical diagnostic motor features of HD, defined as UHDRS Diagnostic Confidence Score = 4; and
- e. Have CAG expansion $\geq 36^*$; and
- f. Have Stage I, II or III HD, defined as UHDRS Total Functional Capacity (TFC) scores between 4 and 13 inclusive.

*Genetic test results must be recorded in a documented report from an accredited genetics laboratory in the medical notes.

4.1.2 Exclusion Criteria

1. For all groups, participants are ineligible if they meet any of the following exclusion criteria:

- a. Use of investigational drugs or participation in a clinical drug trial within 30 days prior to Sampling Visit; or
- b. Current intoxication, drug or alcohol abuse or dependence; or

- c. If using any antidepressant, psychoactive, psychotropic or other medications or nutraceuticals used to treat HD, the use of inappropriate (e.g., non-therapeutically high) or unstable dose within 30 days prior to the Sampling Visit; or
 - d. Significant medical, neurological or psychiatric co-morbidity likely, in the judgment of the Principal Investigator, to impair participant's ability to complete essential study procedures; or
 - e. Needle phobia, frequent headache, significant lower spinal deformity or major surgery; or
 - f. Antiplatelet or anticoagulant therapy within 14 days prior to Sampling Visit, including but not limited to: aspirin, clopidogrel, dipyridamole, warfarin, dabigatran, rivaroxaban and apixaban; or
 - g. Clotting or bruising disorder; or
 - h. Screening blood test results outside the clinical laboratory's normal range for the following: white cell count, neutrophil count, lymphocyte count, hemoglobin (Hb), platelets, Prothrombin time (PT) and activated partial thromboplastin time (APTT); or
 - i. Screening blood test results for C-reactive protein (CRP) $>2\times$ upper limit of normal; or
 - j. Predictable non-compliance as assessed by the Principal Investigator; or
 - k. Inability or unwillingness to undertake any of the essential study procedures; or
 - l. Exclusion during history or physical examination, final decision to be made by the Principal Investigator; including but not limited to:
 - i any reason to suspect abnormal bleeding tendency, e.g. easy bruising, petechial rash; or
 - ii any reason to suspect new focal neurological lesion, e.g. new headache, optic disc swelling, asymmetric focal long tract signs; or
 - iii any other reason that, in the clinical judgment of the operator or the Principal Investigator (including clinically relevant abnormalities on the optional MRI scan), it is felt that lumbar puncture is unsafe.
2. Participants are ineligible for the optional MRI component if they meet any of the following criteria:
- a. Contraindication to MRI, including, but not limited to, MR-incompatible pacemakers, recent metallic implants, foreign body in the eye or other indications, as assessed by a standard pre-MRI questionnaire; or
 - b. Pregnant (as confirmed by urine pregnancy test); or
 - c. Claustrophobia, or any other condition that would make the subject incapable of undergoing an MRI.

4.2 Criteria for Termination of the Study

If the study is prematurely terminated or suspended for any reason, the Principal Investigator/institution will promptly inform the study participants and should assure appropriate follow-up for them. The Principal Investigator will also inform the appropriate Regional Ethics Committee and Trust Joint Research Office.

5. Study Procedures

All procedures are performed at baseline and follow-up (24 months +/- 3 months)

Visit Number	1	2		3	
	CORE BASELINE ACTIVITIES			OPTIONAL BASELINE ACTIVITIES	
Visit Type	Screening	Sampling	Telephone Follow-Up	Optional Repeat Sampling	Telephone Follow-Up ³
Days	-30 to -1	Day 0	Day 1 to 3	Day 28 - 56	+1-3 Days After Optional Sampling Visit
Informed Consent	✓			✓	
Inclusion/Exclusion Criteria review	✓	✓		✓	
Confirm Enroll-HD core assessments completed within last two months; if not, complete Enroll-HD core assessments	✓				
UHDRS motor assessment, diagnostic confidence score, total functional capacity and Independence Scale (if applicable)	✓				
Short Problem behaviours assessment (PBA-S) (if applicable)	✓				
Symbol-digit modality test (if applicable)	✓				
Stroop word reading (if applicable)	✓				
Stroop colour naming (if applicable)	✓				
Categorical verbal fluency (if applicable)	✓				
Brief Physical Exam	✓	✓		✓	
Medical History update	✓	✓		✓	
Prior/Concomitant Medication update	✓	✓	✓	✓	✓
Standard Neurological Examination	✓	✓		✓	
Total Motor Score (TMS)		✓		✓	
Vital Signs (BP, pulse, body temp)		✓		✓	
Safety Laboratory Assessments	✓				
Optional MRI scan	✓				
Adverse Events	✓	✓	✓	✓	✓
Final Eligibility Check		✓		✓	
Lumbar CSF Collection		✓		✓	
Venous Blood Draw ²		✓		✓	
CSF and Blood Sample Processing		✓		✓	
CSF QC Processing		✓		✓	

Visit Number	4	5		6	
	CORE FOLLOWUP ACTIVITIES			OPTIONAL FOLLOWUP ACTIVITIES	
Visit Type	Screening	Sampling	Telephone FollowUp	Optional Repeat Sampling	Telephone FollowUp ³
Time window	Upto 30 days before follow-up sampling	21 to 27 months after baseline sampling	Follow-up sampling + 1-3 days	28 – 56 days after follow-up sampling	+1-3 Days After follow-up optional Sampling Visit
Informed Consent	✓			✓	
Inclusion/Exclusion Criteria review	✓	✓		✓	
Confirm Enroll-HD core assessments completed within last two months; if not, complete Enroll-HD core assessments	✓				
UHDRS motor assessment, diagnostic confidence score, total functional capacity and Independence Scale (if applicable)	✓				
Short Problem behaviours assessment (PBA-S) (if applicable)	✓				
Symbol-digit modality test (if applicable)	✓				
Stroop word reading (if applicable)	✓				
Stroop colour naming (if applicable)	✓				
Categorical verbal fluency (if applicable)	✓				
Brief Physical Exam	✓	✓		✓	
Medical History update	✓	✓		✓	
Prior/Concomitant Medication update	✓	✓	✓	✓	✓
Standard Neurological Examination	✓	✓		✓	
Total Motor Score (TMS)		✓		✓	
Vital Signs (BP, pulse, body temp)		✓		✓	
Safety Laboratory Assessments	✓				
Optional MRI scan	✓				
Adverse Events	✓	✓	✓	✓	✓
Final Eligibility Check		✓		✓	
Lumbar CSF Collection		✓		✓	
Venous Blood Draw ²		✓		✓	
CSF and Blood Sample Processing		✓		✓	
CSF QC Processing		✓		✓	

¹Confirm and record continued consent. ²Obtain venous blood sample immediately after CSF collection is complete. ³For selected subjects only.

5.1 Description of Study Assessments

HD-CSF is a longitudinal study with two assessment blocks – baseline and follow-up. Follow-up activities occur 24 months (+/- 3 months) after baseline.

Each block contains three core activities: a screening visit, a sampling visit and a telephone follow-up. Each block also has two optional activities: an optional repeat sampling visit and a corresponding telephone follow-up.

The Screening and Sampling Visits within each block should be no more than 30 days apart. The screening visit may occur with an Enroll-HD visit. The optional Repeat Sampling Visit will occur within 4-8 weeks of the first sampling visit.

Optional repeat sampling visits will be offered to all subjects and booked after the baseline sampling visit for those who wish to proceed. Optional MRI scans will be offered to all subjects at the baseline and follow-up screening visits.

Information regarding occurrence of adverse events (AEs) will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study procedures will be recorded on the electronic case report form (eCRF).

5.1.1 Screening Visit

- The study will be described in detail to prospective participants then informed consent will be obtained at baseline and reconfirmed at follow-up.
- If Enroll-HD study core assessments have not been performed within the last two months, these will be carried out during the Screening Visit according to the procedures in the Enroll-HD Protocol and study materials. The Enroll core assessments currently include:
 - Height and weight measurement
 - UHDRS motor assessment, diagnostic confidence score, total functional capacity and Independence Scale. (*The UHDRS is a standardised rating scale for assessing clinical features of Huntington's disease. The motor assessment is a brief directed neurological examination and includes a diagnostic confidence score of 1-4 that reflects the assessor's certainty that the person has manifest HD. The TFC and IS both quantify the degree to which a person's functioning is affected by HD.*)
 - Short Problem behaviours assessment (PBA-S). (*The PBA assesses behavioural symptoms of HD in a standardised way using a semi-structured questionnaire*)
 - Symbol-digit modality test (*This is a paper-based cognitive test that involves matching symbols to numbers. It is sensitive to change in early HD.*)
 - Stroop word reading (*This is a paper-based cognitive test that involves reading words. It is sensitive to change in early HD.*)
 - Stroop colour naming (*This is a paper-based cognitive test that involves naming the colours of written words. It is sensitive to change in early HD.*)

- Categorical verbal fluency (*This is a cognitive test in which the participant is asked to name as many items within a particular category as possible within a time limit. It is sensitive to change in early HD.*)
- Medical history update since the last Enroll-HD study visit, including medication history and co-morbidities, is obtained.
- Demographic information update since the last Enroll-HD study visit.
- A standard neurological examination is performed as below, as well as a brief general physical examination. Evidence of possible bleeding tendency such as bruises or petechial rash should be noted.
 - Cranial nerves
 - visual acuity
 - visual fields to confrontation
 - fundoscopy (including appearance of discs and presence / absence of venous pulsations)
 - smooth pursuit and saccadic eye movements
 - facial sensation
 - jaw power
 - facial symmetry and power
 - bedside auditory acuity
 - palatal elevation
 - pharyngeal sensation
 - cough
 - Sternocleidomastoid muscle and trapezius power
 - Upper and lower limbs
 - Tone
 - Proximal and distal power
 - Reflexes (-, +/-, +, ++, +++)
 - Pinprick sensation
 - Plantar responses
 - Coordination
- Up to 15 ml of venous blood is drawn according to local clinical standards and procedures, and routine blood tests performed by UCLH clinical laboratory.
 - Biochemistry panel
 - Full blood count
 - Clotting profiles: PT and APTT
 - CRP
- An optional MRI brain scan, lasting up to 45 minutes, is obtained. This will consist of localiser, 2 T1 volumetric sequences and a diffusion tensor imaging sequence.

If the blood count or clotting profiles are outside normal range, or if CRP is greater than 2× the upper limits of normal the subject will not be booked for a sampling visit. The Principal Investigator will act on any abnormalities according to clinical judgment.

The MRI scan is not intended or sufficient to establish the safety or otherwise of lumbar puncture, which is determined on clinical grounds. Scans will be briefly reviewed for any major abnormalities by the study radiographer and, if necessary, escalated to the Principal Investigator for review and further action including postponing the sampling visit if the PI has concerns about the safety of lumbar puncture on reviewing the scan.

If participants do not fulfil all inclusion criteria, they may be rescheduled to repeat some or all of the screening assessments above within the one-month screening window.

If these assessments confirm all the eligibility requirements are met for the study, a date will be given via a telephone call for the sampling visit.

5.1.2 Sampling Visit

- The sampling visit is scheduled in such a way to allow for the lumbar puncture to be performed between 8:00 and 10:30 am local time. All participants will be asked to fast from midnight the night before their appointment, but are permitted to drink water freely. Compliance with instructions to fast is recorded. If participant did not fast, they will be sent home, and the procedure rescheduled.
- Participant continued consent to participate is confirmed and recorded.
- The results of the routine laboratory examination are reviewed and recorded.
- Medical and concomitant medication history is updated.
- Measurement of vital signs.
- The check-list 'Inclusion and Exclusion Criteria – Sampling Visit' is completed. Any changes to medical history and medication are noted.
- The neurological examination and brief physical exam are repeated for safety.
- The Total Motor Score (TMS) of the UHDRS is performed.
- Lumbar CSF Collection is performed. (See Section 6.1 for full details)
- Venous blood sampling is performed immediately after CSF collection is complete. (See Section 6.2 for complete instructions)
- CSF, Serum and Plasma samples are processed as per Sections 7.1, 7.2 and 7.3
- Samples are stored per Section 8.

5.1.2.1 Participant Discharge

Participants are observed for potential complications for at least an hour and discharged once appropriate. Any AEs are recorded.

Participant is discharged by nurses with instructions for over-the-counter pain medication and hydration in the event of headache.

5.1.3 Follow-up Telephone Call

Participants will be contacted 24 to 72 hours following the Sampling Visit to collect any AE and/or concomitant medication data.

5.1.4 Optional Sampling

- This visit is optional. Participant continued consent to participate is confirmed and recorded.

- This visit should be scheduled 4 - 8 weeks following the initial Sampling Visit.
- All procedures are identical to the sampling visit including sample collection, processing and storage; participant discharge and follow-up telephone call.

6. Sample Collection Procedures

6.1 Lumbar CSF Collection

1. Ensure that all equipment is on hand and that ice is available for CSF collection and transportation of samples to the lab.
2. Ensure availability and settings of centrifuges for appropriate temperatures and timely processing of CSF and blood samples.
3. Pre-cool CSF collection tubes on ice.
4. Prepare a sterile field containing all equipment needed, label tubes.
5. Place participant into lateral decubitus position with pillow between knees.
6. Identify L4/5 or L3/4 space using surface markings.
7. Disinfect skin using pre-filled antiseptic sponge.
8. Inject up to 5ml of 2% lidocaine for local anaesthesia. Use the 25g needle and inject lidocaine to raise a skin wheal. Then inject lidocaine more deeply.
9. Obtain CSF using a 22G spinal needle. If the participant is thin, do not insert the deep infiltration needle all the way. Use only about 2/3 of its length (to prevent entering the subarachnoid space with anything other than the pencil-point spinal needle).
10. If CSF cannot be obtained, up to three needles may be used.
11. An adjacent space may be used (with further lidocaine, max. total 10 ml, if needed).
12. If necessary, CSF space may be located by sitting patient up, but once CSF is seen, it is recommended to have patient lie back in lateral decubitus position for 30 seconds before collection begins. Document positions of patient during puncture and collection in the eCRF.
13. Document the space used for lumbar puncture, the number of attempts and volume of lidocaine used in the eCRF.
14. Omit pressure measurement for all participants (because polypropylene spinal manometers are not available).
15. CSF is collected in 50ml tubes placed on ice in the Styrofoam cup.
16. Collect the first 1 ml of CSF into the supplied tube labelled 'CSF'. If the first 1 ml (approx. 15 drops) is not macroscopically bloody, continue sampling CSF in the same tube up to 20 ml, keeping the tube in the ice cup. If the first 1 ml is macroscopically bloody, stop collecting CSF by reinserting the stylet partially, discard the tube, and collect a second 1 ml in a new pre-cooled 'CSF' tube, and examine it visually for blood contamination. If it is free of blood, continue collecting CSF up to 19 ml. If the second separately collected ml of CSF is also macroscopically bloody, discard the tube, and continue to collect 18 ml of CSF in

a third pre-cooled 'CSF' tube. Stop collecting CSF when sampling time exceeds 20 minutes. Document these details in the eCRF.

17. Place cap on tube and leave on crushed ice until further processing.
18. Reinsert the stylet before withdrawing the needle.
19. Cover the puncture site with sterile dressing.
20. Record time of CSF collection.
21. Participants can mobilise or remain lying for an hour at their discretion.
22. Transport samples immediately to biomarker laboratory for processing.

6.2 Venous Blood Collection

Venous blood is drawn immediately after CSF collection is complete, recording the time. The following samples are acquired:

- 1 × 8.5 ml serum tube.
- 4 × 10 ml blood in lithium heparin tubes. Gently invert each tube 10 times immediately after collection, and place on ice.
- If venepuncture with vacuum tubes proves challenging, a needle and syringe may be used and the blood transferred immediately into the vacuum tubes, observing safety precautions.

7. Sample Processing Procedures

7.1 CSF Sample Processing

1. All CSF processing should be done on ice, beginning within 15 minutes of completion of collection.
2. Agitate the entire CSF sample for 10 seconds to homogenise CSF.
3. Use 200 µl of the CSF to determine white blood cell count and erythrocyte count per µl according to local GLP-approved laboratory practice. This should be done in triplicate within 60 minutes of collection and all values recorded in the eCRF.
4. Centrifuge the 50 ml tube containing residual CSF at $400 \times g$ for 10 min at 4°C to remove cells while preserving cell integrity for potential future use.
5. Pipette supernatant into a single tube labelled "CSF supernatant" and agitate for 10 seconds to homogenise CSF
6. Aliquot the CSF into 300 µl aliquots, using supplied pipette tips and cryovials labelled "CSF".
7. Gently resuspend pellet in 300µL of supplied preservative solution and transfer to the cryovial labelled "Cells from CSF".
8. Freeze CSF aliquots and resuspended cells on dry ice and store at -80°C.
9. Record time of freezing

7.2 Serum Sample Processing

1. Spin serum tube at $2000 \times g$ at room temperature for 10 min immediately upon arrival in the biomarker laboratory

2. Transfer 1500 μ l of the supernatant into each of 2 separate 2 ml cryovials labeled “serum”, freeze on dry ice and store in -80°C .
3. Record time of freezing

7.3 Plasma Sample Processing

1. Spin lithium heparin tubes at $1300\times g$ for 10 min at 4°C immediately on arrival.
2. Discard any tubes whose plasma is pink due to hemolysis.
3. Combine the supernatant in one tube labelled “plasma” and mix by inverting 10 times. Store on crushed ice.
4. Divide lithium heparin plasma into 300 μ l aliquots using supplied pipette tips and cryovials labeled ‘plasma’.
5. Freeze samples on dry ice and store at -80°C .
6. Record time of freezing.

8. Sample storage

- Store samples in a -80°C freezer.
- Log samples in eCRF.

9. Sample Quality Control

The following quality control measures will be carried out to identify and flag samples subject to potential confounders:

- Microscopic erythrocyte count in CSF is performed locally in triplicate and recorded on eCRF. Cut-off for flagging: > 1000 cells/ μ l.
- Microscopic leukocyte count in CSF is performed locally in triplicate and recorded on eCRF. Cut-off for flagging: ≥ 5 cells/ μ l.

10. Analysis

10.1 CSF and plasma samples

CSF and plasma samples will be analysed locally at UCL Institute of Neurology or by collaborators authorised by the Principal Investigator. This may include collaborators outside the EU from academic or commercial entities for the purpose of research (1) to better understand HD or other diseases being studied, (2) that furthers the development of treatments for HD or other diseases or (3) that furthers biomedical research. Any shared samples will be coded and linked-anonymised.

Analyses of huntingtin protein and the kynurenine pathway are specifically planned. Specifically, the levels of the following KP metabolites will be measured in CSF and plasma: kynurenine, kynurenic acid, 3-OH-kynurenine, quinolinic acid and anthranilic acid. In addition, the plasma levels of tryptophan will be determined, which will allow for an additional control for lack of compliance with the stipulation of an overnight fast.

Additional measurements, including but not limited to other KP metabolites or precursors, the levels of soluble HTT, and other putative biomarkers may also be measured at appropriate laboratories.

The primary outcome measurements are of unknown clinical significance. The detailed analysis may include measurements of potential clinical significance in relation to conditions other than HD, such as oligoclonal bands. However, patients with other neurological diagnoses or unexpected examination findings will be excluded. Therefore any abnormal results, obtained on a linked-anonymised basis, will remain of indeterminate clinical significance and will not be fed back to the participant.

A portion of each participant's samples will be shared alongside phenotypic data with CHDI Foundation to augment the collection of CSF and plasma for the complementary HDClarity project (global Chief Investigator: Dr Edward Wild). This shares the aims of HD-CSF in investigating huntingtin protein, the kynurenine pathway and other biomarkers and pathways of relevance to HD.

10.2 MRI data processing

Whole-brain, caudate and white-matter atrophy rates will be calculated using the robust, reproducible methods developed in TRACK-HD, which compared many potential clinical, imaging, cognitive and other biomarkers head-to-head and produced a toolkit for longitudinal clinical trials in HD⁶⁻⁹. Baseline regions will be segmented using MIDAS software and volume change estimated using the boundary shift integral (BSI) method. White matter will be quantified using voxel-based morphometry. Additional analyses may be conducted locally or by authorized collaborators.

10.3 Statistical analysis

Statistical design advice was received from UCL/UCLH Biostatistics Unit. Each biochemical analysis will require its own statistical plan which will be prepared before the analysis is conducted. Broadly, linear regression models, adjusted for age and gender, will compare inter-group cross-sectional differences in primary outcomes (CSF mHTT concentration and CSF ratio of 3HK:KYN) and associations with disease burden score across all groups. Longitudinal analysis will compare rates of change between and across groups. Significantly altered primary outcomes will be taken forward to a second-level analysis and regressed against secondary outcomes (CSF and plasma levels of individual KP metabolites (kynurenine, KA, 3HK, QA) and clinical/ cognitive/ MRI measures. This will identify mHTT species and KP metabolites that predict specific neurobiological or mechanistic features of HD. Multiplicity correction and bootstrapping for non-normally distributed variables will be used where appropriate.

11. Adverse Event Reporting and Documentation

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence during a clinical investigation and that does not necessarily have a causal relationship with study treatments or procedures. An AE is therefore any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of study procedures.

The Principal Investigator or appointed delegate(s) will probe, via discussion with the participant, for the occurrence of AEs during each participant visit, after the screening visit, and record the information in the site's source documents. AEs will be recorded in the patient eCRF. AEs will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study procedures if applicable, or if unrelated, the cause.

11.1.1 AE Severity Grading

The severity of an AE will be graded on a 5-point scale (Common Terminology Criteria for Adverse Events v3.0 (CTCAE);

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm) defined as follows:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE
Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

11.1.2 AE Relationship to study procedures

The relationship of an AE to the study procedures will be evaluated according to the following guidelines:

Probable: This category applies to AEs which are considered with a high degree of certainty to be related to the study procedure. An AE may be considered probably related to the study procedure if:

1. It follows a reasonable temporal sequence from administration of the study procedure;
2. It cannot be reasonably explained by the known characteristics of the participant's clinical state, or by environmental or toxic factors;
3. It follows a known pattern of response to the study procedure;

Possible: This category applies to those AEs in which the connection with the study procedure appears unlikely but cannot be ruled out with certainty. An AE may be considered as possibly related if it has at least two of the following:

1. It follows a reasonable temporal sequence from the study procedure
2. It may readily have been produced by the participant's clinical state, or by environmental or toxic factors;
3. It follows a known response pattern to the study procedure.

Unrelated: This category applies to those AEs which are judged to be clearly and incontrovertibly due to extraneous causes (disease, environment, etc.) and do not meet the criteria for study procedure relationship listed under possible or probable.

11.2 Serious Adverse Events

A Serious Adverse Event (SAE) is defined as any AE that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the participant or require intervention to prevent one of the outcomes listed.

An AE is considered to be life-threatening if, in the view of the Principal Investigator, the participant was at immediate risk of death from the reaction as it occurred. It does not include a reaction that, had it occurred in a more serious form, might have caused death.

11.2.1 Serious Adverse Experience Reporting

SAEs (as defined in Section 11.2) must be reported to the Sponsor immediately and in no case later than within 24 hours of awareness of the event.

All SAEs that occur (whether or not related to study procedures) will be documented. The collection period for all SAEs will begin from the Sampling Visit and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the REC, the Principal Investigator will report SAEs to the REC.

11.3 Post-study Follow-up of Adverse Events

Any AE, including clinically significant physical examination findings, must be followed until the event resolves, the condition stabilises, the event is otherwise explained, or the participant is lost to follow-up. If resolved, a resolution date should be documented on the eCRF and in the source documents. The Principal Investigator is responsible for ensuring that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals as is medically indicated.

12. Statistical Methodology

12.1 Determination of Sample Size

Power calculations were based on a 12-subject CSF analysis of mutant huntingtin using a novel single molecule counting immunoassay¹. Detecting cross-sectional differences between control and HD requires very small numbers (<5 per group for >90% power at 5% significance). 20 subjects per group gives >90% power to detect predicted longitudinal change in mutant huntingtin over two years.

For the biomarkers discovered and analysed, it may be important to understand the stability of the biomarker within participants over relatively short time periods. Thus, approximately 20 participants per cohort will be invited to return for a repeat sampling visit 4-8 weeks after their first visit.

13. Study Management

13.1 Ethics and Regulatory Considerations

The investigator will conduct the study in compliance with the protocol and in accordance with the ICH for GCP and the appropriate regulatory requirement(s). The study will also be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Participants must give informed consent prior to undertaking study procedures and these informed consents must be obtained by clinical site staff using approved processes. Signed consent forms will be maintained in a secure designated location.

13.1.1 Audits and Inspections

The study may be subject to inspection and audit by University College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition). Audits and/or inspections may also be carried out by local authorities, or authorities to which information on this trial has been submitted. All documents pertinent to the trial will be made available for such inspection after an adequate announcement.

13.1.2 Ethics Committee Approval

The Investigator will obtain approval from the Local Research Ethics Committee (REC). The Investigator will require a copy of the R&D/NHS approval letter before accepting participants into the study. Substantial amendments to the protocol will require written approval / favourable opinion from the REC prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects. Deviation from the protocol required to eliminate an immediate hazard(s) to subjects will be fully documented in the CRF and source documentation.

13.1.3 Confidentiality

In order to maintain subject privacy, all case report forms (CRFs), study reports and communications will identify the subject by initials and the assigned Subject number. The investigator will preserve the confidentiality of participants taking part in the study in accordance with the Data Protection Act.

13.1.4 Sponsor

University College London will act as the Sponsor for this study.

13.1.5 Indemnity

University College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

13.2 Informed Consent Procedure

Consent to enter the study will be sought from each participant only after a full explanation of the study has been given, a patient information sheet offered and time allowed for consideration. Signed participant consent will be obtained. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

The right of the participant to refuse to participate without giving reasons will be respected. After the participant has entered the study the investigator remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

13.3 Biological samples (handling, processing and storage)

In the study, blood and CSF will be collected from participants in accordance with the patient consent form and patient information sheet and shall include all biological materials and any derivatives, portions, progeny or improvements as well as all patient information and documentation supplied in relation to them. These biological samples will be stored at UCL Institute of Neurology for the processing described in section 8 of this protocol. This will prepare samples for shipment to collaborators in accordance with the analytical plan agreed with the Principal Investigator. The PI and his delegated representatives will process, store and dispose of samples in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereto.

13.4 Data Collection, Retention and Monitoring

13.4.1 Data transfer (handling, processing and storage)

In the study, Name, date of birth, medical history, ethnicity and cognitive data will be collected from patients in accordance with the patient consent form, patient information sheet and sections 5 and 5.1 of this protocol.

The patient data will be stored securely at UCL Institute of Neurology and collaborators authorized by the principal investigator for statistical analysis, and UCL will act as the data controller of such data for the study.

The PI and his delegated representatives will process, store and dispose of patient data in accordance with all applicable legal and regulatory requirements, including the Data Protection Act 1998 and any amendments thereto. Data held on paper will be stored at the UCL Institute of Neurology Huntington's Disease Research Centre under secure access control, in a locked filing cabinet controlled by the Investigator.

All transfers of data and/or samples will be covered by materials transfer agreements.

13.4.2 Data Entry/Electronic Data Capture System

Data will be entered electronically via secure internet-based technology provided by the Enroll-HD platform. Access to the eCRF is limited by password and can only be authorized by the PI. Monitoring of clinical data will be carried out by the Enroll-HD data monitors. The data managers are responsible for study monitoring and ensuring compliance with the study protocol.

13.4.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports pertaining to data omissions and discrepancies will be forwarded to the Principal Investigator and site investigators for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

13.4.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be

maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

13.4.5 Source Documents

The Principal Investigator will maintain source documents for each participant enrolled in the study. Source documents such as local laboratory ranges and reports, participant charts and doctors' notes will be kept as part of the participants' medical records. For participants who do not have a medical record per se, another method of documentation and record keeping will be employed, along with the obligation to retain source documents, such as laboratory reports, for the period of time specified in the site agreement. Participant files including medical records and signed participant informed consent forms must be available for review in the event the site is selected for monitoring, audits, or inspections.

13.4.6 Monitoring

The Principal Investigator, on behalf of the Sponsor, is responsible for ensuring the proper conduct of the study with regard to ethics, protocol adherence, site procedures, integrity of the data, and applicable laws and/or regulations.

The Principal Investigator will make study data accessible to the clinical monitors, to other authorized representatives of the Sponsor, and to regulatory inspectors.

13.4.7 Intellectual Property Rights

All background intellectual property rights (including licences) and know-how used in connection with the study shall remain the property of the party introducing the same and the exercise of such rights for purposes of the study shall not infringe any third party's rights.

All intellectual property rights and know-how in the protocol and in the results arising directly from the study, but excluding all improvements thereto or clinical procedures developed or used by each participating site, shall belong to UCL. Each participating site agrees that by giving approval to conduct the study at its respective site, it is also agreeing to effectively assign all such intellectual property rights ("IPR") to UCL and to disclose all such know-how to UCL.

Each participating site agrees to, at the request and expense of UCL, execute all such documents and do all acts necessary to fully vest the IPR in UCL.

Nothing in this section shall be construed so as to prevent or hinder the participating site from using know-how gained during the performance of the study in the furtherance of its normal activities of providing or commissioning clinical services, teaching and research to the extent that such use does not result in the disclosure or misuse of confidential information or the infringement of an intellectual property right of UCL. This does not permit the disclosure of any of the results of the study, all of which remain confidential.

13.5 Amendments

Any amendments to the protocol will be written and approved by the Principal Investigator and submitted to the REC for approval prior to implementing the changes. In some instances, an amendment may require changes to the informed consent form, which also must be submitted for REC and JRO approval prior to administration to study participants.

13.6 Record Keeping

13.6.1 Statutory compliance

The Principal Investigator agrees to comply with all applicable laws and regulations relating to the privacy of patient health information.

13.6.2 Retention of Study Documents

Study-related records must be retained for the period of at least five years.

14. Appendix A –Principal Investigator Obligations

The study protocol and the final version of the participant informed consent form will be approved by a REC before enrollment of any participants. The opinion of the IRB/ERB will be dated and given in writing.

The Principal Investigator will ensure that the REC will be promptly informed of all changes in the research activity and of all unanticipated problems including risk to participants. The Principal Investigator will not proceed with changes to the protocol until REC approval has been obtained.

Written informed consent must be given freely and obtained from every participant prior to clinical study participation. The rights, safety, and well-being of the study participants are the most important considerations and should prevail over interests of science and society.

As described in GCP guidelines, study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective task(s). Study personnel will not include individuals against whom sanctions have been invoked after scientific misconduct or fraud (e.g., loss of medical licensure, debarment). Quality assurance systems and procedures will be implemented to assure the quality of every aspect of the study.

REC Review/Approval/Reports

The protocol and informed consent for this study, including advertisements used to recruit participants, must be reviewed and approved by an appropriate REC prior to enrolment of participants in the study. It is the responsibility of the Principal Investigator to ensure that all aspects of the ethical review are conducted in accordance with the current Declaration of Helsinki, ICH, GCP, and/or local laws, whichever provide the greatest level of protection. Amendments to the protocol will be subject to the same requirements as the original protocol.

A progress report with a request for re-evaluation and re-approval will be submitted by the Principal Investigator to the REC at intervals required by the REC.

After completion or termination of the study, the Principal Investigator will submit a final report to the REC. This report should include: deviations from the protocol, the number and types of participants evaluated, and significant AEs, including deaths.

Study Documentation

The Principal Investigator is required to maintain complete and accurate study documentation in compliance with current Good Clinical Practice standards and all applicable federal, state, and local laws, rules, and regulations related to the conduct of a clinical study. Study documentation includes REC correspondence, protocol and amendments, information regarding monitoring activities, participant exclusion records, eCRFs, and data queries.

Confidentiality

The anonymity of study participants must be maintained. Study participants will be identified by an assigned participant number on eCRFs and other documents submitted to the clinical monitor. Documents that will be submitted to the clinical monitor and that identify the participant (e.g., the signed informed consent document) must be maintained

in strict confidence by the Principal Investigator, except to the extent necessary to allow auditing by regulatory authorities or the clinical monitor.

Study Facilities

The Principal Investigator must ensure that there is a robust institutional policy on freezer failure that includes checks, alarms, emergency contact details, backup power supplies, CO₂ cylinders and an infrastructure to transfer samples to an off-site facility if necessary.

15. References

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