Statistical Analysis Plan

Ref: SAP001V04

TRIHEP 3

A comparative phase 2 study assessing the efficacy of triheptanoin, an anaplerotic therapy in Huntington's Disease

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ABBREVIATIONS

31P-MRS 31-Phosphorus Magnetic Resonance Spectroscopy (^{31}P-MRS)

ANOVA Analyse of Variance

ANCOVA Analyse of Covariance

CAG Cytosine-Adenine-Guanine Cr = Creatine

HD Huntington's disease

HTT Huntingtin

MRI Magnetic Resonance Imaging

PBA-S Problem Behavior Assessment Short version

PCr Phosphocreatine

Pi inorganic Phosphate

SAP Statistical Analysis Plan

SDMT Symbol Digit Modality Test

SF-36 Short Form 36

TFC Total Functional Capacity

TMT Trail Making Test

UHDRS-TMS Unified Huntington's Disease Rating Scale Total Motor Score

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1 History of modifications

Version	Author	Modification description	Date
V01	A. DIALLO	Draft SAP creation	08/10/2018
V02	E. VICAUT	Revision	02/01/2019
V03	F. MOCHEL	Edits	06/01/2019
V04	E. VICAUT	Finalization	09/01/2019

2 Introduction

This Statistical Analysis Plan (SAP) is prepared by the URC Lariboisiere for a 2 centers, randomized, double-blind, placebo-controlled study (the TRIHEP3 Trial) to evaluate the benefit of triheptanoin in early affected HD patients compared to placebo, the study entitled: A comparative phase 2 study assessing the efficacy of triheptanoin, an anaplerotic therapy in Huntington's Disease (Project Code: C14-62, ClinicalTrials.gov Identifier: NCT02453061, EUDRACT No.: 2014-005112-4).

The primary objective of this clinical trial is to assess the efficacy of triheptanoin compared to placebo in decreasing of the caudate volume using volumetric magnetic resonance imaging.

The specific structure and content of SAP meets the requirements of the State Food and Drug Administration's biostatistical technical guidelines for clinical trials of chemical drugs and biological products and the technical requirements of ICH on drug registration.

This document describes the statistical analysis plan (SAP) for the TRIHEP3 trial and includes more detailed procedures for executing the statistical analyses of the primary and secondary endpoints. This SAP is based on the protocol version 7.0 (Dated 7 January 2019) and finalized before patient enrolment is completed and the database is locked for analysis. This SAP was written by the trial statistician and principal investigator and approved by the Head of biometry. All changes to the statistical analyses planned in the trial protocol are documented in Section 10.

3 Study objectives and endpoints

3.1 Hypothesis

Our proof-of-concept study strongly suggests that triheptanoin is able to improve the brain metabolic profile of HD patients at an early stage of the disease. It is very unlikely that these changes in brain energy metabolism are observed by chance as we have previously validated that the Pi/PCr changes in HD patients are stable over time, including over a one-month interval. Of note, the correction of the Pi/PCr response during brain activation was particularly notable when compliance to triheptanoin was ensured. We also observed an interesting trend of improved motor functions in HD patients after one month of anaplerotic therapy although these findings have to be interpreted with caution due to a possible placebo effect in this open-label study.
Thanks to neuroimaging biomarkers already validated in HD and the newly identified metabolic brain biomarkers using ^{31}phosphorous magnetic resonance spectroscopy (MRS), we can test for a reduction in neurodegeneration among HD patients resulting from an improvement in brain energy profiles with triheptanoin.

In our follow-up study, TRIHEP 3, we plan to include 100 early affected HD patients (5 ≤ UHDRS ≤ 40) in a randomized, double-blind, controlled study in 2 centers (France and the Netherlands). Patients will receive either triheptanoin at 1g/kg of body weight per day (n = 50), or a control oil (n = 50) for 6 months followed by an open label phase using triheptanoin for another 6 months. Efficacy of triheptanoin will be evaluated by measurements of caudate volume using volumetric magnetic resonance imaging and brain energy metabolism as evaluated by the ratio of inorganic phosphate/phosphocreatine, during visual stimulation, using ^{31}P-MRS. Clinical improvement will be evaluated by UHDRS, TFC, and PBA-S score as well as performance on the neurocognitive battery; patient quality of life will be evaluated with the SF-36 questionnaire before and after treatment; biological tolerance and compliance will be evaluated by routine biochemical blood tests, plasma and urine measurements of triheptanoin oil derivatives and patient report.

3.2 Principal objective

The primary objective of TRIHEP 3 is to evaluate the efficacy of triheptanoin in slowing atrophy in the caudate of early affected HD patients as measured with volumetric magnetic resonance imaging.

3.3 Secondary objective(s)

The secondary objectives are:

- To assess the efficacy of triheptanoin in restoring brain energy profiles using ^{31}phosphorous spectroscopy, and in slowing white matter alterations using diffusion tensor imaging.

- To assess the clinical benefit of triheptanoin on motor function in HD patients using scores on the United Huntington's Disease Rating Scale.

- To assess the clinical benefit of triheptanoin on psychiatric symptoms and cognitive function in HD patients using scores on the PBA-S, SDMT, TMT, Digit span test and Stroop test.

- To assess the effect on quality of life (SF-36) of HD patients of therapeutic use of triheptanoin.

- To confirm long-term clinical and biological tolerance of triheptanoin in HD patients.

3.4 Primary endpoint

The primary efficacy endpoint is the change from baseline to 6 months in caudate atrophy as measured by caudate boundary shift integral (cBSI).

3.5 Secondary endpoints

- Change from baseline to 3, 6 and 12 months in index of brain energy restoration (defined as delta of activation-recovery from the Pi/PCr ratio measured by ^{31}phosphorous MRS).
- Change from baseline to 12 months in caudate atrophy as measured by cBSI.
- Change from baseline to 6 and 12 months in DTI metrics and fiber integrity as measured by FBA.
- Change from baseline to 6 and 12 months in TMS and TFC.
- Change from baseline to 6 and 12 months in PBA-s, SDMT, TMT, Digit-Span, Stroop and SF-36.

3.6 Safety endpoints

Safety and long-term tolerance of Triheptanoin based on review of adverse events and changes in clinical labs and physical examination/vital signs.

4 Experimental plan

4.1 Study design

TRIHEP3 is a phase 2a, multicenter, double-blind, randomized, controlled two-armed study evaluating the efficacy of 1g/kg of body weight triheptanoin per day versus a placebo for a six-month treatment period, followed by a 6-month open-labelled period with all patients treated with triheptanoin.

4.2 Measures to limit bias

Double-blind, randomized design.

5 Number of necessary subjects

The rate of progression of caudate atrophy is linear in HD patients at an early-disease stage (Tabrizi SJ, Scahill RI, Owen G, et al. Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. Lancet Neurol. 2013;12(7):637-649). We hypothesized that, in patients under placebo, the progression of caudate atrophy over 6 months would be reduced by 42%. Based on such hypothesis and data from the TRACK-HD study, fifty patients under placebo and fifty patients treated with triheptanoin would be required to achieve 80% power at the one-sided 0.025 alpha level. Therefore, a total of 100 patients was required.

6 Study population

6.1 Inclusion criteria

- Positive genetic test with CAG repeat length ≥39 in HTT gene
- At least 18 years of age
- Signature of informed consent
- Covered by social security
- UHDRS score between 5 and 40
- Ability to undergo MRI scanning

6.2 Non-inclusion criteria

- Hypersensitivity to triheptanoin or to one of its excipients
- Additional psychiatric or neurological conditions
- Severe head injury
- Participation in another therapeutic trial (3 months exclusion period)
- Pregnancy or breastfeeding
- Inability to understand information about the protocol
- Persons deprived of their liberty by judicial or administrative decision
- Adult subject under legal protection or unable to consent
- Treatment with tetrabenazine
- Neuroleptic treatments other than olanzapine at small doses (≤ 10 mg) and aripiprazole (≤ 15 mg)

6.3 Population to analyze

Intent-to-treat (ITT) Population

The main efficacy analysis will be based on all data in the intention-to-treat population defined as all randomized patients who have signed an informed consent form. In case of consent withdrawal, only data collected before withdrawal will be used. All patients will be analyzed on the basis of their randomized group of treatment.

Per-Protocol (PP) Analysis Set

In addition, an explanatory analysis (PP) of all patients randomized and treated without major protocol violations/deviations will be carried out. Pre-defined major protocol violations/deviations are:

1. Missing data for the primary efficacy endpoints
2. Premature discontinuation of treatment, whatever the reason.
3. Unjustified change in treatment during follow-up
4. Inclusion in another clinical study

7 Variables measured and kept for analysis

All variables of the eCRF and those in additional files reporting data from MRI are kept for analysis. Regarding these latter following variables will be used:

- Index of brain energy restoration:

The delta of activation-recovery from the Pi/PCr ratio measured by ^{31}phosphorous MRS will be taken as the index of brain energy restoration.

- Caudate atrophy:

This will be the caudate volume loss (proportion of baseline volume) as measured by the caudate boundary shift integral (cBSI).

- Diffusion tensor metrics:

Fractional anisotropy (FA), radial diffusivity (RD), mean diffusivity (MD).

- Fiber tracking metrics:

Fiber density (FD), fiber cross-section (FC) and a combination of both - fiber density and cross-section (FDC).

Diffusion tensor metrics and fiber tracking metrics will be analyzed in the following areas from the JHU-atlas:

- Middle cerebellar peduncle
- Pontine crossing tract
- Genu of corpus callosum
- Body of corpus callosum
- Splenium of corpus callosum
- Fornix
- Corticospinal tract
- Medial lemniscus
- Inferior cerebellar peduncle
- Superior cerebellar peduncle
- Cerebral peduncle
- Anterior limb of internal capsule
- Posterior limb of internal capsule
- Retrolenticular part of internal capsule
- Anterior corona radiata
- Superior corona radiata
- Posterior corona radiata
- Posterior thalamic radiation
- Sagittal stratum
- External capsule
- Cingulum (cingulate gyrus)
- Cingulum (hippocampus)
- Fornix (cres) / Stria terminalis
- Superior longitudinal fasciculus
- Superior fronto-occipital fasciculus
- Uncinate fasciculus
- Tapetum

8 Missing data

For the main criterion a multiple imputation technic will be used in case of missing data (SAS PROC MI). No imputation will be used for secondary criteria.

9 Statistical analysis

Tabulated descriptive statistics by treatment will be provided for all parameters recorded in the eCRF.

Analysis of Continuous Data:
Continuous variables will be summarized using number of observations, mean, standard deviation, minimum, maximum, 25%, 50%, 75% quantiles and the two-sided 95% confidence intervals. Means, medians, minimum, maximum and standard deviations will be presented to one further decimal place. In addition, 95% confidence interval of difference between groups will be reported. Exact confidence intervals will be used if required.

Analysis of Categorical Data:
There will be counting of the absolute and relative frequencies (percentages) for categorical variables. Percentages will be rounded to one decimal place and there may be occasions where the total of the percentages does not equal 100% exactly.

General considerations regarding alpha value:
The nature of this study is mainly exploratory. We identified a main criterion for which a 5% two-sided alpha value has been chosen for statistical inference. As regards to the secondary criteria, we did not adjust alpha value for multiplicity since all the statistical inference regarding these numerous criteria should be considered as exploratory.

General considerations regarding parametric or non-parametric procedures:
All variables will be checked for their gaussian distributions by Shapiro-Wilk ‘s test. In case of non-normality, a normalizing transformation will be used. If this procedure failed a rank analysis will be used.

Demographic and other baseline characteristics data will be summarized descriptively as described above and will be summarized using Consort standard tabulations and listings will be generated.

Disposition of patients, patient status and patients excluded from ITT, PP populations will be summarized by treatment group. Descriptive statistics for primary reason for patient’s withdrawal will be also presented by treatment group as well as a list of these patients sorted by treatment group.

Primary endpoints:
The difference between the two groups regarding change from baseline in caudate atrophy at M6 will be analyzed using an ANOVA (SAS PROC MIXED) with Kenward–Roger’s adjusted degrees of freedom. Adjusted analysis will be performed using ANCOVA with age, gender and BMI as covariates. Prior to performing statistical tests, the assumptions of normality and

homogeneity of variances of residuals were tested using Shapiro-Wilk test and Levene's test respectively. If the data meet the normality assumption and do not meet homogeneity assumption, proc mixed allows to estimate separately the variance-covariance matrices in repeated statement using group option and thereby overcome the issue of heterogeneity of variance. If the data do not meet the normality or homogeneity assumptions of the parametric tests and are highly skewed, then a rank analysis was used.

Secondary criteria

- The difference between the two groups regarding change from baseline in index of brain energy restoration at M3, M6 and M12 (defined as delta of activation-recovery from the Pi/PCr ratio measured by ^{31}phosphorous MRS) will be analyzed using ANCOVA with baseline value as covariate. Adjusted analysis will be performed using ANCOVA with baseline value, age, gender and BMI as covariates

- The difference between the two groups regarding change from baseline in caudate atrophy at 1 year will be analyzed using ANOVA. Adjusted analysis will be performed using ANCOVA with age, gender and BMI as covariates.

- Diffusion tensor metrics and fiber tracking metrics will be analyzed using ANCOVA.

- Clinical scores will be analyzed using ANCOVA with baseline value as covariates. Adjusted analyses will be performed using an ANCOVA with baseline value, age, gender and BMI as covariates.

- Long-term compliance will be confirmed by regular biochemical testing from blood and urine samples – especially the levels of plasma propionylcarnitine and urinary 3-hydroxypropionic, 2-methylcitric, propionylglycine, tiglylglycine and/or methylmalonic acid – as well as by oil container accounting at study visits. The number of patients with detectable levels of triheptanoin metabolites will be reported.

- In addition, specific image analysis will be performed by the imaging department to allow for voxel-wise statistical comparisons and visualization of significant brain areas. These voxel-wise analytical methods will include tract-based spatial statistics for the diffusion tensor metrics and connectivity-based fixel enhancement for the fiber tracking metrics.

Safety endpoints:

- Long-term tolerance will be confirmed by clinical exam at study visits and by patient report during phone calls and/or visits. Side effects will be described in both arms of the trial. All Severe Adverse Events will be described by group and body system.

10 Changes to the statistical analyses planned in the protocol

The main analyses were described in the protocol version 4 (dated 7 January 2019) for the trial TRIHEP3. In the protocol version 3 (dated 1 June 2015), the two co-primary endpoints were caudate volume using volumetric magnetic resonance imaging, and brain energy metabolism as evaluated by the delta in Pi/PCr ratio between visual stimulation and recovery using ^{31}phosphorous MRS. To avoid multiplicity issues, the caudate volume will be analyzed as primary endpoint and brain energy metabolism as secondary endpoint.

11 Software and Programs used

All hypotheses testing will be carried out at the 5% (two-sided) significance level and statistical analyses will be performed using SAS 9.4 software. Image analysis and visualization will be performed using FSL and MRtrix.