Official Title: An Open-Label Adaptive Multiple-Dose Study to Investigate the Pharmacokinetics and Pharmacodynamics of RO7234292 in CSF and Plasma, and Safety and Tolerability Following Intrathecal Administration in Patients With Huntington's Disease

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STATISTICAL ANALYSIS PLAN

TITLE: **AN OPEN-LABEL, ADAPTIVE MULTIPLE-DOSE STUDY TO INVESTIGATE THE PHARMACOKINETICS AND PHARMACODYNAMICS OF RO7234292 IN CSF AND PLASMA, AND SAFETY AND TOLERABILITY FOLLOWING INTRATHECAL ADMINISTRATION IN PATIENTS WITH HUNTINGTON'S DISEASE**

PROTOCOL NUMBER: BP40410

STUDY DRUG: RO7234292 (ISIS 443139 or RG6042)

VERSION NUMBER: 1

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SPONSOR: F. Hoffmann-La Roche Ltd

PLAN PREPARED BY: [redacted]

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STATISTICAL ANALYSIS PLAN APPROVAL

Date and Time(UTC)	Reason for Signing	Name
15-Jun-2020 08:54:30	Company Signatory	[redacted]

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STUDY GLOSSARY

Abbreviation	Definition
ADA	Anti-drug antibody
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
ASO	Antisense oligonucleotide
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
BLQ	Below the limit of quantification
CAG	Cytosine, adenine, guanine base sequence found in DNA which is translated into glutamine
CAP	CAG age product
CGI-C	Clinical Global Impression – Change
CGI-S	Clinical Global Impression – Severity
ClinRO	Clinician-reported outcome
CNS	Central nervous system
CSF	Cerebrospinal fluid
C-SSRS	Columbia-Suicide Severity Rating Scale
DNA	Deoxyribonucleic acid
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic data capture
FDA	Food and Drug Administration
HA	Health Authorities
HD	Huntington's disease
HD-DAS	Huntington's Disease -Daily Activities Scale
HTT	Huntingtin gene
IMP	Investigational medicinal product
INR	International normalized ratio
IRB	Institutional Review Board
IT	Intrathecal
MAD	Multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
mHTT	Mutant Huntingtin
MoCA	Montreal Cognitive Assessment

MOE	Methoxyethyl
mRNA	Messenger ribonucleic acid
NfL	Neurofilament light chain
OLE	Open-label extension
PD	Pharmacodynamics(s)
PerfO	Performance outcome
PK	Pharmacokinetic(s)
PT	Prothrombin time
QTc	Corrected QT interval
QTcF	QT interval corrected through use of Fridericia's formula
RBC	Red blood cell
RBR	Research Biosample Repository
RNA	Ribonucleic acid
SAP	Statistical Analysis Plan
SDMT	Symbol Digit Modalities Test
SI	Système International d'Unités
SPA	Statistical Programming & Analysis
SWRT	Stroop Word Reading Test
Study Day 1	Defined as the first day Study Drug is administered to the patient
Study Drug	*RO7234292 (previously known as ISIS 443139)*
TEAE	Treatment-emergent adverse event
TFC	Total Functional Capacity
TMS	Total Motor Scale
UHDRS	Unified Huntington's Disease Rating Scale
ULN	Upper limit of normal
WBC	White blood cell
WES	Whole exome sequencing
WGS	Whole genome sequencing
wtHTT	Wild-type huntingtin

1. BACKGROUND

This Statistical Analysis Plan describes all planned methods of summarizing and analyzing data to be collected in study BP40410. Study BP40410 is an open-label, adaptive multiple-dose clinical study designed to generate time course data on PK, PD, and the PK/PD relationship after intrathecal (IT) administration of RO7234292 to patients with manifest HD (males and females aged 25-65 years, inclusive).

Huntington's disease is an autosomal dominant neurodegenerative disease caused by a CAG repeat expansion in the first exon of the HTT gene located on Chromosome 4 resulting in a polyglutamine expansion in the huntingtin protein (Htt). Based upon non-clinical and clinical evidence, mHTT protein is considered the primary driver of HD pathophysiology [1]. Individuals who carry at least 40 CAG repeats inevitably experience progressive motor, cognitive, and functional decline, usually in adult life, with a mean age of motor onset of 45 years. The average illness course post-motor onset is approximately 10 to 20 years, with pneumonia, heart failure, or other complications frequently cited as the cause of death [2].

2. STUDY DESIGN

Eligible subjects will receive dose assigned to the cohort they are allocated to. Three dose levels are tentatively planned as follows:

- Dose level 1: 120 mg RO7234292 (n=4 patients)
- Dose level 2: 60 mg RO7234292 (n=4 patients)
- Dose level 3: 30 mg RO7234292 (n=4 patients)

This is not a randomization study Patients are allocated to a cohort and hereby dose level according to the way they are sequentially enrolled into the study. Thus, the first 4 subjects will be assigned to 120mg, the next 4 subjects to 60mg and the next 4 subjects to 90mg.

After a review of medical data from the initial 3 dose levels and initial set of patients, a decision will be made whether to enroll additional patients and, in this case, whether to repeat a dose level, or to evaluate a lower or intermediate dose levels. The final number of dose levels and total number of patients assigned to a given dose level will be defined during the study. Up to a maximum of 20 patients will be enrolled in the whole study and the highest tested RO7234292 dose in this study will not exceed 120 mg, which is the highest test dose in the previous Phase I/IIa clinical study (ISIS 443139-CS1).

Study day is calculated relative to the date of first dose administration in Study BP40410. Study Day 1 will be the first treatment dose administration date. There is no day zero, hence the study day for patients who took at least one dose will be computed as follows:

Study Day = assessment date – treatment start date + 1,

if the assessment date is on or after the treatment date; or

Study Day = assessment date – treatment start date,

if the assessment date is earlier than the treatment date.

The end of this study is defined as the date when the last patient, last visit occurs. The end of the study is expected to occur approximately 6 months after the last study drug administration for the last patient has occurred. The total length of the study, from screening of the first patient to the end of the study, is expected to be approximately 1.5 years.

After completing study treatment in the current study, the patient will be eligible to enroll in an open label extension (OLE) study (BN40955) with active RO7234292 compound, provided the data from the ongoing RO7234292 program support continued development, the patient meets the inclusion and exclusion criteria for the OLE, and the OLE meets approval by the relevant competent authorities, IRBs, and ECs.

The SAP language supersedes the language in the protocol and protocol synopsis.

A schematic overview of the study design is presented in Figure 1. For additional details, refer to the Schedule of Assessments in the study protocol (Appendices 1 and 2).

<table>
<tr><td>Screening</td><td colspan="6">Treatment Period</td><td>Follow-up</td></tr>
<tr><td></td><td colspan="2">In-House Periods</td><td colspan="4">Out-Patient Visits</td><td></td></tr>
<tr><td>Day -28 to Day -3</td><td>Day -2 to Day 4*</td><td>Day 28 to Day 29*</td><td>Day 30</td><td>Day 43</td><td>Day 71</td><td>Day 127</td><td>6 months (± 2 weeks) after last study drug administration</td></tr>
<tr><td></td><td>• 1st dose of study drug on Day 1
• CSF sampling from predose to 48 hours postdose</td><td>• 2nd dose of study drug on Day 29
• Single predose CSF sample on Day 29</td><td colspan="4">• Single (equivalent to predose) CSF sample on Day 43, Day 71, Day 127, and FU</td><td></td></tr>
</table>

* Note: Patients will be admitted to the site in the afternoon/evening of Day -2 or in the morning of Day-1 to begin the first in-house period of the study; patients will be discharged on Day 4 after all assessments have been completed. Patients will return to the site for the second in-house period in the afternoon/evening of Day 28 or in the morning on Day 29 and will be discharged on Day 29 after all assessments have been completed.

CSF=cerebrospinal fluid; FU=follow-up.

Figure 1. Diagram of study design

2.1 OBJECTIVES

This study will evaluate the PK, PD, and safety of RO7234292 in patients with HD.

2.1.1 Primary Objectives

- To characterize the PK of RO7234292 in CSF and plasma following administration of multiple (2) IT doses of RO7234292
- To characterize mHTT CSF protein time course following administration of multiple (2) IT doses of RO7234292 in patients with HD
- To investigate the PK/PD relationship of multiple (2) IT doses of RO7234292 on mHTT in CSF

2.1.2 Secondary Objectives

- To assess the safety and tolerability of multiple (2) IT doses of RO7234292 in patients with HD
- To evaluate the immunogenicity of RO7234292
- To characterize the PK of RO7234292 in urine following administration of an IT dose of RO7234292

2.2 EXPLORATORY OBJECTIVES/ENDPOINTS

The exploratory objectives for this study would be to evaluate the effects of RO7234292 compared on the basis of the following endpoints:

- Change from baseline in exploratory biomarkers in CSF (e.g. neurofilament light chain [NfL])
- Relationship between exploratory fluid biomarkers in CSF and blood (e.g. CSF and plasma NfL)
- Relationship between biomarkers, safety (including Holter monitoring), PK, and immunogenicity
- Relationship of biomarkers to clinical severity at baseline using the UHDRS and Clinical Global Impression

Safety, PK, PD and clinical individual endpoints are outlined in the following sub-sections.

2.3 ENDPOINTS

2.3.1 Safety and Tolerability Endpoints

- Adverse events (AEs)
- Vital signs
- Electrocardiograms (ECGs)
- Clinical laboratory results
- Columbia - Suicide Severity Rating Scale (C-SSRS)
- Anti-drug antibodies (ADAs)

2.3.2 Pharmacokinetic Endpoints

PK parameters will be read directly from the (plasma, CSF) concentration-time profiles or calculated using standard non-compartmental methods. These parameters include but not limited to:

- Time to maximum (or peak) plasma concentration, maximum (or peak) plasma concentration
- Area under the plasma concentration-time curve (AUC) from time zero to the last measurable concentration, AUC from time zero to a timepoint, AUC from time zero to infinity, extrapolated AUC, AUC for a dosing interval
- Lambda(z), half-life; apparent total clearance
- Cumulative amount of unchanged drug excreted in urine, fraction of IT administered drug excreted in urine, renal clearance of the drug from plasma

2.3.3 Pharmacodynamic and Clinical Outcomes

- **Biomarkers**
    - CSF levels, including but not limited to:
        - mHtt
        - NFfL
    - Plasma NfL
    - MRI

- **Clinical-Reported Outcomes**
    - Montreal Cognitive Assessment (MoCA)
    - Huntington's Disease Daily Activities Scale (HD-DAS)
    - Independence Scale (IS)
    - Total Motor Scale (TMS)
    - Total Functional Capacity Scale (TFC)
    - Clinical Global Impression-Severity (CGI-S)
    - Clinical Global Impression-Change (CGI-C)
    - Symbol Digit Modalities Test (SDMT)
    - Stroop Wording Reading Test (SWRT)

2.4 DETERMINATION OF SAMPLE SIZE

Up to 20 patients with HD may be enrolled in this study. Due to the exploratory nature of this study, the actual number of patients will be determined during the study.

The sample size of 20 patients (at maximum) has been selected based on prior experience with second generation 2 ASOs given by IT injection to ensure that the PK and PD will be adequately assessed while minimizing patient exposure. The number of patients may be adapted during the study to sufficiently characterize the PK/PD relationship in CSF of RO7234292, while not exceeding 20.

2.5 ANALYSIS TIMING

Despite the tentative safety analyses performed in order to determine the subsequent dose levels, the final analysis will be conducted once all recruited patients have completed the study as defined in the protocol or withdrawn early from the study and data have been cleaned and verified and the database has been locked.

Given the adaptive nature of this study, data may be reviewed at any time by the Sponsor, to inform the decision of eventually enrolling more than the initial planned 12 patients.

Additionally, data may be used before the final analysis to support interactions with health authorities (HA).

3. STUDY CONDUCT

The Guidelines of the World Medical Association Declaration of Helsinki dated October 2002, the applicable regulations and guidelines of current Good Clinical Practice (GCP) as well as the demands of national drug and data protection laws and other applicable regulatory requirements will be strictly followed.

3.1 PATIENT ENROLLMENT

This is a non-randomization study as patients are assigned to a dose level in the order they are enrolled after successfully completing all screening evaluations and after the Investigator has verified that the patient is eligible per inclusion and exclusion criteria outlined in the Protocol.

In the event of a screen-failure, a maximum of 1 re-screening will be allowed within 4 weeks of the initial screening failure (e.g., as a consequence of abnormal laboratory values or general medical status not meeting inclusion or exclusion criteria). If re-screening is required, CAG repeat length testing does not need to be repeated (historical values will not be accepted), MRI and viral serology from the initial screening may be acceptable as part of the re-screening assessments, if performed within 4 weeks of the baseline visit.

4. STATISTICAL METHODS

Descriptive summary statistics including but not limited to n, mean, median, standard deviation, range (minimum, maximum) for continuous variables, and counts and percentages for categorical variables will be used to summarize data by dose level and visit. Where applicable, statistical tests will be conducted using 2-sided tests and 95% confidence intervals will reported.

4.1 ANALYSIS POPULATIONS

4.1.1 Safety Population

All patients who are enrolled and received any dose of RO7234292, whether prematurely withdrawn from the study or not, will be included in the safety analyses.

The Safety Population will be the primary population for all safety analyses and patients will be analyzed by dose received.

4.1.2 Immunogenicity Population

All patients who received any dose of RO7234292 and with at least one post-dose ADA assessment.

4.1.3 Pharmacokinetic Population

All patients who are enrolled and receive at least 1 dose of RO7234292, have not significantly deviated from the protocol, violated inclusion or exclusion criteria. Patients with incomplete data may be excluded if this would undermine the interpretability of the the PK analysis. Excluded cases will be documented together with the reason for exclusion.

4.1.4 Pharmacodynamic Population

All patients who are enrolled and receive at least 1 dose of RO7234292, have not significantly deviated from the protocol, violated inclusion or exclusion criteria. Patients with incomplete dattta may be excluded of this would undermine the interpretability of the PD analysis. Excluded cases will be documented together with the reason for exclusion.

4.2 BASELINE DEFINITIONS

For all assessments, baseline will be defined as the last non-missing measure prior to the first dose administration. For fluid biomarkers, if appropriate time-matched baseline may also be considered to assess diurnal effects.

4.3 ANALYSIS OF STUDY CONDUCT

The number of patients who enroll in the study, discontinue from the study, and complete the study will be summarized overall and by dose level. Reasons for premature study withdrawal will be listed and summarized. All major protocol deviations will be listed and summarized.
Additional analyses will be performed to assess and describe the impact of COVID-19 on the study conduct.

4.4 ANALYSIS OF TREATMENT GROUP COMPARABILITY

Demographic and baseline characteristics will be summarized by dose level using but not limited to n, mean, standard deviation, median and range for continuous variables and proportion for categorical variables, as appropriate. Summaries will be presented overall and by dose level.

4.5 DATA HANDLING RULES

For safety laboratory and measurements that are reported as '<x' or '>x', the analysis value will be replaced by 'x'. For biomarkers (e.g. CSF mHtt, CSF NfL, etc) where entries will be indicated as 'BLQ', the analysis value will be set to BLQ divided by 2 (BLQ*0.5). No imputation will be performed on missing entries without a corresponding BLQ limit. In case of listing, however, the original data entries will be presented.

A summary table for subjects with imputed values by dose level and visit will be presented for each parameter.

For CSF/plasma biomarkers (e.g. NfL, mHtt) and CSF protein, scatter plots to assess relationships will be based on the natural log-transformed parameter.

For PK concentrations, BLQ values will be set to zero if corresponding time was prior to Tmax and set to missing after Tmax.

4.6 MISSING DATA

Incomplete dates for AEs and laboratory data will be handled as described in the Roche data analysis standards (GDSR). Missing values will not be imputed unless otherwise specified (e.g. for BLQs).

4.7 SAFETY ANALYSES

Safety information will be collected, reviewed, and evaluated by the Sponsor or designee in accordance with the Safety Management Plan throughout the conduct of the clinical trial. Data will be summarized using descriptive statistics. Only scheduled visits will be presented in summary tables and both scheduled and unscheduled visits will be included in listings.

4.7.1 Exposure of Study Medication

Duration of treatment exposure (in days) to study drug will be calculated as:

Date of last dose – Date of first dose +1

Summary tables for treatment exposure will be presented by dosing level. Number of doses received will also be reported.

4.7.2 Adverse Events

All treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) will be summarized for each dose level and for all patients using the Medical Dictionary for Regulatory Activities (MedDRA) coding by system organ class, preferred term, relationship to Study Drug, and severity. Missing data will be handled as described in Section 4.6. Percentages presented in AE tables will display the percentage of patients within a treatment dose level experiencing the AE at least once.

For frequency counts by preferred term, multiple occurrences of the same AE in an individual are counted only once. For overall total number of events, multiple occurrences of the same AE in an individual are counted separately.

An adverse event will be regarded as treatment emergent adverse event (TEAE) if the event occurs after the first dose of treatment or worsened after treatment.

Adverse events that occur before first dose administration and are not related to study procedure will be included in data listings only. SAEs and non-serious AEs that lead to treatment discontinuation or treatment interruption will be listed separately. Selected AEs, for instance procedure related AEs, will be summarized and listed separately.

Additional analyses will be considered to describe the impact of COVID-19 on patient safety.

4.7.3 Laboratory Data

Laboratory data including chemistry, hematology, CSF safety labs and urinalysis, will be collected throughout the study.

Summary statistics at each assessment time will be presented using the International System of Units (SI units; Système International d'Unités). Laboratory data not reported in SI units will be converted to SI units before processing.

Descriptive tables will be used to summarize absolute, change and percentage change from baseline by dose level and study day. Measurements reported as '>x' or '<x' will be handled as outlined in Section 4.5.

Spaghetti plots (with overlaid median) for absolute and change and percentage change from baseline values will be presented for all parameters by dose level on the numeric time scale.

In addition, the shifts (relative to the normal range) from baseline to the minimum and maximum post-baseline values will be presented. If a subject is missing a baseline value but had a post-baseline value, then the baseline assessment is labeled as "unknown". Likewise, if a subject had a baseline value but had no post-baseline values, then the minimum and maximum are labeled as "unknown". For each parameter, the incidence of shift to *low* (below the normal range) will be summarized using the minimum post-baseline values; the incidence of shift to *high* (above the normal range) will be summarized using the maximum post-baseline values.

Finally, the number of patients with abnormal absolute values for the CSF safety labs, according to the parameter ranges presented in Table 1 will be summarized by dose level and visit and also as a listing. Red blood and white blood counts cannot be negative and the lower normal range limit is 0, hence no abnormal low values. Roche standard reference ranges, rather than the reference ranges of the Investigator will be used for all parameters.

For most parameters, the measured laboratory test result will be assessed directly using the Roche standard reference range. Certain laboratory parameters will be transformed to Roche's standard reference ranges. A transformation will be performed on certain laboratory tests that lack sufficiently common procedures and have a wide range of Investigator ranges, e.g., enzyme tests that include AST, ALT, alkaline phosphatase, and total bilirubin. Since the standard reference ranges for these parameters have a lower limit of zero, only the upper limits of the ranges will be used in transforming the data.

Standard and marked reference range for CSF laboratory parameters are presented in Table 1, given that there were no internal standards for those parameters.. There is no difference in the standard and marked reference ranges for protein and Leukocytes except for Glucose and Erythrocytes upper limits.

Table 1. CSF Safety Labs Standard (Marked) Reference Ranges

Parameter	Standard Range			Marked Range
	Low	Normal	High	High
Glucose (mmol/L)	<2.2	2.2-3.9	>3.9	>4.4
Protein (g/L)	<0.15	0.15-0.5	>0.5	
Erythrocytes (cells/mm^3)		0	>0	>5
Leukocytes (cells/mm^3)		0-5	>5	

4.7.4 Vital Signs

Primary vital signs (pulse rate, systolic and diastolic blood pressure, temperature and respiratory rate; all supine after resting for approx. 5 minutes) and body weight will be measured at screening and protocol specified visits.

Absolute values and changes from baseline will be summarized with descriptive statistics by dose level and study day.

Spaghetti plots (with overlaid median) for absolute and change from baseline values will be presented for all parameters by dose level on the numeric time scale.

Listings of subjects meeting any of the abnormality criteria (according to Roche Standard ranges) outlined in Table 2 will be provided.

Table 2. Vital Signs (Supine) Abnormality Ranges

Parameter	Low	Normal	High
Pulse rate (beats/min)	<60	60-100	>100
Systolic blood pressure (mmHg)	<90	90-130	>130
Diastolic blood pressure (mmHg)	<60	60-80	>80
Temperature (C)	<36.5	36.5-37.5	>37.5
Respiratory rate (breaths/min)	<8	8-20	>20

4.7.5 Electrocardiogram

A standard 12-lead electrocardiogram (ECG) will be recorded at study visits outlined in the protocol. ECG characteristics recorded include, HR, QRS duration, and PR and QT intervals. Changes in T-wave and U-wave morphology and overall ECG interpretation will also be documented on the eCRF. T-wave information will be captured as normal or abnormal. U-wave information will be captured in two categories: absent/normal or

abnormal. RR and QT corrected according to Frederica's (QTcF) formulas used at sites are defined as following:

- $RR = {}^{60}/_{HR}$ and
- $QTcF = QT/RR^{1/3}$

The absolute values as well the changes from baseline will be summarized with descriptive statistics by dose level and study day. All ECG data will be listed.

In addition, abnormal findings for ECG parameters (according to Table 3, Roche Standard ranges) will be listed. If a patient has an abnormal finding, all parameters will be reported. Abnormal absolute QTc and change in QTc interval, as presented in Table 4 will also be summarized. Abnormal ECG Interpretation, U-wave and T-wave will also be listed.

Spaghetti plots (with overlaid median) for absolute and change from baseline values will be presented for all parameters by dose level on the numeric time scale.

Holter ECG recordings will also be recorded. Details on their collection and analysis will be detailed and provided in a separate document. In addition, details on QTc-concentration analysis will be detailed and provided in a separate document.

Table 3. ECG Parameters Abnormality Ranges

Parameter	Low	Normal	High
HR (beats/min)	<40	40-100	>100
PR Interval (msec)	<120	120-200	>200
RR Interval (msec)	<600	600-1500	>1500
QRS Duration (msec)	<40	40-120	>120
QT Interval (msec)	<200	200-500	>500
QTcF interval (ms)	<350	350-450	>450

Table 4. Criteria for abnormal absolute QTc and change in QTcF interval

Parameter	Criteria
QTcF interval (ms)	>450
	>480
	>500
QTcF change from baseline (ms)	>30
	>60

4.7.6 Anti-drug Antibodies

For patients under treatment, there is a potential for them to develop anti-drug antibodies (ADA) over time. The plasma sampling for anti-drug antibody assessment data will be available for baseline and pre-dose at each time point post-baseline and follow-up visit 6

months after the last dose (or early termination visit). Data will be presented in summary tables (proportions of baseline prevalence and incidence of ADA by dose level and a listing of patients who tested positive for ADA.

The following definitions based on [3] as well as a Sponsor's definition for ADA with neutralizing potential will be used to describe patients' immunogenicity:

- ADA Positive
  - Treatment induced: ADA developed de novo (seroconversion) following drug administration. Patients who have no pre-existing ADA or are missing ADA data before drug administration and who have at least one ADA positive sample following drug administration.
  - Treatment boosted: Patients who have pre-existing ADA and have a ≥4 fold increase in ADA titer following drug administration compared to baseline measurement.

- ADA Negative
  - ADA negative all samples: Patients who have no pre-existing ADA (i.e. antibodies reactive with the drug that are present in patients before treatment) or are missing ADA data before drug administration and who have all negative ADA results following drug administration, and treatment unaffected ADA-negative patients.
  - Treatment unaffected ADA: Patients who had positive sample at baseline and post-baseline were either negative or positive but do not have a ≥4 fold increase in ADA titer following drug administration compared to baseline measurement.

- Transient ADA
  - Treatment-induced ADA detected only at one sampling time point during the treatment or follow-up observation period (excluding the last sampling time point).

- Persistent ADA
  - Treatment-induced ADA detected at 2 or more sampling time points during the treatment or follow-up observation period, or detected on last sampling time point.

4.7.7 Physical/Neurological Examination

A complete physical and neurological examination will be performed at screening and at other visits as specified in the schedule of activities (refer to the study protocol Appendices 1 and 2 for details). Unlike other assessments which will be pre-dose, neurological examination will be pre and post dose on each dosing day.

Any abnormality identified at baseline will be recorded on the General Medical History and Baseline Conditions eCRF. Changes from baseline abnormalities will be recorded in patient notes. New or worsened clinically significant abnormalities (i.e., beyond expected variation or normal age-related change) will be recorded as adverse events on the Adverse Event eCRF.

Respective assessments will be summarized in their respective eCRF aforementioned category.

4.7.8 Columbia-Suicide Severity Rating Scale

The Columbia-Suicide Severity Rating Scale (C-SSRS) is a structured tool to assess suicidal ideation and behavior. Four constructs will be assessed: severity of ideation, intensity of ideation, behavior and lethality of actual suicide attempts.

The C-SSRS collects binary responses to 11 categories: five subtypes of suicidal ideation, five subtypes of suicidal behavior, and self-injurious behavior without suicidal intent. Specifically, the following outcomes are C-SSRS categories and have binary (Yes/No) responses. The categories have been re-ordered from the actual scale to facilitate the definitions of the composite endpoints and to enable clarity in the presentation of the results.

Suicidal Ideation:

- **Category 1** - Wish to Be Dead
- **Category 2** - Non-specific Active Suicidal Thoughts
- **Category 3** - Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act
- **Category 4** - Active Suicidal Ideation with Some Intent to Act, without Specific Plan
- **Category 5** - Active Suicidal Ideation with Specific Plan and Intent

Suicidal Behavior:

- **Category 6** - Preparatory Acts or Behavior
- **Category 7** - Aborted Attempt
- **Category 8** - Interrupted Attempt
- **Category 9** - Actual Attempt (non-fatal)
- **Category 10** - Completed Suicide

Other:

- **Category 11** - Non-suicidal Self-injurious Behavior

In addition, a numerical score, the Suicidal Ideation Score, will be defined as the highest suicide ideation category (1-5) at which the patient responded "Yes" for the given visit. If the patient did not respond “Yes” to any of these categories, the score will be set to zero.

For each of the aforementioned 11 categories, the number and percent of patients with a “Yes” response at any time post-baseline (regardless of baseline response) will be summarized by dose level. Emergent suicidal ideation or behavior will be summarized.

The listed binary categories and the Suicidal Ideation Score will be used to identify a number of composite suicidal endpoints. Note that "recent history" for these composite endpoints is defined as the 12 months prior to Screening and the Screening period. At the Screening visit, CSSRS data is collected for the prior 12 months. Therefore, analyses that utilize data from “recent history'” will include the historical 12-month data collected at Screening as well as all on-study C-SSRS data collected prior to first dose. The endpoints are defined as follows:

- **Suicidal Ideation**: A “Yes" answer at any time post-first-dose to any one of the five suicidal ideation questions (Categories 1-5), regardless of the pre-dose responses
- **Suicidal Behavior**: A “Yes" answer at any time post-first-dose to any one of the five suicidal behavior questions (Categories 6-10), regardless of the pre-dose responses.
- **Suicidal Ideation or Behavior**: A "Yes" answer at any time post-first-dose to any one of the ten suicidal ideation or behavior questions (Categories l-10), regardless of the pre-dose responses.
- **Treatment-Emergent Suicidal Ideation compared to recent history**: A maximum post-first-dose suicidal ideation score that is increased from the maximum suicidal ideation score in recent history.
- **Treatment-Emergent Serious Suicidal Ideation compared to recent history**: A maximum post-first-dose suicidal ideation score of 4 or 5 when the maximum suicidal ideation score during recent history was less than 4 (i.e., scores of 0-3). Only patients with a recent history score of 0-3 will be considered evaluable for this outcome.
- **Emergence of Serious Suicidal Ideation compared to recent history**: A maximum post-first-dose suicidal ideation score of 4 or 5 when the maximum suicidal ideation score during recent history was 0. Only patients with a recent history score of 0 will be considered evaluable for this outcome.
- **Improvement in Suicidal Ideation compared to baseline**: A decrease in the suicidal ideation score at the patient's Study Day 127 (last day for the Treatment Period) C-SSRS assessment compared to the baseline score, defined as the last measurement taken prior to first dose. Only patients with a baseline score >0 will be considered evaluable for these outcomes.
- **Emergence of Suicidal Behavior compared to all prior history**: The occurrence of suicidal behavior (a "Yes" response to one or more of Categories 6-10) post-first dose from not having suicidal behavior prior to first dose (includes the "lifetime" score collected at the Screening Visit as well as all C-SSRS assessments collected from the Screening Visit through pre-dose on Study Day 1).

Each of the composite endpoints will be summarized by dose level and study day. For each treatment emergent outcome listed, only those patients with the specified screening condition will be considered evaluable. In addition, patients who discontinue from the study with no post-first dose C-SSRS assessment will be considered unevaluable for analyses of suicidality. Percentages will be based on the number of evaluable patients for each outcome.

In addition, a shift table will be created to demonstrate the change in suicidal ideation score from recent history to treatment period and/or post-treatment period. The maximum suicidal ideation score in each period will be used to create the shift table. If a patient's recent history suicidal ideation score is missing but has a post-first-dose score, then the recent history assessment will be labeled as "unknown". Likewise, if a patient's recent history suicidal ideation score is available but has no post-first-dose score, then the scores during the treatment and post-treatment period will be labeled as "unknown".

4.8 CLINICAL OUTCOMES AND PHARMACODYNAMICS

Clinical-reported outcomes and pharmacodynamic parameters considered for analysis are outlined in Section 2.3.3 and detailed information is presented in the following sub-sections. Refer to the study protocol (Appendices 3 and 4) for a summary of all clinical endpoints with respect to number of items recorded and duration.

4.8.1 Describing Parameters over Time

All clinical outcomes will only be collected at Screening and follow-up visits, hence, descriptive tables (Table 5) will be presented at these two visits (including change from baseline at follow-up visit), while association analysis (Table 6) involving these parameters will only be presented at baseline.

As presented in Table 5, for PD biomarkers, descriptive tables will be presented per each time point and dose level. Additionally, for biomarkers, spaghetti plots (with overlaid median) for absolute, change and percent change from baseline values using untransformed data will be presented for all parameters by treatment dose level. In addition, individual patient profiles for selected biomarkers on absolute, change and percent change from baseline will be presented on the same page.

4.8.1.1 Unified Huntington's Disease rating Scale

The Composite Unified Huntington's Disease Rating Scale (cUHDRS) [4] is defined as a combination of the following first 4 individual assessments:

- **Total Functional Capacity**

  The Total Functional Capacity (TFC) represents the Investigator's assessment of the patient's capacity to perform a wide range of activities of daily living including working, chores, managing finances, eating, dressing and bathing. The 5-item assessment is based on a brief interview with the patient and the study companion

(if available). The TFC score ranges from 0 to 13, with a higher score representing better functioning.

- **Total Motor Scale**

  The Total Motor Scale (TMS) is the sum of the individual motor ratings obtained during administration of the motor assessment portion of the UHDRS by the investigator. The score ranges from 0 to 124, with a higher score representing more severe impairment.

- **Symbol Digit Modalities Test**

  The Symbol Digit Modalities Test (SDMT) is used to assess attention, visuoperceptual processing, working memory and psychomotor speed. It has been shown to have strong reliability and validity [5] [6]. The patient must pair abstract symbols with specific numbers according to a translation key. The test measures the number of items correctly paired (maximum of 110) in 90 seconds.

- **Stroop Word Reading Test**

  The Stroop Word Reading Test (SWRT) is a measure of processing and psychomotor speed and depends upon quick verbal motor output. Patients are presented with a page of color names (i.e., "BLUE," "RED," or "GREEN") printed in black ink and are asked to read aloud as many words as possible within a given amount of time (typically within 45 seconds). The number of words read correctly is counted and higher scores indicate better cognitive performance.

Based on the aforementioned assessments, the following formula is used to derive the cUHDRS;

$$\mathbf{cUHDRS} = \left(\frac{\mathrm{TFC} - 8.8}{2.8}\right) - \left(\frac{\mathrm{TMS} - 34.4}{17.4}\right) + \left(\frac{\mathrm{SDMT} - 25.2}{12.4}\right) + \left(\frac{\mathrm{SWRT} - 58}{21.2}\right) + 10.0$$

- **Independence Scale**

  The patient's Independence Scale (IS) is the Investigator's assessment of the patient's degree of independence and is a subscale of the UHDRS. The scale consists of 19 discrete levels ranging from 10 to 100 (by 5), in which a score of 100 indicates no special care is needed and a score of 10 indicates the patient is fed by tube and requires total bed care.

Descriptive tables will be provided as described in Section 4.8.1 and Table 5 for cUHDRS, its individual components and IS.

4.8.1.2 Huntington's Disease Daily Activities Scale

The Huntington's Disease Daily Activities Scale (HD-DAS) assesses patients' daily function. Following a semi-structured interview with the patient and/or study companion, the patient's ability to perform daily tasks such as eating or using a telephone will be recorded. Each item is scored on a 4-point Likert-type scale, where 0 indicates no impact and 3 indicates severe impact.

Descriptive tables will be provided as described in Section 4.8.1 and Table 5.

4.8.1.3 Clinical Global Impression-Severity

The Clinical Global Impression-Severity (CGI-S) is a single-item measure of current global severity and is completed by the clinician at clinic visits. The CGI-S is assessed using an 11-point numeric rating scale, where higher scores indicate greater severity.

Descriptive tables will be provided as described in Section 4.8.1 and Table 5.

4.8.1.4 Clinical Global Impression-Change

The Clinical Global Impression-Change (CGI-C) is a single-item measure of change in global status (since starting the study) and is completed by the clinician at the follow-up visit. The CGI-C has seven response options: *"very much worse," "much worse," "minimally worse," "no change," "minimally improved," "much improved,"* and *"very much improved."* To assess the relevance of this change, a follow up question with dichotomous response options ("yes" or "no") asks if the change has had a meaningful impact on the patient's well-being.

Descriptive tables will be provided as described in Section 4.8.1 and Table 5.

4.8.1.5 Montreal Cognitive Assessment

The Montreal Cognitive Assessment (MoCA) is a patient-completed assessment used to detect cognitive impairment. The MoCA contains a series of basic assessments, including attention and visuospatial tasks. The total score ranges from 0 to 30, where lower scores indicate greater impairment.

Descriptive tables will be provided as described in Section 4.8.1 and Table 5.

4.8.1.6 Summary of outputs for describing the parameters over time

Table 5. Overview of planned outputs to describe parameter evolution over time

Parameter	Output Presentation		Data Type			Time points
	Plot	Table	Absolute	Change	%Change	
mHtt	Y	Y	Y	Y	Y	All available
CSF NfL	Y	Y	Y	Y	Y	All available
Plasma NfL	Y	Y	Y	Y	Y	All available
BSI (MRI)*		Y		Y	Y	BSL, FU
Baseline MRI Volume£		Y	Y			BSL only
PK	Y	Y	Y			All available
ADA		Y	Y			All available
UHDRS+		Y	Y	Y		BSL, FU
MoCA		Y	Y	Y		BSL, FU
HD-DAS		Y	Y	Y		BSL, FU
CGI++		Y	Y	Y		BSL, FU

- Absolute=measured data at a given time point
- BSL=Baseline
- FU=Follow-up
- Change (%Change) = change from BSL from a given post-BSL measurement (expressed as a percentage)

\+ The parameters considered are cUHDRS, its individual components as well the Independence Scale (IS)

++ The parameters considered are CGI-S and CGI-C

* BSI=Boundary Shift Integral. BSi is already a measure of change in volume (thus the change is not derived as for the other variables). More specifically, %-Change for BSI is derived as $\left(\frac{BSI}{baseline\ volume}\right) \cdot 100$

£ Baseline MRI Volume will be reported as 'raw' as well as normalized by intra-cranial volume when available

4.8.2 Relationship between selected parameters

Relationship between biomarkers and between biomarkers and clinical outcomes will be assessed as outlined in Table 6 (e.g. to assess the association between CSF mHtt and CSF NfL).

For CSF/plasma biomarkers (e.g. NfL, mHtt) and CSF protein, scatter plots to assess relationships will be based on the natural log-transformed parameter.

Scatter plots between selected pairs of continuous variables will be presented based on data types shown in Table 6 for X (main predictor, e.g. CSF mHtt) and Y (response, e.g. CSF NfL) variables at each indicated time point separately and data points will be colored by dose level.

Due to small sample sizes involved in these data, no formal statistical modeling will be performed, spearman's correlation coefficient over all dose groups will be used to measure the strength of association between two variables. The coefficient and 95% confidence interval will be presented in/around the scatter plots.

Table 6. Overview of planned outputs to describe the association between selected pairs of parameters

Paired Variables	Data Type				Time points
	X-variable		Y-variable		
	Absolute	Change	Absolute	Change	
X=**mHtt**, Y=**CSF-Nfl**	Y		Y		X & Y= BSL
		Y		Y	X & Y= All available
X=**CSF-Nfl**, Y=**Plasma-Nfl**	Y		Y		X & Y= BSL
		Y		Y	X & Y= All available
X=**mHtt**, Y=**Clinical Outcomes**§	Y		Y		X & Y = BSL
X= **CSF-Nfl**, Y= **Clinical Outcomes**§	Y		Y		X & Y = BSL
X= **Plasma-Nfl**, Y= **Clinical Outcomes**§	Y		Y		X & Y = BSL
X= **Baseline MRI Volume***, Y= **Clinical Outcomes**§	Y		Y		X & Y = BSL

- BSL=Baseline

§ Clinical Outcomes will include cUHDRS, TFC, TMS, SDMT, SWRT, MoCA, HD-DAS, CGI-S and CGI-C

* For the analysis, both 'raw' and 'normalized' Baseline MRI Volume will be considered when available

4.9 PHARMACOKINETIC AND PKPD ANALYSES

All PK analyses will be based on the PK analysis population described in Section 4.1.3.

PK parameters will be read directly from the (plasma and CSF) concentration-time profiles, or calculated using standard non-compartmental methods. PK parameters outlines in Section 2.3.2 will be computed for RO7234292 and its metabolite(s), as appropriate. However, other PK parameters might be computed in addition as appropriate.

Individual and mean PK concentration data at each sampling timepoint of RO7234292 (and its metabolite[s] as appropriate) in plasma, CSF, and urine, and calculated PK parameters thereof, will be presented by listings and descriptive summary statistics including arithmetic means, geometric means, ranges, standard deviations, and coefficients of variation. Individual and mean concentration versus time of RO7234292 (and its metabolite[s] as appropriate) will be plotted on linear or semi-logarithmic scales as appropriate.

All PK parameters will be presented by individual listings and summary statistics including arithmetic means, geometric means, medians, ranges, standard deviations, and coefficients of variation.

Nonlinear mixed effects modeling will be used to analyze the plasma and CSF concentrations of RO7234292 and mHTT in CSF. If needed, the PK and PD data collected in this study may be pooled with data from other clinical studies. The influence of covariates, such as age, body weight, baseline mHTT on the PK and PD parameters will be investigated.

Population and individual estimates of primary PK parameters (e.g., clearance, distribution volume) and secondary PK parameters (e.g., AUC and average trough plasma concentration) will be computed and used to describe the relationship between plasma and/or CSF exposure and biomarker, clinical endpoints as well as safety measures. The data from this study may be pooled with data from other studies conducted with RO7234292 to support population PK/PD modeling.

Details of this mixed-effects modeling and exploration of exposure-response analysis and results will be described and reported in a separate document from the Clinical Study Report.

4.10 INTERIM ANALYSES

Given the adaptive nature of this study, data may be reviewed at any time by the Sponsor, to inform the decision of eventually enrolling more than the initial planned 12 patients.

Additionally, data may be used before the final analysis to support interactions with Health Authorities.

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