CONFIDENTIAL

Statistical Analysis Plan (SAP)

Sponsor:	**Isofol Medical AB**
Study code:	**ISO-FF-001**
Study title:	**An adaptive, randomized, double-blind, single-center, placebo-controlled Phase I study evaluating ECG effects, safety, tolerability and pharmacokinetics of single ascending doses of [6R]-5,10-Methylene Tetrahydrofolate (Modufolin® for Injection, 100 mg) in healthy male volunteers**
Date:	**05JUL2017 – Draft 1** **12JUL2017 – Final**

TABLE OF CONTENTS

1 LIST OF ABBREVIATIONS

AE – Adverse Event

ATC – Anatomical-Therapeutic-Chemical

BMI – Body Mass Index

CF – Clean File

CGI-C - Clinical global impression of change

CRF – Case Report Form

CSP – Clinical study protocol

CV - Coefficient of Variation

ECG – Electrocardiography

FAS – Full Analysis Set

L-Dopa – Levodopa

LID – L-dopa Induced Dyskinesia

MedDRA – Medical Dictionary for Regulatory Affairs

PD - Parkinson's disease

PKG - Parkinson's KinetiGraph

PPS – Per Protocol Set

SAE – Serious Adverse Event

SAP – Statistical Analysis Plan

SAS – Statistical Analysis System

SD – Standard Deviation

SOC – System Organ Class

STAT – Biostatistician

2 INTRODUCTION

This Statistical Analysis Plan (SAP) gives details regarding the statistical analyses and data presentation outlined in the final Clinical study protocol (CSP) for the study *ISO-FF-001 Clinical Study Protocol FINAL 2016-12-05.* Any changes from the final CSP are given in Section 8.

3 CLINICAL STUDY DETAILS

3.1 Clinical Study Objectives

3.1.1 Primary objective

To evaluate effects on ECG parameters after single ascending doses of Modufolin® for Injection, 100 mg in healthy male volunteers

3.1.2 Primary endpoint

The primary evaluation parameter is:

- Change-from-baseline QTcF (ΔQTcF)

3.1.3 Secondary objectives

To evaluate safety, tolerability and PK following single ascending doses of Modufolin® for Injection, 100 mg in healthy male volunteers

3.1.4 Secondary endpoints

The secondary evaluation parameters are:

- Change-from-baseline heart rate, PR and QRS interval (ΔHR, ΔPR and ΔQRS)
- Categorical outliers for QTcF, HR, PR interval, QRS interval;
- Categorical analysis for T wave morphology;
- Relationship between Modufolin® plasma concentration and ΔΔQTc
- Frequency, seriousness and intensity of AEs
- Physical examination
- Vital signs
- Safety laboratory measurements
- Plasma PK characteristics of Modufolin® for Injection, 100 mg and its metabolites:

  [6R]-5,10- Methylene Tetrahydrofolate (MTHF; Modufolin® for Injection, 100 mg)
  [6S]-5-Methyl-Tetrahydrofolate (5-Methyl-THF)
  [6S]-Tetrahydrofolate (THF)
  [6S]-5-Formyl-Tetrahydrofolic acid (5-Formyl-THF)

3.2 Clinical Study Design

An adaptive randomised, double-blind, single-centre, placebo-controlled Phase I study evaluating ECG effects, safety, tolerability and PK of single ascending doses of Modufolin® for Injection, 100 mg in healthy male volunteers.

Thirty-three (33) eligible and consenting subjects will be included in 3 cohorts, 11 subjects in each cohort. Within each cohort, subjects will be randomised to receive either placebo (3 subjects) or Modufolin® for Injection, 100 mg (8 subjects).

There will be 3 pre-defined ascending dose-levels. Additional dose levels may be considered if recommended by the internal Safety Review cCommittee. Safety data will be analyzed and evaluated by the iSRC between each dose level. The iSRC will have the choice to decide to escalate the dose as planned, reduce or increase the dose escalation step, repeat the dose, reduce the dose or terminate the study.

The total study duration for the subjects will be approximately 5 weeks and there will be in total 3 visits to the clinic. Subjects will be screened for eligibility according to study-specific inclusion/exclusion criteria within 4 weeks prior to start of study treatment (Visit 1; Screening visit). The subjects will be confined to the research clinic from the evening before dosing (Day -1) until 24 hrs post dose (Days 1 and 2). The subjects should be fasting overnight (8 hrs) before IMP/placebo administration until 4 hrs post-dose. A Follow-up Visit will be performed 5 to 10 days after dose administration of for each cohort.

3.3 Number of Subjects

Thirty-three (33) male subjects will be included in the study.

3.4 Methods of Assigning Subject to IMP

Subjects in each cohort will be randomized to receive either placebo (3 subjects) or active treatment (8 subjects).

The randomization list will be generated by CTC or delegate and provided to the packing company. The original randomization list will be kept in a sealed envelope by the randomizer. Sealed treatment code envelopes will be kept by CTC and the Sponsor.

3.5 Blinding

This is a double-blinded study and the allocation of treatments will not be disclosed until clean file has been declared and the database has been locked.

The IMP and the placebo are not identical in appearance and all efforts will be made at the clinic in order to maintain the blind. Both the IMP and the placebo will be masked in such a way that study subjects and study staff will remain blinded during the study. An un-blinded study nurse will prepare the IMP/placebo and administer the solutions to the study subject. The un-blinded study nurse will not be involved in any study-specific assessments or evaluations.

The final randomization list will be sent to ICardiac after clean file.

4 STATISTICAL AND ANALYTICAL PLANS

All analyses about ECG (including but not limited to QTcF, QT/QTc, PK/QTc) will be performed by iCardiac Technologies, Inc. who will provide a separate SAP.

No reference to any of these analyses will be part of this SAP.

4.1 Sample Size Justification

Assuming a 1-sided 0.05 significance level and a standard deviation of 7 msec for ΔQTcF, a total of 33 evaluable subjects who complete Modufolin® (24 subjects) and placebo (9 subjects), separately will be sufficient to achieve 82% power to exclude a prolongation of 10 msec or longer of the upper 1-sided 95% CI of the mean ΔΔQTcF, assuming that the prolongation is 3 msec at the geometric mean peak Modufolin® concentration. Under the same assumptions, a total of 66 subjects (48 Modufolin® and 18 placebo) will achieve 97.5% power.

Note that this calculation is conservative, since it does not take into account any gain in precision due to the use of all data of each subject with the help of a linear mixed effects model.

4.2 Definition of Analysis Sets

4.2.1 Full Analysis Set

The Full Analysis Set will consist of all subjects who have been randomized and received one dose of IMP/placebo.

4.2.2 Per Protocol Analysis Set

The Per Protocol Analysis Set will consist of all subjects who have been randomized and completed the study period without any major protocol deviations. All protocol violations will be judged as major or minor at the clean file meeting.

4.2.3 Safety Analysis Set

The Safety Analysis Set will include all subjects who receive at least one dose of IMP/placebo and have at least one post-dose safety assessment.

4.2.4 PK Analysis Set

The PK Analysis Set will include all subjects who have evaluable plasma concentration data for Modufolin® and for whom one or more of the designated PK parameters can be determined.

4.3 Definition of Baseline

Baseline measurement is defined as the latest measurement prior to first dose of IMP.

4.4 Summary Statistics

In general, all data collected will be presented with summary statistics and given in patient data listings. Summary statistics will include number of patients, mean, standard deviation, median, minimum and maximum for continuous data and frequency and percentage for categorical data. Table with summary statistics will be divided by treatment group and dose group, and visit where applicable. Patient data listings will be sorted by treatment, subject and timing of assessments.

PK data will be presented using summary statistics. Data will be presented in terms of number (N), arithmetic mean, standard deviation (SD), minimum and maximum value. In addition, for the parameters AUC (0-t) and Cmax the geometric mean and coefficient of variation (CV) will be presented.

4.5 Significance Level

Not applicable

4.6 Multiple Comparisons/Multiplicity

Not applicable

4.7 Handling of Drop-outs, Missing Data and Outliers

Outliers will be included in summary tables and listings, and will not be handled separately in any analyses

4.8 Adjustment for Covariates

Not applicable

4.9 Multicenter Studies

Not applicable.

4.10 Examination of Subgroups

No examination of subgroups is planned.

4.11 Blind Review

Prior to the data base lock of the study, the biostatistician (STAT) will review data in order to determine if any exclusion from analysis data sets will be necessary. Details regarding decisions made with respect to analysis data sets will be given in the CF protocol.

5 SUBJECTS

5.1 Subject Disposition

The number of subjects that entered the study, withdrawn subjects, completed subjects and the number of subjects at each visit will be summarized by treatment.

5.2 Baseline Characteristics and Demographics

The following baseline characteristics will be given by treatment:

- Age
- Gender
- Weight
- Height
- Ethnicity
- BSA (Body Surface Area)
- BMI
- Drug use
- Medical/Surgical history
- Physical examination
- Vital signs (BP, HR)

6 TREATMENT INFORMATION AND EXTENT OF EXPOSURE

6.1 Active Treatment

The number of subjects treated and their individual doses will be tabulated.

6.2 Placebo Treatment

The number of subjects received placebo and their individual doses will be tabulated..

6.3 Prior and Concomitant Medications

Prior and concomitant medication data will be listed only. Prior and concomitant medications will be coded according to the World Health Organization (WHO) Anatomic Therapeutic Chemical (ATC) classification system.

7 STATISTICAL METHODOLOGY

All parameters will be presented by treatment and visit using summary statistics. Additional statistical analyses are specified below.

7.1 Secondary endpoints

7.1.1 Definition

7.1.1.1 Physical examination

All available data will be used in the analysis of physical examination, without any further calculations.

7.1.1.2 Vital signs

Vital signs parameters (systolic/diastolic blood pressure and pulse) will be summarized by treatment and visit. Absolute and relative changes from baseline will be calculated.

7.1.1.3 Safety laboratory analyses

Safety laboratory data will be presented by individual time courses for each parameter

7.1.1.4 Adverse Events (AEs)

AEs and Serious Adverse Event (SAEs) will be recorded from start of IMP administration. Medical events occurring between screening and first treatment with IMP will be reported separately as baseline events.

7.1.1.5 PK

The definition and calculations of the PK parameters will be done by another vendor and are not included in this SAP.

CTC will receive all calculated PK parameters in a PK report that will be appended to the Clinical Study Report (CSR).

7.1.2 Presentation

7.1.2.1 Physical examination

Each item measured will be presented using tables with frequencies and percent. Abnormal findings will be specified and presented by patient and summarized by treatment.

7.1.2.2 Vital signs

All vital signs will be presented by treatment and visit, divided by actual value, absolute change and relative change.

7.1.2.3 Safety laboratory analyses

The laboratory parameters will be presented by treatment and visit using summary statistics. Abnormal laboratory values will be presented using frequency tables.

7.1.2.4 Adverse Events (AEs)

AEs verbatim terms will be encoded using the Medical Dictionary of Regulatory Activities (MedDRA), latest version available when approving the DMP.

AE/SAE:

The following summaries of AEs and SAEs will be given by treatment and in total:

- Total number of AEs
- Total number of unique AEs
- Total number of unique, related AEs
- Total number (%) of subjects with at least one AE
- Total number (%) of subjects with at least one related AE
- Unique AEs by MedDRA System Organ Class (SOC) and preferred term
- Number (%) of subjects with a least one AE by MedDRA
- Unique AEs by relation of study product and MedDRA SOC and preferred term.

Severity, action taken, concomitant therapy started and subject outcome of the AEs will be given in data listings only. AEs, which were reason for premature discontinuation of study product, will be listed separately.

The total number of SAEs and patients with a least one SAE will always be given. Further summaries of SAEs depending on the number of SAEs observed.

7.1.2.5 Discontinuation

Patients who discontinue from IMP treatment will be tabulated. The reason for discontinuation will be given. For discontinuation due to AE, the AEs will be given.

7.2 Interim Analysis

Not applicable.

8 CHANGES FROM THE CSP

9 STATISTICAL DELIVERABLES

The following documents will be delivered:

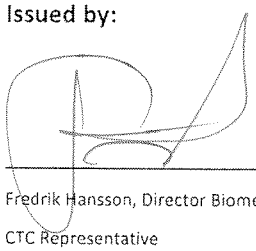
- SAP
- Statistical analyses and summary tables

10 SOFTWARE

All statistical analyses will be performed using SAS Version 9.4 (SAS institute, Cary, NC).

11 APPROVAL

Issued by:

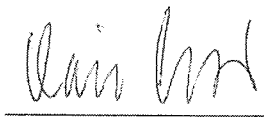


28 NOV 2017

Fredrik Hansson, Director Biometrics
CTC Representative

Date (dd-Mmm-yyyy)

Approved by:



24 AUG 2017

KARIN GÄNLÖV, MD CHIEF MEDICAL OFFICER
Sponsor Representative

Date (dd-Mmm-yyyy)