Clinical Study Protocol

EudraCT No.	2016-004874-16
Investigational Medicinal Product	Modufolin® for Injection, 100 mg
Study code	ISO-FF-001
Protocol Version and date	FINAL, 05 Dec 2016

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

Development phase	Phase I
Test product and dosage	Modufolin® for Injection, 100 mg (dose groups: 200, 350, 500 mg/m^2)
Comparator product	Placebo, 0.9% NaCl sterile solution
Sponsor Signatory	Anders Rabbe, Chief Executive Officer Isofol Medical AB Biotech Center Arvid Wallgrens Backe 20, 5th level SE-413 46 Gothenburg, Sweden Phone: +46 (0)70 764 65 00 E-mail: anders.rabbe@isofolmedical.com
Principal Investigator	Cornelia Lif-Tiberg, MD CTC Clinical Trial Consultants AB
Clinical study conduct and management	CTC Clinical Trial Consultants AB Uppsala University Hospital, Entrance. 85, 2nd level SE-751 85 Uppsala, Sweden

The following amendments have been made to the Final Clinical Study Protocol version 1.0:

Amendment No.	**Date of Amendment**	**Revised protocol version** *(if applicable)*

2 STUDY SYNOPSIS

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	
Study code ISO-FF-001	**EudraCT No** 2016-004874-16
Study period Estimated date of first subject enrolled: Q2 2017 Estimated date of last subject completed: Q2 2018	**Phase of development** Phase I
Coordinating Investigator Cornelia Lif-Tiberg, MD CTC Clinical Trial Consultants AB, Uppsala, Sweden	
Study design Single ascending dose study (SAD)	
Objectives <u>Primary objective</u> To evaluate effects on electrocardiogram (ECG) parameters after single ascending doses of Modufolin® for Injection, 100 mg in healthy male volunteers. <u>Secondary objectives</u> To evaluate safety, tolerability and pharmacokinetics following single ascending doses of Modufolin® for Injection, 100 mg in healthy male volunteers.	
Number of subjects planned Thirty-three (33) male subjects will be included in the study. Subjects who are prematurely withdrawn from the study for any reason except the occurrence of Treatment-emergent AEs (TEAEs) assessed as possibly or probably related to study treatment may be replaced during the course of the study.	
Diagnosis and main eligibility criteria Healthy male volunteers aged 18 to 60 years with a body surface area ≤ 2 m^2.	
Methodology This study is an adaptive randomized, double-blind, single-center, placebo-controlled Phase I study evaluating ECG effects, safety, tolerability and pharmacokinetics of single ascending doses of Modufolin® for Injection, 100 mg in healthy male volunteers. At least 33 eligible and consenting subjects will be included in 3 cohorts, 11 subjects in each cohort. Within each cohort, subjects will be randomized 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 Committee (iSRC). There will be an interval of at least 7 days between each dose level to allow time safety data to be analyzed and evaluated by the iSRC. 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.	

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 until 24 hrs post dose (Visit 2; Days -1, 1 and 2). The study subjects should be fasting overnight (8 hrs) before dose administration until 4 hrs post-dose.

A Follow-up Visit will be performed 5 to 10 days after administration of Investigational Medicinal Product (IMP)/placebo for each cohort.

Investigational Medicinal Product (IMP), dosage and mode of administration

Modufolin® for Injection, 100 mg

The pre-defined dose levels are 200, 350 and 500 mg/m^2.

Intravenous (*i.v.*) bolus injection (injection time 3 min)

Non-investigational Medicinal Product

Placebo: 0.9% NaCl sterile solution

Blinding

IMP (Modufolin® for Injection, 100 mg) and 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 for injection and will administer the IMP/placebo to the study subject. The un-blinded study nurse performing the dose administration will not be involved in any study-specific assessments or evaluations.

Duration of treatment

Each subject will receive a single bolus *i.v.* injection of IMP or Placebo on study Day 1.

Duration of subject involvement in the study

The total study duration will be approximately 5 weeks and there will be in total 3 visits to the clinic (Screening, In-clinic period and Follow-up visit).

Safety assessments

The primary safety evaluation parameter is:

- Change-from-baseline QTcF (ΔQTcF)

The secondary safety evaluation parameters are:

- Change-from-baseline HR, 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® for Injection, 100 mg plasma concentration and ΔΔQTc
- Frequency, seriousness and intensity of AEs
- Physical examination
- Vital signs
- Safety laboratory measurements (Hematology/Clinical chemistry)

Pharmacokinetic (PK) assessments

Plasma PK characteristics will be determined for:

[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)

The PK parameters to be measured/calculated are:

AUC	Area under the plasma concentration-time curve from time 0 to infinity. Calculated using the linear/log trapezoidal rule with extrapolation to infinity.
AUC_{last}	Area under the plasma concentration-time curve from time 0 to the last time point t.
AUC_{τ}	Area under the plasma concentration-time curve in a dosing interval. Calculated using the linear/log trapezoidal rule with extrapolation to τ, if required.
$AUC_{\%Extrap}$	Percentage of AUC that is due to extrapolation from the last concentration to infinity.
AUMC	Area under the first moment curve extrapolated to infinity based on the last observed concentration.
C_0	Back extrapolated concentration at time point 0 after intravenous administration.
C_{max}	Maximum plasma concentration
$C_{(time)}$	Plasma concentration at a certain time
C_{ss}	Steady-state drug concentration in plasma
CL	Total body clearance for intravenous administration
LLOQ	Lower Limit of Quantification
Mean	Arithmetic mean, otherwise specified
MRT	Mean Residence Time extrapolated to infinity.
NCA	Non-Compartmental pharmacokinetic Analysis
PK	Pharmacokinetics
Tau (τ)	Dosing interval
t_{max}	Time at which maximum plasma concentration is observed.
$t_{1/2}$	Terminal half-life
λ_z *(lambda z)*	Terminal rate constant. Calculated by log-linear regression of concentrations versus time.
V_z	Volume of distribution based on the terminal phase.
V_{ss}	Volume of distribution based at steady state.

Statistical methods

A more technical and detailed elaboration of the principal features will be presented in a separate Statistical Analysis Plan (SAP).

All statistical analysis of the study will be performed using the statistical software SAS for Windows Version 9.4. In all calculations, zero will be substituted for concentrations below the quantification limit of the assay. Data collected from all subjects will be presented in data listings. Both absolute

values and change from baseline values for each subject will be given where applicable. All continuous data will be listed with the same precision as will be presented in the database. Data listings will be sorted by treatment, subject ID and time point. A missing value will be represented by an empty cell and no imputation will be made.

Continuous data will be summarized in tables using number of subjects (n), mean, median, standard deviation (SD), standard error (SE), 90% confidence interval (CI; based on a t-distribution if not otherwise stated), minimum, and maximum by study time point. Categorical data will be summarized by time point using frequencies and percentages. Percentages will be rounded to the nearest tenth. Population counts will be used as the denominator in the calculation of percentages unless otherwise specified.

Data Analysis Sets

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.

Safety Analysis Set

The Safety Analysis Set will include all subjects who receive at least one dose of IMP (any dose of Modufolin® or matching placebo) and have at least one post-dose safety assessment.

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.

QT/QTc Analysis Set

The QT/QTc Analysis Set will include all subjects in the Safety Analysis Set with measurements at baseline as well as on-treatment with at least one post-dose time point with a valid ΔQTcF value. The QT/QTc Analysis Set will be used for the by-time point and categorical analyses in the electrocardiogram analysis.

PK/QTc Analysis Set

The PK/QTc Analysis Set will include all subjects who are in both the QT/QTc Analysis Set and PK Analysis Set with at least one pair of post-dose PK and QTcF data from the same time point. The PK/QTc Analysis Set will be used for the exposure-response analysis in the electrocardiogram analysis.

Sample Size Considerations

Assuming a 1-sided 0.05 significance level and a standard deviation of 7 milliseconds (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.

Exposure-Response Analysis (Primary objective)

The relationship between the plasma concentration of Modufolin® and change-from-baseline QTcF (ΔQTcF) will be quantified using a linear mixed-effects modeling approach with ΔQTcF as the dependent variable, plasma concentration of Modufolin® as the covariate, treatment (active or placebo) and time as categorical factors, and a random intercept per subject. The degrees of freedom for the model estimates will be determined by the Kenward-Rogers method. From the model, the slope (*i.e.*, the regression parameter for the concentration) and the treatment effect-specific intercept

(defined as the difference between active and placebo) will be estimated together with 2-sided 90% CI. The estimates for the time effect will be reported with degrees of freedom and standard error SE.

The geometric mean of the individual C_{max} values for subjects in each of the active drug groups will be determined. The predicted effect and its 2-sided 90% CI for placebo-corrected change-from-baseline QTcF (ΔΔQTcF) (*i.e.*, the product with the slope estimate + treatment effect) at this geometric mean C_{max} will be obtained for each Modufolin® dose separately. If the upper bound of the 90% CI of the model predicted QTcF effect is below 10 msec at clinically relevant plasma levels of Modufolin® it will be concluded that Modufolin® does not cause clinically concerning QTc prolongation.

By-Time point Analysis

The analysis for QTcF will be based on a linear mixed-effects model with change-from-baseline QTcF (ΔQTcF) as the dependent variable, time (categorical), treatment (Modufolin® for Injection, 100 mg and placebo), and time-by-treatment interaction as fixed effects, and baseline QTcF as a covariate. Subject will be included as a random effect for the intercept. Subject dosed with placebo will be analyzed as a pooled group. An unstructured covariance matrix will be specified for the repeated measures at post-dose time points for each subject. If the model with unstructured covariance matrix fails to converge, other covariance matrix such as autoregressive and compound symmetry will be considered. From this analysis, the least-squares (LS) mean and 2-sided 90% CIs will be calculated for the contrast "Modufolin® versus placebo" at each dose of Modufolin® for Injection, 100 mg and each post-dose time point, separately.

For HR, PR, and QRS intervals, the analysis will be based on the change-from-baseline values (ΔHR, ΔPR, ΔQRS). The same model will be used as described for QTcF. The LS mean, SE and 90% CI from the statistical modeling for both change-from-baseline and placebo-corrected change-from-baseline values will be listed in the tables and graphically displayed.

Categorical Analyses

The analysis results for categorical outliers, and T-wave morphology will be summarized in frequency tables with counts percentages for both number of subjects and number of time points. For categorical outliers, the number (percentage) of subjects as well as time points who had increases in absolute QTcF values >450 and ≤480 msec, >480 and ≤500 msec, and 500 msec, and changes from pre-dose baseline of >30 and ≤60 msec, and >60 msec; increase in PR from pre-dose baseline >25% to a PR>200 msec; increase in QRS from pre-dose baseline >25% to a QRS >120 msec; decrease in HR from pre-dose baseline >25% to a HR <50 bpm; and increase in HR from pre-dose baseline >25% to a HR >100 bpm will be determined. For T-wave morphology, the analyses will be focused on change from baseline (*i.e.*, treatment-emergent changes).

Pharmacokinetic analysis (Secondary objective)

The PK analysis will be based on the PPAS and subcontracted by the Sponsor to PKxpert. The PK parameters will be calculated by non-compartmental analysis (NCA) using the software Phoenix WinNonlin® version 6.4 or later (Pharsight Corporation, U.S.A.).

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4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or term	Explanation
5-FU	5-Fluorouracil
5-Methyl-THF	[6S]-5-Methyl-Tetrahydrofolate
5-Formyl-THF	[6S]-5-Formyl-Tetrahydrofolate
AUC	Area under the plasma concentration-time curve from time 0 to infinity
AUC_{last}	Area under the plasma concentration-time curve from time 0 to the last time point t
AUC_{τ}	Area under the plasma concentration-time curve in a dosing interval
$AUC_{\%Extrap}$	Percentage of AUC that is due to extrapolation from the last concentration to infinity
AUMC	Area under the first moment curve extrapolated to infinity based on the last observed concentration
ALT	Alanine transaminase
BP	Blood Pressure
C_0	Back extrapolated concentration at time point 0 after intravenous administration
C_{max}	Maximum plasma concentration
$C_{(time)}$	Plasma concentration at a certain time
C_{ss}	Steady-state drug concentration in plasma
CA	Competent Authority
CI	Confidence Interval
CL	Total body clearance for intravenous administration
CRF	Case Report Form
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CTC	Clinical Trial Consultants AB
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of Variation
DLT	Dose Limiting Toxicity
DMP	Data Monitoring Plan
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram

EEA	European Economic Area
FdUMP	5-fluoro-2'-deoxyuridine 5'-monophosphate
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
Hb	Hemoglobin
HDMTX	High Dose Methotrexate
HR	Heart Rate
hrs	Hours
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICF	Informed Consent Form
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IND	Investigational New Drug
iSRC	Internal Safety Review Committee
i.v.	Intravenous
LLOQ	Lower Limit of Quantification
LLV	Levoleucovorin
LS	Least-squares
LV	Leucovorin
lambda z	Terminal rate constant
MedDRA	Medical Dictionary for Regulatory Activities
min	Minute
msec	Millisecond
MTHF	[6R]-5,10-Methylene Tetrahydrofolate
MTHF-HS	[6R]-5,10-Methylene Tetrahydrofolate-Hemi Sulfate
MTX	Methotrexate
n	Number
NCA	Non-compartmental Pharmacokinetic Analysis
PK	Pharmacokinetic
PQ	The PQ interval starts at the beginning of the atrial contraction and ends at the beginning of the ventricular contraction
PR	The PR interval is measured from the beginning of the P wave to the beginning of the QRS complex

PT	Preferred Term
q2w	every 2 weeks
QRS	Corresponds to ventricular depolarizations
QT	The QT interval is the time from start of the Q wave to the end of the T wave
QTc	Corrected QT interval
qwk	every week
RR	The R-R interval is used to calculate heart rate (time between beats)
SAD	Single Ascending Dose
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SMD	Study Maximum Dose
SDV	Source Data Verification
SE	Standard Error
SOC	System Organ Class
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
Tau (τ)	Dosing interval
t_{max}	Time at which maximum plasma concentration is observed
$t_{1/2}$	Terminal half-life
TEAE	Treatment-emergent Adverse Event
TCA	Tricyclic antidepressants
THC	Tetrahydrocannabinol
THF	[6S]-Tetrahydrofolate
TS	Thymidylate Synthase
TQT	Thorough QT
ULN	Upper Limit of Normal
V_z	Volume of distribution based on the terminal phase
V_{ss}	Volume of distribution based at steady state
WHO	World Health Organisation

5 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

5.1 Medical emergencies contacts

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes a Serious Adverse Event (SAE) and is to be reported as such. Detailed SAE reporting procedures are included in Section 12.5.5.

In the case of a medical emergency the Investigator may contact the Medical Responsible Person at Isofol Medical AB, Gothenburg, Sweden.

Name	Function in the study	Telephone number and e-mail
Karin Ganlöv, MD	Sponsor's Medical Officer	Phone: +46 (0)70243 37 50 E-mail: karin.ganlov@isofolmedical.com

5.2 Overdose

An overdose is a dose in excess of the dose specified for each cohort in this Clinical Study Protocol (CSP).

Over-dosing is not likely to occur in this study since Modufolin® for Injection, 100 mg will be administered by site personnel under medical surveillance. Modufolin® for Injection, 100 mg is the active metabolite of Leucovorin (LV) and based on historical data, an overdose of Modufolin® for Injection, 100 mg is unlikely to be life threatening. However, if a subject would be injected with Modufolin® for Injection, 100 mg at a higher dose than the prescribed dose, the Principal Investigator must be informed immediately, the subject should be monitored, and any adverse events (AEs) should be reported.

Overdose should be recorded as follows:

- An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the Case Report Form (CRF).
- An overdose without associated symptoms is only reported in the subject´s medical records.

6 INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor
Isofol Medical AB
Biotech Center
Arvid Wallgrens Backe 20, 5th level
SE-413 46 Gothenburg, Sweden

Sponsor's Chief Medical Officer
Karin Ganlöv, MD
Phone: +46 (0)70 243 37 50
E-mail: karin.ganlov@isofolmedical.com

Sponsor's Chief Scientific Officer
Anders Vedin, MD, PhD
Phone: +46 (0)708 761 570
E-mail: anders.vedin@isofolmedical.com

Sponsor's Chief Executive Officer
Anders Rabbe
Phone: +46 (0)707 646 500
E-mail: anders.rabbe@isosfolmedical.com

Clinical Conduct and Management
CTC Clinical Trial Consultants AB
Uppsala University Hospital,
Entrance. 85, 2nd level
SE-751 85 Uppsala, Sweden

Principal Investigator
Cornelia Lif-Tiberg, MD
Phone: +46 (0)73 978 94 45
E-mail: cornelia-lif-tiberg@ctc-ab.se

Co-Investigator
Fredrik Huss, MD, PhD, Associate Professor
Phone: +46 (0)70 794 15 74
E-mail: fredrik.huss@clinicaltrialconsultants.se

Biostatistics
Devicia
Energigatan 10 B
SE-434 37 Kungsbacka, Sweden

Biostatistician
Fredrik Hansson, MSc
Phone: +46 (0)721 93 55 01
E-mail: fredrik.hansson@devicia.se

Pharmacokinetics
PKxpert AB
Tomtebogatan 32
SE-113 38 Stockholm, Sweden

Pharmacokineticists
Annsofi Nihlén, PhD
Achim Orzechowski, PhD
Phone: +46 (0)765 698 175
E-mail: annsofi.nihlen@pkxpert.com

iCARDIAC
150 Allens Creek Rd
Rochester
NY 146 18, USA
Phone. +1 585295-7610

iCardiac Technologies
Börje Darpö, MD PhD
Chief Scientific Officer
Phone: +46 (0)763 902 130
E-mail: borje.darpo@icardiac.com

Laboratory (Bioanalysis)
Charles River Laboratories
Edinburgh
Elpinstone Research Centre
Tranent, East Lothian,
EH33 2NE
Edinburgh
United Kingdom

Contact Person at Charles Rivers Labs.
Colin Pattison, BSc
Phone: +44(0) 1875 618066
Fax: +44(0) 1875 614555
E-mail: colin.pattison@crl.com

Laboratory (Safety)
Clinical Microbiology
Dag Hammarskjölds väg 17
SE-752 37 Uppsala, Sweden

Laboratory (Safety)
Clinical Chemistry
Uppsala University Hospital, Entrance 85
SE-751 85 Uppsala, Sweden

Signatures required are provided in Appendix 18.1.

7 INTRODUCTION

Isofol Medical AB is developing novel therapeutics for unmet needs in oncology. The lead clinical candidate Modufolin® for Injection, 100 mg ([6R]-5,10-Methylene Tetrahydrofolate [MTHF-HS]) is a novel folate-based compound developed to improve the efficiency of a range of antimetabolite treatments used within oncology. Three different therapeutic areas of special interest have been identified and are included in the development program of Modufolin® for Injection, 100 mg: (i) rescue therapy for High Dose Methotrexate (HDMTX) treatments, (ii) modulation of 5-Fluorouracil (5-FU) activity via Thymidylate Synthase (TS), and (iii) Modulation of antifolate activity.

7.1 Project background

Folate-based pharmaceuticals have been used for years as rescue agents for reduction of toxic plasma levels of Methotrexate (MTX) during HDMTX treatment[1,2] and also in combination with the antimetabolite 5-FU for treatment of gastrointestinal cancers as they significantly improve the efficacy of 5-FU.[3] The most commonly administered folate-based drug is LV.

Isofol Medical AB has in close collaboration with its strategic R&D partner Merck & Cie, a leading manufacturer of reduced folates, successfully manufactured Modufolin® for Injection, 100 mg, an endogenous folate-based compound. Modufolin® for Injection, 100 mg is developed to increase the efficacy and decrease the side effects of chemotherapeutic agents such as the antifolate drug 5-FU and the multi-targeted antifolate Pemetrexed which are used in the treatment of solid tumors.

Folates are also standard of care as rescue treatment in HDMTX treatment regimens for osteosarcoma. The variability of the pharmacokinetics (PK) of folates is considerable and may impact the safety and/or efficacy of HDMTX treatments. The timing of the rescue greatly influences overall tumor-free survival by improving the index of normal vs. malignant cell rescue while still maintaining fairly tolerable toxicity profile.[2,4]

Isofol Medical AB's hypothesis is that the administration of Modufolin® for Injection, 100 mg will result in greater bioavailability and higher intracellular concentrations of the active metabolite compared to LV administration, as several metabolic steps are bypassed by Modufolin® for Injection, 100 mg administration.[5] It is also suggested that Modufolin® for Injection, 100 mg may reduce potential inter- and intrapatient variability and as a consequence allow for safer and more predictable treatments.

The primary objective of the current clinical study is to evaluate potential effects on electrocardiogram (ECG) parameters following single doses administration of Modufolin® for Injection, 100 mg in healthy volunteers. To allow exclusion of a small effect on the QT interval, serial ECG monitoring is implemented in the study, a highly precise ECG methodology will be used and data will be analysed using exposure response analysis. Three dose levels of Modufolin® for Injection, 100 mg will be studied, including two supratherapeutic doses, which will obviate the need for a positive control in the study.[6]

This methodology promises much higher precision in gauging the cardiac safety of compounds in Phase I human studies.

7.2 Investigational Medicinal Product

7.2.1 Product characteristics

The drug product, Modufolin® for Injection, 100 mg, is a lyophilized powder (100 mg Modufolin® per vial, calculated as free acid) for administration after reconstitution with 10 mL of water for intravenous (*i.v.*) injection. Modufolin® for Injection, 100 mg is not authorised for sale in any country.

7.2.2 Mechanism of action

The active drug substance in Modufolin® for Injection, 100 mg, ([6R]-5,10-Methylene Tetrahydrofolic acid hemisulfate [MTHF-HS]), is a stable formulation of the naturally occurring diastereoisomer of MTHF, the endogenous co-substrate of thymidylate synthase (TS) which is involved in the enzymatic pathway of nucleotide substrates necessary for DNA synthesis.[5]

In contrast to other available folates such as LV and Levoeucovorin (LLV), Modufolin® for Injection, 100 mg does not require enzymatic metabolic activation. MTHF is the key metabolite of LV and may be directly involved in the formation of 5-fluoro-2'-deoxyuridine-5'-monophosphate-thymidylate synthase (FdUMP-TS) ternary complex. The hypothesis is that patients which are not capable of metabolizing LV could benefit from treatment with Modufolin® for Injection, 100 mg.

7.2.3 Non-clinical safety

Modufolin® for Injection, 100 mg has been evaluated in preclinical Good Laboratory Practice (GLP) rat and dog studies. In summary, twice daily *i.v.* administration of Modufolin® for Injection, 100 mg for up to 28 days is well tolerated in rats at doses up to 100 mg/kg/day and in beagle dogs at doses up to 50 mg/kg/day. The active pharmaceutical ingredient of Modufolin® for Injection, 100 mg has also been evaluated in various non-GLP mouse models in combination with different anti-metabolites such as Pemetrexed and 5-FU. No safety issues were raised in the non-clinical safety studies.

7.2.4 Clinical experience

Completed clinical studies

ISO-CC-002 was the first phase I/II non-Investigational New Drug (IND) clinical study with Modufolin® for Injection, 100 mg in patients diagnosed with operable colorectal cancer which was performed at Sahlgrenska University Hospital in Gothenburg, Sweden. In this clinical study, 60 or 200 mg/m^2 of Modufolin® or LLV was administered as single doses prior to surgery with the objective of comparing PK/pharmacodynamic profiles in plasma, tumors, and tumor adjacent mucosa. Thirty-two (32) patients were included in this study, of whom 16 were exposed to Modufolin® for Injection, 100 mg. None of the 24 AEs reported in the study were assessed as related to study treatment.

ISO-MC-091 was an extended feasibility phase I/II non-IND clinical study of Modufolin® for Injection, 100 mg and Pemetrexed single agent, given as neoadjuvant treatment in patients with resectable rectal cancer.
Twenty-four (24) patients with operable rectal cancer received Modufolin® for Injection,

100 mg at doses of 10, 50, 100, or 500 mg/m^2 qwk for 10 weeks in combination with Pemetrexed as part of a neoadjuvant setting for evaluation of the feasibility and activity of the treatment.[7,8] The safety data revealed that 20 of 24 patients had at least one AE that was judged to be related to the study drugs. Eleven SAEs were reported by 5 patients. One SAE (fever) was assessed as an SAR related to the study drug Pemetrexed.

On-going clinical studies

ISO-MTX-003 is an open-label, multicenter, phase I/II non-IND clinical study designed to identify the Modufolin® for Injection, 100 mg dose with the most favorable safety prospect and confirmed ability to mitigate HDMTX induced toxicity during treatment of osteosarcoma patients. The study was designed to (i) investigate the safety of Modufolin® rescue therapy, (ii) identify a recommended dose for further study, (iii) and evaluate the feasibility of Modufolin® as rescue therapy for HDMTX treatment.

ISO-CC-005 is an open-label, multi-site, phase I/II non-IND clinical dose cohort study which will investigate the tolerability of Modufolin® for Injection, 100 mg in combination with a fixed dose of 5-FU alone or together with a fixed dose of Oxaliplatin or Irinotecan in patients with stage IV colorectal cancer. Toxicity will be evaluated for 4 dose levels (30, 60, 120 and 240 mg/m^2) in 5 treatment arms, see treatment regimen below:

<u>Modufolin® for Injection, 100 mg:</u>

Arm 1: 30, 60, 120 and 240 mg/m^2.

Arm 2-3: 30 and 60 mg/m^2 *i.v.* bolus injection, twice q2w (*i.e.*, on Day 1 and Day 2 of each chemotherapy cycle)

Arm 4: 60, 120 and 240 mg/m^2 *i.v.* bolus injection, Day 1 only of each chemotherapy cycle). Enrolment in next dose cohort if the lower dose shows favorable tolerability.

Arm 5: Selected Phase 2 dose from Arm 4.

<u>Chemotherapies (4 cycles over 8 weeks):</u>

Arm 1: 5-FU (500 mg/m^2) alone

Arm 2: 5-FU (500 mg/m^2) and Oxaliplatin (85 mg/m^2)

Arm 3: 5-FU (500 mg/m^2) and Irinotecan (180 mg/m^2)

Arm 4: 5-FU (400 mg/m^2+2400 mg/m^2) and Oxaliplatin (85 mg/m^2)

Arm 5: 5-FU (400 mg/m^2+2400 mg/m^2) and Oxaliplatin (85 mg/m^2) + bevacizumab (5 mg/kg)

For further details on completed and on-going clinical studies, refer to the Investigator's Brochure (IB).

A summary of safety and PK data from Isofol Medical AB's previous clinical studies on Modufolin® for Injection, 100 mg in cancer patients is provided in Appendix 18.3.

7.3 Risk/benefit assessment

Modufolin® for Injection, 100 mg is a stable formulation of the naturally occurring diastereoisomer of MTHF, the endogenous co-substrate of thymidylate synthase which is involved in the enzymatic pathway of nucleotide substrates necessary for DNA synthesis.

Based on available data to date, the occurrence of any adverse reactions due to Modufolin® for Injection, 100 mg alone is not expected. Modufolin® for Injection, 100 mg has been safely administered to 72 patients at doses between 7.5 and 500 mg/m^2 with no serious side-effects specifically related to Modufolin® for Injection, 100 mg alone. There is no evidence that Modufolin® for Injection, 100 mg may be of safety concern for patients, either after administration of repeated doses or after administration of single high doses. No SAEs assessed as possibly related to Modufolin® for Injection, 100 mg alone have been reported. Consequently, all SAEs assessed as having a possible relationship to Modufolin® for Injection, 100 mg should be reported as Suspected Unexpected Serious Adverse Reactions (SUSARs).

A review of the safety data from the non-IND clinical studies ISO-MC-091, ISO-CC-002, ISO-MTX-003 and ISO-CC-005 identified fatigue and nausea as the most common AEs assessed as possibly related to study treatment. However, these events were no more frequent than could be expected for the same cytotoxic agents in the presence of other folates.

7.3.1 Summary of risk management

As the healthy volunteers in this study will have no medical benefit from participation, their safety and well-being is of utmost importance.

The subjects will however remain at the research clinic for 24 hrs after the administration of the IMP/placbeo and will be closely monitored by medical staff. A final safety check will take place at a Follow-up visit scheduled 5 to 10 days after dose administration.

The Principal Investigator at the research clinic will ascertain that adequate facilities and procedures are available to handle emergency situations should they occur during the study. The medical staffs at CTC Clinical Trial Consultants AB (hereafter referred to as CTC) have extended experience from early Phase I studies and there are adequate procedures in place to handle unexpected and expected adverse reactions in the study subjects.

There can be risks related to medical devices used in the study *e.g.*, indwelling venous catheters, but they are devices used in routine medical care and the risk is considered low and ethically justifiable. Study specific evaluations and sampling procedures like blood-pressure measurements using a blood pressure cuff and frequent blood-sampling, can cause transient discomfort but the risk is deemed to be low and ethically justifiable.

The use and frequency of the above mentioned risk factors have been kept at a low level that still will meet the scientific and medical goals for the study and at the same time not expose the healthy volunteers participating in the study for risks that would not be ethically justifiable.

8 STUDY OBJECTIVES AND ENDPOINTS

8.1 Primary objective

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

8.1.1 Primary endpoints

The primary evaluation parameter is:

- Change-from-baseline QTcF (ΔQTcF)

8.2 Secondary objectives

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

8.2.1 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)

  The PK parameters to be evaluated are listed in Section 17.7.5.

9 INVESTIGATIONAL PLAN

9.1 Overall study design and schedule of events

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 (iSRC; see Section 9.1.2). There will be an interval of at least 7 days between each dose level to allow time for safety data to be analyzed and evaluated by the iSRC. 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 (see Sections 10.4 and 10.5) 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.

The timing of all study assessments is shown in the Schedule of events provided in Table 1.

Further details about the randomization procedures are presented in Section 10.8, procedures for maintaining the blind in Section 10.9 and treatment administration in Section 11.6.

Table 1 Schedule of events (2 pages)

Visit number	**1**	**2**																	**3**
	Sreening	**In-clinic period**																	**FU**
Day	**-28 to -1**	**-1**	**1**															**2**	**6 to 11**
Time points	**-**		**-2 h**	**-45 min**	**-30 min**	**-15 min**	**0**	**5 min**	**15 min**	**30 min**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**	**8 h**	**12 h**	**24 h**	**-**
Time windows (min)	-	-	-	±2	±2	±2		±2	±2	±2	±5	±5	±5	±5	±5	±5	±5	±5	±60	-
Informed consent	x																			
Demographics	x																			
Medical/surgical history	x																			
Inclusion/exclusion criteria	x	x																		
Physical examination	x																			x
Weight	x	x																		
Height	x																			
Vital signs (BP and pulse)	x					x							x		x		x		x	x
Hematology, clinical chemistry	x		x[1]																x	x
Urine analysis (dip stick)	x	x																		
HIV, hepatitis B and C	x																			
Drugs of abuse	x	x																		
Alcohol screen	x	x																		
12-lead safety ECG[2]	x		x[2]										x		x		x		x	x

Visit number	**1**	**2**																	**3**
	Sreening	**In-clinic period**																	**FU**
Day	**-28 to -1**	**-1**	**1**															**2**	**6 to 11**
Time points	**-**		**-2 h**	**-45 min**	**-30 min**	**-15 min**	**0**	**5 min**	**15 min**	**30 min**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**	**8 h**	**12 h**	**24 h**	**-**
Time windows (min)	-	-	-	±2	±2	±2		±2	±2	±2	±5	±5	±5	±5	±5	±5	±5	±5	±60	-
Holter ECG (Cardiodynamic)[3]			x[3]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Randomisation			x																	
IMP/placebo administration							x[4]													
PK blood sampling[5]						x		x	x	x	x	x	x	x	x	x	x	x	x	
AE reporting		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Prior and concomitant medication	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

FU=Follow-up Visit; min=minutes; h=hour; BP=Blood Pressure; ECG=Electrocardiogram; IMP=Investigational Medicinal Product; PK=Pharmacokinetic; AE=Adverse Event

[1] Within 1 hr prior to dose administration.

[2] Safety ECGs will be printed on-site and interpreted by the Investigator. Pre-dose safety ECGs in triplicate will be extracted within 1 hr prior to dose administration.

[3] Continuous12-lead ECG recording (Holter ECG) will start 2 hrs prior to dose administration on Day 1. Holter ECGs will be extracted at 3 time points before dosing (-45, -30 and -15 min). Subjects will be supinely resting for at least 10 min prior to time points for ECG recordings. When time points for ECG recordings coincide with other assessments, procedures should be carried out in the following order: (1) Holter ECG, (2) safety ECG, (3) vital sign, (4) PK blood sampling and (5) safety blood sampling.

[4] Subjects should be fasting overnight (8 hrs) before IMP/placebo administration and until 4 hrs post-dose.

[5] Time 0 = end of IMP/placebo infusion. For further information on the PK blood sampling procedures, refer to Section 13.1.

9.1.1 Dose levels

The proposed dose levels are 200, 350 and 500 mg/m^2 Modufolin® for Injection, 100 mg.

Each dose will be administered as a single bolus *i.v.* injection.

Depending on results obtained during the study, the number of dose levels, the actual dose, frequency of dosing and planned sampling times might have to be adjusted. These adjustments will be documented in non-substantial amendments to the CSP.

The study maximum dose (SMD) will not exceed 500 mg/m^2 administered as single bolus *i.v.* injection.

9.1.2 Internal Safety Review Committee

The iSCR will consist of:

- The Principal Investigator or delegate
- A Sponsor Medical representative or delegate

Additional internal or external experts may be consulted by the iSCR if needed.

After each completed dose level, the iSCR will evaluate all available safety data. The decision may be to give the next intended dose, a greater or smaller dose increment than the intended dose, a repeated dose, a lower dose or to stop dosing.

A dose escalation beyond 500 mg/m^2 will not be mandated by the iSRC unless authorized by the regulatory authority in a substantial amendment to this CSP.

The randomization code may be broken by the iSRC during the assessment process (partial un-blinding) but only for subjects with severe AEs (≥grade 3) to enable their decision on continued dosing of further cohorts or to stop the dose escalation. The medical staff and the subjects will remain blinded for the treatments (active drug or placebo) to be administered in the subsequent dose groups/cohorts in order to minimize bias.

The decision of the iSRC on the next dose level will be taken in consensus between the iSRC members and documented appropriately in a safety evaluation form provided by CTC.

9.1.3 Stopping criteria for dose escalation

The Principal Investigator and the iSRC will follow the recommendations and grading system of Common Terminology Criteria for Adverse Events (CTCAE) v4.03[9] (see Section 12.5.2.1) but also take into account the recommendations published by M Sibille *et al.* 2010[10] which is an adaptation to FIH studies of the grading systems previously proposed by National Cancer Institute (NCI),[9] World Health Organization (WHO),[11] National Institutes of Health (NIH)[12] and Food & Drug Administration (FDA).[13] The grade, the frequency of AEs and the blinding will be taken into account.

At the individual level, basically, a Grade 2 AE is at minimum a safety alert leading to caution and closer assessment of safety in other subjects. An escalation to Grade 3 applies if rapid worsening, concomitant findings, clinical symptoms and signs occur. A Grade 3 AE always supports stopping the suffering subject from continuing participation in the study.

At the dose group level, the algorithm supporting the stopping rules to be strictly followed is presented in Table 2. An un-blinding, limited to suffering subjects, will be applied. A 33%

frequency of AEs or findings (Grade 3 or 2, as explained above) of the same types will trigger a stop of dose escalation. However, depending on the type and potential risk of the AE, any modulation can be decided, for example, for safety concerns, a lower frequency level. Following the iSRC risk evaluation of AEs (Grade ≥3) it may be decided to, for the next dose level to either repeat the current dose level, lower the dose or stop dosing.

Table 2 Dose escalation and stopping rules

If 0/11[1] subjects have Dose Limiting Toxicity (DLT)	Escalate to the next higher dose level	No un-blinding
If 1/8 subjects on active drug has DLT	Repeat the current dose level or escalate to the next higher dose level	Partial un-blinding (of subjects with DLT)
If ≥2/8 subjects on active drug have DLT	Stop further dosing	Partial un-blinding (of subjects with DLT)

[1] Control subjects included

9.2 Rationale for study design and dose groups

A Single Ascending Dose (SAD) design was chosen for the evaluation of the cardiac safety of Modufolin® for Injection, 100 mg. The adaptive study design allows for flexible dose escalation and involves careful monitoring of the subject's well-being. The study will also provide important PK data to support the design of further studies, both in healthy volunteers and in patients. The time points for PK blood sampling were selected based on data obtained from previous clinical studies (see summary of PK data in Appendix 18.3). After dose administration, the time points for extraction of ECG data are paired with PK blood sampling.

A placebo control will be used to establish the frequency and magnitude of changes in endpoints that may occur in the absence of active treatment.

Randomization will be used to minimize bias in the assignment of subjects to dose groups and to increase the likelihood that known and unknown subject attributes (*e.g.*, demographic and baseline characteristics) are evenly balanced across treatment groups.

Blinded treatment will be used to reduce potential bias during data collection and evaluation of endpoints.

The primary objective of the current clinical study is to evaluate potential effects on ECG parameters following single doses administration of Modufolin® for Injection, 100 mg in healthy volunteers. To allow exclusion of a small effect on the QT interval, serial ECG monitoring is implemented in the study, a highly precise ECG methodology will be used and data will be analysed using exposure response analysis.

Three dose levels of Modufolin® for Injection, 100 mg will be studied (200, 350 and 500 mg/m^2), including two supratherapeutic doses, which will obviate the need for a positive control in the study.[6] Based on current data the expected clinical dosing regimen in future clinical trials in metastatic colorectal cancer patients (lead indication) is 120 mg/m^2 Modufolin® for Injection, 100 mg. Doses of Modufolin® up to 500 mg/m^2/day have been administered to patients in a previous non-IND clinical study with no safety concerns (ISO-MC-091).

10 STUDY POPULATION

10.1 Recruitment

The subjects will be recruited from a database of healthy volunteers at CTC and from advertising in media including social media.

10.2 Screening and enrolment log

A screening number will be allocated to each subject undergoing screening. The Investigator will keep records of all subjects screened and included. The reason for screen failure should be stated for all subjects screened but not included. The reason for withdrawal should be stated for all subjects included but not completed.

If a subject cannot receive the planned dose of IMP/placebo within 28 days after screening (*i.e.*, the time interval between signing informed consent until dose administration, the subject should be rescreened before proceeding in the study).

10.3 Number of subjects

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

To account for potential drop-outs and/or additional cohorts depending on PK data and recommendations from the iSRC, additional male subjects might be included in the study (see Section 9.1.2).

For replacements of subjects discontinuing the study, see Section 10.7.1.

10.4 Inclusion criteria

For inclusion in the study, subjects must fulfil the following criteria:

1. Willing and able to provide a written informed consent for participation in the study.
2. Healthy male subject aged 18-60 years inclusive.
3. Body Mass Index (BMI) $\geq$ 18 and $\leq$ 30 kg/m^2 and weight at least 50 kg and no more than 100 kg at screening and body surface area $\leq$ 2 m^2.
4. Clinically normal medical history, physical findings, vital signs, ECG and laboratory values at the time of screening, as judged by the Investigator.
5. Willing to use condom and highly effective contraceptive methods with a failure rate of < 1% to prevent pregnancy[1] and drug exposure to a partner and refrain from donating sperm from the date of dosing until 3 months after dosing of the IMP/placebo.

[1] Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomized partner/vasectomy, sexual abstinence.

10.5 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled:

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
2. Any clinically significant illness, medical/surgical procedure or trauma within four weeks of the first administration of IMP/placebo.
3. Any planned major surgery within the duration of the study.
4. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).
5. After 10 minutes (min) supine rest at the time of screening, any vital signs values outside the following ranges:
    - Systolic BP > 150 mm Hg
    - Diastolic BP > 90 mm Hg
    - Pulse < 40 or > 85 beats per min
6. Prolonged QTcF (>450 ms), cardiac arrhythmias or any clinically significant abnormalities in the resting ECG at the time of screening, as judged by the Investigator.
7. History of severe allergy/hypersensitivity or on-going allergy/hypersensitivity, as judged by the Investigator, or history of hypersensitivity to drugs with a similar chemical structure or class to Modufolin® (*i.e.*, folate derivatives).
8. Regular use of any prescribed or non-prescribed medication including antacids, analgesics, herbal remedies, vitamins and minerals within two weeks prior to the administration of IMP/placebo, except occasional intake of paracetamol (maximum 2000 mg/day; and not exceeding 3 000 mg/week), at the discretion of the Investigator and nasal decongestants without cortisone or antihistamine for a maximum of 10 days, at the discretion of the Investigator.
9. Regular use of any prescribed or non-prescribed medication which could influence folate and vitamin B12 status within 30 days prior to the administration of IMP/placebo.
10. Administration of another new chemical entity (defined as a compound which has not been approved for marketing) or has participated in any other clinical study that included drug treatment with less than three months between administration of last dose and first dose of IMP/placebo in this study. Subjects consented and screened but not dosed in previous phase I studies are not excluded.
11. Current smokers or users of nicotine products. Irregular use of nicotine (*e.g.*, smoking, snuffing, chewing tobacco) less than three times per week is allowed before screening visit.
12. Positive screen for drugs of abuse or alcohol at screening or on admission to the unit prior to administration of the IMP/placebo.
13. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse.

14. Intake of xanthine and/or taurine containing energy drinks within two days prior to screening.
15. Plasma donation within one month of screening or blood donation (or corresponding blood loss) during the three months prior to dosing.
16. Investigator considers the subject unlikely to comply with study procedures, restrictions and requirements.

10.6 Restrictions during the study

10.6.1 General restrictions

- Contraception Requirements: The male volunteers are expected to use condom and other effective contraceptive methods with a failure rate of < 1% to prevent pregnancy[2] and drug exposure of a partner and refrain from donating sperm from the date of dosing until 3 months after last dosing of the IMP/placebo.
- Meals and Dietary Restrictions: Study subjects will receive standardized meals during study days at the clinic. No breakfast will be served before dose administration. Lunch will be served 4 hrs post-dose, a snack at 6 hrs post-dose and dinner 9 hrs post-dose. Water is allowed ad libitum at the clinic.
- Fasting: The subjects should be fasting overnight (8 hrs) before IMP/placebo administration until 4 hrs post-dose.
- Alcohol: Consumption of alcohol is not allowed within 48 hrs prior to all clinic visits until after the Follow-up Visit.
- Coffee: Consumption of up to five cups of coffee per day will be allowed during the study.
- Xanthine or taurine containing products/beverages: Energy drinks (*e.g.*, Redbull) are not allowed during the study.
- Nicotine: Smoking or use of nicotine-containing products is not allowed during the study.
- Grapefruit and grapefruit containing products: Consumption of grapefruit and/or grapefruit containing products is not allowed during the study.
- Exercise: The subjects must refrain from strenuous exercise (defined as greater than 70% of the maximal pulse rate for one hour or more) during the study.
- Blood donation: The subjects must not donate blood or plasma during the study until three months after the final medical examination at the Follow-up Visit.

[2] Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner, sexual abstinence.

10.6.2 Prior and concomitant therapy

In general, no concomitant medications or therapies, including herbal remedies, vitamin supplements and over-the-counter (OTC) products, will be allowed during participation in the study.

In particular, intake of medications which could influence folate and vitamin B12 status is prohibited during the study.

However, the following will be allowed:

- Paracetamol in doses up to 2000 mg/day for a maximum of 3 consecutive days. If this amount of paracetamol is not sufficient for treatment of the subject, withdrawal should be considered.
- Nasal decongestants without cortisone or antihistamine for a maximum of 10 days.

Other medications considered necessary for the subject's safety and well-being, may be given at the discretion of the Investigator during the residential period. Following consultation with the Sponsor, the Investigator will determine whether or not the subject should continue in the study.

10.7 Criteria for subject withdrawal

10.7.1 General withdrawal criteria

Subjects are free to discontinue their participation in the study at any time and for whatever reason without affecting their right to an appropriate follow-up investigation or their future care. If possible, the reason for withdrawal of consent should be documented.

Subjects may be discontinued from the study at any time at the discretion of the Investigator for any of the following reasons:

- Severe non-compliance to study protocol procedures, as judged by the Investigator and/or Sponsor.
- Subject is lost to follow-up.
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor.
- Withdrawal of informed consent to the use of biological samples.

10.7.1.1 QTc withdrawal criteria

A subject meeting the criteria below will be withdrawn from the study. The same QT correction formula will be used to determine discontinuation throughout the study.

- QTcF > 500 millisecond (msec)
- Change from baseline: QTc >60 msec to QTc > 480 msec

Withdrawal decisions will be based on an average QTc value of triplicate ECGs. If an ECG demonstrated a prolonged QT interval, two more ECGs will be obtained over a brief period and the averaged QTc values of the three ECGs used to determine whether the subject should be discontinued from the study.

10.7.1.2 Liver chemistry withdrawal criteria

Liver chemistry threshold stopping criteria have been designed to assure subject safety and to evaluate liver event etiology.[14]

Study treatment will be stopped if any of the following liver chemistry stopping criteria is met:

- Alanine transaminase (ALT) 3 x Upper Limit of Normal (ULN) and total bilirubin ≥ 2xULN (>35% direct bilirubin); **or** ALT 3xULN and INR > 1.5)

NOTE: serum bilirubin fractionation should be performed if testing is available. If fractionation is unavailable, urinary bilirubin is to be measured via dipstick (a measurement of direct bilirubin, which would suggest liver injury).

- ALT 5xULN.
- ALT 3xULN if associated with symptoms (new or worsening) believed to be related to hepatitis (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness or jaundice) or believed to be related to hypersensitivity (such as fever, rash or eosinophilia).

Subjects with ALT 3xULN **and** < 5xULN **and** bilirubin < 2xULN, who do not exhibit hepatitis symptoms or rash, will be allowed to continue study treatment as long as they are monitored weekly for four weeks.

10.7.2 Procedures for discontinuation of a subject from the study

A subject who prematurely discontinues participation in the study will always be asked about the reason(s) for discontinuation and the presence of any AEs. If possible, they will be seen by the Investigator and assessed according to the procedures scheduled for the follow-up visit. Any ongoing AEs will be followed as described in Section 12.5.6.

10.7.1 Subject replacement

Subjects who are prematurely withdrawn from the study for any reason except the occurrence of Treatment-emergent AEs (TEAEs) assessed as possibly or probably related to study treatment may be replaced during the course of the study.

10.8 Randomisation

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.

10.9 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.

10.10 Emergency decoding of blinded treatment during the study

The treatment code may only be broken by the study medical staff in case of emergency when knowledge of the treatment received is necessary for the proper medical management of the subject. The code breaking procedure should be carefully documented in the CRF.

The randomization code may be broken by the iSRC during the assessment process (partial un-blinding) but only for subjects with severe AEs (≥grade 3) to enable their decision on continued dosing of further cohorts or to stop the dose escalation. The medical staff and the subjects will still be blinded for the treatments (active drug or placebo) to be administered in the subsequent cohorts in order to minimize bias (see Section 9.2).

11 TREATMENTS

11.1 Identity of Investigational Medicinal Product

Test product (Modufolin® for Injection, 100 mg)

Pharmaceutical formulation

Modufolin® for Injection, 100 mg is formulated as a lyophylized white to light yellowish or light beige powder containing 100 mg Modufolin® per vial (calculated as free acid, for composition see Table 3). After reconstitution with 10 mL sterile water, reconstituted Modufolin® Injection, 100 mg is a clear yellowish fluid ready for intravenous (*i.v.*) use as bolus injection (final concentration of 10 mg/mL). The resulting solution for injection is isotonic and has a pH of 8.5.

Table 3 Composition of pharmaceutical formulation

Component	Amount/vial	Function
Sodium hydroxide	0.3 g	For pH adjustment
Trisodium citrate dihydrate	0.2 g	Buffer (to guarantee a defined stable pH after reconstitution in water)
Modufolin® drug substance	0.1 g	Active pharmaceutical ingredient

11.2 Non- Investigational Medicinal Product (Placebo)

The placebo in this study will be 0.9% NaCl sterile solution.

11.3 Packaging and labelling of Investigational Medicinal Product

The drug product, Modufolin® for Injection, 100 mg is manufactured, released and tested in compliance with Good Manufacturing Practice (GMP) by the following companies as summarised in Table 4.

Table 4 Manufacturers of IMP

Name	Address	Function
Cobra Biopharma Matfors AB (previously Unitech Biopharma AB)	Storjordenvägen 2 SE-864 31 Matfors, Sweden	Manufacture Batch release
Merck & Cie	Im Laternenacker 5 8200 Schaffhausen Switzerland	Release testing
Unimedic AB	Storjordenvägen 2 SE-864 31 Matfors, Sweden	Release testing (particulate matter)
Pharmacontrol MQL AB	Virdings Allé 2 SE-754 50 Uppsala Sweden	Release testing (sterility)

Primary packaging (Modufolin® for Injection, 100 mg):

Lyophilized powder: 10 mL type 1 glass vials with lyophilization stopper (20 mm, siliconized, Helvoet FM157.

Secondary packaging (Modufolin® for Injection, 100 mg):

Each box will contain 10 x 10 mL glass vials.

Labels will comply with applicable current Good Manufacturing Practice (GMP) requirements.[15]

The following labels will be used for Study ISO-FF-001:

Primary packaging- Label to be attached to vial

For clinical trial	Study No. ISO-FF-001
Caution: New Drug--Limited by Federal law to investigational use	
Modufolin® for Injection, 100 mg	
Batch No: XXXX	
Store at 2 - 8°C (refrigerator)	
Principal investigator: Cornelia Lif-Tiberg, MD	
Sponsor: Isofol Medical AB, Phone: +46 (0)702 43 37 50	

Secondary packaging- Label to be attached to each box containing 10 vials

For clinical trial	Study No. ISO-FF-001
Principal investigator: Cornelia Lif-Tiberg, MD	
Caution: New Drug--Limited by Federal law to investigational use	
Modufolin® for Injection, 100 mg	
10x100mg	
To be dissolved with Water for Injection according to separate preparation description	
Batch No: XXXX	Expiry date: YYYY-MM-DD
Store at 2 - 8°C (refrigerator)	
Sponsor: Isofol Medical AB, Arvid Wallgrens Backe 20 Gothenburg, Sweden 413 46 Phone: +46 (0)702 43 37 50	

11.4 Conditions for storage

Modufolin® for Injection, 100 mg vials are stored at refrigerated storage conditions 2-8 °C.

Stability data demonstrate that the drug product is stable when stored at 2-8 °C for up to 48 months. The current shelf life is 42 months.

Stability data on the solution for injection (the dissolved powder) show that the reconstituted IMP is stable for up to 2 hrs at room temperature after reconstitution.

11.5 Dispensing and accountability

CTC AB and the Investigator will maintain a *Drug Dispensing Log* detailing the dates and quantities of study medication received, dispensed to and used by each subject and study medication returned or destroyed at the end of the study. Any discrepancies between dispensed and returned IMP must be explained and documented. Products deliberately and/or accidentally destroyed by the Investigator/ pharmacy or the subject must be accounted for.

11.6 Treatment administration

The pre-defined dose levels are 200, 350 and 500 mg/m^2 of Modufolin® for Injection, 100 mg.

There will be 3 cohorts, 11 subjects in each cohort. Subjects will be assigned to a cohort in a consecutive manner. In each cohort, 8 subjects will receive Modufolin® for Injection, 100 mg and 3 subjects will receive placebo. The IMP/placebo will be administered as a single dose on Study Day 1. The IMP/placebo will be administered in the morning and the time will be recorded in the CRF. The study subjects should be fasting overnight (8 hrs) before IMP/placebo administration until 4 hrs post-dose. There will be an interval of at least 7 days between each cohort to allow time for the iSRC to evaluate safety data before proceeding to the next dose level.

Route of administration

Intravenous bolus injection (injection time 3 min).

Preparation and handling of IMP solution (Modufolin® for Injection, 100 mg):

1. The preparation of the solution for injection will be done by trained personnel, for example a site pharmacist or a registered nurse. There will be 2 un-blinded persons working together, one person will handle the IMP and perform the reconstitution of the drug product while the other person will supervise the process. The process will also be recorded on video tape.
2. The reconstitution of each vial of IMP powder in 10 mL of water for injection should be done using aseptic technique.
3. Gently swirl each vial until the powder is completely dissolved. The resulting solution is clear and ranges in color from colorless to yellow.
4. The solution should be inspected visually for particulate matter and discoloration prior to administration. If particles are present, do not administer.
5. The reconstituted solution can be kept at room temperature.
6. Administration should preferably be done directly after reconstitution and must be done within 2 hrs after reconstitution.
7. If administration cannot take place for whatever reason, the vial should be handled in accordance with the study-specific instructions on IMP disposal.

11.7 Continuation of treatment with Investigational Medicinal Product

This is a Phase I study in healthy volunteers who will have no medical benefit from the treatment and thus there will be no treatment with Modufolin® for Injection, 100 mg after end of study participation.

11.8 Treatment compliance

All study products will be administered at the research clinic under medical supervision to ensure compliance.

11.9 Return and destruction of Investigational Medicinal Product

Any unused IMP will be returned to the Sponsor or the Hospital Pharmacy for destruction. Empty containers will be destroyed at the study site. The Monitor will perform final IMP accountability reconciliation at the study end to verify that all unused IMP is adequately destroyed/returned and documented.

12 STUDY ASSESSMENTS

The study assessments are described in the sections below and the timing of these assessments are detailed in the Schedule of events in in Section 9.1 (Table 1).

12.1 Recording of data

The Principal Investigator will provide the Sponsor with all data produced during the study from the scheduled study assessments. He/she ensures the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the CRF and in all required reports.

When time points for ECG recordings coincide with other assessments, procedures should be carried out in the following order:

1. Holter ECG
2. Safety ECG
3. Vital sign
4. PK blood sampling
5. Safety blood sampling.

The actual PK blood sampling time should always be recorded in the CRF and will be used in the calculation of the PK parameters. Pre-dose assessments may be performed up to 60 min prior to dosing if not specified in the Schedule of events in Section 9.1 (Table 1).

12.2 Demographics and other baseline characteristics

12.2.1 Informed consent

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 14.3.

12.2.2 Demographic information

The following demographic data will be recorded: gender, age, and ethnic origin.

12.2.3 Weight and height

Weight and height will be measured without shoes. BMI will be calculated from the height and weight recorded and rounded to the nearest whole number.

12.2.4 Medical/surgical history

Medical/surgical history will be obtained by interview in order to verify that the eligibility criteria are met.

12.2.5 HIV and Hepatitis B/C

Subjects will be tested for HIV and Hepatitis B/C prior to inclusion into the study in order to protect personnel handling the blood samples.

12.2.6 Urine drug screen

Urine will be screened for drugs of abuse at screening and prior to dose administration using the Alere™ Drug Screen Test Panel. Additional random tests can be performed during the study.

The following substances will be included in the screen panel:

Amphetamine	Methadone
Barbiturates	Methamphetamine
Benzodiazepines	Methylenedioxymethamphetamine (MDMA)
Buprenorphine	Morphine
Clonazepam	Opiate
Cocaine	Oxycodone
Fentanyl	Phencyclidine
Ketamine	Propoxyphene
Marijuana (Tetrahydrocannabinol [THC])	Tramadol
	Tricyclic antidepressants (TCA)

12.2.7 Alcohol breath test

An alcohol breath test will be performed at screening and prior to dose administration. Additional random tests can be performed during the study.

12.3 Assessments related to primary endpoint

12.3.1 Cardiodynamic assessment

Twelve-lead ECGs will be extracted from continuous recordings (Holter recordings) prior to and serially after dosing with Modufolin® for Injection, 100 mg at time points as shown in the Schedule of events (Table 1; Section 9.1). Holter ECGs will be extracted at 3 time points before dosing. Subjects will be supinely resting for at least 10 min prior to time points for ECG recordings.

The 12-lead Holter and ECG equipment will be supplied and supported by iCardiac Technologies, Inc.

All ECG data will be collected using a Global Instrumentation (Manlius, NY, USA) M12R ECG continuous 12 lead digital recorder. The continuous 12-lead digital ECG data will be stored onto SD memory cards. ECGs to be used in the analyses will be selected by pre-determined time points as defined in the in the Schedule of events (Table 1; Section 9.1) and will be read centrally by iCardiac Technologies, Inc.

The following principals will be followed in iCardiac's core laboratory:

- ECG analysts are blinded to the subject, visit and treatment allocation.
- Baseline and on-treatment ECGs for a particular subject will be over-read on the same lead and will be analyzed by the same reader.
- The primary analysis lead is lead II. If lead II is not analyzable, then primary lead of analysis will be changed to another lead for the entire subject data set.

The following is a brief description of ECG analysis methods utilized by iCardiac' core laboratory.

12.3.2 Thorough QT plus ECG extraction technique

Ten 14-second digital 12-lead ECG tracings will be extracted from the continuous Holter recordings using the 'Thorough QT (TQT) Plus method', a computer-assisted and statistical process utilized by iCardiac Technologies. The method enables extraction of ECGs with the lowest HR variability and noise within the protocol-specified extraction time window (*e.g.*, the HR and QT changes from beat-to-beat in the range of <10%). At each protocol-specified time point, 10 ECG replicates will be extracted from a 5-min "ECG window" (typically, the last 5 min of the 15-min period when the subject is maintained in a supine or semi-recumbent quiet position).

12.3.3 High-precision QT analysis

High-precision QT analysis will be performed on all analyzable (non-artifact) beats in the 10 ECG replicates. Statistical quality control procedures are used to review and assess all beats and identify "high" and "low" confidence beats using several criteria, including:

- QT or QTc values exceeding or below certain thresholds (biologically unlikely).
- RR values exceeding or below certain thresholds (biologically unlikely).
- Rapid changes in QT, QTc or RR from beat to beat.

Measurements of all primary ECG parameters (QT, QTc, RR) in all recorded beats of all replicates that are deemed "high confidence" is performed using COMPAS software. All low confidence beats are reviewed manually and adjudicated using pass-fail criteria. The final QC assessment is performed by a cardiologist. The beats found acceptable by manual review are included in the analysis. The median QT, QTc, and RR value from each extracted replicate is calculated, and then the mean of all available medians from a nominal time point is used as the subject's reportable value at that time point.

Categorical T-wave morphology analysis and the measurement of PR and QRS intervals will be performed manually in 3 of the 10 ECG replicates at each time point (Table 5). Each fiducial point (onset of P-wave, onset of Q-wave, offset of S-wave, and offset of T-wave) is electronically marked.

In addition to the T-wave categorical analysis, the presence of abnormal U-waves is noted.

Table 5 T-wave morphology categories (assessed manually)

Category	Description
Normal T-wave	Any T-wave not meeting any criterion below
Flat T-waves	T amplitude < 1 mm (either positive or negative) including flat isoelectric line
Notched T-wave (+)	Presence of notch(es) of at least 0.05 mV amplitude on ascending or descending arm of the positive T-wave
Biphasic	T-wave that contains a second component with an opposite phase that is at least 0.1 mV deep (both positive and negative/positive and polyphasic T-waves included)
Normal T-wave (-)	T amplitude that is negative, without biphasic T-wave or notches
Notched T-wave (-)	Presence of notch(es) of at least 0.05 mV amplitude on descending or ascending arm of the negative T-wave

12.4 Assessments related to secondary endpoints

12.4.1 Safety resting 12-lead ECG

Single 12-lead safety ECGs will be recorded in supine position after 10 min of rest using the site's ECG machine or printouts from the GI Holter device. HR and PQ/PR, QRS, QT and QTcF intervals will be recorded.

Safety ECG recordings will be performed at baseline in triplicate, after dose administration (at time points in the vicinity of T_{max}) and before discharge from the unit.

Safety ECGs will be reviewed and interpreted on-site by the investigator.

12.4.2 Adverse events

The frequency, seriousness and intensity of AEs will be collected and reported as described in detail in Section 12.5.

12.4.3 Physical examination

A complete physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities.

12.4.4 Vital signs

Systolic and diastolic blood pressure (BP) and pulse will be measured in supine position after 10 min of rest.

12.4.5 Laboratory safety assessments

Blood samples for analysis of clinical chemistry, hematology and coagulation parameters will be collected through an indwelling venous catheter and sent to the certified clinical chemistry laboratory at Uppsala University Hospital and analyzed by routine analytical methods.

Urine analysis will be performed at the research clinic using dip sticks.

The following safety laboratory parameters will be assessed at time-points defined in Section 9.1:

Clinical Chemistry	**Hematology**
Alanine aminotransferase (ALT)	Hematocrit
Alkaline phosphatase (ALP)	Hemoglobin (Hb)
Albumin	Platelet count
Aspartate aminotransferase (AST)	Red blood cell (RBC) count
Bilirubin (total and conjugated)	
Calcium	White blood cell (WBC) count with differential count
Chloride	
Creatinine	
Magnesium	**Urinalysis (dip stick)**
Phosphorous	Glucose
Potassium	Erythrocytes
Sodium	Nitrite
Urea nitrogen	Protein
Uric acid	Specific gravity
	pH

12.4.6 Pharmacokinetic measurements

Blood sampling for PK measurements of MTHF and its metabolites will be performed as described in Section 13.1 (Sample collection for PK measurement) and Section 13.2 (Bioanalytical method for PK measurements).

The specific PK parameters to be evaluated are listed in Section 17.7.5.

12.5 Adverse Events

The Principal Investigator is responsible for ensuring that all medical staff involved in the study is familiar with the content of this section and the content of the CTC Standard Operating Procedures (SOPs) regarding emergencies and Phase I studies.

12.5.1 Event definitions

12.5.1.1 Adverse Event

An AE is any untoward medical occurrence in a clinical study subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including clinically significant abnormal values from relevant tests, such as clinical safety laboratory tests, ECGs, vital signs), symptom, or disease temporally associated with the use of an IMP, regardless of whether it is considered related to the IMP.

A *baseline event* is any AE in a clinical study subject that occurs after he signed the Informed Consent Form (ICF) up until the first administration of IMP/placebo.

A *treatment emergent AE* (TEAE) is any AE not present prior to the initiation of IMP/placebo administration or any event already present that worsens in either intensity or frequency following exposure to the IMP/placebo.

12.5.1.2 Serious Adverse Event

An SAE is any AE that:

- results in death
- is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is medically important (this refers to an event that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent any of the SAEs defined above)

Examples of medically important events are intensive treatment in an emergency room for allergic bronchospasm or blood dyscrasias, convulsions that do not result in hospitalization, development of drug dependency, and drug abuse.

Planned hospitalizations or surgical interventions for a condition that existed before the subject signed the ICF and that did not change in intensity are not SAEs.

If there is any doubt as to whether an AE meets the definition of an SAE, a conservative viewpoint must be taken, and the AE must be reported as an SAE.

12.5.1.3 Serious Adverse Drug Reaction

The term Serious Adverse Drug Reaction (SADR) is to be used whenever either the Investigator or Sponsor or designee assessed the SAE as possibly or probably related to the IMP.

12.5.1.4 Suspected Unexpected Serious Adverse Reaction

A Serious Unexpected Serious Adverse Reaction (SUSAR) is any SADR whose nature or intensity is not consistent with the current version of the IB.

12.5.2 Adverse Event assessment definitions

12.5.2.1 Assessment of severity/intensity

The grading of the severity/intensity of AEs will follow the CTCAE v4.03.[9] Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline.

The Investigator must assess the *severity/intensity* of an AE using the following definitions, and record it on the *Adverse Event Form* in the CRF:

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self- care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

**Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.*

***Self- care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.*

12.5.2.2 Assessment of causal relationship

The Investigator must assess the *causal relationship* between a TEAE and the IMP using the definitions below and record it on the *Adverse Event Form* in the CRF as well as on the *Serious Adverse Event Report Form*, if applicable:

- *Probable* – the AE has a strong temporal relationship to the IMP or recurs on re-challenge, and another etiology is unlikely or significantly less likely
- *Possible* – the AE has a suggestive temporal relationship to the IMP, and an alternative etiology is equally or less likely
- *Not related* – the AE has no temporal relationship to the IMP or is due to underlying/concurrent illness or effect of another drug (that is, there is no causal relationship between the IMP and the AE).

An AE is considered causally related to the use of the IMP when the causality assessment is *probable* or *possible*.

For a baseline event, a causality assessment is not relevant.

12.5.2.3 Assessment of Outcome

The Investigator must assess the *outcome* of an AE using the definitions below and record it on the *Adverse Event Form* in the CRF:

- *Recovered* – the subject has recovered completely, and no symptoms remain.
- *Recovering* – the subject's condition is improving, but symptoms still remain.
- *Recovered with sequelae* – the subject has recovered, but some symptoms remain (for example, the subject had a stroke and is functioning normally, but has some motor impairment).
- *Not recovered* – the subject's condition has not improved and the symptoms are unchanged (for example, an atrial fibrillation has become chronic).
- *Death*

12.5.3 Collecting Adverse Events

AEs (including baseline events) identified using any of the following methods will be recorded:

- AEs spontaneously reported by the subject
- AEs observed by the Investigator or medical personnel
- AEs elicited based on non-leading questions from the Investigator or medical personnel

Collection of baseline events starts after the subject signs the ICF and continues until the first administration of IMP/placebo.

TEAE collection starts with administration of the IMP/placebo and continues until the last follow-up assessment. Any AE with start date on the day of first IMP/placebo administration must be recorded with start time.

At the Follow-up Visit, information on new AEs or SAEs, if any, and stop dates for AEs recorded and on-going during the dosing period must be recorded.

12.5.4 Recording Adverse Events

AEs (including baseline events) must be recorded on an *Adverse Event Form* in the CRF. The Investigator must provide information on the AE, preferably with a diagnosis or at least with signs and symptoms; start and stop dates, start and stop time; intensity; causal relationship to IMP/placebo; action taken, and outcome.

If the AE is serious, this must be indicated in the CRF. Furthermore, the Investigator must fill out the *Serious Adverse Event Report Form* and report the SAE to the Sponsor as described in Section 12.5.5.

AEs, including out-of-range clinically significant clinical safety laboratory values, must be recorded individually, except when considered manifestations of the same medical condition or disease state; in such cases, they must be recorded under a single diagnosis.

If the severity/intensity of an AE increases a new *Adverse Event Form* must be completed in the CRF.

12.5.5 Reporting Serious Adverse Events

The Investigator must report SAEs to the Sponsor immediately (within 24 hrs) after becoming aware of them, by contacting:

Karin Ganlöv, MD
Isofol Medical AB
Arvid Wallgrens Backe 20
SE-413 46 Gothenburg, Sweden
Telephone (mobile): +46 (0)702 43 37 50
E-mail: karin.ganlov@isofolmedical.com

The same information must also be sent to the CTC SAE email inbox: sae@ctc-ab.se.

To report SAEs, the *Serious Adverse Event Report Form* for clinical studies provided must be used. The first report should contain as much information as possible, and if more information about the subject's condition becomes available a follow-up report must be submitted with the additional information using the same procedure as for the initial report.

The Sponsor or a delegate will assume responsibility for reporting SAEs to the Competent Authorities (CAs) in accordance with local regulations.

The Sponsor is responsible for informing the Investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

12.5.6 Treatment and follow-up of Adverse Events

Subjects with AEs that occur during the study must be treated according to daily clinical practice at the discretion of the Investigator.

AEs must be followed up until resolution or until the last Follow-up visit, whichever comes first. At the Follow-up Visit, information on new AEs, if any, and stop dates for previously

reported AEs must be recorded. AEs on-going at the last Final-up visit may be followed up until assessed as stable or until resolution as judged by the Investigator.

It is the responsibility of the Investigator to follow up on all SAEs until the subject has recovered, stabilized, or recovered with sequelae, and to report to the Sponsor all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.

SAEs spontaneously reported by a subject to the Investigator within 30 days after the last follow-up assessment must be handled in the same manner as SAEs occurring during the study. These SAEs will be reported to the Sponsor.

12.6 Appropriateness of measurements

Standardized methods for measurements of safety, tolerability and PK will be used.

13 PROCEDURE FOR BIOLOGICAL SAMPLES

13.1 Sample collection for pharmacokinetic measurement

Blood samples for PK measurements will be drawn by venipuncture or an indwelling venous catheter (opposite arm from drug administration) into chilled a-Monovette® EDTA K_3EDTA 2.6 mL tubes at time points specified in the Schedule of events (Table 1; Section 9.1).

The time points for PK blood sampling will start from end time of injection post dosing.

The exact sampling time will be recorded in the CRF and used for the PK calculations.

After blood collection, the vacutainer tubes will be inverted and centrifuged within 40 min at 3000 rpm for 10 min at approximately 4° C according to local standard procedures. The separated plasma is transferred into two aliquots of 0.3 mL each in microtubes (polypropylene) and immediately placed at -80° C.

The plasma samples will be shipped to Charles River Laboratories (UK) for determination of MTHF, 5-Formyl-THF, 5-Methyl-THF and THF.

13.2 Bioanalytical method for pharmacokinetic measurements

The bioanalytical method for the determination of levels of MTHF, 5-Formyl-THF, 5-Methyl-THF and THF will be described in a separate bioanalytical laboratory manual.

13.3 Volume of blood

During the ambulatory visits, blood samples will be taken by venipuncture and during the stay in the research clinic through an indwelling intravenous catheter.

The anticipated volume of blood samples collected during the study from each subject will not exceed 450 mL per subject (*i.e.*, less than the volume drawn during a regular blood donation).

13.4 Handling, storage and destruction of laboratory samples

All biological samples will be registered in a tissue-bank at CTC (893).

Any remains from the safety laboratory samples will be disposed of after analyses.

The samples for analyses of PK will be stored at -80°C until analyzed. The samples will be disposed of after the Clinical Study Report (CSR) has been finalized.

13.5 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

CTC keeps full traceability of collected biological samples from the subjects while in storage at the research clinic until shipment and keeps documentation of receipt of arrival.

The sample receiver (the analytical laboratory) keeps full traceability of the samples while in their storage and during use until used or disposed of.

The Sponsor keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

13.6 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of biological samples donated, the samples will be disposed of /destroyed, if not already analyzed and documented.

The Principal Investigator will ensure that:

- Subject withdrawal of informed consent is notified immediately to Sponsor.
- Biological samples from the subject, if stored at the research clinic, are immediately identified, disposed of/destroyed and the action is documented.

The Sponsor has to ensure that the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed or returned to the research clinic and the action is documented.

14 ETHICAL AND REGULATORY REQUIREMENTS

14.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP) E6 (R1), EU Clinical Trials Directive, and applicable local regulatory requirements.

A link to the Declaration of Helsinki is included in Appendix 18.2.

14.2 Ethics and regulatory review

The Principal Investigator is responsible for submission of the CSP, the patient information and ICF, any other written information to be provided to the subjects and any advertisements

used for recruitment of subjects to applicable Independent Ethics Committee (IEC) for approval.

The Sponsor is responsible for submission of study documents to the applicable CA to local regulatory requirements.

Approval must be obtained in writing from both IEC and CA before the first subject can be recruited.

The Sponsor will provide the CA, IEC and Principal Investigators with safety updates/reports according to local requirements. Progress reports and notifications of SUSARs will be provided to the IEC according to local regulations and guidelines.

14.3 Subject information and consent

It is the responsibility of the Investigator or an authorized associate to give each potential study subject adequate verbal and written information before any study specific assessments are performed.

The information will include the objectives and the procedures of the study as well as any risks or inconvenience involved. It will be emphasized that participation in the study is voluntary and that the subject may withdraw from participation at any time and for any reason, without any prejudice. All subjects will be given the opportunity to ask questions about the study and will be given sufficient time to consider participation before signing the ICF.

Before performing any study-related procedures the ICF must be signed and personally dated by the subject (or their legally acceptable representative and/or witness, as applicable) and by the Investigator. A copy of the subject information including the signed ICF will be provided to the subject.

Documentation of the discussion and the date of informed consent must be recorded in the source documentation and in the CRF. The subject information sheet and the signed ICF should be filed by the Investigator for possible future audits and/or inspections.

The final approved version of the subject information and ICF must not be changed without approval from the Sponsor and the applicable IEC.

14.4 Subject information card

The subject will be provided with a Subject Information Card including the following information:

- That he/she is participating in a clinical study
- Subject study ID
- That he is treated with the IMP/placebo
- The name and phone number of the Investigator
- Name and address of the Sponsor

14.5 Subject data protection

The ICF includes information that data will be recorded, collected and processed and may be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union Data Protection Directive (95/46/EC), the data will not identify any persons taking part in the study.

The potential study subject (or the subject's legally acceptable representative and/or witness, as applicable) should be informed that by signing the ICF he approves that authorized representatives from Sponsor and CTC, the concerned IEC and CA have direct access to his/her medical records for verification of clinical study procedures. This agreement is to be substantiated in a separate document, according to local requirements.

The subject has the right to request access to his/her personal data and the right to request rectification of any data that is not correct and/or complete.

The Investigator must file a *Subject Identification List* which includes sufficient information to link records, *i.e.*, the CRF and clinical records. This list should be preserved for possible future inspections/audits but should not be made available to the Sponsor except for monitoring or auditing purposes.

14.6 Changes to the approved Clinical Study Protocol

Any proposed change to the approved Final CSP (including appendices) will be documented in a written and numbered Clinical Protocol Amendment. All amendments including substantial changes to the protocol must be approved by the appropriate IEC and/or CA before implementation according to applicable regulations.

14.7 Audits and inspections

Authorized representatives of Sponsor, a CA, or an IEC may perform audits or inspections at the research clinic, including Source Data Verification (SDV). The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, ICH-GCP guidelines and any applicable regulatory requirements. The Investigator will contact the Sponsor immediately if contacted by a CA about an inspection at the center.

14.8 Insurance

Subjects will be covered under Sponsor's liability insurance policy through the Swedish Pharmaceutical insurance *(Läkemedelsförsäkringen)*. Subjects will also be covered under Isofol Medical AB's liability insurance policy through "IF Skadeförsäkring AB". The certificates of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects are also protected in accordance with national regulations, as applicable. CTC has a company insurance covering services performed by CTC.

15 STUDY MANAGEMENT

15.1 Training of study site personnel

Before enrolment of the first study subject a Sponsor representative or delegate will perform a study initiation visit at the research clinic. The requirements of the CSP and related documents will be reviewed and discussed and the investigational staff will be trained in any study specific procedures and system(s) utilized.

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, and have a detailed knowledge of and training in the procedures that are to be executed by them. Any new information of relevance to the performance of this study must be forwarded to the staff involved in a timely manner.

The Investigator will keep a list of all personnel involved in the study together with their function and study related duties delegated. A Curriculum Vitae will be available for all staff delegated study-specific duties.

15.2 Clinical monitoring

The study site will be periodically visited by a Monitor from an independent group at times agreed on by the Investigator and the Monitor. At the time of each monitoring visit, the function of the Monitor is to:

- provide information and support to the investigational team.
- confirm that facilities and resources remain acceptable.
- confirm that the investigational team is adhering to the CSP, applicable SOPs, guidelines, manuals and regulatory requirements.
- verify that data are being accurately and timely recorded in the CRFs and that IMP accountability checks are being performed.
- verify that data in the CRF are consistent with the clinical records (SDV) in accordance with the Monitoring Plan.
- verify that the correct informed consent procedure has been adhered to for participating subjects.
- ensure that withdrawal of informed consent to the use of the subject's biological samples will be reported and biological samples are identified and disposed of/destructed accordingly, and that this action is documented and reported to the subject.
- verify that AEs are recorded and reported in a timely manner and according to the CSP.

When the study has been completed and all queries have been resolved and the database has been locked, the Monitor will perform a close-out visit.

15.3 Source data document

A separate Source Data Verification List will be generated for each site before start of enrolment, specifying the location of the source of derived information appearing in the CRF.

This document must be signed by the Principal Investigator and the Monitor to confirm agreement before start of recruitment.

The paper CRF is considered source data when data is entered directly into the CRF.

The Investigator should guarantee access to source documents to the Monitor, CAs and the IECs, if required.

15.4 Study agreements

The Principal Investigator must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this study.

Agreements between Sponsor and CTC must be in place before any study-related procedures can take place, or subjects be enrolled.

15.5 Study time table and end of study

The end of the clinical part of the study is defined as the last visit of the last subject participating in the study.

The study is expected to start in Quarter 2, 2017 and to be completed by Quarter 2, 2018.

15.6 Discontinuation of the study

The Sponsor reserves the right to discontinue the study at any time, but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the Investigator must inform all participating subjects and perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused study products and other study materials must be returned and all CRFs completed as far as possible.

15.7 Reporting and publication

15.7.1 Clinical Study Report

A summarizing report must be submitted to the applicable CA and IEC within 12 months after completion of the study (in accordance with LVFS 2011:19, Chapter 9).

A CSR, in compliance with ICH E3; *Structure and content of Clinical Study Reports*, describing the conduct of the study, the statistical analysis performed and the results obtained, will be prepared by CTC. The CSR will be reviewed and approved by, as a minimum, the Principal Investigator, the Statistician and the Sponsor.

15.7.2 Confidentiality and ownership of study data

Any confidential information relating to the IMP or the study, including any data and results from the study, will be the exclusive property of the Sponsor. The Investigator and any other persons involved in the study will protect the confidentiality of this proprietary information belonging to the Sponsor.

15.7.3 Publication

The results from this study will be submitted for publication at the discretion of the Sponsor.

15.8 Archiving

The Principal Investigator is responsible for maintaining essential documents, (as defined in ICH E6 GCP, Section 8) for 10 years after finalization of the CSR. This includes any original source documents related to the study, the Subject Identification List (providing the sole link between named subject source records and anonymous CRF data), the original signed ICFs and detailed records of disposition of IMP.

It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

The Sponsor will archive the Study Master File (SMF) in accordance with ICH E6 GCP, Section 8 and applicable regulatory requirements.

16 DATA MANAGEMENT

16.1 Case Report Form

Data will be collected in paper CRFs specifically designed for this study. The Investigator or an authorized person will record subject data in the CRF in a precise and accurate manner. Abbreviations should not be used. The Investigator is responsible for the data entered and will sign off the CRF at each visit and at the end of the study. The data should be recorded as soon as they are generated. CRF entries must be made with an archive resistant pen. Any correction should be marked by a ~~strike-through~~ and the correct information should be written next to the error. All corrections must be signed and dated. Correction fluid must not be used. Only persons authorized by the Investigator are allowed to make entries to the CRF.

16.2 Database management plan and database design

Detailed information on data management will be described in a study-specific Data Management Plan (DMP). The person entering data into the database is not allowed to attempt any personal interpretation or to make any decisions on the data other than self-evident corrections as listed in the study-specific Data Entry Instructions or Data Handling Report. Single data entry type will be applied.

Data validation/data cleaning procedures are designed to assure validity and accuracy of clinical data. These procedures consist of manual reviewing during data entry and computerized edit checks and queries for identifying data values that are outside the allowed range, protocol violations, incomplete or inconsistent. The Data Validation Plan specifies the checks that are to be performed on subject data for the study. All study-specific and standard data validation programming will be tested in a separate testing environment prior to use on production data.

16.3 External data

External data may be received in electronic format or paper printout. Key variables are defined in order to uniquely identify each sample record. File and data formats are agreed with the external data provider. Any electronically transferred data must contain origin, date created, date sent and number of records at minimum.

The continuous 12-lead digital ECG data will be stored onto Secure Digital memory cards.

16.4 Medical encoding

Medical encoding will be performed by trained personnel at CTC. Adverse events and medical history verbatim terms are encoded using the Medical Dictionary of Regulatory Activities (MedDRA), latest version available when approving the DMP.

Prior and concomitant medications will be coded according to the WHO Anatomic Therapeutic Chemical classification system.

All coding will be approved by Sponsor.

16.5 Database lock

When all data have been entered, discrepancies solved and all reconciliation with the SAE database is complete, the database will be locked and the data will be analyzed and reported in a CSR.

17 STATISTICAL METHOD AND DETERMINATION OF SAMPLE SIZE

The principal features of the statistical analysis to be performed are described in this section. A more technical and detailed elaboration of the principal features will be presented in a separate Statistical Analysis Plan (SAP).

17.1 General

All statistical analysis of the study will be performed using the statistical software SAS for Windows Version 9.4. In all calculations, zero will be substituted for concentrations below the quantification limit of the assay. Data collected from all subjects will be presented in data listings. Both absolute values and change from baseline values for each subject will be given where applicable. All continuous data will be listed with the same precision as will be presented in the database. Data listings will be sorted by treatment, subject ID and time point. A missing value will be represented by an empty cell and no imputation will be made.

Continuous data will be summarized in tables using number of subjects (n), mean, median, standard deviation (SD), standard error (SE), 90% confidence interval (CI; based on a t-distribution if not otherwise stated), minimum, and maximum by study time point. Categorical data will be summarized by time point using frequencies and percentages. Percentages will be rounded to the nearest tenth. Population counts will be used as the denominator in the calculation of percentages unless otherwise specified.

17.2 Determination of sample size

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.

17.3 Analysis data sets

17.3.1 Full Analysis Set

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

17.3.2 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.

17.3.3 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.

17.3.4 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.

17.3.5 QT/QTc Analysis Set

The QT/QTc Analysis Set will include all subjects in the Safety Analysis Set with measurements at baseline as well as on-treatment with at least one post-dose time point with a valid ΔQTcF value. The QT/QTc Analysis Set will be used for the by-time point and categorical analyses in the electrocardiogram analysis.

17.3.6 PK/QTc Analysis Set

The PK/QTc Analysis Set will include all subjects who are in both the QT/QTc Analysis Set and PK Analysis Set with at least one pair of post-dose PK and QTcF data from the same time point. The PK/QTc Analysis Set will be used for the exposure-response analysis in the electrocardiogram analysis.

17.4 Electrocardiogram analysis

17.4.1 Baseline

For all ECG parameters, baseline is defined as the average of the measured QTc intervals from the 3 pre-dose time points (45, 30, and 15 min pre-dose) on Day 1.

17.4.2 QT correction methods and parameters

The QT and RR value for each beat will be used for HR correction.

Replicate ECGs will be extracted in up to 10 replicates from each nominal time point pre-specified in the protocol. The median value from each extracted replicate will be calculated, and then the mean of all available medians (minimum 3 medians) from a nominal time point will be used as the subject's reportable value at that time point.

QTcF:

The Fridericia's correction QTcF is defined as: $QTcF = QT/RR^{1/3}$.

17.4.3 Exposure-response analysis (Primary analysis)

The relationship between the plasma concentration of Modufolin® and change-from-baseline QTcF (ΔQTcF) will be quantified using a linear mixed-effects modeling approach with ΔQTcF as the dependent variable, plasma concentration of Modufolin® as the covariate, treatment (active or placebo) and time as categorical factors, and a random intercept per subject. The degrees of freedom for the model estimates will be determined by the Kenward-Rogers method. From the model, the slope (*i.e.*, the regression parameter for the

concentration) and the treatment effect-specific intercept (defined as the difference between active and placebo) will be estimated together with 2-sided 90% CI. The estimates for the time effect will be reported with degrees of freedom and SE.

The geometric mean of the individual C_{max} values for subjects in each of the active drug groups will be determined. The predicted effect and its 2-sided 90% CI for placebo-corrected change-from-baseline QTcF (ΔΔQTcF) (*i.e.*, the product with the slope estimate + treatment effect) at this geometric mean C_{max} will be obtained for each Modufolin® dose separately. If the upper bound of the 90% CI of the model predicted QTcF effect is below 10 msec at clinically relevant plasma levels of Modufolin®, it will be concluded that Modufolin® does not cause clinically concerning QTc prolongation.

The plot of the observed median-quantile Modufolin® concentrations and associated mean ΔΔQTcF (90% CI) adjusted for diurnal effects together with the regression line presenting the predicted ΔΔQTcF (90% CI) (as described in the publication by Tornoe et al.[16] will be used to evaluate the adequacy of the model fit to the assumption of linearity and the impact on quantifying the exposure response relationship. Additional exploratory analyses (via graphical displays and/or model fitting) will include accounting for a delayed effect (hysteresis) and the justification for the choice of pharmacodynamic model (linear versus nonlinear) as follows.

Investigation of hysteresis:

If a QTc effect (ΔΔQTcF) exceeding 10 msec cannot be excluded in the by-timepoint analysis of the two highest dose groups, hysteresis, *i.e.*, difference in peak QT response and C_{max}, will be explored through visual inspection of overlaid PK and ΔΔQTc time curves and through so called hysteresis loops for each dose of Modufolin® for Injection, 100 mg

Appropriateness of a linear model:

To assess the appropriateness of a linear model, normal QQ-plots for the residuals and plots of weighted residuals vs. concentration and vs. fitted values will be produced. The scatter plot of residuals vs concentration by Loess (*i.e.*, locally weighted scatterplot smoothing as described in the publication by Cleveland[17]) fitting will be also produced with an optimal smoothing parameter selected by the Akaike information criterion with a correction (AICC).[18] In addition, a model with a quadratic term in concentration will be fitted and the quadratic term will be tested on the two-sided 5% level. If there is an indication that a linear model is inappropriate, additional models will be fitted, in particular:

- An E-max model;
- A log-linear model where the plasma concentration C is replaced by $\log(C/C_0)$, C_0 is the limit of quantification of the assay used to determine C and all values below C_0 are replaced by C_0 (*i.e.*, $\log[C_0/C_0] = 0$).

The Exposure-Response analysis will then be repeated for the model found to best accommodate the nonlinearity detected.

17.4.4 By-time point analysis:

The analysis for QTcF will be based on a linear mixed-effects model with change-from-baseline QTcF (ΔQTcF) as the dependent variable, time (categorical), treatment (Modufolin® for Injection, 100 mg and placebo), and time-by-treatment interaction as fixed effects, and baseline QTcF as a covariate. Subject will be included as a random effect for the intercept. Subject dosed with placebo will be analyzed as a pooled group. An unstructured covariance

matrix will be specified for the repeated measures at post-dose time points for each subject. If the model with unstructured covariance matrix fails to converge, other covariance matrix such as autoregressive and compound symmetry will be considered. From this analysis, the least-squares (LS) mean and 2-sided 90 % CIs will be calculated for the contrast "Modufolin® versus placebo" at each dose of Modufolin® and each post-dose time point, separately.

For HR, PR, and QRS intervals, the analysis will be based on the change-from-baseline values (ΔHR, ΔPR, ΔQRS). The same model will be used as described for QTcF. The LS mean, SE and 90% CI from the statistical modeling for both change-from-baseline and placebo-corrected change-from-baseline values will be listed in the tables and graphically displayed.

17.4.5 Categorical analyses

The analysis results for categorical outliers, and T-wave morphology will be summarized in frequency tables with counts percentages for both number of subjects and number of time points. For categorical outliers, the number (percentage) of subjects as well as time points who had increases in absolute QTcF values >450 and ≤480 msec, >480 and ≤500 msec, and 500 msec, and changes from pre-dose baseline of >30 and ≤60 msec, and >60 msec; increase in PR from pre-dose baseline >25% to a PR>200 msec; increase in QRS from pre-dose baseline >25% to a QRS >120 msec; decrease in HR from pre-dose baseline >25% to a HR <50 bpm; and increase in HR from pre-dose baseline >25% to a HR >100 bpm will be determined. For T-wave morphology, the analyses will be focused on change from baseline (*i.e.*, treatment-emergent changes).

17.5 Description of study population

17.5.1 Demographics and baseline characteristics

Descriptive statistics for demographics, weight and height will be presented by treatment.

17.5.2 Medical history and concomitant medication

Medical/surgical history and prior/concomitant medications will be presented by treatment using descriptive statistics and listings.

17.5.3 Treatment compliance

The number of subjects treated in each treatment period and their individual dose will be tabulated.

17.6 Analysis of primary endpoints

17.6.1 Electrocardiogram

All ECG data will be listed for each subject and summarized as the vital signs parameters. In addition, ECGs will be categorized as "normal", "abnormal, not clinically significant", or

”abnormal, clinically significant” (as judged by the Investigator) and summarized by treatment and period using frequency tables.

17.6.2 Telemetry

Ambulatory ECG telemetry will be used for cardiac surveillance up to 24 hrs after dose administration.

17.7 Analysis of secondary endpoints

17.7.1 Physical examination

Abnormal findings will be specified and presented by subject and summarized by treatment and period.

17.7.2 Vital signs

Vital signs (systolic/diastolic blood pressure and pulse will be summarized by treatment and period using descriptive statistics.

17.7.3 Safety laboratory analyses

Safety laboratory data will be presented by individual time courses for each parameter and subject and summarized by treatment and period.

17.7.4 Adverse Events

All AE data will be fully listed by Investigator terms and MedDRA Preferred Term (PT). AE data will be summarized by System Organ Class (SOC) and PT.

17.7.5 Definition and calculation of folate pharmacokinetic parameters

The PK analysis will be based on the PPAS and subcontracted by the Sponsor to PKxpert. The PK parameters will be calculated by non-compartmental pharmacokinetic analysis (NCA) using the software Phoenix WinNonlin® version 6.4 or later (Pharsight Corporation, U.S.A.).

The following non-compartmental PK parameters will be assessed for [6R]-5,10-MTHF, [6S]-5-Methyl-THF, [6S]-THF and [6S]-5-Formyl-THF, if data permit:

AUC	Area under the plasma concentration-time curve from time 0 to infinity. Calculated using the linear/log trapezoidal rule with extrapolation to infinity.

$$AUC(0-t_{max}) = \left(\sum_{i=1}^{n-1} \frac{C(i)+C(i+1)}{2} \cdot (t(i+1)-t(i)) \right)$$

The log trapezoidal rule is used from time t_{max} to the last time point t:

$$AUC(t_{max} - t) = \left(\sum_{i=1}^{n-1} \frac{C(i) - C(i+1)}{\ln\left(\frac{C(i)}{C(i+1)}\right)} \cdot (t(i+1) - t(i)) \right)$$

Extrapolation to infinity is performed using:

$$AUC(t - \infty) = \frac{C(t)}{\lambda_z}$$

AUC_{last}	Area under the plasma concentration-time curve from time 0 to the last time point t.
AUC_{τ}	Area under the plasma concentration-time curve in a dosing interval. Calculated using the linear/log trapezoidal rule with extrapolation to τ, if required
$AUC_{\%Extrap}$	Percentage of AUC that is due to extrapolation from the last concentration to infinity: $AUC_{\%Extrap} = \frac{AUC(t-\infty)}{AUC} \cdot 100\%$
AUMC	Area under the first moment curve extrapolated to infinity based on the last observed concentration. $AUMC = AUMC_{last} + \frac{t_{last} \cdot C_{last}}{\lambda_z} + \frac{C_{last}}{\lambda_z^2}$
C_0	Back extrapolated concentration at time point 0 after intravenous administration
C_{max}	Maximum plasma concentration
$C_{(time)}$	Plasma concentration at a certain time
C_{ss}	Steady-state drug concentration in plasma
CL	Total body clearance for intravenous administration. $CL = \frac{Dose}{AUC}$
LLOQ	Lower Limit of Quantification
Mean	Arithmetic mean, otherwise specified
MRT	Mean Residence Time extrapolated to infinity. $MRT = \frac{AUMC}{AUC}$
NCA	Non-Compartmental pharmacokinetic Analysis
PK	Pharmacokinetics

Tau (τ)	Dosing interval
t_{max}	Time at which maximum plasma concentration is observed.
$t_{1/2}$	Terminal half-life: $t_{½} = \frac{\ln 2}{\lambda_z}$
λ_z *(lambda z)*	Terminal rate constant. Calculated by log-linear regression of concentrations versus time
V_z	Volume of distribution based on the terminal phase. $Vz = \frac{Dose}{AUC \cdot \lambda_z}$
V_{ss}	Volume of distribution based at steady state. $V_{ss} = MRT \cdot CL$

Descriptive statistics for the PK parameters will be presented by treatment group with number of measurements, arithmetic mean, SD, Coefficient of variation (CV), median, minimum, maximum, geometric mean, geometric CV%.

17.8 Statistical/analytical issues

17.8.1 Handling of dropouts or missing data

For information, refer to the SAP.

17.8.2 Interim analyses and data monitoring

Not applicable.

18 APPENDICES

18.1 Signature page

"I agree to the terms of this Clinical Study Protocol."

Sponsor signatories

Anders Rabbe, CEO
Name

Signature

05 DEC 2016
Date

Karin Ganlöv, MD, CMO
Name

Signature

05 DEC 2016
Date

Principal Investigator

Cornelia Lif-Tiberg, MD
Name

Signature

05 DEC 2016
Date

Jan Erik Berglund, MD, PhD
Name

Signature

03 JUL 2017
Date

18.2 Declaration of Helsinki

http://www.up.ac.za/media/shared/Legacy/sitefiles/file/45/2875/declarationofhelsinki_fortaleza_brazil2013.pdf

18.3 Summary of safety and pharmacokinetic data from Isofol Medical AB's previous clinical studies in cancer patients

Therapeutic dose	The ongoing clinical study ISO-CC-005 is investigating 60, 120 and 240 mg/m^2. Based on current data the expected clinical dosing regimen in future clinical trials in metastatic colorectal cancer (lead indication) patients is 120 mg/m^2 Modufolin®.	
Maximum tolerated dose	Doses of Modufolin® up to 500 mg/m^2/day has been administered to patients in study ISO-MC-091. From animal studies: In dogs, the 28-day toxicology studies indicate that doses of 200 mg/m^2 Modufolin® for Injection, 100 mg may be administered twice daily (*i.e.*, total daily dose of 400 mg/m^2) resulting in safety ratios between dogs and humans above 10. Looking at the safety exposure margins for rats, the safety factors would still be above 10 even if 200 mg/m^2 doses were administered up to 6-8 times daily (*i.e.*, total daily doses of 1,200-1,800 mg/m^2).	
Principal adverse events	Modufolin® alone is not anticipated to induce any AEs or DLTs. Modufolin® is a biomodulator of the 5-FU anticancer effect in colorectal cancer or as a rescue treatment after High Dose Methotrexate treatment. In the ISO-CC-005 study in metastatic CRC patients (Modufolin® administered together with drugs such as 5-FU, irinotecan and oxaliplatin): Most common AEs: nausea (25), diarrhea (24), fatigue (24) and vomiting (13). Main DLT AE: Neutropenia (8)	
Maximum dose tested	Single Dose	In the ISO-CC-002 study, colorectal cancer patients have been exposed to single Modufolin® doses of 60 or 200 mg/m^2. In the ISO-MC-091 study, rectal cancer patients have been exposed to single and multiple doses ranging between 10 and 500 mg/m^2/day.
	Multiple Dose	In the ISO-MC-091 study, rectal cancer patients have been exposed to multiple doses ranging between 10 and 500 mg/m^2/day.
Exposures Achieved at Maximum Tested Dose	Single Dose	**Cycle 1 Day 15, 500 mg/m^2 n=6, (ISO-MC-091)** Modufolin® drug substance ([6R]-MTHF) (parent) $C_{(10min)}$ 23300 ±6680 ug/L $AUC_{(0-4h)}$ 13600 ±3430 h*ug/L [6S]-5-tetrahydrofolate ([6S]-THF) Cycle 1 Day 15, 500 mg/m^2 (ISO-MC-091) $C_{(10min)}$ 30200 ±5780 ug/L $AUC_{(0-4h)}$ 66700±12900 h*ug/L [6S]-5-methyl-tetrahydrofolate ([6S]-methyl-THF) $C_{(10min)}$ 1600 ±400 ug/L $AUC_{(0-4h)}$ 17400 ±3120 h*ug/L

	Multiple Dose	**Cycle 3 Day 15, 500 mg/m^2 (ISO-MC-091)** Modufolin® drug substance ([6R]-MTHF) (parent) $C_{(10min)}$ 24200 ±2410 ug/L $AUC_{(0-4h)}$ 14900 ±1450 h*ug/L [6S]-5-Tetrahydrofolate ([6S]-THF) $C_{(10min)}$ 31600 ±6330 ug/L $AUC_{(0-4h)}$ 70800±12500 h*ug/L [6S]-5-Methyl-Tetrahydrofolate ([6S]-Methyl-THF) $C_{(10min)}$ 1400 ±420 ug/L $AUC_{(0-4h)}$ 14900 ±4150 h*ug/L
Range of linear PK	60 and 200 mg/m^2 (ISO-CC-002) and 500 mg/m^2 (ISO-MC-091) AUC_{last}: Average values: 2270 h*ug/L at 60 mg/m^2 (t_{last} = 1-1.5 h, AUC_{0}-2h could not be calculated) AUC_{0-2h}: Average values: 7730 h*ug/L at 200 mg/m^2 and 13200 h*ug/L at 500 mg/m^2. Thus, dose proportional increase was seen comparing 60 to 200 mg/m^2, and a minor sub-proportional increase to dose increase was seen, comparing up to 500 mg/m^2. It should be emphasized that 60 and 200 mg/m^2 was in one study with colon cancer patients (ISO-CC-002) and 500 mg/m^2 was in another study with rectal cancer (ISO-MC-091).	
Accumulation at steady state	• Modufolin® drug substance ([6R]-MTHF) (parent, active metabolite), no accumulation after once daily dosing up to 500 mg/m^2/day.	
Metabolites	Most relevant other metabolites: [6S]-5-Tetrahydrofolate ([6S]-THF) and ([6S]-5-Methyl-Tetrahydrofolate ([6S]-Methyl-THF) Both metabolites can serve as precursors to MTHF, but are not active in itself	
Absorption	Absolute/Relative Bioavailability	N/A, IV administration
	T_{max}	• Median (range) for parent N/A, IV administration • Median (range) for metabolites [6S]-5-tetrahydrofolate ([6S]-THF): The concentration of the metabolite, THF, after IV administration of Modufolin® increased rather rapidly, t_{max} ranged between 5-15 min at 60 mg/m^2 and 15-45 min at 200 mg/m^2. [6S]-5-Methyl-Tetrahydrofolate ([6S]-Methyl-THF): t_{max} ranged between 1.5-4 h at 60 mg/m^2 and 3-6 h at 200 mg/m^2
Distribution	Vd	The distribution volume (V_{ss}) of MTHF was in the range of 9.2-22.7 L in the 60 mg/m^2 dose group and 9.4-29.3 L in the 200 mg/m^2 dose group.

	% bound	Not performed
Elimination	Route	• Primary route; percent dose eliminated IV. Not studied, but it is anticipated that most of the dose will entering the endogenous folate metabolism. • Other routes N/A
	Terminal $t_{½}$	• Mean (± SD) for parent The elimination half-life calculated for two patients in the high dose level (200 mg/m^2, measurable concentrations until 6 h) were 1.5-1.8 h, but the major part of the exposure of the parent compound was eliminated faster, a multi-compartmental elimination curve was seen for Modufolin® drug substance ([6R]-MTHF) . • Mean (± SD) for metabolites [6S]-5-Tetrahydrofolate ([6S]-THF): one phase elimination curve with an elimination half-life less than 1.5 h [6S]-5-Methyl-Tetrahydrofolate ([6S]-Methyl-THF): The concentration of methyl THF could be measured until 6 - 24 h. The PK of methyl THF for two patients at 200 mg/m^2 could be described by a one phase elimination curve with an elimination half-life of 4.3-14.5 h
	CL	The clearance (CL) of methylene THF, parent, was in the range of 31-60 L/h
Intrinsic Factors	Age	Currently no information available (popPK).
	Sex	Currently no information available (popPK).
	Race	Currently no information available (only caucasians studied hitherto).
	Hepatic & Renal Impairment	Currently no information available.
Extrinsic Factors	Drug interactions	Not performed
	Food Effects	Intravenous administration / No food effects expected
Expected High Clinical Exposure Scenario	In the other investigated indication with the treatment of Modufolin® (Osteosarcoma), the drug candidate is used as rescue after High dose methotrexate administration. In scenarios of extreme Methotrexate levels, according to the Children's Oncology Group, doses of folic acids (*e.g.*Leucovorin/Calcium folinate) might be administered up to maximum 1500 mg q6h until Methotrexate levels are <0.1 µM. This corresponds to a maximum dosage of 750 mg q6h Modufolin®. (Modufolin® is the active metabolite of Leucovorin/Calcium folinate, hence half the dosage is needed)	

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