A randomized, multicenter, parallel-group, Phase III study to compare the efficacy of arfolitixorin versus leucovorin in combination with 5-fluorouracil, oxaliplatin, and bevacizumab in patients with advanced colorectal cancer

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Drug Product: arfolitixorin

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2.0	13 November 2018	Administrative change
3.0 USA	25 January 2019	Local amendment
4.0 USA	29 August 2019	Global amendment
5.0 USA	07 October 2019	Administrative change
6.0 USA	20 February 2020	Global amendment
7.0 USA	20 August 2021	Global amendment

8.0 USA	**22 February 2022**	**Global Amendment. Never submitted, changes included in v 9.0**
9.0 USA	**22 February 2022**	**Global Amendment**

For more detailed information regarding the changes, see section 10.3 in this study protocol.

Table of Contents

STATEMENT OF COMPLIANCE 1
CONFIDENTIALITY STATEMENT 1
SIGNATURE PAGE Protocol approval 2
SIGNATURE PAGE Study Coordinators 3
SIGNATURE PAGE Investigator 4
1 PROTOCOL SUMMARY 5
1.1 Synopsis 5
1.2 Schema 14
1.3 Schedule of Activities (SoA) 15
2 INTRODUCTION 17
2.1 Study Rationale 17
2.1.1 Advanced Colorectal Cancer 17
2.1.2 Current standard of care 17
2.1.3 Mechanism of action of Arfolitixorin 18
2.2 Background 19
2.2.1 Non-clinical Background 19
2.2.2 Clinical Background 19
2.3 Risk/Benefit Assessment 21
2.3.1 Known Potential Risks 21
2.3.2 Known Potential Benefits 21
2.3.3 Assessment of Potential Risks and Benefits 21
3 OBJECTIVES AND ENDPOINTS 24
4 STUDY DESIGN 25
4.1 Overall Design 25
4.2 Scientific Rationale for Study Design 26
4.3 Justification for Dose 27
4.4 End of Study Definition 29
5 STUDY POPULATION 29
5.1 Inclusion Criteria 29
5.2 Exclusion Criteria 30
5.3 Lifestyle Considerations 31
5.4 Screen Failures 31
5.5 Strategies for Recruitment and Retention 31
6 STUDY INTERVENTION 32
6.1 Study Intervention(s) Administration 32
6.1.1 Study Intervention Description 32
6.1.2 Dosing and Administration 32
6.2 Preparation/Handling/Storage/Accountability 33
6.2.1 Acquisition and accountability 33
6.2.2 Formulation, Appearance, Packaging, and Labelling 34
6.2.3 Product Storage and Stability 35
6.2.4 Preparation 35
6.3 Other Medications 36
6.3.1 5-FU 36
6.3.2 Oxaliplatin 36

6.3.3 Bevacizumab 37
6.3.4 Dose adjustements 37
6.4 Measures to Minimize Bias: Randomization and Blinding 38
6.4.1 Randomization 38
6.4.2 Blinding 38
6.5 Study Intervention Compliance 38
6.6 Concomitant Therapy 39
6.6.1 Prohibited Therapies 39
6.6.2 Authorized Medication 39
6.6.3 Rescue Medicine 39
7 IMP DISCONTINUATION AND PATIENT DISCONTINUATION 39
7.1 Discontinuation of Study Intervention 39
7.2 Participant Discontinuation/Withdrawal from the Study 40
7.3 Lost to Follow-Up 40
8 STUDY ASSESSMENTS AND PROCEDURES 41
8.1 Efficacy Assessments 41
8.1.1 CT scan / MRI 41
8.1.2 Pharmacokinetics 43
8.1.3 ECOG performance status 45
8.1.4 EQ-5D Quality of life 45
8.1.5 Survival Status 45
8.2 Safety and Other Assessments 46
8.2.1 Laboratory safety assessments 46
8.2.2 Vital signs, ECG and physical findings related to safety 46
8.2.3 PRO CTCAE 47
8.2.4 Pharmacogenetics 47
8.2.5 RESECTION/REMOVAL OF TUMOR LESION(S) 48
8.3 Study Schedule 48
8.3.1 Screening and Enrollment 48
8.3.2 Baseline (Cycle 1) 49
8.3.3 Treatment Period 49
8.3.4 End of treatment visit (EOT) 50
8.3.5 Post-Treatment visits 50
8.4 Adverse Events and Serious Adverse Events 51
8.4.1 Definition of Adverse Events (AE) 51
8.4.2 Definition of Serious Adverse Events (SAE) 51
8.4.3 Classification of an Adverse Event 52
8.4.4 Time Period and Frequency for Event Assessment and Follow-Up 54
8.4.5 Adverse Event Reporting 54
8.4.6 Adverse Event of Special Interest 54
8.4.7 Serious Adverse Event Reporting 54
8.4.8 Reporting Events to Participants 55
8.4.9 Reporting of Pregnancy 55
9 STATISTICAL CONSIDERATIONS 56
9.1 Statistical Hypotheses 56
9.2 Sample Size Determination 56

9.2.1 Primary efficacy endpoint: ORR 56
9.2.2 Secondary efficacy endpoint: PFS 57
9.2.3 Hierarchical testing approach 57
9.2.4 Overall survival 58
9.2.5 Accrual rate 58
9.3 Populations for Analyses 58
9.4 Statistical Analyses 59
9.4.1 General Approach 59
9.4.2 Analysis of the Primary Efficacy Endpoint(s) 59
9.4.3 Analysis of the Secondary Endpoint(s) 59
9.4.4 Safety Analyses 60
9.4.5 Baseline Descriptive Statistics 60
9.4.6 Planned Interim and final Analyses 60
9.4.7 Sub-Group Analyses 63
9.4.8 Tabulation of Individual participant Data 63
9.4.9 Exploratory Analyses 63
10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS 64
10.1 Regulatory, Ethical, and Study Oversight Considerations 64
10.1.1 Informed Consent Process 64
10.1.2 Study Discontinuation and Closure 65
10.1.3 Confidentiality and Privacy 65
10.1.4 Future Use of Stored Specimens and SAMPLES 65
10.1.5 Key Roles and Study Governance 65
10.1.6 Safety Oversight 66
10.1.7 Clinical Monitoring 66
10.1.8 Quality Assurance 67
10.1.9 Data Handling and Record Keeping 68
10.1.10 Protocol Deviations 69
10.1.11 Publication and Data Sharing Policy 70
10.1.12 Insurance 70
10.1.13 Conflict of Interest Policy 70
10.2 Abbreviations 72
10.3 Protocol Amendment History 74
11 REFERENCES 79

Tables

Table 1: Schedule of Activities (SOA) 15
Table 2: Components of arfolitixorin 34
Table 3: Laboratory tests 46
Table 4: Hazard ratio for significance in function of the number of PFS events 57
Table 5: Guidelines for sample size increase at interim analysis 62

Appendices

Appendix 1: ECOG performance status scale

Appendix 2: NCI PRO CTCAE

Appendix 3: EuroQol EQ-5D

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP), the provisions of the Helsinki Declaration, and the relevant legislation in force. For US based clinical sites, the study will be conducted under an IND. For sites based outside of the US (Non-IND sites), the study will be conducted according to GCP and other applicable local laws and regulation. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without written approval by an adequately constituted Institutional Review Board/Ethics Committee and Regulatory Authorities, except where necessary to eliminate an immediate hazard(s) to the study patients. All personnel involved in the conduct of this study have completed ICH GCP Training and have appropriate knowledge about other applicable regulation, including Human Subject Protection and GDPR.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Ethics Committee and/or Regulatory Authorities for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the Ethics Committee and/or Regulatory Authorities before the changes are implemented to the study. All changes to the consent form will be Ethics Committee and/or Regulatory Authorities approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

CONFIDENTIALITY STATEMENT

This Clinical Study Protocol (CSP) contains information that is confidential and proprietary to Isofol Medical AB (publ) (Sponsor). This information is being provided to you for the purpose of conducting a clinical study sponsored by Isofol. You may disclose the contents of this CSP to study personnel under your supervision who need to know the contents for this purpose, as well as to your Independent Ethics Committee/Institutional Review Board (IEC/IRB). The contents of this CSP may not be disclosed to any other person or entity without the prior written permission of Isofol and may not be used for any other purpose than the conduct of this study. The foregoing shall not apply to disclosure required by governmental regulations or laws; however, you will give prompt notice to Isofol of any such disclosure.

Any supplemental information that may be added to this document is also confidential and proprietary to Isofol and must be kept in confidence in the same manner as the contents of this CSP.

Any person who receives this CSP without due authorization from Isofol is requested to return it to Isofol or to promptly destroy it.

SIGNATURE PAGE PROTOCOL APPROVAL

The signatures below constitute the approval of this protocol and the appendices, and provides the necessary assurances that this protocol has been designed and the study will be conducted according to all stipulations of GCP and according to all applicable legal and regulatory requirements.

Sponsor's Representative:

Roger Tell, MD, PhD
Chief Medical Officer
Isofol Medical AB (publ)

Date:

SIGNATURE PAGE STUDY COORDINATORS

I have read and understood this study protocol, and I agree to conduct the study according to the study protocol, GCP and applicable local regulation.

Biostatistician:

Koenraad D`Hollander, MSc
IDDI
Louvain-la-Neuve, Belgium

Date:

Global Coordinating Investigator:

Josep Tabernero, MD, PhD, MSc
Vall D'Hebron Institute of Oncology
Barcelona, Spain

Date:

US Coordinating Investigator:

Heinz-Josef Lenz, MD, FACP
University of Southern California
Norris Comprehensive Cancer Center
Los Angeles, USA

Date:

SIGNATURE PAGE INVESTIGATOR

I have read and understood this study protocol, and I agree to conduct the study according to the study protocol, GCP and applicable local regulation.

Site investigator signature

Principal Investigator (Sign):

Name (Block letters): Date:

Site:

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1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Study title	A randomized, multi-center, parallel-group, Phase III study to compare the efficacy of arfolitixorin versus leucovorin in combination with 5-fluorouracil, oxaliplatin, and bevacizumab in patients with advanced colorectal cancer.
Global Coordinating Investigator	Josep Tabernero, MD, PhD, MSc Vall D'Hebron Institute of Oncology Barcelona, Spain
US Coordinating Investigator	Heinz-Josef Lenz, MD, PhD Division of Medical Oncology, University of Southern California Norris Comprehensive Cancer Center Los Angeles, CA, USA
Study Duration	Recruitment period: Q4 2018 – Q2 2021 Study period: Q4 2018 – Q1 2023
Participant duration	All patients will be treated until progressive disease (PD), or toxicity, and followed for survival until end of study.
Short Summary	This is a multicenter, randomized, parallel-group, Phase III study in at least 440 patients with advanced colorectal cancer to compare the efficacy of treatment with arfolitixorin versus Leucovorin in combination with 5-fluorouracil, oxaliplatin, and bevacizumab according to modified FOLFOX-6 until PD according to RECIST 1.1 criteria.
Primary Endpoint	Overall Response Rate (ORR)
Background	5-fluorouracil (5-FU) in combination with the folate Leucovorin (LV) has been the cornerstone in the treatment of colorectal cancer (CRC) for decades. All folates currently approved for use in the clinical setting need to be metabolically activated to [6R] 5,10-methylenetetrahydrofolic acid ([6R]-MTHF), which is the active thymidylate synthase co-substrate that potentiates the effect of 5-FU. Arfolitixorin does not need multi step metabolic activation like currently available folates. It is therefore hypothesized that the administration of arfolitixorin will result in higher, and more consistent, intracellular concentrations of the active thymidylate synthase co-substrate [6R]-MTHF in all patients compared to LV administration.
Indication	First line advanced CRC patients eligible for treatment with 5-FU, oxaliplatin, and bevacizumab.

Objectives and endpoints

Objective:	Endpoint/Outcome Measure:
Primary	
Overall response rate (ORR)	Best ORR, defined as the best response recorded from the start of the study treatment until the end of treatment. All responses will be confirmed 8 weeks after onset of response. All assessments including confirmation of response will be performed by BICR.
Key Secondary	
Progression-free survival (PFS)	PFS, defined as the time from randomization to first occurrence of tumor progression assessed by BICR (RECIST 1.1) based on CT-scans/MRIs conducted every 8 weeks after start of treatment, or death from any cause.
Duration of Response (DOR)	The duration of overall response (DOR) is measured from the first time point at which criteria are met for complete response (CR) or partial response (PR) through the last time point when overall response has been objectively documented.
Other Secondary (efficacy)	
Overall survival (OS)	OS, defined as time from randomization to death from any cause
Quality of Life	Quality of Life assessed using the EQ-5D patient reported outcome questionnaire (PRO).
Other Secondary (safety)	
Safety and tolerability	Number and severity of adverse events (AEs), including clinically significant abnormal laboratory findings, regardless of causal relationship to arfolitixorin or LV. Specific AEs will be followed using PRO (NCI PRO-CTCAE).
Patients undergoing curative metastasis resection.	Defined as the number of patients qualifying for curative metastasis resection after treatment with study drug.
Exploratory	
Daily living abilities	Daily living abilities as assessed by the Eastern Cooperative Oncology Group (ECOG) Performance Status Scale

To determine the pharmacokinetic (PK) characteristics of arfolitixorin and LV in patients with advanced CRC	The plasma concentration of methylene tetrahydrofolate ([6R]-MTHF), methyl-tetrahydrofolate (methyl-THF), and tetrahydrofolate (THF) will be determined in a limited number of patients in both treatment arms.
To investigate folate metabolism- and transportation-related gene expression levels in patients with advanced CRC	Gene expression levels of folate metabolism- and transportation-related genes, analyzed by sub-group low/high gene expression levels.
Recurrence Free Survival (RFS) for patients undergoing metastatic resection.	Defined as the time between the first surgery with complete removal of the metastasis and recurrence of the disease or death from any cause.

Main Selection Criteria

Inclusion criteria

For randomization in the study, patients must fulfill all the following criteria:

1. Colorectal adenocarcinoma verified by biopsy.
2. Availability of biopsy material, from the primary tumor or metastasis, allowing for analysis of tumor gene expression.
3. Non-resectable metastatic CRC planned for first line therapy with 5-FU, Leucovorin, oxaliplatin, and bevacizumab.
4. Evaluable disease with at least one measurable lesion of metastatic disease (≥10 mm in longest diameter on axial image on CT-scan or alternatively MRI with <5 mm reconstruction interval) or lymph node (≥ 15 mm in shortest axis when assessed by CT) obtained within 28 days of randomization.
5. Life expectancy of more than 4 months.
6. ECOG performance status 0 or 1.
7. Hemoglobin (Hb) > 80 g/L, Absolute neutrophil count (ANC) > $1.5x10^9$/L. Thrombocytes > $100x10^9$/L.
8. Creatinine clearance > 50 mL/min, Total bilirubin < 1.5 x ULN, AST and ALT < 3 x ULN (and < 5 x ULN in case of liver metastases).
9. Male or female ≥18 years of age.
10. Female patients of childbearing potential must have a negative urine pregnancy test and use adequate contraceptive measures[1]. Male patients must use adequate contraceptive measures[2].
11. Voluntarily signed informed consent before performance of any study related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.

Exclusion Criteria

Patients meeting one or more of the following criteria are ineligible to participate in the study:

1. Malignant tumors other than colorectal adenocarcinomas (current or within the previous five years), with the exception for curatively treated non-melanoma skin cancer or in situ carcinoma of the cervix.

[1] Female patients must be post-menopausal for more than one year or must provide a negative urine pregnancy test and use an efficient method of contraception (i.e., a method with less than 1% failure rate [e.g., sterilization, hormone implants, hormone injections, some intrauterine devices, or vasectomized partner]) during the study and for 1 month (or more if requested by non-IMP labels) after the end of the study (last dose of IMP).

[2] Unless the partner of a male patient is post-menopausal or using an efficient method of contraception as described above, male patients must agree to use condoms during the study treatment and for 1 month after the end of the study treatment.

2. Less than 6 months between randomization and completion of the last anti-cancer treatment (chemotherapy/radiotherapy/immunotherapy, etc.). (NB: Rectal cancer treatment shorter than 8 weeks of chemo/radiation therapy is allowed.)
3. Confirmation of progressive disease within 6 months after completion of prior adjuvant anti-cancer treatment.
4. Indication for any metastatic Colo-rectal Cancer (mCRC) surgery or anti-cancer treatment other than study treatment.
5. Prior treatment with arfolitixorin.
6. Indication for treatment with a 5-FU analogue, or 5-FU for a condition other than mCRC.
7. Known Dihydropyrimidine Dehydrogenase Deficiency (DPD) deficiency.
8. Known or suspected central nervous system (CNS) metastases.
9. Unresolved bowel obstruction, uncontrolled Crohn's disease, or ulcerative colitis.
10. History of cardiac disease with a New York Heart Association Class II or greater, congestive heart failure, myocardial infarction, or unstable angina at any time during the 6 months prior to randomization, or serious arrhythmias requiring medication for treatment.
11. Current CTCAE ≥ grade 3 diarrhea.
12. Current chronic infection or uncontrolled serious illness causing immunodeficiency.
13. Known or suspected hypersensitivity or intolerance to arfolitixorin, LV, 5-FU, oxaliplatin, or bevacizumab.
14. Breastfeeding patients.
15. Patient who received investigational drugs in other clinical trials within 28 days, or 5 half-lives of the investigational drug, prior to randomization.
16. Patient with serious medical or psychiatric illness likely to interfere with participation in this clinical study.
17. Ongoing drug or alcohol abuse, as deemed by the Investigator.
18. Any condition that, in the opinion of the Investigator, could compromise the patient's safety or adherence to the study protocol.
19. Involvement, or related to people involved in the planning or conduct of the study (applies to both Isofol Medical AB (publ) staff and staff at the study site)
20. Surgery (excluding previous diagnostic biopsy) in the 28-day period before randomization

Interventions

Investigational Medicinal Product (IMP)

- **Study drug**: Arfolitixorin ([6R] 5,10-methylenetetrahydrofolic acid) is formulated as a lyophilized white to light yellowish or light beige powder containing 100 mg arfolitixorin per vial. Each vial of arfolitixorin must be reconstituted with 10 mL water for injection before administration as rapid IV bolus injections.
- **Comparator**: Leucovorin (LV; folinic acid) 400 mg/m^2 IV infusion.

Non-investigational medicinal products

- 5-FU, oxaliplatin, and bevacizumab.

Treatment regimens

Eligible and consenting patients will be randomized 1:1 to one of two treatment groups (A and B), administered according to the treatment regimens described below. Each new treatment cycle should start on day 14 (+7 days) after the previous treatment cycle (unless delayed due to toxicity):

Group A (ARFOX + bevacizumab):

- Bevacizumab 5 mg/kg, IV infusion in accordance with the label
  ↓ followed by
- Oxaliplatin 85 mg/m^2 IV infusion in accordance with the label
  ↓ followed by
- 5-FU 400 mg/m^2 IV bolus (2–4 minutes)
  ↓ followed by (30 minutes (± 5 minutes) after 5-FU bolus)
- Arfolitixorin 60 mg/m^2 rapid IV bolus (less than 3 minutes)
  ↓ followed by
- 5-FU 2400 mg/m^2 continuous IV infusion over 46 hours
  ↓ followed by (30-60 minutes after first arfolitixorin bolus)
- Arfolitixorin 60 mg/m^2 rapid IV bolus (less than 3 minutes)

Group B (modified FOLFOX-6 + bevacizumab):

- Bevacizumab 5 mg/kg, IV infusion in accordance with the label
  ↓ followed by
- Oxaliplatin 85 mg/m^2 IV infusion in accordance with the label
  ↓ followed by*
- LV 400 mg/m^2 IV infusion
  ↓ followed by
- 5-FU 400 mg/m^2 IV bolus (2–4 minutes)
  ↓ followed by
- 5-FU 2400 mg/m^2 continuous IV infusion over 46 hours

**Oxaliplatin and LV may be administered at the same time through two different injection ports. NB: Oxaliplatin and LV must not be mixed.*

The doses of arfolitixorin and LV should not be adjusted. 5-FU and LV should be administered separately to avoid the formation of a precipitate. Following a treatment-related adverse event the doses of 5-FU, oxaliplatin, and bevacizumab

may be adjusted or delayed in accordance with the label and section 6.3.4 in the protocol. Oxaliplatin cannot be substituted but caution should be taken regarding toxicity and in case of toxicity, dose modifications (decrease in infusion rate and/or dose reductions) according to label should be performed. For patients with platinum hypersensitivity, pre-medication with IV dexamethasone, chlorphenamine, and ranitidine can be given at the Investigator's discretion.

Prohibited concomitant therapies

Treatment with nucleoside analogues, known DPD inhibitors, additional anti-cancer treatment, additional experimental medications, and radiation therapy are prohibited during the screening period or in conjunction with the IMP.

Study visits

Study visits include a screening visit, a randomization/baseline visit, regular treatment visits every 2 weeks, and CT/MRI assessments every 8 weeks. Treatment visits will continue until progressive disease (PD) as assessed by the Investigator. The assessment visits will continue until centrally confirmed PD according to RECIST 1.1 criteria. An end of treatment visit will occur following PD or premature discontinuation of IMP. Follow-up (visit or phone call) for OS occurs every 12 weeks thereafter until end of study.

Study design

This is a randomized, multicenter, parallel-group, Phase III study to compare the efficacy of arfolitixorin versus LV in patients with advanced CRC treated by 5-FU, oxaliplatin, and bevacizumab.

Patients will be randomized in a 1:1 ratio to either the investigational arm (arfolitixorin) or the comparator arm (Leucovorin), following the completion of all screening assessments and after confirmation of their eligibility, using a stratified permuted block randomization. Randomization will be stratified for the following baseline factors:

- Geographic region (Europe *versus* North America *versus* Australia *versus* Japan)
- Primary tumor location (left colon *versus* right colon *versus* rectal cancer)
- Previous neo-adjuvant/adjuvant CRC treatment (yes *versus* no)

Tumor evaluations, based on CT/MRI, will be performed at baseline and every 8 weeks until PD by a blinded independent central review committee (according to RECIST 1.1 definitions). All patients will be treated according to the study protocol until PD as determined by the Investigator, unless prematurely discontinued from treatment for any reason. A BICR review will be performed to assess all tumor responses and progression in accordance with RECIST 1.1 definitions. BICR committee members will be blinded to all treatment data and perform their reviews in closed-meeting sessions. All activities and processes related to the BICR will be outlined in the BICR Charter.

Final analysis of ORR and PFS will be done when at least 235 PFS events are reached. An adaptive design allows for a re-estimation of the sample size based on interim results. If the sample size is increased to 660 patients, the final analysis of ORR and PFS will be done when approximately 450 PFS events have been observed. All patients will be followed for OS after end of treatment (regardless whether due to discontinuation/withdrawal or PD) until 60% OS events have been reached.

Premature discontinuation of treatment

Patients may withdraw consent to participation in the study or voluntarily discontinue treatment with IMP at any time without providing a reason and without any prejudice to future treatment. The Investigator may also, at his/her discretion, discontinue IMP at any time if it is in the best interest of the patients' well-being. The Sponsor can instruct the Investigator to discontinue IMP (e.g. due to severe non-compliance with the protocol). Patients discontinuing treatment will be asked to continue with follow-up assessments, including ongoing CT/MRI assessments until centrally confirmed PD or end of study.

Data and Safety Monitoring Board (DSMB)

An independent DSMB will be established and regularly monitor the safety profile and study progress to ensure that the study is being conducted with the highest safety, scientific and ethical standards. The DSMB Charter specifies the DSMB procedures and constitution, statistically derived interim analysis decision guidelines, as well as time points for DSMB evaluations. Recommendations from the DSMB to the Sponsor will comprise, in addition, increasing sample size, as well as early termination of the study or any other relevant recommendations.

An interim analysis will be performed when 16-week BICR evaluation has been performed for the 330th patient. The objective of the interim analysis is to calculate the conditional power for both ORR and PFS to guide continuing the study as planned or increasing the sample size. The guidelines for sample size increase decisions are defined in the DSMB charter and protocol section 9.4.6. There is no intention to claim efficacy for ORR or PFS at this stage. The interim results will only serve to guide the decision rules regarding sample size re-estimation. Results of this analysis will remain blinded to everyone, except the DSMB. Regardless of the efficacy results of the interim analysis, the trial will continue to accrue patients, until the total planned sample size is reached.

Number of planned patients & replacement

Approximately 490 patients will be randomized 1:1 to arfolitixorin or LV at approximately 80-100 study sites in Europe, Canada, the United States, Australia and Japan. Patients randomized but prematurely discontinuing before receiving any study treatment will be replaced.

The sample size calculation is based on the following assumptions:

- The ORR is 60% in Group A
- The ORR is 45% in Group B

For a two-sided test with α=0.05 and a power of 80%, 440 patients need to be treated to detect this difference, and also allow for an interim analysis on 330 patients. Adjustment of the significance level in the interim analysis and final analysis has been performed, using a Rho spending function, with ρ=5 for the primary endpoint (ORR) at the interim analysis, so that the overall 2-sided significance level does not exceed the desired α of 0.05. Although significance will not be claimed based on the interim results, this small alpha spending is foreseen to take into account the interim look at the results for ORR and PFS.

Initially, approximately 440 were planned to be randomized. However, Japanese authorities requested that approximately 12.7% of the total study population are enrolled from Japan. For this reason, additional patients from Japan were randomized. In the end a total of 490 patients were randomized. This decision to randomize extra Japanese patients was made before the interim analysis. The decision to include the Japanese patients in the ITT population (see Section 9.2.2) for the primary efficacy analysis was made after the interim analysis. The addition of extra Japanese patients leads to a statistical power greater than 80% for ORR.

Statistical Methods

A statistical analysis plan will be in place before including patients in the study. The analysis will be stratified for the following factors:

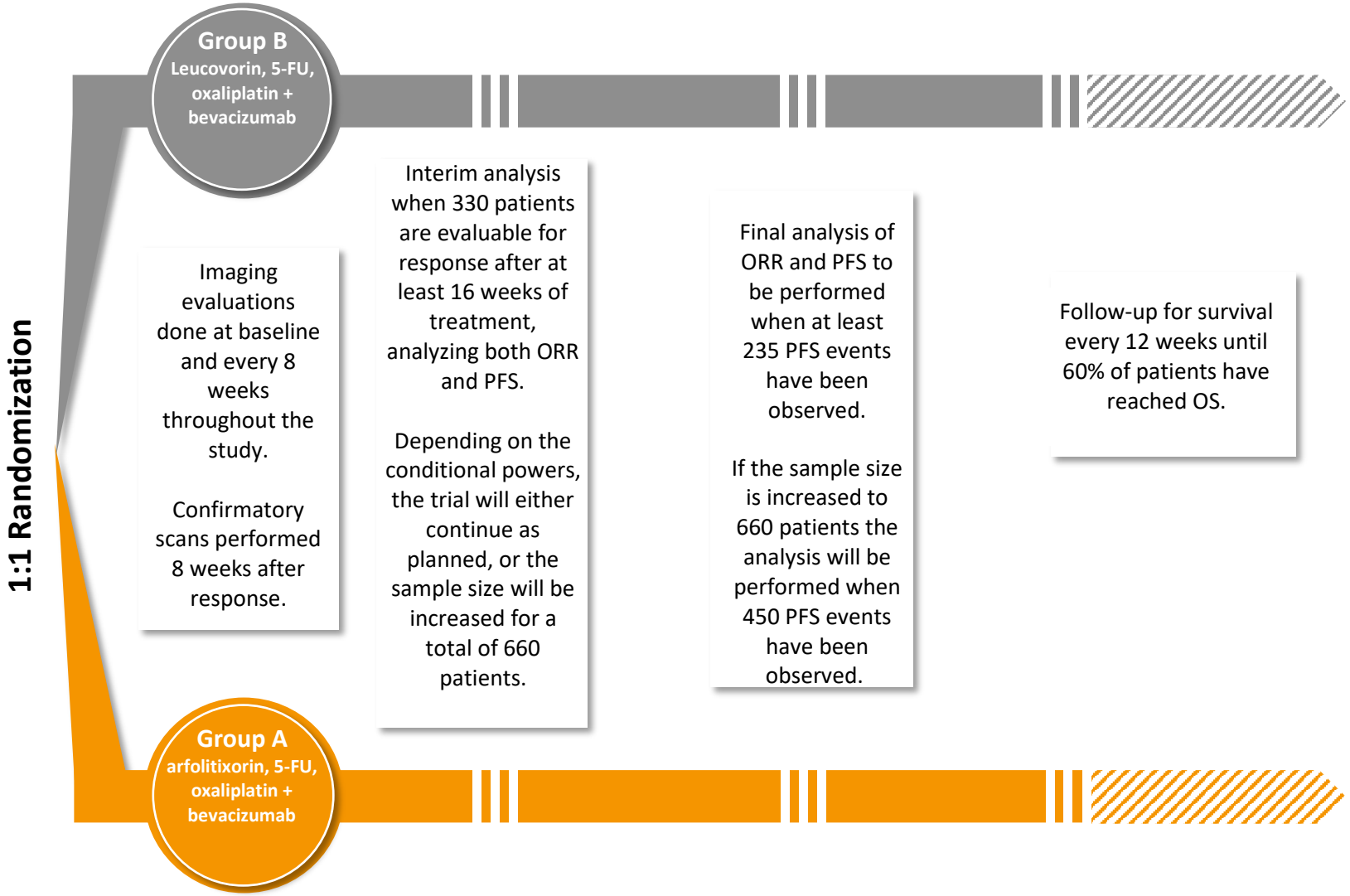
- Geographical region (Europe *versus* North America *versus* Australia *versus* Japan)
- Primary tumor location (left colon *versus* right colon *versus* rectal cancer)
- Previous neo-adjuvant/adjuvant CRC treatment (yes *versus* no)

All efficacy analyses will be performed on both the ITT and the per-protocol analysis sets. The ITT analysis will be the primary basis for interpreting study results.

ORR will be analyzed using a Cochran-Mantel-Haenszel test (CMH), stratified for the stratification factors used for randomization (geographic region, primary tumor location and previous neo-adjuvant/adjuvant CRC treatment).

PFS, DOR and OS will be analyzed using Kaplan-Meier curves, the logrank test and a stratified Cox proportional hazards model, using the same stratification factors as for randomization (geographic region, primary tumor location and previous neo-adjuvant/adjuvant CRC treatment). The assumption of proportional hazards will be tested. For AEs absolute and relative frequencies will be presented in total and by system organ class/preferred term, intensity, and relatedness.

1.2 SCHEMA


1:1 Randomization
Group B
Leucovorin, 5-FU, oxaliplatin + bevacizumab
Group A
arfolitixorin, 5-FU, oxaliplatin + bevacizumab
Imaging evaluations done at baseline and every 8 weeks throughout the study.
Confirmatory scans performed 8 weeks after response.
Interim analysis when 330 patients are evaluable for response after at least 16 weeks of treatment, analyzing both ORR and PFS.
Depending on the conditional powers, the trial will either continue as planned, or the sample size will be increased for a total of 660 patients.
Final analysis of ORR and PFS to be performed when at least 235 PFS events have been observed.
If the sample size is increased to 660 patients the analysis will be performed when 450 PFS events have been observed.
Follow-up for survival every 12 weeks until 60% of patients have reached OS.

1.3 SCHEDULE OF ACTIVITIES (SOA)

Table 1: Schedule of Activities (SOA)

		PRE-TREATMENT	TREATMENT PERIOD					END OF TREATMENT	FOLLOW-UP	
		Screening	Administer IMP		Assessment visit	Administer IMP	Assessment visit	End of treatment visit	Follow-up visits until centrally confirmed PD	Survival follow-up
			Cycle 1	Cycles 2-4		Cycles 5 - PD or discontinuation			*Only patients prematurely discontinuing IMP prior to centrally confirmed PD*	*All patients*
	Timing		Day 1 Baseline	14 days after previous admin visit	Week 8 from day 1	14 days after previous admin visit	Weeks 16, 24, etc. from day 1	Within 30 days after last dose of IMP and before start of new anti-cancer treatment	Every 8 weeks from end of treatment visit until centrally confirmed PD	Every 12 weeks after centrally confirmed PD until death or end of study
CSP Section	**Visit window**	Day -28 to Day -1	-3 days[a,f]	+7 days	±7 days	+7 days	±7 days		±7 days	±7 days
Screening procedures										
10.1.1	Informed consent	X								
8.3	Patient Study ID	X								
5.1/5.2	Eligibility criteria	X	X							
8.3	Demographics/medical history	X								
6.4	Randomization		X[a]							
General procedures										
6.6	Concomitant therapy	X	X	X	X	X	X	X		
8.2	Physical examination	X	X		X		X	X		
8.2	Vital signs	X	X	X	X	X	X	X		
8.2	Height	X								
8.2	Weight[e]	X	X	X	X	X	X	X		
8.2	Biopsy available for Pharmacogenetics	X								
6.1/6.2	Administer and record study treatment		X	X		X				
6.6	Record other anti-cancer treatment							X	X	X
8.1	ECOG	X	X		X		X	X	X	

		PRE-TREATMENT	TREATMENT PERIOD					END OF TREATMENT	FOLLOW-UP	
		Screening	Administer IMP		Assessment visit	Administer IMP	Assessment visit	End of treatment visit	Follow-up visits until centrally confirmed PD	Survival follow-up
			Cycle 1	Cycles 2-4		Cycles 5 - PD or discontinuation			*Only patients prematurely discontinuing IMP prior to centrally confirmed PD*	*All patients*
	Timing		Day 1 Baseline	14 days after previous admin visit	Week 8 from day 1	14 days after previous admin visit	Weeks 16, 24, etc. from day 1	Within 30 days after last dose of IMP and before start of new anti-cancer treatment	Every 8 weeks from end of treatment visit until centrally confirmed PD	Every 12 weeks after centrally confirmed PD until death or end of study
CSP Section	**Visit window**	Day -28 to Day -1	-3 days[a,f]	+7 days	±7 days	+7 days	±7 days		±7 days	±7 days
Efficacy assessments										
8.1	Plasma PK sample		X[b]	X[b]						
8.1	CT-scan/MRI[c]	X			X		X	X	X	
8.1	PRO EQ-5D		X		X		X	X		
8.1	Survival Status									X[g]
Safety assessments										
8.4	AEs		X	X	X	X	X	X[d]	X[d]	
8.2	PRO CTCAE		X	X	X		X	X		
8.2	Hematology and clinical chemistry[e]	X[f]		X		X		X		
8.2	Urinalysis[e]	X[f]		X		X		X		
8.2	ECG[h]		X	X						
8.2	Pregnancy test	X[f]						X		

[a] Randomisation can occur on the same day as study treatment initiation, or up to 3 days before study treatment initiation. Study treatment must be initiated within 28 days from start of screening.

[b] Plasma PK samples will be collected during Cycle 1 and Cycle 2.

[c] CT-scans/MRIs must be performed on thorax, abdomen and pelvis. Post-randomisation CT-scans/MRIs should be performed every 8 weeks (±7 days) from Day 1 to PD confirmed by BICR. End of Treatment visit scans will be taken if the previous scan is >14 days.

[d] AEs are to be recorded until the day of the End of Treatment Visit. After the End of Treatment Visit, only SAEs related to study specific procedures or considered to be at least possibly related to study drug should be recorded.

[e] Laboratory, urine samples and weight are to be collected according to local practice (7 days before study treatment). The laboratory and urinalysis reports must be obtained and reviewed prior to administration of chemotherapy for each cycle.

[f] Haematology, clinical chemistry, urinalysis, and pregnancy testing must be performed within 7 days prior to study treatment initiation and must be assessed before randomization

[g] Survival follow-up visit could be conducted either by phone. ordinary visits, hospital records or other means found suitable.

[h] 12-lead ECG should be performed during administration visits 1-4. Directly prior to and 10 min (±5 min) after each arfolitixorin dose, and directly prior to and 10 min (±5 min) after initiation of Leucovorin infusion, during administration visits 1-4.

2 INTRODUCTION

2.1 STUDY RATIONALE

2.1.1 ADVANCED COLORECTAL CANCER

Colorectal cancer (CRC) is the third most common cancer in men (10% of the total) and the second in women (9.2%), with over 1.3 million cases (746 000 men and 614 000 women) reported worldwide during 2012. The geographic incidence of CRC varies widely across the world, and the geographical patterns are very similar in men and women. Incidence rates vary ten-fold in both sexes worldwide, the highest estimated rates being in Australia/New Zealand (the age standardized rate (ASR) 44.8 and 32.2 per 100 000 in men and women respectively), and the lowest in Western Africa (4.5 and 3.8 per 100 000). The incidence increases with age and is highest amongst the elderly, i.e., 60-64 years: 67.4; 65-69 years: 95.1; 70-74 years: 127.8; and ≥ 75 years: 196.2 per 100 000 [1].

Approximately 40-50% of the affected patients develop metastatic disease and more than half a million deaths are reported annually as a consequence of CRC [2]. CRC accounted for 694 000 deaths worldwide solely during 2012 (8.5% of total cancer deaths) [1].

2.1.2 CURRENT STANDARD OF CARE

CRC patients are usually treated surgically and, in most circumstances, with curative intent. Surgery, in fact, remains the primary modality of treatment for malignancies of the lower gastrointestinal tract, and standard resection is the only therapy required for early-stage cancer [3].

As the stage of the tumor advances, in terms of depth of penetration and lymph node involvement, the chance of cure with surgery alone diminishes and the rate of local recurrence increase. In such cases, surgery may either be combined with adjuvant treatment or be performed for palliative control of symptoms only.

Standard first-line systemic therapy for metastatic colorectal cancer (mCRC), in accordance with current European Society for Medical Oncology (ESMO) guidelines, is chemotherapy with 5-fluorouracil (5-FU) + Leucovorin (LV) + oxaliplatin/irinotecan ± bevacizumab [4].

2.1.2.1 CYTOTOXIC AGENTS

The backbone of first-line palliative chemotherapy alone, as well in combination with targeted agents, consists of a fluoropyrimidine (FP; intravenous [IV] 5-FU or the oral FP capecitabine) in various combinations and schedules [5, 6].

LV is a part of a standard treatment regimen for advanced CRC in combination with the anti-metabolite 5-fluorouracil (5-FU) as it greatly improves the efficacy of 5-FU [7, 8]. Arfolitixorin is an endogenous folate-based compound designed to replace LV

The oral FP capecitabine is an alternative to IV 5-FU/LV [7, 8]. Combination chemotherapy with 5-FU/LV/oxaliplatin (FOLFOX) or 5-FU/LV/irinotecan (FOLFIRI) provides higher response rates (RRs), longer PFS and better survival than 5FU/LV alone [5, 6, 9, 10]. FOLFOX and FOLFIRI as chemotherapy alone have similar activity and are both partners for biologics but have a different toxicity profile:

more alopecia and, in most trials, more severe diarrhea for irinotecan and more polyneuropathy for oxaliplatin [5, 11]. They also have potentially different interactions with biologicals.

2.1.2.2 BIOLOGIC AGENTS

Bevacizumab, an antibody that binds circulating VEGF-A, increases the activity of any active cytotoxic regimen. Bevacizumab has been shown to improve RR, PFS and/or OS when used as first-line treatment in combination with 5-FU/LV/irinotecan and in combination with 5-FU/LV or capecitabine alone [12, 13, 14, 15]. Bevacizumab has also been shown to improve the PFS but not RR or OS in combination with an FP plus oxaliplatin in the first-line treatment of mCRC [16, 17]. Bevacizumab is usually continued in combination with a cytotoxic agent/combination until PD or toxicity.

2.1.3 MECHANISM OF ACTION OF ARFOLITIXORIN

The Active ingredient of the Drug substance is arfolitixorin or [6R]-5,10-methylene-tetrahydrofolic acid ([6R]-5,10-MTHF). It is present as such in the reconstituted solution from the Drug product lyophilizate. The principal mechanism of action is ultimately the same for arfolitixorin as for LV or L-LV [18], used together with 5-FU as [6R]-5,10-MTHF is the key endogenous active metabolite of these drugs, and is a necessary co-substrate in the synthesis of deoxy-thymidine monophosphate (dTMP) from deoxy-uridine monophosphate (dUMP), a reaction catalyzed by the enzyme thymidylate synthase (TS). dTMP is in turn a necessary substrate for DNA synthesis and repair and is thus essential for cell division and life [19]. Indeed, the antitumor agent 5-FU is known to exert its cytotoxic activity, to a major extent, through inhibition of the TS enzyme [18].

For either synthesis of dTMP from dUMP via TS catalysis, or inhibition of TS by 5-FdUMP (a metabolite of 5-FU), a ternary complex is formed between TS, [6R]-5,10-MTHF, and the respective third component, either dUMP or 5-FdUMP. In the (normal) synthesis situation dUMP picks up the methylene (the 1-carbon unit) from [6R]-5,10-MTHF while bound in the ternary complex to form dTMP. dTMP is then released during complete decomposition of the ternary complex. The transformation of dUMP into dTMP occurs simultaneously with a 5-H abstraction from the dUMP molecule. If instead 5-FdUMP has become bound into the ternary complex, the 5-F atom cannot be abstracted. The ternary complex instead remains stable and the TS enzyme is inhibited. This is because the enzyme TS cannot be released from the 5F-containing ternary complex for a renewed catalytic reaction [18].

The relatively stable FdUMP- containing “inhibitory” ternary complex can, however, release its [6R]-5,10-MTHF moiety and initiate complete decomposition of this ternary complex. Since the release step is an equilibrium reaction, increased concentration of [6R]-5,10-MTHF will stabilize the (“inhibitory”) ternary complex and thereby strengthen the TS inhibition. This means that the higher the concentration of [6R]-5,10-MTHF, the higher the stability of the FdUMP-containing “inhibitory” ternary complex.

In vivo tetrahydrofolic acid (THF) and 5-methyl-THF are rapidly formed after administration of both arfolitixorin and LV/L-LV and these two metabolites together constitute a “metabolite pool”, which can re-generate [6R]-5,10-MTHF [18]. Plasma concentration data from a PK/PD study, showing very high (transient) plasma levels of [6R]-5,10-MTHF immediately after the administration of arfolitixorin and hardly any after L-LV [20]. These much higher levels during the first 30 minutes after arfolitixorin may be particularly relevant for efficacy. Our selected dose regime for arfolitixorin, involving two bolus doses separated by 30 minutes, is thought to be supported by the PK/PD situation.
Arfolitixorin, [6R]-5,10-MTHF, circumvents the genetically influenced multistep metabolic activation necessary for biological activity of LV/L-LV and consequently, is thought to be efficacious in a larger

proportion of patients and with significantly less inter- and possibly intra-individual variability [18]. This together with all the direct PK/PD advantages is thought to serve to make arfolitixorin a better candidate for improved outcome of 5-FU-based chemotherapy regimens in advanced CRC provided that an acceptable benefit/risk ratio can be maintained.

2.2 BACKGROUND

2.2.1 NON-CLINICAL BACKGROUND

The nonclinical data are described in the current version of arfolitixorin Investigator's brochure.

Arfolitixorin has been evaluated in preclinical GLP (Good Laboratory Practice) rat and dog studies. In summary, twice daily IV administration of arfolitixorin 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 (API) of arfolitixorin has also been evaluated in various non-GLP mouse models in combination with different anti-metabolites such as pemetrexed and 5-FU. No indications of safety issues related to arfolitixorin have been observed in these studies.

A 13-week (3 months) toxicology program in rats and beagle dogs concluded that the twice daily intravenous administrations of arfolitixorin to Sprague-Dawley rats at doses of up to 300 mg/kg (150 mg/kg b.i.d) for 13 weeks was well tolerated, causing non-adverse histopathological changes at the site of administration and the spleen (extramedullary hematopoiesis) The majority of findings showed clear evidence of recovery during the two-week off-dose period, and those without recovery were all considered non-adverse. Therefore, the highest dose of 300 mg/kg (150 mg/kg b.i.d) was considered to be the no-observed-adverse-effect level (NOAEL).

In Beagle dogs for a 13 weeks administration in cycles of 5 out of 7 days during 3 weeks on and 1 week off was well tolerated and resulted in findings in the kidneys of females, and a non-toxicologically relevant higher ALT and AST activity and an increase in urea and creatinine level, at 375 mg/kg/day. All renal changes had resolved after a 2-week recovery period. Therefore, the dose of 100 mg/kg/day arfolitixorin was considered the NOAEL (No Observed Adverse Effect Level).

2.2.2 CLINICAL BACKGROUND

The complete clinical data are described in the current version of the arfolitixorin Investigator's brochure.

One phase I/II clinical trial with arfolitixorin in patients diagnosed with operable CRC was performed with the aim of comparing PK/PD profiles in plasma, tumors, and tumor adjacent mucosa. In this clinical trial (ISO-CC-002), 60 or 200 mg/m^2 of arfolitixorin or L-LV was administered as single doses prior to surgery. Thirty-two patients were included in this trial, of whom 16 were exposed to arfolitixorin. The report from Clinical Trial ISO-CC-002 has been finalized and the results have been published [21]. The study explored four folate metabolites: [6R]-MTHF, [6S]-5-methyl-tetrahydrofolate (5-methyl-THF), and [6S]-5-THF (THF). The distribution/PK results from the final report show that arfolitixorin administration resulted in higher levels of [6R]-MTHF and THF in tumors and the surrounding mucosa than did L-LV. Arfolitixorin to L-LV ratios in the tissues were 2.5-4.0 for

[6R]-MTHF and 2.4-5.6 for THF. Arfolitixorin and L-LV administration resulted in [6S]-5-methyl-THF levels of the same magnitude in tumors and the surrounding mucosa. Arfolitixorin and L-LV gave similar concentrations of [6S]-5-methyl-THF in plasma.

In another phase I/II clinical trial in 24 patients with operable rectal cancer (ISO-MC-091) arfolitixorin was administered at a dose of 10, 50, 100, or 500 mg/m^2 once every week for 10 weeks in combination with Pemetrexed in a neo-adjuvant setting. The primary objective was to evaluate the feasibility of arfolitixorin and Pemetrexed as neo-adjuvant treatment and to identify the optimal dose of arfolitixorin. Secondary aims were to evaluate pathological complete response rate, sphincter-saving surgery, and levels of arfolitixorin and its metabolites in tumors, normal mucosa and plasma. The study was terminated prematurely after four of the five intended doses of arfolitixorin had been administered (10, 50, 100, and 500 mg/m^2). The results obtained by then did not indicate any improvement in Pemetrexed efficacy with any dose level of arfolitixorin and it was concluded that no further information would be gained by administering the 200 mg/m^2 dose. The findings on safety and adverse events (AEs) have been published [22]. There were no AEs in the study that could specifically be ascribed to arfolitixorin.

Another clinical trial, ISO-MTX-003, with arfolitixorin as a rescue agent after high dose methotrexate (HDMTX) administration in osteosarcoma patients has been completed. ISO-MTX-003 was a multi-center study designed to (i) investigate the safety of arfolitixorin rescue therapy, (ii) identify a recommended dose for further study, (iii) and evaluate the feasibility of arfolitixorin as rescue therapy for HDMTX treatment. Eight (8) patients in two dose cohorts, 15 mg/m^2 and 7.5 mg/m^2 were exposed to arfolitixorin. The study findings suggest that the arfolitixorin dose of 15 mg/m^2 works as rescue treatment at least as well as the standard of care (with calcium folinate) of 15 mg/m^2 while the lower arfolitixorin dose of 7.5 mg/m^2 seems to involve a slightly lower recue ability in relation to standard of care. The dose of 15 mg/m^2 was selected for further clinical development.

Yet another trial in colorectal cancer has been initiated, the ISO-CC-005. The trial investigates the tolerability of arfolitixorin in patients with stage IV CRC simultaneously treated with 5-FU alone or together with oxaliplatin (85 mg/m^2) or irinotecan (180 mg/m^2) and also to define a dose for subsequent trials. Toxicity will be evaluated for four dose levels (30, 60, 120 and 240 mg/m^2). The selected dose from the arfolitixorin, 5-FU and oxaliplatin treatment arm will then be evaluated for safety together with bevacizumab prior to study start. 105 patients in this trial have been exposed to arfolitixorin.

According to IB version 14, dated 1 Oct 2021, seven hundred and thirteen (713) patients have been enrolled in Isofol Medical AB's clinical development program and four hundred and twenty (420) have received at least one administration of arfolitixorin in doses of 7.5 to 500 mg/m^2 alone or in combination with different cytostatic agents. The 420 patients include 388 patients with rectal/colon cancer, 8 patients with osteosarcoma and 24 adult healthy male subjects. A total of 226 serious adverse events (SAEs) have been reported for patients exposed to arfolitixorin, 25 of which were assessed as related to arfolitixorin.

Consistent with the outcomes reported from previous studies, in particular ISO-CC-005, a review of reported events in study ISO-CC-007 to date indicates that fatigue and gastrointestinal adverse events (AEs), such as nausea and diarrhea, were the most commonly reported AEs among those patients exposed to arfolitixorin.

The collected knowledge on arfolitixorin indicates that the drug is safe and well tolerable at all doses tested (7.5 to 500 mg/m^2). Treatment emergent AEs are often attributable to the co-administered chemotherapy agents rather than to arfolitixorin itself, in fair agreement with the known safety

profiles for other folates. The results show that treatment with the chosen Selected phase 2 dose (SP2D) 120 mg/m^2 arfolitixorin is safe and feasible in dosing settings similar to the well-established mod-FOLFOX-6 and FOLFIRI.

Based upon existing non-clinical and clinical data to date, there are no safety concerns identified.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

No toxicity directly related to arfolitixorin was observed in toxicity studies performed in rat and dog.

In a healthy volunteer study (ISO-FF-001) to investigate QT effects, 33 subjects were randomized to placebo (n=9) and three doses of arfolitixorin, given as a 2-minute bolus injection in the following doses: 200 mg/m^2 (n=8); 350 mg/m^2 (n=8); or 500 mg/m^2 (n=8). The study data indicate that in relation to baseline, arfolitixorin causes a dose-dependent increase in QTcF that is transient. For the lowest dose level 200 mg/m^2 the effect is small and not considered to add risks or warrant increased surveillance for patients.

Safety data from two initial investigations in CRC patients show no evidence of any safety concerns for patients after either single high-doses or multiple doses of arfolitixorin. These clinical data indicate that arfolitixorin, in doses of up to 500 mg/m^{2}are well tolerated as bolus IV administration in CRC patients.

Arfolitixorin, in doses of 30, 60, 120, or 240 mg/m^2, were administered to CRC patients in combination with 5-FU every 2 weeks for at least 8 weeks in a Phase I/II dose-finding and safety study (ISO-CC-005). Interim data from this study indicate that all doses are well tolerated with no indications of safety concerns.

Further details from the non-clinical and clinical studies can be found in the Investigator's Brochure (IB).

2.3.2 KNOWN POTENTIAL BENEFITS

Arfolitixorin is the active metabolite of LV and in contrast to LV does not require genetically regulated enzymatic activation. There is a statistically significant correlation between the level of expression of certain key genes and the rate of metabolic activation, as measured by tissue concentrations of [6R]-MTHF [20]. The level of expression of folate-relevant genes correlates with outcome and survival in advanced CRC patients treated with 5-FU and folates. Approximately two thirds of advanced CRC patients fall into the category of low gene expression having a worse prognosis [23].

It has been demonstrated that the administration of arfolitixorin will result in greater bioavailability and higher intracellular concentrations of the active metabolite than does LV administration in all patients, as several metabolic steps are bypassed by arfolitixorin administration and varying genetic profiles are rendered unimportant [23]. Further it is anticipated that arfolitixorin may reduce potential interpatient variability and as a consequence allow for safe and more adaptable and predictable treatments. The hypothesis is therefore that administration of arfolitixorin compared to LV in combination with 5-FU will result in better efficacy.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Arfolitixorin is expected to be more effective than LV in potentiating the effect of 5-FU, particularly in patients with low folate-relevant gene expression. An exploratory endpoint of this study is to compare the impact of certain folate transportation and metabolism related genes on treatment outcome for arfolitixorin and LV, to establish the magnitude of the hypothesized improvement.

To minimize the number of patients exposed to the investigational drug, the design of the study is adaptive, and contains interim analysis and a final analysis (see section 9.4.6). The interim analysis includes an opportunity to increase the sample size of the trial adaptively, based on the difference in the primary endpoint results.

A DSMB consisting of at least three experienced members including at least an oncologist and one biostatistician will be appointed to review, on a regular basis, accumulating safety data as well as efficacy data according to the interim analysis plan. The DSMB will review unblinded data that will not be shared with anyone else (Sponsor or Investigators). At each of their meetings, the DSMB will recommend a course of action: stop the trial, amend the trial protocol, or continue the trial unchanged. The DSMB recommendation will be based on safety considerations and, when appropriate, on efficacy considerations as outlined in the interim analysis plan.

The sponsor (or designee) will be responsible to safeguard the ethical and safety interests of the patients, Investigators and the sponsor; to assess the safety of the study's interventions; to monitor the overall conduct of the study and to protect its validity and credibility. The sponsor (or designee) will meet at predefined times (or also ad hoc, if deemed necessary), to assess at intervals the progress of the clinical study and the critical efficacy endpoints (e.g. ORR and PFS) and will decide whether to continue, modify or stop the study for safety.

The study will be reviewed and not initiated before approval from relevant IRB/IEC have been obtained. The study will also be monitored, according to GCP and local requirements, securing the trial patients' well-being and the integrity of data collected.

The patients in this study will be closely monitored at the participating study sites following the visit schedule (see section 8.3).

Arfolitixorin is the active metabolite of the commercially available prodrug LV and is intended as an improvement of established care. The efficacy is presumed to be as good as or better than that of LV. A comparative study is necessary to assess the magnitude of efficacy improvement. The study design, the close monitoring of the study patient from the Investigators, the DSMB and the study monitors contributes to preserving the well-being of the trial patients minimizes the risk of study participation.

2.3.3.1 ADVERSE EVENTS OF SPECIAL INTEREST

The hypothesis is that arfolitixorin in combination with 5-FU will increase tumor cell killing by increasing the active metabolites MTHF and THF within the tumor. However, the increases in these metabolites may also occur in the normal tissue. The two tissues that may have increased toxicity with arfolitixorin in combination with 5-FU are bone marrow and the mucous membranes. Therefore, these will be monitored and assessed as Adverse Events of Special Interest (AESI) during the trial and by the DSMB.

Seventy patients have been treated with arfolitixorin in study ISO-CC-005 and are evaluable after a 30-day cut off from their first dose of therapy across the arfolitixorin dosage administered which ranged from 7 patients treated at 240 mg/m^2, 13 subjects treated at 30 mg/m^2, 20 patients treated at 60 mg/m^2 and 35 patients treated at 120 mg/m^2. The incidence of mucositis in the combination of

arfolitixorin + 5-FU with or without other chemotherapeutic agent's ranges from 1-5% of all reported AEs. Similarly, bone marrow adverse events are also between 1-5% of all reported AEs in in patients treated with arfolitixorin + 5-FU combinations. The incidence of either mucositis or bone marrow suppression (neutropenia and thrombocytopenia) appear to be similar to what is reported in clinical practice for patients treated with FOLFOX + Bevacizumab.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Overall response rate (ORR)	Best ORR, defined as the best response recorded from the start of the study treatment until the end of treatment. All responses will be confirmed 8 weeks after onset of response. All assessments including confirmation of response will be performed by BICR.	The antitumor effect attributable directly to the drug, not the natural history of the disease.
Key Secondary		
Progression-free survival (PFS)	PFS, defined as time from randomization to first occurrence of tumor progression (RECIST 1.1) assessed by BICR based on CT-scans/MRIs conducted every 8 weeks after treatment, or death from any cause.	PFS is a relevant endpoint to measure clinical effect as it also includes stable disease.
Duration of Response (DOR)	The duration of overall response (DOR) is measured from the first time point at which criteria are met for complete response (CR) or partial response (PR) through the last time point when overall response has been objectively documented.	DOR measures the duration of confirmed CR and PR, which is an important aspect of the clinical significance of DOR.
Secondary		
Overall survival (OS)	OS, defined as time from randomization to death from any cause.	OS measures potential detrimental effects of therapy, and possibly a direct benefit.
Quality of Life	Quality of Life assessed using the EQ-5D patient reported outcome questionnaire.	PRO EQ-5D measures patients self-experienced quality of life.
Safety and tolerability	Number and severity of adverse events (AEs), including clinically significant abnormal laboratory findings, regardless of causal relationship to arfolitixorin or LV. Specific AEs will be followed using patient reported outcome questionnaire (NCI PRO CTCAE).	Relevant endpoint to measure safety and tolerability.
Patients undergoing curative metastasis resection.	Defined as the number of patients qualifying for curative	To understand study treatment effects on

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	metastasis resection after treatment with study drug.	metastasis become curatively resectable.
Exploratory		
Daily living abilities	Daily living abilities as assessed by the Eastern Cooperative Oncology Group (ECOG) Performance Status Scale	Physicians professional estimation of the patient's status.
To determine the pharmacokinetic (PK) characteristics of arfolitixorin and LV in patients with advanced CRC	The plasma concentration of [6R]-MTHF, methyl-THF, THF, will be determined in a limited number of patients in both treatment arms.	To increase the understanding of the study drug, the study treatment and also in relation to the comparator arm.
To investigate folate metabolism- and transportation-related gene expression levels in patients with advanced CRC	Gene expression levels of folate metabolism- and transportation-related genes, analyzed by sub-group low/high gene expression levels.	To increase the understanding of the study drug.
Recurrence Free Survival (RFS) for patients undergoing metastatic resection.	Defined as the time between the first surgery with complete removal of the metastasis and recurrence of the disease or death from any cause.	To increase the understanding of disease recurrence after metastasis resection.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a randomized, multicenter, parallel-group, Phase III study to compare the efficacy of arfolitixorin versus LV in patients with advanced CRC treated by 5-FU, oxaliplatin, and bevacizumab. To be eligible for the study, patients must have advanced CRC, without indication for resection, with at least one measurable lesion of metastatic disease according to RECIST 1.1 criteria [24] within 28 days of randomization.

Approximately 490 patients will be randomized 1:1 to receive either arfolitixorin or LV as a component of standard first line therapy for advanced CRC. The study will consist of a screening visit, a randomization visit during which patients will receive their first course of IMP, regular treatment visits every 2 weeks thereafter, and CT/MRI assessments every 8 weeks from baseline. Treatment visits are to continue until PD, as identified by the Investigator based on locally-performed assessment of CT-scans/MRIs. In the event of PD, Investigators are to discontinue IMP and manage the patient according to local routine. Patients will be followed thereafter until death.

Randomization minimizes potential bias. Multiple sites across geographic regions allows for widely applicable results. Baseline factors considered in the randomization will include:

- Geographical region (Europe *versus* North America *versus* Australia *versus* Japan)
- Primary tumor location (left colon *versus* right colon *versus* rectal cancer)

- Previous neo-adjuvant/adjuvant CRC treatment (yes *versus* no)

Patient and Investigator blinding is not possible as there is a difference in the color of the injection fluids and the method of administration. However, as a measure to minimize any bias arising either from the inability to blind patients and Investigators or from potential inconsistencies in local tumor response assessments across study sites, analyses of the tumor response endpoints will be based on blinded, retrospective, centrally-adjudicated assessment of CT-scans/MRIs according to a pre-specified Image Review Charter.

An interim analysis will be performed when 16-week BICR evaluation has been performed for the 330th patient. The objective of the interim analysis is to calculate the conditional power for ORR and PFS to continue the study as planned or increasing the sample size. The guidelines for sample size increase decisions is clearly defined in the DSMB charter and in section 9.4.6. Regardless of the efficacy results of the interim analysis, the trial will continue to accrue patients, until the total planned sample size is reached.

Initially, approximately 440 were planned to be randomized. However, Japanese authorities requested that approximately 12.7% of the total study population are enrolled from Japan. For this reason, additional patients from Japan were randomized. In the end a total of 490 patients were randomized. This decision to randomize extra Japanese patients was made before the interim analysis. The decision to include the Japanese patients in the ITT population (see Section 9.2.2) for the primary efficacy analysis was made after the interim analysis.

The decision-making process to terminate a trial early due to evidence of harm or benefit is complex and is defined in section 9.4.6.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Standard first-line systemic therapy for mCRC, is chemotherapy with 5-FU + LV + oxaliplatin/irinotecan ± bevacizumab. LV is a prodrug of the active substance MTHF, which has a central role in the potentiation of the 5-FU-mediated inhibition of TS and subsequently halting DNA synthesis and cell proliferation.

Arfolitixorin is the active metabolite of LV and in contrast to LV does not require genetically influenced multistep metabolic activation. Distribution results have shown that arfolitixorin versus LV administration of equimolar doses resulted in higher levels of [6R]-MTHF and THF in tumors and the surrounding mucosa for arfolitixorin [20].

Arfolitixorin is therefore expected to be more effective than LV, particularly in patients with low folate-relevant gene expression. Further it is suggested that arfolitixorin may reduce potential interpatient variability and as a consequence allow for safe and more adaptable and predictable treatments. The purpose of this study is to compare arfolitixorin to LV to establish the magnitude of the hypothesized improvement.

The study is designed as a superiority study. To minimize the number of patients exposed to the investigational drug, the design of the study is adaptive, and contains interim analysis and a final analysis (see section 9.4.6 for details). The interim analysis includes an opportunity to increase the sample size of the trial adaptively, based on the conditional power for ORR and PFS.

Patient and Investigator blinding is not possible as there is a difference in color of the injection fluids and the method of administration (intravenous bolus injection versus infusion). However, as a

measure to minimize any bias arising either from the inability to blind patients and Investigators or from potential inconsistencies in local tumor response assessments across study sites, analyses of the tumor response endpoints will be based on blinded, retrospective, centrally-adjudicated assessment of CT-scans/MRIs according to a pre-specified Image Review Charter.

4.3 JUSTIFICATION FOR DOSE

The mechanism of action and the rationale for combining folate derivatives with 5-FU is universally accepted and applied [18, 19]. In treatment of colorectal cancer with cytotoxic combinations comprising 5-FU together with folate derivatives, the principal role of the folate component is to potentiate and prolong the inhibitory effect of 5-FdUMP (a 5-FU metabolite) on TS and thereby reduce the supply of thymidine for DNA synthesis and cell replication whilst maintaining an acceptable clinical benefit-risk ratio. Despite universal use of this combination for decades, the literature is scarce regarding systematic studies aiming to define optimal doses of each drug in the combination. Instead the focus is on the nature of the disease and the intrinsic statistical variance with ensuing large sample sizes on one hand and the demand for therapeutic progress on the other necessitated a different route of advancing. The folate predominantly used in this context has been LV, which requires multistep enzymatic conversion to the active metabolite [6R]-5,10-methylenetetrahydrofolate. In this pivotal study, ISO-CC-007, the logical reference therapy is LV and the reference treatment regimen is modified FOLFOX-6 plus bevacizumab, which is a frequently used standard regimen in the treatment of advanced CRC patients. For the experimental arm, the LV infusion of the modified FOLFOX-6 therapy is exchanged to arfolitixorin. Arfolitixorin is however not suitable for infusion due to a short half-life. Arfolitixorin is therefore administered as bolus injection.

The Sponsor has considered the following aspects when selecting the dose for future development of arfolitixorin:

1. The Sponsor has taken previous efforts by Adventrx Inc in developing the dual diastereoisomers [6R,S]-5,10-methylenetetrahydrofolate into account. The development was unsuccessful for various reasons, particularly due to the infusion administration of [6R,S]-5,10-methylenetetrahydrofolate, which is highly unstable in aqueous solution over 2 hours.

2. Comparison of dose levels with marketed reduced folates (prodrugs of arfolitixorin) i.e., LV and L-LV and failed attempts to develop [6R]-MTHF. LV is and has been used in doses from 15 mg/m^2 every six hours IV to 1500 mg/m^2 every six hours IV as rescue after high dose methotrexate treatment [25, 26]. Furthermore, in combination with 5-FU in colorectal cancer, doses from 20 mg/m^2 IV to 500 mg/m^2 IV over 2 hours every second week have been used [4, 20].

3. PK and tissue concentration data of arfolitixorin and LV collected from previous studies, i.e. the ISO-CC-002 and ISO-CC-005 studies. The results obtained demonstrate that arfolitixorin administration leads to much greater exposure and levels of MTHF and THF compared to administration of an equimolar L-LV dose. The identified differences in PK/PD with respect to [6R]-MTHF and THF levels in tumor, surrounding mucosa, and plasma after administration of arfolitixorin and L-LV suggest that it might be possible to modify the outcome of present well-established treatments within different cancer therapeutic areas that are based on chemotherapy regimens including folates.

4. The amalgamated pre-clinical safety experience for arfolitixorin shows no toxicity or adverse effect directly related to arfolitixorin in either the preliminary or 28-day toxicity studies performed in rats and dogs. At that time, the highest dose tested was considered to be the maximum feasible dose. Roughly determined, maximum exposure in rats and dogs was more than 50-fold compared to estimated exposure at 120 mg/m^2. The initiated 3-month toxicity studies in the same species are expected to further confirm the safety of arfolitixorin and will also investigate higher concentrations with the aim of reaching the maximum tolerated dose (MTD).

5. The arfolitixorin Investigators Brochure was last updated in 9 July 2018. Up until 20 August 2018, arfolitixorin has been administered to over 145 patients and administered more than 1000 times in dosages varying from 7.5 to 500 mg/m^2 alone or in combination with different cytostatic agents. Results from the ongoing ISO-CC-005 study, designed to investigate the tolerability of various doses, has evaluated dose levels of 30, 60, 120 and 240 mg/m^2 of arfolitixorin in combination with 5-FU and oxaliplatin with monitoring of clinical and laboratory safety data. Preliminary results show that arfolitixorin in a dose range of 30-240 mg/m^2 is not linked to worsened tolerability compared to historical experience.

6. The main mechanism of action is a folate-enhanced and prolonged inhibition of TS. Plasma deoxyuridine levels (pdUrd) is a marker for the global (all cells and organs in the organism) inhibition of TS. The influence on pdUrd of Leucovorin, an equimolar and several incremental doses of arfolitixorin have now been established.

Arfolitixorin gives enhanced levels of pdUrd compared to an equimolar standard dose of Leucovorin and a dose dependent increase of pdUrd with incremental doses of arfolitixorin which levels off between the doses 60 and 240 mg/m^2 BSA. Doses above 120 mg are therefore unlikely to result in much higher efficacy and the doses of arfolitixorin of both 60 and 120 m/m^2 are deemed adequate for efficacy under the above hypothesis.

The decision to use two separate injections is supported by the following data based on both safety and efficacy considerations:

ISO-CC-005 study data demonstrates no dose-related increase in toxicity when 28 patients were dosed with 120 mg/m^2 administered with two separate injections of 60 mg/m^2 each.

PK and metabolism data from the ISO-CC-002 study suggest attaining a high C_{max} on two occasions achieved with the two injections of 60 mg/m^2 may move more arfolitixorin into the cells. In the ISO-CC-002 study, a bolus dose of 60 mg/m^2 arfolitixorin provides a pronounced transient increase of the [6R]-5,10-MTHF levels in plasma but disappears within 30 min.

Furthermore, 5-FU given as a bolus is known to be rapidly (in 10 to 20 minutes) converted into 5-FdUMP, which binds highly efficiently to the TS enzyme in tumors under the formation of a binary complex. The binary complex can be further strengthened by supplying arfolitixorin at the early stage after 5-FU administration [18]. For this reason, the high transient plasma level of [6R]-5,10-MTHF should co-exist with the high concentration of 5-FdUMP in the tumor. An overall optimal treatment should therefore be obtained by dividing the 120 mg/m^2 dose of arfolitixorin into two bolus doses, and to achieve a high [6R]-5,10-MTHF intracellular concentrations over a longer period of time the bolus doses should be separated by 30-60 minutes.

LV 400 mg/m^2 IV infusion has been chosen as the comparator as this is the agent recommended in advanced CRC treatment guidelines [4].

Arfolitixorin or LV will be administered in combination with 5-FU, oxaliplatin, and bevacizumab. This is standard first-line adjuvant therapy according to current ESMO and NCCN guidance. All patients in this study will receive bevacizumab as it appears to improve PFS when used in combination with 5-FU/LV/oxaliplatin [4, 27]. The doses of 5-FU, oxaliplatin, and bevacizumab specified in this protocol are those commonly used in clinical practice and according to their labels.

4.4 END OF STUDY DEFINITION

A patient is considered to have completed the study if he or she has completed all phases of the study including the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3, i.e. the last contact (phone, hospital records or other means found suitable) to collect the death date.

The final analysis of ORR and PFS will be performed when at least 235 PFS events have been observed. If the sample size is increased to 660 patients, the final analysis of ORR and PFS will be performed when approximately 450 PFS events have been observed. All study patients will be followed for OS a until 60% of OS events are achieved for the study population, i.e. 294 OS events in case the sample size is not increased, and 396 OS events in case the sample size is increased to 660 patients.

At the time of final analysis of ORR and PFS, patients still on IMP treatment will continue IMP treatment until locally assessed progressive disease or discontinuation of IMP for other reasons. After completed End of Treatment visit these patients have reached the End of Study definition and will thereafter move into survival follow-up until 60% of OS events are achieved for the study population.

Patients already in follow-up phase at the time of final analysis of ORR and PFS have reached the End of Study definition and will move into survival follow-up until 60% of OS events are achieved for the study population.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

For randomization in the study, patients must fulfill all of the following criteria:

1. Colorectal adenocarcinoma verified by biopsy.
2. Availability of biopsy material, from the primary tumor or metastasis, allowing for analysis of tumor gene expression.
3. Non-resectable metastatic CRC planned for first line therapy with 5-FU, Leucovorin, oxaliplatin, and bevacizumab.
4. Evaluable disease with at least one measurable lesion of metastatic disease (≥10 mm in longest diameter on axial image on CT-scan or alternatively MRI with <5 mm reconstruction interval) or lymph node (≥15 mm in shortest axis when assessed by CT) within 28 days of randomization.
5. Life expectancy of more than 4 months.
6. ECOG performance status 0 or 1.

7. Hemoglobin (Hb) > 80 g/L, Absolute neutrophil count (ANC) > $1.5x10^9$/L. Thrombocytes > $100x10^9$/L.

8. Creatinine clearance > 50 mL/min, Total bilirubin < 1.5 x ULN,
   AST and ALT < 3 x ULN (and < 5 x ULN in case of liver metastases).

9. Male or female ≥18 years of age.

10. Female patients of childbearing potential must have a negative urine pregnancy test and use adequate contraceptive measures[1]. Male patients must use adequate contraceptive measures[2].

11. Voluntarily signed informed consent before performance of any study related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.

[1] Female patients must be post-menopausal for more than one year or must provide a negative pregnancy test and use an efficient method of contraception (i.e., a method with less than 1% failure rate [e.g., sterilization, hormone implants, hormone injections, some intrauterine devices, or vasectomized partner]) during the study and for 1 month (or more if requested by non-IMP labels) after the end of the study (last dose of IMP).

[2] Unless the partner of a male patient is post-menopausal or using an efficient method of contraception as described above, male patients must agree to use condoms during the study treatment and for 1 month after the end of the study treatment.

5.2 EXCLUSION CRITERIA

Patients meeting one or more of the following criteria are ineligible to participate in the study:

1. Malignant tumors other than colorectal adenocarcinomas (current or within the previous five years), with the exception for curatively treated non-melanoma skin cancer or in situ carcinoma of the cervix.

2. Less than 6 months between randomization and completion of the last anti-cancer treatment (chemotherapy/ radiotherapy/immunotherapy, etc.). (NB: Rectal cancer treatment shorter than 8 weeks of chemo/radiation therapy is allowed.)

3. Confirmation of progressive disease within 6 months after completion of prior adjuvant anti-cancer treatment.
4. Indication for any mCRC surgery or anti-cancer treatment other than study treatment.

5. Prior treatment with arfolitixorin.

6. Indication for treatment with a 5-FU analogue, or 5-FU for a condition other than mCRC.

7. Known DPD deficiency.

8. Known or suspected central nervous system metastases.

9. Unresolved bowel obstruction, uncontrolled Crohn’s disease, or ulcerative colitis.

10. History of cardiac disease with a New York Heart Association Class II or greater, congestive heart failure, myocardial infarction or unstable angina at any time during the 6 months prior to randomization, or serious arrhythmias requiring medication for treatment.

11. Current CTCAE ≥ grade 3 diarrhea.

12. Current chronic infection or uncontrolled serious illness causing immunodeficiency.

13. Known or suspected hypersensitivity or intolerance to arfolitixorin, LV, 5-FU, oxaliplatin, or bevacizumab.

14. Breastfeeding patients.

15. Patient who received investigational drugs in other clinical trials within 28 days, or 5 half-lives of the investigational drug, prior to randomization.

16. Patient with serious medical or psychiatric illness likely to interfere with participation in this clinical study.

17. Ongoing drug or alcohol abuse, as deemed by the Investigator.

18. Any condition that, in the opinion of the Investigator, could compromise the patient's safety or adherence to the study protocol.

19. Involvement, or related to people involved in the planning or conduct of the study (applies to both Isofol Medical staff and staff at the study site).
20. Surgery (excluding previous diagnostic biopsy) in the 28-day period before randomization

5.3 LIFESTYLE CONSIDERATIONS

Not applicable

5.4 SCREEN FAILURES

Screening failures are patients who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study due to a failure to meet inclusion/exclusion criteria. These patients should have the reason for study withdrawal recorded as 'Eligibility Criteria Not Fulfilled'. Screening failure patients will not be allowed to be rescreened into the study.

If a patient does not meet all the eligibility criteria but is randomized in error, or incorrectly started on treatment, the Investigator should inform the Sponsor immediately, before a decision is made to continue or discontinue the patient from treatment.

All AEs that occur after the consent form is signed and before treatment allocation/randomization must be reported by the Investigator if the AE causes the patient to be excluded from the study, or are the result of a protocol-specified intervention.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Approximatively 80-100 sites across Europe, Canada, the US, Australia and Japan will participate in the study. The recruitment will be competitive between the study sites. With an estimated recruitment rate of 0.36 patients per site and month, 440 patients should be recruited in approximately 19 months including 6 months ramp up period until all sites are activated. The patients will be recruited from the sites ordinary pool of patients. No study site should include more than 10 % of the total number of patients in the study (i.e. not exceeding 44 patients, or 66 patients if the sample size is increased).

The recruitment will continue during the interim analysis. Depending on the conditional powers, the trial will either continue as planned, or the sample size of the trial will be increased for a total of 660 randomized patients.

Initially, approximately 440 were planned to be randomized. However, Japanese authorities requested that approximately 12.7% of the total study population are enrolled from Japan. For this reason, additional patients from Japan were randomized. In the end a total of 490 patients were randomized. This decision to randomize extra Japanese patients was made before the interim analysis. The decision to include the Japanese patients in the ITT population (see Section 9.2.2) for the primary efficacy analysis was made after the interim analysis.

Study sites should keep up to date contact information for all study patients in their records to ensure patient retention throughout the study, including the survival follow up phase.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Eligible and consenting patients will be randomized 1:1 to one of two treatment groups (A and B) and administered one of the following treatment regimens starting within 3 days of randomization. Following treatment cycles should be started 14 (+ 7) days after the previous administration.

- Group A “Experimental”: ARFOX (Arfolitixorin + 5-FU + Oxaliplatin) + Bevacizumab
- Group B “Comparator”: mFOLFOX-6 (Leucovorin + 5-FU + Oxaliplatin) + Bevacizumab

6.1.2 DOSING AND ADMINISTRATION

Group A (ARFOX + bevacizumab):

- Bevacizumab 5 mg/kg, IV infusion in accordance with the label

  ↓ followed by

- Oxaliplatin 85 mg/m^2 IV infusion in accordance with the label

  ↓ followed by

- 5-FU 400 mg/m^2 IV bolus (2–4 minutes)

  ↓ followed by (30 minutes (± 5 minutes) after 5-FU bolus)

- Arfolitixorin 60 mg/m^2 rapid IV bolus (less than 3 minutes)

  ↓ followed by

- 5-FU 2400 mg/m^2 continuous IV infusion over 46 hours

  ↓ followed by (30-60 minutes after the first arfolitixorin bolus)

- Arfolitixorin 60 mg/m^2 rapid IV bolus (less than 3 minutes)

Group B (modified FOLFOX-6 + bevacizumab):

- Bevacizumab 5 mg/kg, IV infusion in accordance with the label

  ↓ followed by

- Oxaliplatin 85 mg/m^2 IV infusion in accordance with the label

  ↓ followed by*

- LV 400 mg/m^2 IV infusion

  ↓ followed by

- 5-FU 400 mg/m^2 IV bolus (2–4 minutes)

  ↓ followed by

- 5-FU 2400 mg/m^2 continuous IV infusion over 46 hours

**Oxaliplatin and LV may be administered at the same time through two different injection ports. NB: Oxaliplatin and LV must not be mixed.*

The doses of arfolitixorin and LV should not be adjusted.

5-Fluorouracil and LV should be administered separately to avoid the formation of a precipitate.

Following a treatment-related adverse event the doses of 5-FU, oxaliplatin, and bevacizumab may be adjusted or delayed in accordance with the label and section 6.3.4 in the protocol. Oxaliplatin cannot be substituted but caution should be taken regarding toxicity and in case of toxicity, dose modifications (decrease in infusion rate and/or dose reductions) according to label should be performed [4, 28].

For patients with platinum hypersensitivity, pre-medication with IV dexamethasone, chlorphenamine, and ranitidine can be given at the Investigator's discretion.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The IMP (arfolitixorin and LV) will be sent from Creapharm, with depots in France and in the US, direct to the study sites. All shipments of IMP to site will be managed through an Interactive Web Response System (IWRS).

At all times, the number of supplied, used and remaining treatment doses should match. The Investigator (or designee) will be responsible for IMP accountability, reconciliation, and record maintenance. In accordance with all applicable regulatory requirements, the Investigator (or designee) must maintain the following inventory records:

- Receipt of IMP boxes at the study site;
- IMP administered to each patient (including vial numbers);
- Inventory of IMP at study site;
- Log of any discarded/unused/expired IMP;
- Monitoring of the storage conditions.

These records will be monitored and verified by the local monitor regularly, as part of the routine monitoring procedure.

Any unused or expired IMP should be destroyed at site if possible only after Sponsor approval. If returned to the supplier locally approved safety procedures and documentation should be used.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELLING

6.2.2.1 STUDY DRUG: ARFOLITIXORIN

The active pharmaceutical ingredient (API) of arfolitixorin is the hemisulfate salt of [6R]5,10-methylene-tetrahydrofolic acid ([6R]-MTHF-HS). The API is manufactured in accordance with Good Manufacturing Practice (GMP) by Merck & Cie (Schaffhausen, Switzerland).

The drug product, arfolitixorin powder for injection 100 mg, is a lyophilized white to light yellowish or light beige powder (100 mg arfolitixorin per vial, calculated as free acid) for administration after reconstitution with 10 mL of water for injection. The drug product is manufactured in accordance with GMP by Recipharm (Wasserburg, Germany)

Table 2: Components of arfolitixorin

Component	Amount/vial	Function
Sodium hydroxide	0.03[1] g	For pH adjustment
Trisodium citrate dihydrate	0.2 g	Buffer (to guarantee a defined stable pH after reconstitution in water)
Arfolitixorin	0.1 g	Active Ingredient

1) The listed amount of Sodium hydroxide (2 N in WFI) can vary slightly since the final amounts are dependent on the pH-adjustment. During the lyophilization process, water is removed.

Reconstitution is performed prior to administration (see section 6.2.4.1). The resulting solution for injection is isotonic and has a pH of 8.5. The final API concentration is 10 mg/mL calculated as the free acid.

The primary packaging is 10 mL type 1 glass vials with lyophilization stopper (20 mm, siliconized, Helvoet FM157/1, light grey) and flip-off cap (20 mm with white synthetic disc).

Arfolitixorin vials are labeled by Creapharm in accordance with GMP and local regulatory guidelines. The labels fulfil GMP Annex 13 requirements for labelling. Label text will be translated into local language.

6.2.2.2 COMPARATOR DRUG: LEUCOVORIN

The comparator, Leucovorin (calcium folinate) 10 mg/mL is a commercialized specialty which will be provided to the sites by Creapharm.

Each vial of 20 mL solution contains 10 mg/mL of folinic acid provided as calcium folinate.

List of excipients

- Sodium Chloride
- Water for Injection

Calcium folinate vials are labeled by Creapharm in accordance with GMP and local regulatory guidelines. The labels fulfil GMP Annex 13 requirements for labelling. Label text is translated into local language.

6.2.3 PRODUCT STORAGE AND STABILITY

6.2.3.1 STUDY DRUG: ARFOLITIXORIN

Stability tests according to ICH (International Conference on Harmonization) guidelines are ongoing. The study drug product should be stored at 2 – 8°C. Reconstituted powder (for details see 6.2.4.1) can be handled at room temperature and should be administrated within 2 hours.

6.2.3.2 COMPARATOR DRUG: LEUCOVORIN

The comparator is commercialized Leucovorin (calcium folinate) and should be stored at 2 – 8°C, protected from light.

6.2.4 PREPARATION

The study drug is allocated using the IWRS. The doses are based on the patient's body surface area and is calculated using the DuBois formula:

$BSA = 0.007184 \times Height^{0.725} \times Weight^{0.425}$

6.2.4.1 STUDY DRUG: ARFOLITIXORIN

1. Arfolitixorin solution for injection should be prepared by trained personnel, for example at the hospital pharmacy or by a registered nurse.

2. Use aseptic technique.

3. Reconstitute each vial (100 mg) with 10 mL water for injection and note the time point for reconstitution. Gently swirl each vial until the powder is completely dissolved. The solution

should be clear and range in colour from colourless to yellow. The solution contains 10 mg/mL arfolitixorin.

4. Inspect the solution visually for particles. If particles are present, discard the solution.

5. The solution can be kept in the vial, alternatively, directly after the visual inspection transferred to a syringe according to standard procedure.

6. Storage conditions for the solution:

    a. If the solution is stored at room temperature it must be administered within 2 hours from the time of reconstitution. Document the expiry time.

    b. If the solution is stored in the refrigerator (2-8 °C) it must be administered within 6 hours from the time of reconstitution. Document the expiry time.

    c. If the solution is not administered before it expires, it must be discarded.

6.2.4.2 COMPARATOR DRUG: LEUCOVORIN (LV)

The preparation should be performed according to the product preparation instructions.

For intravenous infusion, LV may be diluted with 0.9% sodium chloride solution or 5% glucose solution before use. Refer also to section 6.3.

5-Fluorouracil and LV should be administered separately to avoid the formation of a precipitate.

As the LV used in this study is Bendafolin which contains the excipient trometamol, LV may not be mixed with oxaliplatin during the administration of the study drugs.

6.3 OTHER MEDICATIONS

6.3.1 5-FU

5-FU is formulated as injection solution.

<u>5-FU Dosing:</u>
Per label, 5-FU will be administered as a 400 mg/m^2 IV bolus (2–4 minutes) on Day 1 followed by continuous 2400 mg/m^2 IV infusion over 46 hours in each treatment cycle. The treatment will be repeated every two weeks until PD or premature discontinuation of IMP.

An administered 5-FU IV bolus dose should not surpass the maximum recommended daily dose of 1000 mg, regardless of the body surface area.

6.3.2 OXALIPLATIN

Oxaliplatin is formulated as a concentrated infusion solution.

<u>Oxaliplatin Dosing:</u>

Per label, Oxaliplatin will be administered as 85 mg/m^2 IV infusion over 15 – 120 minutes on Day 1 in each treatment cycle and repeated every two weeks until PD or premature discontinuation of IMP. Caution will be taken regarding toxicity and in case of toxicity, dose modifications (decrease in infusion rate and/or dose reductions) according to label should be performed.

6.3.3 BEVACIZUMAB

Bevacizumab is formulated as a concentrated infusion solution.

US licensed bevacizumab biosimilars are allowed for treatment. A biosimilar is a biological product that is highly similar to and has no clinically meaningful differences from an existing biologic therapy. Several biosimilars are available on the U.S. market, including bevacizumab-awwb and -bvzr that have been FDA approved for the treatment of mCRC. The biosimilars are however not interchangeable and no patient may therefore change between bevacizumab and any biosimilar treatment or between different biosimilars during the course of the study.

Bevacizumab Dosing:
Per label, Bevacizumab administered as an intravenous infusion as 5 mg/kg of body weight given once every 2 weeks. It will be administered on Day 1 of every 2-week cycle and repeated every two weeks until PD, premature discontinuation of IMP or unacceptable toxicity. The initial dose should be delivered over 90 minutes as an intravenous infusion. If the first infusion is well tolerated, the second infusion may be administered over 60 minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be administered over 30 minutes. It should not be administered as an intravenous push or bolus.

6.3.4 DOSE ADJUSTEMENTS

On the planned day of treatment, chemotherapy may be administered only if:

- ANC is ≥ 1.0 × 10^3/µL (1.0 × 10^9/L)†
- Platelet count is ≥ 75 × 10^3/µL (75 × 10^9/L)
- Chemotherapy-related gastrointestinal toxicity is ≤ Grade 1

† In situations where ANC is < 1.5 × 10^3/µL (1.5 × 10^9/L) but ≥ 1.0 × 10^3/µL (1.0 × 10^9/L) and institutional guidelines (or Investigator's discretion) recommend not administering chemotherapy, then chemotherapy may be delayed.

General dose-modification considerations are as follows:

- All study patients should receive standard approaches to dosing and dose modification for adverse reactions that are consistent with approved product labeling for the individual drugs within the combination chemotherapy regimen.
- If 5-FU administration is delayed, oxaliplatin, bevacizumab, arfolitixorin or LV must also be postponed
- If 5-FU treatment is discontinued the patient will be withdrawn from the study treatment and followed for OS.
- 5-FU dose reductions are allowed in case of toxicity, but the 5-FU bolus may not be removed if the 5-FU treatment continues. Instead both the 5-FU bolus and 5-FU infusion dose should be reduced according to the rates described in the 5-FU summary of product characteristics [34].

- If oxaliplatin or bevacizumab administration is delayed or discontinued, 5-FU/arfolitixorin or LV may be continued.
- Dose reductions for arfolitixorin or LV are not allowed; however, arfolitixorin or LV will always be withheld when 5-FU is withheld.

All dose modifications and treatment discontinuations will be captured in the eCRF.

6.4 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

6.4.1 RANDOMIZATION

Patients will be randomized in a 1:1 ratio to either the experimental arm (arfolitixorin) or the comparator arm (Leucovorin), following the completion of all screening assessments and after confirmation of their eligibility, using a stratified permuted block randomization. Randomization will be stratified for the following baseline factors:

- Geographical region (Europe *versus* North America *versus* Australia *versus* Japan)
- Primary tumor location (left colon *versus* right colon *versus* rectal cancer)
- Previous neo-adjuvant/adjuvant CRC treatment (yes *versus* no)

Randomization will be performed via an interactive web response system (IWRS). The system enables time and date stamped entries that in turn allow real time compliance monitoring.

The system is compliant with the FDA 21 CFR Part 11 standards ensuring the system's secured access and integrity of data collected. In this respect, any person involved with the IWRS will be identified by a personal username and will need a secret code to access the system.

6.4.2 BLINDING

Patient and Investigator blinding of the study treatment is not possible as there is a difference in the color of the injection fluids and the method of administration. To minimize any bias arising either from the inability to blind patients and Investigators or from potential inconsistencies in local tumor response assessments across study sites, analyses of the tumor response endpoints will be based on BICR, i.e. blinded, retrospective, centrally-adjudicated assessment of CT-scans/MRIs according to a pre-specified Image Review Charter.

6.5 STUDY INTERVENTION COMPLIANCE

Date and procedures of each study visit will be entered in the electronic case report form (eCRF) to document patient compliance to the study schedule as well as to the study procedures (for instance complies with the prohibited concomitant therapies).

In addition, during monitoring visit done at the Investigator's site, the monitor will check that:

- All IMPs and procedures for the trial are to be used exclusively within the limits defined by the protocol;
- Inventory records of study products are in order and that there are sufficient supplies;
- The expiry dates are not likely to be, or have not been, exceeded;

- The storage conditions for study products are adequate;
- Procedures and records of returned and/or unused study products are complete.

6.6 CONCOMITANT THERAPY

6.6.1 PROHIBITED THERAPIES

The following treatments are not permitted:

- nucleoside analogues (e.g., sorivudine) or known DPD inhibitors (e.g., brivudin)
- additional anti-cancer treatment (e.g., chemotherapy, biologics, etc.) except what is described in Section 6.3
- experimental medications other than arfolitixorin
- therapeutic radiation therapy. Palliative radiation can be approved by sponsor on a case by case basis.

If any of the foregoing treatments are considered essential during the study the patient is to discontinue IMP as per section 7.1.

6.6.2 AUTHORIZED MEDICATION

Throughout the study, patients will receive medication required for their health condition at the discretion of the Investigator or treating physician, if not included in the list of prohibited medication (section 6.6.1). All concomitant treatment administered at any time, from the screening visit until the End of Treatment visit, will be recorded in the eCRF on a medication form (including the generic name of the medication, the medical indication/AE, the daily dose, the route of administration and the start and end dates). After IMP discontinuation, further anti-cancer therapies will be recorded in the eCRF, until death or end of study. After the last administration of study treatment, the patients will be treated according to local clinical practice.

Also, the use of vitamins and dietary supplements will be recorded in the eCRF.

6.6.3 RESCUE MEDICINE

Not applicable

7 IMP DISCONTINUATION AND PATIENT DISCONTINUATION

7.1 DISCONTINUATION OF STUDY INTERVENTION

The IMP treatment can be discontinued based on the decision of the patient, the Investigator, or the Sponsor, as follows:

- Patients may voluntarily discontinue the IMP at any time without providing a reason and without any prejudice to future treatment.

- The Investigator may, at their discretion, discontinue the IMP at any time if deemed to be in the best interest of the patient (including AEs, complete response, or symptomatic deterioration), or due to incorrect randomization, or pregnancy.

- The Sponsor may also instruct the Investigator to discontinue treatment with IMP in the event of severe non-compliance with the protocol (e.g., refusal to adhere to the treatment schedule or other protocol related assessments), or a protocol deviation that could affect the safety or well-being of the patient, or if new safety information showing unfavorable data becomes available.

Patients prematurely discontinuing the IMP are to be treated according to local routine. Patients will be asked to complete an end of treatment visit within 30 days of last dose of IMP and before start of new anti-cancer treatment.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Patients are free to withdraw from the study at any time, without necessarily providing a reason and without prejudice to further treatment.

A patient who withdraws consent will always be asked the reason(s) for the withdrawal and the presence of any AEs. Patients will also be asked the following:

- To complete an End of Treatment visit.

- To permit the Investigator to follow-up (including by telephone) and provide data to the Sponsor regarding OS and anti-cancer therapy.

- If the patient does not have PD at the time of withdrawal, to permit the Investigator to continue to provide data to the Sponsor regarding PD (either or both the Investigator's determination of PD or any future CT-scans/MRIs) and anti-cancer therapy.

Every effort will be made to minimize the number of patients withdrawing from the trial or lost to follow-up. A patient may withdraw from study treatment, however still continue to be followed until PD and/or death. Only patients who explicitly withdraw their consent or who are completely lost to follow-up will be considered "dropouts". Data for these patients will be kept in the data base, unless the patient request that all his/her data to be deleted from the data base.

Randomized patients who have received IMP and withdraws from participation in the study will not be replaced.

7.3 LOST TO FOLLOW-UP

To minimize lost to follow-up in the study, each site should ensure contact details (including relative) are updated when the patient is entering the study. A study patient will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the study site staff for six months after last study visit. Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

Before declaring a patient to be "Lost to follow-up", reasonable effort should be spent in following actions:

- The site will attempt to contact the participant and reschedule the missed visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, the Investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's medical record or study file.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

8.1.1 CT SCAN / MRI

CT-scans or MRIs will be performed of the thorax, abdomen, and pelvis at screening and every 8 weeks after randomization until centrally confirmed PD. A radiologist at each study site/hospital (preferably the same radiologist for all patients at that hospital) is to evaluate each scan for target lesions. The following information is to be collected:

- Patient ID
- Date of procedure
- Type of procedure
- Progressive disease (Yes/No)

It is preferred that the scans for a patient is taken with the same technique (CT or MRI) throughout the study. On occasions where it is not possible, for whatever reason, to use the same technique as for the screening scan the other technique should be used. The technique used for each scan will be documented in the eCRF.

To minimize bias and have an objective assessment of the tumor response, a central imaging vendor (Banook Group) will perform independent and blinded response evaluations retrospectively.

The central reading process will be done from screening until centrally confirmed PD by two different Central Readers (double review with adjudication). All the images will be sent to the Central Readers in a continues chronological sequence as outlined in the Site Procedure Manual. In order to remove the bias of interpolation and ensure the reader concentrates on the latest time-point images and has no idea that there are more to follow, each time-point images will be displayed, scored and locked in the database before the assessment of the following time point.

The methodology applied at baseline regarding the identification of the target and non-target lesions is based on a common lesions selection process and an identical usage of those selected lesions by both Central Readers.

The rationale for this lesion selection process at baseline is based on the fact that readers can potentially identify different target and non-target lesions that each considers representative of the patient's overall extent of disease. There is also the potential for the Central Readers to identify the

same lesions but classify them differently between target and non-target lesions. Discordance in lesion selection can reach 40% or even more. In addition, since lesions do not respond or progress at the same rate, differences between readers with regard to response or progression of lesions can be observed in the subsequent time-points.

To minimize such important risk of discordance, the following process for the initial CT-scan or MRI will be applied.

One of the two primary Central Readers is randomly selected to perform lesions selection on initial images:

- Determines the number of target lesions and non-target lesions based on RECIST 1.1,
- Communicates the target and not target lesions identification to the second primary Central Reader, via the central imaging vendor to preserve the independence of the two Central Readers.
- Tracks lesions locations and labelling for up to 5 (2 per organ) target lesions and 10 non-target lesions on the subsequent time points.

After having defined the type of targets and others lesions to be selected, the two primary Central Readers will carry out a separate and blinded measurement of the target lesions at Baseline:

- If there is an agreement between Central Readers (Δ ∑ <20% and Δ <15% for each lesion), Central Reader will keep his own ∑ for the next time points,

In case of discrepancies between Central Readers (Δ ∑ >20% or Δ >15% for any lesion), the Central Chair/adjudicator) will adjudicate the result of the two previous Central Readers and will provide the reference values for the baseline (for each target lesion). This value will be considered as reference by both Central Readers for the next time point analyses.

8.1.1.1 INITIAL CT-SCAN OR MRI

Tumor imaging at screening must be performed within 28 days prior to the date of randomization. Scans performed as part of routine clinical management are acceptable for use as screening tumor imaging if they are of diagnostic quality and performed within 28 days prior to the date of randomization and fulfill the requirements stipulated by the central imaging vendor.

Overall review of the TAP (thorax, abdomen, pelvis) CT-scan or MRI for response assessment will only be performed on patients delivering an evaluable baseline time point and at least one evaluable follow-up time point.

8.1.1.2 SUBSEQUENT TIME POINTS

The two Central Readers will carry out a continuous reading at each complete patient in a blind way one versus the other. Initial images are intended to act as a lesion reference map for lesion assessments made during subsequent time point review.

- The Central Readers perform lesion(s) measurements at each subsequent time point as described in the Central Review Charter,
- The Central Readers assess the outcome of non-target lesions,
- The Central Readers determine, if any, the number and location of new lesion(s),
- The Central Readers provide an overall response assessment.

8.1.1.3 RECIST CRITERIA

Tumor response will be evaluated according to the following definitions, as per RECIST 1.1 criteria. *(NB: Investigators are to rely on the locally-performed assessment for treatment guidance)*. The centrally-performed assessment will be the basis for evaluation of the study endpoints:

Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.
Progressive Disease (PD)	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

8.1.2 PHARMACOKINETICS

The plasma concentration of [6R]-MTHF, methyl-THF, THF will be determined in approximately 20 patients per treatment group. The sample size is not based on pop-PK modelling, but on feasibility as the sampling requires access to laboratory facilities at the site. Blood samples for determination of folate PK characteristics will be collected from those patients randomized at clinical sites qualified to ensure correct handling of samples in accordance with study-specific instructions.

PK sampling procedures

Plasma sampling will occur during Cycle 1 (Week 1) and Cycle 2 (Week 3).

PK sampling for the experimental arm (Timings of PK samplings are approximate and actual sampling times will be captured in the PK sample log):

1. Directly prior to the first arfolitixorin administration

2. 10 minutes after the first arfolitixorin administration

3. 1 hour after the first arfolitixorin administration (NB: This sample will be taken after the second arfolitixorin dose)

4. Directly prior to the second arfolitixorin administration

5. 10 minutes after the second arfolitixorin administration

6. 1 hour after the second arfolitixorin administration

PK sampling schedule for the comparator arm (Timings of PK samplings are approximate and actual sampling times will be captured in the PK sample log):

1. Directly prior to the initiation of the Leucovorin infusion

2. 10 minutes after completion of the Leucovorin infusion

3. 10 min after the 5-FU Bolus

4. 1 h after initiation of 5-FU infusion

In total 12/8 PK samples will be collected from each patient in the arfolitixorin/Leucovorin group participating in the PK part of the study yielding approximately 60/40 mL blood collected for this purpose.

The study site should strive to take the blood samples as close to the suggested time-points as possible. The exact sampling time will be recorded in the eCRF and will be used for the PK calculations.

Plasma samples will be collected, handled, stored, and sent to Charles River Laboratories, (UK and USA) for analysis as described in study-specific laboratory instructions. All samples collected for each patient will be analyzed at the same time.

Bioanalytical method

A validated bioanalytical method for the determination of levels of [6R]-MTHF, methyl-THF and THF is described in a separate bioanalytical method protocol.

8.1.2.1 POPULATION PHARMACOKINETIC ANALYSIS

The pharmacokinetics of [6R]-MTHF will be analyzed using a population PK approach. The specific objectives of the analysis are to:

- Characterize the single and multiple dose pharmacokinetics, including associated variability, of [6R]-MTHF.
- Evaluate the impact of potential covariates (body size, age, sex, race, ethnicity and renal function) on the pharmacokinetics of [6R]-MTHF.

In addition to the concentration data collected in study ISO-CC-007 (see section 8.1.2 for the sampling schedule and anticipated number of patients), data from three Phase I/II studies in patients (ISO-MC-091, ISO-CC-002 and ISO-CC-005) and from one Phase I study in healthy volunteers (ISO-FF-001) will be included in the population PK analysis.

The population PK analysis will be performed in a stepwise manner as outlined below.

1. Exploratory graphical analysis of plasma concentrations versus time data and covariate distributions, for identification of potential outliers and to inform the further analysis.
2. Develop a population PK model for [6R]-MTHF:
    a. Evaluate alternative structural and stochastic models to describe the typical [6R]-MTHF PK profile.
    b. Investigate and characterize covariates that influence the volume of distribution, and clearance for [6R]-MTHF.
    c. Evaluate, and if necessary refine, the candidate final model.

The population PK analysis will be described in a population PK report. The report will contain a thorough description of the data used in the analysis, including any data exclusions and handling of missing data; the key components of the model development, including the base model, covariate analysis and model evaluation; and full description and discussion of the final model.

8.1.2.2 EXPOSURE-RESPONSE ANALYSIS

The aim of the exposure-response analysis is to evaluate the suitability of the dose of 120 mg/m^2. The individual [6R]-MTHF exposure ($AUC_{average}$ and C_{max}), as established from the population PK analysis described above, will be used in the exposure-response analysis (see section 8.1.2 for anticipated number of patients). The following efficacy and safety parameters will be evaluated for a correlation with exposure:

- ORR
- Dose limiting toxicities
- Adverse events

In addition to the exposure-response data collected in this study (ISO-CC-007), data from study ISO-CC-005 will be included in the exposure-response analysis. The analysis will be described in a separate report. The exposure-response report will contain a thorough description of the data used in the analysis, including any data exclusions and handling of missing data.

8.1.3 ECOG PERFORMANCE STATUS

The ECOG performance status scale will be used to quantify the extent to which the disease affects the daily living abilities of the patient on a scale from 0 to 4 (Appendix 1). The assessment will be performed at screening, baseline (cycle 1), each assessment visit, at the end of treatment visit, and at CT/MRI Follow-up visits until centrally confirmed PD. The scored values will be recorded in the eCRF.

8.1.4 EQ-5D QUALITY OF LIFE

EQ-5D (Appendix 3) patient reported outcome questionnaire will be used to assess the disease effects on the patient's quality of life. The EQ-5D questionnaire will be completed by the patient prior to any study related activity at the visit for first administration of IMP, the assessment visits until PD and at the End of Treatment visit. The EQ-5D questionnaire will be completed by the patient on paper and then transferred to the eCRF by the study site staff.

8.1.5 SURVIVAL STATUS

Patients discontinuing study treatment and/or with centrally confirmed PD will move into the survival follow-up phase. Survival status will be assessed by telephone, ordinary visits, hospital records or other means found suitable every 12 weeks until death or the end of the study, whichever occurs first. Information regarding post-study anti-cancer therapies will be collected if new treatment is initiated.

8.2 SAFETY AND OTHER ASSESSMENTS

8.2.1 LABORATORY SAFETY ASSESSMENTS

The laboratory safety samples (hematology, clinical chemistry, and urinalysis), including pregnancy test, will be collected according to the protocol Schedule of Activities and in accordance with local practice and analyzed by local laboratories.

The observed values will be recorded and assessed as “normal” or “abnormal” in the eCRF. Abnormal values will be assessed by the Investigator as “clinically significant” or “not clinically significant”. Clinically significant values will be captured as Medical history or AEs, as applicable.

The laboratory tests are to be performed within 7 days before next treatment and the results must be reviewed by the Investigator or qualified designee and found to be acceptable prior to the administration of study treatment.

All required laboratory tests are specified in Table 3 .

Table 3: Laboratory tests

HEMATOLOGY	CLINICAL CHEMISTRY	URINALYSIS
Hemoglobin	Sodium	Protein
Hematocrit	Potassium	Glucose
WBC	Calcium	Blood
Neutrophils	Glucose	
Lymphocytes	BUN or urea	
Monocytes	Creatinine	
Eosinophils	AST	
Basophils	ALT	
Platelet count	Alkaline phosphatase	
	Total bilirubin	
	Total protein	
	Albumin	
	LDH	
	gGT	

8.2.2 VITAL SIGNS, ECG AND PHYSICAL FINDINGS RELATED TO SAFETY

Vital signs collected will include resting heart rate (beats per minute), systolic and diastolic blood pressure (mmHg; to be measured after at least five minutes of supine rest), weight (within 7 days prior to each study drug administration), and height (screening only).

The physical examination is to be performed according to local clinical practice, and include neurological status, skin, and mucous membranes.

A 12-lead ECG will be performed at treatment cycles 1 to 4. Information about the ECG examination must be present in the source documentation at the study site. Single 12-lead safety ECGs will be

recorded in supine or in reclining position (should be the same position for all measurements during the same day) using the site's ECG machine.

- Group A (Arfolitixorin): ECG should be assessed directly prior to each arfolitixorin administration, and 10 min (± 5 min) after the completion of each administration (i.e. 4 ECG per treatment cycle).

- Group B (Leucovorin): ECG should be assessed directly prior to Leucovorin infusion, and 10 min (± 5 min) after start of Leucovorin infusion (i.e. 2 ECG per treatment cycle).

Safety ECGs will be reviewed and interpreted on-site by the investigator. Significant findings present prior to the start of study treatment administration must be recorded in the Medical history section of the eCRF. Clinically significant findings made after the start of study treatment administration, and which meet the definition of an AE, must be recorded in the eCRF. QTc abnormalities should be reassessed after 10 minutes to ensure normalization. If a patient develops a change-from-baseline in QTcF > 60 ms or a QTcF interval of > 500 ms, ECG should be monitored until normalized, and the patient should be discontinued from IMP treatment.

8.2.3 PRO CTCAE

The PRO CTCAE (Appendix 2) adverse event self-assessment questionnaire will be completed by the patient immediately after completion of the EQ-5D questionnaire during the first 4 IMP administration visit, during the assessment visits until PD and at the End of Treatment visit. The PRO CTCAE will be completed by the patient on paper, and then transferred to the eCRF by the site staff. The PRO CTCAE questionnaire will only be used in countries where there is a validated translation available at the time of study submission to IRB/IEC.

The Investigator must always conduct an ordinary AE assessment during all study visits independently of the information provided by the patient in the PRO CTCAE.

AEs to be assessed by PRO CTCAE is:

- Mucositis
- Nausea
- Vomiting
- Diarrhea
- Pain in the abdomen
- Neuropathy
- Fatigue

The above AEs are the most frequently reported AEs from the previously conducted and ongoing Phase I/II studies with arfolitixorin in combination with 5-FU, and also including the most commonly reported AEs for the anti-cancer treatment combination used in this protocol (modified FOLFOX-6 + bevacizumab) [14, 29].

8.2.4 PHARMACOGENETICS

Level of gene expression will be analyzed at a central laboratory (TATAA Biocenter, Gothenburg, Sweden) to determine drug metabolism- and transportation-related gene expression levels in all patients. The specifications and logistics for the gene analysis (preparation and shipment of FFPE tissue slices from tumor biopsies to the central laboratory) are described in a separate study-specific

laboratory instruction provided to the sites. The relative gene expression levels will be analyzed for the following genes: ABCC1, ABCC3, ABCC5, CES2, DPYD, ERCC1, FGF7, MTHFD2, SHMT1, SHMT2, SLC46A1, SLC19A1, SLC24A32, TGFB1, TYMS, ACTB and PPIA. The gene expression analysis will be performed retrospectively.

8.2.5 RESECTION/REMOVAL OF TUMOR LESION(S)

For patients assessed by the investigator as eligible for metastasis and/or primary tumor resection during the IMP treatment phase, treatment with the study regimen may be continued at the investigator discretion. The therapy will be registered in the eCRF including if continued study treatment is intended as well as planned delay until continued treatment. The EoT visit will be conducted when the IMP treatment has been completed. If the patient discontinues IMP treatment in conjunction with the resection, the EoT visit will be conducted within 30 days after last dose of IMP. Patients undergoing metastasis and/or primary tumor resection will be followed for disease recurrence by central imaging and Overall Survival until death or the end of the study.

8.3 STUDY SCHEDULE

8.3.1 SCREENING AND ENROLLMENT

Investigators must keep a record of all patients enrolled in to the study.

During the screening period, Investigators will:

- Obtain signed informed consent from the patient before any study specific procedures are performed
- Assign the patient a unique Patient Study Identifier (ID)
- Determine patient eligibility
- Record patient demographic data (date of birth, gender and race) and gene mutations (BRAF, KRAS, NRAS and other) where this information is available in the patient´s medical records.
- Record medical history
- Record all concomitant therapies
- Conduct physical examination and record vital signs
- Assess ECOG status
- Record weight and height
- Collection of blood sample for hematology (within 7 days before IMP administration)
- Collection of blood sample for clinical chemistry (within 7 days before IMP administration)
- Collection of urine sample for urinalysis (within 7 days before IMP administration)
- Conduct pregnancy test for all women of childbearing potential (within 7 days before IMP administration)
- Conduct CT scan / MRI
- Ensure availability of tumor tissue biopsy

If a patient withdraws from participation in the study, then his/her ID code must not be reused.

Investigators must file Patient Identification Lists of all patients enrolled that include sufficient information to link eCRF data with clinical records. This list should be available at the study site for possible future inspections/audits. Sponsor staff or representatives should only have access to the list for monitoring or auditing purposes.

If a CT-scan/MRI was performed before enrolment a new scan is not required, provided that the scan occurred no more than 28 days before start of treatment and can be assessed by the central imaging vendor.

Study treatment should be initiated within 28 days after enrolment.

8.3.2 BASELINE (CYCLE 1)

During the first treatment visit the following will occur:

- Collect PRO EQ-5D (prior to any other study specific activity)
- Collect PRO CTCAE (immediately after EQ-5D)
- Assessment of the inclusion/exclusion criteria
- Randomization (should be done within three days before study treatment initiation)
- Record adverse event assessment between visits
- Record all concomitant therapies added and/or changed
- Conduct physical examination and record vital signs
- Record weight
- Conduct ECG
- Assess ECOG status
- Administer and record study treatments (i.e. 1st cycle)
- Collection of blood sample for PK (only at pre-selected sites)

8.3.3 TREATMENT PERIOD

8.3.3.1 TREATMENT VISITS

Treatment visits will occur every 14 days (+7 days) until PD.

During all following treatment visits, the following will occur:

- Collect PRO CTCAE, treatment visits 2-4 (prior to any other study specific activity)
- Record adverse event/toxicity assessment between visits and during IMP administration
- Record all concomitant therapies added and/or changed
- Record vital signs
- Record weight
- Conduct ECG, treatment visits 2-4
- Collection of blood sample for hematology (results evaluated prior to IMP administration)
- Collection of blood sample for clinical chemistry (results evaluated prior to IMP administration)
- Collection of urine sample for urinalysis (results evaluated prior to IMP administration)
- Administer and record study treatments
- Collection of blood sample for PK at cycle 2 (only at pre-selected sites)

At each treatment visit, the collection of blood and urine samples and the recording of vital signs must occur before start of treatment. Following a treatment-related adverse event the doses of 5-FU, oxaliplatin, and bevacizumab can be adjusted or delayed in accordance with the label and protocol section 6.3.4. Oxaliplatin cannot be substituted but caution should be taken regarding toxicity and in

case of toxicity, dose modifications (decrease in infusion rate and/or dose reductions) according to label should be performed. In case the 5-FU is delayed the IMP will be delayed.

8.3.3.2 ASSESSMENT VISITS (EVERY 8 WEEKS UNTIL PD)

Assessment visits are to occur every 8 weeks (±7 days) after the baseline visit (1st IMP administration day). During these visits, the following will occur:

- Collect PRO EQ-5D (prior to any other study specific activity)
- Collect PRO CTCAE (immediately after EQ-5D)
- Record adverse event/toxicity assessment between visits
- Record all concomitant therapies added and/or changed
- Conduct physical examination and record vital signs
- Record weight
- Assess ECOG status
- Conduct CT scan / MRI
- Evaluate disease status

Assessment visits should continue until PD or premature discontinuation of IMP.

8.3.4 END OF TREATMENT VISIT (EOT)

All patients discontinuing IMP should complete an EOT visit within 30 days of discontinuation (or as soon as possible thereafter). The EOT visit should preferably be conducted before initiation of new anti-cancer therapy. During this visit, the following will occur:

- Collect PRO EQ-5D (prior to any other study specific activity)
- Collect PRO CTCAE (immediately after EQ-5D)
- Record adverse event/toxicity assessment prior this visit.
- Record all concomitant therapies added and/or changed
- Conduct physical examination and record vital signs
- Record weight
- Collection of blood sample for hematology
- Collection of blood sample for clinical chemistry
- Conduct pregnancy test for all women of childbearing potential
- Collection of urine sample for urinalysis
- Assess ECOG status
- Conduct CT scan / MRI (if previous scan is >14 days)

8.3.5 POST-TREATMENT VISITS

At the time of final analysis of ORR and PFS, patients still on IMP treatment will continue IMP treatment until locally assessed progressive disease or discontinuation of IMP for other reasons. After completed End of Treatment visit these patients have reached the End of Study definition and will thereafter move into survival follow-up until 60% of OS events are achieved for the study population.

Patients already in follow-up phase at the time of final analysis of ORR and PFS have reached the End of Study definition and will move into survival follow-up until 60% of OS events are achieved for the study population.

8.3.5.1 FOLLOW-UP VISITS UNTIL PROGRESSIVE DISEASE

Patients who discontinue IMP for reasons other than centrally confirmed PD will move into the Follow-up phase and will be continuing to be assessed every 8 weeks. During this visit, the following will occur:

- Conduct CT scan / MRI
- Assess ECOG status
- Post-study anti-cancer therapies will be collected if new treatment is initiated
- Collect SAEs related to study procedures
- Collect SAEs considered to be at least possibly related to IMP

Every effort should be made to collect information regarding disease status until PD, death, or the end of the study.

8.3.5.2 SURVIVAL FOLLOW-UP

Patients discontinuing study treatment and/or with centrally confirmed PD will move into the survival follow-up phase. Patient survival status will be assessed every 12 weeks until death or the end of the study, whichever occurs first. Patient survival data can be collected by phone, visit, medical records, contact with relatives etc. Date of death, or the Investigators best estimate of the date of death, should be entered in the eCRF.

Information regarding post-study anti-cancer therapies will be collected if new treatment is initiated.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 DEFINITION OF ADVERSE EVENTS (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Progression of the cancer under study is not considered an adverse event since the development of the cancer will be followed and reported separately.

8.4.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A serious adverse event is any AE occurring at any dose that results in any of the following outcomes:

- Death
- Is life-threatening
    - A life-threatening adverse drug experience is any AE that places the patient, in the view of the Investigator, at immediate risk of death from the reaction as it occurred. This does not include a reaction that, had it occurred in a more severe form, might have caused death.

- A persistent or significant disability/incapacity
- Inpatient hospitalization or prolongation of existing hospitalization, except that planned hospitalization during days in clinic and surgery or hospitalization for the social reasons or the convenience of the patient or physician shall not be considered an SAE
- A congenital anomaly/birth defect
- Important medical event
    - Important medical events that may not result in death, be life-threatening, or require hospitalization may be serious when, based upon appropriate medical judgment, the event may jeopardize the patient and require medical or surgical intervention to prevent one of the outcomes listed above.

Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

The following events are **not** considered to be SAE:

- progression of the cancer under study since the development of the cancer will be followed and reported separately.
- hospitalizations for the routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- hospitalizations for the treatment, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under trial and did not worsen
- hospitalizations for the admission to a hospital or other institution for general care, not associated with any deterioration in condition
- hospitalizations for the treatment on an emergency, outpatient basis for an event **not** fulfilling any of the definitions of serious given above and **not** resulting in hospital admission.

8.4.3 CLASSIFICATION OF AN ADVERSE EVENT

8.4.3.1 SEVERITY OF EVENT

The severity (intensity) of AE in physical signs or symptoms will be graded by the investigator according to the NCI-CTCAE Version 5.0 [30]. For AEs not listed in NCI-CTCAE, the severity of AE in physical signs or symptoms will be classified as follows:

- Grade 1: Mild asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living*.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living**.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

*Instrumental activities of daily living refer to preparing meals, shopping, using the telephone, managing money, etc.
**Self-care activities of daily living refer to: bathing, dressing and undressing, eating, using the toilet, taking medications, and ability to get out of the bed.

8.4.3.2 RELATIONSHIP TO STUDY INTERVENTION

Causality regarding relationship to administration of any of the medications in the study treatment regimen, including both IMP (arfolitixorin/Leucovorin) and non-IMP (5-FU, oxaliplatin, and bevacizumab) will be assessed by the investigator as:

Related	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the treatment, unlikely to be attributed to concurrent disease or other drugs or chemicals, which follows a clinically reasonable response on withdrawal (de-challenge), and which is definitive pharmacologically or phenomenologically (i.e., an objective and specific medical disorder or a recognised pharmacological phenomenon). Re-challenge information may be required to fulfil this definition.
Probable	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the treatment, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfil this definition.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to treatment, but which could also be explained by concurrent disease or other drugs or chemicals. Information on IMP withdrawal may be lacking or unclear.
Unlikely	A clinical event, including laboratory test abnormality, with a temporal relationship to treatment which makes a causal relationship improbable, and in which other drugs, chemicals, or underlying disease provide plausible explanations.
Not Applicable	Expected only when the drug has not been administered.

For SUSAR reporting, the three following criteria, *Related*, *Probable* and *Possible* will correspond to the criteria “Reasonable possibility of a causal relationship”. The *Unlikely* criteria will correspond to the criteria “No reasonable possibility of a causal relationship”.

8.4.3.3 EXPECTEDNESS

Expected adverse reactions are AEs that are known to occur for the study intervention being studied. Expectedness is assessed based on the awareness of AEs previously observed, not on the basis of what might be anticipated from the properties of the study intervention.

An adverse reaction is considered as unexpected if the nature or severity of this is not consistent with the applicable product information presented in the Investigator's Brochure or in a summary of product characteristics (SPC) for an authorized product.

The IB presents a review of the safety data from clinical trials which identified fatigue and nausea as the most common ARs. However, they were no more frequent than could be expected for the same cytotoxic agents in the presence of other folates.

The Sponsor will be responsible for determining whether an SAE is ***expected*** or ***unexpected***. An SAE will be considered unexpected if the nature, severity or frequency of the event is not consistent with the risk information previously described.

8.4.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

AEs and SAEs will be reported in the eCRF from the time of signing informed consent form until End of Treatment visit, whether or not considered to be drug related.

- From the time of signing informed consent form until randomization, only AEs causing the patient to drop out of the study or being related to study procedure will be reported in the eCRF.

- After the End of Treatment visit, only SAEs related to study specific procedures or considered to be at least possibly related to study drug should be recorded

All SAEs and AESIs will be entered in the eCRF and followed until satisfactory resolution or until the site Investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the study sponsor and should be provided as soon as possible.

Before database closure, reconciliation between the SAEs entered in the safety database and the study database will be performed.

8.4.5 ADVERSE EVENT REPORTING

AEs reported by the patient or observed by the Investigator will be individually listed in the eCRF. Including diagnosis, signs and symptoms (if a diagnosis is not available), date of onset, duration, action taken, severity, outcome to date, relationship to any of the treatment regimen drugs or study procedure and seriousness will be recorded. The severity of AEs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE Version 5.0) toxicity criteria.

At each visit, the Investigator will be prompted to report causality of AEs as “Related”, “Probable,” “Possible” or “Unlikely” related to the IMP, any anti-cancer treatment regimen or study procedure.

8.4.6 ADVERSE EVENT OF SPECIAL INTEREST

AEs related to mucositis and bone marrow toxicity will be followed as AESIs in this study, to ensure that there are no significant safety issues when arfolitixorin, instead of Leucovorin, is combined with 5-FU in the mFOLFOX-6 + Bevacizumab regimen. AESI will be followed as ordinary AEs but reported separately in the study report as described in the SAP.

8.4.7 SERIOUS ADVERSE EVENT REPORTING

The reporting of SAEs will be done through the eCRF. Only in the event of the eCRF not being available SAEs should be reported by fax or e-mail on the paper SAE report form found in the Study File.

Fax: +33 (0) 467 107 253
or
Email: isofol-safety@vigipharm.fr

The Investigator is responsible for ensuring that all SAEs are reported to the Sponsor immediately, but in any event no later than 24 hours of any site staff becoming aware of the event. Initial reports should be followed as soon as possible by detailed reports. The initial and follow-up reports should identify patients by unique study code (Study ID). The patients' names, personal identification numbers, and addresses must not be included in the report. The following information is mandatory for the initial report:

- Patient Study ID
- Study treatment
- Start date (time, if relevant) of the study treatment
- Brief description of the event (diagnosis, or signs/symptoms if diagnosis is not available)
- Start date (time, if relevant) of the event
- Seriousness criteria
- Causality assessment

Detailed instructions describing the procedure of reporting SAEs including SUSARs will be distributed to the site and all involved parties.

For reported deaths, the Investigator should supply the Sponsor and the IRB/IEC (if applicable) with any additional requested information (e.g., autopsy reports and terminal medical reports).

The investigator is responsible to report the events to the Sponsor according to the protocol to allow the Sponsor to meet their SUSAR reporting obligations. The Sponsor is responsible for reporting SUSARs to the competent authorities. The IRB/IEC will be informed according to local requirements by either the Sponsor or the PI.

8.4.8 REPORTING EVENTS TO PARTICIPANTS

Sponsor will inform Investigators of any SUSAR, discovered in this or in any other study during the study period. It is the investigators responsibility to inform the patients if deemed necessary.

8.4.9 REPORTING OF PREGNANCY

Female patients will be instructed to notify the Investigator immediately if they become pregnant during the study. Male patients will be instructed to notify the Investigator immediately if their partner becomes pregnant. Pregnant patients will be withdrawn from further study treatment. The patients will also be instructed to report pregnancies discovered after the last visit if they believe that conception occurred during their participation in the study.

A pregnancy is not an AE, unless there is a possibility that the IMP has interfered with the efficiency of any contraceptive measures. However, the Investigator should report pregnancies according to the procedures and timelines described for reporting of SAEs. The pregnancy report form should be used instead of the SAE form.

Pregnant patients or partners will be followed until the end of the pregnancy. Any complication during the pregnancy will be reported as an AE. The outcome of the pregnancy must be reported on the pregnancy report form. Any spontaneous abortion, stillbirth, birth defect/congenital anomaly, death, or other serious infant condition must be reported and followed up as an SAE.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

The primary end-point is ORR. Null and alternative hypotheses are expressed in terms of ΔORR, the difference in ORR between the randomized treatment arms:

- H0: ΔORR=0
- H1: ΔORR≥15%

9.2 SAMPLE SIZE DETERMINATION

9.2.1 PRIMARY EFFICACY ENDPOINT: ORR

For this adaptive design, the initial sample size calculation is based on an improvement by 15% in ORR; specifically, ORR is assumed to be 45% in the control arm vs. 60% in the experimental arm. It is assumed that up to 10% of patients will be unevaluable for response in both treatment arms. These patients will be considered non-responders and will not contribute to the difference between experimental and control (hence the expected difference is 13.5% rather than 15%). For a two-sided test with α=0.05 and a power of 80% to detect this difference, at least 440 patients need to be randomized. At a sample size of 330 (75% of the information fraction for ORR), interim results will be used for adaptive sample size re-estimation, based on the conditional power for both ORR and PFS, without a formal interim analysis of efficacy or futility. This is detailed in the interim analysis plan. The statistical design allows for a 50% sample size increase (to 660 patients), should interim results be promising enough to warrant such a sample size increase. In case the sample size is increased, this provides 94% power to detect the same difference, i.e. 13.5%, for ORR.

Initially, approximately 440 were planned to be randomized. However, Japanese authorities requested that approximately 12.7% of the total study population are enrolled from Japan. For this reason, additional patients from Japan were randomized. In the end a total of 490 patients were randomized. This decision to randomize extra Japanese patients was made before the interim analysis. The decision to include the Japanese patients in the ITT population (see Section 9.2.2) for the primary efficacy analysis was made after the interim analysis. The addition of extra Japanese patients leads to a statistical power greater than 80%.

Blinded Independent Central Review (BICR) is the process by which radiographic exams obtained as part of a clinical study are submitted to a central location for review by independent physicians who are not involved in the clinical study. Regulatory authorities have recommended BICR of all patients (or a cohort of patients) for oncology registration studies when the primary study endpoint is based on tumor measurements, such as PFS, DOR or ORR [31].

As the ISO-CC-007 study is an open-label study, it is essential to keep the assessors for the primary endpoint blinded to treatment allocation.

The comparator arm in the ISO-CC-007 study consist of Leucovorin, administered together with 5-FU, oxaliplatin (the mFOLFOX-6 regimen) + bevacizumab. The efficacy for this treatment combination has been assessed by blinded independent central review only in a few clinical Phase III studies [14, 27]. The ORR in these trials range from 38 to 48 %. For the ISO-CC-007 study, we have therefore assumed an ORR of 45% for the comparator arm.

9.2.2 SECONDARY EFFICACY ENDPOINT: PFS

The study is also powered to detect a hazard ratio of 0.725, which corresponds to a clinically meaningful difference in PFS (median PFS equal to 10 months in the control arm vs. 13.8 months in the experimental arm). For a power of 80%, 300 PFS events need to be observed using a 1-sided $\alpha = 0.025$. The statistical design allows for a 50% sample size increase (to 660 patients), should interim results be promising enough to warrant such a sample size increase. In case the sample size is increased, the target number of events will be increased to 450 PFS events, which provides 80% power to detect a hazard ratio of 0.77 (median PFS equal to 10 months in the control arm vs. 13 months in the experimental arm).

At the time of writing of this protocol amendment, the interim analysis with the possibility of an adaptive sample size increase, already took place. The DSMB did not advice to increase the sample size. Furthermore, as the number of patients censored because of new anti-cancer therapies, was higher than expected, it became clear during the study that the 300 PFS events could not be reached. Therefore it was decided to include all Japanese patients in the ITT population, increasing the total sample size from the planned 440 to 490 patients in total, and to decrease the number of PFS events at the final analysis from 300 to at least 235.
Table 4 shows, for 300 and 235 PFS events, the hazard ratio for which the trial would have 80% power, the hazard ratio for which the trial would just reach statistical significance, and the corresponding median PFS in the treatment arm, assuming a 10-month median PFS in the control arm.

Table 4: Hazard ratio for significance in function of the number of PFS events

		Design		Final analysis	
Number of PFS events	**Median PFS Control arm**	**Hazard ratio**	**Median PFS Treatment arm**	**Hazard ratio for significance**	**Median PFS Treatment arm**
300	10 months	0.725	13.8 months	0.80	12.5 months
235	10 months	0.692	14.5 months	0.77	12.9 months

A hazard ratio of 0.77 was considered a clinically relevant threshold for statistical significance. The final PFS analysis will therefore be performed when at least 235 PFS events will be reached.

PFS analysis will be based on the randomized treatment. The censoring rules and details of the sensitivity analyses are specified in the study specific statistical analysis plan.

9.2.3 HIERARCHICAL TESTING APPROACH

A fixed sequence hierarchical test procedure will be applied, with a pre-defined order in which the hypotheses are to be tested. Each test will be performed without a multiplicity adjustment for multiple endpoints, i.e. at the nominal α-level foreseen by the group sequential design. The secondary endpoint PFS can only be tested formally after the first hypothesis, i.e. the null hypothesis for ORR, is rejected at the final analysis.

9.2.4 OVERALL SURVIVAL

An analysis of OS at the same time as the final PFS analysis has a 30% power to demonstrate a hazard ratio of 0.8 (corresponding to a median OS equal to 25 months in the control arm vs. 31.25 months in the experimental arm), when approximately 180 events are observed, using a 1-sided α=0.025. If the sample size is increased to 660 patients, analyzed at the same time as the final PFS analysis, this power is increased to 45%, when approximately 270 events are observed. The addition of the extra Japanese patients (see Section 9.2.2) to the 440 initial patients, resulting in a total number of 490 randomized patients, leads to a statistical power slightly greater than 30%. No interim efficacy analysis is foreseen for OS. Note that this trial is underpowered for OS, and that there is no intention to claim efficacy for OS.

Given the key relevance of this endpoint in an overall assessment of benefit/risk, it is important to power the OS analysis to exclude a significant detrimental effect of arfolitixorin as compared to leucovorin in terms of OS. The OS analysis is based on the following considerations:

1) Collection of vital status will continue in all randomized patients after the final PFS analysis, until the OS analysis. Of note, the aim for this continued follow-up is to monitor the patients' safety, rather than showing superior efficacy of arfolitixorin in terms of OS.
2) Arfolitixorin is not expected to cause serious toxicity. It is therefore reasonable to assume that if superior efficacy of arfolitixorin is demonstrated, OS is unlikely to be inferior in the arfolitixorin arm compared to the leucovorin arm. The magnitude of the OS difference is hard to predict, for example due to the multiple lines of active therapies currently available for the treatment of patients with advanced colorectal cancer. Assuming similar post-progression survival in both trial arms, a reasonable assumption is that the difference in median PFS between arms will "carry over" to the difference in median OS.
3) A "significant detrimental effect on OS" will be taken equal to the benefit of leucovorin estimated in the meta-analysis of all trials comparing 5-fluroruracil alone with 5-fluroruracil + leucovorin [33]. The overall OS HR of leucovorin was equal to 0.9 in these trials; hence, an OS hazard ratio equal to 1/0.9 = 1.11 in the current trial may be considered a significant detriment, to the extent that it will outweigh the benefit observed historically with leucovorin.
4) Collection of vital status will continue after the ORR and PFS analysis, until 60% of the patients have had a death event. If the trial accrues 490 patients, 294 deaths, or 60% of 490, will be observed.

9.2.5 ACCRUAL RATE

The accrual rate will be assumed to ramp up as follows: 2, 3, 7, 11, 14, 22 and 25 patients respectively in the 1st to 7th months after study start, and 28.8 patients monthly thereafter.

9.3 POPULATIONS FOR ANALYSES

All enrolled patients who were randomized will be included in the analyses. The following analysis datasets will be considered:

• **Intention-to-Treat (ITT) analysis set** consists of all randomized participants. Analyses will be based on the randomized treatment.
• **Intention-to-Treat Excluding Additional Japanese Patients (ITTE) analysis set** consists of all randomized participants excluding additional Japanese patients. Analyses will be based on the randomized treatment.
• **Per-Protocol (PP) analysis set** defines a subset of the participants in the ITT analysis set without major protocol deviations. The definition of major protocol deviations will be agreed upon, and all cases of such major deviations adjudicated prior to database lock. Analyses will be based on the randomized treatment.
• **Safety analysis set** defines the subset of participants for whom safety analyses will be conducted. It includes all patients who received at least one dose of study medication. Analysis will be based on the actual treatment.
• **PK analysis set** defines a subset of the participants in the Safety analysis set for whom PK samples were analyzed. Analyses will be based on the actual treatment.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Categorical data will be summarized in contingency tables presenting frequencies and percentages. Continuous data will be summarized using number of non-missing values (n), mean, standard deviation, median, minimum and maximum values. Significance tests will be performed at the $\alpha = 0.05$ significance level for two-tailed tests and at $\alpha = 0.025$ for one-tailed tests. Methods to calculate confidence intervals will be specified in Statistical Analysis Plan.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary end-point is ORR based on BICR assessment of CT/MRI scans, and will be analyzed both for the ITT and PP population.

ORR will be analyzed using a Cochran-Mantel-Haenszel test (CMH), stratified for the stratification factors used for randomization (geographic region, primary tumor location and previous neo-adjuvant/adjuvant CRC treatment).

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Secondary efficacy endpoints are PFS, DOR, OS, quality of life (EQ-5D questionnaire), curative metastasis resection, and safety and tolerability (including PRO CTCAE).

PFS and DOR will be based on BICR assessment of the patients CT/MRI scans. PFS, DOR and OS will be analyzed using Kaplan-Meier curves, the logrank test and a stratified Cox proportional hazards model, using the same stratification factors as for randomization (geographic region, primary tumor location and previous neo-adjuvant/adjuvant CRC treatment). The assumption of proportional hazards will be tested. In addition to the p-value, based on a stratified logrank test, and the Hazard Ratio (with 95%CI), descriptive statistics will also be presented for time to event endpoints (Number of patients used for analysis, number of events, median months to event and 95%CI).

The EQ-5D questionnaire results will be analyzed in a descriptive way, e.g. by means of graphical representation. Number of patients undergoing curative metastasis resection will be compared

between the treatment groups. Further evaluation of the results will be specified in the statistical analysis plan which will be in place before including the first patient in the study.

9.4.4 SAFETY ANALYSES

Safety variables include the reported AEs, SAEs, laboratory tests, vital signs and physical examination. AEs will be coded and evaluated for severity using NCI-CTCAE Version 5.0 and will be summarized by MedDRA system organ class and preferred term. Separate summaries and listings will include the following:

- All adverse events
- Adverse events of CTC grade ≥3
- Related adverse events
- Fatal adverse events
- Discontinuations due to adverse events or deaths
- Serious adverse events
- Deaths on IMP treatment or within 30 days of study drug discontinuation
- Patient reported outcome on specific AEs on PRO CTCAE (See Appendix 2) for countries where valid translations are available (See Appendix 2)

The NCI PRO-CTCAE questionnaire results will be analyzed in a descriptive way, e.g. by means of graphical representation. Further evaluation of the results will be specified in the statistical analysis plan.

Laboratory tests will be summarized at each timepoint, including change from baseline. Summaries will be presented separately for hematology, blood chemistry and urinalysis parameters. Other safety data (vital signs, physical examination) will also be tabulated and listed.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

The study treatment groups will be compared on baseline characteristics, including demographics and laboratory measurements, using descriptive statistics. No inferential statistics will be used.

9.4.6 PLANNED INTERIM AND FINAL ANALYSES

9.4.6.1 ANALYSIS PLAN

The trial design will include the following analyses (and adaptations if indicated):

- Based on the interim results of 330 patients (75% information fraction for ORR), who have a follow-up of at least 16 weeks, the DSMB may recommend a 50% sample size increase (from 440 to 660 patients) according to the guidelines in Table 5.
- if the sample size is not increased:
    - the final analysis of ORR and PFS will take place after approximately 34 months with 300 PFS events
- if the sample size is increased:
    - the final analysis of ORR and PFS will take place after approximately 38 months with 450 PFS events

At the time of writing of this protocol amendment, the interim analysis with the possibility of an adaptive sample size increase, already took place. The DSMB did not advice to increase the sample size. Furthermore, as the number of patients censored because of new anti-cancer therapies, was higher than expected, it became clear during the study that the 300 PFS events could not be reached. Therefore it was decided to include all Japanese patients in the ITT population, increasing the total sample size from the planned 440 to 490 patients in total, and to decrease the number of PFS events at the final analysis from 300 to at least 235 (see also Section 9.2.2).

9.4.6.2 INTERIM ANALYSIS

An interim analysis will be performed when 16-week BICR evaluation has been performed for the 330th patient. For the interim analysis of ORR, the significance level will be determined using a Rho spending function, with $\rho = 5$, resulting in a very conservative boundary. Assuming an information fraction of 75% for ORR at this interim analysis, the 1-sided significance level for efficacy will be 0.006, and it will be reached if the difference in ORR is larger than 13.8%. This small alpha spending is foreseen to take into account the interim look at the efficacy primary endpoint, i.e. ORR. However, there is no intention to claim efficacy for ORR at this stage. The interim results, i.e. the conditional power for both ORR and PFS, will only serve to guide the decision rules regarding sample size re-estimation. Results of this analysis will remain blinded to everyone, except the DSMB. Regardless of the efficacy results of the interim analysis, the trial will continue to accrue patients, until the total planned sample size is reached.

As for ORR, there is no intention to claim efficacy for PFS at this early stage either. A small alpha spending is foreseen to take into account the interim look. For this, a very conservative Rho spending function boundary, with $\rho = 5$, is used. Assuming an information fraction of 37% for PFS at this interim analysis, the 1-sided significance level for efficacy will be 0.000173, and it will be reached if the hazard ratio is less than 0.51.

9.4.6.3 SAMPLE SIZE ADAPTATION

The conditional power for both ORR and PFS will be calculated at the interim analysis, assuming that the estimated treatment difference at interim analysis is the true effect. Depending on the conditional powers, the trial will either continue as planned to accrue 440 patients, or the sample size of the trial will be increased by 50%, for a total sample size of 660 patients. The guidelines provided in Table 5 will be used by the DSMB to make a recommendation to the Sponsor. Two possible decisions can be communicated to the Sponsor by the DSMB: “sample size increase” or “no sample size increase”.

Table 5: Guidelines for sample size increase at interim analysis

ORR		PFS		Recommendation	CP660 for	
CP440	Observed ORR difference	CP440	Observed HR		ORR	PFS
≥ 80%	≥11.5%	≥ 75%	≤0.75 (≥3.3 m)	Continue trial unchanged	NA	NA
		50-75%	>0.75; ≤0.8 (≥2.5<3.3m)	Increase sample size	≥ 90%	70-90%
		25-50%	>0.8; ≤0.85 (≥1.8;<2.5m)	Increase sample size*	≥ 90%	>40-<70%
		<25%	>0.85 (<1.8m)	Continue trial unchanged	NA	NA
60-80%	≥ 10%;< 11.5%	≥ 75%	≤0.75 (≥3.3 m)	Increase sample size	80-90%	≥90%
		50-75%	>0.75; ≤0.8 (≥2.5<3.3m)	Increase sample size	80-90%	70-90%
		25-50%	>0.8; ≤0.85 (≥1.8;<2.5m)	Increase sample size*	80-90%	>40-<70%
		<25%	>0.85 (<1.8m)	Continue trial unchanged	NA	NA
< 60%	<10%	≥ 75%	≤0.75 (≥3.3 m)	Continue trial unchanged	NA	NA
		50-75%	>0.75; ≤0.8 (≥2.5<3.3m)	Continue trial unchanged	NA	NA
		25-50%	>0.8; ≤0.85 (≥1.8;<2.5m)	Continue trial unchanged	NA	NA
		<25%	>0.85 (<1.8m)	Continue trial unchanged	NA	NA

ORR = overall response rate; PFS = progression-free survival S=Success, F=Failure, CP=Conditional Power assuming that the estimated treatment difference at interim analysis is the true effect, CP440= conditional power at N=440, CP660= conditional power at N=660. m=months, NA=not applicable
** Including type I error rate adjustment, using the Cui, Hung and Wang method [32]*

The conditional power for ORR would be equal to approximately 80% if the observed difference in ORR, at the time of the interim analysis (75% information fraction) were equal to 11.5%. The conditional power for PFS would be equal to 25% if the observed hazard ratio, at the time of the interim analysis (37% information fraction) were equal to 0.85. Note that a HR=0.85 corresponds with a Median survival time Control/Experiment of 10/11.75, which is considered clinically meaningful. The conditional power for PFS would be equal to 50% if the observed hazard ratio, at the time of the interim analysis (37% information fraction) were equal to 0.80. The conditional power for PFS would be equal to 75% if the observed hazard ratio, at the time of the interim analysis (37% information fraction) were equal to 0.75.

9.4.6.4 FINAL ANALYSIS OF ORR WITHOUT SAMPLE SIZE INCREASE

The final analysis of ORR will be performed when at least 235 PFS events have been observed. The 1-sided significance level for efficacy will be 0.024.

9.4.6.5 FINAL ANALYSIS OF ORR WITH SAMPLE SIZE INCREASE

If the sample size is increased to 660 patients, the final analysis of ORR will be performed when 450 PFS events have been observed. The 1-sided significance level for efficacy will be 0.025.

9.4.6.6 FINAL ANALYSIS OF PFS WITHOUT SAMPLE SIZE INCREASE

The final analysis of PFS will be performed when at least 235 PFS events have been observed. The 1-sided significance level for efficacy will be 0.025.

9.4.6.7 FINAL ANALYSIS OF PFS WITH SAMPLE SIZE INCREASE

If the sample size is increased to 660 patients, the final analysis of PFS will be performed when 450 PFS events have been observed. The 1-sided significance level for efficacy will be 0.025.

9.4.7 SUB-GROUP ANALYSES

Subgroup analyses will be performed for ORR and PFS with respect to the trial's stratification factors (geographic region, primary tumor location and previous adjuvant CRC treatment) and other prognostic factors, which will be specified in the SAP.

The consistency of the treatment effect across prognostic subgroups will be assessed visually using forest plots.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant data will be listed by measure and timepoint

9.4.9 EXPLORATORY ANALYSES

Exploratory analyses are:

- Daily living abilities: analyses will be descriptive.
- Pharmacokinetic characteristics of arfolitixorin
- To determine folate metabolism- and transportation-related gene expression levels in patients with advanced CRC
- Evaluation of Recurrence Free Survival for patients undergoing complete metastatic resection.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms, in local language and in layman's terms, describing in detail the study interventions, study procedures, and risks and possible benefits are given to the patient and written documentation of informed consent is required prior to any study specific procedures are performed.

The protocol, consent form(s), recruitment materials and all patient material (and any changes to these documents) will be submitted to the IRB/IEC for review and approval. If the Patient Information/Informed Consent Form is amended, a determination will be made regarding whether previously consented patients need to re-consent.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the patient's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB/IEC-approved, and the patient will be asked to read and review the document. Furthermore, the consent forms will include any requirements from the ethics committee. The Investigator will explain the research study to the patient, be the primary contact person and answer any questions that may arise. A verbal explanation will be provided in terms suited to the patient's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research patients. Patients will have the opportunity to carefully review the written consent form and ask questions prior to signing. The patients should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate.

Patients must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice.

The patients (or their legally acceptable representative or witness, as applicable) will sign and personally date the informed consent document prior to any study specific procedure. A copy of the informed consent document will be given to the patients. Another copy of the signed consent form will be saved in the Investigator's file. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the patient undergoes any study specific procedures. The rights and welfare of the patients will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

The informed consent includes information that data will be recorded, collected, processed and may be transferred to countries outside the patient's country of residence. The informed consent shall comply, in all respects, all applicable laws and regulations concerning personal data protection in the country in which the study takes place.

The informed consent includes information about the intended purposes of the processing of personal data, the right to obtain access to personal data, the right to correct incorrect personal data, the right

to withdraw their consent of the processing of personal data at any time and the right to delete personal data already collected in the study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to patients, Investigator, and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform, the IRB/IEC and, patients will be contacted, if applicable.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the Sponsor, IRB/IEC and/or Regulatory Authorities.

10.1.3 CONFIDENTIALITY AND PRIVACY

Patient confidentiality and privacy is strictly held in trust by the participating Investigators, their staff, and the Sponsor. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

The study monitor, other authorized representatives of the Sponsor, representatives of the IRB/IEC, regulatory authorities may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The patient's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB/IEC, Institutional policies, or Sponsor requirements.

10.1.4 FUTURE USE OF STORED SPECIMENS AND SAMPLES

No specimens and samples will be used or kept after the end of the study.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Sponsor
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Chairman of DSMB
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10.1.6 SAFETY OVERSIGHT

An independent Data and Safety Monitoring Board (DSMB) will be constituted. The DSMB will protect the safety and well-being of the patients participating in the study and ensuring the ethical conduct of this clinical trial, through a regular review of the accumulated unblinded safety data reported by the independent DSMB statistician. The DSMB will make recommendations to the Sponsor or designee after each meeting, regarding the continuation of the trial, modification of the protocol or any other changes they will judge appropriate. Closure of the study may be recommended if the DSMB deems there is an unacceptable risk / benefit ratio of the participants.

In addition, for Japan the DSMB will review PK data, safety and tolerability after completion of two treatment cycles for the first ten randomized Japanese patients, including patients from both treatment arms. In case of safety concerns or substantial differences in PK profile compared to the other study patients, the DSMB may halt the inclusion of study patients in Japan to gather more information. After review of the data, the DSMB will provide a recommendation to continue, or stop, inclusion of Japanese study patients.

The DSMB will be composed of at least three members with the appropriate expertise, including at least one oncologist and one biostatistician. Members of the DSMB are independent from the study conduct and free of conflicts of interests. A DSMB Charter, finalized before the start of the study, specifies the DSMB procedures, constitution and statistically derived guidelines for sample size increase. The safety monitoring analyses by the DSMB will be conducted every six months or more often if needed, commencing with completion of the first three months of study accrual. Recommendations from the DSMB to Sponsor will comprise, in addition, considerations on sample size increase at interim analysis, as well as early termination of the study or any other relevant recommendations.

10.1.7 CLINICAL MONITORING

In accordance with applicable regulations including GCP, study monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements and facilities, and their responsibilities to satisfy regulatory, ethical, and Sponsor requirements.

Study monitors will be delegated responsibility to monitor the study and site activity to verify that the:

- Data are authentic, accurate, and complete;

- Safety and rights of patients are being protected; and

- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

- Data collection is accurate and reliable which will be assured by verification and cross-check of selected data in the eCRFs against the Investigator's records by the study monitor (source document verification).

- Location of source documents are clearly identified.

The monitor will contact and visit the Investigator regularly and must be permitted access to all source documents needed to verify the entries on the eCRF and other protocol-related documents, provided that patient confidentiality is maintained in accordance with local regulations. It will be the monitor's responsibility to inspect the eCRFs at regular intervals throughout the study, to verify adherence to the protocol and the completeness, consistency and accuracy of the data being entered on the eCRFs.

Sponsor or CRO monitoring standards require full verification that informed consent has been provided, and verification of adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of the main efficacy, safety and tolerability endpoints. Additional checks of the consistency of the source data with the eCRFs will be performed according to the Clinical Monitoring Plan (CMP). Any data reported in the eCRF must be verifiable in the site source documents, if not clearly specified that the eCRF can be the source for a specific data point.

Details of clinical site monitoring are documented in a CMP. The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of risk-based monitoring and SDV will be performed, and the distribution of monitoring reports

The Investigator must ensure that patient anonymity is maintained. On CT-scans, MRIs, lab reports or other documents submitted to Sponsor or to other parties outside the study site, patients must be identified by assigned study ID number only, and never by name or other identifiable information. The Investigator must keep a patient identification code list showing the patient's name, date of birth, or any other locally accepted identifiers. Documents identifying the patients (e.g., signed informed consent forms) should not be sent to Sponsor, and must be kept in strict confidence by the Investigator.

The Investigator and co-Investigators agree to cooperate with the monitor(s) to ensure that any issues detected in the course of these monitoring visits are resolved. If the patient is hospitalized or dies in a hospital other than the study site, the Investigator is responsible for contacting that hospital in order to document the SAE.

The Investigator must on request supply the Sponsor with any required background data from the study documentation or clinical records. This is particularly important when errors in data transcription are suspected. In the case of special problems and/or governmental queries, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

10.1.8 QUALITY ASSURANCE

This study will be conducted in compliance with the protocol, the ICH Guideline for GCP, and any applicable local regulations (for US sites, the study will be conducted under an IND). Sponsor is

responsible for independent quality assurance (QA) audits of the clinical study processes, if deemed appropriate. Audit of the study sites may be conducted to assess and help assure compliance with GCP and applicable regulatory requirements. The study sites may be subject to a QA audit by Sponsor or its representatives, may be reviewed by an independent QA department, or may be inspected by regulatory authorities. Auditors/inspectors will have the right to inspect the study sites at any time during and/or after completion of the study and will have access to source documents, including patients' medical records. By participating in this study, the Investigator agrees to this requirement.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 PERSONAL DATA PROTECTION

Patient personal data will be collected and processed in this study to answer the scientific questions investigated. The legal basis for the processing of personal data is the informed consent collected from all patients. Patients are informed about their rights connected to the processing of their personal data in the informed consent process.

The Sponsor is the controller of all personal data recorded, collected and processed in this study. The Sponsor follows applicable laws and regulations concerning personal data protection in the countries where the study is being conducted. All personal data processed by the Sponsor is coded, and the Sponsor does not know the identity of the individual patients (this information will only be available at the study site of the respective patient).

The sites are responsible for handling of their patients' personal data collected as part of the patient's treatment. The Sponsor is responsible for the processing of the coded data received from the sites. Both sites and the Sponsor will take the necessary measures in order to comply with all applicable Data Protection laws.

Coded patient data for statistical analysis and scientific reporting, will be transmitted to various recipients (Sponsor, and sponsor representatives, Health Authorities, IRB/IEC, or other participating clinical study sites). The transferred data will not include the participant's contact or identifying information. Individual participants and their research data will be identified by a unique identification number. The Sponsor is not able to un-identify the code, as the key to the code is available to designated study staff at each clinical site only. The study data entry and study management systems used by clinical sites and by the Sponsor will be secured and protected with a personal password. Isofol Medical has set up appropriate restrictions and controls to ensure that patient data remains protected. If study data is transferred to the US, it will only be transferred to companies who participate in and comply with the so-called Privacy Shield, which is an agreement between EU and USA for the protection of personal data in the US.

The Sponsor has an integrity policy for clinical studies. This policy includes details of the legal basis for processing of personal data and the Data Protection Officer appointed at the Sponsor (contact information can be found in this protocol and on the Sponsor's website).

10.1.9.2 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

An eCRF will be used to record all data required by the protocol,

Prior to the start of the study, the Investigator will complete a "Delegation of significant study-related duties" form, defining any person who is authorized to make or change entries in the eCRF and any person authorized to sign the eCRF.

Role-specific training sessions will be completed before a person will be granted access to the eCRF (e.g. Investigators and site staff, Sponsor staff and contract research organization staff, including project managers, clinical research associates and data managers).

Support is available in the form of general eCRF user manuals (monitor and site manuals) and study specific Data Entry Instructions.

The eCRF used for this study is validated and fulfils the ICH GCP requirements, and European, US FDA (21 CFR Part 11), Australian and Japanese regulations. The data capture system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly, or from defined source documents by an authorized person.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Hardcopies of the study visit worksheets may be provided for use as source document worksheets for recording data for each patient enrolled in the study. Data recorded in the eCRF derived from source documents should be consistent with the data recorded on the source documents. The Investigator is responsible for the management and accuracy of the information in the eCRF. At each monitoring visit, the eCRF should be at the monitor's disposal for review.

Changes to the data in the eCRF must be made at the site by an authorized person. The eCRF will have an audit trail with appropriate functionality for data capture, tracking, and documentation of any queries or changes. Electronic signatures will be used to lock the data and identify the person entering or changing the data.

Before database closure, reconciliation between the SAEs entered in the safety database and the study database will be performed.

10.1.9.3 STUDY RECORDS RETENTION

Following closure of the study, the Investigator or the head of the medical institution (where applicable) must maintain all site study records, except for those required by local regulations to be maintained by someone else, in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff.

Sponsor will inform the Investigator/institution of the required time period for retaining these records in order to be compliant with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that study site, as dictated by ICH GCP E6 Section 4.9, any institutional requirements or local laws and regulations, or Sponsor standards/procedures.

The Investigator must notify Sponsor of any changes in the archival arrangements, including, but not limited to, the following: archival at an off-site facility, transfer of ownership of the records in the event the Investigator leaves the site. In addition, the Investigator should seek the written approval of the Sponsor prior to disposing any of the archived records.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or ICH GCP. The noncompliance may be either on the part of the patient, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site Investigator to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation. All deviations must be documented and reported to Sponsor. If relevant, protocol deviations must be sent to the reviewing IRB/IEC per their policies. The site Investigator is responsible for knowing and adhering to such IRB/IEC requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

Upon finalization of the ORR and PFS analysis, an integrated clinical and statistical study report will be written by the Sponsor in consultation with the Coordinating Investigators. This report will be based on the items detailed in this study protocol. When the clinical study report is completed, Sponsor will provide the Investigators with a full summary of the study results. The Investigators are encouraged to share the summary results with the patients, as appropriate.

An update of the study report will be done when the follow-up is completed including overall survival results.

The first resulting publication will be a full publication of all data from all participating sites, coordinated by Sponsor. Any secondary publications by the Investigators (abstracts in journals, oral presentations etc.) will reference the original publication and will require pre-submission review by the Sponsor. Note that the Sponsor is entitled to delay any proposed secondary publication, in order to obtain patent protection, if required.

Authorship for Investigators will be assigned on the basis of their recruitment contribution, as well as intellectual and administrative input. Ranking will be according to the number of patients randomized as well as contribution to the study conduct and preparation of final manuscript.

10.1.12 INSURANCE

Patients will be covered under Sponsor’s liability insurance policy throughout the course of the study. The certificate of insurance will be available in the Investigator Study File at each study site and may be provided upon request. The participating patients are also protected in accordance with national regulations as applicable. Essential information about the insurance coverage will be provided to the participating patients through the Informed Consent process.

10.1.13 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study

leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest. Certification and disclosure of any applicable financial interests of all clinical investigators shall be done per the requirements of 21 CFR 54 (Financial Disclosure by Clinical Investigators).

10.2 ABBREVIATIONS

5-FU	5-fluoroucacil
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine Transaminase
ANC	Absolute Neutrophil Count
API	Active Pharmaceutical Ingredient
AST	Aspartate Transaminase
BICR	Blinded Independent Central Review
BOR	Best Overall Response
CFR	Code of Federal Regulations
CR	Complete Response
CRA	Clinical Research Associate
CRF	Case Report Form
CSP	Clinical Study Protocol
CTCAE	Common Terminology Criteria for Adverse Events
DOR	Duration of Response
DPD	Dihydropyrimidine Dehydrogenase
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Forms
EGFR	Epidermal Growth Factor Receptor
EMA	European Medicines Agency
EORTC	European Organization for Research and Treatment of Cancer
EOT	End of Treatment Visit
ESMO	European Society for Medical Oncology
FDA	Food and Drug Administration
FFPE	Formalin-Fixed Paraffin-Embedded
FP	Fluoropyrimidine
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HDMTX	High Dose Methotrexate
IB	Investigator's Brochure
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IRC	Independent Review Committee
ITT	Intention-To-Treat
IWRS	Interactive Web Response System
LV	Leucovorin
mCRC	Metastatic Colorectal Cancer
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging

MTHF	Methylene Tetrahydrofolate
ORR	Overall Response Rate
OS	Overall Survival
PD	Progressive Disease
PFS	Progression-Free Survival
PI	Principal Investigator
PK	Pharmacokinetic
PR	Partial Response
QA	Quality Assurance
PRO	Patient Reported Outcome
RECIST	Response Evaluation Criteria In Solid Tumours
RR	Response Rate
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Stable Disease
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAP	Thorax Abdomen Pelvis
THF	Tetrahydrofolate
TS	Thymidylate Synthase
ULN	Upper Limit of Normal
UP	Unanticipated Problem
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

10.3 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.0	21Sep2018	Initial version	Initial version
2.0	21Sep2018	In case of Oxaliplatin caused toxicity, dose modifications according to label should be performed. Update of OS analysis to exclude a significant detrimental effect of arfolitixorin as compared to leucovorin in terms of OS.	FDA SPA request
3.0 USA	13Nov2018	Inclusion criteria 3: Addition of leucovorin. Inclusion criteria 7: Change Hgb level from 100 g/L to 80 g/L Add collection of baseline mutations in the eCRF (KRAS, BRAF, NRAS) at screening if available. Update of interim analysis conditions. Clarifying CT/MRI follow-up after metastasis resection.	To clarify the components of standard FOLFOX treatment. To align protocol inclusion criteria regarding Hgb levels with similar phase III mCRC studies. To enable sub-group analysis. To avoid increasing sample size in case of poor interim study results. Protocol clarification.
4.0 USA	29Aug2019	Exclusion criteria 2 and 20: Change from >6 months to >28 days from surgery to randomization Exclusion criteria 3: adding “adjuvant” anti-cancer treatment Oxaliplatin and Leucovorin should be administered in sequence or in two different injection ports.	To align protocol exclusion criteria regarding pre-study surgery with clinical praxis. Clarifying the intention of the exclusion criteria. To ensure study patients get full benefit from their oxaliplatin treatment as described in the SPC.
5.0 USA	07Oct2019	Section 8.2.2. Clarifying ECG assessments according to the previously updated Oxaliplatin and Leucovorin administration.	To correct the information according to the Summary of Changes document dated 29 Aug 2019.
6.0 USA	20Feb2020	2.2.1 Addition of non-clinical data. 2.2.2 Addition of clinical data. 3. Update of the outcome measure for Duration of Response objective. 3. New exploratory objective investigating relevant tumor biomarkers levels.	Additional data available. Additional data available. To align wording with recommendations from competent authorities. Increase understanding of the relation between biomarker

			levels and the treatment outcome.
		3. New exploratory objective investigating Recurrence Free Survival for patients undergoing metastatic resection.	To compare Recurrence Free Survival data between the treatment groups.
		4.1 Clarifying geographical stratification.	Addition on Australia and Japan.
		5.1 Inclusion criteria 4: Addition of lymph nodes.	Protocol clarification in accordance with RECIST 1.1
		5.5 Update of number of sites and regions	Protocol clarification.
		6.1.2 and 6.3.1 Defining 5-FU bolus duration.	Protocol clarification.
		6.2.4 Body Surface Area calculated according to DuBois formula.	Protocol clarification.
		6.2.4.2 Leucovorin administration	The supplied Bendafolin contains trometamol.
		6.3.3 Allowing use of bevacizumab biosimilars.	Protocol clarification.
		6.3.4 5-FU dose reduction	Protocol clarification
		6.4.1 Geographical region update	Protocol clarification
		8.1.2 PK sampling timing.	Protocol clarification
		8.2.2 Clarifying patients position during blood pressure and ECG measurements.	Protocol clarification
		8.2.5 Clarifying how to manage patients becoming eligible for metastasis resection.	Protocol clarification
		8.3.4 and 8.4 Update of safety section	Protocol clarification
		9.2.4 Overall Survival analysis updated.	Clarifying according to FDA request.
		9.4.9 Addition of recurrence free survival.	Protocol clarification
		10.1.5 Update of contact details	Protocol clarification
		10.1.9.2 Addition of study specific Data Entry Instructions and update reg eCRF validation.	Protocol clarification

		10.1.6 Adding details to the DSMB review.	Japan added to the study.
7.0 USA	20Aug2021	1.2 Schema Updated	Schema updated as ORR and PFS analysis are performed at the same timepoint
		2.1.3 Minor clarifications	Minor clarifications done to improve understanding of mechanism of action
		4.4 Follow-up process for patients post primary endpoint analysis	Process clarified as time point for ORR and PFS analysis is updated
		8.1.2 Update of patients included in PK analysis	Number of patients needed in PK evaluation is adjusted
		8.1.2.1 Update of analyte in population PK analysis and studies included in the analysis	Population PK analysis will only be done on the active compound and not on the metabolites. One previous mentioned study will not be included in the analysis
		8.2.2 Patients experiencing a QTc prolongation should be taken of study drug	Previous version stated that patient should be excluded from study but patient should only be taken off study drug
		8.2.4 Section updated and clarified	Genes included in the analysis are updated
		8.2.5 Section updated and clarified	Updated as not only metastases are resected during study
		8.3.5 Follow-up process for patients post primary endpoint analysis	Process clarified as time point for ORR and PFS analysis is updated
		9.2.2 Section updated and clarified	Correction to ensure wording is not contradicting the SAP
		9.2.4 Section updated	ORR included as the analysis of ORR and PFS will be done at the same timepoint
		9.3 Section updated and clarified	Correction to align wording with SAP
		9.4.6.1 Section updated and clarified	Correction to align wording with SAP
		9.4.6.3 Corrections in Table 5 of errors	To be aligned with SAP and DSMB charter
		9.4.6.4 Section updated	ORR included as the analysis of ORR and PFS will be done at the same timepoint

		9.4.6.5 Section updated	ORR included as the analysis of ORR and PFS will be done at the same timepoint
		9.4.7 Section updated and clarified	To be aligned with SAP and DSMB charter
		10.1.11 Section clarified related to Clinical Study Report	CSR will be written when the analysis for the primary endpoints are available. An update of the CSR will done when OS data are available
		Minor editorial changes	
8.0 USA (Never submitted, changes included in version 9.0)	22Feb2022	Number of patients included in the study updated in multiple sections	Change from 440 patients to 490 patients as all Japanese patients are included
		Number of PFS events updated in multiple sections	Change from 300 PFS events to 230 PFS events
		1.1 Synopsis	Study recruitment period updated to reflect the actual recruitment period
		2.2.2 Section updated	Updated with latest safety information from IB V14.0 1Oct2021
		4.1 and 5.5 Sections updated	Information added clarifying that the addition of patients to the ITT population was taken after the interim analysis
		9.2.1 Section updated	Addition of information on how statistical analysis of ORR is affected after study population is changed to 490
		9.2.2 Section updated	Addition of information on how statistical analysis of PFS is affected after change in number of PFS events
		9.2.4 Section updated	Approximate timelines removed. Considerations for OS analysis is updated, and wording is deleted as PFS hazard ratio has been updated but timepoint of OS analysis at 60% remains unchanged
		9.3 Section updated	“Main study” removed as all 490 patients are included in the analysis

		9.4.6 Section updated 9.4.7 Section updated 10.1.5 CMO phone number updated Minor editorial changes	Text updated and added as this protocol update is done after the Interim analysis Information updated in accordance with current version of the SAP Phone number changed
9.0 USA	22Apr2022	Number of PFS events updated in multiple sections 9.3 Addition of ITTE analysis set	Change from 300 PFS events to at least 235 PFS events, to align with statistical analysis plan and regulatory authority feedback. Intention-to-Treat Excluding Additional Japanese Patients (ITTE) analysis set added, to be in line with current SAP.

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