A Phase 1/2 Study to Investigate the Safety, Pharmacokinetics and Efficacy of Tinostamustine, a First-in-Class Alkylating Histone Deacetylase Inhibition (HDACi) Fusion Molecule, in Patients with Advanced Solid Tumors

Sub-study to Characterize the Effects of Tinostamustine at a Dose of 60 mg/m^2 Administered during a 60-minute Infusion on Cardiac Repolarization in 6 Patients with Advanced Solid Tumors

NCT NUMBER **NCT03345485**

TABLE OF CONTENTS

TABLE OF TABLES

ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Explanation
AE	Adverse event
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
ASCO	American Society of Clinical Oncology
AST	Aspartate aminotransferase
AUC	Area under the curve
BSA	Body surface area
BUN	Blood urea nitrogen
CA	Competent Authority
CAP	College of American Pathologists
CFR	Code of Federal Regulations
C_{max}	Maximum plasma concentration
CNS	Central nervous system
CR	Complete response
CRP	C-reactive protein
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose-limiting toxicity
DME	Dose-modifying event
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EDC	Electronic data capture
ELISA	Enzyme-linked immunoassay
FA	Full analysis
FDA	Food and Drug Administration
FFPE	Formalin fixed paraffin embedded
GCP	Good Clinical Practice

Abbreviation	Explanation
GGT	Gamma-glutamyl transaminase
GIST	Gastrointestinal stromal tumors
HDACi	Histone deacetylase inhibitor
HER2	Human epidermal growth factor receptor
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HPβCD	Hydroxyl-propyl-β-cyclodextrin
i.v.	Intravenous; Intravenously
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IHC	Immunohistochemistry
IME	Important medical event
INN	International Nonproprietary Name
IRB	Institutional Review Board
ISH	In situ hybridization
LDH	Lactate dehydrogenase
MAD	Maximum administered dose
MedDRA	Medical Dictionary for Regulatory Activities
miRNA	Micro ribonucleic acid
MM	Multiple myeloma
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NYHA	New York Heart Association
ORR	Objective response rate
OS	Overall survival
PARP	Poly (ADP-ribose) polymerase
PFS	Progression free survival

Abbreviation	Explanation
Ph. Eur.	Pharmacopoeia Europaea
PI	Principal Investigator
PK	Pharmacokinetic
PMN	Polymorphonuclear
PO QD	orally, once daily
QT	A measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle. The QT interval represents electrical depolarization and repolarization of the ventricles.
QTc	Corrected QT interval
RBC	Red blood cell count
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic acid
RP2D	Recommended Phase 2 dose
SADR	Serious Adverse Drug Reactions
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCLC	Small cell lung cancer
SD	Stable disease
SJS	Stevens-Johnson syndrome
SmPC	Summary of Product Characteristics
SRC	Safety Review Committee
STS	Soft tissue sarcoma
SUSAR	Suspected unexpected serious adverse reaction
TdP	Torsades de pointes
TEAE	Treatment-emergent adverse event
TEN	Toxic epidermal necrosis
TLS	Tumor lysis syndrome
T_{max}	Time to maximum concentration
TNBC	Triple negative breast cancer
ULN	Upper limit of normal
US, USA	United States, United States of America

Abbreviation	Explanation
USP	United States Pharmacopeia
WBC	White blood cell count

1 PROTOCOL SYNOPSIS

Title	**Sub-study to Characterize the Effects of Tinostamustine at a Dose of 60 mg/m^2 Administered during a 60-minute Infusion on Cardiac Repolarization in 6 Patients with Advanced Solid Tumors.**
Protocol Number	EDO-S101-1002
Trial Sponsor	**Mundipharma Research Limited**
Objectives	Primary objective • To characterize the effect of tinostamustine at a dose of 60 mg/m^2 on cardiac repolarization (QTcF) and other ECG parameters in 6 patients with solid tumors who have progressed after at least one line of therapy and for whom no other standard therapy with proven clinical benefit is available. Tinostamustine will be administered intravenously (i.v.) on Day 1 and 15 of each 4-week treatment cycle. Secondary objective: • To evaluate safety and tolerability of tinostamustine. • To establish the pharmacokinetic (PK) profile of tinostamustine 60 mg/m^2 administered over 1 hour on Day 1 and 15 of each 4-week treatment cycle. • To determine overall response (ORR), the clinical benefit rate (CBR) and stable disease at 4 months and duration of response (DR). • To determine the progression free survival (PFS). • To determine the overall survival time (OS). • To determine duration of response. Exploratory objective: • To correlate the extent of gene expression changes in tumor samples with anti-tumor activity.
Clinical Phase	Phase 1 sub-study to Phase 1/2 clinical trial EDO-S101-1002
Trial Drug	Tinostamustine (EDO-S101)
No. of Patients	Maximum 6 patients
Number of Centers	Up to three centers; 6 patients will be enrolled in the United States.

Trial Design	This sub-study is designed to better characterize the effect of tinostamustine on cardiac repolarization (QTc) and other ECG parameters, for the treatment of solid tumors as investigated in the Phase 1 portion of the EDO-S101-1002 trial. An additional subgroup of six (6) patients treated with a dose of 60 mg/m^2 tinostamustine administered over 60 minutes will be studied with intense ECG measurements, including 24-h Holter monitoring, and PK sampling. The PK sampling will occur at the first 2 treatment infusions in cycle 1 assuming no Grade 3 or higher (CTCAE v.5) QTcF prolongation in any 2 subjects or any fatal cardiac event occur in 1 or more subjects. The relationship between Cmax, and AUC will be examined relative to any prolongation of the QTcF seen on central ECG reading and simple descriptive statistics will be applied as appropriate. In particular, the distribution of the Cmax levels and the variability of the QTcF in this population will be described as well as the apparent relationship between Cmax and QTcF changes for the assessment of further dose escalation to ensure subject safety. These 6 patients will then be treated in accordance with the main trial protocol and treatment will continue as foreseen up to trial completion. Following enrollment of 6 patients the study will be stopped. Additionally, if any two or more patients have grade 3 or greater centrally confirmed QTc prolongation or QTcF increased >60 ms from baseline or ≥1 patient has a fatal cardiac event related to tinostamustine, the trial will be stopped. Additional patients beyond the initial 6 patients at the 60 mg/m^2 dose or new cohorts evaluating higher doses can only be enrolled following review of the data with regulatory authorities.
Sub-Study Stopping Rules	**Stopping Rules for sub-study for administering Tinostamustine** Stopping rules in this sub-study apply for patients who experience QTc prolongations >500 ms or change from baseline >60 ms (Grade 3) that are not transient or occur in more than 1 treatment cycle. If the QTcF value on the electrocardiogram (ECG) machine printout is >500 ms or represents an increase > 60 ms from baseline, 2 additional ECGs are to be performed approximately 1 minute apart. If the average QTcF of the 3 ECGs is >500 ms or increased > 60 ms from baseline, the tinostamustine infusion must be stopped. The patient should stay in the unit until the QTcF has decreased to baseline. In addition, the patient is to be continuously observed for syncope or other clinically relevant cardiac events. A thorough evaluation of ECGs, including expedited central review of Grade 3 QTc prolongations by an independent assessor, will be performed. The decision

	will then be made by the Sponsor in consultation with the Medical Monitor, whether tinostamustine treatment is to continue or be postponed. If any two or more patients have grade 3 or greater centrally confirmed QTc prolongation or QTcF increased >60 ms from baseline or ≥1 patient has a fatal cardiac event related to tinostamustine , the trial will be stopped. (Refer to Section 8.7, Dose modification guidelines and stopping rules, for the management of patients who experience clinically significant QTcF prolongations). **General stopping rules for sub-study**: If 2 patients from the cohort of 6 experience the following AEs at Grade 3 judged to be causally related to study drug or application procedure, the sub-study will be stopped for enrollment and the patients already recruited stopped for further treatment. • G3 deep venous thrombosis • G3 serum creatinine • G3 nervous system disorders excluding headache
Sub-study Population	**Inclusion Criteria for sub-study:** • Signed informed consent. • Patients age ≥18 years at signing of the informed consent. • Histologically confirmed diagnosis of advanced or metastatic solid tumors, disease should have progressed following at least 1 line of therapy and no other standard therapy with proven clinical benefit is available or recommended based on the investigator's individual risk-benefit assessment for the patient. Women with triple negative breast cancer must have had at least 3 prior lines of therapy and there remains no other standard therapy with proven clinical benefit. • Patients with secondary metastasis to the central nervous system (CNS) are eligible if they have had brain metastases resected or have received radiation therapy ending at least 4 weeks prior to trial day 1 and they meet all the following criteria: (1) Residual neurological symptoms ≤ Grade 1 (2) No glucocorticoid requirements or patients may be receiving low doses of glucocorticoids providing the dose has been stable for at least 2 weeks prior to starting the trial medication

(3) Follow-up imaging does not show progression of treated lesions and no new lesions

- Evaluable disease; either measurable on imaging or with informative tumor marker as assessed by RECIST version 1.1.
- Discontinuation of previous cancer therapies at least 3 weeks or 5 half-lives, whichever is shorter, as long as the patient has recovered to eligibility levels prior to treatment in this trial.
- Eastern Cooperative Oncology Group (ECOG) performance status ≤2 (Section 12.1).
- Absolute neutrophil count (ANC) (polymorphonuclear [PMN] cells plus bands) >1,000 µL.
- Platelets ≥100,000 µL.
- Aspartate aminotransferase/alanine aminotransferase (AST/ALT) ≤3× upper limit of normal (ULN). In cases with liver involvement ALT/ AST ≤5× ULN.
- Total bilirubin ≤1.5 mg/dL unless elevated due to known Gilbert's syndrome.
- Creatinine ≤1.5 ULN.
- Serum potassium and magnesium within normal range, at baseline (supplementation is permissible).
- Men and women of child-bearing potential, and their partners, must be willing to use at least 2 effective forms of birth control during the trial drug administration and for at least 90 days after the administration of the trial drug to be eligible to participate. Vasectomized partners and patients must be willing to use a secondary method of effective birth control. Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the trial treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient.

Exclusion Criteria for sub-study:

- Patients with primary central nervous system (CNS) cancer.
- Patients with QTc interval (Fridericia's formula) >450 ms.
- Patients who are on treatment with drugs known to prolong the QT/QTc interval. Refer to CredibleMeds list of drugs with known risk of Torsade des pointes (TdP): http://crediblemeds.org/new-drug-list.

	• Patients who are being treated with Valproic Acid for any indication (epilepsy, mood disorder) must be excluded or must stop using the medication and have a wash out period of 3.5 days prior to first dose of tinostamustine in this trial. • Any serious medical condition that interferes with adherence to trial procedures. • Prior history of another solid tumor malignancy diagnosed within the last 3 years of trial enrollment excluding adequately treated basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or *in situ* cervical cancer, *in situ* breast cancer, *in situ* prostate cancer (patients must have shown no evidence of active disease for 2 years prior to enrollment) • Pregnant or breast-feeding women. • New York Heart Association (NYHA) stage III/IV congestive heart failure (Section 12.2). The following arrhythmias: atrial fibrillation/flutter with poor rate control, documented sustained ventricular tachycardia (defined as >30 seconds or requiring cardioversion before 30 seconds have elapsed) or TdP, ventricular preexcitation (Wolff Parkinson White syndrome) Brugada Syndrome, Complete LBBB, QRS > 120 ms, • Implanted pacemaker or implantable cardiac defibrillator (ICD) • Significant co-morbidities (e.g., active infection requiring systemic therapy, history of human immunodeficiency virus [HIV] infection, or active Hepatitis B or Hepatitis C). • Use of other investigational agents within 30 days or 5 half-lives prior to the first dose of tinostamustine, provided the patient has recovered from any related toxicities ≥Grade 1. • Steroid treatment within 7 days prior to trial treatment. Patients that require intermittent use of bronchodilators, topical steroids, or local steroid injections will not be excluded from the trial. Patients who have been stabilized to 10 mg prednisolone orally (PO) once daily (QD) (or equivalent), daily (or less) at least 7 days prior to trial drug administration are allowed.
Dose and Schedule	The dose of 60 mg/m^2 tinostamustine administered over 60 minutes. Administration D1 and D15 in a 28-day cycle. There will be a maximum of 6 cycles of treatment according to the Sub study protocol. At the Investigators' discretion and Sponsor approval, treatment can be continued beyond 6 cycles in responding patients or patients who have experienced clinical benefit up to a maximum of 12 cycles.

Duration of sub-study / Patient Participation	The number of patients participating in this sub-study is 6 at maximum. An estimated three months for patient enrollment and 12 months for follow-up.
Safety Evaluations	Safety assessments include physical examinations, ECOG performance status determinations, electrocardiograms (ECGs), pregnancy testing for women of childbearing potential, documentation of treatment- emergent adverse events (TEAEs), clinical laboratory evaluations including hematology, blood chemistry and urinalysis, vital signs, and documentation of concomitant medication usage. Toxicities in both stages of the trial are assessed for severity using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03, June 2010, with the exception that assessment of QTc prolongations constituting AEs of special interest are based on CTCAE version 5.0.
Efficacy Evaluations	Efficacy evaluations in this sub-study will include ORR (i.e., patients with a CR plus patients with a PR of any duration), rate of patients with SD of at least 4 months duration, duration of response, PFS, and OS. Radiologic response assessment by computed tomography (CT) or magnetic resonance imaging (MRI) will be performed at baseline and every 2 cycles during treatment and every 2 months after stop of treatment. Tumor response will be evaluated according to RECIST version 1.1 (Section 12.3).
Pharmacokinetic Assessments	Plasma samples will be collected to determine the concentrations of tinostamustine, and its 2 metabolites M2 and M8, by a method fully validated according to the relevant guidelines. PK samples are to be collected from the arm opposite of that used for tinostamustine administration. Blood sampling will occur in Cycle 1 only at each drug administration and should be collected following the 10-minute supine resting periods described for the continuous Holter recordings. The schedule is provided in Section 7.16 of this protocol.
Pharmacodynamic Assessments	Patient participation in the gene expression analysis is not mandatory for enrollment into the trial. If the patient agrees to participate in the gene expression analysis, a fresh or archival tissue sample will be collected during screening. Formalin fixed paraffin embedded (FFPE) biopsy samples will be used. The Affymetrix chip technology for analyzing total ribonucleic acid (RNA) will be used. For formalin fixed paraffin embedded samples, RecoverAll method from Ambion® for isolating total RNA will be used. The isolated RNA is total RNA and will contain both messenger RNA (mRNA) and micro RNA (miRNA). The

	expression profile will be analyzed for correlation with response or resistance to therapy. Details of sample preparation and shipping are described in the Laboratory Manual.
Sample Size Determination, Analyses	No formal sample size determination

2 SCHEDULE OF ASSESSMENTS

Table 1 Schedule of Assessments

Procedure	Screening 28 days from Baseline (First Day Trial Drug Administration); Scans 28 days from Baseline[15]	Cycle 1 and Subsequent Cycles[1]					Trial Drug Discontinuation (at any time or Day 28 of last cycle)[14]	Follow-up[12,13]
		Day 1[14]	Day 2[14]	Day 15[14]	Day 16[14]	Day 22[14]		
Informed Consent	X							
Eligibility Criteria	X							
Demographics and Medical History (including prior cancer therapies)	X							
Complete Physical Examination	X	X					X	
Abbreviated Physical Examination[15]				X		X		
Weight and Height[2]	X	X						
Vital Signs[3]	X	X		X		X	X	
ECOG Performance Status	X	X		X		X	X	
12-lead ECG Assessments (Safety and Holter)[4]	X	X		X				
PK Assessments[5]		X[5]	X[1]	X[5]	X[1]			
Gene Expression Profiling[6]	X							

Procedure	Screening 28 days from Baseline (First Day Trial Drug Administration); Scans 28 days from Baseline[15]	Cycle 1 and Subsequent Cycles[1]					Trial Drug Discontinuation (at any time or Day 28 of last cycle)[14]	Follow-up[12,13]
		Day 1[14]	Day 2[14]	Day 15[14]	Day 16[14]	Day 22[14]		
Hematology[7]	X	X		X		X	X	
Serum Chemistry[8]	X	X		X			X	
Urinalysis	X						X	
Pregnancy Test (urine or serum)[9]	X	X					X	
Baseline and Response Assessments[10, 12]	X						X	
Record TEAEs		X	X	X	X	X	X	
Assessment of Infusion Site and potential allergic reactions[11]		X		X				
Record Concomitant Therapies and Procedures	X	X		X		X	X	
Trial Drug Administration		X		X				
Obtain PFS Information[12]								X
Survival Follow-up								X[13]

[1] Visits on Day 2 and 16 in Cycle 1 only: 24h (±2 hr) from the start of infusion the day before.

[2] Height will be measured at screening or baseline only. The weight will be measured at screening and on Day 1 of each cycle. The documentation of weight will be used for trial drug calculations of BSA. BSA will be calculated using the DuBois formula for each patient at the site.

[3] Resting supine blood pressure, pulse, respiratory rate, and temperature will be measured at Screening, Day 1, 15, and 22, and at trial discontinuation. On each treatment day blood pressure, pulse, and respiratory rate will be recorded pre-dose, 3 (±10 minutes), and 6 (±10 minutes) hours from the start of the tinostamustine infusion. Temperature

will be recorded at pre-dose on each treatment day. After Cycle 1 the vitals for the 3- and 6-hour time points are considered optional assessments and should be done at the Investigator's discretion. If the 3- and 6-hour time points are not done, the Investigator must provide adequate instruction to the patient regarding potential allergic reactions, and this should be clearly documented in the patient chart.

[4] All ECGs will be obtained digitally using a Global Instrumentation (Manlius, NY, USA) M12R ECG and are to be performed after the patient is supine for 10 minutes A triplicate ECG will be performed during Screening (at least 1-2 minutes between each measurement) and will be read centrally to determine patient eligibility for the trial. A triplicate ECG is to be performed before tinostamustine administration on D1 (i.e., day of tinostamustine dosing) in each cycle. Furthermore, patients are to have single ECGs performed at 30 and triplicate at 60 minutes from the start of tinostamustine administration on D1 and D15 each treatment cycle. Additional single ECGs will be conducted at 90, 120 and 180 min and at study drug discontinuation. Additional ECGs may be conducted as clinically indicated.

Holter monitoring will commence 60 minutes prior to the start of the infusion on C1D1 and will continue through 24 hours from the start of infusion. Replicate 10 second, 12-lead ECGs will be extracted from the continuous recording at each of the following time points on C1D1 and C1D15:

- C1D1: -45, -30, -15 minutes predose, and 15, 30, 45, 60, 75, 90, 120, 180, 360 minutes and 24 hours from the start of infusion.
- C1D15: prior to the start of infusion, and 15, 30, 45, 60, 75, 90, 120, 180, 360 minutes and 24 hours from the start of infusion.

[5] The blood sampling schedule for the PK assessment is conducted on Day 1 and 15 of Cycle 1 only. Multiple samples are taken as follows: up to 0.5 hours prior to dose administration and at 15, 30 and 45 minutes, 1 hour, 75 and 90 minutes, and 2, 3, 6, and 24 hours from the start of the tinostamustine infusion, from the start of drug administration. Samples will be taken at the same time and as close to the exact time point as possible, with sample draw windows: 15, 30, 45, 60, 75, and 90 minutes (±5 minutes); 2, 3 and 6 hr (±10 minutes), and 24 hr (±2 hr). In Phase 1, the PK profiles of tinostamustine in plasma were assessed in each patient during the escalation phase and in Phase 2, at centers participating a maximum of 55 patients consecutively enrolled will participate in the PK analysis. The remaining patients will have only one PK assessment at the end of infusion.

[6] Best efforts will be undertaken to obtain specimens of tumor from patients. Information on the purpose of genetic research in the gene-expression sub-trial is provided, either in the main ICF or a separate ICF, based on applicable regulatory requirements, to allow the patient to decide whether he or she want to participate in this part of the trial. Participation in this genetic research is voluntary. See Section 7.9 and the Laboratory Manual for tumor sample requirements.

[7] Hematology will include white blood cell count (WBC) and differential, RBC, hemoglobin, hematocrit, platelets and absolute neutrophil count (ANC). Blood samples will be collected at Screening, Days 1, 15 and 22 of each cycle, from cycle 1 to cycle 6, and at the time of trial drug discontinuation (at any time or Day 28 of the last treatment cycle). Patients continuing on trial until PD or intolerable toxicity, will have blood samples collected on days of drug administrations and at the time of trial drug discontinuation (at any time or Day 28 of the last treatment cycle).

[8] Serum chemistry will include albumin, total protein, creatinine, uric acid, blood urea nitrogen (BUN), sodium, potassium, calcium, glucose, total bilirubin, alkaline phosphatase, AST, ALT, gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) and C-reactive protein (CRP).

[9] Women of childbearing potential.

[10] Patients will have a baseline tumor assessment done within the 28 days (with a 7-day window) prior to Cycle 1, Day 1. Response assessment by imaging after Cycle 2, 4 and 6. In addition, the response assessment may be performed at any time according to symptoms and clinical judgment of the treating physician.

[11] Assessment of infusion site reactions must be performed on each treatment day at pre-dose, 1 hr (±15 min). The patient will be observed at 1 hr (±15 min post dose) for potential allergic reactions (See Section 8.10 for possible infusion reactions). The Investigator must provide adequate instruction to the patient regarding potential allergic reactions, and this should be clearly documented in the patient chart.

[12] For patients who discontinue trial treatment for reasons other than PD, tumor assessments per RECIST will be performed every 8 weeks (± 2 weeks) until documentation of disease progression or the initiation of a subsequent anti-cancer therapy, whichever comes first.

[13] Patients will be contacted every 3 to 4 months for the subsequent use of anti-cancer therapy as well as survival until 1 year after the last patient's first treatment (C1D1).

[14] Scans should be done within 28 days of baseline (with a 7-day window). The window for visits on Day 15 and 22 in cycle 1 is +/-1 day; the window for visits in cycle 2 onwards is +/-2 days, unless the tinostamustine dose is delayed due to toxicity (see Section 8.6).

[15] Abbreviated physical examination is directed by disease site and symptoms.

3 INTRODUCTION

Tinostamustine (formerly EDO-S101) is a first-in-class alkylating deacetylase inhibiting molecule (DACi), a fusion of an alkylator, bendamustine, and a histone-deacetylase inhibitor (HDACi), vorinostat.

The compound was designed to be a fully bi-functional molecule with the hypothesis that HDAC inhibition would relax chromatin by increased acetylation of histones and make it more accessible to deoxyribonucleic acid (DNA) damaging moiety. Additionally, the assumption was that a molecule that would not be cleaved in the mammalian organism might be the optimal approach to exploit the synergies of both modes of action. This way, differences caused by the route of administration and pharmacokinetic (PK) profile of two independent drugs would be irrelevant and maximum activity and a clear safety profile would be expected to be seen.

Tinostamustine has been investigated for the treatment of patients with hematologic malignancies (EDO-S101-1001) and solid tumors (EDO-S101-1002) at doses ranging from 20 mg/m^2 to 120 mg/m^2 once every 3 or 2 weeks, respectively. The most common toxicities observed have been hematologic in nature, including Grade 3/4 neutropenia, thrombocytopenia, and febrile neutropenia, including dose-limiting cases. Based on the experience with bendamustine, which has a kaliuretic effect and might lead to hypokalemia, potassium levels were evaluated before every tinostamustine infusion, with potassium supplementation considered if levels were <3.5 mmol/L. Mild (Grade 1) hypokalemia was observed in 2 patients in the initial clinical study. Clinically significant QTc prolongation was not observed in either trial.

Based on these findings, the utility of tinostamustine as a conditioning agent prior to autologous stem cell transplantation (ASCT) was considered plausible. Study EDO-S101-1004 (TITANIUM) was therefore designed to explore the potential efficacy and safety of tinostamustine used as conditioning agent for ASCT in patients with relapsed/refractory multiple myeloma who had undergone a prior ASCT, a population of patients for whom ASCT was indicated. The initial dose of tinostamustine in the Phase 1 portion of the study was 180 mg/m2, with escalation to 220, 260, and then 300 mg/m2 (or higher) planned, using a standard 3+3 design.

In study EDO-S101-1004, 3 subjects have been dosed with tinostamustine 180 mg/m2 and 3 subjects with 220 mg/m2 as a 1-hour infusion. In the 180 mg/m2 cohort, one subject had a >60 ms QTcF increase, and a second had a 30-60 ms QTcF increase. In the 220 mg/m2 cohort, one subject had a nearly 60 ms increase in QTcF, and one subject had a > 90 ms increase in QTcF to a peak QTcF of 509 ms, 60 minutes after dosing. In all cases, the peak increase in QTc occurred 45-90 minutes after dosing, with QTc returning to baseline within 90-180 minutes. Three of the four subjects with large QTcF increases (nearly 60ms increase over baseline or higher) had tinostamustine plasma concentrations > 3000 ng/mL.

Based in the QTc effects observed in the first two cohorts in TITANIUM, the trial has been cancelled, and the sponsor plans to amend the two ongoing trials, EDO-S101-1001 and EDO-S101-1002, to include more intense ECG and PK collection in order to allow a precise characterization of the effects of tinostamustine, at the dosages being investigated for the treatment of hematologic and solid tumors, on cardiac repolarization (QTc) and other ECG parameters.

3.1 Background

3.2 Preclinical Pharmacology and Toxicology

Tinostamustine is a small molecule with molecular weight 415 Dalton, and is an alkylating histone-deacetylase inhibitor (HDACi) fusion molecule with full bi-functional properties. The compound is made of bendamustine as alkylator and vorinostat as HDACi, covalently linked together in one molecule.

Tinostamustine has been administered as a 1-hour IV infusion to beagle dogs at doses up to 15 mg/kg. The half-life of tinostamustine is short, approximately 5-10 min. The plasma concentration declines rapidly after the infusion is stopped. In dogs administered 15 mg/kg, tinostamustine was not detectable by 0.5 hours post infusion.

An *in vitro* metabolic study was performed with human, rat and dog hepatocytes in which tinostamustine at the concentrations of 1 and 10 µM was incubated with cryopreserved hepatocytes. Tinostamustine is primarily metabolized by i) oxidation of the hydroxy amide moiety to carboxylic acid; ii) hydrolysis of 1 of 2 chlorine atoms; iii) oxidative shortening of the aliphatic side-chain; iv) loss of one chloroethyl group; and v) combinations of these biotransformations. For all but 2 metabolites found in the samples, the elemental composition could be proposed. Tinostamustine was stable in negative control sample. After 90 minutes incubation in the medium alone at the concentration of 10 µM, tinostamustine accounted for 94% of total drug-related material. The functional moieties of tinostamustine were the most sensitive to metabolic degradation. Accordingly, all metabolites would be considered less- active than the parent compound.

The main metabolite in the human 90-minute sample was M2 (hydrolysis of 2 Cl atoms at the alkylating site), accounting for 15% of the total metabolites. All relevant metabolites found in human were qualitatively present in other species, while in different distributions. M2 metabolite was present only in a limited amount (2%) after 90 minutes incubation with both, rat and dog hepatocytes. Hence, within the non-clinical toxicology program, in these species the contribution of M2 to the overall toxicology of tinostamustine was limited.

The excretion profile of tinostamustine was evaluated in a radiolabeled study following IV administration of [^{14}C]-tinostamustine as bolus and as 1-hour infusion in male rats. Two doses were investigated, 15 and 30 mg/kg. Main blood and plasma PK parameters were also calculated. No relevant differences in excretion profile were observed between the 2 modes of administration. After a single IV administration 35.6-39.8% radioactivity was eliminated in faeces, 8.0-13.3% with urine, and 25% with expired air as [^{14}C]-CO2. At the end of the excreta collection period, the radioactive dose recovered from the carcasses of the animals after IV dosing ranged between 9.4- 12.3%. The total cumulative amount of radioactivity recovered within 72 hours after dosing was between 60.6-67.2% of the administered dose.

The non-clinical toxicology study program for tinostamustine was performed according to the International Council for Harmonisation S9 Guidance "*Nonclinical Evaluation for Anticancer Pharmaceuticals."* in the standard rodent species rat (Sprague Dawley) and in the standard non- rodent species dog (Beagle). Single dose toxicology/toxicokinetic studies were conducted in both species and a repeat-dose study in rats.

Overall, the non-clinical toxicology single dose studies showed that tinostamustine has a toxicity profile similar to alkylators; bone marrow toxicity and depletion of white blood cells with nadir at Day 7 and recovery by Day 21 were demonstrated in both species. Other observed toxicities included emesis and liquid feces in dogs and inappetence in rats. No organ toxicity was observed within the single dose studies apart of reversible reduced cellularity of the bone marrow and other lymphatic tissues (lymph nodes, thymus and spleen).

Toxicity reported in the repeat dose study was similar to the toxicity observed in single dose studies. However, minimal to moderate secondary treatment-related findings linked to immunosuppression were present in the lumbar lymph nodes, lungs, heart (minimal to slight inflammatory cell infiltrates in the myocardium), and at injection sites. Following a 28-day treatment-free period, there was complete recovery in the thymus, lungs and liver, while partial recovery was observed in the spleen, lymph nodes, Peyer's patches, bone marrow, heart, and injection sites.

3.3 Preclinical Cardiac Safety

A GLP in vitro hERG safety assay determined IC50 for the inhibitory effect of tinostamustine on hERG potassium current at 0.38 µM, a concentration lower than needed for preclinical efficacy. The tinostamustine effect on cardiac activity was also evaluated in dogs in an exploratory toxicology MTD (maximum tolerated dose) study and in the subsequent telemetric study in which tinostamustine was administered by 1-hour IV infusion. Both studies showed that tinostamustine had no effect on the ECG parameters, including the QTc interval. Only a moderate decrease of systolic and diastolic pressure was observed in the telemetric study, resulting in overall decreases in mean arterial and pulse pressure.

3.4 Clinical Data

Tinostamustine has been evaluated in 3 clinical studies to date, Study EDO-S101-1001 in 55 patients (46 patients in the escalation part and 9 patients in the expansion part) with hematologic malignancies and Study EDO-S101-1002 in 45 patients with solid tumors (22 patients in phase I and 23 patients in phase II), and as a conditioning agent prior to autologous stem cell transplantation to 6 patients in EDO-S101-1004. A total of 106 patients have been treated to date in the 3 clinical studies.

3.4.1 Clinical Cardiac Safety

3.4.1.1 EDO-S101-1001

EDO-S101-1001 is a Phase 1 study designed to evaluate the safety, pharmacokinetics (PK), and efficacy of tinostamustine in patients with relapsed/refractory hematologic malignancies. Tinostamustine was administered at doses ranging from 20 -120 mg/m^2 on D1 of 21-day cycles with infusion durations ranging from 30-60 minutes. The MTD was identified as 100 mg/m^2 administered over 1 hour. 12-lead ECGs were collected at screening and in triplicate one hour prior to dosing on Day 1 of Cycle 1. Additional ECGs were performed at 15, 30, 45, 60, +/-5min; 90 and 120 minutes +/-15min and at 3, 4, and 24 hours +/-30min from the start of EDO-S101 infusion, in Cycle 1 only and study discontinuation, and were not centrally measured. However, all ECGs which were reported to have prolonged QTc based on the ECG machine generated measurements were reviewed by an independent cardiac safety expert. Although the ECG machine generated measurements indicated that several subjects had marked QTc prolongation, an independent review of these ECGs revealed that no subjects had QTcF > 500 ms, only 2 subjects had QTcF > 480 ms, and only 2 subjects had

QTcF increases of > 60 ms. The most extreme example of an ECG machine QTc over-measurement occurred for a subject in EDO-S101-1001, who had ECG machine reported QTcB and QTcF measurements of 677 ms and 644 ms, respectively, at the discontinuation visit (a 208 ms increase from the baseline QTcF, 434 ms). The manually measured QTcF was 463 ms (a 46 ms increase from the baseline QTcF of 417 ms).

3.4.1.2 EDO-S101-1002

EDO-S101-1002 is a Phase 1/2 study designed to evaluate the safety, pharmacokinetics (PK), and efficacy of tinostamustine in patients with advanced solid tumors. Tinostamustine was administered at doses of 60 -100 mg/m^2 with infusion durations of 30 or 60 minutes. ECGs were collected in triplicate at screening, C1D1, C1D15, and study discontinuation while single ECGs were collected during subsequent cycles. ECG measurements were not centralized. However, all ECGs which were reported to have prolonged QTc based on the ECG machine generated measurements were reviewed by an independent cardiac safety expert. Although the ECG machine generated measurements indicated that several subjects had marked QTc prolongation, an independent review of these ECGs revealed that 1 subject had QTcF > 500 ms, and only 1 subject had QTcF 480-500 ms.

3.4.1.3 EDO-S101-1004

- EDO-S101-1004 was designed as a 2-part, international, multi-center, open-label study of salvage treatment with tinostamustine conditioning followed by autologous stem cell transplant (ASCT) in patients with relapsed/ refractory multiple myeloma (MM). The total dose of tinostamustine is administered on Day -1, with ASCT on Day 1. The initial tinostamustine dose was 180 mg/m^2, and the second cohort received 220 mg/m^2. Continuous 12 lead holter ECGs which allowed collection of both safety ECGs, for standard ECG measurements and holter, were collected on Day -1, the day of tinostamustine administration, for 24 hours. Replicate ECGs were extracted from the continuous 12 lead ECG recordings and were analyzed by ERT using the high precision QT method. Baseline time points were obtained on Day -1 directly prior to dosing 0 minutes [before start of infusion]. Post-dose time points occurred at the following times after start of the infusion: 15, 30, 45, 60 and 75 minutes and 3, 6, and 24 hours following the start of the infusion.
- Prior to study termination, 3 subjects were dosed with 180 mg/m^2, and 3 subjects with 220 mg/m^2.
- In the 180 mg/m^2 cohort, one subject had a >60 ms QTcF increase, and a second had a 30-60 ms QTcF increase.
- In the 220 mg/m^2 cohort, one subject had a nearly 60 ms increase in QTcF, and one subject had a > 90 ms increase in QTcF to a peak QTcF of 509 ms, 60 minutes after dosing. In all cases, the peak increase in QTc occurred 45-90 minutes after dosing, with QTc returning to baseline within 90-180 minutes. Three of the four subjects with large QTcF increases (nearly 60 ms increase over baseline or higher) had tinostamustine C_{max} > 3000 ng/mL.

3.5 Sub-study Rationale

This sub-study is designed to better characterize the effect of Tinostamustine on cardiac repolarization (QTc) and other ECG parameters, at the dosages investigated for the treatment of solid tumors in the phase 1 portion of EDO-S101-1002 trial.

4 SUB-STUDY OBJECTIVES

4.1 Primary Objectives

- To characterize the effect of tinostamustine at a dose of 60 mg/m^2 on cardiac repolarization (QTcF) and other ECG parameters in 6 patients with solid tumors who have progressed after at least one line of therapy and for whom no other standard therapy with proven clinical benefit is available. Tinostamustine will be administered intravenously (i.v.) on Day 1 and 15 of each 4-week treatment cycle.

4.2 Secondary Objectives

- To evaluate safety and tolerability of tinostamustine.
- To establish the pharmacokinetic (PK) profile of tinostamustine 60 mg/m^2 administered over 1 hour on Day 1 and 15 of each 4-week treatment cycle.
- To determine overall response (ORR), the clinical benefit rate (CBR) and stable disease at 4 months and duration of response (DR).
- To determine the progression free survival (PFS).
- To determine the overall survival time (OS).
- To determine duration of response.

4.3 Exploratory Objective

- To correlate the extent of gene expression changes in tumor samples with anti-tumor activity.

5 TRIAL DESIGN

5.1 Overall Sub-Study Design

This sub-study is designed to better characterize the effect of tinostamustine on cardiac repolarization (QTc) and other ECG parameters, for the treatment of solid tumors as investigated in the Phase 1 portion of the EDO-S101-1002 trial.

An additional subgroup of six (6) patients treated with a dose of 60 mg/m^2 tinostamustine administered over 60 minutes will be studied with intense ECG measurements, including 24-h Holter monitoring, and PK sampling. The PK sampling will occur at the first 2 treatment infusions in cycle 1 assuming no Grade 3 or higher (CTCAE v.5) QTcF prolongation occurs in any two or more patients or QTcF increased >60 ms from baseline or ≥1 patient has a fatal cardiac event related to tinostamustine, the trial will be stopped

The relationship between Cmax, and AUC will be examined relative to any prolongation of the QTcF seen on central ECG reading and simple descriptive statistics will be applied as appropriate. In particular, the distribution of the Cmax levels and the variability of the QTcF in this population will be described as well as the apparent relationship between Cmax and QTcF changes for the assessment of further dose escalation to ensure subject safety.

These 6 patients will then be treated in accordance with the main trial protocol and treatment will continue as foreseen up to trial completion.

Following enrollment of 6 patients the study will be stopped. Additionally, if any two or more patients have grade 3 or greater centrally confirmed QTc prolongation or QTcF increased >60 ms from baseline or ≥1 patient has a fatal cardiac event related to tinostamustine, the trial will be stopped.

Additional patients beyond the initial 6 patients at the 60 mg/m2 dose or new cohorts evaluating higher doses can only be enrolled following review of the data with regulatory authorities.

Stopping Rules for sub-study for administering Tinostamustine

Stopping rules in this sub-study apply for patients who experience QTc prolongations >500 ms or change from baseline >60 ms (Grade 3) that are not transient or occur in more than 1 treatment cycle.

If the QTcF value on the electrocardiogram (ECG) machine printout is >500 ms or represents an increase > 60 ms from baseline, 2 additional ECGs are to be performed approximately 1 minute apart. If the average QTcF of the 3 ECGs is >500 ms or increased > 60 ms from baseline, the tinostamustine infusion must be stopped. The patient should stay in the unit until the QTcF has decreased to baseline. In addition, the patient is to be continuously observed for syncope or other clinically relevant cardiac events.

A thorough evaluation of ECGs, including expedited central review of Grade 3 QTc prolongations by an independent assessor, will be performed. The decision will then be made by the Sponsor in consultation with the Medical Monitor, whether tinostamustine treatment is to continue or be postponed.

If any two or more patients have grade 3 or greater centrally confirmed QTc prolongation or QTcF increased >60 ms from baseline or ≥1 patient has a fatal cardiac event related to tinostamustine, the trial will be stopped.

(Refer to Section 8.7, Dose modification guidelines and stopping rules, for the management of patients who experience clinically significant QTcF prolongations).

General stopping rules for sub-study:

If 2 patients from the cohort of 6 experience the following AEs at Grade 3 judged to be causally related to study drug or application procedure, the sub-study will be stopped for enrollment and the patients already recruited stopped for further treatment.

- G3 deep venous thrombosis
- G3 serum creatinine
- G3 nervous system disorders excluding headache

6 SELECTION AND WITHDRAWAL OF PATIENTS

6.1 Inclusion Criteria for sub-study

- Signed informed consent.
- Patients age ≥18 years at signing of the informed consent.
- Histologically confirmed diagnosis of advanced or metastatic solid tumors, disease should have progressed following at least 1 line of therapy and no other standard therapy with proven clinical benefit is available or recommended based on the investigator's individual risk-benefit assessment for the patient. Women with triple negative breast cancer must have had at least 3 prior lines of therapy and there remains no other standard therapy with proven clinical benefit.
- Patients with secondary metastasis to the central nervous system (CNS) are eligible if they have had brain metastases resected or have received radiation therapy ending at least 4 weeks prior to trial day 1 and they meet all the following criteria:

  (1) Residual neurological symptoms ≤Grade 1.

  (2) No glucocorticoid requirements or patients may be receiving low doses of glucocorticoids providing the dose has been stable for at least 2 weeks prior to starting the trial medication.

  (3) Follow-up imaging does not show progression of treated lesions and no new lesions
- Evaluable disease; either measurable on imaging or with informative tumor marker as assessed by RECIST version 1.1.
- Discontinuation of previous cancer therapies at least 3 weeks or 5 half-lives, whichever is shorter, as long as the patient has recovered to eligibility levels prior to treatment in this trial.
- Eastern Cooperative Oncology Group (ECOG) performance status ≤2 (Section 12.1).
- Absolute neutrophil count (ANC) (polymorphonuclear [PMN] cells plus bands) >1,000 µL.
- Platelets ≥100,000 µL.
- Aspartate aminotransferase/alanine aminotransferase (AST/ALT) ≤3× ULN. In cases with liver involvement ALT/ AST ≤5× ULN.
- Total bilirubin ≤1.5 mg/dL unless elevated due to known Gilbert's syndrome.
- Creatinine ≤1.5 ULN.
- Serum potassium and magnesium within normal range, at baseline (supplementation is permissible).
- Men and women of child-bearing potential, and their partners, must be willing to use at least 2 effective forms of birth control during the trial drug administration and for at least 90 days after the administration of the trial drug to be eligible to participate. Vasectomized partners and patients must be willing to use a secondary method of effective birth control. Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the trial treatment.

The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient.

6.2 Exclusion Criteria for sub-study

To be eligible to participate in the trial, a patient cannot meet any of the following exclusion criteria:

- Patients with primary CNS cancer.
- Patients with QTc interval (Fridericia's formula) >450 ms.
- Patients who are on treatment with drugs known to prolong the QT/QTc interval. Refer to CredibleMeds list of drugs with known risk of Torsade des pointes (TdP): http://crediblemeds.org/new-drug-list.
- Patients who are being treated with valproic acid for any of its indication (epilepsy, mood disorder) must be excluded or must stop using the medication and have a wash out period of 3.5 days prior to first dose of tinostamustine in this trial.
- Any serious medical condition that interferes with adherence to trial procedures.
- Prior history of another solid tumor malignancy diagnosed within the last 3 years of trial enrollment excluding adequately treated basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or *in situ* cervical cancer, *in situ* breast cancer, *in situ* prostate cancer (patients must have shown no evidence of active disease for 2 years prior to enrollment).
- Pregnant or breast-feeding women.
- New York Heart Association (NYHA) stage III/IV congestive heart failure (Section 12.2). The following arrhythmias: atrial fibrillation/flutter with poor rate control, documented sustained ventricular tachycardia (defined as >30 seconds or requiring cardioversion before 30 seconds have elapsed) or TdP, ventricular preexcitation (Wolff Parkinson White syndrome) Brugada Syndrome, Complete LBBB, QRS > 120 ms,
- Implanted pacemaker or implantable cardiac defibrillator (ICD)
- Significant co-morbidities (e.g., active infection requiring systemic therapy, history of human immunodeficiency virus [HIV] infection, or active Hepatitis B or Hepatitis C).
- Use of other investigational agents within 30 days or 5 half-lives prior to the first dose of tinostamustine, provided the patient has recovered from any related toxicities ≥Grade 1.
- Steroid treatment within 7 days prior to trial treatment. Patients that require intermittent use of bronchodilators, topical steroids, or local steroid injections will not be excluded from the trial. Patients who have been stabilized to 10 mg prednisolone orally (PO) once daily (QD) (or equivalent), daily (or less) at least 7 days prior to trial drug administration are allowed.

6.3 Trial Termination

6.3.1 Withdrawal of Patient from Trial Treatment

Patients can withdraw from the trial at any time for any reason if they wish to do so without any consequences for their further medical treatment. The investigator can decide to withdraw a patient

from the trial for urgent medical reasons. Furthermore, patients will need to be discontinued from further trial treatment in the event of any of the following:

- Unmanageable toxicity.
- Pregnancy.
- Physician decision if continuation is not in the patient's best interest.
- Termination of the trial by the Sponsor.
- Other reasons (e.g. major protocol violation, non-compliance).
- Progressive disease.

If a patient is withdrawn from the trial, the primary reason must be recorded in the electronic case report form (eCRF) and the Investigator should make every effort to perform the assessments listed in the Schedule of Assessments (Table 1) under the Investigational product Discontinuation column (at any time or Day 28 of the last treatment cycle).

7 TRIAL ASSESSMENTS

A tabular schedule of evaluations and procedures is provided in Table 1 (Schedule of Assessments).

7.1 Screening

The Investigator at each trial site is responsible for maintaining a record of all patients screened, including both those who enter the trial and those who are excluded. Screening procedures will be performed no more than 28 days prior to baseline, with the exception for scans that will be performed no more than 28 days prior to baseline (with 7-day window). The baseline is defined as the first day on which investigational product is administered. Screening procedures are listed in Table 1 (Schedule of Assessments).

7.2 Informed Consent

Each potential patient must sign a written ICF prior to performing any trial specific procedures. A copy of the signed informed consent form will be provided to the patient. Separate consent must be provided for collection of samples for determination of gene expression.

7.3 Inclusion/Exclusion Criteria

Inclusion and exclusion criteria will be reviewed for each potential patient during Screening. All eligible patients will be treated with tinostamustine employing sequential enrollment (i.e. as they qualify for participation).

7.4 Demographics and Medical History

Each patient's medical history will be documented at Screening, including demographic information, relevant medical history, current primary cancer diagnosis, and prior cancer treatments (chemotherapies and immunotherapies, radiation therapy, surgeries, etc.).

7.5 Physical Examination

A complete physical examination will be performed at Screening, at Day 1 of each cycle and at the time of investigational product discontinuation (at any time or Day 28 of the last treatment cycle). On Day 15 and Day 22, abbreviated physical examination directed by disease site and symptoms will be performed. The abbreviated physical examination includes vitals and ECOG performance status.

The findings of each examination will be recorded on the source documents and in the eCRF. The complete physical examination will include:

- General appearance
- Head, eyes, ears, nose, and throat
- Respiratory
- Cardiovascular
- Musculoskeletal
- Abdomen
- Neurologic

- Extremities
- Dermatologic
- Lymphatics

Interim or symptom-directed physical examinations will be performed at other times, if necessary, at the discretion of the Investigator to evaluate potential adverse events or clinical laboratory abnormalities.

7.6 Vital Signs, Height and Weight

Vital signs will include resting supine blood pressure, pulse, respiratory rate, and temperature. Vital sign determinations will be performed at Screening, Days 1, 15 and 22. On each treatment day blood pressure, pulse, and respiratory rate will be recorded pre-dose, 3 hours (±10 minutes), and 6 hours (±10 minutes) from the start of the tinostamustine infusion, and at the time of investigational product discontinuation (at any time or Day 28 of the last treatment cycle). Due to the burden on the patients, after Cycle 1 the vitals for the 3- and 6-hour time points are considered optional assessments and should be done at the Investigator's discretion. If the 3- and 6-hour time points are not done, the Investigator must provide adequate instruction to the patient regarding potential allergic reactions, and this should be clearly documented in the patient chart.

Temperature will be recorded at pre-dose on each treatment day. Height will be measured at Screening or baseline only. Weight will be measured at Screening and in conjunction with vital sign determinations at Day 1 of each treatment cycle. The weight measured on Day 1 of each cycle will be used for investigational product calculations of body surface area (BSA). BSA will be calculated using the same formula for each patient at the site. The DuBois formula will be used to calculate the BSA.

7.7 ECOG Performance Status

ECOG performance status of each patient will be assessed at Screening and at every visit when a physical exam is performed including the time of investigational product discontinuation (at any time or Day 28 of the last treatment cycle) using the criteria provided in Section 12.1.

7.8 12-Lead ECG (Safety and Holter)

All ECGs will be obtained digitally using a Global Instrumentation (Manlius, NY, USA) M12R ECG and are to be performed after the patient is supine for 10 minutes.

A triplicate ECG will be performed during Screening (at least 1-2 minutes between each measurement) to determine patient eligibility for the trial and will be reviewed centrally.

During tinostamustine treatment, 2 types of ECGs are to be performed: Holter and safety ECGs.

Holter ECGs are stored continuously on a digital medium and will not be available for review until the data is received by ERT and analyzed (Section 7.8.1.1). Holter ECG readings will be used for the final data analysis from selected predetermined time points as detailed below and will be read centrally using a high-resolution manual on-screen caliper semiautomatic method with annotations.

Safety ECGs (standard digital 12-lead) will be immediately available to site staff for assessment (Section 7.8.1.2).

7.8.1.1 Holter ECGs

Holter monitoring will commence 60 minutes prior to the start of the infusion on C1D1 and 15 minutes prior to the start of infusion in C1D15 and will continue through 24 hours from the start of infusion.

Replicate 10 second, 12-lead ECGs will be extracted from the continuous recording at each of the following time points on C1D1 and C1D15:

- C1D1: -45, -30, -15 minutes predose, and 15, 30, 45, 60, 75, 90, 120, 180, 360 minutes and 24 hours from the start of infusion.
- C1D15: prior to the start of infusion, and 15, 30, 45, 60, 75, 90, 120, 180, 360 minutes and 24 hours from the start of infusion.

The central reader interpretation of ECGs extracted from Holter monitoring will be used to determine all ECG data for trial endpoints including baseline QTcF interval for cardiac safety analyses.

7.8.1.2 Safety ECGs

A triplicate ECG is to be performed before tinostamustine administration on D1 (i.e., day of tinostamustine dosing) in each cycle. Furthermore, patients are to have single ECGs performed at 30 and triplicate ECGs at 60 minutes from the start of tinostamustine administration on D1 and D15 of each treatment cycle. Additional single ECGs will be conducted at 90, 120 and 180 min and at study drug discontinuation.

In addition, additional ECGs may be conducted as clinically indicated during the tinostamustine infusion.

Expedited central reading of safety ECGs will be requested in all cases of Grade 3 or higher QTcF prolongations that occur within 6 hours from start of infusion. The results of the expedited central review will be made available to the site within 6 hours.

The Investigator's interpretation of ECGs will be used for patient safety management during the trial. For SAE reporting: initial assessment and reporting will be based on the local reading of the safety ECG and may be corrected if not confirmed by central reading.

7.9 Pharmacodynamics (Gene Expression)

Patient participation in the gene expression analysis is not mandatory for enrollment into the trial. Information on the purpose of genetic research in the gene-expression sub-trial is provided, either in the main ICF or a separate ICF, based on applicable regulatory requirements, to allow the patient to decide whether he or she want to participate in this part of the trial. Participation in this genetic research is voluntary.

If the patient agrees to participate in the gene expression analysis, a fresh or archival tissue sample will be collected during Screening. Formalin fixed paraffin embedded (FFPE) biopsy samples will be used. The Affymetrix chip technology for analyzing total ribonucleic acid (RNA) will be used. For formalin fixed paraffin embedded samples, RecoverAll method from Ambion® for isolating total RNA will be used. The isolated RNA is total RNA and will contain both messenger RNA (mRNA) and micro RNA (miRNA). The expression profile will be analyzed for correlation with response or resistance to therapy. Details of sample preparation and shipping are described in the Laboratory Manual.

Samples will be stored in accordance with the IRB/IEC approved ICF and applicable laws. Several steps will be taken to keep the patients identity and the genetic test result confidential throughout the trial (e.g., double coding of the samples; restricted access to the samples and testing results).

7.10 Clinical Laboratory Tests (Hematology, Chemistry and Urinalysis)

Certified local laboratories will perform all clinical laboratory tests and results will be provided to the Investigator. Blood samples for hematology determinations will be collected at Screening, Days 1, 15 and 22 of treatment cycles 1 to 6, and at the time of investigational product discontinuation (at any time or Day 28 of the last treatment cycle). On tinostamustine administration days, blood samples for laboratory tests are to be collected before the start of tinostamustine infusion. Patients continuing on trial beyond Cycle 6 will have blood samples collected on days of investigational product administrations and at the time of investigational product discontinuation (at any time or Day 28 of the last treatment cycle).

Hematology tests will include white blood cell count (WBC) plus differential, red blood cell count (RBC), hemoglobin, hematocrit, platelets and an ANC determination.

Blood samples for serum chemistry determinations will be collected at Screening, Day 1 and 15 of each treatment cycle, and at the time of investigational product discontinuation (at any time or Day 28 of the last treatment cycle).

On days of drug administrations, blood samples for hematology and serum chemistry determinations will be collected prior to administration of the investigational product. Additional samples can be collected, and determinations performed if clinically indicated.

Blood chemistry tests will include albumin, total protein, creatinine, uric acid, blood urea nitrogen, sodium, potassium, calcium, glucose, total bilirubin, alkaline phosphatase, AST, ALT, gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), and C-reactive protein (CRP). In addition, an evaluation of potassium levels before every tinostamustine infusion will be performed and if lower than normal this would need to be corrected before the infusion proceeds.

Urine for routine urinalysis will be collected at Screening and at the time of investigational product discontinuation (at any time or Day 28 of the last treatment cycle). Urine microscopic examination will be performed if there are any positive findings upon dipstick assessment.

In the event of a clinically significant laboratory toxicity that is greater than or equal to Grade 2, more frequent laboratory tests should be performed until resolution or stabilization to less than or equal to Grade 1.

7.11 Pregnancy Testing

A serum or urine pregnancy test will be performed for female patients of childbearing potential at Screening, on D1, of each treatment cycle (prior to tinostamustine administration), and at the time of discontinuation of tinostamustine. The test results at Screening and D1 must be negative for the patient to be enrolled in the trial. A positive urine pregnancy test result observed following enrollment should be confirmed with a repeat serum pregnancy test and if confirmed positive, the patient must be withdrawn from treatment immediately. See Section 9.6.5 for details regarding the pregnancy reporting procedure.

Males and female patients of child-bearing potential and their partners, must be willing to use at least 2 effective forms of birth control during tinostamustine administration and for at least ninety (90) days after the administration.

Vasectomized partners and patients must be willing to use a secondary method of effective birth control. Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the trial treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient.

7.12 Treatment-Emergent Adverse Events (TEAEs)

Monitoring and recording of TEAEs will be conducted from the time of start of first dose administration to discontinuation of tinostamustine (D28 of last treatment cycle). TEAEs, including serious adverse events (SAEs), will be captured in the eCRFs from the first day of tinostamustine dosing through discontinuation of tinostamustine. Only AEs ongoing at Tinostamustine Discontinuation Visit are required to be followed to resolution or stabilization. In the event of a clinically significant laboratory toxicity that is ≥Grade 2, more frequent laboratory tests should be performed until resolution to Grade 1 or stabilization (i.e., the CTCAE grade remains the same for at least 14 days). The final resolution/stabilization date is recorded in the AE eCRF. During Follow-up, any new SAE commencing within 30 days of Tinostamustine Discontinuation should be recorded and followed to resolution.

7.13 Infusion Site and Allergic Reaction Assessment

All injection site reactions will be considered TEAEs. However, the nature and severity of each injection site reaction will be determined using the CTCAE criteria, version 4.03 (June 2010) in Phase 2.

In Phase 2, a 2 mg/mL tinostamustine infusion solution after reconstitution will be utilized as outlined in the IB V5.0. Assessment of injection site reactions must be performed on each treatment day at pre-dose and 1-hour (± 15 minutes) post dose.

7.14 Prohibited Concomitant Medications and Procedures

7.14.1 Steroids

Use of bronchodilators, topical steroids, or local steroid injections will only be allowed for patients who require intermittent therapy. Treatment with steroids will be allowed for patients who have been stabilized to oral daily administration of prednisone 10 mg PO QD (or equivalent) or less, 7 days prior to tinostamustine administration.

7.14.2 Valproic Acid

Patients receiving valproic acid for any indication (epilepsy, mood disorder) must be excluded from the trial or must stop using the medication and have a wash out period of 3.5 days.

7.14.3 Allopurinol

Pre-treatment with allopurinol is contraindicated. There may be increased risk of severe skin toxicity when tinostamustine and allopurinol are administered concomitantly. As prevention in patients with high risk for developing tumor lysis syndrome (TLS) or for the treatment of established TLS, patients should receive rasburicase.

7.14.4 Serotonin 5-HT3 receptor antagonists

Palonosetron (Aloxi®) is the only serotonin 5-HT3 receptor antagonist that does not cause significant QTc changes; therefore, if required, it could be used for antiemetic prevention.

Ondansetron (Zofran®) is associated with QTc prolongation and cardiac arrhythmias, with a dose related effect. Consequently, it is contraindicated during the administration of tinostamustine. Given the short half-life of tinostamustine, ondansetron can be used, if needed, for the prophylaxis of delayed nausea and vomiting. A minimal interval of 24 hours should be respected between the last intake of ondansetron and the next tinostamustine infusion. This is related to the half-life of 4-6 hours of ondansetron. This interval should be extended to 48 hours in patients with mild biological hepatic impairment but who are still eligible (see inclusion and exclusion criteria). In this category of patients, a significant prolongation of ondansetron's half-life has been reported.

7.14.5 NK1 receptor antagonists

Aprepitant (Emend®) is not allowed, since interactions with alkylating agents have been demonstrated. Aprepitant has additional interactions that could lead to an increase of side effects or decrease in efficacy of tinostamustine.

7.14.6 Investigational agents

Use of other anti-cancer investigational agents within 30 days or 5 half-lives prior to the first dose of tinostamustine provided the patient has recovered from any related toxicities ≥Grade 1.

Use of any other investigational medicinal product or non-approved experimental therapy is not allowed.

7.15 Efficacy Evaluations

7.15.1 Baseline Disease Assessment

Baseline (Screening Visit) tumor assessments will be performed using CT scan. Patients will have a baseline tumor assessment done within the 28 days (+7-day window) prior to Cycle 1, Day 1.

7.15.2 Disease Response Assessments

Radiologic response assessment by computed tomography (CT) or magnetic resonance imaging (MRI) will be performed at baseline and every 2 cycles. Tumor response will be evaluated according to RECIST version 1.1 (Section 12.3).

7.15.3 Follow-up Assessments

PFS Response Follow-up

Patients who have discontinued trial treatment for reasons other than PD will be assessed per RECIST 1.1 every 8 weeks (± 2 weeks) until documentation of PD or the initiation of a subsequent anti-cancer therapy, whichever comes first.

Survival Follow-up

Patients will be contacted every 3 to 4 months for the subsequent use of anti-cancer therapy as well as survival until 1 year after the last patient's first treatment (C1D1).

7.16 PK Assessments

Plasma samples will be collected for determination of tinostamustine concentrations as well as its 2 metabolites, M2 and M8, using a method fully validated according to the relevant guidelines. The PK profile of tinostamustine, M2 and M8 in plasma will be assessed by analyzing parameters such as C_{max}, AUC, and time to maximum concentration (T_{max}), and elimination half-life at each drug administration of Cycle 1 in each patient. The blood sampling schedule for the PK assessment is conducted in Cycle 1 only on Day 1 and 15 during tinostamustine administration. Samples are taken as follows: up to 0.5 hours prior to dose administration and at 15, 30 and 45 minutes, 1 hour, 75 and 90 minutes, and 2, 3, 6, and 24 hours from the start of the tinostamustine infusion.

The following time windows are permissible for all PK blood draws:

Sampling Time	Time from Scheduled Sampling Allowed
From > 0 up to ≤ 90 minutes post dose	± 5 minutes
From > 2 hours to 6 hours post dose	± 10 minutes
At 24 hours post dose	± 2 hours

The PK assessments together with the ECG Holter results will be used to perform a concentration QTc-analysis following a separate statistical analysis plan (SAP).

7.17 Missed Visits

If a patient misses a scheduled visit to the trial site, the patient will continue on protocol and attend the next scheduled visit. In this case, the treating medical team should at least contact the patient by phone to establish patient status. If a patient misses 2 scheduled visits, his or her continued trial participation will be re-evaluated for possible non-compliance.

8 TRIAL TREATMENT

8.1 Investigational Drug Description

Other Names: The International Nonproprietary Name (INN) for tinostamustine is Tinostamustine. Tinostamustine is a first in class alkylating HDACi fusion molecule that is being investigated for the treatment of relapsed/refractory hematologic malignancies. The active pharmaceutical ingredient is insoluble in water and having its optimal solubility in an acidic medium. The drug substance is sensitive to degradation at pH values below 4.5 and precipitates in blood at pH 7. In addition, it rapidly hydrolyses in water and is sensitive to ambient temperature. The chemical structure of tinostamustine is comprised of 3 chemical moieties that include a DNA alkylation moiety, a purine- like benzimidazole ring and a vorinostat group.

8.2 Storage and Dispensing

Tinostamustine should be stored at 2 to 8°C in a secure area with access limited to the Investigator and authorized site staff. Before administration each vial tinostamustine needs to be reconstituted with 20 mL of 0.9% saline and the solution must be further diluted with 0.9% saline to a total volume of 50mL. The reconstituted and diluted solution should be stored for not more than 8 hours at 2 - 8ºC. Intravenous administration should take place after subsequent equilibration at room temperature (25°C) within 2 hours including infusion duration. Tinostamustine is compatible with infusion materials indicated in the Pharmacy Manual.

Only patients enrolled in the trial may receive investigational product. At the beginning of each 21-day treatment cycle, the single dose of tinostamustine is to be dispensed only by the Principal Investigator (PI), sub-Investigators, or authorized personnel at the institution(s) specified on the US Food and Drug Administration (FDA) Form 1572 (relevant for USA) and listed on the delegation of authority log.

Under no circumstances is the investigational drug to be used other than as directed within this trial protocol.

8.3 Supply, Packaging and Labeling

Tinostamustine is provided as a lyophilized powder in single dose, sealed glass vials. Each 50 mL vial will contain 100 mg tinostamustine. Hydroxyl-propyl-β-cyclodextrin (HPβCD) is present as the main functional excipient to keep the drug in solution at physiological pH after reconstitution. The vials are of clear glass Type I and stopper (V10 F597 W4432/50 WESTAR RS, Westar Pharmaceuticals Services) as well as Aluminium flip-off cap (20 mm, Westar Pharmaceuticals Services) to ensure container closure. All materials are in conformance with United States Pharmacopeia (USP) and Pharmacopoea Europaea (Ph. Eur.).

8.4 Treatment Allocation

Trial number allocation for eligible patients will be completed according to a process defined by the Sponsor.

8.5 Investigational Product Administration

Following Screening, all patients who are eligible to participate in the sub study protocol will receive a single dose of tinostamustine 60 mg/m^2 on Day 1 and Day 15 of each treatment cycle. The investigational product (reconstituted and diluted solution as described in Section 8.2) will be administered by i.v. infusion through a peripheral vein or port over a 60-minute infusion time.

Instructions for preparation of each dose of investigational product during each phase of the trial are provided in the Pharmacy Manual. The PI or qualified site personnel will administer tinostamustine by i.v. infusion.

Preparation and dispensing of the investigational product will be handled by the investigational site pharmacy. Instructions for safe handling of the investigational product are provided in the Pharmacy Manual. The requirements for maintaining drug accountability are provided in Section 8.8 of this protocol.

8.6 Treatment Criteria Beyond Cycle 1, Day 1

For a patient to receive the next dose of therapy, the following criteria must be met:

- ANC ≥1,000/mm^3
- platelets ≥75,000/mm^3
- non-hematologic treatment related toxicities have improved to ≤Grade 1 or to the patient's baseline values (except alopecia)

If a patient does not meet these criteria, dosing will be delayed, and the patient should be evaluated within 48-72 hours. If the next cycle is delayed by more than 3 weeks, the patient should not receive further treatment with tinostamustine. In these situations, if the patient is experiencing clinical benefit and has a favorable benefit/risk profile, trial participation may continue with a reduced dose of tinostamustine (50 mg/m^2) or resume at the patient's current dose, if agreed upon by the Sponsor and the Investigator. Additionally, after Cycle 1 the Investigator and Sponsor may decide to reduce a patient's dose in case of safety concerns. The reduced dose will be maintained as such, based on the Sponsor's and Investigator's determination of any ongoing safety concerns.

8.7 Dose Modification Guidelines and Stopping Rules for Patients who Experience Clinically Significant QTcF Prolongations

If the QTcF value on the ECG machine printout is >500 ms or represents an increase >60 ms from baseline, 2 additional ECGs are to be performed approximately 1 minute apart. If the average QTcF of the 3 ECGs is >500 ms or increased >60 ms from baseline, the tinostamustine infusion must be stopped. The patient should stay in the unit until the QTcF has decreased to baseline. In addition, the patient is to be continuously observed for syncope or other clinically relevant cardiac events.

A thorough evaluation of ECGs, including the expedited central reading of Grade 3 QTc prolongations, needs to be performed. The decision will then be made by the investigator in accordance with the Sponsor, whether treatment can continue, and administration of tinostamustine is to be postponed.

If any two or more patients have grade 3 or greater centrally confirmed QTc prolongation or QTcF increased >60 ms from baseline or ≥1 patient has a fatal cardiac event related to tinostamustine, the trial will be stopped.

As a general rule:

- Administration of ≥50% of the planned dose will be considered as a full dose.
- If a dose of up to 50% of the planned dose was administered, the remainder to a full planned dose can be administered the following day(s).
  - If the centrally reviewed QTcF value confirms the local finding and is >500 ms or increased >60 ms from baseline, the dose of the subsequent cycles should be reduced.
  - If a centrally confirmed QTc prolongation (>500 ms or increased >60 ms from baseline) occurs again with the reduced dose, the patient will be taken off trial treatment.
  - If the QTc prolongation is not confirmed by a central assessor, meaning a central read of ≤500 ms or increased ≤60 ms from baseline, the patient can continue the treatment with the initially planned dose within the judgement of the investigator.

Summary Guidance:

If centrally reviewed QTcF value confirms local measurement of >500ms or increase >60 ms from baseline (Grade 3)	1st occurrence: Reduce dose to 50 mg/m^2 in subsequent cycles
If centrally reviewed QTcF value confirms local measurement of >500ms or increase >60 ms from baseline	2nd occurrence: Investigational treatment should be discontinued
If centrally reviewed QTcF does not confirm local measurement of > 500 ms or increased >60 ms from baseline	Investigational treatment can continue as planned according investigator's judgement

8.8 Investigational Product Accountability

Investigational drug accountability records will be maintained throughout the course of the trial. The Investigator or designee will document the amount of tinostamustine received, the amount dispensed to trial patients and the amount destroyed locally.

Unused tinostamustine remaining at the completion of the trial will be destroyed at the site per institutional standard operating procedures, provided that destruction is documented. Destruction of drug supplies will take place only when drug accountability has been completed and the Sponsor/Contract Research Organization (CRO) has given written approval for destruction.

8.9 Supportive Care

Unless otherwise prohibited (see Section 7.14), supportive therapy for optimal medical care may be administered per institutional standard of care at the trial centers. Such supportive therapies may include:

1. For neutropenia: Growth factor support is allowed in Cycle 1 only beyond Day 7. There are no restrictions for subsequent cycles.

2. For diarrhea: Appropriate treatment is allowed, e.g. loperamide, atropine-diphenoxylate, or octreotide.
3. For nausea and/or vomiting, see guidelines in Section 7.14.4.
4. For rash and/or allergic reactions: Steroids like hydrocortisone, dexamethasone, and antihistamines.

8.10 Drug Interactions/Precautions

There are no known drug interactions with tinostamustine

As a precaution, patients who are on treatment with drugs known to prolong QT/QTc interval and those who have QTc interval longer than 450 ms are excluded. An updated list of drugs with known risk of TdP issued by Credible Meds: https://crediblemeds.org/new-drug-list will be provided to all participating centers every time a new list is issued. Precautions of use of the 5HT3 receptor antagonists are discussed in Section 7.14.4.

- Tinostamustine is a fusion molecule composed of an alkylating agent with a backbone similar to bendamustine and a histone deacetylase inhibitor similar to vorinostat. Based on the clinical experience with these 2 agents, potential toxicities that may be seen with tinostamustine include some of the more common toxicities outlined below in addition to other, rare but serious toxicities such as TLS seen with bendamustine and thromboembolism observed with vorinostat.
- TLS was reported in patients with a large tumor burden. Onset typically occurs within the first treatment cycle with bendamustine and, without intervention may lead to acute renal failure and death. Preventive measures include vigorous hydration and close monitoring of blood chemistry. Pretreatment with allopurinol is contraindicated. Rasburicase is recommended instead of allopurinol. There may be increased risk of severe skin toxicity when bendamustine and allopurinol are administered concomitantly. Skin reactions including rash, toxic skin reactions and bullous exanthema. Cases of Stevens-Johnson syndrome (SJS) and toxic epidermal necrosis (TEN), some fatal, have been reported when bendamustine was administered concomitantly with allopurinol and other medications known to cause these syndromes. Patients with skin reactions must be closely monitored. If skin reactions are severe or progressive treatment must be discontinued.
- Patients treated with bendamustine are at risk for reactivation of infections including (but not limited to) hepatitis B, cytomegalovirus, Mycobacterium tuberculosis, and herpes zoster. Patients should undergo appropriate measures (including clinical and laboratory monitoring, prophylaxis, and treatment) for infection and infection reactivation prior to administration.
- The most common serious drug-related adverse reactions associated with vorinostat were pulmonary embolism and anemia. Physicians should be alerted to the signs and symptoms of these events, particularly in patients with a prior history of thromboembolic events.
- Hyperglycemia has been observed in association with vorinostat; therefore, serum glucose should be monitored, especially in diabetic or potentially diabetic patients. Adjustment of diet and/or therapy for increased glucose may be necessary.

- Infusion reactions to bendamustine have occurred commonly in clinical trials. Symptoms include fever, chills, pruritus, and rash. In rare instances severe anaphylactic and anaphylactoid reactions have occurred, particularly in the second and subsequent cycles of therapy.

  Assessment of infusion site reactions must be performed on each treatment day at pre-dose, 1 hr (±15 minutes). The patient will be observed at 1 hr (± 15 minutes post-dose) for potential allergic reactions (See Section 8.10 for possible infusion reactions).

  The Investigator must provide adequate instruction to the patient regarding potential allergic reactions, and this should be clearly documented in the patient chart.

  Allergic reactions during or shortly after the infusion may cause skin itching, rash, reddening of the skin, swelling of the face, hands, feet, shortness of breath or anaphylactic reactions. These reactions are generally transient and disappear after symptomatic treatment is applied. Patients should be carefully monitored for all reactions after the infusions and take appropriate prophylactic measures with corticosteroids and/or antihistamines to prevent such or more severe reactions in subsequent treatment cycles.

8.11 Overdose

Investigational product will be administered by the Investigator or qualified site personnel. Therefore, it is highly unlikely that an overdose will occur. However, in the event of an investigational product overdose due to pharmacy error, the PI and Sponsor/Sponsor's designee should be immediately notified and, if signs or symptoms are present, the overdose recorded as a TEAE. The patient should be carefully monitored for potential adverse reactions and symptomatic treatment instituted as per institutional standards of care.

9 TEAES AND SAES

9.1 Definition of a Treatment Emergent Adverse Event (TEAE)

A TEAE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. A TEAE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a drug, without any judgment about causality. A TEAE can arise from any use of the drug from any route of administration, formulation, or dose, including an overdose.

TEAEs also include:

- An exacerbation of a pre-existing illness.
- An increase in frequency or intensity of a pre-existing episodic event or condition.
- A condition detected or diagnosed after tinostamustine administration even though it may have been present prior to the start of the trial.

TEAEs do not include:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is a TEAE.
- Pre-existing diseases or conditions present or detected at the start of the trial that do not worsen in severity or frequency.
- Situations where an untoward medical occurrence has not occurred (e.g. hospitalization for elective surgery, social and/or convenience admissions).
- Overdose of either Investigational or concomitant medication ***without*** any signs or symptoms.

A clinically significant laboratory assessment (as determined by the Investigator) is considered an AE and must be recorded in patients' source documents and the eCRF. As the CTCAE are used for laboratory parameters, as a general rule all ≥Grade 3 events are to be considered TEAEs.

Disease progression is a worsening of a patient's condition attributable to the disease for which the trial medication is being given. This may be an increase in severity of the disease or an increase in the symptoms of the disease.

New or increasing symptoms related to disease progression should be reported as TEAEs, however, disease progression itself and death from disease progression should not be recorded as a TEAE.

9.2 Recording Adverse Events

All TEAEs must be reported from the start of dosing of the first dose of tinostamustine through the time of tinostamustine discontinuation. Only AEs ongoing at time of Tinostamustine Discontinuation Visit are required to be followed to resolution or stabilization of event and then final resolution date is recorded in the AE eCRF. During Follow-up, any new SAE commencing within 30 days of Tinostamustine Discontinuation should be recorded and followed to resolution.

Whenever possible, a diagnosis should be given when signs and symptoms are due to common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as 'upper respiratory infection'). TEAE reporting and severity grading will be assessed using the NCI

CTCAE, version 4.03 (June 2010), with the exception that in Phase 2, QTc prolongations will be assessed for severity using CTCAE version 5.0. For those events without assigned CTCAE grades, the recommendation on page 1 of the CTCAE that converts mild, moderate, and severe into CTCAE grades should be used. A copy of the NCI CTCAE is available online at: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

The causal relationship of all TEAEs to investigational treatment will be determined by the Investigator according to best medical judgment, as follows:

- Definitely related: This category applies when, after careful medical consideration, there is almost no consideration of other causation.
- Probably related: There is a clinically plausible time sequence between onset of the AE and trial treatment administration. The AE is unlikely to be caused by a concurrent and/or underlying illness, other drugs, or procedures. If applicable, the AE follows a clinically consistent resolution pattern upon withdrawal of tinostamustine.
- Possibly related: There is a clinically plausible time sequence between onset of the AE and trial treatment administration, but the AE could also have been caused by the concurrent/underlying illness, other drugs, or procedures. Information regarding tinostamustine withdrawal may be lacking or unclear. “Possible” should be used when trial treatment administration is one of several biologically plausible causes of the AE.
- Unlikely related: The AE is most likely due to a non-trial-treatment-related cause. However, association with the trial treatment cannot be completely ruled out.
- Unrelated: Another cause of the AE is most plausible and a clinically plausible temporal sequence is inconsistent with the onset of the AE and trial treatment administration and/or a causal relationship is considered biologically implausible.

For the causality assessment of QTc prolongations, see Section 9.4.

For the purpose of regulatory reporting requirements, causal relationship criteria given as definite, probable, and possible will be considered treatment-related, while unlikely and unrelated will be considered not treatment-related.

9.3 Serious Adverse Events

A SAE is any TEAE that is considered ‘serious’ if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Is fatal;
- Is life-threatening (defined as an immediate risk of death from the event as it occurred);
- Requires in-patient hospitalization or prolongation of existing hospitalization (Exception: Hospitalization for elective treatment of a pre-existing condition that did not worsen during the trial and is not considered an adverse event. Note: Complications that occur during hospitalization are adverse events and if a complication prolongs hospitalization, then the event is serious);
- Results in persistent or significant disability/incapacity, or substantial disruption of the ability to conduct normal life functions;

- Is a congenital anomaly/birth defect;
- Though not included in the above definitions, may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Examples of important medical events which may meet the definition of a SAE include: intensive treatment in the emergency room or at home for allergic bronchospasm, certain abnormalities (e.g., blood dyscrasias), convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.

All events related to disease progression, including events resulting in death, are not considered SAEs. They need to be nevertheless clearly documented as events due to disease progression in the eCRF.

9.4 Events of Special Interest

Based on data obtained across the development program of tinostamustine, the Sponsor has identified QTc prolongations as events of special interest for which the following reporting requirements apply based on local ECGs:

1) All QTc prolongations Grade 2 need to be reported as AEs by entering them in the AE section of the eCRF
2) All QTc prolongations Grade 3 (per the most recent CTCAE 5.0 criteria) are to be considered clinically significant and need to be reported as SAEs if they fulfill the following criteria:

   QTcF >500 ms or

   QTcF increase from baseline >60 ms

Note that either 1 or >1 occurrence within 1 treatment cycle of 28 days will be regarded as 1 single event. Occurrences in more than 1 treatment cycle will be regarded as separate events in 1 patient.

For events meeting criterion 2, an SAE report form needs to be submitted as for all other SAEs.

Based on available PK data, a causal relationship with tinostamustine has to be assumed, if the QTc prolongation occurs within 6 hours from the start of tinostamustine infusion. Therefore, all SAEs identified as per point 2 above will be considered Serious Adverse Drug Reactions (SADR).

9.5 Suspected Unexpected Serious Adverse Reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions qualify as SUSARs if the following 3 conditions are met:

1. the event must be serious;
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Investigator’s Brochure for an unauthorized medicinal product:

- For this trial, Section 7. 4 (Table 7.2) of the most current IB version contains the Reference Safety Information for the expectedness assessment

9.6 Reporting of TEAEs, SAEs, Serious and Unexpected Adverse Experiences

9.6.1 Reporting TEAEs and SAEs to the Sponsor

All TEAEs must be reported in the eCRF from the start of administration of the first dose of tinostamustine through the time of tinostamustine discontinuation. Only AEs ongoing at time of Tinostamustine Discontinuation Visit are required to be followed to resolution or stabilization of event and then final resolution date is recorded in the AE eCRF. During Follow-up, any new SAE commencing within 30 days of tinostamustine discontinuation should be recorded and followed to resolution. If the Investigator becomes aware of safety information that appears to be drug related, involving a patient who participated in the trial, even after an individual patient has completed the trial, this should also be reported to the Sponsor or the designated vendor. In addition, all treatment related SAEs should be followed until resolution or stabilization.

All SAEs, regardless of relationship to tinostamustine, must be additionally reported to the Sponsor or the designated vendor within 24 hours of the Investigator becoming aware of the event using the SAE form. Follow-up SAE reports must be submitted by the Investigator as new information becomes available or as requested by the Sponsor or designated vendor. Supportive source documents have also to be provided together with the SAE form.

9.6.2 Reporting Suspected Unexpected Serious Adverse Reactions

The responsibility for expedited reporting of SUSARs within 7 days (life-threatening and death cases) or 15 days (all other SUSARs) is with the Sponsor or its delegated vendor.

The Sponsor will report to the regulatory authorities as per national regulations of the countries where the ongoing tinostamustine trials are conducted and will provide the case documentation to the CROs for reporting to the applicable IRBs/IECs.

Investigators will be informed as per national requirements.

9.6.3 Reporting of Grade 4 and Grade 5 TEAEs

All grade 4 AEs (per definition "life-threatening") and grade 5 ("death"), as per CTCAE, version 4.03, that occur during the trial are to be reported as SAEs, with the exception of Grade 4 laboratory abnormalities, which are to be reported as SAEs only if, in the Investigator’s judgement, they are considered immediately life-threatening.

9.6.4 Reporting of Important Medical Events of Special Interest

Important medical events (IME) of a clinically significant severity grade (≥Grade 3) may also qualify as SAEs. To establish consistency across trials with tinostamustine, the IME list published by the European Medicines Agency and regularly updated (current version April 2019) serves as a reference.

9.6.5 Reporting Pregnancy

If a female patient or the female partner of a male patient becomes pregnant during the course of the trial, the Investigator must report the pregnancy to the Sponsor or its designated vendor, using the **Pregnancy Reporting Form** within **24 hours** of becoming aware of the event. The Investigator must

obtain consent to collect pregnancy information (including the status of the newborn, if applicable). If possible, pregnancy needs to be followed up until its termination (birth, abortion, miscarriage)

If some of the information required for completion of the Pregnancy Reporting Form is unavailable at the time of the initial report, follow-up reports will be completed and submitted within 24 hours of becoming aware of the new information. The Investigator is required to follow the pregnancy through delivery. The outcome of the pregnancy and the status of the newborn (if applicable) will be reported on the Pregnancy Reporting Form within 24 hours of becoming aware.

9.6.6 Reporting to the IRB/IEC

SAEs will be reported to their IRB/IEC by the Investigator according to the IRB/IEC's policy and procedures.

9.6.7 Annual Safety and Progress Reports (DSUR)

In addition to the expedited reporting of SUSARs, the Sponsor will submit, once a year throughout the clinical trial, a safety and progress report in DSUR format to applicable competent authorities. The DSUR or an Executive Summary of the DSUR will be submitted to the IRBs/IECs as applicable per local requirements.

10 TRIAL ADMINISTRATION

10.1 Case Report Forms and Source Documentation

In order to provide the Sponsor/CRO with accurate, complete, and legible data, the following criteria are to be maintained:

- Source documents will be completed according to a source document agreement outlining all the data that is to be collected in the source documents throughout the trial.

Electronic data capture (EDC)/eCRF entries should be made as close to the visit of the subject as possible.

10.2 Good Clinical Practice Statement

This trial is to be performed in full compliance with the protocol, the Declaration of Helsinki, ICH, and all applicable local Good Clinical Practices (GCP) and regulations. All required trial documentation will be archived as required by competent authorities.

10.3 Investigator Documentation

The Investigator will provide the Sponsor with a fully executed FDA form 1572 (or applicable alternative Investigator statements) including the Investigator's curriculum vitae.

10.4 Record Retention

The circumstances of completion or termination of the trial notwithstanding, the Investigator has the responsibility to retain all trial documents, including but not limited to the protocol, copies of eCRFs/EDC, Investigator's Brochure/Summary of Product Characteristics (SmPC), regulatory agency registration documents, ICFs, and IEC correspondence.

The site should plan on retaining trial documents for approximately 15 years after completion of the trial. This will include copies of the eCRF/EDC.

It is requested that at the completion of the required retention period, or should the Investigator retire or relocate, the Investigator contact the Sponsor, allowing the Sponsor the option of permanently retaining the trial records. Records retained will be stored independently of the Sponsor, and the Sponsor will not be permitted direct access to this data.

10.5 Protocol Deviations and Amendment

The Investigator is not permitted to alter or deviate from the protocol. All deviations should be reported by the Investigator to their IRB/IEC. An immediate and unapproved deviation is permitted if immediate health care concerns mandate it.

All protocol revisions (Amendments) must originate with and be documented by the Sponsor. In the US, the Investigator must submit all amendments to his/her IRB/IEC for review and approval prior to implementation; documentation of approval signed by the chairperson or designee must be sent to the Sponsor.

10.6 Institutional Review Board and Independent Ethics Committee

Federal regulations and ICH require that approval be obtained from an IRB/IEC prior to participation of patients in research trials. Approval by the Competent Authority, if applicable, or as required by local laws and regulations, is also required in Europe. Prior to the trial onset, the protocol, any protocol amendments, ICFs/assent forms, advertisements to be used for patient recruitment, and any other written information regarding this trial to be provided to a patient or patient's legal guardian, must be approved by the IRB/IEC.

All IRB/IEC approvals must be dated and signed by the IRB/IEC Chairperson or designee and must identify the IRB/IEC by name and address, the clinical protocol by title and/or protocol number, and the date approval or favorable opinion was granted for the clinical research.

No drug will be released to the site to dose a patient until written IRB/IEC authorization has been received by the Sponsor or designee.

The Investigator is responsible for obtaining continuing review of the clinical research at least annually or more often if specified by the IRB/IEC. The Investigator must supply the Sponsor or designee with written documentation of the approval of the continued clinical research.

The Investigator, sponsor, or its designee as applicable, will make all attempts to ensure that the IRB/IEC is constituted and operates in accordance with Federal and ICH GCP and any local regulations.

In the case of early termination/temporary halt of the trial, the Investigator should notify the IRB/IEC and Competent Authority (CA) within 15 days and a detailed written explanation of the reasons for the termination/halt should be given. If the IEC decides to suspend or terminate the trial, the Investigator will immediately send the notice of trial suspension or termination by the IRB/IEC to the CRO.

At the end of the trial, the Sponsor should notify the IRB/IEC and CA within 90 days. The end of the trial will be the date of the last scheduled trial visit for the last Subject in the trial. The Sponsor will always also provide the IRB/IEC/CA with a summary of the trial's outcome.

10.7 Sponsor Monitoring and Auditing

After satisfactory receipt of the Clinical Trial Agreement and all other necessary regulatory paperwork, the Sponsor's monitor will arrange that all trial material be delivered to the trial site at a mutually convenient time. An initiation visit by the Sponsor representative and its monitoring personnel will be made. At this meeting, all personnel expected to be involved in the conduct of the trial will undergo an orientation to include review of trial protocol, instruction for eCRF completion and overall responsibilities, including those for drug accountability and trial file maintenance.

Throughout the course of the trial, the Sponsor's representative monitor will make frequent contact with the Investigator. This will include telephone and/or on-site visits. During these visits, eCRFs will be reviewed for completeness and adherence to the protocol. As part of the data verification process, it is expected that source documents (e.g., hospital records, office records) will be made available for review by the monitor. The monitor also will perform drug accountability checks and may periodically request review of the Investigator's trial file to ensure completeness of documentation in all respects of trial conduct.

Upon trial completion, the monitor will arrange for a final review of the site trial files, after which the file should be secured by storage for the appropriate period as specified in Section 10.4.

Audits will be conducted on a frequency which is based on risk and proportionate to the complexity of the trial. Additional audits may be performed if there is cause for concern or when requested by Sponsor, CRO, or competent authority. Regular audits will usually be performed with advance notice. Audits may be performed without notice. Authority inspections may occur at any time as deemed appropriate by the responsible authority in the country.

Audits and authority inspections may be performed without notice, especially where the Sponsor or competent authority deems necessary to investigate patient safety, welfare, scientific integrity, compliance and/or fraud (a for-cause audit). The Investigator is required to support audit or authority inspections, to be available to the auditors/inspectors upon request and to permit the auditor/inspector direct access to source data/documents.

10.8 General Informed Consent and Sub-study Informed Consent for Genetic Samples

The Investigator will explain the nature of the trial as described in the general ICF and will inform the patient that participation is voluntary and that they can withdraw at any time and that withdrawal consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Information on the purpose of genetic research in the gene-expression sub-trial is provided, either in the main ICF or a separate ICF to allow the patient to decide whether he or she want to participate in this part of the trial. Participation in this genetic research is voluntary.

The applicable ICFs must be approved by the IRB/IEC before use in the clinical trial.

The subject will be asked to sign and date the ICF prior to any trial-specific procedures being performed. The subject should understand the ICF before signing and dating the ICF. The Investigator or person obtaining consent must also sign and date the form. Each subject will be given a copy of the signed informed consent and written information. No subject can enter the trial before his/her informed written consent, and in the USA the Health Insurance Portability and Accountability Act (HIPAA) authorization] has been obtained. Each patient's signed ICF, including additional ICFs signed (e.g. for re-consent, and the pharmacogenomic sub-set) must be kept on file by the Investigator for possible inspection by regulatory authorities and/or the Sponsor personnel.

Each patient's signed informed consent form must be kept on file by the Investigator for possible inspection by regulatory authorities and/or the Sponsor personnel.

10.9 Confidentiality

The Investigator and his staff shall maintain the confidentiality of all patient records. Patient data will be made available to CRAs and auditors commissioned by the Sponsor, and to FDA and other competent authorities during inspections.

Data that is transmitted by the Investigator to the Sponsor, competent authority, or IRB/IEC will not be directly traceable to the patient. In the event that a publication of this research incorporates a patient's medical data, that data will not identify the patient. The subject's name will not appear on documents transmitted to the Sponsor in order to maintain confidentiality. Additional anonymization/pseudonymization laws as applicable by country will also be adhered to.

Processing of data and/or samples will be carried out in accordance with federal and local regulations. This applies to all trial data in whatever format it is collected and recorded.

10.10 Financial Disclosure

The Investigator and sub-investigators, as noted on the Form FDA 1572 (or applicable alternative Investigator statement), shall provide the Sponsor with accurate financial disclosure information as required under 21 Code of Federal Regulations (CFR) 54. The Investigator shall promptly update this information if any relevant changes occur during the trial and for 1 year following the completion of the trial.

10.11 Reporting and Publication Policy

The Sponsor will determine the identity of the Co-ordinating Investigator for the trial who will review and sign off the Clinical Trial Report. This decision will be based on involvement in the trial including, but not limited to, trial design, Subject recruitment and interpretation of trial data.

Clinical trials will be registered in public databases and summary results released / disseminated via publically available clinical trials databases according to the Sponsor's standard operating procedures (SOPs) and local requirements. As a general rule, both Phase 1 Healthy Volunteer studies and trials using a medicinal product in the normal course of medical practice (for example Non Interventional trials and Post Marketing Surveillance trials), are excluded from the above public registration and reporting requirements. If such trials and trials do require public registration and/or reporting, this will be undertaken according to local requirements.

The Sponsor registers clinical trials and posts the summary results as required by local and federal regulations.

Following the end of the clinical trial, the summary results should be made publicly available according to accepted timelines and requirements, usually within 12 months of trial completion. Special note should be taken to ensure timelines for the release of pediatric trial results are met, which may be 6 months from trial completion.

For multi-site trials, it is mandatory that the first publication be based on data obtained from all analyzed Subjects; therefore, Investigators participating in multi-site trials must not present data gathered individually or by a subgroup of sites prior to the full, initial publication, unless this has been agreed to by all other Investigators and the Sponsor. Publication of clinical trial results may include the presentation of such work at national and international congresses, symposia, professional meetings, peer-reviewed journals, and via other appropriate channels. Named authors and contributors to such publications shall be determined by the Sponsor in accordance with both the Company Publication Policy (which can be found at: http://www.mundipharma-rd.eu/research-areas/publications.html) and the generally accepted criteria for authorship as outlined by the ICMJE authorship guidelines. The data associated with any publication will be and shall remain the sole property of the Sponsor; the copyright of the document may be transferred to the scientific peer-reviewed journal prior to and as part of the publication process, as appropriate.

Subject to the paragraph above, the site may publish or present the results of the clinical trial subject to the protection of the Sponsor or its nominee(s) intellectual property rights, know- how, and its proprietary information. The Sponsor must be furnished with a copy of any proposed publication or presentation at least 60 days prior to submission for review and comment. Upon notice by the

Sponsor, however, that the Sponsor intends to secure its intellectual property rights (for example, file a patent application relating to the trial) or that Sponsor requires for its know-how or proprietary information to be removed prior to such publication, such publication may be delayed for a further 6 months or until its intellectual property rights have been secured, whichever is the later. The site further agrees that Sponsor's reasonable comments in relation to the proposed publication will be incorporated into the publication.

10.12 Insurance

The Sponsor shall have clinical trial insurance in accordance with applicable national regulations. This insurance provides cover for damage to research participants through injury or death caused by trial participation and is independent of investigational drug causality.

11 REFERENCES

1. Storer B.E. Design and Analysis of Phase I Clinical Trials Biometrics. 45, no. 3 (1989): 925-937.

2. Bose P, Da Y, Grant S. Histone deacetylase inhibitor (HDACI) mechanisms of action: Emerging insights. Pharmacology & Therapeutics Volume 143, Issue 3, September 2014, Pages 323–336.

3. Cai B, Lyu H, Huang J, Wang S, Lee CK, Gao C, Liu B. Combination of bendamustine and entinostat synergistically inhibits proliferation of multiple myeloma cells via induction of apoptosis and DNA damage response. Cancer Lett. 2013 Jul 28;335(2):343-50.

4. Schoeffski P, et al. “Weekly administration of bendamustine: a phase I study in patients with advanced progressive solid tumors.” Ann Oncol 2000; 11:729–734.

5. Schoeffski P, et al. “Repeated administration of short infusions of bendamustine: a phase I study in patients with advanced progressive solid tumors.” J Cancer Res Clin Oncol 2000; 126:41–47.

6. Rasschert M, et al. “A Phase 1 study of Bendamustine hydrochloride administred on Day 1 and 2 every 3 weeks in patients with solid tumors.” Br J Cancer 2007;96 1692-1698.

7. Rasschert M, et al. “A phase I study of bendamustine hydrochloride administered once every 3 weeks in patients with solid tumors.” Anti-Cancer Drugs 2007, 18:587–595.

8. Data on file at Mundipharma Research Limited.

9. Jagannath S, Richardson PG, Barlogie B, Berenson JR, Singhal S, Irwin D, Srkalovic G, Schenkein DP, Esseltine DL, Anderson KC; SUMMIT/CREST Investigators. “Bortezomib in combination with dexamethasone for the treatment of patients with relapsed and/or refractory multiple myeloma with less than optimal response to bortezomib alone.” Haematologica. 2006 Jul;91(7):929-34.

10. Oken MM, Creech RH, Tormey DC, et al. “Toxicity and response criteria of the Eastern Cooperative Oncology Group.” Am J Clin Oncol. 1982; 5:649-655.

11. Viet Hong Phan, Cindy Tan, Anneliese Rittau, Hongmei Xu, Andrew J McLachlan, Stephen J Clarke. "An Update on Ethnic Differences in Drug Metabolism and Toxicity from Anti-Cancer Drugs." Expert Opinion on Drug Metabolism & Toxicology 7, no. 11 (2011): 1395-1410.

12. Dolan, Peter H. O’Donnell and M. Eileen. "Cancer Pharmacoethnicity: Ethnic Differences in Susceptibility to the Effects of Chemotherapy." Clin Cancer Res. 15, no. 15 (2009): 4806–4814.

13. Simon R (1989). “Optimal Two-Stage Designs for Phase II Clinical Trials.” Controlled Clinical Trials 10: 1-10.

12 APPENDICES

12.1 Appendix A: Eastern Cooperative Oncology Group (ECOG) Performance Status Scale[10]

ECOG Performance Status	
Grade	**Description**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

12.2 Appendix B:NYHA Functional Classification

NYHA Class	Symptoms
I	Cardiac disease, but no symptoms and no limitation in ordinary physical activity, e.g. no shortness of breath when walking, climbing stairs, etc.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20 to 100 meters). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients.

12.3 Appendix C:New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1)

QUICK REFERENCE

Eligibility

Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

— **Measurable disease** - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

— **Measurable lesions** - lesions that can be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm)
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)
- 20 mm by chest X-ray.

Malignant lymph nodes: to be considered pathologically enlarged and measureable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At Baseline and follow-up, only the short axis will be measured and followed.

— **Non-measurable lesions** - all other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

- For special considerations regarding lesion measurability for bone lesions, cystic lesions and lesions with prior local treatment, consult the RECIST 1.1 guidelines in the Study Reference Manual.
- All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before beginning of treatment.
- The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.
- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, either a CT scan or documentation by color photography, including a ruler to estimate the size of the lesion, is to be done.

Methods of Measurement

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm

contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

- Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- When the primary endpoint of the trial is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.
- The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.
- Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response when all lesions have disappeared.
- Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Baseline documentation of "Target" and "Non-Target" lesions

— **Target Lesions** - all measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs should be identified as ***target lesions*** and recorded and measured at baseline.

  - Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).
  - A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.

— **Non-target lesions** - all other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria:

Evaluation of target lesions

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 LD of target lesions, taking as reference the baseline sum LD
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions from the smallest sum of the LD recorded since the treatment started; the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of ≥1 new lesion 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 LD since the treatment started

Evaluation of non-target lesions

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)
Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions*

* Although a clear progression of “non target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or trial chair).

Evaluation of best objective response

- The best objective response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria

Target Lesions	Non-Target Lesions	New Lesions	Objective Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD

PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Confirmation

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such trials that the responses are not confirmed.
- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the trial protocol may also be appropriate.
- In the case of SD, follow-up measurements must have met the SD criteria at least once after trial entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the trial protocol

Duration of objective response

- The duration of objective response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of stable disease

- SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.
- The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specifies the minimal time interval required

between 2 measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Definition of CT tumor response by RECIST 1.1 criteria:

The following table outlines the response categories by RECIST 1.1 criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
Complete Response (sum of diameters=0 mm)	Complete response	No	Complete Response
Complete Response	Non-complete response, non-progressive disease	No	Partial Response
Complete Response	Not evaluated	No	
Partial Response (decrease in sum of target lesions by ≥30%)	Non-progressive disease OR Not evaluated	No	
Stable Disease	Non-progressive disease OR Not evaluated	No	Stable Disease
Not all evaluated	Non-progressive disease	No	Not Evaluable
Progressive Disease (increase in sum of target lesions by ≥20% with an absolute increase in summed diameters by 5mm)	Any	Yes or No	Progressive Disease
Any	Progressive Disease	Yes or No	
Any	Any	Yes	

E.A. Eisenhauer, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1) European Journal of Cancer 45 (2009) 228–247 doi:10.1016/j.ejca.2008.10.026.

12.4 Appendix D:ASCO and CAP Guidelines on HER2 Testing in Breast Cancer

To determine HER2-negativity, IHC or in situ hybridization can be used. Below are the CAP guidelines as a reference:

HER2 Testing by Immunohistochemistry (IHC):

Negative (Score 0): No staining observed or Incomplete, faint/barely perceptible membrane staining in ≤10% of invasive tumor cells

Negative (Score 1+): Incomplete, faint/barely perceptible membrane staining in >10% of invasive tumor cells

HER2 Testing by In Situ Hybridization:

Reporting Results of HER2 Testing by In Situ Hybridization (single-probe assay):

Negative (not amplified): Average HER2 copy number <4.0 signals/cell

Reporting Results of HER2 Testing by In Situ Hybridization (dual-probe assay):

Negative (not amplified): HER2/CEP17 ratio <2.0 AND average HER2 copy number <4.0 signals/cell