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 80 mg/m^2 Administered during a 80-minute Infusion on Cardiac Repolarization in Patients with Advanced Solid Tumors

NCT number **NCT03345485**

PROTOCOL SIGNATURE PAGE

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By signing below, the Investigator agrees to adhere to the protocol as written and agrees that any changes to the protocol will be agreed to and approved by Mundipharma Research Limited. Prior to instituting those changes, the Investigator will obtain approval from the Institutional Review Board (IRB) or Independent Ethics Committee (IEC).

This trial will be conducted in accordance with the current International Council for Harmonization (ICH) guidance, the Good Clinical Practice (GCP) guidance, the Declaration of Helsinki, the United States (US) Food and Drug Administration (FDA) regulations and local IRB and legal requirements.

Investigator Signature _____ Date: _____

Name of Investigator (print) _____

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
IMP	Investigational Medicinal Product
INN	International Nonproprietary Name
IRB	Institutional Review Board
ISH	In situ hybridization
LDH	Lactate dehydrogenase
LLN	Lower Limit of Normal
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

Abbreviation	Explanation
PARP	Poly (ADP-ribose) polymerase
PFS	Progression free survival
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
QTcF	QT corrected by Fredericia
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

Abbreviation	Explanation
TNBC	Triple negative breast cancer
ULN	Upper limit of normal
US, USA	United States, United States of America
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 80 mg/m^2 Administered during a 80-minute Infusion on Cardiac Repolarization in 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 80 mg/m^2 on cardiac repolarization (QTcF) and other ECG parameters in 6-12 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 determine the plasma concentrations of tinostamustine and metabolites (M2 and M8) following tinostamustine administration of 80 mg/m^2 over 80 minutes on Day 1 and 15 of a 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: • Not applicable for the sub study.
Clinical Phase	Phase 1 sub-study to Phase 1/2 clinical trial EDO-S101-1002
Investigational Medicinal Product	Tinostamustine (EDO-S101)
No. of Patients	Maximum 12 patients. Six patients will be recruited initially; recruitment may be extended to 12 (see sub study stopping rules)
Number of Centers	Up to three centers

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 EDO-S101-1002 trial. A subgroup of 6 to 12 patients treated with a dose of 80 mg/m^2 tinostamustine administered over 80 minutes will be studied with intense ECG measurements, including 30 hr Holter monitoring, and PK sampling. The PK sampling will occur on Day 1 and Day 15 in cycle 1,only. 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. Following enrollment of 6-12 patients the sub study will be stopped.
Sub-Study Stopping Rules	**Patient level Stopping Rules for sub-study for administering Tinostamustine** <u>QTc stopping rules</u> 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. Baseline QTc is defined as the average of the triplicate QTcF interval collected prior to D1 dosing for each 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 ≤450 ms. 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. <u>Non-QTc stopping rules</u> Patients will be discontinued from further treatment if they experience a Grade 4 non-hematologic toxicity that has been deemed as related to tinostamustine treatment. **Trial level Stopping Rules** Six patients will be recruited to the sub study. If ≥ 2 or more patients have centrally confirmed QTc prolongation >500 ms or change from baseline >60 ms (Grade 3), or 1 patient has a QTc prolongation clinical related event (e.g., a 4-beat run of ventricular tachycardia or repeating couplets on the 24-hour

	Holter monitor), then up to 6 additional patients will be recruited. If ≥2 patients have a centrally confirmed QTc prolongation clinical related event (e.g. a 4-beat run of ventricular tachycardia or repeating couplets on the 24-hour Holter monitor) or ≥4 patients have centrally confirmed QTcF prolongation >500 ms, or change from baseline >60 ms (Grade 3), then further patient recruitment will be stopped and the sub study will be assessed by the Sponsor and regulatory agency. If ≥2 patients enrolled in the sub-study experience a Grade 4 QTc prolongation, that is confirmed by central reading and no underlying cause for the event is identified, this will result in the sub-study stoppage. Any treatment related death reported on the sub-study will result in the stoppage of the sub-study.
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. • 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. Platelet transfusions within the 14 days before Day 1 of Cycle 1 is prohibited. • 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 x upper limit of normal (ULN), or patients diagnosed with Gilbert's syndrome, that have been reviewed and approved by the medical monitor • Renal function: estimated creatinine clearance by Cockcroft-Gault formula ≥ 45 mL/min • Serum potassium and magnesium at least at the lowest limit of normal (LLN) range, before every IMP administration. If it is below LLN, supplementation is permissible. • 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 after the administration of IMP to be eligible to participate. Female study participants of child-bearing potential must continue using contraception for at least six months after the last administration of the IMP. Female study participants should be willing to have a pregnancy test performed at screening, ≤ 1 day prior to day 1 of each IMP administration and at study treatment discontinuation. Male study participants who are sexually active with a woman of child-bearing potential should also use a condom during treatment and for at least ninety (90) days after the last administration of IMP. 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): https://crediblemeds.org/index.php/drugsearch Patients who are being

	treated with Valproic Acid for any indication (epilepsy, mood disorder) must be excluded. • 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. Myocardial infarction or acute coronary syndrome within previous 6 months; or severe COPD or severe uncontrollable hypertension. • 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 or previous anticancer therapies within 28 days prior to the first dose of tinostamustine, provided the patient has recovered from any related toxicities ≥Grade 1 (except alopecia). • 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 Investigational Medicinal Product administration are allowed.
Dose and Schedule	The dose of 80 mg/m^2 tinostamustine will be administered over 80 minutes. Administration is on Day 1 and Day 15 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 initially but may be expanded to 12. An estimated four 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 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	Not applicable for sub study.

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 IMP Administration); Scans 28 days from Baseline[15]	Cycle 1 and Subsequent Cycles[1]					IMP 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]	n/a							

Procedure	Screening 28 days from Baseline (First Day IMP Administration); Scans 28 days from Baseline[15]	Cycle 1 and Subsequent Cycles[1]					IMP 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 AEs		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: 30h 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 IMP 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 80 minutes from the start of tinostamustine administration on D1 and D15 each treatment cycle. Additional single ECGs will be conducted at IMP discontinuation and as clinically indicated.

Holter monitoring will commence 60 minutes prior to the start of the infusion on C1D1 and will continue through 30 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 5, 10, 20, 30, 45, 60, 80, 95, 110, 150 minutes, 3h30, 5h, 7h, 10h, 24h, 30 hours from the start of infusion.
- C1D15: prior to the start of infusion, and 5, 10, 20, 30, 45, 60, 80, 95, 110, 150 minutes, 3h30, 5h, 7h, 10h, 24h, 30 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 5, 10, 20, 30, 45, 60, 80, 95, 110, 150 minutes, 3h30, 5h, 7h, 10h, 24h, and 30 hours from the start of the tinostamustine infusion. See section 7.16 for sampling windows.

[6] not applicable for sub study

[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). Windows for haematology are Days 1 (- 2 days), 15 (-2 days) and 22 (+/- 2 days)

[8] Serum chemistry will include albumin, total protein, creatinine clearance by Cockcroft-Gault formula, uric acid, blood urea nitrogen (BUN), sodium, potassium, magnesium, calcium, glucose, total bilirubin, alkaline phosphatase, AST, ALT, gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) and C-reactive protein (CRP). Windows for biochemistry are Days 1 (- 2 days), 15 (-2 days) and 22 (+/- 2 days)

[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, 80 mins (±15 min). The patient will be observed at 80 mins (±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 for a maximum of 12 months after the end of their last cycle of treatment.

[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

Initially regarded as “epigenetic modifiers” acting predominantly through chromatin remodeling by maintaining histone acetylation, histone deacetylase (HDAC) inhibitors (HDACi) are recognized to exert multiple cytotoxic actions in cancer cells, often through acetylation of non-histone proteins. Some well-recognized mechanisms of HDACi lethality include, in addition to relaxation of DNA and de-repression of gene transcription, interference with chaperone protein function, free radical generation, induction of deoxyribonucleic acid (DNA) damage, up-regulation of endogenous inhibitors of cell cycle progression, e.g., p21, and promotion of apoptosis. This class of agents is relatively selective for transformed cells, at least in nonclinical trials. In recent years, additional mechanisms of action of these agents have been uncovered. For example, HDACi compounds interfere with multiple DNA repair processes, as well as disrupt cell cycle checkpoints, critical to the maintenance of genomic integrity in the face of diverse genotoxic insults. Despite their nonclinical potential, the clinical use of HDAC inhibitors remains restricted to certain subsets of T-cell lymphoma. Currently, it appears likely that the ultimate role of these agents will lie in rational combinations, only a few of which have been pursued in the clinic to date.[3]

Multiple lines of recent data have begun to suggest that there is biologically important synergy that exists between alkylating agents and HDAC inhibitors. For example, in one trial the combination of bendamustine, an alkylating agent, and entinostat, a HDAC inhibitor, synergistically inhibits proliferation of multiple myeloma (MM) cells via induction of apoptosis and DNA damage response. In this trial, cell growth assays showed that bendamustine or entinostat inhibited proliferation in a dose-dependent manner, and their combinations synergistically induced growth inhibition in all MM cells tested. An apoptotic enzyme-linked immunoassay (ELISA) and western blot assays on poly (ADP-ribose) polymerase (PARP) cleavage and caspase-8 and caspase-3 revealed that bendamustine in combination with entinostat exhibited a much more potent activity than either agent alone to promote the MM cells undergoing apoptosis in a dose-dependent manner. Flow cytometric analysis found that entinostat exhibited distinct effects on cell cycle progression in different lines and bendamustine mainly arrested the cells at S phase, whereas their combinations dramatically blocked the S cells entering G2/M phase. Furthermore, trials on DNA damage response indicated that phosphohistone H2A.X (P-H2A.X), a hallmark of DNA double strand break, along with phosphorylated CHK2 (P-CHK2) was significantly enhanced by the combinations of bendamustine and entinostat as compared to either agent alone. These molecular changes were correlated with the increases in mitotic catastrophe.[4]

Tinostamustine is a first in class alkylating HDAC inhibitor that is being developed for the treatment of relapsed/refractory hematologic malignancies and solid tumors. The compound underwent broad evaluation in nonclinical models for human cancer. In in vitro and in vivo trials demonstrate efficacy in models of Hodgkin lymphoma, aggressive non-Hodgkin lymphoma, multiple myeloma, T-cell lymphoma and acute myeloid leukemia. In solid tumors activity was seen in models of sarcoma, small cell lung cancer (SCLC), non-small cell lung cancer, breast cancer, ovarian cancer and glioblastoma. The efficacy was independent from p53 status and cell lines resistant to other chemotherapy agents still responded to tinostamustine, including cell lines particularly resistant to bendamustine. Mechanistically, tinostamustine induces a strong DNA damage response, evidenced by a rise of ɣ-pH2AX

and p53, while DNA damage response was suppressed. Consequently, in vitro experiments showed synergy with DNA repair influencing agents such as PARP inhibitors.

3.1 Background

3.2 Clinical Data

Please refer to the Investigators Brochure for full details of supporting clinical data.

Tinostamustine has been investigated in 4 industry sponsored clinical trials in patients, with haematologic malignancies or solid tumours as follows:

- Study EDO-S101-1001, a phase 1 study to investigate the safety, pharmacokinetic (PK) profiles and efficacy of tinostamustine in patients with relapsed/refractory haematologic malignancies. Stage 1 determined the safety, tolerability, MTD and recommended phase 2 doses (RP2D). Stage 2 is ongoing and is the cohort expansion phase utilising RP2D from Stage 1.
- Study EDO-S101-1002, a phase 1/2 study to investigate the safety, PK and efficacy of tinostamustine in patients with advanced solid tumours. Phase 1 was the dose escalation phase to determine the safety, tolerability, MTD, and RP2D. Phase 2 is ongoing and is the cohort expansion phase using the RP2D from phase 1. In addition, Study EDO S101-1002 sub-study (n=6) (protocol version 6.2) was completed using a dose of 60 mg/m^2 infused over 60 minutes in the United states (US). The sub study was completed and 6 patients were recruited.
- Study EDO-S101-1003, a phase 1 clinical study of tinostamustine in Japanese patients with relapsed/refractory haematologic malignancies. This study is complete.
- Study EDO-S101-1004, a phase 1/2, international, multi-centre, open-label study of salvage treatment with tinostamustine conditioning followed by autologous stem cell transplant (ASCT) in patients with relapsed/refractory MM (N=6). This study was terminated in March 2019.

The main toxicities associated with tinostamustine are haematologic abnormalities, primarily thrombocytopenia, anaemia, neutropenia, leukopenia, and lymphopenia. Haematologic abnormalities are the most common Grade 3/4 toxicities. Gastrointestinal symptoms, namely nausea, with or without vomiting, and fatigue are also common.

One of the clinical trials, EDO-S101-1004, was put on hold on 5 March 2019 due to the occurrence of clinically significant Frederica corrected QT interval (QTcF) prolongations detected in 3 out of 6 patients. Although the QTc prolongations were transient and asymptomatic, the sponsor terminated the trial on 13 March 2019 and decided not to pursue further development of this high-dose indication.

The sponsor also put recruitment for all other studies on hold to allow an in-depth analysis of clinically relevant ECG findings and a consequential benefit risk assessment. Occurrence of QTc observations within 6 hours of the start of infusion was used to infer a potential causal relationship with tinostamustine. Data from this analysis were included in the Benefit and Risk Assessment Report (BRAv2.0, 2019).

The benefit risk assessment (112 patients) concluded that benefit in terms of response rates outweighs the risks of a QTc prolonging effect of tinostamustine, which has been shown to be transient in nature, does not increase with re-exposure and has not been associated with high-risk clinical symptoms. Therefore, the trials continued after implementation of risk mitigation measures.

Nineteen patients were enrolled after the re-start of the studies till the DLP of the Investigator's Brochure (IB) version 6.0. Patients were treated with tinostamustine doses of 60 to 100 mg/m^2 administered over a 60-minute infusion. Digital 12-lead ECG recorders were used to monitor cardiac safety during each tinostamustine administration in 16 of these patients enrolled to EDO-S101-1001 and the sub-study of EDO-S101-1002. Three patients enrolled to EDO-S101-1003 trial had ECGs performed on local ECG machines.

None of these patients developed G3 or higher QTc prolongations confirmed by central reading. Enrolment to studies EDO-S101-1001 and EDO-S101-1002 continues; enrolment to study EDO-S101-1003 is complete.

Evidence of anti-tumour activity has been seen in patients with haematologic malignancies and solid tumours. Among patients treated thus far at various dose levels, disease response assessments are available for 79 patients. Among patients with haematological malignancies (HM) (N=52) (from EDO-S101-1001 and EDO-S101-1003 studies), 3 experienced complete response (CR), 11 experienced partial response (PR), 1 experienced minor response (MR), and 14 experienced stable disease (SD). In addition, 3 patients experienced PR and 3 patients experienced SD in the expansion phase of EDO-S101-1001 (9 patients were still ongoing as of DLP). In patients with solid tumours (N=22) 1 patient experienced PR and 10 patients experienced SD (in EDO-S101-1002). In addition, 1 patient experienced PR and 8 patients experienced SD in the expansion phase (phase 2) (N=23 ongoing as of DLP).

From the prematurely terminated trial (EDO-S101-1004), 1 PR, 1 very good partial response (VGPR) and 2 cases of SD were recorded (N=6).

A summary of findings to date in tinostamustine clinical trials is presented in Section **Error! R eference source not found.** of the IB.

The benefit risk assessment concluded that benefit in terms of response rates outweighs the risks of a QTc prolonging effect of tinostamustine, which has been shown to be transient in nature, does not increase with re-exposure and has not been associated with high risk clinical symptoms.

Therefore, the trials continued at planned doses of up to 100 mg/m^2 with implementation of adequate risk mitigation measures.

The benefit risk assessment proposed risk mitigation measures which were introduced via substantial protocol amendments before recruitment to the trials on hold was resumed.

3.3 Sub-study Rationale

A sub study designed to characterize the effect of Tinostamustine 60 mg/m^2 infused over 60 minutes on cardiac repolarization (QTc) and other ECG parameters was completed in Dec 2020. None of the patients who were dosed exhibited G3 or higher QTc prolongations. Therefore, this study supported continued investigation of tinostamustine and a further sub study utilizing a higher dose infused over a longer infusion time is now proposed.

This new sub-study will utilize the methodology used in the previous Sub study (EDO-S101-1002 version 6.2) to characterize the effect of Tinostamustine 80 mg/m^2 infused over 80 minutes on cardiac repolarization (QTc) and other ECG parameters. As the dose has been increased from the recently completed sub study it was decided to prolong the infusion duration accordingly.

4 SUB-STUDY OBJECTIVES

4.1 Primary Objectives

- To characterize the effect of tinostamustine at a dose of 80 mg/m^2 on cardiac repolarization (QTcF) and other ECG parameters in 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 determine the plasma concentrations of tinostamustine and metabolites (M2 and M8) following tinostamustine administration of 80 mg/m^2 over 80 minutes on Day 1 and 15 of a 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

- not applicable for the sub study.

5 TRIAL DESIGN

5.1 Overall Sub-Study Design

This sub-study is designed to 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.

A subgroup of 6-12 patients treated with a dose of 80 mg/m^2 tinostamustine administered over 80 minutes will be studied with intense ECG monitoring, including 30-h Holter monitoring, and PK sampling at cycle 1 Day 1 and Day 15 (Day 1 and Day 15).

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.

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 6-12 patients, the sub study will be stopped. Additional patients beyond the initial 6-12 patients at the 80 mg/m^2 dose or new cohorts evaluating higher doses can only be enrolled following review of the data.

Patient level Stopping Rules for sub-study for administering Tinostamustine

QTc stopping rules

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. Baseline QTcF is defined as the average of the triplicate QTcF interval collected prior to D1 dosing for each 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 ≤450 ms. 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.

Non-QTc stopping rules

Patients will be discontinued from further treatment if they experience a Grade 4 non-hematologic toxicity that has been deemed as related to tinostamustine treatment.

Trial Level Stopping Rules

Six patients will be recruited to the sub study. If ≥ 2 or more patients have centrally confirmed QTc prolongation >500 ms, or change from baseline >60 ms (Grade 3), or 1 patient has a QTc prolongation clinical related event (e.g., a 4-beat run of ventricular tachycardia or repeating couplets on the 24-hour Holter monitor), then up to 6 additional patients will be recruited.

If ≥2 patients have centrally confirmed QTc prolongation clinical related event (e.g. a 4-beat run of ventricular tachycardia or repeating couplets on the 24-hour Holter monitor) or ≥4 patients have centrally confirmed QTc prolongation >500 ms, or change from baseline >60 ms (Grade 3) then further patient recruitment will be stopped, and the sub study will be assessed by the Sponsor and regulatory agency.

If ≥2 patients enrolled in the sub-study experience a Grade 4 QTc prolongation, that is confirmed by central reading and no underlying cause for the event is identified, this will result in the sub-study stoppage.

Any treatment related death reported on the sub-study will result in the stoppage of the sub-study.

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

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.
- 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. Platelet transfusions within the 14 days before Day 1 of Cycle 1 is prohibited
- Aspartate aminotransferase/alanine aminotransferase (AST/ALT) ≤3× ULN. In cases with liver involvement ALT/ AST ≤5× ULN.
- Total bilirubin ≤1.5 x upper limit of normal (ULN), or patients diagnosed with Gilbert's syndrome, that have been reviewed and approved by the medical monitor.
- Renal function: Estimated creatinine clearance by Cockcroft-Gault formula ≥ 45 mL/min.
- Serum potassium and magnesium at least at the lowest limit of normal (LLN) range, before every IMP administration. If it is below LLN, supplementation is permissible.
- 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 after the administration of IMP to be eligible to participate. Female study participants of child-bearing potential must continue using contraception for at least six months after the last administration of the IMP. Female study participants should be willing to have a pregnancy test performed at screening, ⩽ 1 day prior to day 1 of each IMP administration and at study treatment discontinuation. Male study

participants who are sexually active with a woman of child-bearing potential should also use a condom during treatment and for at least ninety (90) days after the last administration of IMP. 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): https://crediblemeds.org/index.php/drugsearch.
- Patients who are being treated with valproic acid for any of its indication (epilepsy, mood disorder) must be excluded. 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. Myocardial infarction or acute coronary syndrome within previous 6 months, or severe COPD or severe uncontrollable hypertension.
- 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 or previous anticancer therapies within 28 days prior to the first dose of tinostamustine, provided the patient has recovered from any related toxicities ≥Grade 1 (except alopecia).
- 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..

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 (-2 days) 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 tinostamustine infusion on C1D1 and up to 15 minutes prior to the start of tinostamustine infusion in C1D15 and will continue through 30 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 5, 10, 20, 30, 45, 60, 80, 95, 110, 150 min, 3h30, 5h, 7h, 10h, 24h, and 30h from the start of tinostamustine infusion.
- C1D15: prior to the start of infusion, and 0 (predose, up to 0.5h prior to the start of the infusion), 5, 10, 20, 30, 45, 60, 80, 95, 110, 150 min, 3h30, 5h, 7h, 10h, 24h, and 30h from the start of tinostamustine 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 minutes (+/- 5 minutes) and triplicate ECGs at 80 minutes (+/- 5 minutes) from the start of tinostamustine administration on D1 and D15 of each treatment cycle.

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)

Not applicable for the sub study.

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 (-2 days), 15 (-2 days) and 22 (+/- 2 days) of treatment cycles 1 to the last treatment cycle, 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 and reviewed by the investigator before the start of tinostamustine infusion. Serum potassium and

magnesium should be at least at the LLN before the start of tinostamustine infusion. If it is below LLN, they would need to be corrected and rechecked before the infusion proceeds.

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 (- 2 days) and 15 (-2 days) 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 clearance by Cockcroft-Gault formula, uric acid, blood urea nitrogen, sodium, potassium, magnesium, 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 and magnesium levels at screening and 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 medicinal 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. Urinalysis parameters will be pH, Specific gravity, blood, total protein, ketones, leukocytes, nitrites and glucose.

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 and Contraception

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 9.6.4 for details regarding the pregnancy reporting procedure. A woman of child-bearing potential is defined as a female who does not meet the criteria for "Women Not of Childbearing Potential": women $\geq$55 years of age and 12 consecutive months without menstrual bleeding or <55 years of age after surgical sterilization.

Female study participants must be willing to use at least TWO highly effective forms of contraception. This should start from the time of study enrollment and continue throughout tinostamustine administration and for at least six months after the last administration of IMP

Male subjects who intend to be sexually active with a woman of childbearing potential must use TWO highly effective forms of contraception including a condom during treatment and for at least 90 days after the last administration of IMP. 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 Adverse Events (AEs)

Monitoring and recording of AEs will be conducted from the time of ICF signature through to point of discontinuation of tinostamustine (D28 of last treatment cycle). 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.

A 2 mg/mL tinostamustine infusion solution after reconstitution will be utilized. Assessment of injection site reactions must be performed on each treatment day at pre-dose and end of infusion. Additional evaluation must be performed in patients who present signs and symptoms of injection site reactions at the end of infusion. (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.

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 (except for patients with secondary metastasis to CNS disease as permitted in the inclusion criteria Section 6.1 and patients with rash and/or allergic reactions as permitted in Section 8.9).

7.14.2 Valproic Acid

Patients receiving valproic acid for any indication (epilepsy, mood disorder) must be excluded from the trialas valproic acid is the same class of drug as tinostamustine .

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 from 24 hrs before tinostamustine infusion until 24 hrs after the end of the tinostamustine infusion. Given the short half-life of tinostamustine, ondansetron can be used, if needed, for the prophylaxis of delayed nausea and vomiting.. 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 28 days 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 for a maximum of 12 months after the end of their last cycle of treatment.

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, and the 2 metabolites, M2 and M8 in plasma will be characterized at Cycle 1, Day 1 and Day 15. The blood sampling schedule for the PK assessment is conducted at Cycle 1, Day 1 and 15.

Samples are taken as follows: 0 (predose, up to 0.5h prior to the start of the infusion), 5, 10, 20, 30, 45, 60, 80, 95, 110, 150 min, 3h30 m, 5h, 7h, 10h, 24h, and 30h 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
Pre dose	≤ 30 min before infusion start
5 minute to 80 minute samples	± 2 minutes
95 minute to 10 hr samples	± 5 minutes
24h and 30 h samples	± 15 minutes

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: Tinostamustine is the International Nonproprietary Name (INN) of the Investigational Medicinal Product (IMP). Tinostamustine is a first in class alkylating HDACi fusion molecule that is being investigated for the treatment of relapsed/refractory hematologic malignancies and advanced solid tumours. 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 histone-‑deacetylase inhibiting moiety.

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 diluted solution is stored in an infusion container (bottle or bag) for a maximum of 10 hours, of which a maximum of 4 hours may be at room temperature, with the remaining storage period at 2-8°C. The storage period at room temperature must include equilibration at room temperature and the infusion duration. Tinostamustine is compatible with infusion materials indicated in the Pharmacy Manual.

Only patients enrolled in the trial may receive investigational product. At Day 1 and Day 15 of each 28-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 (if applicable) 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 80 mg/m^2 on Day 1 and Day 15 of each treatment cycle. The investigational product (reconstituted and diluted solution as described in Section **Error! Reference source not found.**) will be administered by i.v. infusion through a peripheral vein or port over a precise 80-minute (+/- 2 minute) infusion time.

Instructions for preparation and administration of the IMP infusion are provided in the Pharmacy Manual. The PI or qualified site personnel will administer tinostamustine by i.v. infusion using a calibrated infusion pump. A full record of the infusion process will be kept using the source data document included in the Pharmacy manual.

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:

- No active infection
- ANC ≥1,000/mm^3
- platelets ≥ 100,000/ µL or >65% of platelet baseline level (whichever is higher). Baseline for D15 is the level of platelets before tinostamustine administration on D1 of the same cycle. Baseline for D1 administration is the level of platelets on D1 of the previous treatment cycle.
- non-hematologic treatment related toxicities have improved to ≤Grade 1 or to the patient's baseline values (except alopecia).

In the event of toxicity leading to dose delay, tinostamustine dose will be reduced to 60 mg/m^2. If toxicity issues are resolved, the original dose can be administered at the next cycle, at investigator's discretion.

If a patient does not meet these criteria, next dosing will be delayed, by up to 14 days and the patient should be evaluated within 48-72 hours. If the next administration is delayed by more than 14 days, the investigator will determine if there is clinical benefit for patient to continue treatment when the patient's toxicity returns to Grade 1 or the patient's pre-event baseline. The medical monitor must be informed of any treatment delay (email: EDOS101-medical-monitor@mundipharma-rd.eu).

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 ≤450ms. 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. Please also refer to the sub-study specific patient level stopping criteria as detailed in Section 5.1 Overall Sub-Study Design.

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 60 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 but are not limited to:

1. For neutropenia: Growth factor support is allowed per institutional standards..
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. The most current list of drugs with known risk of TdP issued by Credible Meds: https://crediblemeds.org/new-drug-list should be re-viewed by the Investigator (or delegated site staff) prior to dosing a patient. Precautions of use of the 5HT3 receptor antagonists are discussed in Section 7.14.4.

- Tinostamustine is a molecule composed of an alkylating moiety similar to bendamustine and a histone- deacetylase inhibiting moiety 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 chemotherapeutic agent 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 as co-administration with alkylating agents is contraindicated as it increases the likelihood of mild to severe skin toxicity. 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 chemo therapeutic agents were 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. Rasburicase is recommended instead of allopurinol.
- Patients treated with many alkylating agents 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 HDAC inhibitors 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 HDAC inhibitors' treatment; 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 (hypersensitivity) to alkylating agents may be immediate /or delayed. Symptoms include fever, chills, pruritus, and rash. In rare instances severe anaphylactic and anaphylactoid reactions have occurred.

  Assessment of infusion site reactions must be performed on each treatment day at pre-dose, 80 minutes (±15 minutes). The patient will be observed at 80 minutes (± 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 AES AND SAES

9.1 Definition of an Adverse Event (AE)

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related and is any event arising during participation in the clinical trial from the point of signature on the ICF. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug from any route of administration, formulation, or dose, including an overdose.

AEs also include:

- A new disease or exacerbation of a pre-existing disease e.g., increase in frequency or worsening in nature.
- Any deterioration in measurements of laboratory values or other clinical tests (e.g., ECG, vital signs or X-ray) that results in symptoms, a change in treatment, or discontinuation from tinostamustine.
- Other medical events regardless of their relationship to tinostamustine, such as accidents, falls and any injuries resulting from them.

AEs do not include:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure is an AE.
- 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. Note: Overdose should be recorded in special situation dedicated forms and reported to sponsor or designated vendor within 24 hours.

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.

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. Disease progression itself and death from disease progression should not be recorded as an AE.

However, new or increasing symptoms not related to disease progression should be reported as AEs.

9.2 Recording Adverse Events

All AEs must be reported from the time of ICF signature through the point 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'). AE 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.

· **Seriousness**

For definition of seriousness criteria refer to Section 9.3. All seriousness criteria that apply have to be recorded.

· **Action Taken with tinostamustine**

The action taken with tinostamustine as a result of the AE has to be documented. In the situation that the AE leads to permanent discontinuation of tinostamustine, this meets the definition of an AE leading to Subject's withdrawal from the study.

· **Treatment for the AE**

Any treatment for an AE, whether pharmacological or other (e.g., surgical) treatment, has to be recorded in the eCRF.

· **Outcome**

The outcome recorded should be reflective of the outcome at the time of reporting the AE. The following categories should be used:

Not recovered/Not Resolved

- Indicates that the event is ongoing and there has been no recovery.

Recovering/Resolving

- Indicates that the event is in the process of recovery but has not yet fully resolved.

Recovered/Resolved

- Indicates that the event has fully resolved.

Recovered/Resolved with sequelae

- Indicates that there is a residual, possibly permanent consequence of the event (e.g., residual hemiparesis subsequent to stroke).

Fatal

- Indicates that the Subject died due to the event. The outcome “fatal” applies only to the event(s) that were the cause(s) of death. For other adverse events that were ongoing at the time of death, the outcome must be recorded as “not recovered” and not “fatal”.

Unknown

Indicates that the outcome of the AE cannot be determined despite the best efforts of the Investigator. This may be due to the Subject being ‘Lost to Follow-up’ and therefore the Safety Follow-Up visit could not be performed.

· **Causal Relationship of AE**

The causal relationship of all AEs to tinostamustine 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 un-derlying 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 concur-rent/underlying illness, other drugs, or procedures. Information regarding tinostamustine withdrawal may be lacking or unclear. “Possible” should be used when trial treatment ad-ministration 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.

· **Follow up of AE**

Adverse events should be followed up to determine the outcome. The cut off for information collec-tion in the eCRF for AEs and SAEs including any follow up lab information is according to Section 9.2. Any AE that is still ongoing at this visit will have an outcome of ‘Recovering/Resolving’ or ‘Not Recov-ered/Not Resolved’ in the eCRF. After that all information still needs to be collected in the source and for SAEs the information needs to be forwarded to the Sponsor.

- All efforts to collect follow-up information must be documented in the Subject’s source data as soon as it is received.

- All AEs must be followed up by the Investigator until:

- the AE is resolved or resolved with sequelae and all other queries related to the AE have been clarified, or
- the end of the period of observation (= last study visit), or
- the Investigator considers it medically justifiable to stop further follow-up

- If the Subject had an AE with fatal outcome, an autopsy report should be provided if possible.
- If SAEs are ongoing at the time of the Subject's last study visit, an additional safety follow-up visit should be scheduled for those Subjects. This visit will be documented in the source notes and not in the eCRF.

The Investigator should set the interval to the additional safety follow-up visit according to his/ her medical judgement. If the EDC system is closed, information from this visit should be forwarded to the Sponsor using the paper SAE form.

The Investigator should respond to any queries raised by the Sponsor in relation to adverse events, including provision of supporting documentation within the requested timeline.

In case of fatal or life-threatening SAEs the Sponsor may request urgent clarification within one business day.

Subjects who were treated with IMP but did not complete the study as per protocol, should receive all the examinations and investigations scheduled for the last study visit. The Investigator should make all efforts to contact Subjects lost to follow-up and document the attempts in the Subject's source data.

9.3 Serious Adverse Events

A SAE is any AE that is considered 'serious' if, in the view of either the Investigator or Sponsor, it results in any of the following:

- 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;
- Important medical events 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.

Events exempt from immediate reporting as SAEs.

Hospitalization for pre-existing conditions e.g. elective procedures planned prior to study entry, which has not worsened do not require SAE reporting. 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, the most current IB version contains the Reference Safety Information for the

expectedness assessment

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

9.6.1 Reporting AEs and SAEs to the Sponsor

All AEs must be reported in the eCRF from the time of ICF signature to the point 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.

- All SAEs have to be reported via e-mail or fax by using the Mundipharma **SAE Report Form** and a cover page which are available in the Investigator site file. The SAE reporting contact of the Sponsor is Mundipharma Drug Safety & PV:

Mundipharma Drug Safety and PV	Tel: +44 (0) 1223 424444 (Not 24-hour coverage; for 24-hour details please see the Investigator Site file) Fax: +44 (0)1223 426002 E-Mail: drugsafetycentral@mundipharma-rd.eu

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 or the designated vendor (their agent) will report to the regulatory authorities, institutional review boards (IRBs) and investigators (as applicable) as per national regulations of the countries where the ongoing tinostamustine trials are conducted,

9.6.3 Reporting of Grade 4 and Grade 5 AEs

All grade 4 AEs (per definition "life-threatening") and grade 5 ("death"), as per CTCAE, version 5.0, 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 Pregnancy

If a female patient or the female partner of a male patient becomes pregnant during the course of the trial and within 6 months from the last dose of IMP administration, 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.

9.6.5 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.

9.6.6 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.
- Investigator/institution should maintain adequate and accurate source documents that include all pertinent observations on the trial patients. Source data should be attributable, legible, contemporaneous, original, accurate and complete. Changes to the source data should be traceable, should not obscure the original entry and should be explained if necessary.

Electronic data capture (EDC)/eCRF entries should be made as close to the visit of the subject as possible. Data reported on the eCRF should be completed accurately and in a timely manner according to the eCRF guidelines and ICH E6 R2 4.9.2 and 4.9.3

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. The measures implemented for the archiving in a separate location from the sponsor (i.e. an archiving depot) and the access of the site to these records, will be provided to the site if necessary.

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.

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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