Clinical Trial Protocol

Phase I multicenter clinical trial evaluating the combination of trastuzumab emtansine (T-DM1) and non-pegylated liposomal doxorubicin in HER2-positive metastatic breast cancer.

THELMA Study

Code: MedOPP038

Study Drug(s):	T-DM1 (trastuzumab emtansine) Non-pegylated liposomal doxorubicin
EudraCT#:	2014-001056-28
Clinical Trials.gov#:	NCT02562378
Protocol#:	MedOPP038
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Declaration of Investigators

Protocol Title: **Phase I multicenter clinical trial evaluating the combination of trastuzumab emtansine (T-DM1) and non-pegylated liposomal doxorubicin in HER2-positive metastatic breast cancer – THELMA Study.**

Version date: **version 9 : 27th June 2017**

Protocol number: **MedOPP038**

I have received, reviewed and understand the following:

a) Protocol: Phase I multicenter clinical trial evaluating the combination of trastuzumab emtansine (T-DM1) and non-pegylated liposomal doxorubicin in HER2-positive metastatic breast cancer, THELMA Study, version v9, dated 27th June 2017

b) Current Investigator's Brochure for T-DM1 with details of clinical and nonclinical data on T-DM1 that are relevant to the study of the product in human subjects

c) Current Summary of Product Characteristics on Non-pegylated liposomal doxorubicin

I have been adequately informed about the development of the investigational product to date. I will confirm the receipt of updated Investigator's Brochure. I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol.

I fully understand that any changes instituted by the investigator(s) without previous agreement with the sponsor would constitute a violation of the protocol, including any ancillary studies or procedures performed on study patients (other than those procedures necessary for the well being of the patients). I am aware I may only implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB/IEC approval/favourable opinion and /or before sponsor's agreement and, in this case, and as soon as possible, I will submit in written the implemented deviation or change and the reasons for it to the sponsor.

I will not enrol the first subject in the study until I have received approval from the appropriate Institutional Review Board or Independent Ethics Committee (IRB/IEC) and until all legal and regulatory requirements in my country have been fulfilled.

The study will be conducted in accordance with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and its amendments, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines (ICH E6 GCP) and applicable regulations and laws.

I agree to obtain, in the manner described in this protocol and in ICH E6 GCP, written informed consent by the subject or the subject's legally acceptable representative or witnessed verbal informed consent to

participate for all subjects whose participation in this study is proposed to and before any subject's study specific procedure is done.

I will ensure that the study drug(s) supplied by the sponsor are being used only as described in this protocol.

I am aware of the requirements for the correct reporting of serious adverse events, and I commit to document and to report such events as required by the sponsor and in accordance with Health Authority Regulatory requirements.

I agree to supply – upon request – the Sponsor or Sponsor's representative with evidence of current laboratory accreditation, the name and address of the laboratory, and a list of normal values and ranges.

I agree with the use of results of the study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals.

I agree to keep all source documents and case report forms as specified in the relevant sections of this protocol.

I will provide all required Regulatory Authority forms, up-to-date curriculum vitae of myself, sub-investigators and of any member of my study team (if requested) before the study starts, which may be submitted to regulatory authorities.

I am aware of the possibility of being audited by the sponsor or its delegate or inspected by regulatory authorities for the performance of this study. I will permit monitoring, auditing and inspection and provide direct access to source data/documents and reports for these purposes.

Furthermore, I confirm herewith that the sponsor is allowed to enter and utilize my professional contact details and function in an electronic database for internal purposes and for submission to Health Authorities worldwide.

Name: _____

Signature: _____ Date: _____

Protocol synopsis

Product:	Trastuzumab emtansine (T-DM1) and non-pegylated liposomal doxorubicin
Protocol Number:	MedOPP038
EudraCT Number:	2014-001056-28
Protocol Title:	Phase I multicenter clinical trial evaluating the combination of trastuzumab emtansine (T-DM1) and non-pegylated liposomal doxorubicin in HER2-positive metastatic breast cancer – THELMA Study
Target Disease:	HER2-positive metastatic breast cancer
Subjects:	Subjects age ≥ 18 years with HER2-positive metastatic breast cancer that have relapsed or progressed on or after taxanes and trastuzumab-based therapy. Subjects must have histologic or cytologic confirmation of the HER2-positive metastatic breast cancer. Evidence of measurable or evaluable metastatic disease is required. There are 3 planned cohorts of patients.
Number of subjects:	A minimum of 12 and up to 24 patients will be enrolled
Study Objectives	Primary objective: To determine the maximum tolerated dose (MTD) of the combination of T-DM1 and non-pegylated liposomal doxorubicin in metastatic breast cancer (mBC) patients previously treated with taxanes and trastuzumab-based therapy. Secondary objectives: - To determine the efficacy of the combination of T-DM1 and non-pegylated liposomal doxorubicin, defined by the overall response rate (ORR), clinical benefit rate (CBR), number of progressions and number and reasons for deaths. - To assess the safety profile of the combination of T-DM1 and non-pegylated liposomal doxorubicin, defined by all toxicities reported during the study. - To evaluate the cardiac safety of the combination of T-DM1 and non-pegylated liposomal doxorubicin measured by LVEF as assessed by echocardiography, cardiac troponin I and B-type natriuretic peptide (BNP) levels. - To evaluate the potential role of single nucleotide polymorphisms (SNP)

	in the predisposition for developing cardiotoxicity. - To analyze the pharmacokinetics (PK) profile of T-DM1 and its metabolites and non-pegylated liposomal doxorubicin.
Type of study	This is a prospective dose-finding, multicenter and open-label phase I clinical trial. This clinical trial is designed with a dose escalation following a "modified" 3 by 3 design where 3 patients will be included at a given cohort and followed to observe if they experience any dose-limiting toxicity (DLT) during the first two cycles of treatment. If none of the 3 first patients included in each cohort experience a DLT (0/3), the next patients will be enrolled in the subsequent cohort. If 1 of these 3 first patients experience a DLT, 3 more patients will be included at the same cohort to determine the number patients who experience DLTs in the total group of 6 patients. If 2 or more patients treated at a given cohort of six patients experience a DLT, the cohort of one dose level below will be established as the maximum tolerated dose (MTD). No dose escalation between cohorts will be permitted. Patients assigned on each cohort will remain at their study cohort during all the study period. Planned number of cohorts is currently 3 (3+3 design), including an expansion cohort of an additional 6 patients at the maximum tolerated dose (MTD) or recommended phase 2 dose (RP2D). The Steering Committee will review toxicities and may decide to add the expansion cohort to level -1 in the situation where it is necessary to have a dose level -1.
Treatment	Trastuzumab emtansine (T-DM1) will be administered at a fixed dose of 3.6 mg/kg IV on Day 1 every 3 weeks and three cohorts of patients with three different dose levels of conventional non-pegylated liposomal doxorubicin (45 mg/m^2, 50 mg/m^2 and 60 mg/m^2) IV on Day 1 in cycles of 21 days each are planned. The initial dose of T-DM1 will be administered over 90 minutes (±10 minutes) and, in the absence of any signs or symptoms of infusion reactions with the first dose, subsequent doses of T- DM1 may be administered over 30 minutes (±10 minutes). Non-pegylated liposomal doxorubicin will be administered over approximately 60 minutes starting 60 minutes after the end of T-DM1 infusion. After the two first cycles, the combination of study (T-DM1 and non-pegylated liposomal doxorubicin) will be administered for up to 6 cycles

	(including the first two cycles). T-DM1 treatment will continue as a single agent until disease progression or development of intolerable toxicity, whichever occurs first. All patients will be followed up until improvement to grade 1 or complete recovery from all ≥ grade 2 adverse events, or withdrawn consent, death or up to a maximum of 12 months after the first dose of study combination whichever occurs first.
Primary endpoint	The primary endpoint of the study will be to determine the Maximum-tolerated dose (MTD), defined as the highest dose level at which no more than one of six patients or 0 of 3 patients experiences dose-limiting toxicity (DLT) during the first two cycles of study treatment.
Inclusion criteria	Patients must meet the following criteria for study entry: - Signed informed consent prior to any study specific procedure. - Patient to be able and willing to comply with protocol. - Patients with cytologically or histologically confirmed carcinoma of the breast. - Patients with incurable locally advanced or metastatic disease who have previously received up to two previous chemotherapy regimens in this setting (patient starting the first, second or third line of treatment are eligible). Patient must have progressed or relapsed on or after taxane and trastuzumab-based therapy. - HER2-positive disease immunohistochemistry (IHC) 3+ or *in situ* hybridization (FISH) positive assayed at local laboratories and according to updated ASCO/CAP criteria. - At least one measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1; or patients with non measurable lesions could be included with these exceptions: o patients with only blastic bone lesions are not eligible o patients with only pleural, peritoneal or cardiac effusion, or meningeal carcinomatosis are not eligible - Patient ≥ 18 years of age. - ECOG performance status of 0 or 1. - Life expectancy ≥ 3 months. - Adequate bone marrow function: o Hemoglobin ≥ 10 g/dl. o Absolute neutrophil count ≥ 1.5 x 10^9/L. o Platelets ≥ 100 x 10^9/L without transfusions within 21 days before 1st study treatment.

	○ International normalized ratio (INR) < 1.5 × the upper limit of normal (ULN). - Adequate hepatic and renal function: ○ Total bilirubin ≤ 1.5 x ULN, except for Gilbert disease patients. Gilbert's syndrome is suspected in people who have persistent, slightly elevated levels of unconjugated bilirubin without any other apparent cause. A diagnosis of Gilbert's syndrome will be based on the exclusion of other diseases based on the following criteria: unconjugated hyperbilirubinemia noted on several occasions, no evidence of hemolysis (normal hemoglobin, reticulocyte count, and LDH), normal liver function tests, and absence of other diseases associated with unconjugated hyperbilirubinemia. For patients with Gilbert disease, total bilirubin must be ≤ 3 x ULN ○ Alkaline phosphatase ≤ 2.5 × the ULN (≤ 5 × the ULN if liver and/or bone metastases are present). ○ AST (SGOT)/ALT (SGPT) ≤ 1.5 x ULN (< 3 x ULN if liver metastases are present). ○ Creatinine ≤ 1.5 x ULN and calculated creatinine clearance ≥ 50 mL/min per the Cockcroft and Gault formula. - Adequate cardiovascular function with LVEF ≥ 55% as assessed by echocardiography. - Recovery from all reported toxicities of previous anti-cancer therapies to baseline or grade ≤ 1 (CTCAE version 4.0), except for alopecia. - For women of childbearing potential (including pre menopausal women who have had a tubal ligation) and for all women not meeting the definition of postmenopausal (≥ 12 months of amenorrhea), and who have not undergone surgical sterilization with a hysterectomy and/or bilateral oophorectomy, and men with partners of childbearing potential, agreement by the patient and/or partner to use a highly effective, non-hormonal form of contraception or two effective forms of non-hormonal contraception and to continue its use for the duration of study treatment and for 7 months after the last dose administered while being on study. For all other women, documentation must be present in medical history confirming that the patient is not of childbearing potential.
Exclusion	Patients must not meet the following criteria for study entry:

criteria	- Previous treatment with T-DM1 or anthracyclines, either in the (neo)adjuvant or in the metastatic setting. - More than two chemotherapeutic regimens for locally advanced incurable disease or metastatic disease. - Patients who have received prior anti-cancer treatment with chemotherapy, immunotherapy or radiotherapy within 3 weeks (6 weeks for nitrosoureas or mitomycin-C), hormonal therapy or lapatinib within 7 days, prior trastuzumab within 21 days (7 days if weekly trastuzumab) or any other targeted therapy within the last 21 days prior to starting study treatment. - Previous radiotherapy for the treatment of unresectable, locally advanced/recurrent or mBC is not allowed if: o The last fraction of radiotherapy has been administered within 21 days prior to first study drug administration (except for brain irradiation; at least 28 days will be required). o More than 25% of marrow-bearing bone has been irradiated. - History of intolerance (including Grade 3 or 4 infusion reaction) or hypersensitivity to the active substance or to any of the excipients of T-DM1 or non-pegylated liposomal doxorubicin. - Patients with CNS involvement. However, patients with metastatic CNS tumors may participate in this trial if the patient is > 4 weeks from radiotherapy completion, is clinically stable with respect to CNS tumor at the time of study entry and is not receiving steroid therapy for brain metastases. - Severe/uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, or psychiatric illness/social situations that would limit compliance with study requirements. - Cardiopulmonary dysfunction as defined by any of the following: o History of NCI CTCAE (Version 4.0) Grade ≥ 3 symptomatic CHF or NYHA criteria Class ≥ II. o Angina pectoris requiring anti-anginal medication, serious cardiac arrhythmia not controlled by adequate medication, severe conduction abnormality, or clinically significant valvular disease. o High-risk uncontrolled arrhythmias (i.e., atrial tachycardia with a heart rate > 100/min at rest, significant ventricular arrhythmia [ventricular tachycardia], or higher-grade atrioventricular [AV]-

	block [second degree AV-block Type 2 [Mobitz 2] or third degree AV-block]). o Significant symptoms (Grade ≥ 2) relating to left ventricular dysfunction, cardiac arrhythmia, or cardiac ischemia. o Myocardial infarction within 12 months prior to randomization. o Uncontrolled hypertension (systolic blood pressure > 180 mmHg and/or diastolic blood pressure >100 mmHg). o Requirement for oxygen therapy. - Current peripheral neuropathy of Grade ≥ 3 per the NCI CTCAE, v4.0. - History of a decrease in LVEF to < 40% or symptomatic CHF with previous trastuzumab treatment. - Patients who have had a prior malignancy, other than carcinoma in situ of the cervix, or non-melanoma skin cancer, unless the prior malignancy was cured ≥ 5 years before first dose of study drug with no subsequent evidence of recurrence. - Current known active infection with HIV, hepatitis B, and/or hepatitis C virus. For patients who are known carriers of hepatitis B virus (HBV), active hepatitis B infection must be ruled out based on negative serologic testing and/or determination of HBV DNA viral load per local guidelines. - Women who are pregnant or breast-feeding.
Pharmacokinetic (PK) assessments	Pharmacokinetic (PK) assessments will be only performed in cycle 1 and 2 and cycle 4 during the dose-finding period, defined as the period between the first patient in the study being treated and the MTD definition. Pharmacokinetic data will be compared with historical data of T-DM1 pharmacokinetics and non-pegylated liposomal doxorubicin pharmacokinetics and will include serum concentrations of T-DM1, total trastuzumab, free DM1 (maytansinoid, derivative of maytansine) and non-pegylated liposomal doxorubicin. Approximately 6 ml of whole blood will be drawn at each time PK point. The following PK parameters will be calculated: AUC (main PK data), clearance (CL), distribution volume (Vd), apparent half-life ($t_{1/2}$) and maximal serum concentration (C_{max}). Serum levels of HER2 Extracellular Domain (ECD) will be also measured, as this biomarker has been shown to be a relevant covariate in the Population PK model for T-DM1.
Efficacy and	The safety and efficacy of combination will be evaluated in all treated

Safety assessments	patients. However, a patient leaving the study for reasons other than toxicity before the end of first 2 cycles will be replaced by another patient. The occurrence and maximal grade of toxicity for the whole duration of treatment will be listed and tabulated by type and dose level. Adverse events reported as non-drug related by the responsible investigator will be reported as well. Toxicity will be evaluated in this study using the Common Terminology Criteria for Adverse Events by the NCI (CTCAE version 4.0) and the New York Heart Association (NYHA) criteria for cardiotoxicity.

Table of abbreviations

Abbreviation	Definition
ACE	Angiotensin I converting enzyme
ADL	Activities of daily living
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine transaminase
AML	Acute myelogenous leukemia
ANC	Absolute neutrophil count
ALP	Alkaline phosphatase
aPTT	Activated partial thromboplastin time
ARDS	Acute respiratory distress syndrome
ARO	Academic Research Organization
AST	Aspartate transaminase
AT	Aminotransferase
AUC	Area under the serum concentration-time curve
AV	Atrioventricular
BC	Breast cancer
BML	Below measurable limit
BoR	Best overall response
BSA	Body surface area
BUN	Blood urea nitrogen
CBR	Clinical benefit rate
CDER	Center for Drug Evaluation and Research
CHF	Congestive heart failure
CI	Confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CL	Clearance
Cmax	Maximum concentration
CNS	Central Nervous System
CR	Complete response
CRF	Case Report Form
CRO	Contract research organization
CSF	Colony-stimulating factors
CSR	Clinical study report
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DPD	Dihydropyrimidine dehydrogenase
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
DoR	Duration of response
dV	Distribution Volume
DSUR	Development safety update report
EC	Ethics Committee
ECD	Extracellular domain
ECG	Electrocardiogram
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group

eCRF	Electronic Case Report Form
EDC	Electronic data capture
EMA	European Medicines Agency
EOS	End of Study
ESA	Erythropoieses stimulating agents
FDA	Food and Drug Administration
FDG-PET	Fluorodeoxyglucose-Positron Emission Tomography
FISH	Fluorescence in situ hybridization
GCP	Good Clinical Practice
HBV	Hepatitis B virus
β-HCG	Human chorionic gonadotropin
HER2	Human epidermal growth factor receptor 2
HIV	Human immunodeficiency virus
HR	Hazard Ratio
5-HT	5-hydroxytryptamine
ICH	International Conference on Harmonisation
IHC	Immunohistochemistry
INR	International normalized ratio
IRB	Independent Review Board
ISH	In situ hybridation
ITT	Intention to Treat
LDH	Lactate dehydrogenase
LC-MS/MS	Liquid chromatography–mass spectrometry
LVEF	Left ventricular ejection fraction
mBC	Metastatic Breast Cancer
MDS	Myelodysplastic Syndromes
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
MUGA	multi-gated radionuclide angiography
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NRH	Nodular regenerative hyperplasia
NYHA	New York Heart Association
ORR	Overall Response Rate
PCR	Polymerase chain reaction
PD	Progression Disease
PET	Positron Emission Tomography
PFS	Progression Free Survival
PK	Pharmacokinetics
PP	Protocol compliant population
PR	Partial Response
PVC	Polyvinyl chloride
QTcF	Corrected QT interval using the Fridericia formula
qw	Once weekly
q3w	Every 3 weeks
RBC	Red Blood Cells
RECIST	Response Evaluation Criteria In Solid Tumors
RNA	Ribonucleic acid

SADR	Serious Adverse Drug Reaction
SAP	Statistical Analysis Plan
SC	Steering Committee
SD	Stable Disease
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SmPC	Summary of Product Characteristics
SNP	Single nucleotide polymorphisms
SOC	System-organ class
TBL	total bilirubin
T-DM1	Trastuzumab emtansine
ULN	Upper Limit of Normal
Vss	Volume of distribution at steady state
WBC	White blood cell

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1. BACKGROUND AND RATIONALE

1.1. HER2-Positive Metastatic Breast Cancer

Breast cancer is the most common tumor among women. In 2012, around 1.7 million new cases were diagnosed with breast cancer around the world. Approximately 232,340 new cases of invasive breast cancer and 39,620 breast cancer deaths are expected to occur among US women in 2013 (DeSantis et al. 2014). Approximately 7,900 of these deaths were related to HER2-positive breast cancer. Human epidermal growth factor receptor 2 (HER2) is a growth factor receptor gene that is amplified in approximately 20% of breast cancers (Pegram, Konecny, and Slamon 2000)(Pegram et al. 2000). Studies have shown that women whose tumors exhibit either amplification of the HER2 gene or overexpression of its encoded protein have a more aggressive form of cancer that is associated with significantly shortened disease-free and overall survival compared with women whose tumors do not over express HER2. The incorporation of trastuzumab has significantly altered the natural history of HER2-positive breast tumors, converting them from an aggressive tumor subtype to one with improved prognostic outcomes (Dawood et al. 2010; Verma et al. 2013).

For patients with HER2-positive mBC, the combination of trastuzumab and a taxane is a globally accepted first-line treatment, based on the survival advantage demonstrated in two large pivotal trials(Marty et al. 2005; D. Slamon et al. 2011). More recently, the final overall survival analysis of the CLEOPATRA study with a dual blockade of the HER2 protein plus docetaxel, made the authorities to approve this combination as the first line treatment (Swain et al. 2013). However, virtually all patients with HER2-positive mBC develop progressive disease (PD) and require additional therapies. Importantly, such tumors continue to express high levels of HER2 (Spector et al. 2005), and neither the process of internalization nor the level of surface expression is altered when HER2 is bound by trastuzumab (Austin et al. 2004). HER2-directed combination therapy beyond progression for HER2-positive mBC is an accepted palliative treatment approach.

1.2. Trastuzumab Emtansine (T-DM1) in HER2-Positive Metastatic Breast Cancer

A novel approach to HER2-targeted breast cancer therapy is trastuzumab emtansine (T-DM1), an antibody-drug conjugate that combines intracellular delivery of the potent cytotoxic agent, DM1 (maytansinoid, a derivative of maytansine that causes apoptosis through inhibition of microtubule assembly, with greater potency than vinka alkaloids and paclitaxel) (Widdison et al. 2006) with the antitumor activity of trastuzumab. T-DM1 uses a non reducible thioether linker (MMC) to combine the antibody and the cytotoxic (Junttila et al. 2011). The stability of MCC was shown to strongly contribute the favorable activity and toxicty profiles of T-DM1, delivering selectively DM1 to HER2- positive cells whereas exposure of normal tissue is minimized. Indeed clinical studies have shown

that DM1 plasma levels are consistently low (generally <10 ng/ml), transient and with no evidence of DM1 accumulation with repeated T-DM1 doses (consistent with a half life of 3.5 days) (I. E. Krop et al. 2010; Girish et al. 2012; Hurvitz et al. 2013). However, T-DM1 provides more than just targeted delivery of a cytotoxic, since once bound T-DM1 retains the hypothesized mechanisms of action of trastuzumab including flagging HER2-positive tumor cells for destruction by antibody- dependent cellular toxicity and inhibiting HER2 signalling (Phillips et al. 2014).

T-DM1 has significant antitumor potency in vitro and in vivo, which is maintained in tumors resistant to trastuzumab or lapatinib. In Phase I and II trials, T-DM1 provided objective tumor responses and was well tolerated across various lines of therapy in patients with HER2-positive metastatic breast cancer (I. E. Krop et al. 2010): the study TDM3569g was a Phase I, dose-escalation study that evaluated the safety and efficacy of trastuzumab emtansine as a single agent in 52 patients with HER2-positive mBC, whose disease progressed on a trastuzumab containing chemotherapy regimen. 24 patients received trastuzumab emtansine q3w and 28 patients received trastuzumab emtansine on a weekly (qw) schedule. On the q3w dosing schedule, dose-limiting toxicities of Grade 4 thrombocytopenia were seen in 2 of 3 patients treated at 4.8 mg/kg. Therefore, 3.6 mg/kg was determined to be the maximum tolerated dose (MTD) of trastuzumab emtansine given q3w, and the cohort was expanded to 15 patients. On the basis of these data, the recommended dose schedule for the Phase II studies was 3.6 mg/kg q3w. On the qw schedule, 2.4 mg/kg was identified as the MTD. Treatment with trastuzumab emtansine was well tolerated, and toxicity was generally mild, reversible, and non-cumulative. No drug-related cardiac toxicity was noted. Trastuzumab emtansine administration demonstrated considerable activity in this Phasel study. The confirmed overall response rate (ORR) in patients with measurable disease at the 3.6 mg/kg q3w schedule was 44% (4 of 9 patients), as assessed by investigators. The median PFS among the 15 patients receiving 3.6 mg/kg q3w was 10.4 months.

Clinical efficacy and safety of T-DM1 has been established in several phase II and III trials. T-DM1 is active in metastatic breast cancer patients with ≥ 1 previous HER2-directed therapy in 2 single-arm phase II trials (Study TDM4258g and Study TDM4374g) with an ORRs: 25.9% and 34.5% respectively (Burris et al. 2011; I. E. Krop et al. 2012), and prolonged PFS compared with trastuzumab plus docetaxel among patients without prior HER2-targeted therapy in a randomized phase II trial (Study TDM4450g) (median PFS: 14.2 vs 9.2 months; HR: 0.59; P = 0.035) (Hurvitz et al. 2013).

In the Study TDM4258g trastuzumab emtansine was administered at a dose of 3.6 mg/kg (IV) q3w in patients with HER2-positive mBC who had progressed on previous HER2-directed therapy and conventional chemotherapy. The final analysis of ORR was 37.5% (95% confidence interval [CI]:

28.6%–46.6%) by investigator assessment and 25.9% (95% CI: 18.4%–34.4%) by Independent Review Board (IRB). The clinical benefit rate (CBR) was 46.3% by investigator assessment and 39.3% by independent review. The median PFS was 4.6 months as assessed by both the investigators and the IRB. In the subset of patients whose archival primary tumors were retrospectively confirmed to be HER2-positive (74 of 95 patients with submitted tumor samples), the ORR was 33.8% by independent review and 47.3% based on investigator assessment. The most common adverse events (AEs) (occurring in≥ 20% of patients) were fatigue (65.2%), nausea (50.9%), headache (40.2%), epistaxis (35.7%), pyrexia (34.8%), constipation (30.4%), cough (27.7%), hypokalemia (26.8%), diarrhea (25.9%), vomiting (24.1%), arthralgia (22.3%), pain in extremity (22.3%), anemia (20.5%), and dyspnea (20.5%). Most of these AEs were Grade 1–2. The three most common Grade 3–4 AEs observed in this trial were hypokalemia (8.9%), thrombocytopenia (8.0%), and fatigue (4.5%). There was one reported Grade 5 event in a patient who died of respiratory failure attributed by the investigator to underlying disease. No Grade≥ 3 left ventricular systolic dysfunction events (symptomatic congestive heart failure [CHF] and/or left ventricular ejection fraction [LVEF] of < 40%) were observed (Burris et al. 2011).

The other single-arm study of trastuzumab emtansine has been the study TDM4374g where the drug was administered at 3.6 mg/kg by IV infusion q3w to patients with HER2-positive mBC. Patients must have received an anthracycline, trastuzumab, a taxane, lapatinib, and capecitabine given in the neoadjuvant, adjuvant, or metastatic setting or as treatment for locally advanced disease. Patients must have been treated with two HER2-directed therapies in the metastatic or locally advanced setting and have progressed on their most recent treatment. A total of 110 patients were enrolled and treated in the study. An efficacy analysis (data cut-off date: 21 June 2010) with a median follow-up of 17.4 months demonstrated an ORR (complete and PR) of 34.5% (95% CI: 26.1%–43.9%; 38 of 110 patients) by independent review and 32.7% (95% CI: 24.1%–42.1%; 36 patients) by investigator assessment. The CBR was 48.2% by independent review and 46.4% by investigator assessment. The median duration of response (DoR) and PFS by independent review was 7.2 months and 6.9 months, respectively. In the subset of patients whose archival primary tumors were retrospectively confirmed to be HER2-positive (80 of 95 patients with submitted tumor samples), the ORR was 41.3% by independent review and 40.0% based on investigator assessment. The most common AEs (occurring in≥ 20% of patients) were fatigue (61.8%), thrombocytopenia (38.2%), nausea (37.3%), increased aspartate transaminase (AST, 26.4%), constipation (23.6%), pyrexia (22.7%), epistaxis (22.7%), headache (21.8%), hypokalemia (20.9%), decreased appetite (20.9%), dry mouth (20.0%), and anemia (20.0%). Most of these AEs were Grade 1–2. Fifty-two patients (47.3%) experienced at least one Grade≥ 3 AE, the most common being thrombocytopenia (9.1%) and fatigue (4.5%). Serious AEs (SAEs) were recorded by 28 patients (25.5%), the most common being cellulitis (3.6%), pyrexia (2.7%), and pneumonia

(2.7%). One patient recorded a Grade 5 AE of hepatic dysfunction, which was recorded as possibly related to trastuzumab emtansine. The patient had pre-existing non-alcoholic fatty liver disease as well as multiple other comorbidities, including renal insufficiency (I. E. Krop et al. 2012).

A randomized multicenter phase II trial (Study TDM4450g) of trastuzumab emtansine versus trastuzumab plus docetaxel in patients with metastatic HER2-positive BC who had not received prior chemotherapy for metastatic disease was conducted. Seventy patients were randomized to the control arm and 67 patients to the trastuzumab emtansine arm. The median duration of follow-up was 13.5 months for the control arm and 13.8 months for the trastuzumab emtansine arm. As of 15 November 2010, the PFS was 14.2 months in the trastuzumab emtansine arm versus 9.2 months in the trastuzumab plus docetaxel arm. The HR for PFS was 0.594 (95% CI: 0.364–0.968; p= 0.0353). The ORR in the trastuzumab emtansine arm was 64.2% (95% CI: 51.8%–74.8%) compared with 58.0% (95% CI: 45.5%–69.2%) in the control arm (based on 69 evaluable patients). The CBR was 74.6% (95% CI: 63.2%–84.2%) in the trastuzumab emtansine arm versus 81.2% (95% CI: 70.7%–89.1%) in the trastuzumab plus docetaxel arm (based on 69 evaluable patients). Based on safety data analyzed at the data cut-off date, single-agent trastuzumab emtansine appears to have a favorable overall safety profile compared with trastuzumab and docetaxel in first-line mBC. The incidence of Grade≥ 3 AEs in the control arm (89.4%; n= 66) was nearly twice that of trastuzumab emtansine (46.4%; n = 69). The rates of SAEs for both arms were similar (control arm 25.8% versus trastuzumab emtansine 18.8%). One patient in the trastuzumab emtansine group died as a result of an AE (sudden death). This patient was randomized to receive trastuzumab plus docetaxel but mistakenly received a single dose of 6 mg/kg trastuzumab emtansine instead of 6 mg/kg trastuzumab. One patient in the trastuzumab plus docetaxel group died due to cardiopulmonary failure. With respect to cardiotoxicity, based on local assessments of LVEF, trastuzumab emtansine was not associated with an increase in cardiotoxicity compared with trastuzumab plus docetaxel (Hurvitz et al. 2013).

The first phase III study to evaluate T-DM1 in HER2-positive metastatic breast cancer (mBC) was EMILIA study (Verma et al. 2012). This trial compared T-DM1 with capecitabine plus lapatinib in HER2-positive breast cancer treated in first-, second-, or third-line, but previously treated with taxanes and trastuzumab. PFS and OS are investigated as co-primary endpoints in this study with PFS based on modified RECIST (Therasse et al. 2000) and conducted by the IRB. An additional tumor assessment of PFS is performed after investigator documented disease progressionT-DM1. Significantly prolonged PFS (median PFS: 9.6 vs 6.4 months; HR: 0.65 (0.55-0.77), p < 0.0001), and overall survival (median PFS: 30.9 vs 25.1 months; HR: 0.68 (0.55-0.85), p < 0.001). Based on safety data analyzed at the data cut-off date, single-agent trastuzumab emtansine appears to have a favorable overall safety profile compared with lapatinib and capecitabine in mBC. The incidence of Grade≥ 3 AEs in the control arm (56.9%; n= 488) was 278; that of trastuzumab emtansine

(40.8%; n= 490) was 200. The rates of SAEs for both arms were similar (control arm 18% versus trastuzumab emtansine 15.5%).It was concluded that overall, there is no concern regarding the clinical safety of trastuzumab emtansine for the patient population that was studied, based on the currently available data (Verma et al. 2012).

T-DM1 has a favorable safety profile as a single agent. There have been no special cardiac safety concerns in T-DM1 trials to date. Side effects that would be expected with DM1 such as substantial incidence of grade 3-4 peripheral neuropathy, have not been observed in clinical trials with T-DM1 as single agent, which confirms that there is a low systemic exposure to DM1 (Krop and Winer 2014).

1.3. Pharmacokinetics of T-DM1

The PK of trastuzumab emtansine and its analytes (total trastuzumab and DM1) were characterized in one Phase I study (TDM3569g) and three Phase II studies (TDM4258g, TDM4374g, and TDM4688g).

For study TDM3569g, the final PK parameter estimates based on noncompartmental PK analysis for q3w and qw regimens of trastuzumab emtansine administration are presented in the table below (Cycle 1 mean (s.d) Pharmacokinetic parameters for T-DM1 following T-DM1 administration every 3 weeks and weekly in study TDM3569g).

Dose (mg/kg)	No. of Patients	C_{max} (µg/mL)	AUC_{inf} (day µg/mL)	$T_{1/2}$ (day)	V_d (mL/kg)	CL (mL/day/kg)
Every 3 Week Dosing						
0.3	3	9.6 (1.7)	14.5 (3.4)	1.3 (0.2)	35.7 (7.5)	21.1 (4.5)
0.6	1	13.3	24.5	1.3	43.8	24.5
1.2	1	20.3	42.9	1.3	51.8	27.8
2.4	1	76.3	330.0	2.2	30.7	7.2
3.6	15	76.2 (19.1)	300.3 (65.8)	3.1 (0.7)	58.4 (12.4)	12.7 (3.6)
4.8	3	130.3 (7.8)	673.0 (12.2)	4.1 (0.7)	41.2 (6.2)	7.1 (0.1)
Weekly Dosing						
1.2	3	29.6 (5.7)	76.2 (10.4)	2.3 (0.6)	47.5 (6.0)	15.9 (2.4)
1.6	3	34.3 (4.8)	130.3 (39.7)	3.4 (0.8)	59.8 (16.6)	13.0 (3.4)
2.0	3	48.0 (9.6)	175.0 (41.0)	3.1 (0.3)	51.0 (8.1)	11.8 (2.4)
2.4	16	54.8 (12.6)	198.5 (54.5)	3.3 (1.1)	55.4 (13.0)	13.1 (4.1)
2.9	3	78.1 (33.9)	212.0 (39.0)	2.9 (0.5)	57.7 (2.2)	14.0 (2.6)

AUC_{inf} = area under the serum concentration-time curve from time 0 extrapolated to infinity;
C_{max} = maximum serum concentration; CL = clearance; s.d. = standard deviation;
$T_{1/2}$ = terminal half-life; V_d = volume of distribution.

Dose intensity, defined as percentage of the planned trastuzumab emtansine dose that was actually received, was higher with the 3.6 mg/kg q3w regimen (median 99.7%, range 88%–106%) than with the 2.4 mg/kg qw schedule (median 82%, range 54%–101%). However, since the PK of trastuzumab emtansine is linear at doses≥ 2.4 mg/kg, an almost 2-fold higher cumulative dose can be administered within a q3w cycle with a 2.4 mg/kg qw regimen compared with 3.6 mg/kg q3w.
Based on a population PK analysis, trastuzumab emtansine has a consistent PK profile with low inter-individual variability (21%–48%) in PK parameters among patients with mBC. Greater

baseline tumor burden and lower serum albumin levels, potential indicators of disease severity, resulted in small increases (< 13%) in trastuzumab emtansine clearance (CL). However, trastuzumab emtansine PK was not affected by baseline residual trastuzumab (from prior treatment) or by differences in serum concentrations of HER2 extracellular domain (Gupta et al. 2012).

An aggregate PK assessment of trastuzumab emtansine was performed with samples from studies TDM3569g, TDM4258g, TDM4374g, and TDM4688g (Girish et al. 2012). PK parameters for trastuzumab emtansine, total trastuzumab, and DM1 were consistent across the four studies at Cycle 1 and steady state. Trastuzumab emtansine PK was not affected by residual trastuzumab from prior therapy or circulating extracellular domain of HER2. No significant correlations were observed between trastuzumab emtansine exposure and efficacy, thrombocytopenia, or increased concentrations of transaminases. Across the four studies, the incidence of anti-therapeutic antibodies to trastuzumab emtansine was low and detected in 4.5% (13/286) of evaluable patients receiving trastuzumab emtansine q3w.

The PK profile (i.e., maximum concentration [Cmax], area under the serum concentration-time curve [AUC], terminal half-life [T½], apparent volume of distribution at steady state[Vss], and CL) of single-agent trastuzumab emtansine (3.6 mg/kg q3w) is predictable, well characterized, and unaffected by circulating levels of HER2 extracellular domain or residual trastuzumab. Trastuzumab emtansine exposure does not correlate with clinical responses or key AEs. Weekly administration of trastuzumab emtansine in study TDM3568g at a dose of 2.4 mg/kg showed consistent PK data with the q3w dosing schedule.

1.4. Mechanisms of cardiotoxicity of study drugs

Cardiac disfunctions are among the most common side effects of anthracyclines. Endomyocardial biopsy and troponin I measurements suggest that myocyte injury may occur early after exposure to the drug, however clinical manifestation becomes apparent later due to cardiac reserves and activation of compensatory mechansims. Clinically, early cardiac events are reversible and include arhytmia, repolarization changes, pericarditis and less frequent myocarditis.Late anthracyclcine cardiotoxicity includes cardiomyopathy and systolic heart failure. Patients treated with doxorubicin are five times more likely to develop chronic heart failure or reduction in LVEF compared to those treated with non-antracyclin regimens. Moreover, cardiotoxicity induced by doxorubicin is dose-dependent, being 300mg/m*2 the tolerated cumulative dose, although there is substantial heterogeneity among patients. The mechanism of doxorubicin-induced cardiotoxicity is not fully understood. The drug enters myocits where it causes mitochondrial dysfunction with consecutive

changes in calcium and contractile function. Further increase in drug concentration causes myocyte cell death (Suter and Ewer 2013). A strategy to avoid the increased cardiac risk of doxorubicin is pharmacokinetic modification by non-pegylated liposomal encapsulation, as discussed in section 1.5.

On the other hand, trastuzumab cardiotoxicty has been widely studied, specially when administered in combination of anthracyclines. Based on the observations in phase III trials of trastuzumab plus anthracyclines (discussed in section 1.6), a correlation between time of administration of anthracyclines and trastuzumab was observed, suggesting a high risk of cardiotoxicity in the concomitant administration. From a mechanistic point of view, trastuzumab may act as a modulator of anthracyclin toxicity when administered during a period of myocyte vulnerability following anthracyclin exposure. Based on these observations, the following risk factors for trastuzumab-associated cardiotoxicity have been identified: prior treatment with anthracyclines, borderline limit LVEF, preexisting arterial hypertension and advanced age.

1.5. Non-pegylated liposomal doxorubicin

Non-pegylated liposomal doxorubicin is a nanotechnology product intended to passively accumulate into solid malignancies through gaps in the tumor microvasculature while circumventing cardiac uptake. In the clinical setting, two randomized clinical trials comparing non-pegylated liposomal doxorubicin versus conventional doxorubicin either alone or in combination with cyclophosphamide in first line mBC have been shown a statistically significant reduction in cardiac toxicity while preserving similar antitumor efficacy (Mayer et al. 1990; Harris et al. 2002; Batist et al. 2001). On the other hand, combination therapy with non-pegylated liposomal doxorubicin and trastuzumab has been shown to be an active regimen in the clinical setting with no increase in cardiac toxicity, as discussed in section 1.6.

1.6. Rationale to Study the Combination of T-DM1 and non-pegylated liposomal doxorubicin

Albeit T-DM1 is a major conceptual and clinical advance in the treatment of HER2-positive metastatic breast cancer, there are two observations that emphasize the need to explore combination strategies: first, the response rate of T-DM1 when given alone in metastatic disease is less than 50%, and second, despite the median duration of the response is prolonged, progression inevitably occurs. Some of the combinations tested to date include T-DM1 with taxanes +/- pertuzumab, T-DM1 with capecitabine or T-DM1 with pertuzumab, that have the potential to result in increased activity with an acceptable tolerance to treatment related toxicity.

In this context, it is appealing to study T-DM1 and doxorubicin for a number of reasons:

Doxorubicin is one of the most active chemotherapeutic agents against breast cancer;

In preclinical models the combined antitumor activity of trastuzumab plus doxorubicin arm was superior than trastuzumab and paclitaxel arm (Baselga et al. 1998).

In the pivotal phase III trial of trastuzumab in first-line HER2-positive metastatic breast cancer, the highest antitumor effect was observed in the anthracycline arm compared to the taxane arm (D. Slamon et al. 2011; Rayson et al. 2012; Buzdar et al. 2013). In that initial phase III randomized registration trial, trastuzumab was combined with an anthracycline (doxorubicin or epirubicin) and cyclophosphamide (AC) in patients who had not previously received anthracycline therapy, or with paclitaxel (P) in patients who had received adjuvant anthracycline (D. J. Slamon et al. 2001; Dawood et al. 2010; Verma et al. 2013). Addition of trastuzumab significantly prolonged time to disease progression and overall survival (OS) as compared to chemotherapy alone.

Cardiotoxicity, however, was significant in the pivotal trial, with 16% New York Heart Association (NYHA) Class III-IV heart failure on the trastuzumab/AC arm compared to 3% with AC alone (D. J. Slamon et al. 2001). The high cardiotoxicity of the combination of trastuzumab and doxorubicin observed in the first pivotal trial has been significantly reduced in subsequent trials with cardiac monitoring measures that took advantage of the observations, unexpected at that time, of the increased cardiotoxic potential of this combination. Subsequent trials, using careful cardiac monitoring, defined safer ways to combine trastuzumab and anthracyclines, but this combination has no clear role outside of the context of clinical trials (Romond et al. 2012; Buzdar et al. 2013; Baselga et al. 2014). In this clinical trial and in order to minimize the potential cardiotoxicity of the combination of TDM-1 and doxorubicin, the non-pegylated liposomal form of the drug will be administered which decreases considerably the risk of cardiotoxicity. The combination of non-pegylated liposomal doxorubicin, trastuzumab, and paclitaxel (MTP) was investigated in a phase I–II trial, as first-line treatment of patients with HER2-overexpressing locally advanced BC or MBC and no prior exposure to anthracyclines, taxanes, or trastuzumab (Cortes et al. 2009). No patient developed treatment related symptomatic congestive heart failure (CHF). Asymptomatic protocol-defined cardiac dysfunction was found in 11 (17%) of 54 patients at the recommended dose. Left ventricular ejection fraction (LVEF) recovered to ⩾50% in eight patients and to >45% in the remaining three patients. Among 26 patients with MBC, 25 responded, median time to progression was 22.1 months and median OS was 40.4 months. On the basis of the above results, a prospective, randomized phase III study (STM01-102) was designed in patients with HER2- overexpressing MBC and no prior chemotherapy for metastatic disease. The phase III trial included
181 to receive MTP, and 183 to TP, with a median PFS of 16.1 and 14.5 months with MTP and TP, respectively [hazard ratio (HR) 0.84; two-sided P = 0.174]. Although the frequency of adverse

events was higher with MTP, there was no significant difference in cardiac toxicity between treatment arms (Baselga et al. 2014).

On the other side, only one study has assessed the effect of trastuzumab emtansine (3.6 mg/kg q3w) on the QT interval in patients with HER2-positive recurrent locally advanced BC or mBC was evaluated in study TDM4688g, and had no meaningful effect on the corrected QT interval in these patients (Gupta et al. 2013). At Cycle 1 Day 1, 15 minutes post-infusion, the baseline-adjusted mean heart rate-corrected QT interval using the Fridericia formula (QTcF) increased by 1.2 ms. By 60 minutes post-infusion, the baseline-adjusted mean QTcF interval decreased by 1.0 ms, and by Day 8 of Cycle 1, the baseline-adjusted mean QTcF interval decreased by 4.0 ms. By Cycle 3 Day 1, prior to trastuzumab emtansine infusion, the mean QTcF interval had reverted back to baseline. Following the third infusion of trastuzumab emtansine, the baseline-adjusted mean QTcF interval at both the 15 minute and the 60 minute post-infusion time points was increased by 4.7 ms. No patient exhibited a mean change in QTcF interval from baseline exceeding 30 ms at any of the protocol-specified time points.

The relationship between trastuzumab emtansine pharmacokinetic (PK) and electrocardiogram (ECG) data was also assessed. While there appears to be a trend between trastuzumab emtansine drug concentration and its effect on QT interval, at the observed concentration ranges of trastuzumab emtansine, DM1, and total trastuzumab, there is reasonable assurance that the true increase in mean baseline-adjusted average QTcF does not exceed 5 ms.

Moreover, because trastuzumab emtansine, total trastuzumab, and DM1 achieve steady state levels by Cycle 3, the likelihood of progressively longer QTcF with repeated trastuzumab emtansine dosing is low.
In a pharmacokinetic study in patients treated with doxorubicin and trastuzumab, the exposure to the doxorubicin metabolites doxorubicinol and 7-deoxy-13-dihydro-doxorubicinone was increased in the presence of trastuzumab (Bianchi et al. 2003) but the clinical significance of this increase was felt to be minor, if any. However, pharmacokinetic data on the combination of doxorubicin (Gianni et al. 1997) and T-DM1 are not available. Another issue of interest is to study genetic factors that may predispose to cardiac toxicity. Importantly, polymorphism of HER2 gene coding for the transmembrane domain of HER2 [Ile655Val] may predict risk of trastuzumab cardiotoxicity (Roca et al. 2013).

Since T-DM1 is highly active as single agent due to its dual mechanism of action and being its overall safety profile acceptable, with an expected low cardiotoxicity similar to lapatinib, it is

expected that T-DM1 and non-pegylated liposomal doxorubicin may be safely combined together and offer the potential of increased antitumor effect compared to each agent given alone.

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2. OBJECTIVES

2.1. Primary Objective

- To determine the maximum tolerated dose (MTD) of the combination of T-DM1 and non-pegylated liposomal doxorubicin in HER2-positive metastatic breast cancer (mBC) patients previously treated with taxane and trastuzumab-based therapy.

PrimaryEndpoint: Maximum-tolerated dose (MTD) is defined as the highest dose level at which no more than one of six patients or 0 of three patients experiences dose-limiting toxicity (DLT) during the first two cycles of study treatment.

2.2. Secondary Objectives

- To explore efficacy of the combination of T-DM1 and non-pegylated liposomal doxorubicin, defined by the overall objective response rate (ORR), clinical benefit rate (CBR), number of progressions and number and reasons for deaths.

SecondaryEndpoints:

Overall response rate (ORR). Overall response rate (ORR) is defined as the proportion of patients with best overall response of confirmed complete response (CR) or partial response (PR) based on local investigator's assessment according to RECIST criteria guidelines (version 1.1)(Eisenhauer et al. 2009). An objective response needs to be confirmed at least 4 weeks after the initial response.

Clinical benefit rate (CBR) Clinical benefit rate is defined as the proportion of patients with a best overall response of complete response (CR) or partial response (PR) or stable disease (SD) lasting more than 24 weeks based on local investigator's assessment.

Number of patients with progressions and number of patients who died.

- To assess the safety profile of the combination of T-DM1 and non-pegylated liposomal doxorubicin, defined by all toxicities reported during the study. The NCI CTCAE version 4.0 and the New York Heart Association (NYHA) criteria (for cardiotoxicity) will be used to evaluate the clinical safety of the treatment in this study. Patients will be assessed for adverse events at each clinical visit and as necessary throughout the study.

- To evaluate the cardiac safety of the combination of T-DM1 and non-pegylated liposomal doxorubicin measured by LVEF as assessed by echocardiography, cardiac troponin I and B-type natriuretic peptide (BNP) levels (if feasible). The number of patients who have

defined LVEF decline >10 percentual points or LVEF <50%, develop left ventricular dysfunction IV NYHA, discontinue any of the study drugs due to cardiac function or die due to cardiac cause will be summarized. Cardiac troponin I elevation will be assessed according to CTCAE v4.0 criteria and the segmental wall-motion abnormalities (not described in CTCAE v4.0) will be also recorded.

- To explore the potential role of single nucleotide polymorphisms (SNP) in the predisposition for developing cardiotoxicity. Polymorphism of HER2 gene coding for the transmembrane domain of HER2 [Ile655Val] will be tested at baseline and correlated with LVEF changes and overall cardiac toxicity.
- To analyze the pharmacokinetics (PK) profile of T-DM1, non-pegylated liposomal doxorubicin and each ones metabolites. The following PK parameters will be calculated: AUC, clearance (CL), distribution volume (dV), apparent half life (t1/2) and maximal serum concentration (Cmax). Pharmacokinetic data will be compared with historical data of T-DM1 alone and non-pegylated liposomal doxorubicin alone.

3. STUDY OVERVIEW

3.1. Study Design

This is a prospective dose finding, multicenter and open-label phase I clinical trial. There are three planned cohorts. T-DM1 will be administered at a fixed dose of 3.6 mg/kg IV on Day 1 every 3 weeks and three cohorts of patients with three different dose levels of non-pegylated liposomal doxorubicin (45 mg/m^2, 50 mg/m^2 and 60 mg/m^2) IV on Day 1 in cycles of 21 days each are planned.

Study Cohort	T-DM1	Non-pegylated liposomal doxorubicin
Cohort 1 (level 1)	3.6 mg/kg IV D1	45 mg/m^2 IV D1
Cohort 2 (level 2)	3.6 mg/kg IV D1	50 mg/m^2 IV D1
Cohort 3 (level 3)	3.6 mg/kg IV D1	60 mg/m^2 IV D1
Note: *level -1 will be considered with T-DM1 (3.6 mg/kg IV) and weekly non-pegylated liposomal doxorubicin (15 mg/m^2 IV) if more than one of six patients experiences dose-limiting toxicity (DLT) in the level 1*		

3.2. T-DM1 drug dose schedule

Clinical activity has been observed at a dose of 3.6 mg/kg q3w in two Phase II studies of single-agent trastuzumab emtansine in patients with advanced heavily pre-treated HER2-positive mBC

(study TDM4258g and study TDM4374g) and in patients who had not received prior chemotherapy for metastatic disease (TDM4450g).

The initial dose of T-DM1 will be administered over 90 minutes (±10 minutes) and, in the absence of any signs or symptoms of infusion reactions with the first dose, subsequent doses of T-DM1 may be administered over 30 minutes (±10 minutes). T-DM1 will be administered on Day 1 of a 3-week cycle at a dose of 3.6 mg/kg IV. If the timing of study drug administration coincides with a holiday or any other organizational circumstance that does not allow to administer the study drugs on the scheduled date, the treatment should be performed within 3 days of the scheduled date on the earliest possible following date.

The total dose will be calculated based on the patient's weight on Day 1 (or up to 3 days before) of each cycle. Infusions may be slowed or interrupted for patients experiencing infusion-associated symptoms. Any stop and/or change in the infusions rate must be recorded providing data about the time the infusion was stopped, restarted, volumes already administered and/or pending to be administered and new infusion rate.

Vital signs must be assessed before and at any time within 60 min after the end of T-DM1 administration. Following the initial dose, patients will be observed for at least 60 minutes for fever, chills, or any other infusion-associated symptoms. If prior infusions were well tolerated (without any signs or symptoms of infusion reactions), subsequent doses of T-DM1 may be administered over 30 minutes (±10 minutes), with a minimum 60 minutes observation period following the end of the infusion. Local health authority guidelines must be followed with regard to further observation and monitoring, if applicable.

No premedication is necessary prior to administration of T-DM1. Pre-medication for nausea and infusion reactions (e.g. acetaminophen or other analgesics, antihistamines such as diphenhydramine, or corticosteroids) may be given at the investigator's discretion (see Sections 3.7, 3.9 and 9.1).

3.3. Non-pegylated liposomal doxorubicin drug dose schedule

Non-pegylated liposomal doxorubicin doses have been selected as being in the range of efficacious doses for single-agent or combination use.
Non-pegylated liposomal doxorubicin will be administered approximately over 60 minutes starting 60 minutes after the end of T-DM1 infusion. Standard antiemetics according to each center's policy will be given for non-pegylated liposomal doxorubicin.

3.4. Dose escalation schema: description of cohorts

This clinical trial is designed with a dose escalation following a “modified” 3 by 3 design where 3 patients will be included at a given cohort and followed to observe if they experience any dose-limiting toxicity (DLT) during the first two treatment cycles. If none of the 3 first patients included in each cohort experience a DLT (0/3), the next patients will be enrolled in the subsequent cohort. If 1 of these 3 first patients experience a DLT, 3 more patients will be included at the same cohort to determine the number patients who experience DLTs in the total group of 6 patients. If 2 or more of the 6 patients at a given cohort experience a DLT, the inferior cohort will be evaluated and established as the maximum tolerated dose (MTD) if correct (note: if 2 or more of the 3 first patients included at cohort 1 experience a DLT, level -1 will be explored). Once the MTD is established, 6 additional patients will be enrolled at the recommended phase 2 dose (RP2D) defined per the MTD as the highest dose level at which no more than one of six patients or 0 of three patients experiences dose-limiting toxicity (DLT) during the first two cycles of study treatment. The Steering Committee will review toxicities and may decide to add the expansion cohort to level -
1 in the situation where it is necessary to have a dose level -1.

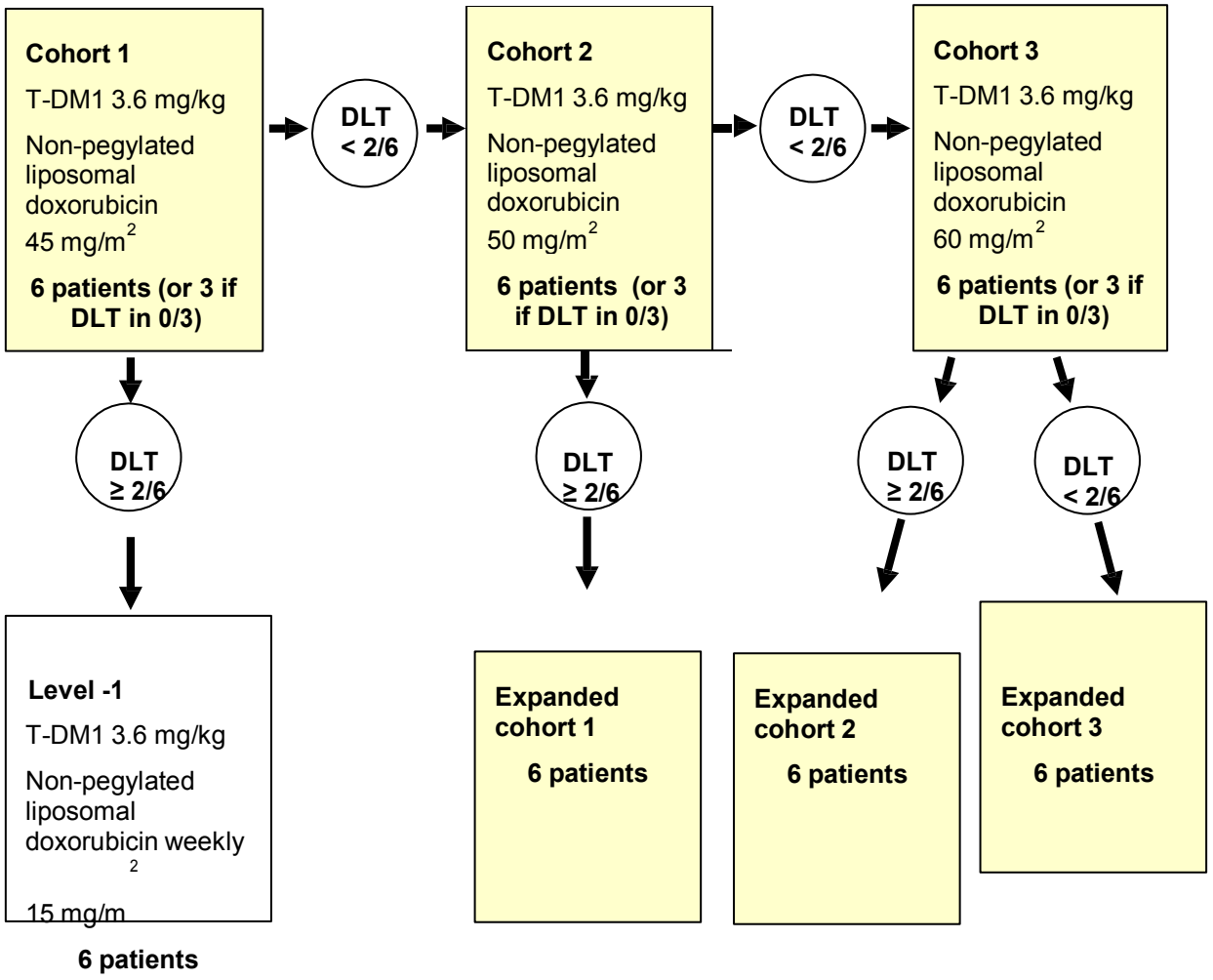
No dose escalation between cohorts will be permitted. Patients assigned on each cohort will remain at their study cohort during all the study period.

Dose escalation of non-pegylated liposomal doxorubicin to next level will not occur until all patients in a cohort have completed cycle 2 and the Steering Committee and study site Investigator(s) have been able to review all toxicities and agreement is reached on the next dose escalation step.

After the two first cycles, the study drug combination (T-DM1 and non-pegylated liposomal doxorubicin) will be administered for up to 6 cycles (including the first 2 cycles). After that, T-DM1 treatment may continue as a single agent until disease progression or development of intolerable toxicity, whichever occurs first.

A simplified overview (not explicity showing the “modified” 3 by 3 design) of the dose escalation scheme is shown below.

Figure 1. Design of study cohorts



The detailed rules for dose escalation are detailed below:

Cohort 1: Patients will be treated with a fixed dose of T-DM1 of 3.6 mg/kg IV every 3 weeks. Non-pegylated liposomal doxorubicin will be administered at a dose of 45 mg/m^2. If none of the 3 first patients included experience a DLT (0/3), the next patients will be enrolled in the subsequent cohort. If 1 of the 3 first included patients experiences a DLT, 3 more patients will be included for a maximum of 6. If less than 2 patients in the total group of 6 patients experience any DLT (0/3 or 1/6), then the following patients will be included in Cohort 2. Otherwise, if 2 or more patients suffer any DLT (≥ 2/6), then cohort 1 will be declared too toxic and level -1 will be opened.

Cohort 2: Patients will be treated with a fixed dose of T-DM1 of 3.6 mg/kg IV every 3 weeks. Non-pegylated liposomal doxorubicin will be administered at a dose of 50 mg/m^2. If none of the 3 first patients included experience a DLT (0/3), the next patients will be enrolled in the subsequent cohort. If 1 of the 3 first included patients experiences a DLT, 3 more patients will be included for a maximum of 6. If less than 2 patients in the total group of 6 patients experience any DLT (0/3 or 1/6), then the next patients will be included in Cohort 3. Otherwise, if 2 or more patients suffer any

DLT (≥ 2/6), then cohort 2 will be declared too toxic, the schedule of cohort 1 will be declared the

recommended dose for phase II trials (RP2D) and an expansion cohort of 6 more patients will be added to cohort 1.

Cohort 3: Patients will be treated with a fixed dose of T-DM1 of 3.6 mg/kg IV every 3 weeks. Non-pegylated liposomal doxorubicin will be administered at a dose of 60 mg/m^2. If none of the 3 first patients included experience a DLT (0/3), then 6 more patients will be included in an expansion cohort. If 1 of the 3 first included patients experiences a DLT, 3 more patients will be included for a maximum of 6. If less than 2 patients in the total group of 6 patients experience any DLT (0/3 or 1/6), then 6 more patients will be included in an expansion cohort. Otherwise, if 2 or more patients suffer any DLT (≥ 2/6), then cohort 3 will be declared too toxic, and the schedule of cohort 2 will be declared the recommended dose for phase II trials (RP2D) and an expansion cohort of 6 more patients will be added to cohort 2. If the MTD is not established by the envisaged dose escalation for the combination regimen in cohort 3, further escalations may be considered, based on the safety profile; alternatively, the established dose in cohort 3 may be declared as the recommended phase 2 dose (RP2D).

Level -1: Three patients will be treated with a fixed dose of T-DM1 of 3.6 mg/kg IV every 3 weeks. Non-pegylated liposomal doxorubicin will be administered at a weekly dose of 15 mg/m^2. If 0 or 1 of the 3 first included patients experiences a DLT, 3 more patients will be included for a maximum of 6. If less than 2 patients in the total group of 6 patients experience any DLT (0/6 or 1/6), then this schedule will be declared the recommended dose for phase II trials (RP2D). The Steering Committee will review toxicities and may decide to add the expansion cohort to level -1 in the situation where it is necessary to have a dose level -1. Otherwise, if 2 or more of 6 patients show a DLT (≥ 2/6), then level -1 will be declared too toxic and the study will finish.

No dose escalation between cohorts will be permitted. Patients assigned on each cohort will remain at their study cohort during all the study period. The recommended phase II dose (RP2D) will not exceed the highest dose level of the combination of T-DM1 and non-pegylated liposomal doxorubicin at which $\leq$ 1 of 6 patients experienced a DLT. In case MTD is exceeded during escalation, de-escalation may be not considered.

3.5. Toxicity Criteria

All patients who receive any study treatment are evaluable for toxicity. Toxicities will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4 (NCI CTCAE v4.0) and the NYHA criteria for cardiotoxicity.

Cardiac segmental wall-motion abnormalities (not explicity described in CTCAE v4.0) will be graded under the category of "investigations – other, specify" with a grading according to:

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Investigations – other, specify	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death

Toxicity of T-DM1 alone and non-pegylated liposomal doxorubicin alone has been well established in previous studies. Unexpected adverse reactions will be considered attributable to T-DM1 and not to non-pegylated liposomal doxorubicin, unless exacerbation of known adverse reactions is observed that could be considered related to the combination. It may only be possible to finally attribute an adverse reaction to either T-DM1 or non-pegylated liposomal doxorubicin or their combination after discontinuation of one of the drugs and continuation of the therapy with the other drug alone. If adverse reactions occur that are considered may be related to the combination of T-DM1 with non-pegylated liposomal doxorubicin or to non-pegylated liposomal doxorubicin alone, non-pegylated liposomal doxorubicin treatment will be discontinued, and T-DM1 treatment may be continued at the discretion of the treating physician who will follow the guidelines for T-DM1 use. Patients still not tolerating T-DM1 after discontinuation of non-pegylated liposomal doxorubicin and developing unacceptable (Grade 3 or 4) adverse reactions to T-DM1 should be treated using the established dose modification scheme for T-DM1 as described in section 6.2 or discontinued from T-DM1 treatment at the discretion of the treating physician who must clearly document the reason for the discontinuation.

Patients are evaluated for safety at the end of cycle 2. After the two first cycles, the study drug combination (T-DM1 and non-pegylated liposomal doxorubicin) will be administered for up to 6 cycles (including the first 2 cycles). T-DM1 treatment may continue as a single agent until disease progression or development of intolerable toxicity, whichever occurs first. Dose of T-DM1 administered as single agent will be the dose level used at the end of study treatment (combination T-DM1+non-pegylated liposomal doxorubicin).

3.6. Definition of Dose-Limiting Toxicities (DLTs)

A Dose-Limiting Toxicity (DLT) is defined as any of the drug-related adverse events described in the Table 1 occurring during the first two cycles of study treatment.
Reminder: Drug-related is an adverse event for which a causal relationship with the study treatment (T-DM1 and non-pegylated liposomal doxorubicin combination) cannot be ruled out.

If the second dose of study treatment has to be delayed for any reason and the delay has not exceeded 42 days from the first dose, the patient is also assessed for DLTs occurrence.

When third dose of study treatment is going to be administered, patient will be no longer evaluated for DLTs in all subsequent cycles.

Any patient who does not complete the DLT assessment (at end of cycle 2 or pre-dose at cycle 3 day 1) will be replaced, except for patients who go end of study due to any DLT that does not allow to start cycle 2.

For this study, the following toxicities will be defined as DLTs:

Table 1 Summary of Dose-Limiting Toxicities (DLTs)

Hematological toxicities: - Grade 4 neutropenia (i.e. absolute neutrophil count (ANC) < 0.5 x 10^9 cells/L for minimal duration of 7 days). - Grades 3 and 4 febrile neutropenia (i.e. ANC < 1.0 x 10^9 cells/L with a single temperature of >38.3°C or a sustained temperature of ≥ 38°C for more than one hour). - Uncomplicated Grade 4 thrombocytopenia (< 25.0 x 10^9 cells/L) which does not recover to ≥ 75.0 x 10^9 cells/L before next planned dose administration. - Thrombocytopenia (any grade) complicated with clinically significant bleeding requiring medical intervention, such as platelet transfusion or cauterization. However, patients with Grade 1 or 2 epistaxis may have cauterization and this should not be considered as a DLT.

Cardiac toxicity:
- Level I cardiotoxicity defined as:
  - Sudden death (defined as within 24 hours; unexplained)
  - Heart failure NYHA criteria class III-IV and LVEF decline defined as an absolute drop ≥10% resulting in a final LVEF <50%

Hepatic toxicity: - Increase in AST (SGOT)/ALT (SGPT) values to >5x ULN - Increase in total bilirubin value to > 3xULN

- Hy's Law, defined by The U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) as the rule of thumb that a drug is at high risk of causing a fatal drug-induced liver injury (DILI) when given to a large population, if it caused cases of liver injury that satisfied certain criteria when given to a smaller population. Hy's Law cases have the following three components:
  - The drug causes hepatocellular injury, generally shown by more frequent 3-fold or greater elevations above the upper limits of normal (ULN) of ALT or AST than the (nonhepatotoxic) control agent or placebo.
  - Among subjects showing such aminotransferase (AT) elevations, often with ATs much greater than 3xULN, some subjects also show elevation of serum total bilirubin (TBL) to >2xULN, without initial findings of cholestasis (serum alkaline phosphatase (ALP) activity >2xULN).
  - No other reason can be found to explain the combination of increased AT and serum TBL, such as viral hepatitis A, B, or C, pre-existing or acute liver disease, or another drug capable of causing the observed injury.

OtherGrade≥3non-hematological toxicities with the exception of:

- Grade ≥ 3 diarrhea that recovers to Grade ≤ 2 after 24 hours of starting recommended anti-diarrheal treatment
- Grade 3 nausea, vomiting or diarrhea without appropriate treatment.
- Grade 3 or 4 nausea or anorexia that resolves to grade 1 prior to the start next cycle.
- Infusion-related reactions (IRR). They are not considered to be DLTs since, based on experience with monoclonal antibodies, IRRs are not dose-related events. Precautions will be taken if IRR grade ≥ 3 occur. If described precautions are not sufficient, other options will be discussed between sponsor and investigator.
- Laboratory values of ≥ Grade 3 which are judged not clinically significant by the investigator.

The following non-hematological toxicities are considered as DLTs:

- Any other treatment-related non-hematological toxicity Grade ≥ 3 preventing the start of the 3rd cycle on Day 42 (6 weeks cycle length)
- Grade 2 non-hematological toxicity requiring interruption of treatment for > 21 days
- Patient not able to receive 100% of the dose level going into Cycle 3, Day 1

NOTE: *If a Grade 2 event requires a dose delay, it will not be considered as a DLT. However, if the toxicity does not resolve to Grade 1 or baseline by Day 42, requiring study treatment discontinuation, will be considered as a DLT. The SC will adjudicate in cases of DLTs that are not covered by the existing DLT criteria.*

Finally, failure to recover from any toxicity adequately treated which results in a dose delay of more than 21 days will be considered a DLT or any toxicity at cycles 1 and/or 2 that compels to reduce next T-DM1 and/or non-pegylated liposomal doxorubicin dose/s or to discontinue the patient's treatment (e.g. Hy's Law, nodular regenerative hyperplasia, interstitial lung disease (ILD) including pneumonitis).

In addition, those cases of elevations of Troponin I and BNP values consisting of an increase >10% from screening values will be considered AESIs and will be reported to Steering Committee for being assessed.

3.7. General Concomitant Medication and Supportive Care Guidelines

Concomitant therapy and pre-medications are defined as non-IMPs. Concomitant therapy includes any prescription medication, over-the-counter preparation, or herbal therapy between the 21 days preceding first treatment and the safety follow up visit. All concomitant therapies will be registered. Afterwards, only information about further anti-cancer therapies received by the patient once he/she goes off study will be collected.

No pre-medication for the first infusion of T-DM1 is required; however, pre-medication is allowed at the investigator's discretion. Additional antiemetics (aprepitant, 5HT3 antagonists) may also be given prior to non-pegylated liposomal doxorubicin at investigator's discretion.

Except for cycles 1 and 2, erythropoieses stimulating agents (ESAs) (such as Procrit, Aranesp, Epogen) and/or colony-stimulating factors (CSFs) (such as Neupogen, Neulasta, Leukine) may be used in accordance with National Comprehensive Cancer Network (NCCN) guidelines. At cycle 3 and beyond, these agents are allowed if clinically indicated in accordance with local prescribing guidelines.

Refer to section 9.1 and 9.2 for potential interactions on the use of concomitant medication with T-DM1 and non-pegylated liposomal doxorubicin.

Once the patient is on study treatment, palliative radiotherapy may be permitted to treat pre-existing painful bone metastases or to treat brain metastases (for patients who have disease control outside of the brain). The schedule of palliative radiotherapy will start 48 h after last dose of non-pegylated liposomal doxorubicin. Please contact the Medical Monitor or Scientific Global Coordinator for approval.

Other medications considered necessary for the patient's safety and wellbeing may be given at the discretion of the investigator. Use of bisphosphonates or denosumab is permitted for the control of bone pain, prevention and/or treatment of bone metastases, and treatment of osteoporosis. If bisphosphonates are required for the treatment of symptomatic malignancy-associated hypercalcemia, tumor assessments should be performed to assess for potential disease progression.

3.8. Women of Childbearing Potential and mandatory use of contraceptive methods

Women of childbearing potential (defined as women with regular menses, women with amenorrhea for less than 12 months, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum pregnancy test within 7 days prior to the first dose of either study medication.

All heterosexually active patients are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 7 months following the last dose of T-DM1 and/or non-pegylated liposomal doxorubicin.

If a patient is suspected to be pregnant, T-DM1 and non-pegylated liposomal doxorubicin should be immediately discontinued. If pregnancy is determined by a positive urine test, the pregnancy must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a patient or a patient's partner becomes pregnant during study treatment or within 7 months after the last dose of T-DM1 and/or non-pegylated liposomal doxorubicin, the Medical Monitor must be notified and the pregnant patient will be withdrawn from the study. The Medical Monitor should also be notified of any pregnancy occurring during the study but known/confirmed after completion of the study. In the event that a patient or a patient's partner is found to be pregnant during study treatment or within 7 months after the last dose of T-DM1 and/or non-pegylated liposomal doxorubicin, the pregnancy will be followed and the status of mother and/or child will be reported to the sponsor after delivery. A pregnancy notification form will be completed.

Fetal harm has been identified as an important potential risk for T-DM1. Pregnant or lactating women have been excluded from all trastuzmab emtansine trials, and the use of effective contraception is required in the study protocols and the prescribing information.

Additional follow up information on any trastuzumab emtansine-exposed pregnancy and infant will be requested at specific time points (i.e., after having received the initial report, at the end of the second trimester, 2 weeks after the expected date of delivery, and at 3, 6, and 12 months of the infant's life).

Follow-up queries may be sent, asking for further information, if required for a comprehensive assessment of the case.

Please note that a serum β-HCG test must be performed during screening, every 3 cycles and at 3 and 7 months following the last dose of T-DM1 and/or non-pegylated liposomal doxorubicin for women of childbearing potential (including pre-menopausal women who have had a tubal ligation) and for women not meeting the definition of postmenopausal.

3.9. Prohibited therapies

Use of the therapies described below is prohibited during the study prior to discontinuation of study treatment (collectively, these will be referred to as non-protocol therapy):

Erythropoieses stimulating agents (ESAs) (such as Procrit, Aranesp, Epogen), colony-stimulating factors (CSFs) (such as Neupogen, Neulasta, Leukine) and/or corticosteroids (except those needed to treat acute hypersensitivity or infusion related reactions or as pre-medication for T-DM1 and Myocet administration) are prohibited during cycles 1 and 2.

Any therapies intended for the treatment of cancer, other than T-DM1 and non-pegylated liposomal doxorubicin, whether they are approved by national health authorities or experimental, including cytotoxic chemotherapy, immunotherapy, hormonal therapy (other than megestrol acetate), and biologic or targeted agents (other than granulocyte colony-stimulating factor and erythropoiesis stimulating agents), are prohibited.

Radiotherapy for unequivocal disease progression is not permitted while on study treatment, with the following exception of new central nervous system (CNS) metastases or isolated progression of previously treated CNS lesions. Patients who have disease control outside of the CNS, defined as confirmed PR or CR of any duration, or SD for ≥ 3 months, but who have developed CNS metastases that are treatable with radiation will be allowed to continue to receive study therapy until they either experience systemic progression of their disease outside of the CNS and/or further progression in the CNS that cannot be treated with additional radiation. Patients must not miss more than one cycle of study treatment for the treatment of their CNS metastases and must have an ECOG performance status of 0, 1, or 2 to continue on study treatment. The Medical Monitor

should be informed before a decision is made to resume study treatment after radiotherapy for CNS metastases.

3.10. Duration of study treatment

The study treatment period is defined as the time between the study entry and the last dose of study combination (T-DM1 + non-pegylated liposomal doxorubicin) therapy. T-DM1 administration may continue as a single agent until disease progression or development of intolerable toxicity, whichever occurs first.

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue with the protocol therapy, the study treatment should be discontinued and the reason(s) for discontinuation documented in the clinical records of the patient and corresponding case report form.

Study treatment may continue until one of the following criteria applies:

- Radiologically confirmed and documented unequivocal disease progression, with the following exception of new CNS metastases or isolated progression of previously treated CNS lesions. Patients who have disease control outside of the CNS, defined as confirmed PR or CR of any duration, or SD for ≥ 3 months, but who have developed CNS metastases that are treatable with radiation will be allowed to continue to receive study therapy until they either experience systemic progression of their disease outside of the CNS and/or further progression in the CNS that cannot be treated with additional radiation
- Adverse event(s) that according to the protocol or in the judgment of the investigator may cause severe or permanent harm or which rule out continuation of study drug. *Note: see detailed criteria for study treatment discontinuation due to toxicity in section 6*.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- Suspected patient's pregnancy.
- Serious non-compliance with the study protocol.
- Investigator removes the patient from study.
- Death.
- Lost to follow-up.
- Patient withdraws consent.
- The study site or the sponsor decides to close the study.

All patients who have not progressed and are still in receipt of T-DM1 therapy at the close of the study, as defined here in section 3.12, who are not eligible to receive the T-DM1 treatment in a reimbursement setting, will continue to receive the drug per study procedures.

3.11. Duration of period of study follow-up

Follow-up period is defined as the time between last dose of study combination (T-DM1 + non-pegylated liposomal doxorubicin) up to 12 months after first dose of study treatment.

3.12. End of Study (EoS)

EoS is defined as the LPLV at the end of the follow-up period, as defined in previous section. This will be the last data collection point, which can be a clinic visit or a laboratory sample. LPLV is expected to occur approximately up to one year after the last patient has been enrolled into the study.

4. PATIENT SELECTION

4.1. Target study population

This study will enroll patients with histologically or cytologically confirmed HER2-positive mBC that have relapsed or progressed on or after both taxane and trastuzumab-based therapy Only patients whose HER2 tumor status was locally scored as IHC 3+ or ISH positive will be eligible. Evidence of measurable or evaluable metastatic disease is required.

4.2. Inclusion Criteria

Patients must meet the following criteria for study entry:

1. Signed informed consent prior to any study specific procedure.
2. Patient to be able and willing to comply with protocol.
3. Patients with cytologically or histologically confirmed carcinoma of the breast.
4. Patients with incurable locally advanced or metastatic disease who have previously received up to two previous chemotherapy regimens in this setting (patient starting the first, second or third line of treatment are eligible). Patient must have progressed or relapsed on or after taxane and trastuzumab-based therapy
5. HER2-positive disease immunohistochemistry (IHC) 3+ or *in situ* hybridization (FISH) positive assayed at local laboratories and according to updated ASCO/CAP criteria (Wolff et al. 2013).

6. At least one measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1; or patients with non measurable lesions could be included with these exceptions:
   - patients with only blastic bone lesions are not eligible
   - patients with only pleural, peritoneal or cardiac effusion, or meningeal carcinomatosis are not eligible
7. Patient ≥ 18 years of age.
8. ECOG performance status of 0 or 1.
9. Life expectancy ≥ 3 months.
10. Adequate bone marrow function:
    a. Hemoglobin ≥ 10 g/dl.
    b. Absolute neutrophil count ≥ 1.5 x 10^9/L.
    c. Platelets ≥ 100 x 10^9/L without transfusions within 21 days before 1st study treatment.
    d. International normalized ratio (INR) < 1.5 × the upper limit of normal (ULN).
11. Adequate hepatic and renal function:
    a. Total bilirubin ≤ 1.5 x ULN, except for Gilbert disease patients. Gilbert's syndrome is suspected in people who have persistent, slightly elevated levels of unconjugated bilirubin without any other apparent cause. A diagnosis of Gilbert's syndrome will be based on the exclusion of other diseases based on the following criteria: unconjugated hyperbilirubinemia noted on several occasions, no evidence of hemolysis (normal hemoglobin, reticulocyte count, and LDH), normal liver function tests, and absence of other diseases associated with unconjugated hyperbilirubinemia. For patients with Gilbert disease, total bilirubin value must be ≤ 3 x ULN.
    b. Alkaline phosphatase ≤ 2.5 × the ULN (≤ 5 × the ULN if liver and/or bone metastases are present).
    c. AST (SGOT)/ALT (SGPT) ≤ 1.5 x ULN (< 3 x ULN if liver metastases are present).
    d. Creatinine ≤ 1.5 x ULN and calculated creatinine clearance ≥ 50 mL/min per the Cockcroft and Gault formula.
12. Adequate cardiovascular function with LVEF ≥ 55% as assessed by echocardiography,
13. Recovery from all toxicities of previous anti-cancer therapies to baseline or grade ≤ 1 (CTCAE version 4.0), except for alopecia.
14. For women of childbearing potential (including pre menopausal women who have had a tubal ligation) and for all women not meeting the definition of postmenopausal (≥ 12 months of amenorrhea), and who have not undergone surgical sterilization with a hysterectomy and/or bilateral oophorectomy, and men with partners of childbearing

potential, agreement by the patient and/or partner to use a highly effective, non-hormonal form of contraception or two effective forms of non-hormonal contraception and to continue its use for the duration of study treatment and for 7 months after the last dose of study. For all other women, documentation must be present in medical history confirming that the patient is not of childbearing potential.

4.3. Exclusion Criteria

Patients must not meet the following criteria for study entry:

1. Previous treatment with T-DM1 or anthracyclines, either in the (neo)adjuvant or in the metastatic setting.
2. More than two chemotherapeutic regimens for locally advanced incurable disease or metastatic disease.
3. Patients who have received prior anti-cancer treatment with chemotherapy, immunotherapy or radiotherapy within 3 weeks (6 weeks for nitrosoureas or mitomycin-C), hormonal therapy or lapatinib within 7 days, prior trastuzumab within 21 days (7 days if weekly trastuzumab) or any other targeted therapy within the last 21 days prior to starting study treatment.
4. Previous radiotherapy for the treatment of unresectable, locally advanced/recurrent or mBC is not allowed if:
    a. The last fraction of radiotherapy has been administered within 21 days prior to first study drug administration (except for brain irradiation; at least 28 days will be required).
    b. More than 25% of marrow-bearing bone has been irradiated.
5. History of intolerance (including Grade 3 or 4 infusion reaction) or hypersensitivity to the active substance or to any of the excipients of T-DM1 or non-pegylated liposomal doxorubicin.
6. Patients with CNS involvement. However, patients with metastatic CNS tumors may participate in this trial if the patient is > 4 weeks from radiotherapy completion, is clinically stable with respect to CNS tumor at the time of study entry and is not receiving steroid therapy for brain metastases.
7. Severe/uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, or psychiatric illness/social situations that would limit compliance with study requirements.
8. Cardiopulmonary dysfunction as defined by any of the following :
    a. History of NCI CTCAE (Version 4.0) Grade ≥ 3 symptomatic CHF or NYHA criteria Class ≥ II.

b. Angina pectoris requiring anti-anginal medication, serious cardiac arrhythmia not controlled by adequate medication, severe conduction abnormality, or clinically significant valvular disease.
c. High-risk uncontrolled arrhythmias (i.e., atrial tachycardia with a heart rate > 100/min at rest, significant ventricular arrhythmia [ventricular tachycardia], or higher-grade atrioventricular [AV]-block [second degree AV-block Type 2 [Mobitz 2] or third degree AV-block]).
d. Significant symptoms (Grade ≥ 2) relating to left ventricular dysfunction, cardiac arrhythmia, or cardiac ischemia.
e. Myocardial infarction within 12 months prior to randomization.
f. Uncontrolled hypertension (systolic blood pressure > 180 mmHg and/or diastolic blood pressure >100 mmHg).
g. Requirement for oxygen therapy.

9. Current peripheral neuropathy of Grade ≥ 3 per the NCI CTCAE, v4.0.
10. History of a decrease in LVEF to < 40% or symptomatic CHF with previous trastuzumab treatment.
11. Patients who have had a prior malignancy, other than carcinoma in situ of the cervix, or non-melanoma skin cancer, unless the prior malignancy was cured ≥ 5 years before first dose of study drug with no subsequent evidence of recurrence.
12. Current known active infection with HIV, hepatitis B, and/or hepatitis C virus. For patients who are known carriers of hepatitis B virus (HBV), active hepatitis B infection must be ruled out based on negative serologic testing and/or determination of HBV DNA viral load per local guidelines.
13. Women who are pregnant or breast-feeding.

5. ASSESSMENTS AND STUDY PROCEDURES

5.1. Registration

It should be obtained written patient informed consent prior to undergoing any study related procedures. Prior to the first dose of study drugs it is mandatory to register the patient at the Study Site. The following steps must be taken before registering patients to this study:

1. Completion of patient eligibility checklist
2. Registration of patient in the sponsor study database
3. Confirmation of patient allocation by sponsor

Completion of patient eligibility checklist and Registration of patient in the sponsor study database

The investigator's team must complete the patient eligibility checklist, sign and date it. This checklist must be sent to sponsor for the allocation of the patient and his/her registration in the study database.

Confirmation of patient allocation by sponsor

Each patient will be identified with a unique patient number (UPN) for this study by the sponsor. All data will be recorded with this identification number on the appropriate CRFs.

5.2. Study assessments

Medical History and Demographic Data: Medical history includes clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), smoking history, use of alcohol and drugs of abuse, and all medications (e.g., prescription drugs, over-the-counter drugs, herbal/homeopathic remedies, nutritional supplements) used by the patient within 21 days prior to the screening visit. Demographic data will include age, sex, and self-reported race/ethnicity.

Vital Signs: Vital signs will include measurements of weight, respiratory rate, heart rate, blood pressure, and temperature. Abnormal or significant changes to vital signs from baseline should be recorded as adverse events, if appropriate.

Physical Examinations: A complete physical examination should include the evaluation of head, eye, ear, nose, and throat; cardiovascular; dermatological; musculoskeletal; respiratory; gastrointestinal; and neurological systems. Changes from baseline abnormalities should be recorded at each subsequent physical examination. New or worsened abnormalities should be recorded as adverse events if appropriate.

As part of tumor assessment, physical examinations should also include the evaluation of the presence and degree of enlarged lymph nodes, hepatomegaly, and splenomegaly. Limited physical examinations will be symptom-directed.

Tumor and Response Evaluations: All patients are evaluable for disease response unless they come off study due to treatment related adverse events prior to the completion of cycle 2 and have not had any acceptable complete disease assessment.

Measurable and unmeasurable disease must be documented at screening and re-assessed at each subsequent tumor evaluation. Tumor assessments with computed tomography (CT) or

magnetic resonance imaging (MRI) scans of the chest, abdomen and pelvis are to be performed as described in *Appendix 1: Schedule of assessments and study procedures*.

CT or MRI of the brain and bone scan must be obtained at screening. If an isotope-based bone scan was performed > 28 days but ≤ 60 days prior to the first study treatment the bone scan does not need to be repeated and non-isotopic radiographic modalities should be utilized to document the extent of bony metastatic disease. In the event a positron emission tomography (PET)/CT scanner is used for tumor assessments, the CT portion of the PET/CT must meet criteria for diagnostic quality. Tumor assessments should include an evaluation of all known and/or suspected sites of disease, whenever possible. Patients should have lesions selected that can be evaluated at every tumor assessment.

The same radiographic procedure used at screening must be used throughout the study (e.g., the same contrast protocol for CT scans). Initial tumor response assessment will be performed at the end of cycle 2. Afterwards, tumor response assessment will be performed at the end of 4thcycle and cycles 6. Thereafter, the tumor response assessment will be performed every 9 weeks up to progression or up to 12 months after first dose of study treatment. Response assessments will be assessed by the investigator, based on physical examinations, CT or MRI scans, and bone scans using RECIST v. 1.1 (see *Appendix 2: Response Evaluation Criteria in Solid Tumors* (RECIST criteria) guidelines (version 1.1)). For patients who continue study treatment after isolated brain progression, the frequency of follow-up scans is at the discretion of the investigator. At the investigator's discretion, CT scans, MRI scans, and/or bone scans may be obtained at any time when clinically indicated or if progressive disease is suspected.

If a bone scan cannot be performed during the course of the study because of the unavailability of the Tc-99m isotope, the investigator may choose an alternative imaging modality (see *SNP).*

Radiographic imaging should always be performed rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical examination. In applying RECIST v.1.1, documentation by color photography including a ruler to estimate the size of the lesion is recommended.

LaboratoryAssessments

Local Laboratory Assessments: Prospective HER2 status Hematology, pregnancy testing, biochemistry, coagulation, and cardiac troponin I and B-type natriuretic peptide (BNP) levels (note: for those studies where BNP determination is not be feasible this determination will not be done). Patients will be enrolled based on local results.

Central Laboratory Assessments: Serum T-DM1 and serum non-pegylated liposomal doxorubicin concentrations and total trastuzumab metabolite and serum levels of HER2 ECD using a validated immunoassay. Plasma concentration of DM1 using a validated liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS) method. Polymorphism of HER2 gene coding for the transmembrane domain of HER2 [Ile655Val]. The complete description of this procedure will be recovered in the "Secondary sub-studies Manual".

Electrocardiograms: A 12-lead ECG should be obtained at baseline and be printed and kept with the patient's record. After the screening visit electrocardiograms will be performed as described in *Appendix 1: Schedule of assessments and study procedures*.

Echocardiograms: To be performed as described in *Appendix 1: Schedule of assessments and study procedures*.

ECOG Performance Status: Performance status will be measured using the ECOG performance status scale (see below). It is recommended, where possible, that a patient's performance status be assessed by the same person throughout the study.

Table 1. Scale of ECOG Performance Status

Grade	Scale
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, i.e., light housework, 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

(http://www.ecog.org/general/perf_stat.html)

5.3. Schedule of assessments

Study assessments are outlined in Appendix 1: Schedule of assessments and study procedures. Written informed consent for participation in the study must be obtained before performing any study specific screening tests or evaluations. Informed Consent Forms for enrolled patients and for patients who are not subsequently enrolled will be maintained at the study site.

Results of standard of care tests or examinations performed prior to obtaining informed consent and within 28 days prior to study start may be used; such tests do not need to be repeated for screening.

All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before study start. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

Visits are based on scheduled 21-day cycles (if no treatment delay due to toxicity occurs). Dose delays and dose reductions will be allowed as outlined in Section 6. All visits must occur within ± 3 business days from the scheduled date, unless otherwise noted in the schedule of assessments. All assessments will be performed on the day of the specified visit unless a time window is specified. Assessments scheduled on the day of study treatment administration should be performed prior to study treatment administration unless otherwise noted. If the timing of a protocol-mandated procedure coincides with a holiday and/or weekend, it should be performed on the nearest following date (i.e., within 3 business days).

Local laboratory assessments scheduled for Day 1 of all cycles must be performed within 72 hours prior to study treatment administration unless otherwise specified. In addition, local laboratory assessments scheduled for Days 8 and 15 of cycles 1 and 2 must be performed ± 2 business days. Results of local laboratory assessments must be reviewed and the review documented prior to study treatment administration.

All patients will be closely monitored for safety and tolerability during study treatment and at the follow up period. Patients should be assessed for toxicity prior to any study treatment administration; dosing will occur only if the clinical assessment and local laboratory test values are acceptable.

Efficacy follow up: Initial tumor response assessment will be performed at the end of cycle 2. Afterwards, tumor response assessment will be performed at the end of 4thcycle and cycles 6. Thereafter, all patients will be followed up for efficacy every 9 weeks for up to 12 months after the first dose of study treatment or until progression or the patient withdraws consent or death, whichever occurs first. Response and progression will be evaluated in this study using the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1).

Safetyfollowup: All patients will be followed up for up to 12 months after the first dose of the study combination treatment or up to study termination, whichever occurs first. Cardiac safety will be included.

The first safety follow up visit for the combination treatment will be scheduled for all patients 28 days (+/- 7 days) after the last study treatment (T-DM1 + non-pegylated liposomal doxorubicin) administration in order to follow up toxicities and changes in concomitant medication. Subsequent safety visits will be done every 9 weeks. Last safety follow-up visit will be 28(+/- 7 days) after last dose of any IMP.

Note: for patients that discontinue the study treatment due to the delay of T-DM1 administration for 42 days, the safety follow up visit will be done at 42 days (±5 days) after the last study treatment administration.

All ≥ grade 2 adverse events will be followed up until improvement to baseline levels, grade 1 or complete recovery, the patient withdraws consent, patient's death or up to a maximum of 12 months after the first dose of study combination treatment whichever occurs first.

Survivalfollowup: Patients will be followed for survival every 6 months for up to 12 months after the first dose of study treatment or the patient withdraws consent or until death, whichever occurs first. During survival follow up patients will be assessed at least every six months by means of a visit on the site or other means (e.g., phone calls) to assess the current status of patients and the possible initiation of other treatments.

5.4. Polymorphism of HER2 gene (SNP) assessment

Blood samples must be drawn consistently either from any peripheral vein or from the port-a-cath for any time point, preferable before the first study treatment will be administered. Details will be provided in the Secondary sub-studies Manual. All samples will be labelled with patient study number, date and time of collection.

Technical properties: Next-generation sequencing technologies allow obtaining sequences from multiple loci in a group of samples at reduced costs. Sequences can be produced for the whole-genome or for targeted regions, from one or a few genes or regions to the whole-exome. Briefly, DNA is sheared by sonication followed by 5' ends repair. The resulting fragments are then ligated to adaptors and purified size-selected DNA is then amplified by polymerase chain reaction (PCR) and hybridized to the capture library containing the targeted sequences. Hybridization is followed

by washing and capture of the hybridized DNA through magnetic bead selection, PCR and purification. Quantification of the library is assessed using a Bioanalyzer machine and qPCR. Sequencing will be performed using Illumina massive parallel sequencing technology (MiSeq or NextSeq).

Commercially available array include sets of genes from pathways related to cancer. As an example, the GeneRead DNAseq Breast Cancer Gene Panel (Quiagen, http://www.sabiosciences.com/NGS_product/HTML/NGHS-001Z.html) includes the coding and UTR regions of the 20 most commonly mutated genes in human breast cancer samples. We will produce a high-coverage at the targeted regions for all the individuals in the study. This coverage ensures a very low false negative discovery rate. Deep-resequencing will allow detecting nucleotidic changes, short indels and also structural mutations. Using the barcoding system several individuals are pooled to optimize the achieved coverage. Thus, for a capture array including 115 Kb of sequences, the expected average coverage is higher to 500X for 12 samples included in a MiSeq run. Sequence reads will be aligned to the human genome reference sequence (hg19, downloaded from http://genome.ucsc.edu). Sequence analysis will result with a list of genetic variants for every analyzed individual, including known polymorphisms previously related to cancer (e.g. the Ile655Val change at the gene coding for transmembrane domain of HER2) as well as new genetic variants not previously described. Newly described genetic variants can be ultimately validated by Sanger sequencing.

Specificprocedures: Purified DNA samples will be shipped on dry ice for analysis to Barcelona Biomedical Research Park (Spain). Further details are given in the Secondary sub-studies Manual.

5.5. Pharmacokinetic assessment

Rationale and Pharmacokinetic Outcome Measures

The PK sampling rationale is to characterize the PK of T-DM1, total trastuzumab, and DM1, to assess potential drug-drug interaction when T-DM1 is given in combination with non-pegylated liposomal doxorubicin, and to explore potential correlations between drug exposure and measures of both efficacy (ORR) and toxicity (troponin I, transaminases (ALT, AST), platelets, etc.), if possible. The following PK parameters of T-DM1 and non-pegylated liposomal doxorubicin (including but not limited to those listed below) will be determined for all cohorts in all patients who receive study treatment during the dose-finding period, defined as the period between the first patient in the study being treated and the MTD definition, using either non-compartmental and/or population methods, if possible:

- Serum concentrations of T-DM1 (conjugate) and total trastuzumab

- Plasma concentrations of DM1, non-pegylated liposomal doxorubicin and its active metabolite doxorubicinol
- Total exposure (e.g., AUC)
- C_{max}
- Clearance (CL)
- Distribution volume (Vss)
- $T_{1/2}$

The PK of T-DM1, total trastuzumab and DM1 will be compared with historical T-DM1 single-agent PK data to evaluate the potential effect of non-pegylated liposomal doxorubicin on the PK of T-DM1 and related analytes.

All PK parameters will be listed and tabulated by treatment dose and by cohort. Descriptive summary statistics including arithmetic mean, geometric mean (e.g., AUC and C_{max}), median, range, and coefficient of variation will be presented for each cohort. Nonlinear mixed effects modelling will be also used but details will be presented in the statistical analysis plan (SAP).

PK samples can be obtained ad hoc in case of SAE or unexpected toxicities which may suggest a potential drug-drug interaction.

The exact time of PK sampling will be recorded for all samples. Patients will have approximately 6 ml of peripheral blood collected at each sampling time point.

Extracellular domain of HER2 receptor will be also measured, as it has been shown to represent a relevant covariate in the population PK modeling for T-DM1.

Samples will be shipped to QPS Laboratory (Netherlands) or to PPD Laboratory (USA). Samples will be analyzed according to methods previously published.

Further details are given in the Secondary sub-studies Manual.

5.6. Patient, study, and site discontinuation

Patient discontinuation: Patients have the right to withdraw from the study at any time for any reason. The investigator has the right to discontinue a patient from study treatment or from the study for any medical condition that the investigator determines may jeopardize the patient's safety

if he or she continues in the study, for reasons of non-compliance (e.g., missed doses, visits), if the patient becomes pregnant, or if the investigator determines it is in the best interest of the patient.

Patients must be withdrawn from study treatment if they become pregnant or experience disease progression defined using RECIST v. 1.1. The exception to this is patients who develop isolated progression in the brain as described in *Appendix 2: Response Evaluation Criteria in Solid Tumors* (RECIST criteria) guidelines (version 1.1).

Details of discontinuation due to toxicity are given in section 6.

Patients who discontinue from study treatment prematurely for any of the above reasons will continue to be followed according to section 3.11. The primary reason for discontinuation must be recorded on the appropriate CRF page.

Study discontinuation: Section 3.12 describes the EOS definition and discontinuation of study. Prior to that, the Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to patients
- Patient enrollment is unsatisfactory
- Data recording is inaccurate or incomplete

6. DOSE DELAYS/DOSE MODIFICATIONS

Since the potential adverse events associated to T-DM1 and non-pegylated liposomal doxorubicin partly overlap (for instance, cardiac events, liver toxicity, haematological toxicity or mucositis), dose delays or modifications will generally apply to both drugs. Exceptions will be discussed with the Medical Monitor for special adverse events that can be accurately related only to one of the two agents, such as infusion reactions or extravasations.

6.1. Criteria for Recycling and Dose Delays study drugs

Patients should be assessed for toxicity prior to each dose; dosing will occur only if the results of clinical assessments and laboratory test values are acceptable.

T-DM1 and non-pegylated liposomal doxorubicin will be administered every 21 days only if the following criteria are met:

- ANC ≥ 1.5 x 10^9/L.
- Platelets ≥ 75 x 10^9/L.

- AST/ALT/bilirubin equal to baseline levels or grade ≤ 1.
- Recovery or improvement of other treatment related toxicities (except alopecia) equal to baseline levels or to grade ≤ 1.

Each new cycle may be delayed to a maximum of 3 weeks (maximal duration of cycle of 42 days). Dose delays and reductions are designed to maximize treatment for those patients who respond to or present clinical benefit from treatment while ensuring patient's safety. Dose delays due to T-DM1 related toxicities and/or non-pegylated liposomal doxorubicin-related toxicities other than infusion reactions, thrombocytopenia, hepatotoxicity, neurotoxicity, cardiotoxicity, and ILD or pneumonitis are as follows:

- If significant treatment-related toxicities (other than infusion reactions, thrombocytopenia, hepatotoxicity, neurotoxicity, and cardiotoxicity) have not recovered to Grade 1 or baseline, the next scheduled dose may be delayed for up to 42 days from the last dose received. "Significant" and "related" will be based on the judgment of the investigator (in consultation with the Sponsor's Medical Monitor or designee when appropriate). For example, alopecia even if considered related would most likely not be considered to be significant. Fatigue may or may not be considered either related or significant.

- In general, when the significant and related toxicity or any other toxicity that the investigator chooses to delay dosing for (other than infusion reactions, thrombocytopenia, hepatotoxicity, neurotoxicity, and cardiotoxicity) resolves to Grade 1 or baseline, the patient may resume T-DM1 and non-pegylated liposomal doxorubicin if the delay has not exceeded 42 days from the last received study treatment. In general, patients should be re-evaluated weekly during the delay, whenever possible. In the case of patients who experience a Grade 3 or 4 hematologic event, it is mandatory that the re-evaluation is done at least weekly until recovery. If dosing resumes, the patient may receive T-DM1 and non- pegylated liposomal doxorubicin either at the same dose level as before or at one lower dose level (see Table 2 for T-DM1 dose reductions and decrease non-pegylated liposomal doxorubicin dose according to national prescribing guidelines).

- Non-pegylated liposomal doxorubicin should be held for any Grade 3–4 toxicity attributable to non-pegylated liposomal doxorubicin until resolution to Grade ≤ 1 or baseline grade

- **Dose reductions must be discussed previously with the Sponsor's Medical Monitor or designee**.

- If a patient requires a dose reduction, T-DM1 and /or non-pegylated liposomal doxorubicin dosing will be reduced by one dose level. No dose re-escalation will be allowed.

Table 2. Dose Reduction for T-DM1

Dose Level	every 3 weeks schedule
0	3.6 mg/kg
-1	3.0 mg/kg
-2	2.4 mg/kg
Indication for further dose reduction	Off study treatment

The non-pegylated liposomal doxorubicin dose will be reduced to one dose level in the event of grade 3 or 4 mucositis or grade 2 mucositis persisting at day 21; febrile neutropenia or infection of more than grade 2 after a 1-week delay, and after an additional 1-week delay. Non-pegylated liposomal doxorubicin dose will be also reduced one dose level for any adverse event leading to dose reduction of T-DM1, unless the Medical Monitor concludes that non-pegylated liposomal doxorubicin role in such adverse event is fully ruled out.

Non-pegylated liposomal doxorubicin will be also reduced one dose level for the following:

- ANC < 0.500 x 10^9/L for > 7 days
- ANC < 1.0 x 10^9/L with fever or infection
- Platelets < 25 x 10^9/L
- Platelets < 50 x 10^9/L requiring transfusion

If non-pegylated liposomal doxorubicin is delayed, T-DM1 will be also held until both drugs can be administered, unless one or both are permanently discontinued.

- If toxicity does not resolve within 42 days from the last study treatment received, the patient will discontinue study treatment and will be followed for disease progression and survival outcome.

6.2. Criteria for T-DM1 Dose Modifications in case of specific toxicities

T-DM1DoseModificationforThrombocytopenia

Introduction: Thrombocytopenia, or decreased platelet counts, was reported in patients in clinical trials of trastuzumab emtansine. The majority of these patients had Grade 1 or 2 events (≥ 50 x 10^9/L), with the nadir occurring by Day 8 and generally improving to Grade 0 or 1 (≥ 75 x 10^9/L), by the next scheduled dose. In clinical trials, the incidence and severity of thrombocytopenia were higher in Asian patients. Severe cases of both non-fatal and fatal hemorrhagic events including

central nervous system hemorrhage have been reported in clinical trials with trastuzumab emtansine; these events were independent of the patients' ethnicity. In some of the observed cases the patients were also receiving anti-coagulation therapy. The need for platelet transfusions has been reported.

Patients with thrombocytopenia and on anti coagulant treatment have to be monitored closely during treatment with trastuzumab emtansine. Platelet counts should be obtained no less frequently than weekly to evaluate recovery whenever any of the events listed below occurs, at least prior to each trastuzumab emtansine dose.

Monitoring follow up of thrombocytopenia episodes:

- If platelet counts do not recover to Grade ≤ 1 within 42 days from the last dose received, the patient will be discontinued from study treatment. No re-escalation of the T-DM1 dose is allowed.

Note: although complete blood counts with platelets are required within 72 hours prior to study treatment administration at each cycle, the investigator may monitor platelet counts (or any other laboratory test) more frequently as clinically indicated.

- In the event of decreased platelet count to Grade 3 (< 50 x 10^9/L), do not administer T-DM1 until platelet counts recover to Grade 1 (≥ 75 x 10^9/L), then treat at the same dose level.
- Patients receiving T-DM1 who experience a first Grade 4 thrombocytopenia event may, after adequate recovery to a platelet count of Grade ≤ 1 or baseline, continue treatment with T-DM1 at a dose of 3 mg/kg in subsequent treatment cycles. Patients at the 3 mg/kg dose level who experience a Grade 4 thrombocytopenia event may, after adequate recovery as defined above, continue treatment with T-DM1 at a dose of 2.4 mg/kg in subsequent treatment cycles. Patients who experience a Grade 4 thrombocytopenia event at the 2.4 mg/kg dose level will be discontinued from study treatment.

Use of erythropoiesis stimulating agents will be allowed as consistent with prescribing guidelines. Transfusion of red blood cells and/or platelets will be allowed according to and at the discretion of the treating physician.

T-DM1DoseModificationforHepatotoxicity

Concurrent elevations of ALT/AST and bilirubin meeting Hy's Law laboratory criteria: Regardless of dose level, T-DM1 must be permanently discontinued in patients with ALT and/or AST >3 × ULN and concurrent increase of total bilirubin to > 2 × ULN.

Nodular regenerative hyperplasia (NRH): T-DM1 must be permanently discontinued in patients who are diagnosed with NRH.

Transaminase elevations or bilirubin elevation requiring dose adjustment: Patients who experience a ≥ Grade 3 elevation of liver function should be checked twice weekly for the recovery of transaminases and/or total bilirubin. If a patient's transaminases and/or total bilirubin do not recover as per Table 3 and Table 4, within 42 days from the patient's last dose of study treatment received, the patient will be discontinued from study treatment.
No re-escalation of the T-DM1 dose is allowed.

Table 3 and Table 4 describe the dose modification guidelines for increases in serum bilirubin and transaminases, respectively.

Table 3. Trastuzumab Emtansine Dose Modification: Total Serum Bilirubin

Grade 2 **(> 1.5 to ≤ 3 x ULN)**	**Grade 3** **(> 3 to ≤ 10 × ULN)**	**Grade 4** **(> 10 × ULN)**
Do not administer T-DM1 until total bilirubin recovers to Grade ≤ 1, and then treat at the same dose level	Do not administer T-DM1 until total bilirubin recovers to Grade ≤ 1 and then reduce one dose level	Discontinue T-DM1

ULN = upper limit of normal.
Note: A maximum of two trastuzumab emtansine dose reductions is allowed. A patient requiring more than two dose reductions must discontinue study treatment

Table 4. Trastuzumab Emtansine Dose Modification: Serum ALT or AST

Grade 2 **(> 3 to ≤ 5 × ULN)**	**Grade 3** **(> 5 to ≤ 20 × ULN)**	**Grade 4** **(> 20 × ULN)**
Treat at the same dose level	Do not administer trastuzumab emtansine until AST/ALT recovers to Grade ≤ 2, and then reduce one dose level	Discontinue trastuzumab emtansine

ALT = alanine transaminase; AST = aspartate transaminase; ULN = upper limit of normal.
Note: A maximum of two trastuzumab emtansine dose reductions is allowed. A patient requiring more than two dose reductions must discontinue study treatment.

T-DM1 Dose Modification for Neurotoxicity: Patients receiving T-DM1 who experience Grade 3 or 4 peripheral neuropathy that does not resolve to Grade ≤ 2 within 42 days after the last dose received will be discontinued from study treatment.

T-DM1 Dose Modification/Management for Infusion-related Reactions, Hypersensitivity Reactions

T-DM1 treatment should be interrupted in patients with severe infusion-related reactions. T-DM1 should be permanently discontinued in the event of life-threatening infusion-related reactions. Infusion of T-DM1 should be interrupted for patients who develop dyspnea or clinically significant hypotension. The infusion should be slowed to ≤ 50% or interrupted for patients who experience any other infusion-related symptoms. When the patient's symptoms have completely resolved, the infusion may be continued at ≤ 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes as tolerated. Infusions may be restarted at the full rate during the next cycle.

Patients who experience T-DM1 infusion-related temperature elevations to > 38.5°C and/or other infusion-related symptoms may be treated symptomatically with acetaminophen and/or diphenhydramine hydrochloride. Serious infusion-related events manifested by dyspnea, hypotension, wheezing, bronchospasm, tachycardia, reduced oxygen saturation, or respiratory distress should be managed with supportive care, such as oxygen, beta agonists, antihistamines, or antipyretics, at the investigator's discretion. Antihistamines and antipyretics may be used before subsequent infusions of T-DM1 at the investigator's discretion. Medication with corticosteroids may be used after cycle 2. Patients should be monitored until complete resolution of symptoms. In the event of a true hypersensitivity reaction (i.e., if the severity of reaction increases with subsequent infusions), T-DM1 treatment must be permanently discontinued. Patients who experience a Grade ≥ 3 hypersensitivity reaction or acute respiratory distress syndrome (ARDS) will be discontinued from the study. Patients who experience a severe delayed infusion reaction will be discontinued from study treatment.

T-DM1 Dose Modification for Pulmonary Toxicity: Cases of interstitial lung disease (ILD), including pneumonitis (including severe, life-threatening cases) and some leading to ARDS or fatal outcome have been reported with T-DM1. Treatment with T-DM1 has to be permanently discontinued in patients who are diagnosed with ILD or pneumonitis.

T-DM1 Dose Modification for Extravasation: During the clinical development of T-DM1, reactions secondary to extravasation have been observed. These reactions were usually mild and comprised of erythema, tenderness, skin irritation, pain or swelling at the infusion site. Although T-DM1 is not

considered as a vesicant, close monitoring of the infusion site for possible subcutaneous infiltration during drug administration is recommended.

6.3. Study drug dose modification for Cardiotoxicity

Patients without significant cardiac history and with a baseline LVEF ≥ 55% as determined by ECHO are eligible for study participation.

LVEF will be monitored during the last week of cycles 1, 2, 3, 4, 5, and 6, and after every 9 weeks afterwards. If the LVEF is reported as a range, the median of the range should be taken.

On this protocol cardiotoxicity is defined as follows:

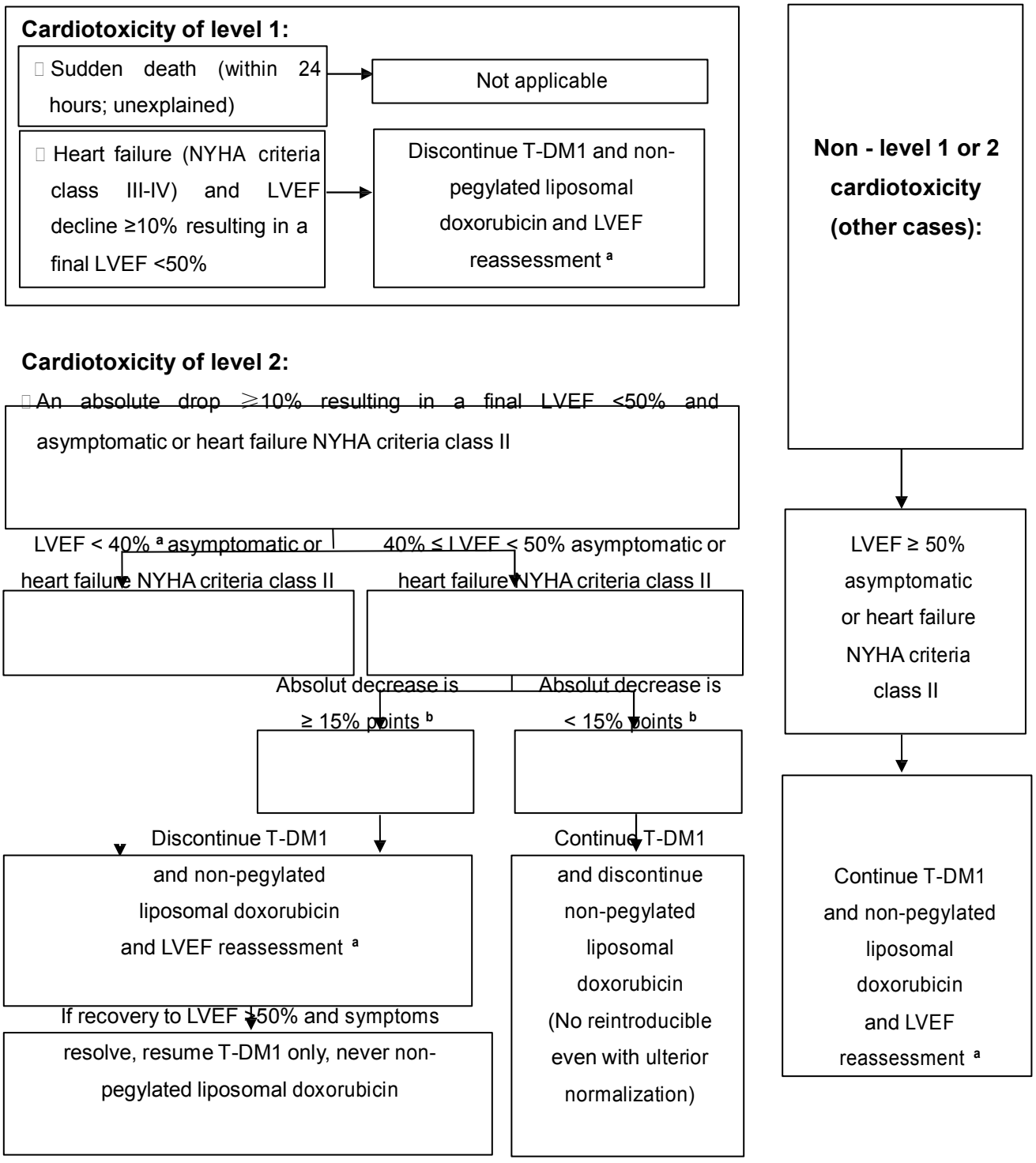
- Cardiotoxicity of Level 1 defined as:
  - Sudden death (defined as within 24 hours; unexplained)
  - Heart failure NYHA criteria class III-IV and LVEF decline defined as an absolute drop ⩾ 10% resulting in a final LVEF <50%

- Cardiotoxicity of Level 2 defined as:
  - An absolute drop ⩾10% resulting in a final LVEF <50% and asymptomatic or heart failure NYHA criteria class II

For cardiotoxicity cases, algorithm for continuation and discontinuation of combination of study drugs will be as follows:

- For patients with cardiotoxicity of level 1 (as defined as DLT in Table 1) -> Discontinue T-DM1 and non-pegylated liposomal doxorubicin according to the algorithm of Figure 2.
- For patients with cardiotoxicity of level 2, defined as asymptomatic or heart failure NYHA criteria class II and an absolute drop ⩾10% resulting in a final LVEF <50% -> Continue or not discontinue T-DM1 and non-pegylated liposomal doxorubicin according to the algorithm of Figure 2.

Figure 2 Algorithm for continuation and discontinuation of combination of study drugs based on Left Ventricular Ejection Fraction assessments



CHF= congestive heart failure; LVEF = left ventricular ejection fraction.
Note: LVEF assessment results must be reviewed before the next scheduled T-DM1 and non-pegylated liposomal doxorubicin infusion.
[a] LVEF can be repeated within 21 days.
[b] a second LVEF monitoring within 21 days consecutive confirmatory result

Previous paragraph and previous figure summarizes the management of the combination of T-DM1 and non-pegylated liposomal doxorubicin on the basis of measured LVEF and changes in LVEF from baseline in patients, the decision to stop or continue the combination of study should be based

on this algorithm. Both, T-DM1 and non-pegylated liposomal doxorubicin, must be withheld

in all patients for whom a confirmed drop of LVEF to below 45%. T-DM1 treatment can be resumed if LVEF reassessed within 21 days is recovered to values >50%. Non-pegylated liposomal doxorubicin should be discontinued permanently. Similar approach should be followed for patients whose LVEF drops to values between 45% and 50% with an absolute decrease in LVEF of ≥ 15% points from baseline. For these patients, study treatment should be temporarily discontinued, the LVEF should be repeated within 21 days, and only T-DM1 should be resumed if the LVEF has recovered to within 15% absolute difference below baseline. For patients whose LVEF drops to values between 45% and 50% with an absolute decrease in LVEF of < 15% points from baseline, non-pegylated liposomal doxorubicin should be discontinued permanently but treatment with T- DM1 can continue without interruption. If an investigator is concerned that an adverse event may be related to cardiac dysfunction, an additional LVEF measurement may be performed.

If clinically significant cardiac dysfunction or cardiac failure develops or persists or if significant medical management is required to maintain ejection fraction, the patient should be discontinued from study treatment. T-DM1 and non-pegylated liposomal doxorubicin will be discontinued as well it is summarized in the Figure 2.

In addition, those cases of elevations of Troponin I and BNP values consisting of an increase >10% from screening values will be considered AESIs and will be reported to Steering Committee for being assessed and confirmed as DLTs or not, and T-DM1 and non-pegylated liposomal doxorubicin will be discontinued and monitored according to decision of the Steering Committee.

7. SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

Safety assessments will consist of monitoring and recording protocol-defined AEs, adverse events of special interest (AESIs) and SAEs; measurement of protocol-specified hematology, clinical chemistry, measurement of protocol-specified vital signs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study drug(s).

The Sponsor or its designee is responsible for reporting relevant SAEs to the Competent Authority, other applicable regulatory authorities, and participating investigators, in accordance with International Conference on Harmonisation (ICH) guidelines, European Clinical Trials Directive (Directive 2001/20/EC), and/or local regulatory requirements.

The Sponsor or its designee is responsible for reporting unexpected fatal or life-threatening events associated with the use of the study drug to the regulatory agencies and competent authorities by telephone or fax within 7 calendar days after being notified of the event. The Sponsor or its designee will report other relevant SAEs associated with the use of the study medication to the appropriate competent authorities (according to local guidelines), investigators, and central Institutional Review Boards/Ethics Committees (IRBs/ECs) by a written safety report within 15 calendar days of notification.

7.1. Adverse Events Definitions

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

The causal relationship between an adverse event and the Investigational Medicinal Product (IMP) will be defined as below:

Not related: The temporal association between the adverse event and the IMP makes a causal relationship unlikely, or the patient's clinical state or the study procedure/conditions provide a sufficient explanation for the adverse event.

Related: The temporal association between the adverse event and the IMP makes a causal relationship possible and the patient's clinical state or the study procedure/conditions do not provide a sufficient explanation for the adverse event.

Each adverse event must be assessed by the investigator as to whether or not there is a reasonable possibility of causal relationship to T-DM1, non-pegylated liposomal doxorubicin and/or their combination.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf).

The intensity (severity) of an adverse event will be recorded as one of the following:

- Mild - Easily tolerated and does not interfere with normal daily activities, CTCAE Grade 1.
- Moderate - Causes some interference with daily activities, intervention or treatment may be needed. CTCAE Grade 2.
- Severe - Normal daily activities are substantially impaired, hospitalization and/or intervention or treatment is required, CTCAE Grade 3 or 4.
- Fatal - Death, CTCAE Grade 5.
- Not applicable (Clinically significant and asymptomatic laboratory test abnormalities or abnormal assessments, for which no CTCAE grading guidance is applicable but which are considered as AEs).

A mild, moderate or severe AE may or may not be serious. These terms are used to describe the intensity of a specific event. However, a severe event (such as severe headache) may be of relatively minor medical significance and is not necessarily serious. For example, nausea lasting several hours may be rated as severe, but may not be clinically serious. Fever of 39 °C that is not considered severe may become serious if it prolongs hospital discharge by a day. Seriousness rather than severity serves as a guide for defining regulatory reporting obligations.

Adverse Drug Reactions: All noxious and unintended responses to an IMP (i.e. where a causal relationship between an IMP and an adverse event is at least a reasonable possibility), related to any dose should be considered adverse drug reactions. For marketed medicinal products, a response to a drug which is noxious and unintended and which occurs at doses normally used in

man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function, is to be considered an adverse drug reaction.

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

Serious Adverse Events

Per definition, a Serious Adverse Event is defined as any adverse event that either:

- results in death (i.e., the AE actually causes or leads to death)
- is life threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death when it occurs),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person ability to conduct normal life functions),
- is a congenital anomaly/birth defect (in a neonate/infant born to a mother exposed to the investigational product(s),
- considered a significant medical event by the investigator (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above) (see below definition of clinically/medically significant event).

Definition of Life Threatening: An adverse event is life threatening if the patient was at immediate risk of death from the event as it occurred, i.e. does not include a reaction that might have caused death if it had occurred in a more serious form. For instance, drug induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though drug induced hepatitis can be fatal.

Definition of Hospitalization: Adverse events requiring hospitalization should be considered serious. In general, hospitalization signifies that the patient has been detained (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment which would not have been appropriate at the study site. When in doubt as to whether hospitalization occurred or was necessary, the adverse event should be considered as serious.

Hospitalization for elective surgery or routine clinical procedures, which are not the result of an adverse event, need not to be considered adverse events. If anything untoward is reported during the procedure, this must be reported as an adverse event and either ‘serious’ or ‘non-serious’ attributed according to the usual criteria.

Definitionofclinically/medicallysignificantevent:

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience 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 such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Clinically/medically significant events MUST be reported as SAEs

In this clinical trial and as defined in this protocol, serious adverse events and hospitalizations unequivocally and solely related to established tumor disease progression will NOT be treated as serious adverse events for reporting obligations.

Serious adverse events, if brought to the attention of the Investigator at any time after the cessation of the study treatment and considered by the Investigator to be possibly related to the study treatment (so, in fact serious adverse reactions), will be reported to the Sponsor.

7.2. Adverse Event Reporting

Adverse events will be collected from the first study-mandated procedure until the safety follow up visit to be done 28 days (+/- 7 days) after the last day of study treatment. All study patients will be carefully monitored for the occurrence of adverse events during this period.

Clearly related signs, symptoms and abnormal diagnostic procedure results should be grouped together and reported as a single diagnosis or syndrome whenever possible. Any additional events that fall outside this definition should also be reported separately.

All adverse events must be recorded in the CRF.

Serious Adverse Event Reporting and Timeframe

Europeansites:

Reporting requirements will comply with all EU safety reporting requirements as detailed in “Directive 2001/20/EC of the European Parliament and of the council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating

to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use" and the associated guideline CT-3.

All protocol defined SAEs and AESIs will be reported to the Sponsor (MedSIR ARO) within 24 hours of when the Investigator or anyone of the site study team becomes aware of it as follows:

- Report all SAEs and AESIs (as defined in this protocol), irrespective of the study drug received by the patient, whether or not this event is considered by the Investigator to be related to study drug, to MedSIR ARO immediately, but in any event no later than 24 hours of any site study team staff becoming aware of the event.
- The full details of the SAE and AESI should be collected and fully documented using the Serious Adverse Event (SAE) form and sent to MedSIR ARO.
- Follow-up information, copies of the results of any tests, the outcome of the event plus the investigator's opinion of IMP relationship to the SAE(s) and AESI(s), and other document when requested and applicable, will accompany the SAE form as available on the day of reporting or provided as soon as possible thereafter.
- The original SAE Report Form and the fax confirmation sheet from the sponsor must be kept with the CRF documentation at the study site(s).

All SAE forms will be sent by the investigator or investigator's team to the Sponsor (MedSIR ARO) by email and fax as noted below:
email: pharmacovigilance@medsir.org
fax: + 34 93 299 23 82

SAEs and AESIS will be followed until resolved, a stable outcome is reached, patient is lost to follow-up or dies.

MedSIR ARO will be responsible for ensuring that events are reported within the mandated timeframe to the EMA and other Competent Authorities, IECs/IRBs and investigator(s), as necessary.

Adverse Events of Special Interest (AESIs) for T-DM1

AESIs must be reported by the Investigator to the Sponsor expeditiously (see section 7.2), regardless of their seriousness (i.e. no more than 24 hours after learning of the event). AESIs for this study include:

- Elevations of Troponin I and BNP values consisting of an increase >10% from screening values.

- Potential drug-induced liver injury as assessed by laboratory criteria for Hy's law. The following laboratory abnormalities define potential Hy's law cases and must be reported as an AESI:
  - Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) elevations that are >3 × upper limit of normal (ULN)
  - Concurrent elevation of total bilirubin >2 × ULN (or clinical jaundice if total bilirubin measures are not available), except in patients with documented Gilbert's syndrome. For patients with Gilbert's syndrome, elevation of direct bilirubin >3 × ULN should be used instead.
- Suspected transmission of an infectious agent by a medication, whereby any organism, virus or infectious particle (e.g. prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non pathogenic, is considered an infectious agent. Transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings indicating an infection in a patient exposed to a medicinal product. This term ONLY applies when contamination of a medication is suspected and DOES NOT apply to infections supported by the mode of action, e.g. immunosuppression.

Pregnancy Reporting

Irrespective of the treatment received by the patient, any patient's or patient's partner pregnancy occurring during study treatment or during the 7 months following study drug discontinuation must be reported within 24 hours of investigator's knowledge of the event.

Pregnancies will be treated as SAEs and the investigator will complete a pregnancy form, and forward it to the sponsor (by e-mail).
email: pharmacovigilance@medsir.org
fax: + 34 93 299 23 82

The patient will be asked to provide follow-up information on the outcome of the pregnancy, including premature termination should the case arise. Spontaneous miscarriage and congenital abnormalities will also be reported as SAEs.

The follow-up period will be deemed to have ended when the health status of the child has been determined at 12 months of the infant's life.

Additional follow up information on any trastuzumab emtansine-exposed pregnancy and infant will be requested at specific time points (i.e., after having received the initial report, at the end of the

second trimester, 2 weeks after the expected date of delivery, and at 3, 6, and 12 months of the infant's life).

Follow-up queries may be sent, asking for further information, if required for a comprehensive assessment of the case.

Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects' research to follow the determinations of the IRBs/IECs. Non compliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB/IECs.

Report non-compliance immediately, within 24hours to MedSIR ARO, to the study site Principal Investigator and, as necessary, to the IRB/IEC.

Serious Noncompliance

Definition: noncompliance that materially increases risks that result in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

Report serious non-compliance immediately, within 24hours to MedSIR ARO, to the study site Principal Investigator and, as necessary, to the IRB/IEC.

Expedited Reporting to Health Authorities, Investigators, Institutional Review Boards, and Ethics Committees

To determine reporting requirements for single AE cases, the Sponsor will assess the expectedness of these events using the following reference documents:

- Trastuzumab emtansine Investigator's Brochures
- Current Summary of Product Characteristics on Non-pegylated liposomal doxorubicin

The Sponsor will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.
Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

7.3. Safety Reporting Flowcharts

See the following flowcharts for the safety information exchange between MedSIR ARO as sponsor of this clinical trial and F. Hoffmann – La Roche as the owner of T-DM1, the investigators, IRB/IEC, Competent Authorities and any other applicable regulatory authorities.

Non-serious AEs reporting responsibilities:

Investigational Center (completing CRF)	⇨	CRO Data Management & MedSIR ARO (review, assessment and processing of safety information): Quaterly non-serious AE listings	⇨	Quarterly non-serious AE listings to F.Hoffmann – La Roche

Pregnancy cases, AESIs & SAEs (as defined by protocol) reporting responsibilities:

Investigational Center (SAE, pregnancy and AESI report, 24 hrs)	MedSIR ARO (review, assessment and processing of safety information): MedWatch/CIOMS report	A. F. Hoffmann – La Roche (copy): • SADRs & AESIs requiring expedited reporting within 15 days • Unrelated SAEs & other AESIs that do not require expedited reporting & pregnancy reports within 30 days B. FDA, EMA and any regulatory authorities, CEIC/IRB, Investigators: • Expedited safety reports & six monthly SUSAR reports according to regulatory reporting obligations (including timelines)

DSUR for the combination of T-DM1 plus non-pegylated liposomal doxorubicin (DSUR model: Non-Commercial Sponsor):

MedSIR ARO (review, assessment and processing of safety information): DSUR development	F. Hoffmann – La Roche (copy): asap Regulatory authorities, CEIC/IRB according to regulatory reporting obligations

Note: Roche to forward to MedSIR an executive summary of the Roche DSUR upon request from MedSIR. MedSIR may cross-reference the executive summary of the Roche DSUR

Investigational Brochure (IB), Medical Alert letters (“Dear Dr.Letters”) & all new information that may modify or supplement known data regarding T-DM1,in particular all new AEs and data relating to T-DM1 tolerance that is likely to reveal a danger to patients:

F. Hoffmann - La Roche (review, assessment and processing of safety information from any other sources different of this clinical trial): IB latest version and IB updates, Medical Alert letters & all new information that may modify or supplement known data regarding T-DM1, in particular all new AEs and data relating to T-DM1 tolerance that is likely to reveal a danger to patients	⇨	MedSIR ARO: asap

Safety Crisis Management & safety queries from regulatory authorities or for publications:
In case of a safety crisis, e.g. where safety issues have a potential impact on the indication(s), on the conduct of the Study, may lead to labeling changes or regulatory actions that limit or restrict the way in which T-DM1 is used, or where there is media involvement, the Party (F. Hoffmann – La Roche or MedSIR ARO) where the crisis originates will contact the other Party asap. Roche shall have the final say and control over safety crisis management issues relating to T-DM1
MedSIR ARO shall not answer any safety query relating to T-DM1 received from regulatory authorities, nor from media and other sources (publications) but shall redirect such queries to Roche taking into account and informing about timelines for required answers

8. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

8.1. Sample size and statistics methods

The study employed a conventional 3 + 3 dose-escalation design and had no formal sample size calculation or hypothesis testing. The total sample size was dependent on the number of dose levels required to determine the MTD. A minimum of 12 and up to 24 patients will be enrolled. Safety assessment was the primary objective and efficacy assessment was an exploratory objective. All data will be presented with listings and summarized using descriptive statistics within each dose level and/or dosing schedule, and overall in all treated patients.

8.2. Analysis populations

The following populations will be analyzed:

1. DLT population: all patients who complete the first two cycles of treatment or who stop treatment during this time because of DLT.
2. The intention to treat (ITT)/safety population: all included patients receiving any dose of treatment.
3. The protocol compliant population (PP): all patients who receive the protocol required study drug exposure and required protocol processing.
4. The pharmacokinetic (PK) population: all patients with a complete treatment concentration-time profile.

8.3. Safety Analyses

Primaryoutcome:

The number and the proportion of patients with DLTs (with corresponding 95% Clopper Pearson confidence intervals) are the primary outcomes. They will be used as the measure for the MTD determination. The definition of DLTs has been presented in Section 3.6. DLTs will be summarized by treatment dose. Confidence intervals will be calculated, according to Clopper-Pearson (exact binomial intervals). The primary outcome will be analysed in DLT population.

Safetyoutcomes:

Safety endpoints will be analysed in intention to treat population. Patients who received at least one dose of both study medications (T-DM1 plus non-pegylated liposomal doxorubicin) and patients who received one treatment alone (T-DM1 or non-pegylated liposomal doxorubicin), will be reported separately. They will be summarized by treatment dosage and will be assessed via total AEs, AEs Grade ≥ 3, SAEs (as described in Section 7), premature withdrawal from study

medication, laboratory parameters, LVEF, exposure to study medication, concomitant medications, vital signs, ECOG performance status, and physical examination.

The incidence of AEs and SAEs will be summarized according to the primary system-organ class (SOC) and within each SOC, by the Medical Dictionary for Regulatory Activities (MedDRA) preferred term. Additional summaries by frequency tables will also be provided for the AEs. Patients who died will be listed together with the cause of death.

Laboratory parameters, hematology and biochemistry will be presented in shift tables of NCI-CTC grade at baseline *versus* worst grade during treatment. LVEF will be summarized over time by means of mean, median and range (minimum and maximum) and will be presented graphically. Vital signs will be analyzed in a similar way.

Other safety variables, such as exposure to study medication, concomitant medications and physical examinations, will be analyzed in a similar way. Frequency tables will summarize the exposure of study medication.

ECOG performance status will be summarized over time and the percentage of patients in different categories will be presented by bar charts at different time points.

8.4. Efficacy Analyses

Efficacy endpoints will be analysed in ITT and PP populations.
The efficacy analyses are exploratory endpoints and they will be investigated as follow:

Overall response rate (ORR). Overall response rate (ORR) is defined as the proportion of patients with best overall response of confirmed complete response (CR) or partial response (PR) based on local investigator's assessment according to RECIST 1.1). An objective response needs to be confirmed at least 4 weeks after the initial response.

Clinical benefit rate (CBR) Clinical benefit rate is defined as the proportion of patients with a best overall response of complete response (CR) or partial response (PR) or stable disease (SD) lasting more than 24 weeks based on local investigator's assessment.

Number of patients with progressions and number of patients who died.

Confidence intervals will be calculated for efficacy data, according to Clopper-Pearson.
Estimates for efficacy data, 95% confidence intervals (CIs) were constructed based on an exact binary distribution.

For the purposes of this study, patients should be re-evaluated for response at the end of cycle 2, 4 and cycle 6. After that, the tumor assessment will be performed every 9 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

8.5. Pharmacokinetic Analyses

Pharmacokinetic Analyses will be analysed in PK population.
The following PK parameters of T-DM1 and non-pegylated liposomal doxorubicin (including but not limited to those listed below) will be determined in all patients who receive study treatment using either non-compartmental and/or population methods, if data allow:

- Serum concentrations of T-DM1 (conjugate) and total trastuzumab
- Plasma concentrations of DM1, non-pegylated liposomal doxorubicin and its active metabolite non-pegylated liposomal doxorubicinol
- Total exposure (e.g. AUC)
- C_{max}
- CL
- V_d
- $T_{1/2}$

The PK of trastuzumab emtansine, total trastuzumab and DM1 will be compared with historical single-agent PK data to evaluate the potential effect of non-pegylated liposomal doxorubicin on the PK of T-DM1 and related analytes. All PK parameters will be listed and tabulated by treatment dose. Descriptive summary statistics including arithmetic mean, geometric mean (e.g., AUC and C_{max}), median, range, s.d., and coefficient of variation will be presented for each cohort. Nonlinear mixed effects modeling will also be used; details will be presented in the SAP.

PK samples can be obtained ad hoc in case of a SAE or unexpected toxicities which may suggest a potential drug-drug interaction.

Extracellular domain of HER2 receptor will be also measured, as it has been shown to represent a relevant covariate in the population PK modeling for T-DM1.

8.6. Steering Committee Review

A Steering Committee (SC) has been established for this study. Initially, it is composed of the investigators, the study medical monitor, the Scientific Global Coordinator, one physician specialising in T-DM1 management and one cardiologist. The study design and DLT definition have been developed by the SC.

The SC will meet at the end of each treatment cohort to review, discuss and evaluate all of the gathered safety data. In case of any arising safety concern, these meetings can also be called at any time at request of a participating investigator. At these meetings, MedSIR ARO and the participating investigators must reach a consensus on whether to escalate the dose any further, or whether to de-escalate and/or expand recruitment into particular cohorts. MedSIR ARO will prepare minutes from these meetings and circulate them to each investigator for comment prior to finalization.

The study site Investigators and MedSIR ARO will review all patient data of their site at least monthly (or before each dose-escalation if occurring sooner than monthly). Each study site Investigator will monitor patients data for serious toxicities on an ongoing basis.

In due course, the SC will also review all available efficacy data and PK information to ensure that safety information is considered in the context of risk-benefit and that dosing is also related to exposure to the drugs.

The SC will adjudicate in the event of DLTs that are not covered by the existing DLT criteria.

9. PHARMACEUTICAL INFORMATION

9.1. Trastuzumab emtansine (T-DM1)

Complete description of T-DM1 will be documented in the Investigator Brochure, located in the Site/Investigator's file.

Summarized information is reported in this section but the Investigator Brochure is the source document for the study drug.

Chemical name: The product name and INN is the following: Trastuzumab emtansine. The chemical name is the following: Immunoglobulin G1, anti-(human receptor tyrosine-protein kinase erbB-2 (EC 2.7.10.1, p185erbB2, MLN 19 or CD340)); humanized mouse monoclonal rhuMab HER2γ1 heavy chain (223-214')-disulfide with humanized mouse monoclonal rhuMab HER2 κlight chain, dimer (229-229":232-232")-bisdisulfide dimer; conjugated on an average of 3 to 4 lysyl, to

maytansinoid DM1 via a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker. CAS Registry Number: 1018448-65-1.

Physico-chemical characteristics: Trastuzumab emtansine exhibits potent in vitro cytotoxic activity against a number of cultured cell lines that over-express *p185*HER2. In addition, T-DM1 is effective in several murine models of HER2-positive breast cancer, including ones that do not respond to unconjugated trastuzumab. Although the primary mechanism of action of T-DM1 is distinct from that of unconjugated trastuzumab, the two molecules are similar with respect to a number of biological activities including binding to recombinant HER2 ECD, C1q, and the neonatal Fc receptor (FcRn). Trastuzumab emtansine also showed similar binding to FcγRI and moderately increased (2- to 3-fold) binding to FcγRIIa and IIb, compared with unconjugated trastuzumab. Additionally, T-DM1 showed similar FcγRIIIa binding.

Structure: Trastuzumab emtansine is an antibody-drug conjugate that contains the humanized anti-HER2 IgG1, trastuzumab, linked to the microtubule-inhibitory maytansinoid, DM1, via a thioether bond. The drug is linked to antibody lysine residues using the heterobifunctional reagent, SMCC.

Clinical Pharmacology: Clinical pharmacological evaluations of T-DM1 are based on PK data obtained from 6 clinical studies (TDM3569g, TDM4258g, TDM4374g, TDM4450g, TDM4688g, EMILIA and TH3RESA)

PharmacokineticsandDrugMetabolism

Patients who received 3.6 mg/kg of T-DM1 intravenously every 3 weeks had a mean maximum serum concentration (Cmax) of T-DM1 of 83.4 (± 16.5) µg/mL. Based on population pharmacokinetic (PK) analysis, following intravenous administration of T-DM1 in patients with HER2-positive metastatic breast cancer, the clearance of T-DM1 was 0.68 L/day and the elimination half-life (t1/2) was of approximately 4 days. No accumulation of T-DM1 was observed after repeated dosing of intravenous infusion every 3 weeks. Trastuzumab emtansine is expected to undergo deconjugation and catabolism by means of proteolysis in cellular lysosomes.

In vitro metabolism studies in human liver microsomes suggest that DM1 is metabolised mainly by CYP3A4 and to a lesser extent by CYP3A5. DM1 did not inhibit major CYP450 enzymes in vitro. In human plasma, T-DM1 catabolites MCC-DM1, Lys-MCC-DM1, and DM1 were detected at low levels. In vitro, DM1 was a substrate of P-glycoprotein (P-gp).

Distribution: Based on population PK analysis, following intravenous administration, the central volume of distribution of T-DM1 was (3.13 L) and approximated that of plasma volume.

The mean volume of distribution at steady-state (Vss) of T-DM1 observed across the six clinical studies in which the drug was administered at 3.6 mg/kg q3w, ranged from 28.4 mL/kg and 58.4 mL/kg after the first T-DM1 dose (Cycle 1) and from 33.3 mL/kg and 43.6 mL/kg at steady state.

Metabolism Half-life: Mean clearance values ranged from 7 to 13 mL/day/kg, the volume of distribution was limited to the plasma volume, and terminal half-life was approximately 4 days.

Excretion: Based on population PK analysis, body weight, albumin, sum of longest diameter of target lesions by Response Evaluation Criteria In Solid Tumors (RECIST), HER2 shed extracellular domain (ECD), baseline trastuzumab concentrations, and aspartate aminotransferase (AST) were identified as statistically significant covariates for T-DM1 PK parameters. However, the magnitude of effect of these covariates on T-DM1 exposure, suggests that with the exception of body weight, these covariates are unlikely to have any clinically meaningful effect on T-DM1 exposure. In addition, exploratory analysis showed that the impact of covariates (i.e., renal function, race and age) on the pharmacokinetics of total trastuzumab and DM1 was limited and was not clinically relevant. In nonclinical studies, T-DM1 catabolites including DM1, Lys-MCC-DM1, and MCC-DM1 are mainly excreted in the bile with minimal elimination in urine.

Immunogenicity: Of all patients treated with T-DM1, a total of 836 patients from six studies had at least one post-dose ATA timepoint evaluable for ATA response. Overall, confirmed positive ATA responses were detected in 44 of 836 (5.3%) patients across the six studies; 28 of these patients had negative baseline samples. Positive responses in all 44 patients were confirmed and characterized by competitive binding immunodepletion with T-DM1 and trastuzumab. The majority (37 of 44) of patients had a positive response immunodepleted by T-DM1 only. The positive response from one or more timepoints from 6 patients was immunodepletable by both T-DM1 and trastuzumab, and the response from 1 patient was immunodepleted by trastuzumab only.
The clinical significance of antibody development against T-DM1 is unknown; however, the impact of ATA response on pharmacokinetics was assessed. There was no obvious change in the pharmacokinetics of patients who tested ATA positive to T-DM1 when compared with data from patients who tested ATA negative.

Supplier: Trastuzumab emtansine is provided as a single-use, lyophilized formulation in a colourless 20 mL Type I glass vial containing 160 mg or 100 mg of T-DM1, closed by means of a FluroTec coated stopper and an overseal with flip-off cap. Upon receipt of T-DM1, vials should be refrigerated at 2–8°C (36–46°F) until use. THE VIAL MUST NOT BE FROZEN OR SHAKEN. Trastuzumab emtansine must be stored in the original carton (as the expiry date will only be

indicated on the carton) Do not use the product beyond the expiration date provided by the manufacturer. The reconstituted product contains no preservative and is intended for single use only. Any remaining medication should be discarded. All vials of T-DM1 should be handled by appropriately trained site staff wearing gloves and using appropriate procedures in place at the clinical site for preparation of chemotherapeutic drugs. Vials should be visually inspected upon receipt to ensure that they are intact without exterior contamination. Discard any cracked vials and report vials with surface contamination to the clinical site manager for assessment. The lyophilized product should be reconstituted using sterile water for injection (SWFI). Using a new syringe, 8 mL SWFI should be added to the vial and the vial swirled gently until the product is completely dissolved. The vial should not be shaken. The resulting product contains 20 mg/mL T-DM1, 10 mM sodium succinate, pH 5.0, 60 mg/mL sucrose, and 0.02% (w/v) polysorbate 20. Each 20 mL vial contains enough T-DM1 to allow delivery of 160 mg T-DM1. The reconstituted product contains no preservative and is intended for single use only. The vial should be inspected to ensure the reconstituted product is a clear colorless solution, and is free of particulates before proceeding. Drug from any vial that appears abnormal upon inspection should not be administered to patients.

Storage and Stability: Using a new syringe, the indicated volume of T-DM1 solution should be removed from the vial(s) and added to the IV bag containing at least 250 mL of 0.45% sodium chloride (preferred) or 0.9% sodium chloride injection and gently inverted to mix the solution. A 0.22 micron non-protein adsorptive polyethersulfone inline filter is recommended when using 0.45% sodium chloride and required when using 0.9% sodium chloride injection. The solution of T-DM1 should not be shaken. The solution of T-DM1 for infusion should be used immediately. If not used immediately, storage times should not be longer than 24 hours at 2–8°C (36–46°F) for solutions of T-DM1 diluted in polyvinyl chloride (PVC) or latex free PVC-free polyolefin, polypropylene, or polyethylene bags containing 0.45% or 0.9% Sodium Chloride for Injection. For additional details, please refer to the current version of the T-DM1 Investigator's Brochure.

SafetyofT-DM1

Full details regarding the clinical safety of T-DM1 are presented in the Investigator's Brochure.

Cardiotoxicity: Patients treated with T-DM1 are at increased risk of developing left ventricular dysfunction. LVEF < 40% has been observed in patients treated with T-DM1. Patients without significant cardiac history and with a LVEF≥ 55% determined by ECHO are eligible for study participation. LVEF will be monitored at screening and regularly throughout the study until the assessment at the safety follow-up visit. Patients with symptomatic cardiac dysfunction will be discontinued from study treatment.

Hematologic Toxicity (Thrombocytopenia): Thrombocytopenia, or decreased platelet counts, was reported in patients in clinical trials of T-DM1. The majority of these patients had Grade 1 or 2 events (≥ 50,000/mm^3), with the nadir occurring by Day 8 and generally improving to Grade 0 or 1 (≥ 75,000/mm^3) by the next scheduled dose. In clinical trials, the incidence and severity of thrombocytopenia were higher in Asian patients. Severe cases of both non-fatal and fatal hemorrhagic events including central nervous system hemorrhage have been reported in clinical trials with T-DM1; these events were independent of the patients' ethnicity. In some of the observed cases the patients were also receiving anti-coagulation therapy. The need for platelet transfusions has been reported. Patients with thrombocytopenia and on anti-coagulant treatment have to be monitored closely during treatment with T-DM1. Platelet counts will be monitored prior to each T-DM1 dose. Use of erythropoiesis-stimulating agents will be allowed as consistent with prescribing guidelines. Transfusion of red blood cells and/or platelets will be allowed according to and at the discretion of the treating physician.

Hepatotoxicity: Hepatotoxicity, predominantly in the form of asymptomatic increases in the concentrations of serum transaminases (Grade 1 − 4 transaminitis), has been observed in patients while on treatment with T-DM1 in clinical trials. Transaminase elevations were generally transient. The incidence of increased AST was substantially higher than that for ALT. The proportion of patients with Grade 1 or 2 increases in transaminases increased with successive cycles, suggesting a modest cumulative effect of trastuzumab emtansine on transaminases; however, no increase over time in the proportion of Grade 3 abnormalities was observed. Patients with elevated transaminases improved to Grade 1 or normal within 30 days of the last dose of T-DM1 in the majority of the patients. Rare cases of severe hepatotoxicity, including death due to drug-induced liver injury and associated hepatic encephalopathy, have been observed in patients treated with T- DM1. Some of the observed cases of acute liver injury may be confounded by concomitant medications with known hepatotoxic potential and/or underlying conditions. Acute severe liver injury (potential Hy's law case) has the following laboratory components:

- Aminotransferase enzymes (ALT/AST) greater than 3× ULN with concurrent elevation of serum total bilirubin to> 2 × ULN,

Patients must have adequate and stable liver function: hepatic transaminases (AST/ALT) and total bilirubin must be within acceptable range, as defined in the protocol, within 4 weeks prior to the first dose of T-DM1. Liver function will be monitored prior to each T-DM1 dose.

Cases of nodular regenerative hyperplasia (NRH) of the liver have been identified from liver biopsies in patients treated with T-DM1 and presenting with signs and symptoms of portal hypertension. NRH was also observed in one fatal case of hepatic failure. NRH is a rare liver

condition characterized by widespread benign transformation of hepatic parenchyma into small regenerative nodules; NRH may lead to non-cirrhotic portal hypertension. Diagnosis of NRH can only be confirmed by histopathology. NRH should be considered in patients who develop clinical symptoms of portal hypertension and/or a cirrhosis-like pattern seen on CT scan of the liver but with normal transaminases and no other manifestations of cirrhosis or liver failure following long- term treatment with T-DM1. Upon diagnosis of NRH, T-DM1 treatment must be permanently discontinued.

PulmonaryToxicity: Cases of interstitial lung disease (ILD), including pneumonitis, some leading to acute respiratory distress syndrome or fatal outcome, have been reported in clinical trials with T-DM1. Signs and symptoms include dyspnea, cough, fatigue, and pulmonary infiltrates. These events may or may not occur as sequelae of infusion reactions. Treatment has included administration of steroids and oxygen, and study drug discontinuation. Patients with dyspnea at rest due to complications of advanced malignancy and comorbidities may be at increased risk of pulmonary events. Treatment with T-DM1 has to be permanently discontinued in patients who are diagnosed with ILD or pneumonitis.

Infusion-related Reactions/Hypersensitivity: Infusion-related reactions characterized by one or more of the following symptoms, flushing, chills, pyrexia, dyspnea, hypotension, wheezing, bronchospasm, and tachycardia may occur with the administration of monoclonal antibodies and have been reported with T-DM1. In general, these symptoms were not severe. In most patients, these reactions resolved over the course of several hours to a day after the infusion was terminated. Patients should be observed closely for infusion-related reactions, especially during the first infusion. Patients should be observed closely for hypersensitivity. Serious, allergic/anaphylactic-like reactions have been observed in clinical trials with treatment of T-DM1. Administration of trastuzumab emtansine will be performed in a setting with access to emergency facilities and staff who are trained to monitor and respond to medical emergencies. Patients will be observed closely for infusion-related/hypersensitivity during and after each T-DM1 infusion for a minimum of 90 minutes after the first infusion and for a minimum of 30 minutes after subsequent infusions in the absence of infusion-related AEs. Pre-medication is allowed according to standard practice guidelines. In the event of a true hypersensitivity reaction (in which severity of reaction increases with subsequent infusions), T-DM1 treatment must be permanently discontinued. Patients who experience a Grade ≥ 3 allergic reaction or acute respiratory distress syndrome will be discontinued from study treatment.

Neurotoxicity: DM1, an anti-microtubule agent, can potentially cause peripheral neuropathy. Peripheral neuropathy, mainly Grade 1 and predominantly sensory, has been reported in clinical

trials of T-DM1. Treatment with T-DM1 should be temporarily discontinued in patients experiencing Grade 3 or 4 peripheral neuropathy until symptoms resolve or improve to≤ Grade 2. Patients should be examined for signs of peripheral neuropathy prior to each dose of T-DM1. Patients who experience Grade≥ 3 neurotoxicity in the form of peripheral neuropathy that does not resolve to Grade≤ 2 within 42 days after last dose received will be discontinued from study treatment.

Extravasation: In T-DM1 clinical studies, reactions secondary to extravasation have been observed. These reactions were usually mild and comprised erythema, tenderness, skin irritation, pain, or swelling at the infusion site. Rare reports of more severe events such as cellulitis, pain (tenderness and burning sensation), and skin irritation have been received as part of the continuing surveillance of T-DM1 safety. These reactions have been observed more frequently within 24 hours of infusion.

Specific treatment for T-DM1 extravasation is unknown at this time. The infusion site should be closely monitored for possible subcutaneous infiltration during drug administration.

Precautions and warnings

A Population PK analysis was performed, which identified several covariates with a significant effect on Population PK parameters. Yet, with the exception of body weight. The impact of these identified covariate on exposure was low. Body weight based dose of 3.6 mg/kg every 3 week without correction for other covariates is considered appropriate.

Elderlypatients: A population pharmacokinetic analysis indicates that age does not have a clinically meaningful effect on the pharmacokinetics of T-DM1. There are insufficient data to provide dosing recommendations in patients≥ 75 years due to limited data in this subgroup, therefore no dose adjustment is proposed.

Renal impairment: T-DM1 derived DM1 and metabolites MCC-DM1 and lys-MCC-DM1 has only been partially characterised in humans. However, the concentration of DM1 were always low, often lower than the LLOQ, which precluded a formal PK analysis. The impact of renal impairment on the PK of T-DM1 was investigated through a Population PK analysis. The results showed a lack of effect of renal impairment on the exposure to T-DM1, which is consistent with the protein moiety of drug being eliminated by ubiquitary proteolysis. The nonclinical data, a mass balance study in rats, and exploratory PK analyses in patients with and without renal impairment in Study TDM4688g that renal impairment has a minimal effect on the exposure to the DM1-containing catabolites, namely MCC-DM1 and Lys-MCC-DM1.

No adjustment to the starting dose is needed in patients with mild or moderate renal impairment. The potential need for dose adjustment in patients with severe renal impairment cannot be determined due to insufficient data and therefore patients with severe renal impairment should be monitored carefully.

A dedicated study of the PK of T-DM1 and relevant catabolites in metastatic breast cancer (mBC) patients with mild to moderate hepatic impairment is ongoing (see Risk-management plan in the Investigator Brochure).

Hepatic impairment: Liver function should be monitored prior to initiation of treatment and each dose. Patients with baseline elevation of ALT (e.g due to liver metastases) may be predisposed to liver injury with a higher risk of a Grade 3-5 hepatic event or liver function test increase.

Interactions: The potential for interaction has been sufficiently investigated. In vitro metabolism studies in human liver microsomes suggest that DM1 is metabolised mainly by CYP3A4 and, to a lesser extent, by CYP3A5. Concomitant use of strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole) with T-DM1 should be avoided due to the potential for an increase in DM1 exposure and toxicity. Consider an alternate medicinal product with no or minimal potential to inhibit CYP3A4. If concomitant use of strong CYP3A4 inhibitors is unavoidable, consider delaying T-DM1 treatment until the strong CYP3A4 inhibitors have cleared from the circulation (approximately 3 elimination half-lives of the inhibitors) when possible. If a strong CYP3A4 inhibitor is coadministered and T-DM1 treatment cannot be delayed, patients should be closely monitored for adverse reactions.

9.2. Non-pegylated liposomal doxorubicin

Complete description of non-pegylated liposomal doxorubicin will be documented in the Summary of Product Characteristics (SmPC), located in the Site/Investigator's file.

9.3. Supplying and Handling Investigational Products

All investigational medicinal products (IMPs) required for the conduct of this study (T-DM1 or non-pegylated liposomal doxorubicin) will be provided by the MedSIR ARO/Roche.

T-DM1 will be released and distributed to study centres by Roche, while non-pegylated liposomal doxorubicin will be released and distributed by sponsor's designee.

The sponsor will ensure supply of the study IMP and appropriate labeling of study IMP provided that it complies with the legal requirements of each country where the study is to be performed.

9.4. Investigators Responsibilities over the Investigational Products

The Investigator must ensure the following:

- acknowledgement of receipt of the study IMP by a designated staff member at the site, including confirmation that IMP was received in good condition. Any damaged shipments will be replaced.
- proper storage of study IMP, including storage in a secure, locked, temperature-controlled location and according to the instructions specified on the labels. IMP that have been stored differently from the manufacturer's indications **must not** be used unless the sponsor provides written authorization for use
- appropriate record keeping and inventory of the study IMP, including regular documentation of adequate storage temperature.
- appropriate use of the study IMP, including use only in accordance with the approved protocol
- proper handling, including confirmation that IMP has not expired prior to administration
- appropriate documentation of IMP administration to study patients including:
- date, dosage, batch/serial numbers, expiration dates, unique identifying numbers assigned to patients and study IMP, and time of administration. This information will be maintained in an accountability log that will be reviewed by the site monitor.
- proper reconciliation of all study IMP received from the sponsor. Reconciliation is defined as maintaining records of which and how many IMP was received, administered and destroyed at site and returned, as applicable.
- IMPs will be either disposed of at the study site according to the study site's institutional standard operating procedure or returned to the Sponsor with the appropriate documentation. The site's method of IMP destruction must be agreed upon by the Sponsor. The site must obtain written authorization from the Sponsor before any IMP is destroyed, and IMP destruction must be documented on the appropriate form
- proper adherence to the local institutional policy with respect to destruction of study including documentation.

10. ETHICAL CONSIDERATIONS

10.1. Regulatory and Ethics Compliance

The study will be performed and reported in accordance with the guidelines of the International Conference on Harmonization (ICH), and the ethical principles laid down in the Declaration of Helsinki. The study will be also compliance with European Directive 2001/20/EC and any applicable local regulations.

10.2. Institutional Review Board / Independent Ethic Committee:

Conduct of the study must be approved by an appropriately constituted IRB/IEC. Approval is required for the study protocol, protocol amendments, informed consent forms, study subject information sheets, and advertising materials. The IRB/IEC must also be contacted in the event of any major protocol violation or any SAE.

It is the Investigator's responsibility to communicate with their local IRB/IEC to ensure accurate and timely information is provided at all phases during the study.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Investigators are also responsible for promptly informing the IRB/EC of any protocol amendments.

In addition to the requirements to report protocol-defined AEs to the Sponsor, investigators are required to promptly report to their respective IRB/EC all unanticipated problems involving risk to human patients. Some IRBs/ECs may want prompt notification of all SAEs, whereas others require notification only about events that are serious, assessed to be related to study treatment, and are unexpected. Investigators may receive written safety reports or other safety related communications from the Sponsor. Investigators are responsible for ensuring that such reports are reviewed and processed in accordance with regulatory requirements and with the policies and procedures established by their IRB/EC and archived in the site's study file.

10.3. Informed Consent

For each study subject, written informed consent will be obtained prior to any protocol related activities. As part of this procedure, the study site Investigator or designee must explain orally and in writing the nature, duration, and purpose of the study, and the action of the drugs in such a manner that the study subject is aware of the potential risks, inconveniences, or adverse effects that may occur. The study subject should be informed that he/she is free to withdraw from the

study at any time. The subject will receive all information that is required by local regulations and ICH guidelines.

The Consent Form must be signed and dated by the patient or the patient's legally authorized representative before her participation in the study. The case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study.

A copy of each signed Consent Form must be provided to the patient or the patient's legally authorized representative, if applicable.

All signed and dated Consent Forms must remain in each patient's study file and must be available for verification by study monitors at any time.

The Informed Consent Form should be revised whenever there are changes to procedures outlined in the informed consent or when new information becomes available that may affect the willingness of the patient to participate.

For any updated or revised Consent Forms, the case history for each patient shall document the informed consent process and that written informed consent was obtained for the updated/revised Consent Form for continued participation in the study. The final revised IRB/EC-approved Informed Consent Form must be provided to the Sponsor for regulatory purposes.

10.4. Data Protection

The sponsor will ensure the confidentiality of patient's medical information in accordance with all applicable laws and regulations.

The sponsor as Data Controller according to the European Directive on the protection of individuals with regard to the processing of personal data and on the free movement of such data [95/46/EC] confirms herewith compliance to Directive 95/46/EC in all stages of Data Management.

Data generated by this study must be available for inspection upon request by representatives of national and local health authorities, the Sponsor monitors, representatives, and collaborators, and the IRB/EC for each study site, as appropriate.

11. SOURCE DOCUMENTATION, STUDY MONITORING, AND QUALITY ASSURANCE

11.1. Source Data Documentation

Source data refers to all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source documents are original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial).

Sponsor's Quality Assurance group may assist in assessing whether electronic records generated from computerized medical record systems used at investigational sites can serve as source documents for the purposes of this protocol.

If a site's computerized medical record system is not adequately validated for the purposes of clinical research (as opposed to general clinical practice), applicable hardcopy source documents must be maintained to ensure that critical protocol data entered into the eCRFs can be verified.

At a minimum, source documentation must be available to substantiate subject identification, eligibility, and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; adequate reporting and follow-up of AEs; administration of concomitant medication; study receipt/dispensing/return records; study administration information; and date of completion and reason.

Data recorded on the CRF will be verified by checking the CRF entries against source documents (i.e., all original records, laboratory reports, medical records) in order to ensure data completeness and accuracy as required by study protocol. The Investigator and/or site staff must make CRFs and source documents of subjects enrolled in this study available for inspection by MedSIR or its representative at the time of each monitoring visit.

The source documents must also be available for inspection, verification, and copying, as required by regulations, officials of the regulatory health authorities (e.g., FDA, EMEA, and others), and/or ECs/IRBs. The Investigator and study site staff must comply with applicable privacy, data

protection, and medical confidentiality laws for use and disclosure of information related to the study and enrolled subjects.

The patient must also allow access to the patients' medical records. Each patient should be informed of this prior to the start of the study.

11.2. Study Monitoring and Source Data Verification

Study progress will be monitored by MedSIR ARO or its representative (e.g., a CRO) as frequently as necessary to ensure:

That the rights and well-being of human subjects are protected;

- the reported trial data are accurate, complete, and verifiable from the source documents; and
- the conduct of the trial is in compliance with the current approved protocol/amendment(s), GCP, and applicable regulatory requirements.

Contact details for the team involved in study monitoring will be identified in a handout located in the Investigator Site File.

Data recorded on the CRF will be verified by checking the CRF entries against source documents (i.e., all original records, laboratory reports, medical records, subject diaries) in order to ensure data completeness and accuracy as required by study protocol. The Investigator and/or site staff must make CRFs and source documents of subjects enrolled in this study available for inspection by the sponsor or its representative at the time of each monitoring visit.

11.3. Retention of records

Investigators must retain all study records required by the applicable regulations in a secure and safe facility. The Investigator must consult a sponsor representative before disposal of any study records and must notify the sponsor of any change in the location, disposition, or custody of the study files.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. "Essential documents" are defined as documents that individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents should be retained for a longer period, however, if required by the applicable e regulatory requirements or by an agreement with the sponsor. The CHMP requires

retention for the maximum period of time permitted by the institution, but not less than 15 years (ICH E6, 4.9.5). It is the responsibility of the sponsor to inform the Investigator/institution as to when these documents no longer need to be retained (ICH E6, 5.5.12).

The study site Investigator must not dispose of any records relevant to this study without either (1) written permission from the Sponsor or (2) providing an opportunity for the Sponsor to collect such records. The study site Investigator shall take responsibility for maintaining adequate and accurate electronic or hard copy source documents of all observations and data generated during this study. Such documentation is subject to inspection by the Sponsor and the FDA and/or EMA (or respective individual EU country regulatory authorities).

These principles of record retention will also be applied to the storage of laboratory samples, provided that the integrity of the stored sample permits testing.

11.4. Data Quality Assurance

During and/or after completion of the study, quality assurance auditor (s) named by the MedSIR ARO or the regulatory authorities may wish to perform on-site audits. The Investigators will be expected to cooperate with any audit and provide assistance and documentation (including source data) as requested.

The Sponsor's representatives are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the clinical study (e.g., CRFs and other pertinent data) provided that patient confidentiality is respected.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing CRFs, are resolved.

In accordance with ICH E6 Good Clinical Practice (GCP) and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor's (or designee's) Quality Assurance Department. Inspection of site facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the study conduct and compliance with the protocol, ICH GCP (ICH E6), and applicable country regulatory requirements.

12. DATA MANAGEMENT

12.1. Data Entry and Management

In this study, all data will be entered onto CRFs in a timely fashion by the Investigator and/or the Investigator's dedicated site staff.

The Investigator must review data recorded in the CRF to verify their accuracy.

Reconciliation of the data will be performed by the designated CRO. At the conclusion of the study, the occurrence of any protocol violations will be identified and recorded as part of the clinical database. After these actions have been completed and the database has been declared to be complete and accurate, it will be locked and will become available for statistical data analysis.

12.2. Data Clarification

As part of the conduct of the trial, MedSIR ARO may have questions about the data entered by the site, referred to as queries. The monitors and the sponsor are the only parties that can generate a query.

12.3. Data Coding Procedures

Coding of AEs, medical history, and prior and concomitant medications will be performed using standard dictionaries as described in the Data Management Plan.

13. STUDY MANAGEMENT

13.1. Discontinuation of the Study

MedSIR ARO reserves the right to discontinue the study for safety or administrative reasons at any time. Should the study be terminated and/or the site closed for whatever reason, all investigational drugs pertaining to the study must be returned to MedSIR ARO. Any actions required to assess or maintain study subject safety will continue as required, in spite of termination of the study.

13.2. Changes to the Protocol

Any change or addition to this protocol requires a written protocol amendment or administrative letter that must be approved by MedSIR ARO, the Scientific Global Coordinator, the study site Investigator and the IRB/IEC before implementation. This requirement for approval should in no way prevent any immediate action from being taken by the study site Investigator or MedSIR ARO

in the interests of preserving the safety of all subjects included in the trial. If an immediate change to the protocol is felt to be necessary by the study site Investigator and is implemented for safety reasons, MedSIR ARO should be notified as soon as possible (within 24 hours if possible) and the IRB/IEC should be informed as necessary.

13.3. Publication Policy Protection of Trade Secrets

All information generated in this study must be considered highly confidential and must not be disclosed to any persons not directly concerned with the study without prior written permission from the Scientific Global Coordinator and MedSIR ARO However, authorized regulatory officials, the Scientific Global Coordinator or the study site Investigator, and MedSIR ARO personnel (or their representatives) will be allowed full access to inspect and copy the records. All clinical investigational drug, patient bodily fluids, and/or other materials collected shall be used solely in accordance with this protocol, unless otherwise agreed to in writing by Scientific Global Coordinator or the study site Investigator and MedSIR ARO.

The sponsor will ensure that as far as possible results of this study will be published as scientific/clinical papers in high-quality peer-reviewed journals. Preparation of such manuscripts will be made with full collaboration of principal Investigators and in accordance with the current guidelines of Good Publication Practice.

The sponsor must be notified of any intent to publish data collected from the study and prior approval from sponsor must be obtained prior to publication

Appendix 1: Schedule of assessments and study procedures

Study Period	Screening	Study Treatment (T-DM1 + non-pegylated liposomal doxorubicin)				End of Study Treatment	Follow-up		
		Cycles 1-2		Cycles 3-6			While on T-DM1 treatment	28 (-42) days after last T-DM1 dose[a]	End of Study visit
Day	-28 to -1	1	21	1	21	28±7 last dose TMD1 + Non-pegylated liposomal doxorubicin	Every 3 weeks		
Informed Consent	X								
HER2 status	X								
Medical History	X								
Physical Examination and ECOG status	X	X		X		X	X	X	
Weight	X	X		X			X		
Vital signs[b]	X	X		X		X	X	X	
Concomitant Medication Reporting	----------								
AE reporting	----------								
ECHO	X		X		X		X[c]	X[c]	
12-lead ECG	X		X		X		X[c]	X[c]	
DLT assessment			X						
Tumor Assessments	X		X[d]		X[d]	X[do]	X[d]	X[d]	

Overall survival ----------

Study Period	Screening	Study Treatment (T-DM1 + non-pegylated liposomal doxorubicin)			End of Study Treatment	Follow-up		
		Cycles 1-2		Cycles 3-6		While on T-DM1 treatment		End of Study visit
Day	-28 to -1	1	21	1	21	28±7 last dose TMD1 + Non-pegylated liposomal doxorubicin	Every 3 weeks	28 (-42) days after last T-DM1 dose[a]	
Standard Laboratory Procedures:									
Pregnancy test[e]	X						X[e]	X[e]	
Hematology[f]	X	X[g] (Days 8 and 15)		X[g]			X[g]		
Biochemistry[h]	X	X[g] (Days 8 and 15)		X[g]			X[g]		
INR/aPTT	X	As clinically indicated					As clinically indicated		
Troponin I determination	X	X[ij] (Days 8 and 15)		X[ij]			X[ij]		
B-type natriuretic peptide (BNP)	X	X[kl] (Days 8 and 15)		X[kl]			X[kl]		
Experimental laboratory (to be performed in a central lab)									
PK samples[m]		X		X					
Single Nucleotide Polymorphisms (SNP)	X								
Drug Administrations									
Trastuzumab Emtansine		X		X			X[no]		

Study Period	Screening	Study Treatment (T-DM1 + non-pegylated liposomal doxorubicin)				End of Study Treatment	Follow-up		
		Cycles 1-2		Cycles 3-6			While on T-DM1 treatment		End of Study visit
Day	-28 to -1	1	21	1	21	28±7 last dose TMD1 + Non-pegylated liposomal doxorubicin	Every 3 weeks	28 (-42) days after last T-DM1 dose[a]	
(T-DM1)									
Non-pegylated liposomal doxorubicin		X[p]		X[p]					

AE = Adverse Event; aPTT = Activated Partial Thromboplastin Time; ECG = Electrocardiogram; ECHO = Echocardiogram; ECOG = Eastern Cooperative Oncology Group; INR = International Normalized Ratio

[a] Patients will be followed for new or worsening adverse events for 28 (+/- 7 days) days following the last dose of any study IMP. All ≥ grade 2 adverse events will be followed up until improvement to baseline levels, grade 1 or complete recovery, initiation of another anti-cancer therapy, the patient withdraws consent, patient's death or up to a maximum of 24 months after the first dose of study combination treatment whichever occurs first. Additionally, patients will be contacted regarding the occurrence of any new SAE considered to be treatment-related at 60 and 90 days following the last study treatment administration or until initiation of another anti-cancer therapy, whichever occurs first.

[b] Vital signs will include measurements of respiratory rate, heart rate, blood pressure, and temperature. Abnormal or significant changes to vital signs from baseline should be recorded as adverse events, if appropriate.

[c] ECHO and ECG will be performed every 1 cycle (cycles 1-6) during treatment with T-DM1 + non-pegylated liposomal doxorubicin. Thereafter, ECHO and ECG will be performed every 9 weeks until 12 months since last dose of study treatment (T-DM1+ non-pegylated liposomal doxorubicin)

[d] During study treatment (T-DM1+ non-pegylated liposomal doxorubicin) tumor assessment wil be performed at the end of cycle 2, cycle 4, and cycle 6. Thereafter, the tumor response assessment will be performed every 9 weeks up to progression or up to 12 months after the first dose of the study combinated treatment for patients who discontinue all study IMP for reasons other than PD. Response assessments will be assessed by the investigator, based on physical examinations, CT or MRI scans, and bone scans using RECIST v. 1.1

[e] Serum β-HCG test must be performed during screening, every 3 cycles and at 3 and 7 months following the last dose of T-DM1 and/or non-pegylated liposomal doxorubicin for women of childbearing potential (including pre-menopausal women who have had a tubal ligation) and for women not meeting the definition of postmenopausal. Testing should be performed at a local laboratory within 7 days prior to the first administration of study treatment. For all other women, documentation must be present in medical history confirming that the patient is not of childbearing potential.

[f] Hematologic assessments include hemoglobin (Hb), hematocrit, red blood cell count, platelet count, and white blood cells (WBC) with differential (including neutrophils, lymphocytes, monocytes, eosinophils and basophils)

[g] Assessments should be performed at screening, within 72 hours prior to any IMP administration, at days 8 and 15 after any IMP administration during the first 2 cycles, weekly following any hematologic adverse event. If non-pegylated liposomal doxorubicin is discontinued due to toxicity, assessments should be performed weekly (minimum) until resolution of the adverse event (Grade ≤ 1 or baseline levels), and on Day 1 of subsequent cycles thereafter. All assessments at Day 1 should be performed within 72 hours preceding administration of any study IMP; results must be reviewed and documented prior to administration of study treatment. Assessments at days 8 and 15 should be performed 7 (±2) and 14 (±2) days after any IMP administration

[h] Biochemistry assessments include: sodium, potassium, chloride, calcium, magnesium, glucose, urea or blood urea nitrogen (BUN), creatinine, uric acid, total protein, albumin, alkaline phosphatase, ALT (SGPT), AST (SGOT), G-GT, LDH, total bilirubin (and direct bilirubin where total bilirubin > ULN).

[i] TPN I determination will be performed 7 days (with a window of ± 2 days) after every infusion of every cycle and also 14 days (with a window of ± 2 days) after infusion of cycles 1 and 2.

[j] TPN I determination will be performed every 1 cycle (cycles 1-6) during treatment with T-DM1 + non-pegylated liposomal doxorubicin. Thereafter, TPN I will be performed every 9 weeks until 12 months since first dose of study treatment (T-DM1+ non-pegylated liposomal doxorubicin).

[k] BNP determination will be performed 7 days (with a window of ± 2 days) after every infusion of every cycle and also 14 days (with a window of ± 2 days) after infusion of cycles 1 and 2.

[l] BNP determination will be performed every 1 cycle (cycles 1-6) during treatment with T-DM1 + non-pegylated liposomal doxorubicin. Thereafter, BNP will be performed every 9 weeks up to 12 months since first dose of study treatment (T-DM1+ non-pegylated liposomal doxorubicin)

[m] Pharmacokinetic assessments will be performed during the Dose Finding part of the study on Cycles 1 and 2 and also on cycle 4.

[n]: Every 3 weeks from last dose of study treatment (combination T-DM1+ Non-pegylated liposomal doxorubicin) until disease progression or development of intolerable toxicity, whichever occurs first.

[o] Optional as per investigator's criteria

[p] At the Level -1, non-pegylated liposomal doxorubicin will be administered in a weekly schedule.

Appendix 2: Response Evaluation Criteria in Solid Tumors (RECIST criteria) guidelines (version 1.1)

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Appendix 3: Instructions for Scans in the Event of Isotope Shortage

Two key suppliers of Tc-99m generators (Chalk River Reactor, Canada and High Flux Reactor, the Netherlands) are expected to close. Supplies from other reactor sources will be unable to meet the expected world wide patient-care needs. As a result, significant shortages of Tc 99m are expected, and the instructions listed below should be followed:

- Tc-99m bone scans should be obtained as part of the baseline tumor assessment in all patients and should be repeated to confirm a CR or if progression of existing bone lesions and/or the development of new bone lesions is clinically suspected.

If a bone scan cannot be performed at baseline or if the investigator suspects that a bone scan may not be able to be repeated during the course of the study because of the Tc-99m shortage, the investigator may choose F-18 NaF or FDG-PET scan as an alternative.

- If bone lesions are selected as index non-target lesions, they must be apparent on baseline CT scans or other radiographic modalities (e.g., skeletal X-rays that can be repeated in subsequent tumor assessments). Additional scans may be obtained to follow clinically important bone lesions if not visualized on the chest, abdomen, or pelvic CT scan.

These measures are intended to ensure that the same method of assessment and the same imaging technique is used throughout the study for each patient. If there is a question regarding the choice of alternatives in the event that a standard bone scan cannot be obtained during screening and/or during the study, please contact the Medical Monitor.