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Programa de Doctorado en
Ciencias de la Salud

Safety and pharmacokinetic study of the combination of trastuzumab emtansine and non-pegylated liposomal doxorubicin for the treatment of advanced HER2 positive breast cancer

Tesis Doctoral presentada por

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Alcalá de Henares (2021)

Son varias las personas e instituciones que han participado en este proyecto, a las cuales quería expresar mi más sincero agradecimiento en estas breves líneas.

En primer lugar, a mis directores de tesis, el Dr. Javier Cortés Castán y el Dr. José Pérez García por su confianza, entusiasmo, ejemplo de esfuerzo y por su generosidad al haberme dado la oportunidad de desarrollar este trabajo.

También quiero agradecer a MedSir y a A.F. Hoffmann – La Roche su confianza y el soporte económico del proyecto.

Al Dr. Alfredo Carrato por la confianza que siempre ha depositado en mi.

A la Dra. Enriqueta Arias Fernández por su tutela.

A mis padres por su tesón y apoyo incondicional.

A mi marido y a nuestros hijos porque son la base sobre la que se sustenta mi vida, por su paciencia y por alegrarse de mis logros profesionales.

Safety and pharmacokinetic study of the combination of trastuzumab emtansine and non-pegylated liposomal doxorubicin for the treatment of advanced HER2 positive breast cancer

TABLE OF CONTENTS

Table of abreviations 11

Summary in English 15

Summary in Spanish 17

1. Background 21

1.1 HER2-positive metastatic breast cancer 21

1.2 Trastuzumab emtansine (T-DM1) in HER2-positive metastatic breast cancer 22

1.3 Pharmacokinetics of T-DM1 25

1.4 Mechanisms of cardiotoxicity of study drugs 27

1.5 Non-pegylated liposomal doxorubicin 28

1.6 Rationale to study the combination of T-DM1 and non-pegylated liposomal doxorubicin 28

2. Work hypothesis 33

3. Objectives 37

3.1 Primary objective 37

3.2 Secondary objectives 37

4. Material and methods 41

4.1 Study design 41

4.2 Dose escalation schema: description of cohorts 43

4.3 Duration of study treatment 43

4.4 Duration of the follow-up period 44

4.5 End of study (EoS) 45

4.6 Study population 45

4.6.1 Target study population 45

4.6.2 Inclusion criteria 45

4.6.3 Exclusion criteria 45

4.7 T-DM1 drug dose schedule 48

4.8 Non-pegylated liposomal doxorubicin drug dose schedule 49

4.9 Dose delays/dose modifications 50

4.9.1 Criteria for recycling and dose delays study drugs 50

4.9.2 Criteria for T-DM1 dose modifications in case of specific toxicities 52

4.9.3 Study drug dose modification for cardiotoxicity 55

4.10 Toxicity criteria 57
4.10.1 Definition of dose-limiting toxicities (DLTs) 58
4.10.2 Adverse events of special interest (AESIs) for T-DM1 61
4.11 Study assessments 61
4.11.1 Schedule of assessments 63
4.11.2 Serum HER2 ECD assessment 65
4.11.3 Pharmacokinetic assessment 65
4.12 Statistical considerations and analysis plan 66
4.12.1 Sample size and statistical methods 66
4.12.2 Analysis populations 66
4.12.3 Safety analyses 67
4.12.4 Efficacy analyses 68
4.12.5 Pharmacokinetics Analyses 68
4.13 General concomitant medication and supportive care guidelines 70
4.14 Women of childbearing potential and mandatory use of contraceptive methods 70
4.15 Prohibited therapies 71

5. Results 75
5.1 Study population 75
5.1.1 Dates of the study 75
5.1.2 Patients disposition 75
5.1.3 Demographic and baseline characteristics 75
5.1.4 Concomitant treatment 77
5.2 Treatment exposure 78
5.3 Concomitant medication 80
5.4 MTD determination 80
5.5 General safety 80
5.6 Cardiac safety 82
5.7 Antitumor efficacy 83
5.8 Pharmacokinetics analysis 83

6.Discussion 89

7.Conclusions 93

8.References 97

Safety and pharmacokinetic study of the combination of trastuzumab emtansine and non-pegylated liposomal doxorubicin for the treatment of advanced HER2 positive breast cancer

TABLE OF ABBREVIATIONS

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: Erythropoiesis 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 Harmonization

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

LPLV: Last patient, last visit

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 cells

Safety and pharmacokinetic study of the combination of trastuzumab emtansine and non-pegylated liposomal doxorubicin for the treatment of advanced HER2 positive breast cancer

SUMMARY

Title

Study to evaluate the safety and preliminary activity of the combination of trastuzumab-emtansine (T-DM1) and non-pegylated liposomal doxorubicin for the treatment of Her2-positive advanced breast cancer

Objectives

Trastuzumab emtansine (T-DM1) has been shown to be effective in previously treated HER2-positive (HER2+) metastatic breast cancer (MBC). However, less than half of the patients achieve an objective response and all the patients will eventually progress and require a new line of treatment. In view of the overall favorable safety profile of T-DM1 and the synergistic effects observed with the combination of anthracyclines and HER2-targeting agents, it is hypothesized that T-DM1 and non-pegylated liposomal doxorubicin (NPLD) may be safely combined for enhanced antitumor activity.

Patients and methods

This single-arm, open-label, multicenter, phase Ib study (NCT02562378) enrolled subjects with anthracycline-naïve HER2+ MBC that had progressed on trastuzumab and taxanes. A standard 3+3 dose-escalation design was used, followed by a dose-expansion cohort. Patients received a maximum of 6 cycles of NPLD intravenously (IV) at various dose levels (45, 50, and 60 mg/m^2) in the dose-escalation part and at 60 mg/m^2 during expansion every 3 weeks (Q3W) plus standard doses of T-DM1. The primary endpoint was to determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of this combination.

Results

A total of 15 patients were included (12 patients during the dose-escalation part and three additional patients in the dose-expansion cohort). One patient experienced a DLT at the 60 mg/m^2 dose level (grade 4 neutropenia lasting 13days). The MTD was T-DM1 3.6 mg/kg plus NPLD 60 mg/m^2 administered IV Q3W. No clinically relevant worsening of cardiac function was reported. The overall response rate among all evaluable patients was 40.0%, with a median duration of responseof 6.9months. The clinical benefit rate was 66.7% and median progression-free survival was 7.2 months (95%CI, 4.5–9.6). No significant influence of NPLD on T-DM1 pharmacokinetics was observed.

Conclusions

The combination of NPLD and T-DM1 is feasible. Unfortunately, the addition of NPLD does not seem to improve the antitumor efficacy of T-DM1 in patients with HER2+ MBC.

Título

Estudio para evaluar la seguridad y la actividad preliminar de la combinación de trastuzumab-emtansina (T-DM1) y doxorrubicina liposomal no pegilada para el tratamiento del cáncer de mama avanzado HER2-positivo

Objetivos

Trastuzumab-emtansina (T-DM1) ha demostrado ser eficaz en el tratamiento del cáncer de mama HER2-positivo (HER2 +) metastásico. Sin embargo, en menos de la mitad delos pacientes se logra una respuesta objetiva y todos ellos progresarán y requerirán una nueva línea de tratamiento. Teniendo en cuenta el perfil de seguridad favorable del T-DM1 y el efecto sinérgico observado con la combinación de antraciclinas y agentes anti-HER2, se genera la hipótesis de que el T-DM1 y la doxorubicina liposomal no pegilada podrían ser combinados de manera segura logrando mejorar su actividad antitumoral.

Material y métodos

Este estudio es un ensayo de fase Ib, de un solo brazo, abierto (NCT02562378) en el que se incluyeron pacientes con cáncer de mama metastásico HER2- positivo que no habían recibido tratamiento previo con antraciclinas, y que además habían progresado a trastuzumab y taxanos. Tiene un diseño 3+3 de escalada de dosis seguido de una fase de expansión. Los pacientes recibieron un máximo de 6 ciclos de doxorubicina liposomal no pegilada intravenosa con varios niveles de dosis (45, 50 y 60 mg/m^2) en la parte de escalada de dosis y con 60 mg/m^2 durante la fase de expansión, cada 3 semanas, junto con T-DM1 a una dosis estándar. El objetivo principal fue establecer la máxima dosis tolerada y las toxicidades limitantes de dosis de esta combinación.

Resultados

Se incluyeron un total de15 pacientes (12 pacientes en la parte de escalada de dosis y tres pacientes adicionales en la parte de expansión). Un paciente sufrió una toxicidad limitante de dosis con 60 mg/m^2 (neutropenia grado 4 durante 13 días). La máxima dosis tolerada de T-DM1 fue de 3.6 mg/kg en combinación con 60 mg/m^2 de doxorrubicina liposomal no pegilada, administrados vía intravenosa cada 3 semanas. No se reportó un deterioro clínicamente relevante de la función cardiaca. Entre todos los pacientes evaluables, la tasa de respuesta global fue el 40,0% con una mediana de duración de la respuesta de 6,9 meses; la tasa de beneficio clínico fue del 66,7% y la mediana de supervivencia libre de progresión fue de 7,2 meses (IC 95%,4,5–9,6). No se observó una influencia significativa de la doxorrubicina liposomal no pegilada en la farmacocinética del T-DM1.

Conclusiones

La combinación de T-DM1 con doxorrubicina liposomal no pegilada es factible. Sin embargo, la adición de doxorrubicina liposomal no pegilada no parece mejorar la eficacia antitumoral del T-DM1 en pacientes con cáncer de mama metastásico HER2-positivo.

Safety and pharmacokinetic study of the combination of trastuzumab emtansine and non-pegylated liposomal doxorubicin for the treatment of advanced HER2 positive breast cancer

1. BACKGROUND

1. Background

1.1 HER2-positive metastatic breast cancer

Breast cancer is the most common cancer among women. In 2012 around 1.7 million new cases of breast cancer were diagnosed around the world. Approximately 232,340 new cases of invasive breast cancer and 39,620 breast cancer deaths were expected to occur among US women in 2013 (DeSantis et al. 2014). Approximately 7,900ofthesedeaths wererelatedtoHER2-positivebreast cancer. Human epidermal growth factor receptor 2 (HER2) is a growth factor receptor gene that is amplified in approximately 20% of breast cancers (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 etal.2005;D.Slamon etal.2011).

More recently, the final overall survival analysis of the CLEOPATRA study with a dual blockade of the HER2 protein plus docetaxel led the authorities to approve this combination as 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-targeted combination therapy beyond progression for HER2-positiveMBCis 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 induces apoptosis through inhibition of microtubule assembly, with greater potency than vinca alkaloids and paclitaxel) (Widdison et al. 2006) with the antitumor activity of trastuzumab. T-DM1 uses a non-reducible thioether linker (MCC) to combine the antibody and the cytotoxic agent (Junttila et al. 2011). The stability of MCC was shown to strongly contribute to the favorable activity and toxicity profiles of T-DM1, by selectively delivering 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 following 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 agent since, once bound, T-DM1 retains the hypothesized mechanisms of action of trastuzumab including the flagging of HER2-positive tumor cells for destruction by antibody-dependent cellular toxicity and inhibiting HER2 signaling (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 TDM3569g study 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 had progressed on a trastuzumab-containing chemotherapy regimen. A total of 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 Phase I study. The confirmed overall response rate (ORR) in patients with measurable disease at the 3.6 mg/kg Q3Wschedule 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.

The clinical efficacy and safety of T-DM1 has been established in several phase II and III trials. T-DM1 was active in metastatic breast cancer patients with ≥ 1 prior HER2-directed therapies in 2 single- arm phase II trials (Study TDM4258g and Study TDM4374g) with ORRs of 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 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%) according to the investigator assessment and 25.9% (95% CI: 18.4%–34.4%) according to the Independent Review Board (IRB). The clinical benefit rate (CBR) was 46.3% as per the investigator assessmentand39.3%according to the independent review. The median PFS was 4.6months 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% based on independent review and 47.3% based on investigator assessment. The most common adverse events (AEs) (occurring in ≥20% of patients) were fatigue 5.2%),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 was 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: 21June 2010) withamedianfollow-upof17.4 months demonstrated an ORR (complete or 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% according to the independent review and46.4% according to investigator assessment. The median duration of response (DoR) and PFS as per 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% based on 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 reported by 28 patients (25.5%), the most common being cellulitis (3.6%), pyrexia (2.7%), and pneumonia (2.7%). One patient reported 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 failure (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, 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 for the first-line treatment of 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 as-

sociated 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 MBC was the EMILIA study (Verma et al. 2012). This trial compared T-DM1 with capecitabine plus lapatinib in HER2-positive breast cancer patients who had been previously treated with taxanes and trastuzumab as first, second or third-line therapy. PFS and OS were 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 was performed after investigator documented disease progression. T-DM1significantly 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 could be expected with DM1, such as a 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 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) was characterized in one Phase I study (TDM3569g) and three Phase II studies (TDM4258g, TDM4374g and TDM4688g).

For study TDM3569g, the final PK parameters estimates based on non-compartmental PK analysis for Q3W and QW regimens of trastuzumab emtansine administration are presented in Table 1 (Cycle 1, mean (SD)).

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,

Table 1: 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.

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 (Girishetal.2012).PK parameters for trastuzumabemtansine, 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 [T1⁄2], 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 dysfunctions are among the most common side effects of anthracyclines. Endomyocardial biopsy data and troponin I measurements suggest that myocyte injury may occur early after exposure to these drugs; however, clinical manifestation may only become apparent later due to cardiac reserves and the activation of compensatory mechanisms. Clinically, early cardiac events are reversible and include arrhythmias, repolarization changes, pericarditis and, less frequently, myocarditis. Late anthracycline cardiotoxicity includes cardiomyopathy and systolic heart failure. Patients treated with doxorubicin are five times more likely to develop chronic heart failure or a reduction in LVEF compared to those treated with non-anthracycline regimens. Moreover, cardiotoxicity induced by doxorubicin is dose-dependent, with 300 mg/m^2 being the maximum tolerated cumulative dose, although there is substantial heterogeneity among patients. The mechanism of doxorubicin-induced cardiotoxicity is not fully understood. The drug enters myocytes where it causes mitochondrial dysfunction with consecutive changes in calcium and contractile function. Further increase in the drug concentration causes myocyte cell death (Suter and Ewer 2013). One strategy to avoid the increased cardiac risk of doxorubicin is pharmacokinetic modification using non-pegylated liposomal encapsulation.

On the other hand, trastuzumab cardiotoxicity has been widely studied, especially when administered in combination of anthracyclines. Based on observations from phase III trials of trastuzumab plus anthracyclines, a correlation between time of administration of anthracyclines and trastuzumab has been found, which suggests a high risk of cardiotoxicity in the concomitant administration. From a mechanistic point of view, trastuzumab may act as a modulator of anthracycline toxicity when administered during a period of myocyte vulnerability following anthracycline exposure. Based on these observations, the following risk factors for trastuzumab-associated cardiotoxicity have been identified: prior treatment with anthracyclines, borderline LVEF, pre-existing 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, for first line treatment of MBC have shown a statistically significant reduction in cardiac toxicity while preserving similar antitumor efficacy (Mayer etal.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.

1.6 Rationale to study the combination of T-DM1 and non-pegylated liposomal doxorubicin

T-DM1 is a major conceptual and clinical advance in the treatment of HER2-positive metastatic breast cancer but there are two observations that emphasize the need to investigate combination strategies: first, the response rate of T-DM1 when given alone in metastatic disease is less than 50%, and second, although the median duration of response is prolonged, progression inevitably occurs. Some of the combinations tested to date include T-DM1 and a taxane +/-pertuzumab, T-DM1 plus capecitabine or T-DM1 plus pertuzumab, which 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 was superior to trastuzumab and paclitaxel (Baselga et al. 1998).
- In the pivotal phase III trial of trastuzumab for first-line treatment of 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 in the trastuzumab/AC arm compared to 3% with AC alone (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 following these observations, unexpected at the time, of the increased cardiotoxic potential of this combination. Subsequent trials, using careful cardiac monitoring, have 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, which considerably decreases the risk of cardiotoxicity, was administered. The combination of non-pegylated liposomal doxorubicin, trastuzumab and paclitaxel (MTP) was investigated in a phase I–II trial, as first-line treatment for patients with HER2-overexpressing locally advanced BC or MBC and no prior exposure to anthracyclines, taxanes, or trastuzumab (Cortes et al. 2009). No patients 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 assigned 181 patients to receive MTP and 183 to TP, with a median PFS of 16.1 and 14.5 months in the MTP and the TP arms, 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 another hand, only one study, TDM4688g, 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 and it had no meaningful effect on the corrected QT interval in these patients (Gupta et al. 2013). At Cycle 1 Day 1 and 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 15 minutes and 60 minutes 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 a 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 to be expected that T-DM1 and non-pegylated liposomal doxorubicin may be safely combined and thus potentially provide increased antitumor activity compared to either agent alone.

2. WORK HYPOTHESIS

2. Work hypothesis

T-DM1 has shown significant antitumor activity and favorable toxicity profile in HER2-positive metastatic breast cancer (MBC). HER2-targeted therapy plus anthracyclines have proven to be highly active, despite concerns about cardiotoxicity. Unfortunately, current cardiac imaging only detects cardiotoxicity when myocardial damage is fully established and the use of serum cardiac markers for early detection of cardiotoxicity before functional impairment occurs needs further clinical validation.

Trastuzumab emtansine (T-DM1) has been shown to be effective in previously treated HER2-positive metastatic breast cancer (MBC). However, less than half of the patients achieve an objective response and all the patients will eventually progress and require a new line of treatment. In view of the overall favorable safety profile of T-DM1, and the synergistic effects observed with the combination of anthracyclines and HER2-targeting agents, it is hypothesized that T-DM1 and non-pegylated liposomal doxorubicin (NPLD) may be safely combined for enhanced antitumor activity.

3. OBJECTIVES

3. Objectives

3.1 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 a taxane and trastuzumab-based therapy.

Primary Endpoint: 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 a dose-limiting toxicity (DLT) during the first two cycles of study treatment.

3.2 Secondary objectives

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

Secondary Endpoints:

- Overall response rate (ORR). Overall response rate (ORR) is defined as the proportion of patients with the best overall response of confirmed complete response (CR) or partial response (PR) based on the 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 the local investigator's assessment.

- Number of patients with progression and number of patients who die.

- To assess the safety profile of the combination of T-DM1 and non-pegylated liposomal doxorubicin, as defined by all toxicities reported during the study. NCI-CTCAE version 4.0 and the New York Heart Association (NYHA) criteria (for cardiotoxicity) were used to evaluate the clinical safety of the treatment in this study. Patients were 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 percentage points or LVEF <50%, develop left ventricular dysfunction IV NYHA, discontinue any of the study drugs due to cardiac function or die due to a 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.

- o 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 PK profile of T-DM1, non-pegylated liposomal doxorubicin and each one of their 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 datawillbecomparedwithhistoricaldataof T-DM1alone and non-pegylated liposomal doxorubicin alone.

4. MATERIAL AND METHODS

4. Material and methods

4.1 Study design

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

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 would be considered as 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 experienced a DLT during level 1.

4.2 Dose escalation schema: description of cohorts

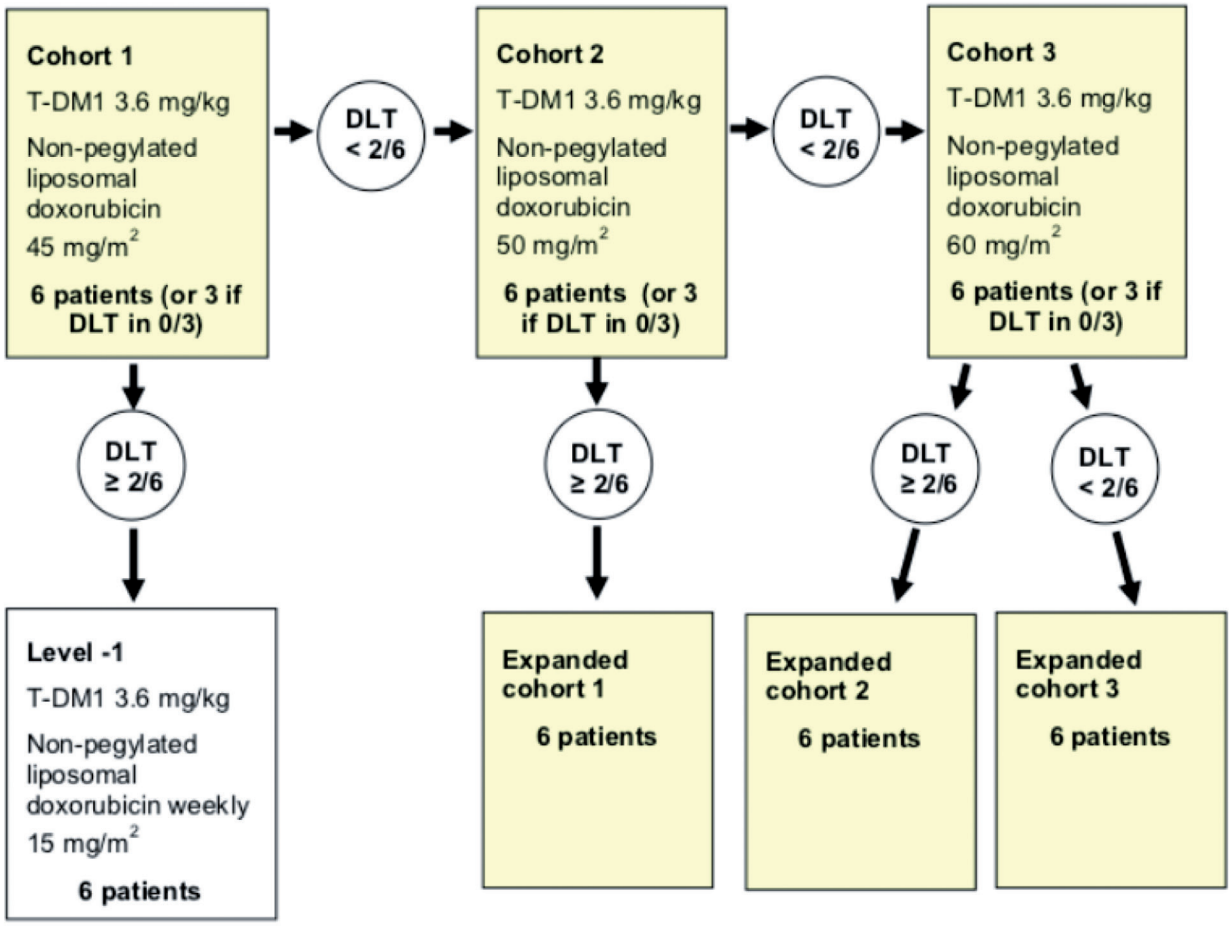


Figure 1: Design of study cohorts.

Cohort 1: Patients would be treated with a fixed dose of T-DM1 of 3.6 mg/kg IV every 3 weeks. Non- pegylated liposomal doxorubicin was administered at a dose of 45 mg/m2. If none of the first 3 patients included experienced a DLT (0/3), the next patients would be enrolled in the subsequent cohort. If1 of the first 3 patients included experienced a DLT, 3 more patients would be included for a maximum of 6. If less than 2 patients in the total group of 6 patients experienced any DLT (0/3 or 1/6), then the following patients would be included in Cohort 2. However, if 2 or more patients suffered any DLT (≥2/6), then cohort 1 would be declared too toxic and level -1 would be opened.

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

next patients would be included in Cohort 3. However, if 2 or more patients suffered any DLT (≥2/6), then cohort 2 would be declared too toxic, the schedule of cohort 1 would be declared the recommended dose for phase II trials (RP2D) and an expansion cohort of 6 more patients would be added to cohort 1.

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

Level -1: Three patients would be treated with a fixed dose ofT-DM1 of3.6 mg/kg IV every 3 weeks. Non-pegylated liposomal doxorubicin would be administered at a weekly dose of 15 mg/m^2. If 0 or 1 of the first 3 included patients experienced a DLT,3 more patients would be included for a maximum of6.Ifless than 2 patients in the total group of 6 patients experienced any DLT (0/6 or 1/6), then this schedule would be declared the recommended dose for phase II trials (RP2D). The Steering Committee would review toxicities and could decide to add the expansion cohort to level -1 in the event it was necessary to have a dose level -1. However, if 2 or more of 6 patients showed a DLT (≥ 2/6), then level -1 would be declared too toxic and the study would end.

No dose escalation within the cohorts was permitted. Patients assigned to a cohort remained in their study cohort for the duration of the study. The recommended phase II dose (RP2D) would not exceed the highest dose level of the combination of T-DM1 and non-pegylated liposomal doxorubicin at which <1 of 6 patients experienced a DLT. If the MTD was exceeded during escalation, de-escalation could not be considered.

4.3 Duration of study treatment

The study treatment period was defined as the time between study entry and the last dose of the study combination (T-DM1 + non-pegylated liposomal doxorubicin) therapy.T-DM1 ad-

ministration could continue as a single agent until disease progression or development of intolerable toxicity, whichever occurred first.

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

Study treatment could continue until one of the following criteria applied:

- Radiologically confirmed and documented unequivocal disease progression, with the exception of new CNS metastases or isolated progression of previously treated CNS lesions. Patients with controlled disease outside of the CNS, defined as confirmed PR or CR of any duration, or SD for ≥3 months, but who had developed CNS metastases that were treatable with radiation, would be allowed to continue receiving study therapy until they either experienced systemic progression of their disease outside of the CNS and/or further progression in the CNS that could not be treated with additional radiation.
- Adverse event(s) that, according to the protocol or in the judgment of the investigator, could cause severe or permanent harm or which ruled out continuation of study drug.
- General or specific changes in the patient's condition that rendered the patient unacceptable for further treatment in the judgment of the investigator.
- Suspected patient's pregnancy.
- Serious non-compliance with the study protocol.
- Investigator removed the patient from study.
- Death.
- Lost to follow-up.
- Withdrawal of consent.
- The study site or the sponsor decided to close the study.

All patients who had not progressed and were still receiving T-DM1 therapy at the close of the study but who were not eligible to receive the T-DM1 treatment in a reimbursement setting, could continue to receive the drug according to study procedures.

4.4 Duration of the follow-up period

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

4.5 End of study (EoS)

EoS was defined as the "last patient, last visit" (LPLV) at the end of the follow-up period. This was the last data collection point. LPLV was expected to occur at approximately one year after the last patient has been enrolled in the study.

4.6 Study population

4.6.1 Target study population

This study enrolled patients with histologically or cytologically confirmed HER2-positive MBC that had 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 were eligible. Evidence of measurable or evaluable metastatic disease was required.

4.6.2 Inclusion criteria

Patients met the following criteria for study entry:

1. Signed informed consent prior to any study specific procedure.

2. Able and willing to comply with protocol.

3. Cytologically or histologically confirmed carcinoma of the breast.

4. Incurable locally advanced or metastatic disease previously treated with up to two previous chemotherapy regimens in this setting (patients starting first, second or third line of treatment were eligible). Patients should 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, 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. Patients with non- measurable lesions could be included with these exceptions:
   - Patients with only blastic bone lesions
   - Patients with only pleural, peritoneal or cardiac effusion, or meningeal carcinomatosis

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 patients with Gilbert's syndrome. Gilbert's syndrome was suspected in people who had persistent, slightly elevated levels of unconjugated bilirubin without any other apparent cause. A diagnosis of Gilbert's syndrome had to 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's syndrome, the total bilirubin value had to be ≤3 x ULN.
    b. Alkaline phosphatase ≤2.5 × the ULN (≤5 × the ULN if liver and/or bone metastases were present).
    c. AST (SGOT)/ALT (SGPT) ≤1.5 x ULN (<3 x ULN if liver metastases were present).
    d. Creatinine 1.5 x ULN and calculated creatinine clearance ≥50 mL/min per the Cockcroft-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. Women of childbearing potential (including premenopausal women who had had a tubal ligation) and all women not meeting the definition of postmenopausal (≥12 months of amenorrhea) and who had not undergone surgical sterilization with a hysterectomy and/or bilateral oophorectomy and men with partners of childbearing potential had to agree(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 7months after the last dose of study. For all other women, there had to be documentation present in the medical history confirming that the patient was not of childbearing potential.

4.6.3 Exclusion criteria

Patients must not meet any of the following criteria in order to participate in the study:

1. Prior 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 had 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 within21days (7days 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 was not allowed if:
   a. The last fraction of radiotherapy had been administered within 21 days prior to the first study drug administration (except for brain irradiation: at least 28 days were required).
   b. More than 25% of marrow-bearing bone had 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 were allowed to participate in this trial if the patient was >4 weeks from radiotherapy completion, was clinically stable with respect to CNS tumor at the time of study entry and was 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 NCI-CTCAE, v4.0.

10. History of a decrease in LVEF to <40% or symptomatic CHF with previous trastuzumab treatment.

11. 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 the 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 were known carriers of hepatitis B virus (HBV), active hepatitis B infection should be ruled out based on negative serologic testing and/or determination of HBV DNA viral load per local guidelines.

13. Women who were pregnant or breast-feeding.

4.7 T-DM1 drug dose schedule

Clinical activity had been observed at a dose of 3.6 mg/kg Q3W in two Phase II studies of single- agent trastuzumabemtansine 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 would 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 might be administered over 30 minutes (±10 minutes). T-DM1 would 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 coincided with a holiday or any other organizational circumstance that did not allow admin-

istration of the study drugs on the scheduled date, the treatment would have to be performed within 3 days of the scheduled date on the earliest possible following date.

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

Vital signs were assessed before and at any time within 60 min after the end of T-DM1 administration. Following the initial dose, patients were 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 ofT-DM1 could be administered over 30 minutes (±10 minutes), with a minimum 60 minute observation period following the end of the infusion. Local health authority guidelines were followed with regard to further observation and monitoring, where applicable.

No premedication was 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) could be given at the investigator's discretion.

4.8 Non-pegylated liposomal doxorubicin drug dose schedule

Non-pegylated liposomal doxorubicin doses were selected as being in the range of efficacious doses for single-agent or combination use. Non-pegylated liposomal doxorubicin was administered over approximately 60 minutes starting 60 minutes after the end of T-DM1 infusion. Non-pegylated liposomal doxorubicin was given with standard antiemetics, in accordance with each site policies

This clinical trial was designed using a modified 3+3 dose escalation design where3patients were included in a given cohort and followed to observe if they experienced any DLT during the first two treatment cycles. If none of the first 3 patients included in a cohort experienced a DLT (0/3), the next patients were to be enrolled in the subsequent cohort. If 1 of these first 3 patients experienced a DLT, 3 more patients would be included in the same cohort to determine the number of patients who experienced DLTs in the total group of 6 patients. If 2 or more of the 6 patients in a given cohort experienced a DLT, the previous cohort would be evaluated and the dose given to this cohort established as the maximum tolerated dose (MTD) (note: if 2 or more of the first 3 patients included in cohort 1 experienced a DLT, level -1 would be explored). Once the MTD was established, 6 additional patients would 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 6 patients or 0 of 3 patients experienced a DLT during the first two cycles of the study treatment. The Steering Committee would review toxicities and could decide to add the expansion cohort to level -1 in the event it was necessary to have a dose level -1.

No dose escalation within the cohorts was permitted. Patients assigned to a cohort remained in their study cohort for the duration of the study.

Dose escalation of non-pegylated liposomal doxorubicin to the next level did not occur until all patients in the cohort had completed cycle 2 and the Steering Committee and study site Investigator(s) had been able to review all toxicities and approval was given for the next dose escalation step.

After the two first cycles, the study drug combination (T-DM1 and non-pegylated liposomal doxorubicin) was administered for up to 6 cycles (including the first 2 cycles).After that,T-DM-1treatmentcouldcontinueas a single agent until disease progression or development of intolerable toxicity, whichever occurred first.

4.9 Dose delays/dose modifications

Since the potential adverse events associated with T-DM1 and non-pegylated liposomal doxorubicin partly overlap (for instance, cardiac events, liver toxicity, hematological toxicity or mucositis), dose delays or modifications were applied to both drugs.

4.9.1 Criteria for recycling and dose delays study drugs

Patients were assessed for toxicity prior to each dose; dosing occurred only if the results of the clinical assessments and laboratory test values were acceptable.

T-DM1 and non-pegylated liposomal doxorubicin were administered every 21 days only if the following criteria were 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 could be delayed for a maximum of 3 weeks (maximum duration of a cycle was 42 days). Dose delays and reductions were designed to maximize treatment for those patients who responded to or derived clinical benefit from treatment while ensuring patient safety. Dose delays due to T- DM1 related toxicities and/or non-pegylated liposomal doxorubicin-related toxicities other than infusionreactions, thrombocytopenia, hepatotoxicity, neurotoxicity, cardiotoxicity and interstitial lung disease (ILD) or pneumonitis were as follows:

- If significant treatment-related toxicities (other than infusion reactions, thrombocytopenia, hepatotoxicity, neurotoxicity and cardiotoxicity) did not recover to Grade 1 or baseline, the next scheduled dose could be delayed for up to 42 days from the last dose received. "Significant" and "related" were based on the judgment of the investigator (in consultation with the Sponsor's Medical Monitor or designee, when appropriate).

- In general, when the significant and related toxicity or any other toxicity that the investigator chose to delay dosing for (other than infusion reactions, thrombocytopenia, hepatotoxicity, neurotoxicity and cardiotoxicity) resolved to Grade 1 or baseline, the patient could resume T-DM1 and non-pegylated liposomal doxorubicin if the delay did not exceed 42days from the last study treatment received. In general, patients were to be re- evaluated weekly during the delay, whenever possible. In cases of patients who experienced a Grade 3 or 4 hematologic event, it was mandatory for the re-evaluation to be done at least weekly until recovery. If dosing resumed, the patient could receive T-DM1 and non-pegylated liposomal doxorubicin either at the same dose level as before or at one lower dose level.

- Non-pegylated liposomal doxorubicin had to be held in the event of any Grade 3–4 toxicities attributable to non-pegylated liposomal doxorubicin until resolution to Grade ≤1 or baseline grade

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

- If a patient required a dose reduction, T-DM1 and/or non-pegylated liposomal doxorubicin dosing were reduced by one dose level. No dose re-escalation was allowed (see Table 2 below).

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 had to be reduced by one dose level in the event of grade 3 or 4 mucositis or grade 2 mucositis persisting at day 21, febrile neutropenia or an infection of more than grade 2 after a 1-week delay. Non-pegylated liposomal doxorubicin dose was also to be reduced by one dose level for any adverse event leading to a dose reduction of T-DM1.

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

- ANC <0.500 x 10^9/L for >7days
- 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 was delayed, T-DM1 also had to be held until both drugs could be administered, unless one or both were permanently discontinued.

If toxicity did not resolve within 42 days from the last study treatment received, the patient had to be discontinued from the study treatment and followed for disease progression and survival outcome.

4.9.2 Criteria for T-DM1 dose modifications in case of specific toxicities

T-DM1 dose modification for thrombocytopenia

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 anticoagulant treatment have to be monitored closely during treatment with trastuzumab emtansine. Platelet counts had to be obtained no less frequently than weekly to evaluate recovery whenever any of the events listed below occurred, prior to each trastuzumab emtansine dose.

Monitoring follow up of thrombocytopenia events:

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

  Note: although complete blood counts with platelets were required within 72 hours prior to study treatment administration at each cycle, the investigator could 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), T-DM1 was not administered until platelet counts had recovered to Grade 1 (≥75 x 10^9/L). Then the patient was treated at the same dose level.

- Patients receiving T-DM1 who experienced a first Grade 4 thrombocytopenia event could, 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 experienced a Grade 4 thrombocytopenia event could, 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 experienced a Grade 4 thrombocytopenia event at the 2.4 mg/kg dose level were discontinued from the study treatment.

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

T-DM1 dose modification for hepatotoxicity

Concurrent elevations of ALT/AST and bilirubin meeting Hy's Law laboratory criteria: regardless of dose level, T-DM1 had to 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 had to be permanently discontinued in patients who were diagnosed with NRH.

Transaminase elevations or bilirubin elevation requiring dose adjustment: patients who experienced a ≥Grade 3 elevation of liver function had to be checked twice weekly for the recovery of transaminases and/or total bilirubin. If a patient's transaminases and/or total bilirubin did not recover within 42 days from the patient's last dose of study treatment received, the patient was discontinued from the study treatment.

No re-escalation of the T-DM1 dose was 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 ≤ 10x ULN	Grade 4 >10 x 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 was allowed. A patient requiring more than two dose reductions had to discontinue study treatment

Table 4. Trastuzumab emtansine dose modification: serum ALT or AST

Grade 2 >3 to ≤5 x ULN	Grade 3 >5 to ≤20 x ULN	Grade 4 >20 x ULN
Treat at the same dose level	Do not administer T-DM1 until total bilirubin recovers to Grade ≤2, and then reduce one dose level	Discontinue T-DM1

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

T-DM1 dose modification for neurotoxicity

Patients receiving T-DM1 who experienced Grade 3 or 4 peripheral neuropathy that did no-tresolveto Grade ≤2 with in 42 days after the last dose received had to be discontinued from study treatment.

T-DM1 dose modification/management for infusion-related reactions

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

Patients who experienced T-DM1 infusion-related temperature elevations to > 38.5°C and/or other infusion-related symptoms could 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 were to be managed with supportive care, such as oxygen, beta agonists, antihistamines or antipyretics, at the investigator's discretion. Antihistamines and antipyretics could be used before subsequent infusions of T-DM1 at the investigator's discretion. Medication with corticosteroids might be used after cycle 2. Patients had to be monitored until complete resolution of symptoms. In the event of a true hypersensitivity reaction (i.e., if the severity of the reaction increased with subsequent infusions), T-DM1 treatment had to be permanently discontinued. Patients who experienced a Grade ≥3 hypersensitivity reaction or acute respiratory distress syndrome (ARDS) had to be discontinued from the study. Patients who experienced a severe delayed infusion reaction had to be discontinued from study treatment.

T-DM1 Dose Modification for pulmonary toxicity: cases of 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 had to be permanently discontinued in patients who were diagnosed with ILD or pneumonitis.

4.9.3 Study drug dose modification for cardiotoxicity

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

LVEF was monitored during the last week of cycles 1, 2, 3, 4, 5 and 6, and every 9 weeks thereafter. If the LVEF was reported as a range, the median of the range was to be taken.

In this protocol cardiotoxicity was defined as follows:

- Level 1 cardiotoxicity was 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%
- Level 2 cardiotoxicity was defined as:

- An absolute drop ≥10% resulting in a final LVEF <50% and asymptomatic or heart failure NYHA criteria class II

For cardiotoxicity cases, the algorithm for continuation or discontinuation of the combination of study drugs was as follows:

- For patients with level 1 cardiotoxicity (as defined as DLT in Table 2) -> Discontinue T-DM1 and non-pegylated liposomal doxorubicin according to the algorithm in Figure 2.

- For patients with level 2 cardiotoxicity, defined as asymptomatic or heart failure NYHA criteria class II and an absolute drop ≥10% resulting in a final LVEF <50% -> Continue or discontinue T-DM1 and non-pegylated liposomal doxorubicin according to the algorithm in Figure 2.

The previous paragraph and above figure summarize 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 study combination was based on this algorithm. Both T-DM1 and non-pegylated liposomal doxorubicin had to be withheld in all patients who had a confirmed drop of LVEF to below 45%. T-DM1 treatment could be resumed If LVEF reassessed within 21 days had recovered to values >50%. Non-pegylated liposomal doxorubicin had to be discontinued permanently. A similar approach was to be followed for patients whose LVEF dropped to values between 45% and 50% with an absolute decrease in LVEF of ≥15% points from baseline. For these patients, the study treatment was to be temporarily discontinued, measurement of the LVEF was to be repeated within 21 days and only T-DM1 could be resumed if the LVEF had recovered to within a15% absolute difference below baseline. For patients whose LVEF dropped to values between 45% and 50% with an absolute decrease in LVEF of <15% points from baseline, non-pegylated liposomal doxorubicin had to be discontinued permanently but treatment with T-DM1 could continue without interruption. If an investigator was concerned that an adverse event could be related to cardiac dysfunction, an additional LVEF measurement could be performed.

If clinically significant cardiac dysfunction or cardiac failure developed or persisted or if significant medical management was required to maintain the ejection fraction, the patient had to be discontinued from study treatment. T-DM1 and non-pegylated liposomal doxorubicin were discontinued as well, as summarized in Figure 2.

In addition, cases in which the elevation in the levels of Troponin I and BNP were >10% over the screening levels were to be considered as AESIs and had to be reported to Steering Committee for assessment and confirmation as to whether or not they were DLTs, in which case T-DM1 and non-pegylated liposomal doxorubicin were to be discontinued and monitored according to Steering Committee's decision.

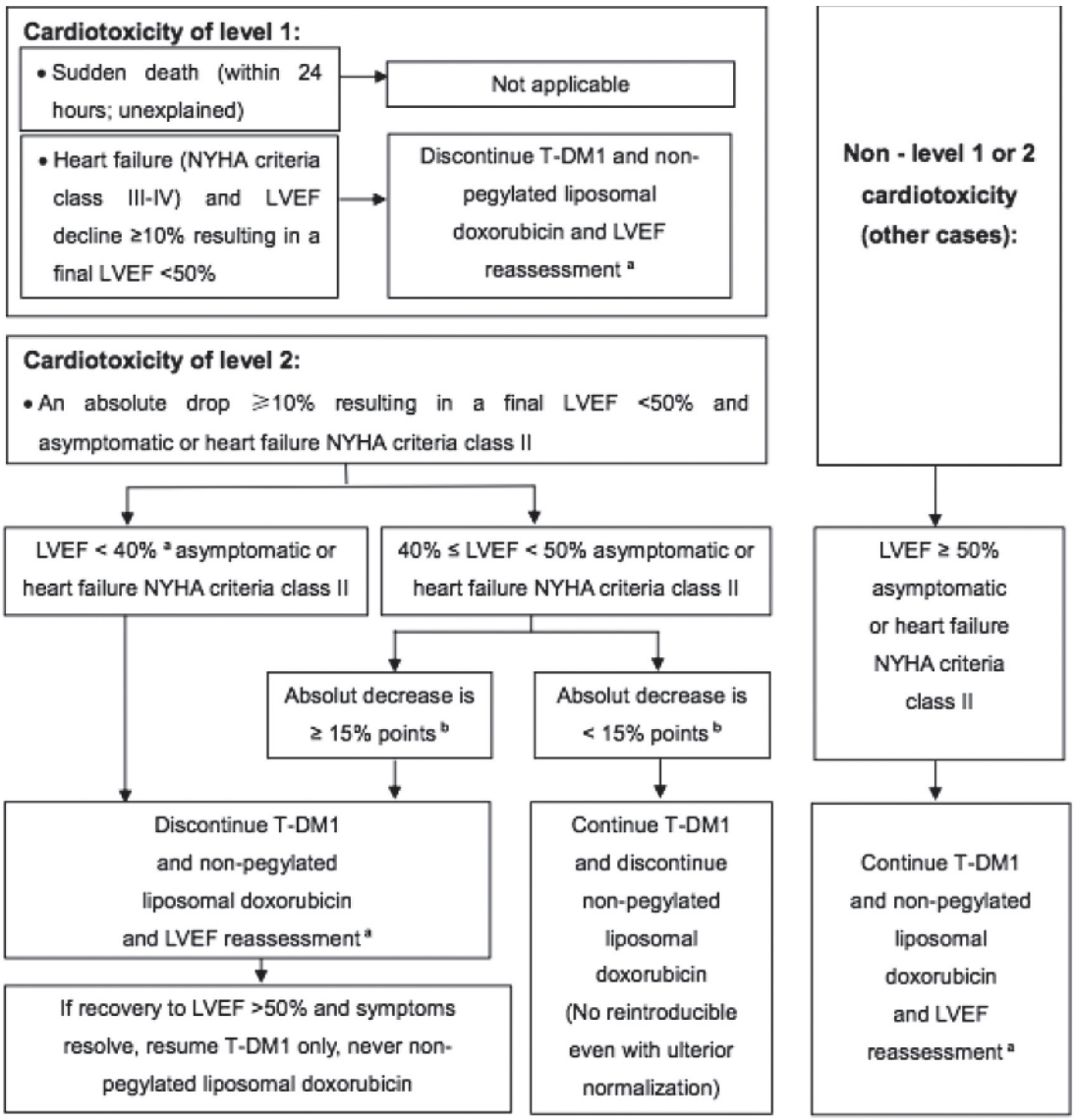


Figure 2. Algorithm for continuation and discontinuation of the combination of study drugs based on Left Ventricular Ejection Fraction assessments.

4.10 Toxicity criteria

All patients who received any study treatment were evaluable for toxicity. Toxicities were 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 explicitly described in CTCAE v4.0) were graded under the category of "investigations – other, specify" with a grading according to Table 5.

Patients were evaluated for safety at the end of cycle 2. After the first two cycles, the study drug combination (T-DM1 and non-pegylated liposomal doxorubicin) was administered for up to

Table 5. Grading of cardiac segmental wall-motion abnormalities

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

6 cycles (including the first2 cycles).T-DM1 treatment could continue as a single agent until disease progression or development of intolerable toxicity, whichever occurred first. Dose of T-DM1 administered as a single agent was the dose level used at the end of study treatment (combination T-DM1 + non-pegylated liposomal doxorubicin).

4.10.1 Definition of dose-limiting toxicities (DLTs)

A Dose-Limiting Toxicity (DLT) was defined as any of the drug-related adverse events described below occurring during the first two cycles of study treatment.

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

When a third dose of study treatment was going to be administered, patients would no longer be evaluated for DLTs in all subsequent cycles.

Any patient who did not complete the DLT assessment (at the end of cycle 2 or pre- dose at cycle 3 day 1) would be replaced, except for patients who ended the study due to a DLT that did not allow them to start cycle 2.

For this study, the following toxicities were defined as DLTs:

1. Hematological toxicities:

- Grade 4 neutropenia (i.e., absolute neutrophil count (ANC) <0.5 x 10^9 cells/L for a duration of at least 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 109 cells/L) which does not recover to ≥75.0 x 109 cells/L before the 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 might have cauterization and this should not be considered as a DLT.

2. 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%.

3. Hepatic toxicity:

- Increase in AST (SGOT)/ALT (SGPT) values to >5 x ULN

- Increase in total bilirubin (TBL) value to >3 x ULN

- 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 (non-hepatotoxic) control agent or placebo.

  - Among subjects showing such aminotransferase(AT) elevations, often with ATs much greater than3 x ULN, some subjects also show cholestasis (serum alkaline phosphatase (ALP) activity >2 x ULN).

- 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.

4. Other Grade ≥3 non-hematological toxicities with the exception of:

- Grade ≥3 diarrhea that recovers to Grade ≤2 after 24 hours of starting recommended antidiarrheal 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 of next cycle.
- Infusion-related reactions (IRR). These are not considered to be DLTs since, based on experience with monoclonal antibodies, IRRs are not dose-related events. Precautions had to be taken if an IRR grade ≥3 occurred. If the described precautions were not sufficient, other options had to be discussed by the sponsor and the investigator.
- Laboratory values of ≥Grade 3 which were judged not clinically significant by the investigator. The following non-hematological toxicities were 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.

If a Grade 2 event required a dose delay, it would not be considered as a DLT. However, if the toxicity did not resolve to Grade1or baseline by Day 42, therefore requiring study treatment discontinuation, it would be considered as a DLT. The SC would adjudicate in cases of DLTs that were not covered by the existing DLT criteria.

Finally, failure to recover from any toxicity adequately treated which resulted in a dose delay of more than 21 days or any toxicity at cycles 1 and/or 2 that compelled a reduction in the 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, ILD including pneumonitis) would be considered a DLT.

In addition, those cases in which the elevation of the levels of Troponin I and BNP was >10% over the screening levels were to be considered as AESIs and had to be reported to Steering Committee for assessment.

4.10.2 Adverse events of special interest (AESIs) for T-DM1

AESIs were reported by the Investigator, regardless of their seriousness (i.e., no more than 24 hours after learning of the event). AESIs for this study included:

- Elevation of Troponin I and BNP values consisting of an increase >10% over 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 had to be reported as an AESI:
    - Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) elevations that were >3 × upper limit of normal (ULN)
    - Concurrent elevation of total bilirubin >2 ×ULN(or clinical jaundice if total bilirubin measurements were not available), except in patients with documented Gilbert's syndrome. For patients with Gilbert's syndrome, elevation of direct bilirubin >3 × ULN was to 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 nonpathogenic, was considered an infectious agent. Transmission of an infectious agent might be suspected from clinical symptoms or laboratory findings indicating an infection in a patient exposed to a medicinal product.

4.11 Study assessments

Medical History and Demographic Data: Medical history included 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 included age, sex, and self-reported race/ethnicity.

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

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

As part of tumor assessment, physical examinations also included the evaluation of the presence and degree of enlarged lymph nodes, hepatomegaly, and splenomegaly.

Tumor and Response Evaluations: All patients were to be evaluable for disease response unless they withdrew from the study due to treatment- related adverse events prior to completion of cycle 2 and had not had any acceptable complete disease assessment.

Measurable and unmeasurable disease was 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 were performed.

CT or MRI of the brain and bone scan had to 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 did not need to be repeated and non-isotopic radiographic modalities could be used to document the extent of bone metastatic disease. In the event a positron emission tomography (PET)/CT scanner was used for tumor assessments, the CT portion of the PET/CT had to meet criteria for diagnostic quality. Tumor assessments had to include an evaluation of all known and/or suspected sites of disease, whenever possible. Patients had to have had lesions selected that could be evaluated at every tumor assessment.

The same radiographic procedures used at screening were used throughout the study (e.g., the same contrast protocol for CT scans). Initial tumor response assessment was performed at the end of cycle 2. Subsequent tumor response assessment was performed at the end of cycles 4 and 6. Thereafter, the tumor response assessment was performed every 9 weeks up to progression or up to 12 months after the first dose of study treatment. Response assessments were assessed by the investigator, based on physical examinations, CT or MRI scans, and bone scans usingRECISTv.1.1.For patients who continued study treatment after isolated brain progression, the frequency of follow-up scans was at the discretion of the investigator. At the investigator's discretion, CT scans, MRI scans, and/or bone scans could be obtained at any time when clinically indicated or if progressive disease was suspected.

If a bone scan could not be performed during the course of the study because of the unavailability of the Tc-99m isotope, the investigator could choose an alternative imaging modality.

Radiographic imaging had to be performed instead of clinical examination unless the lesion(s) being followed could not be imaged but was(were) assessable by clinical examination. In applying RECIST v.1.1, documentation by color photography including a ruler to estimate the size of the lesion was recommended.

Laboratory Assessments

Local Laboratory Assessments: Prospective HER2 status, hematology, pregnancy testing, biochemistry, coagulation and cardiac troponin I, and BNP levels.

Central Laboratory Assessments: Serum T-DM1 and serum non-pegylated liposomal doxorubicin concentrations and total trastuzumab metabolized, 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].

Electrocardiograms and echocardiograms were performed every cycle (cycles 1-6) during treatment with T-DM1 + non-pegylated liposomal doxorubicin.

Thereafter, ECHO and ECG were performed every 9weeks until12months after the last dose of study treatment (T-DM1+ non-pegylated liposomal doxorubicin).

ECOG Performance Status: Performance status was measured using the ECOG performance status scale.

4.11.1 Schedule of assessments

Written informed consent for participation in the study was obtained before performing any study specific screening tests or evaluations. Informed Consent Forms for enrolled patients and for patients who were not subsequently enrolled were 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 could be used; such tests did not need to be repeated for screening.

All screening evaluations were completed and reviewed to confirm that patients met all eligibility criteria before study start.

Visits were based on scheduled 21-day cycles (if no treatment delay due to toxicity occurred). Dose delays and dose reductions were allowed.

Assessments scheduled on the day of study treatment administration had to be performed prior to study treatment administration.

Local laboratory assessments scheduled for Day1 of all cycles had to 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 had to be performed within ± 2 business days. Results of local laboratory assessments were to be reviewed and the review documented prior to study treatment administration.

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

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

Safety follow-up: All patients were followed up for up to 12 months after the first dose of the study combination treatment or up to study termination, whichever occurred first. Cardiac safety was included.

The first safety follow-up visit for the combination treatment was 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 were done every 9 weeks. The last safety follow-up visit took place 28 (+/- 7 days) after the last dose of any investigational medical product.

> *Note: for patients that discontinued the study treatment due to a delay in T- DM1 administration of more than 42 days, the safety follow up visit was done at 42 days (± 5 days) after the last study treatment administration.*

All ≥ grade 2 adverse events were followed until improvement to baseline levels, grade 1 or complete recovery, until the patient withdrew consent, patient's death or up to a maxi-

mum of 12 months after the first dose of study combination treatment, whichever occurred first.

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

4.11.2 Serum HER2 ECD assessment

Peripheral blood was collected at baseline and at cycle 4 in a sterile test tube and following centrifugation serum samples were stored at 20 °C until the time of the assay. After collection of all samples, serum HER2 ECD concentrations were determined by Enzyme-Linked Immunosorbent Assay (ELISA) using the ADVIA® Centaur XP Immunoassay System (Siemens Diagnostics®, Tarrytown, NY,USA) with a detection range of 0.5–350 ng/mL. The assay was conducted in accordance with the manufacturers' instructions and blinded to both patients' characteristics and clinical outcomes.

4.11.3 Pharmacokinetic assessment

The PK sampling rationale was to characterize the PK of T-DM1, total trastuzumab and DM1, in order to assess potential drug-drug interaction when T-DM1 was 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) were 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) - Cmax
- Clearance (CL)
- Distribution volume (Vss)
- T1/2

The PK of T-DM1, total trastuzumab and DM1 was 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 were listed and tabulated by treatment dose and by cohort.

Descriptive summary statistics including arithmetic mean, geometric mean (e.g., AUC and Cmax), median, range, and coefficient of variation were presented for each cohort. Nonlinear mixed effects modeling was also used.

PK samples could be obtained ad hoc in case of SAE or unexpected toxicities which could suggest a potential drug-drug interaction. The exact time of PK sampling was recorded for all samples. Patients had approximately 6 ml of peripheral blood collected at each sampling time point. The extracellular domain of HER2 receptor was also measured, as it has been shown to be a relevant covariate in the population PK modeling for T-DM1. Samples were shipped to QPS Laboratory (Netherlands) or to PPD Laboratory (USA). Samples were analyzed according to methods that have been previously published.

4.12 Statistical considerations and analysis plan

4.12.1 Sample size and statistical methods

The study used 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 could be enrolled. Safety assessment was the primary objective and efficacy assessment was an exploratory objective. All data were presented with listings and summarized using descriptive statistics within each dose level and/or dosing schedule and, overall, in all treated patients.

4.12.2 Analysis populations

The following populations were analyzed:

1. DLT population: all patients who completed the first two cycles of treatment or who stopped treatment during this time because of a DLT.
2. Intentiontotreat(ITT)/safety population: all included patients receiving any dose of treatment.

3. Protocol compliant population (PP): all patients who received the protocol required study drug exposure and required protocol processing.
4. Pharmacokinetics (PK) population: all patients with a complete treatment concentration-time profile.

4.12.3 Safety analyses

Primary outcome:

The number and the proportion of patients with DLTs (with corresponding 95% Clopper Pearson confidence intervals) were the primary outcomes. They were used as the measure for MTD determination. DLTs were summarized by treatment dose. Confidence intervals were calculated, according to Clopper-Pearson (exact binomial intervals). The primary outcome was analyzed in DLT population.

Safety outcomes:

Safety endpoints were analyzed in the 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) were reported separately. They were summarized by treatment dosage and were assessed by total AEs, AEs Grade ≥3, SAEs, 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 were 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 were also provided for the AEs. Patients who died were listed, together with the cause of death.

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

Other safety variables, such as exposure to study medication, concomitant medications and physical examinations, were analyzed in a similar way.

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

4.12.4 Efficacy analyses

Efficacy endpoints were analyzed in ITT and PP populations.

The efficacy analyses included exploratory endpoints and they were investigated as follows:

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

- Clinical benefit rate (CBR). Clinical benefit rate was 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 progression and number of patients who died.

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

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

Response and progression were 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 were used according to the RECIST criteria.

4.12.5 Pharmacokinetics Analyses

Pharmacokinetic analyses were done in the PK population.

The following PK parameters ofT-DM1 and non-pegylated liposomal doxorubicin (including but not limited to those listed below) were determined in all patients who received study treatment, using either non-compartmental and/or population methods, if data allowed:

- 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) - Cmax
- CL - Vd
- T1/2

The PK of trastuzumab emtansine, total trastuzumab and DM1 were 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 were listed and tabulated by treatment dose. Descriptive summary statistics including arithmetic mean, geometric mean (e.g., AUC and Cmax), median, range, SD, and coefficient of variation were presented for each cohort. Nonlinear mixed effects modeling was also used.

The extracellular domain ofHER2 receptor was also measured, as it has been shown to be a relevant covariate in the population PK modeling for T-DM1.

PK analysis was performed with the data of all subjects using Phoenix WinNonlin® version 8.0 (Pharsight, St. Louis, MO, USA) and SAS version 9.3 (SAS Institute, Cary, NC, USA) software. All concentration values that were below the limit of quantification were considered as zero. Missing values were not included in the PK analysis. The estimated Cmax, Tmax, T1/2, and the elimination rate constant (Lambda_z) were calculated. The area under the curve of plasma concentration versus time was also calculated from time zero to the AUClast.

The area under the curve of concentration versus AUCinf was calculated using the linear trapezoidal rule, and the extrapolated AUC percentage of total AUC was calculated as

[AUCinf- AUClast/AUCinf] X 100 (AUCext)

Total CL was calculated as the total dose (mg) divided by AUCinf (CL), and the Vd based on the terminal was calculated as

[CL/Lambda_z]

When AUCext was greater than 20%, AUCinf and its associated parameters (T1/2, CL and Vd) were set as missing, and AUClast was reported. A non-compartmental method (Model 200 of Phoenix WinNonlin® 5.2, Pharsight, St. Louis, MO, USA) was used to estimate the PK parameters of T-DM1, total trastuzumab and DM1.

4.13 General concomitant medication and supportive care guidelines

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

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

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

Once the patient was on study treatment, palliative radiotherapy was permitted to treat pre-existing painful bone metastases or brain metastases (for patients who had disease control outside of the brain).The schedule of palliative radiotherapy would start 48 h after the last dose of non-pegylated liposomal doxorubicin.

Use of bisphosphonates or denosumab was permitted for the control of bone pain, prevention and/or treatment of bone metastases and treatment of osteoporosis. If bisphosphonates were required for the treatment of symptomatic malignancy-associated hypercalcemia, tumor assessments were to be performed to assess for potential disease progression.

4.14 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) were required to have a negative serum pregnancy test within 7 days prior to the first dose of either study medication.

All heterosexually active patients were 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 suspected to be pregnant, T-DM1 and non-pegylated liposomal doxorubicin had to be discontinued immediately. If pregnancy was determined by a positive urine test, the pregnancy had to be confirmed by a serum pregnancy test. If it was confirmed that the patient was not pregnant, the patient could resume dosing.

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

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 required by the study protocols and the prescribing information.

Additional follow-up information on any trastuzumab emtansine-exposed pregnancy and infant would 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).

A serum β-HCG test was 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.

4.15 Prohibited therapies

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

- Erythropoiesis 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) were 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 were 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), were prohibited.

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

Safety and pharmacokinetic study of the combination of trastuzumab emtansine and non-pegylated liposomal doxorubicin for the treatment of advanced HER2 positive breast cancer

5. RESULTS

5. Results

5.1 Study population

5.1.1 Dates of the study

Between October 2015 and December 2017, a total of 15 patients with anthracycline-naïve HER2-positive, unresectable, locally advanced or MBC were enrolled at seven sites.

5.1.2 Patients disposition

Of the 15 patients, 12 patients (80.0%) were distributed into three cohorts during the dose-escalation part (cohorts 1 and 2: three patients in each cohort; cohort 3: six patients), and three patients (20.0%) were included in the dose-expansion part.

5.1.3 Demographic and baseline characteristics

The median age was 50 years (range, 31–62 years), 86.7% had Eastern Cooperative Oncology Group (ECOG) performance status 0, 73.3% had estrogen-receptor positive tumors, 60.0% presented with "de novo" metastatic disease and 73.3% had visceral disease (40.0% with liver metastases). A total of 11 (73.3%), 3 (20.0%) and 1 (6.7%) patients had received prior treatment for advanceddiseaseinthefirst,secondandthird-linesetting,respectively. All patients had previously been treated with a taxane and trastuzumab, and 80.0% had also previously received pertuzumab. Table 6 summarizes the patients' baseline characteristics

Table 6. Demographic and baseline patient characteristics.

Characteristic	Cohort 1 (n = 3)	Cohort 2 (n = 3)	Cohort 3 (n = 9)	Overall (n = 15)
Age, median (range), years	50.0 (39.0– 62.0)	58.0 (57.0– 61.0)	42.0 (31.0– 62.0)	50.0 (31.0– 62.0)
ECOG performance status, *n* (%)				
0	3 (100)	3 (100)	7 (77.8)	13 (86.7)
1	0 (0)	0 (0)	2 (22.2)	2 (13.3)
HER2 expression, *n* (%)				
IHC 3+	2 (66.7)	2 (66.7)	7 (77.8)	11 (73.3)
IHC 2+ and ISH+	1 (33.3)	1 (33.3)	2 (22.2)	4 (26.7)
Hormone receptor status, *n* (%)				
ER-positive	2 (66.7)	3 (100)	6 (66.7)	11 (73.3)
ER-negative	1 (33.3)	0 (0)	3 (33.3)	4 (26.7)
PR-positive	1 (33.3)	2 (66.7)	4 (44.4)	7 (46.7)
PR-negative	2 (66.7)	1 (33.3)	5 (55.6)	8 (53.3)
Disease stage at initial diagnosis, *n* (%)				
I	0 (0)	0 (0)	1 (11.1)	1 (6.7)
II	0 (0)	0 (0)	2 (22.2)	2 (13.3)
III	0 (0)	0 (0)	3 (33.3)	3 (20.0)
IV	3 (100)	3 (100)	3 (33.3)	9 (60.0)
De novo metastatic disease, *n* (%)				
Yes	3 (100)	3 (100)	3 (33.3)	9 (60)
No	0 (0)	0 (0)	6 (66.7)	6 (40)
Sites of metastases, *n* (%)				
Lymph node	2 (66.7)	1 (33.3)	7 (77.8)	10 (66.7)
Bone	3 (100)	3 (100)	4 (44.4)	10 (66.7)
Liver	0 (0)	2 (66.7)	4 (44.4)	6 (40.0)
Lung	0 (0)	1 (33.3)	4 (44.4)	5 (33.3)
Brain	1 (33.3)	0 (0)	1 (11.1)	2 (13.3)
Skin	0 (0)	0 (0)	2 (22.2)	2 (13.3)
Others	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)
Lines of previous treatment for advanced disease, *n* (%)				
1	0 (0)	3 (100)	8 (88.9)	11 (73.3)
2	3 (100)	0 (0)	0 (0)	3 (20.0)
3	0 (0)	0 (0)	1 (11.1)	1 (6.7)
Prior taxane treatment, *n* (%)				
	3 (100)	3 (100)	9 (100)	15 (100)
Prior anthracycline treatment, *n* (%)				
	0 (0)	0 (0)	0 (0)	0 (0)
Prior trastuzumab treatment, *n* (%)				
	3 (100)	3 (100)	9 (100)	15 (100)
Prior pertuzumab treatment, *n* (%)				
	1 (33.3)	3 (100)	8 (88.9)	12 (80.0)

Electrocardiogram showed normal values for QRS duration (80-100; 90.1±8.8), as well as QT duration(normal <420msec;mean385.9±30) and QTc duration(<470 msec;414.5±15.9).Two women in Cohort3 presented bradycardia or incomplete rbbb.

Left ventricular ejection fraction (LVEF) was normal (55-70%; 64.3±4.7). One patient in Cohort 3 had moderate aortic stenosis (not clinically relevant).

5.1.4 Concomitant treatment

Table 7. Concomitant treatment

	Cohort 1 (n=3)	Cohort 2 (n=3)	Cohort 3 (n=9)	Overall (N=15)
Prior Medication				
No	0 (0.0%)	0 (0.0%)	5 (55.6%)	5 (33.3%)
Yes	3 (100.0%)	3 (100.0%)	4 (44.4%)	10 (66.7%)
Prior Medication				
Omeprazole	2 (66.7%)	1 (33.3%)	3 (33.3%)	6 (40.0%)
Paracetamol	0 (0.0%)	1 (33.3%)	2 (22.2%)	3 (20.0%)
Hydrochlorothiazide	1 (33.3%)	1 (33.3%)	0 (0.0%)	2 (13.3%)
Ibuprofen	1 (33.3%)	0 (0.0%)	1 (11.1%)	2 (13.3%)
Alprazolam	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)
Calcium carbonate	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)
Calcium, combinations with vitamin d and/or other drugs	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)
Clopidogrel	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
Denosumab	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)
Dexamethasone	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
Dexketoprofen	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (6.7%)
Ebastine	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)
Enoxaparin	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
Exemestane	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)
Fentanyl	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
Furosemide	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
Levetiracetam	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (6.7%)
Lorazepam	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
Macrogol, combinations	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)
Metoclopramide	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)
Morphine	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (6.7%)
Opioids	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
Other therapeutic products	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
Paroxetine	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (6.7%)
Potassium chloride	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (6.7%)
Quetiapine	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
Ramipril	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)
Tenofovir disoproxil	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (6.7%)
Tramadol	0 (0.0%)	1 (33.3%)	0 (0.0%)	1 (6.7%)

5.2 Treatment exposure

A total of 11 patients (73.3%) completed six cycles of T-DM1 and NPLD: two patients in cohort 1, two patients in cohort 2, four patients in cohort 3 and three patients in the dose-expansion part. The median relative dose intensity for T-DM1 and NPLD was 90.6% and 85.9%, respectively, and the median duration of treatment was 6.3 and 3.7 months, respectively. At the time of the analysis (December 2018), all 15 patients had discontinued study treatment, most commonly because of disease progression (80.0%). Additional reasons for treatment discontinuation were AEs (6.7%), patient request (6.7%), and investigator decision (6.7%). Tables 8 and 9 show the extent of exposure of T-DM1 and NPLD.

Table 8. Extent of exposure of T-DM1 (Safety - ITT)

	Cohort 1 (N=3)	Cohort 2 (N=3)	Cohort 3 (N=9)	Overall (N=15)
Relative Dose Intensity (%)				
Median	99.2	80.7	83.6	85.3
IQR	6.2	20.2	13.5	18.9
Range	6.2	20.2	29.5	29.5
≥50%	3 (100.0%)	3 (100.0%)	9 (100.0%)	15 (100.0%)
≥70%	3 (100.0%)	3 (100.0%)	9 (100.0%)	15 (100.0%)
≥80%	3 (100.0%)	2 (66.7%)	7 (77.8%)	12 (80.0%)
≥90%	3 (100.0%)	1 (33.3%)	3 (33.3%)	7 (46.7%)
≥100%	1 (33.3%)	0 (0.0%)	1 (11.1%)	2 (13.3%)
Total Number of Cycles				
Median	6.0	6.0	6.0	6.0
IQR	4.0	0.0	0.0	0.0
Range	4.0	0.0	4.0	4.0
Total Number of Cycles				
2	1 (33.3%)	0 (0.0%)	1 (11.1%)	2 (13.3%)
4	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
6	2 (66.7%)	3 (100.0%)	7 (77.8%)	12 (80.0%)
Treatment Duration (days)				
Median	106.0	131.0	127.0	113.0
IQR	91.0	28.0	26.0	26.0
Range	91.0	28.0	125.0	126.0
Days on Drug				
Median	6.0	6.0	6.0	6.0
IQR	4.0	0.0	0.0	0.0
Range	4.0	0.0	4.0	4.0
Treatment Delays, Interruptions and Reductions				
Delays	1 (33.3%)	2 (66.7%)	7 (77.8%)	10 (66.7%)
Interruptions	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Reductions	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 9. The Extent of Exposure of NPLD (Safety – ITT)

	Cohort 1 (n=3)	Cohort 2 (n=3)	Cohort 3 (n=9)	Overall (N=15)
Relative Dose Intensity (%)				
N	3	3	9	15
Median	98.6	79.3	83.5	85.9
IQR	13.5	22.0	13.0	20.2
Range	13.5	22.0	30.5	30.5
≥50%	3 (100.0%)	3 (100.0%)	9 (100.0%)	15 (100.0%)
≥70%	3 (100.0%)	3 (100.0%)	9 (100.0%)	15 (100.0%)
≥80%	3 (100.0%)	1 (33.3%)	5 (55.6%)	9 (60.0%)
≥90%	2 (66.7%)	1 (33.3%)	3 (33.3%)	6 (40.0%)
≥100%	1 (33.3%)	1 (33.3%)	1 (11.1%)	3 (20.0%)
Total Number of Cycles				
Median	6.0	6.0	6.0	6.0
IQR	4.0	0.0	0.0	0.0
Range	4.0	0.0	4.0	4.0
Total Number of Cycles				
2	1 (33.3%)	0 (0.0%)	1 (11.1%)	2 (13.3%)
4	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)
6	2 (66.7%)	3 (100.0%)	7 (77.8%)	12 (80.0%)
Treatment Duration (days)				
Median	106.0	131.0	127.0	113.0
IQR	91.0	28.0	26.0	26.0
Range	91.0	28.0	125.0	126.0
Days on Drug				
Median	6.0	6.0	6.0	6.0
IQR	4.0	0.0	0.0	0.0
Range	4.0	0.0	4.0	4.0
Treatment Delays, Interruptions and Reductions				
Delays	1 (33.3%)	2 (66.7%)	7 (77.8%)	10 (66.7%)
Interruptions	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Reductions	0 (0.0%)	0 (0.0%)	1 (11.1%)	1 (6.7%)

5.3 Concomitant medication

Regarding concomitant medication, all women in Cohort 1, one out of three in Cohort 2 and six out of nine in Cohort 3 was treated with proton pump inhibitors (mainly Omeoprazole). Two in Cohort 2 and four in Cohort 3 were treated with anilides (Paracetamol). A total of 33% of the patients in each cohort received benzodiazepine derivatives. Other frequent treatments were colony stimulating factors (33.3%),serotonin antagonists (33.3%) or other antiemetics (26.7%), propionic acid derivatives (26.7%), fluoroquinolones (20%), glucocorticoids (20%), magnesium (20% and propulsives (20%).The rest of concomitant medication was administered in less than 20% of the patients.

5.4 MTD determination

No patient in cohorts 1 and 2 (45 and 50 mg/m^2 NPLD dose levels, respectively) developed a DLT. One patient in cohort 3 (60 mg/m^2 NPLD dose level) experienced a DLT consisting of grade 4 neutropenia lasting13 days. This cohort was expanded to include three additional patients to confirm the safety and tolerability of the MTD with no other DLTs. As a result, the MTD was determined to be3.6 mg/kg ofT-DM1and60 mg/m^2 of NPLD IV on day 1 of each three-week cycle.

5.5 General safety

All 15 patients received at least one dose of study treatment and were included in the safety analysis. All patients experienced at least one AE (grades 1–4). The most common treatment-related toxicities were neutropenia (n = 11, 73.3%), thrombocytopenia(n= 9,60.0%),asthenia(n= 9,60.0%),nausea(n= 9,60.0%), elevation of liver transaminases (n = 8, 53.3%), decreased appetite (n = 5, 33.3%) and anemia (n = 4, 26.7%). These AEs were generally mild (grade 1/2) and reversible. Treatment-related AEs of any grade reported in 10% of patients are listed in Table 10.

Grade 3 treatment-related AEs occurred in nine patients (60.0%), and neutropenia was the most frequent (n = 8, 53.3%), but there were no instances of febrile neutropenia. Other grade 3 treatment-related AEs that occurred in 10% of patients included thrombocytopenia (n = 2, 13.3%) and elevation of liver transaminases (n = 2, 13.3%). One patient developed a hepatobiliary disorder (veno-occlusive liver disease), although it was not clear whether it was related to the study drugs, and led to treatment discontinuation. No grade 5 AEs or other unexpected safety issues were observed. Treatment-related AEs of grade 3 are summarized in Table 11.

Table 10. Treatment-related adverse events of any grade occurring in more than 10% of patients

Adverse Event	Cohort 1 (*n* = 3) *n* (%)	Cohort 2 (*n* = 3) *n* (%)	Cohort 3 (*n* = 9) *n* (%)	Overall (*n* = 15) *n* (%)
Hematological				
Neutropenia	1 (33.3)	3 (100)	7 (77.8)	11 (73.3)
Thrombocytopenia	1 (33.3)	2 (66.7)	6 (66.7)	9 (60.0)
Anemia	1 (33.3)	1 (33.3)	4 (44.4)	4 (26.7)
Leukopenia	0 (0)	1 (33.3)	2 (22.2)	3 (20.0)
Lymphopenia	0 (0)	1 (33.3)	2 (22.2)	3 (20.0)
Decreased hemoglobin	1 (33.3)	1 (33.3)	0 (0)	2 (13.3)
Decreased lymphocyte count	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)
Non-Hematological				
Asthenia	3 (100)	2 (66.7)	4 (44.4)	9 (60.0)
Nausea	2 (66.7)	3 (100)	4 (44.4)	9 (60.0)
Increased aspartate aminotransferase	1 (33.3)	1 (33.3)	6 (66.7)	8 (53.3)
Increased alanine aminotransferase	1 (33.3)	1 (33.3)	4 (44.4)	6 (40.0)
Increased brain natriuretic peptide	0 (0)	2 (66.7)	4 (44.4)	6 (40.0)
Increased gamma-glutamyl transferase	2 (66.7)	2 (66.7)	2 (22.2)	6 (40.0)
Increased troponin I	0 (0)	1 (33.3)	4 (44.4)	5 (33.3)
Decreased appetite	1 (33.3)	1 (33.3)	3 (33.3)	5 (33.3)
Alopecia	0 (0)	1 (33.3)	3 (33.3)	4 (26.7)
Epistaxis	0 (0)	1 (33.3)	2 (22.2)	3 (20.0)
Rhinorrhea	0 (0)	1 (33.3)	2 (22.2)	3 (20.0)
Headache	0 (0)	2 (66.7)	0 (0)	2 (13.3)
Fatigue	0 (0)	0 (0)	2 (22.2)	2 (13.3)
Mucosal inflammation	0 (0)	0 (0)	2 (22.2)	2 (13.3)
Increased blood alkaline phosphatase	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)
Aphthous ulcer	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)
Constipation	1 (33.3)	0 (0)	1 (11.1)	2 (13.3)
Diarrhea	1 (33.3)	0 (0)	1 (11.1)	2 (13.3)
Dry mouth	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)
Gingival bleeding	0 (0)	0 (0)	2 (22.2)	2 (13.3)
Vomiting	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)
Hypoalbuminemia	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)
Rash	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)

Table 11. Grade 3–5 treatment-related adverse events occurring in the safety population

Adverse event	Cohort 1 (n = 3)	Cohort 2 (n = 3)	Cohort 3 (n = 9)	Overall (N = 15)
Hematological				
Neutropenia	0 (0)	2 (66.7)	6 (66.7)	8 (53.3)
Thrombocytopenia	0 (0)	0 (0)	2 (22.2)	2 (13.3)
Leukopenia	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)
Lymphopenia	0 (0)	1 (33.3)	1 (11.1)	2 (13.3)
Non-Hematological				
Increased aspartate aminotransferase	0 (0)	0 (0)	2 (22.2)	2 (13.3)
Fatigue	0 (0)	0 (0)	1 (11.1)	1 (6.7)

5.6 Cardiac safety

The median LVEF values at baseline were 64.1% (range, 59.3–71.0%), 67.0% (range, 60.0–72.0%) and 62.7% (range, 60.0–71.9%) in cohorts 1, 2 and 3, respectively. At the end of cycle 6, the median changes in LVEF values were 11.6% (range, 9.8–13.4%), 4.0% (range, 22.0–4.0%), and 0% (range, 5.0–5.0%), respectively. Table 12 shows the evolution of median left ventricular ejection fraction (LVEF) values at baseline and cycle 6 in the three study cohorts.

Table 12. Evolution of median left ventricular ejection fraction (LVEF) values at baseline and cycle 6 in the three study cohorts

LVEF (%)	T-DM1 3.6 mg/kg plus NPLD 45 mg/m2 (n = 3)	T-DM1 3.6 mg/kg plus NPLD 50 mg/m2 (n = 3)	T-DM1 3.6 mg/kg plus NPLD 60 mg/m2 (n = 3)
Baseline			
Valid n	3	3	9
Mean (SD)	64.8 (5.9)	66.3 (6.0)	63.4 (4.2)
Median (Min, Max)	64.1 (59.3–71.0)	67.0 (60.0–72.0)	62.7 (60.0–71.9)
Overall assessment			
Normal, n (%)	3 (100)	3 (100)	9 (100)
Change from Baseline to Cycle 6 Day 21			
Valid n	2	3	6
Mean (SD)	11.6 (2.5)	-7.3 (13.3)	
Median (Min, Max)	11.6 (9.8–13.4)	-4.0 (-22.0–4.0)	0.1 (3.9)
Overall assessment			0.0 (-5.0–5.0)
Normal, n (%)	3 (100)	3 (100)	6 (100)

No cases of LVEF decline to<50.0%or symptomatic heart failure were observed.

There was an increase in cardiac markers (serum troponin I and BNP) during the study treatment with respect to the baseline, although the elevations were not clinically significant. Overall, 13 patients (86.7%) had at least one marker level above the upper limit of normal (ULN), and both levels were above the ULN in three patients (20.0%). Analyses of serum HER2 extracellular domain levels did not reveal a relationship with either LVEF changes or elevation of cardiac markers.

5.7 Antitumor efficacy

With a median follow-up time of 9.8 months (range, 2.3–24.4 months), objective partial responses (PRs) were observed in six of 15 patients (40.0%). No patient attained complete response (CR). Overall response rate (ORR) was 33.3% (95% confidence interval (CI), 0.8–90.6) in cohort 1, 66.7% (95% CI, 9.4–99.2) in cohort 2, and 33.3% (95% CI, 7.5–70.1) in cohort 3 as per Response Evaluation Criteria In Solid Tumors version 1.1 (RECIST v.1.1). A total of four patients had stable disease for 24 weeks or longer, one patient in cohort 1 and three patients in cohort3,leading to a CBR of 66.7% (95% CI,38.4–88.2). Among responders, the median duration of response (DoR) was 6.9months (95% CI,4.8– 9.1). Of a total of 15 patients, only one patient (6.7%) in cohort 1 experienced progressive disease as the best response.

Of 11 patients in cohorts 2 and 3 who had received one prior treatment for advanced disease, five (45.5%) had PR and three (27.3%) had stable disease for 24 weeks or longer, with a CBR of 72.7% (95% CI, 39–94). Among responders with one prior line of treatment, the median DoR was 8.3 months (95% CI, 5.9– 10.7) and median progression-free survival (PFS) was 7.2 months (95% CI, 6.6– 7.8).

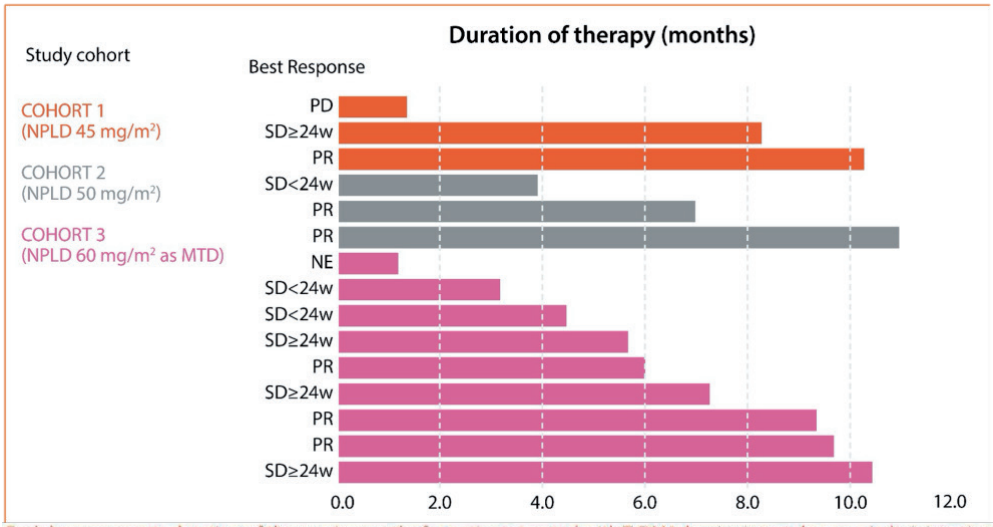
Median PFS was 8.2 months (95% CI, 1.3–10.3) in cohort 1, 7.0 months (95% CI, 3.8–not evaluable) in cohort 2, 7.2 months (95% CI, 4.5–9.6) in cohort 3, and 7.2 months in the overall study population (95% CI, 4.5–9.6).

A summary of the antitumor clinical activity of the study treatment based on investigators' review is provided in Figure 3.

5.8 Pharmacokinetics analysis

Three subjects from each treatment cohort were included in the pharmacokinetics (PK) population.

The PK parameters of the main drugs (T-DM1 and doxorubicin) indicated that the mean plasma concentrations declined quickly in an exponential manner after the first infusion of the study treatment at each dose level. The mean PK parameters, that included the maximum concentration of drug observed in plasma (C_{max}) and time from time zero to infinity (AUC_{inf}) for T-DM1, were similar for each dose level of NPLD after the first administration of the study treatment, with low inter-subject variability (coefficient of variation (CV) 4.0–30.0%). The mean Cmax ranged from 67.8 to 79.6 µg/mL and AUC_{inf} variability (coefficient of variation (CV) 4.0–30.0%). The mean T-DM1 C_{max} ranged from 67.8 to 79.6 µg/mL and the mean NPLD Cmax ranged from 321 to 380 µg x day/mL. The median time required to reach the maximum concentration



Each bar represents duration of therapy in months for patients treated with T-DM1, beginning at day zero (administration of first dose of T-DM1). Orange, gray, and pink bars indicate patients from cohort 1, 2, and 3, respectively. All responses were confirmed at 6 or 9 weeks.

Parameter	Cohort 1 (*n* = 3)	Cohort 2 (*n* = 3)	Cohort 3 (*n* = 9)	Overall (*n* = 15)
Best Overall Response, *n* (%)				
PR	1 (33.3)	2 (66.7)	3 (33.3)	6 (40.0)
SD ≥24 weeks	1 (33.3)	0 (0)	3 (33.3)	4 (26.7)
SD <24 weeks	0 (0)	1 (33.3)	2 (22.2)	3 (20.0)
PD	1 (33.3)	0 (0)	0 (0)	1 (6.7)
NE	0 (0)	0 (0)	1 (11.1)	1 (6.7)
Overall Response Rate, % (95% CI)	33.3 (0.8–90.6)	66.7 (9.4–99.2)	33.3 (7.5–70.1)	40.0 (16.3–67.7)
Duration of Response, median (95% CI), months	–	–	–	6.9 (4.8–9.1)
Clinical Benefit Rate, % (95% CI)	66.7 (9.4–99.2)	66.7 (9.4–99.2)	66.7 (29.9–92.5)	66.7 (38.4–88.2)
Progression-free survival, median (95% CI), months	8.2 (1.3–10.3)	7.0 (3.8–NE)	7.2 (4.5–9.6)	7.2 (4.5–9.6)

NE: Not evaluable. PD: Progressive disease. PR: Partial response. SD: Stable disease.
–: The number of responding patients was not enough to estimate the 95% confidence interval for the duration of the response

Figure 3. Antitumor clinical activity of the combination of T-DM1 and NPLD.

and AUC_{inf} ranged from 321 to 380 µg x day/mL. The median time required to reach the maximum of drug in plasma (T_{max}), mean time taken by the plasma concentration to reduce to 50% during the concentration of drug in plasma (T_{max}), mean time taken by the plasma concentration to reduce to 50% elimination phase (T1/2), body clearance (CL), and volume of distribution (V_d) of T-DM1 were similar during the elimination phase ($T_{1/2}$), as well as body clearance (CL), and volume of distribution (Vd) of T-DM1 for each treatment cohort.

The mean total serum exposures of trastuzumab were approximately 1.6 to 2.3 times higher for cohort 2 than the exposures for the other cohorts. The mean total plasma exposures to DM1 ranged between 10.1 and 23.1 ng x d/mL among cohorts. Table 13, Table 14 and Table 15 summarize the PK results for T-DM1, total trastuzumab and DM1.

Table 13. Pharmacokinetic parameters of trastuzumab emtansine (T-DM1) and doxorubicin by treatment dose level

Treatment dose level	T-DM1 3.6 mg/kg plus NPLD 45 mg/m² (*n* = 3)		T-DM1 3.6 mg/kg plus NPLD 50 mg/m² (*n* = 6)		T-DM1 3.6 mg/kg plus NPLD 60 mg/m² (*n* = 9)	
CYCLE 1						
Parameter	**T-DM1 mean** (% CV)	**Doxorubicin** mean (% CV)	**T-DM1** mean (% CV)	**Doxorubicin** mean (% CV)	**T-DM1** mean (% CV)	**Doxorubicin** mean (% CV)
AUC_{inf} (µg × h/mL)[a]	355	NE (15.2)	380	NE (29.1)	321	NE (18.6)
AUC_{last} (µg × h/mL)	348	2.14 (89.6) (14.4)	372	19.9 (126.6) (30.3)	317	10.8 (68.3) (18.9)
C_{max} (µg/mL) 73.3 (3.6)	0.957 (88.2)	79.6 (28.5)	4.23 (91.5)	67.8 (17.8)	2.68 (47.5)	
T_{max} (h)[b] 1.95 (1.83–2.00)	1.08 (1.08–1.17)	1.83 (1.83–2.02)	1.17 (1.08–1.43)	1.95 (1.80– 2.08)	1.17 (1.13–1.33)	
$T_{1/2}$ (days)[a] 3.57 (33.8)	NE	4.25 (13.2)	NE	3.48 (11.7)	NE	
V_d (mL/kg)[a] 50.1 (10.1)	NE	56.4 (19.5)	NE	55.9 (21.6)	NE	
Cl (mL/kg/day) 10.0 (12.1)[a]	NE	10.1 (32.1)	NE	11.0 (16.3)	NE	
CYCLE 2						
Parameter	**T-DM1 mean (% CV)**	**Doxorubicin mean (% CV)**	**T-DM1 mean (% CV)**	**Doxorubicin mean (% CV)**	**T-DM1 mean (% CV)**	**Doxorubicin mean (% CV)**
AUClast (µg × h/mL)	NA	3.85 (45.6)	NA	21.5 (122.4)	NA	8.27 (42.7)
C_{max} (µg/mL)	NA	1.58 (52.6)	NA	4.71 (86.2)	NA	2.57 (28.3)
T_{max} (h) [a]	NA	1.15 (1.10–1.35)	NA	1.17 (1.08–1.17)	NA	1.33 (1.13–1.37)

[a] Lambda z-dependent parameter (time from time zero to infinity (AUC_{inf}). Mean time taken by the plasma concentration to reduce to 50% during the elimination phase ($T_{1/2}$), body clearance (Cl) and volume of distribution (V_d) were not estimated for doxorubicin. [b] Median (minimum and maximum) is reported for the median time required to reach the maximum concentration of drug in plasma (T_{max}). NE: Not estimated. NA: Not applicable.

Table 14. Pharmacokinetic parameters for trastuzumab by treatment dose level.

	T-DM1 3.6 mg/kg plus NPLD 45 mg/m² (*n* = 3)	T-DM1 3.6 mg/kg plus NPLD 50 mg/m² (*n* = 3)	T-DM1 3.6 mg/kg plus NPLD 60 mg/m² (*n* = 3)
	Trastuzumab mean (% CV)	**Trastuzumab mean (% CV)**	**Trastuzumab mean (% CV)**
AUC_{inf} (µg x h/mL)	569[1] (26.5)	1290[2] (NA)	709 (23.2)
AUC_{last} (µg x h/mL)	612 (26.3)	1000 (7.9)	625 (19.2)
C_{max} (µg/mL)	94.6 (16.8)	114 (2.8)	78.3 (7.6)
T_{max} (h)[a]	2.00 (1.95–25.2)	1.83 (1.83–2.02)	1.95 (1.80–2.08)
$T_{1/2}$ (days)	5.03[1] (45.6)	11.2[2] (NA)	7.03 (19.3)

Table 15. Pharmacokinetic parameters for DM1 by treatment dose level

	T-DM1 3.6 mg/kg plus NPLD 45 mg/m² (*n* = 3)	T-DM1 3.6 mg/kg plus NPLD 50 mg/m² (*n* = 3)	T-DM1 3.6 mg/kg plus NPLD 60 mg/m² (*n* = 3)
	DM1 mean (% CV)	DM1 mean (% CV)	DM1 mean (% CV)
AUC_{inf} (µg x h/mL) C_{max} (µg/mL) T_{max} (h)[a]	23.1 (143.1) 3.76 (18.2 2.00 (1.95–481)	10.1 (25.0) 8.03 (67.3) 1.83 (1.83–2.02)	5.63 (24.9 5.13 (59.1) 1.95 (1.80–2.08)

Table 16. Pharmacokinetic parameters for doxorubicinol by treatment dose level.

	T-DM1 3.6 mg/kg plus NPLD 45 mg/m² (*n* = 3)	T-DM1 3.6 mg/kg plus NPLD 50 mg/m² (*n* = 3)	T-DM1 3.6 mg/kg plus NPLD 60 mg/m² (*n* = 3)
	Doxorubicinol mean (% CV)	Doxorubicinol mean (% CV)	Doxorubicinol mean (% CV)
CYCLE 1			
AUC_{inf} (ng x h/mL) AUC_{last} (ng x h/mL) C_{max} (ng/mL) T_{max} (h) [a] $T_{1/2}$ (days) AUC(m/p)[b] C_{max} (m/p) [c]	1050 (26.4) 982 (28.4) 14.8 (8.4) 3.75 (3.58–3.75) 93.4 (37.9) 0.0231 (57.8) 0.819 (73.3)	966 (36.4) 888 (27.8) 9.19 (42.1) 3.58 (3.58–3.92) 78.5 (6.0) 0.00295 (50.0) 0.106 (64.4)	1360 (61.5) 1340 (65.0) 15.2 (70.6) 3.63 (3.50–3.83) 69.3 (11.7) 0.00511 (42.4) 0.140 (21.0)
CYCLE 2			
AUC_{inf} (ng x h/mL) AUC_{last} (ng x h/mL) C_{max} (ng/mL) T_{max} (h) [a] $T_{1/2}$ (days) AUC(m/p)[b] C_{max} (m/p) [c]	792 [1] (34.7) 899 (40.7) 16.0 (25.2) 4.02 (4.00–4.23) 51.91 (0.5) 0.0123 (63.4) 0.289 (60.5)	907 [1] (30.4) 763 (25.4) 10.1 (38.9) 3.58 (1.17–4.15) 64.01 (23.6) 0.00276 (38.2) 0.0997 (74.9)	1210 [1] (71.7) 1100 (50.3) 15.6 (54.5) 3.78 (3.75–4.00) 49.41 (1.9) 0.00591 (44.7) 0.153 (21.3)

Observations of the doxorubicin concentration–time curves were limited because concentrations fell below the quantification level at the collection point of 72 h post-infusion. The last measurable concentration time using the mean linear trapezoidal method (AUC_{last}) ranged between 2.14 and 19.9 µg x h/mL, and the mean C_{max} was between 0.957 and 4.23 µg/mL with moderate to high inter-subject variability ranging from 48% and 127%. The median T_{max} values were similar for each treatment cohort.

In cycle 2, the results for mean plasma concentration versus time were similar to those observed in cycle 1 (Table 13). The PK parameters of the metabolite doxorubicinol are provided in the Table 16. The potential association between T-DM1 (or its unconjugated components), systemic exposure (C_{max} and AUCinf) and antitumor efficacy (ORR, CBR and PFS) was analyzed by logistic regression. Neither statistically significant associations nor clear positive or negative trends were observed.

Safety and pharmacokinetic study of the combination of trastuzumab emtansine and non-pegylated liposomal doxorubicin for the treatment of advanced HER2 positive breast cancer

6. DISCUSSION

6. Discussion

In the metastatic setting, T-DM1 is approved as a single-agent to treat patients with HER2positive, unresectable, locally advanced or MBC who previously received trastuzumab and a taxane, either separately or in combination. Although T-DM1 has shown significant antitumor activity, less than half of the patients achieve an objective response and all the patients eventually progress and require a new line of treatment.

Over the past few years, T-DM1 in combination with other agents has been explored because of its manageable safety profile, which makes it ideal for combination treatment. Potential chemotherapy combinations have long been examined to improve T-DM1 efficacy in a metastatic setting but results have been negative. Although the efficacy of the combination of T-DM1 plus docetaxel (with or without pertuzumab) was encouraging, this regimen was associated with significant toxicity, leading to dose reductions in nearly half of the study patients. Another study demonstrated that the addition of capecitabine to T-DM1 did not significantly improve patient outcome.

In this phase Ib study, the selected doses of T-DM1 and NPLD were 3.6 mg/kg and 60 mg/m^2 every three weeks, respectively. These doses are the same as the recommended doses for either drug given alone. Moreover, based on comparison with historical controls, no PK interaction was observed between NPLD and T-DM1 and T-DM1 PKs have been consistent with those reported for T-DM1 given as monotherapy.

Unfortunately, the addition of NPLD does not appear to increase the antitumoractivity ofT-DM-1significantly more than as a single agent with a median PFS of7.2months, an ORR of 40.0% and a CBR of 66.7% in a trastuzumab- and taxane-pretreated population. These findings do not significantly differ from those achieved with T-DM1 in the EMILIA trial (median PFS of

9.6 months and ORR of 43.6%). Nevertheless, in contrast to the EMILIA trial, most of the patients included in this study had previously received pertuzumab, which has been associated with reduced T-DM1 efficacy. This fact, along with the limited number of patients, does not allow us to draw definite conclusions.

The safety profiles of T-DM1 and NPLD were consistent with previous reports, with no new safety findings for either agent and AEs were generally manageable. Myelosuppression was the most frequent toxicity, but the addition of NPLD did not significantly increase the incidence of severe thrombocytopenia typically associated with T-DM1. However, hepatotoxicity was slightly higher with the combination of NPLD and T-DM1 than previously reported with T-DM1 as a single-agent, and one patient discontinued the study treatment due to veno-occlusive liver disease, although it was probably not related to the study drugs.

The addition of NPLD was not associated with significant cardiotoxicity and no patients developed asymptomatic LVEF declines or symptomatic heart failure. However, some patients presented an elevation of cardiac markers (troponin I and BNP) that was not clinically significant during the study treatment nor was correlated with a decrease in LVEF. It is important to emphasize that prior treatment with anthracyclines was not allowed and this patient selection criterion may have helped to obtain this favorable cardiac safety profile. No relevant correlation with cardiotoxicity was observed in the analysis of serum HER2 ECD levels.

7. CONCLUSIONS

7. Conclusions

1. The maximum tolerated dose (MTD) of T-DM1 in combination with non-pegylated liposomal doxorubicin in HER2-positive metastatic breast cancer (MBC) patients previously treated with a taxane and trastuzumab-based therapy has been determined to be 60mg/m^2.

2. No PK interaction was observed between NPLD and T-DM1, based on comparison with historical controls, and T-DM1 PKs have been consistent with those reported for T-DM1 given as monotherapy.

3. The combination of T-DM1 with NPLD is feasible; the most frequently reported G3 toxicities were neutropenia (66,7%), thrombocytopenia (22,2%) and increased aspartate aminotransferase (22,2%), all of them reversible.

4. The combination of T-DM1 with NPLD does not increase cardiac toxicity. Over the course of the study, no LVEF decline to <50.0% or symptomatic heart failure were observed. Although there was an elevation of the cardiac markers with respect to their baseline value in most patients, it was not clinically significant. There was also no correlation between this elevation and LVEF decline as early predictors. Analyses of serum HER2 extracellular domain levels did not reveal a relationship with either LVEF changes or cardiac markers elevation.

5. The addition of NPLD does not seem to enhance the antitumor efficacy of T-DM1 in patients with HER2-positive MBC, with median PFS of 7.2 months, ORR of 40.0% and CBR of66.7% in a trastuzumab and taxane pretreated population.

These results are close to those obtained with T-DM1 as monotherapy in the EMILIA trial.

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