Protocol GEICAM/2021-08

"Phase II trial of TRAstuzumab deruxtecaN in firSt-line treatment of HER2-positive locally advanCEd or metastatic breast caNcer (MBC) patients considered resistant to trastuzumab + pertuzumab + taxane Due to Early Relapse."

"TRANSCENDER Study"

SPONSOR:

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SUMMARY OF THE STUDY PROTOCOL

Study Title: Phase II trial of trastuzumab deruxtecan in first-line treatment HER2-positive locally advanced or metastatic breast cancer (MBC) patients considered resistant to trastuzumab + pertuzumab + taxane due to early relapse. "TRANSCENDER Study"

Sponsor Study Code: GEICAM/2021-08

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Indication: Human Epidermal Growth Factor Receptor 2 (HER2)-positive locally advanced or MBC patients who have not received prior chemotherapy or HER2 targeted therapy for advanced disease and with a disease-free interval (DFI) of <12 months from the end of prior (neo) adjuvant anti-HER2 therapy.

Countries and approximate number of sites: The trial is expected to be run in Spain in approximately 19 sites.

Number of patients: approximately 41 patients will be enrolled in the study.

Study Rationale:

The HER2-positive breast cancer subtype (that represents around 20% of all breast cancers) was classically associated with poor prognosis. However, new therapeutic advances (particularly targeted therapies) have significantly increased the cure rate of patients in early stages. In the metastatic setting, anti-HER2 targeted therapies have significantly improved overall survival (OS) with good quality of life, however there is still a substantial group of patients who die, and therefore additional drugs need to be investigated.

In early, locally advanced or inflammatory HER2-positive breast cancer, neoadjuvant/preoperative chemotherapy in combination with trastuzumab and pertuzumab is standard of care in patients with tumors ≥T2 and/or ≥N1, based on the results of several studies. For patients who have received neoadjuvant chemotherapy plus HER2-targeted therapy, the risk of disease recurrence or death is higher in patients with residual invasive disease at surgery than the risk in patients with a pathological complete response (pCR).

Recently, T-DM1 has been approved for the adjuvant treatment for patients with HER2-positive early BC who have residual invasive disease following neoadjuvant trastuzumab and chemotherapy based on results of the KATHERINE study.

In patients not receiving neoadjuvant chemotherapy, the use of adjuvant trastuzumab in HER2-positive early-stage BC improves patient outcomes as demonstrated in several large, randomized trials. In addition, the results of the APHINITY study led to the approval of the double blockage

with trastuzumab plus pertuzumab in combination with chemotherapy in the adjuvant setting of patients with node positive disease.

At disease relapse, the current standard of care for patients with HER2-positive MBC consists of treatment with pertuzumab plus trastuzumab plus a taxane as first-line treatment based on the CLEOPATRA data, followed by trastuzumab-emtansine (T-DM1) in second line, based on the EMILIA data. Recently, results reported in the phase 3 DESTINYBreast-03 trial will probably change clinical practice in the second line setting, with trastuzumab deruxtecan (T-DXd) showing much better results compared to T-DM1.

The CLEOPATRA study included patients with late recurrences (more than 12 months from the completion of all therapy and the diagnosis of metastatic disease), so no data of the combination of trastuzumab plus pertuzumab plus a taxane in first line in patients recurring earlier are available from this study. For T-DM1, the EMILIA trial included some patients in first line, only those relapsing within the first 6 months after completing adjuvant treatment. Besides, a retrospective real-world cohort of early-relapsing patients demonstrated PFS and OS superiority of trastuzumab pertuzumab and chemotherapy over T-DM1 in patients with relapses between 6 and 12 months and even in the first 6 months.

Thus, currently there is not enough data concerning the better strategy in patients with relapse within one year after completing adjuvant treatment, especially in those that have previously received prior trastuzumab and pertuzumab in early stage.

There is an ongoing study exploring T-DXd with or without pertuzumab versus taxane plus trastuzumab plus pertuzumab in HER2-positive MBC, the DESTINY-Breast09 study (NCT04784715), but again, patients with a short disease-free interval will not be included in the study.

Based on all of these it is quite reasonable to study the use of T-DXd in early relapse breast cancer patients that have previously received trastuzumab and pertuzumab and or T-DM1 in early stage.

Study Treatment:

Dose/Route/Regimen

Eligible patients will be enrolled and treated with:

- ✓ Trastuzumab Deruxtecan 5.4 mg/kg IV every 3 weeks.

The T-DXd dose will be recalculated in the event that patients experience body weight variations greater than 10% during the treatment period.

All patients enrolled will receive study therapy until radiographic or symptomatic progressive disease, unacceptable toxicity or withdraw of the informed consent, whatever occurs first.

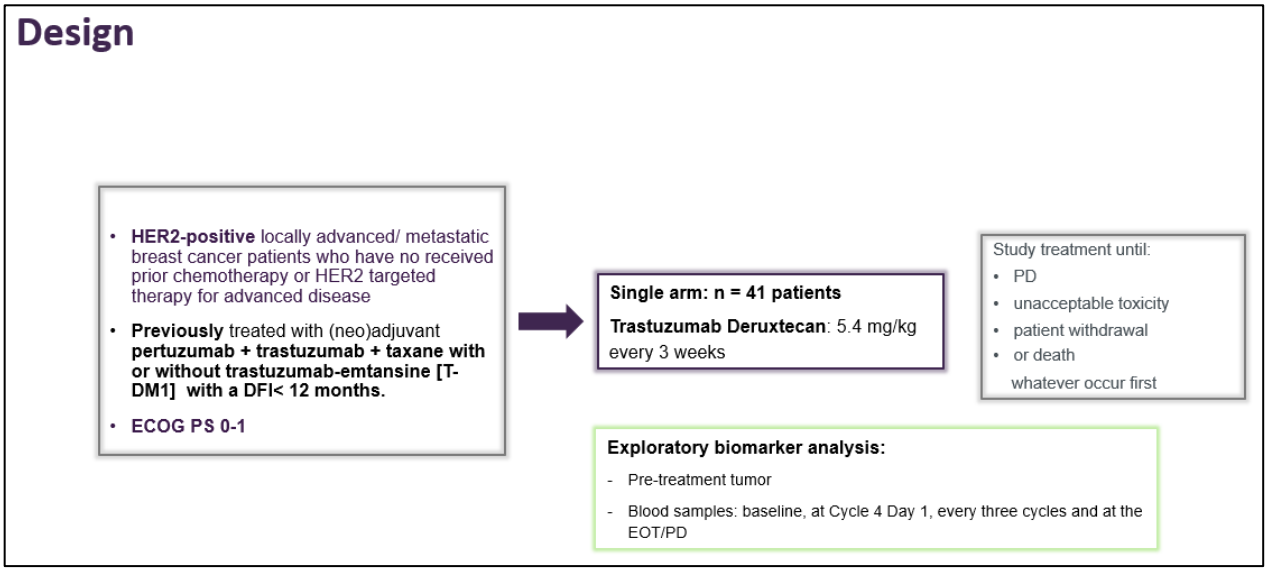
Study Design and Treatment:

Study Design

This is a national, multicenter single arm phase II clinical trial to study the efficacy, safety and tolerability of the administration of T-DXd in HER2-positive locally advanced or MBC patients resistant to trastuzumab plus pertuzumab plus taxane due to early relapse.

Forty-one evaluable patients will be enrolled in the study and treated with T-DXd according to the following study design:

Figure 1: Study design



Primary Objective:

- To evaluate the antitumor activity of T-DXd in the first-line treatment of HER2-positive breast cancer patients resistant to trastuzumab-pertuzumab based therapy.

Primary Endpoint:

- Objective Response Rate (ORR) is defined as the rate of complete response (CR) plus partial response (PR) based on the investigator's assessment using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1., out of the patients who received at least 1 dose of treatment.

Secondary Objectives:

- To assess other efficacy measures.

- To evaluate safety and tolerability in all patients enrolled in the study.
- To evaluate health-related quality of life (HRQoL).

Secondary Endpoints:

- Other efficacy endpoints:
  - Progression-Free Survival (PFS) is defined as the time from the date of enrollment to the date of disease progression, based on the investigator's assessment using RECIST version 1.1., or death from any cause, whichever occurs first.
  - Overall Survival (OS) is defined as the time from the date of enrollment to the date of death from any cause.
  - Time to treatment response (TTR) is defined as the time from the date of enrollment to the date of first documentation of objective tumor response (CR or PR).
  - Duration of response (DoR) is defined as the time from the date of first documentation of objective tumor response (CR or PR) to the date of first documented progressive disease based on the investigator's assessment using RECIST version 1.1., or death from any cause, whichever occurs first.
- Safety: Incidence and severity of Adverse Events (AEs) and clinical lab abnormalities. AEs grades will be defined by the National Cancer Institute Common Terminology Criteria for AEs (NCI-CTCAE) version 5.0. AE terms will be coded according to the MedDRA dictionary.
- Tolerability: Incidence of T-DXd dose modifications, discontinuations due to AEs, number of administered cycles, dose intensity, etc.
- Changes (mean score) and time to deterioration on EORTC QLQ-C30 Global Health Status/QoL from baseline.

Exploratory Objectives:

- To assess other efficacy measures
- To assess T-DXd efficacy according to HER2-expression and HER2 spatial distribution.
- To explore T-DXd efficacy based on the intrinsic tumor subtypes and evaluate if patients with HER2-Enriched intrinsic tumor subtype benefit most from T-DXd.
- To improve the understanding of the mechanism of action and resistance to T-DXd in HER2-positive advanced breast cancer patients. Explore, among others, disease-related inflammatory, immune, or microenvironment-related biomarkers and their correlation with the study treatment, and other potential parameters of clinical utility in blood and tumor samples.

- To explore the possibility of monitoring response to T-DXd by using longitudinal ctDNA assessment.
- To improve the understanding of molecular mechanisms of the metastatic breast cancer.

Exploratory Endpoints:

- PFS2 and PFS3: are defined as the time from the date of enrollment to the date of disease progression to the second and third lines of therapy, respectively.
- Investigate the predictive value of HER2 expression levels and HER2 spatial distribution in baseline tumors.
- Intrinsic molecular subtypes of breast cancer.
- Genomic profiles and molecular biomarkers (e.g. PIK3CA mutations, tumor infiltrated lymphocytes, PD-1, PD-L1, gH2AX, CD3, CD4, CD8, CD68, FoxP3, CK/SOX10), analyzed in tumor tissue and blood samples, associated with treatment response or resistance, and the outcome of patients. Biological samples of patients considered as 'exceptional responders' or as 'rapid progressors' based on the clinical follow-up could be subjected to deeper molecular characterization.
- ctDNA alterations related to response or resistance to treatment.
- Explore tumor subtypes and other biological subgroups, defined by biomarkers involved in immune response, proliferation, cell cycle regulation, DNA-repair, apoptosis, signal transduction pathways and oncogenic dependency, among others.

Study population and main inclusion and exclusion criteria:

HER2-positive locally advanced or MBC patients who have not received prior chemotherapy or HER2 targeted therapy for advanced disease and with a Disease-Free Interval (DFI) of <12 months from the end of prior (neo)adjuvant anti-HER2 therapy.

Inclusion Criteria:

Patients are eligible to be enrolled in the study only if they **meet all** of the following criteria:

1. Written and signed informed consent obtained prior to any study-specific procedure.
2. Male or female patients of at least 18 years of age.
3. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 1.
4. Life expectancy ≥ 12 weeks.
5. Recurrent breast cancer that is unresectable locally advanced or metastatic.
6. Pathologically documented HER2-positive status by local laboratory determination, preferably on the most recent available FFPE tumor sample, according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP)

international guidelines valid at the time of the assay. In case of discordance in HER2 status in different biopsies, the result from the most recent biopsy will be used.

7. Pathologically documented Hormone Receptor (HR)-positive or -negative by local laboratory determination, preferably on the most recent available FFPE tumor sample, and according to ASCO/CAP international guidelines valid at the time of the assay. In case of discordance in HR status in different biopsies, the result from the most recent biopsy will be used.
8. Prior anti-HER2 based therapy (with trastuzumab plus pertuzumab plus taxane with or without trastuzumab-emtansine [T-DM1]) in the (neo)adjuvant setting with a relapse while on therapy or within 12 months from the end of last anti-HER2 therapy.
9. Measurable disease assessed by the investigator based on RECIST version 1.1.
10. Left ventricular ejection fraction (LVEF) ≥ 50% measured by multiple-gated acquisition scan (MUGA) or echocardiogram (ECHO).
11. Adequate organ and marrow function defined as follows:
    a. Absolute Neutrophil Count (ANC) ≥ 1,500/mm^3 ($1.5x10^9$/L).
    b. Platelet count ≥ 100,000/mm^3 ($100x10^9$/L).
    c. Hemoglobin ≥ 9g/dL (90g/L).
    d. Creatinine clearance ≥ 30 mL/min as calculated using the standard method for the institution.
    e. Total serum bilirubin ≤ 1.5 × ULN if no liver metastases or < 3 × ULN in the presence of documented Gilbert's syndrome (unconjugated hyperbilirubinemia) or liver metastases at baseline.
    f. Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) ≤ 3.0 x ULN (< 5.0 × ULN in participants with liver metastases).
    g. Alkaline phosphatase (ALP) ≤ 2.5 x ULN (≤ 5.0 x ULN if bone or liver metastases are present).
    h. Serum albumin ≥ 2.5 g/dL
12. International normalized ratio (INR) and activated partial thromboplastin time (aPTT) ≤ 1.5 x ULN.
13. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.
14. Negative serum pregnancy test with a sensitivity of at least 25 mIU/mL (unless permanent previous sterilization procedure such as bilateral salpingectomy, bilateral oophorectomy, or complete hysterectomy) for premenopausal women, and for women who have experienced menopause onset < 12 months prior to first dose of therapy.

Exclusion Criteria:

Patients will be excluded from the study if they **meet any** of the following criteria:

1. Prior chemotherapy or HER2-targeted therapy for locally advanced or MBC (one prior endocrine therapy regimen for MBC without concurrent anti-HER2 therapy or radiotherapy is allowed).
2. Ineligible for treatment with T-DXd.
3. Any substance abuse or other medical conditions that, in the investigator's opinion, may interfere with patient's participation or study results.
4. Patients with spinal cord compression, leptomeningeal disease or clinically active central nervous system (CNS) metastases. Participants with clinically inactive brain metastases or treated brain metastases that are no longer symptomatic, and no needing corticosteroids or anticonvulsants may be enrolled in the study.
5. Active or prior documented interstitial lung disease (ILD)/pneumonitis or suspected ILD/pneumonitis that cannot be ruled out by imaging at screening.
6. Lung criteria:
    a. Lung-specific intercurrent clinically significant illnesses including, but not limited to, any underlying pulmonary disorder (e.g. pulmonary emboli within three months of the study enrollment, severe asthma, severe Chronic obstructive pulmonary disease (COPD), restrictive lung disease, pleural effusion etc.).
    b. Any autoimmune, connective tissue or inflammatory disorders (e.g. Rheumatoid arthritis, Sjogren's, sarcoidosis etc.) where there is documented, or a suspicion of pulmonary involvement at the time of screening. Full details of the disorder should be recorded in the electronic Case Report Form (eCRF) for patients who are enrolled in the study.
    c. Prior pneumonectomy.
7. Medical history of myocardial infarction within 6 months before registration, symptomatic congestive heart failure (CHF), troponin levels consistent with myocardial infarction as defined according to American College of Cardiologists (ACC) guidelines, unstable angina, or serious cardiac arrhythmia requiring treatment. QT interval corrected using Fridericia's formula (QTcF) > 470 msec (females) or > 450 msec (males) based on average of the screening triplicate 12-lead ECG.
8. History of active primary immunodeficiency, known Human Immunodeficiency Virus (HIV), active Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV).
9. Patients who received before treatment starts:

a. Any investigational agent within 4 weeks.

b. Chemotherapy within a period of time that is shorter than the cycle duration used for that treatment (e.g. < 3 weeks for fluorouracil, doxorubicine, epirubicine or < 1 week for weekly chemotherapy).

c. Targeted therapy (e.g., antibodies): up to 4 weeks prior to starting study treatment.

d. Endocrine therapy: tamoxifen or aromatase inhibitor within 2 weeks prior to starting study treatment.

e. Radiotherapy within 2 weeks prior to starting study treatment. Patients who received prior radiotherapy to >25% of bone marrow are not eligible regardless of when it was administered.

f. Major surgery or other anti-cancer therapy not previously specified within 4 weeks prior to starting study treatment.

In any case, resolution of all acute toxic effects of prior anti-cancer therapy or surgical procedures to NCI-CTCAE version 5.0 grade ≤ 1 (except alopecia or other toxicities not considered a safety risk for the patient at investigator´s discretion) is mandatory.Patients may be enrolled with chronic, stable grade 2 toxicities (defined as no worsening to > grade 2 for at least 3 months prior to enrollment and managed with standard of care treatment) that the investigator deems related to previous anticancer therapy, such as: chemotherapy-induced neuropathy or fatigue and immunotherapy-induced toxicities (e.g. endocrinopathies as hypothyroidism/hyperthyroidism, type 1 diabetes, hypoglycemia, adrenal insufficiency, adrenalitis and skin hypopigmentation [vitiligo]).

10. Have a diagnosis of any other malignancy within 3 years prior to inclusion, except for adequately resected non-melanoma skin cancer, curatively treated in-situ disease, other solid tumors curatively treated and contralateral breast cancer.
11. Receipt of live, attenuated vaccine within 30 days prior to the first dose of T-DXd.
12. Prior treatment with T-DXd or allergic reaction to trastuzumab.
13. Patient is pregnant or breastfeeding or planning to become pregnant within the projected duration of the trial, starting at screening and through 7 months after the last dose of the study treatment. Male patients whose partners plan to become pregnant within the duration of the trial, starting at screening and through 4 months after the last dose of the study treatment.

    ✓ For premenopausal women it is necessary an agreement to remain complete abstinent or use single or combined non-hormonal contraceptive methods that result in a failure rate of < 1% per year during the treatment period and for at least 7 months after the last dose of study treatment.

- Examples of non-hormonal contraceptive methods with a failure rate of < 1% per year include bilateral tubal litigation, male sterilization, and certain intrauterine devices (provided coils are copper banded).
- Alternative, two methods (e.g. two barrier methods such as a condom and a cervical cap or combined with estrogen and progestogen) may be combined to achieve a failure rate of < 1% per year. Barrier methods must always be supplemented with the use of a spermicide.

Female patients must not donate, or retrieve for their own use, ova from the time of enrollment and throughout the study treatment period, and for at least 7 months after the final study drug administration. They should refrain from breastfeeding throughout this time. Preservation of ova may be considered prior to enrollment in this study.

✓ For men it is necessary an agreement to remain complete abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and to refrain from donating sperm during the same period, as defined below with female partners of childbearing potential or pregnant female partners, men must remain abstinent or use a condom during the treatment period and for at least 4 months after the last dose of study treatment to avoid exposing the embryo.

Abstinence is only acceptable if it is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

14. Uncontrolled intercurrent illness including uncontrolled infection requiring intravenous (IV) antibiotics, antivirals, or antifungals.
15. Has substance abuse or any other medical/psychological conditions that may, in the opinion of the investigator, interfere with the patient's participation in the clinical study or evaluation of the clinical study results.

Justification of Sample size determination

Assuming an ORR of 35%, and to achieve a precision of ±15% using two-sided 95% normal asymptotic Confidence Interval (CI) for a single proportion, we will need to enroll 39 evaluable patients.

With a drop-out rate of 5% we will need 41 patients to be enrolled in the study.

Statistical Analyses:

For descriptive analyses, frequencies, percentages and 95% CI of interest will be calculated for categorical variables wherever possible. For continuous variables, standard descriptive statistics, such as total number of observations, number of available data, mean, standard deviation, minimum, percentile 25, median, percentile 75 and maximum will be calculated.

Percentage and two-sided 95% CI will be calculated for the primary endpoint (ORR).

Kaplan-Meier Method will be used to estimate the survival functions, median time to events and probabilities of occurrence of an event at a certain time point (PFS, OS, TTR, DOR, PFS2, PFS3).

Cox Proportional Hazards Regression Model could be used to estimate the hazard ratio and assess the association between the different risk factors and survival times.

Study Duration:

The start date of the study is the date of the first site activation. Recruitment period will occur during approximately 24 months from the first patient in.

The end date of the study is the date of the last visit of the last patient (LPLV), including follow-up. The duration of the study will be approximately 68 months from the first patient in.

Performing exploratory objectives will be independent of the date of the end of the study.

Table of Contents

Abbreviations and Definitions

ACC	American College of Cardiologists
AE	Adverse Event
AEMPS	Spanish Agency for Medicines and Health Products
AESI	Adverse Event of Special Interest
ALT/ALAT (SGPT)	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AP	Alkaline Phosphatase
AR	Adverse Reaction
AST/ASAT (SGOT)	Aspartate Aminotransferase
AUC	Area Under the Curve
BC	Breast Cancer
cDNA	Circulating free DNA
CHF	Congestive Heart Failure
CI	Confidence Interval
CNS	Central Nervous System
Compliance	Adherence to all the trial-related requirements, good clinical practice (GCP) requirements, and the applicable regulatory requirements.
COPD	Chronic Obstructive Pulmonary Disease
CR	Complete Response
CTCAE	Common Terminology Criteria for Adverse Events
CT Scan	Computed Tomography Scan
DNA	Deoxyribonucleic Acid
DSUR	Development Safety Update Report
eCRF	Electronic Case Report Form (sometimes referred to as Clinical Report Form). An electronic form for recording study participants' data during a clinical study, as required by the protocol.
ECG	Electrocardiogram

ECOG	Eastern Cooperative Oncology Group
End of Study (Trial)	The end of study (trial) is the date of the last visit or last scheduled procedure shown in the Study Schedule for the last active patient in the study (*to be modified according to the definition of end of study in the protocol)*
Enroll	The act of assigning a patient to a treatment. Patients who are enrolled in the trial are those who have been assigned a registration number and treatment.
Screen	The act of obtaining informed consent for participation in a clinical trial from patients deemed eligible or potentially eligible to participate in the clinical trial. Patients screened into a trial are those who sign the informed consent document directly or through their legally acceptable representatives.
ER	Estrogen Receptor
EC/IRB	Ethics Committee/Institutional review board: A board or committee (institutional, regional, or national) composed of medical professional and nonmedical members whose responsibility is to verify that the safety, welfare, and human rights of the patients participating in a clinical trial are protected.
FDA	Food and Drug Administration
FFPE	Formalin-Fixed Paraffin-Embedded
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GEICAM	Fundación Grupo Español de Investigación en Cáncer de Mama
G-CSF	Granulocyte Colony-Stimulating Factor
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HER2	Human Epidermal Growth Factor Receptor 2
Hb	Hemoglobin
HR	*Hormone Receptor*
HRQoL	Health-related quality of life
ICD	Informed Consent Document
IHC	Immunohistochemistry

ILD	Interstitial Lung Disease
IMP	Investigational Medicinal Product
Investigator	A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator.
ISH	In Situ Hybridization
ITT	Intent To Treat
IV	Intravenous
Legal Representative	An individual, judicial, or other body authorized under applicable law to consent, on behalf of a prospective patient, to the patient's participation in the clinical trial.
MBC	Metastatic Breast Cancer
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
NCI	National Cancer Institute
NSAI	Non-Steroidal Aromatase Inhibitor
OR	Objective Response
OS	Overall Survival
Patient	A subject with a defined disease.
PD	Progressive Disease or Pharmacodynamic depending on the context
PFS	Progression-Free Survival
PgR	Progesterone Receptor
PK	Pharmacokinetic
PR	Partial Response
PS	Perfomance Status
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RECIST	Response Evaluation Criteria in Solid Tumors
RR	Response Rate

SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAP	Statistical Analysis Plan
SD	Stable Disease
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T-DXd	Trastuzumab deruxtecan
TTP	Time To Progression
ULN	Upper Limit of Normal

Phase II trial of TRAstuzumab deruxtecaN in firSt-line treatment of HER2-positive locally advanCEd or metastatic breast caNcer (MBC) patients resistant to trastuzumab + pertuzumab + taxane Due to Early Relapse

"TRANSCENDER Study"

1. Introduction

1.1. Overview of Breast Cancer

Breast cancer is the most common form of cancer and the second leading cause of cancer-related death in women in Spain, the United States and worldwide. In the last 30 years, the female breast cancer death rate has experienced a decline by 41% because of screening programs and treatment improvements. Because of those improvements, long-term survival can be achieved[1]. However, still many of the patients diagnosed with a breast cancer will present with a metastatic disease at diagnosis or will eventually develop it in spite of all therapeutic efforts. Metastatic disease is incurable in a vast majority of patients; consequently, nowadays the primary goal of treatment in these patients is to extend life and palliate potential symptoms. Thus, further research is needed in order to improve metastatic breast cancer outcomes.

1.2. HER2 overexpression in BC

The epidermal growth factor receptor family was discovered in the 1960s, and includes four different members: ErbB1 or EGFR, ErbB2 or HER2/neu, ErbB3, and ErbB4. The structure of these receptors consists of an extracellular domain, a juxtamembrane domain and an intracellular domain. The intracellular domain is made of two parts: a tyrosine kinase domain and a carboxyl terminal domain. The binding of the ligand to the extracellular domain promotes dimerization of this receptor with other members of the EGFR family[2].

Up to 20-25% of breast cancers overexpress the Human Epidermal Growth Factor Receptor 2 (HER2) [3,4] Tumors that overexpress HER2 are usually more aggressive than hormone receptor (HR)-positive, HER2-negative breast cancer, and traditionally have been associated with poorer outcomes. However, therapeutic developments in the form of targeted therapies have dramatically changed outcomes in this particular type of breast cancer. Thus, targeted therapies have increased curation rates in early disease[5,6] and prolonged survival in advance disease, as well as granting patients a better quality of life[7-10].

1.3. Treatment options for HER2-positive Metastatic BC (MBC) patients

As previously stated, the introduction of HER2-targeted therapy using either antibody-based therapy or a small molecule tyrosine kinase inhibitor (TKI) has led to significant and ongoing improvements in disease-free survival (DFS), progression-free survival (PFS), and OS in both the adjuvant and metastatic settings.

Nowadays, patients with HER2-positive disease who are diagnosed as metastatic or who relapse 1 year after completing adjuvant treatment, the combination of a taxane plus trastuzumab and pertuzumab (THP) is the standard of treatment, based on the CLEOPATRA study[9]. It was a phase III trial for HER2-positive MBC patients naive to previous chemotherapy or biological therapy

for metastatic disease. Patients could have received chemotherapy with or without trastuzumab in the neoadjuvant or adjuvant settings as long as there was a minimum 12-month interval between completion of all therapy and metastatic disease diagnosis. Patients were treated with trastuzumab and docetaxel and were randomly assigned to receive pertuzumab or placebo. The study had a potential limitation, since patients who received adjuvant or neoadjuvant trastuzumab was also small, because trastuzumab was not widely available for this indication during the recruitment of the trial. The study met its primary endpoint in terms of OS and PFS, and the combination of taxane plus trastuzumab and pertuzumab was established as the first line option.

In the second-line setting, ado-trastuzumab emtansine (T-DM1) demonstrated an improvement in progression-free survival (PFS) and OS in comparison with lapatinib/capecitabine in the EMILIA trial[8]. The study was a phase 3 randomized, open-label, international trial involving patients with HER2-positive, unresectable, locally advanced or metastatic breast cancer who were previously treated with trastuzumab and a taxane. Patients had progressed during or after the most recent treatment for locally advanced or metastatic disease or within 6 months after treatment for early-stage disease. They were randomized to receive either T-DM1 or lapatinib plus capecitabine. Based on the results of this study, T-DM1 was stablished as the standard of care in second line.

For the third line and beyond, treatment options included combinations of an anti-HER2 therapy such as trastuzumab, lapatinib or neratinib in combination with cytotoxic therapy. But recently two new drugs have shown better results in this setting, tucatinib and trastuzumab deruxtecan (T-DXd).

Tucatinib is a potent, highly selective, small-molecule kinase inhibitor of HER2. Its efficacy was tested in HER2CLIMB[11]. It was an international, randomized, double-blind trial in which 612 patients with pretreated (prior trastuzumab, pertuzumab and T-DM1) HER2-positive MBC (with or without active/stable brain metastases) were randomized to receive capecitabine plus trastuzumab plus tucatinib or capecitabine plus trastuzumab plus placebo. PFS at 1 year was 33.1% in the investigational arm in contrast to 12.3% in the control arm (Hazard Ratio 0.54; $p<0.001$), with a median PFS of 7.8 vs 5.6 months, respectively. OS at 2 years was 44.9% in the tucatinib arm and 26.6% in placebo arm. Consequently, tucatinib has been approved by the FDA and EMA in patients that have received at least two prior anti-HER2 treatment regimens since 2020.

T-DXd is a next generation antibody-drug conjugate composed of a monoclonal anti-HER2 antibody and a topoisomerase I inhibitor (DX-8951f). T-DXd has been granted approval by the FDA and EMA, and in Europe it is indicated as monotherapy for the treatment of adult patients with unresectable or metastatic HER2-positive breast cancer who have received two or more prior anti-HER2 based regimens (for more information about T-DXd see Section 1.4).

1.4. Overview of Trastuzumab deruxtecan

T-DXd is a HER2-targeted antibody and topoisomerase I inhibitor conjugate. T-DXd is composed of a recombinant humanized anti-HER2 immunoglobulin G1 (IgG1) monoclonal antibody (mAb) that has the same amino acid sequence of trastuzumab, covalently conjugated to a drug-linker, deruxtecan. The released drug, DXd, inhibits topoisomerase I and leads to apoptosis of the target cells.

1.4.1 Preclinical Data

The information in this section is taken from the Investigator Brochure (IB)[12]. Refer to this document for further details on the preclinical data.
The pharmacokinetics and drug metabolism of T-DXd were investigated in cynomolgus monkeys to support toxicology studies and to support ongoing and planning clinical studies.

1.4.2 Human Pharmacokinetic (PK) Data

The information in this section is taken from the IB[12]. Refer to this document for further details on the human PK data.

The clinical pharmacology of T-DXd has been analyzed in a majority of completed clinical studies.

The clinical pharmacology of T-DXd has been well characterized with concentrations of all 3 analyte components of the ADC (ie, intact T-DXd, total anti-HER2 antibody, and released drug, DXd).

Pharmacokinetic (PK) parameters for T-DXd and total anti-HER2 antibody were comparable. Serum concentrations of DXd gradually increased and reached peak concentrations with longer median time to maximum serum concentration (Tmax; approximately 6 hours) compared to T-DXd (approximately 2 hours). On a molar basis, the T-DXd Cmax and trough concentrations across the 5.4 mg/kg to 7.4 mg/kg doses were approximately 50-fold to 53-fold and 69-fold to 94-fold higher, respectively, than those for DXd, demonstrating in vivo stability of the ADC.

The exposure of T-DXd, total anti-HER2 antibody, and DXd increased in a dose-related manner, with T-DXd exposure proportional to dose in the 3.2 mg/kg to 8.0 mg/kg dose range. In the 0.8 mg/kg to 8.0 mg/kg dose range, the maximum serum concentration (Cmax) increased proportional to dose, while the area under the serum concentration-time curve (AUC) for T- DXd and total anti-HER2 antibody increased slightly more than proportional to dose. Exposures (Cmax and AUC) for DXd increased proportional to dose in the 0.8 mg/kg to 8.0 mg/kg dose range.

PK data from the Dose Expansion part of the Study DS8201-A-J101, as of 01 Feb 2019 showed that the T-DXd, total anti-HER2 antibody, and DXd exposures were similar between HER2-

positive and HER2-low breast cancer. The T-DXd and total anti-HER2 antibody exposures appeared to be numerically lower in HER2-positive gastric/gastroesophageal junction (GEJ) cancer than in breast cancer. DXd exposures were consistent across all tumor types evaluated. PK data as of 21 Mar 2019 showed that PK parameters were comparable for FL DP2 and Lyo-DP following administration of 5.4 mg/kg of T-DXd (DS8201-A-U201 [DESTINY-Breast01]). The mean accumulation ratio for area under the plasma concentration-time curve during dosing interval (AUCtau) at Cycle 3 was 1.35 for T-DXd, 1.36 for total anti-HER2 antibody, and 1.09 for DXd (DS8201-A-J102).

The administration of T-DXd together with OATP1B, CYP3A y gp-P inhibitors did not produced a clinically significant increase in the concentration of T-DXd or DXd (approximately 10-20%).

1.4.3 QTc Evaluation Data

QTc evaluation was performed in non clinical and clinical studies. In cynomolgus monkeys during the IV administration of 3 doses of T-DXd over a 6-week period, 1 female was euthanized moribund at 78.8 mg/kg, the highest dose level tested. At this dose level electrocardiogram (ECG) abnormalities (shortened PR interval and QTc prolongation) were found.

DS8201-A-J102 was a Phase 1, multicenter, open-label, multiple-dose study of T-DXd designed to assess the effect on the QTc interval and PK after multiple dosing in subjects with HER2 overexpressing metastatic and/or unresectable breast cancer. A total of 51 subjects received T-DXd in this study. The DCO date for the primary analysis occurred after all subjects had completed at least 3 cycles. T-DXd 6.4 mg/kg administration was not associated with a clinically meaningful (change from baseline >10 milliseconds) prolongation of QT interval corrected for heart rate by Fridericia's formula (QTcF) in subjects with unresectable and/or metastatic HER2 overexpressing BC. The upper bound of the 90% CI for change in QTcF at the observed mean Cmax for each analyte (T-DXd and DXd) in the linear model of concentration vs change in QTcF for Cycles 1 and 3 was under 10 milliseconds.

1.4.4 Trastuzumab deruxtecan Dose Rationale

The DESTINY-Breast01 is a phase 2 multicenter trial evaluating T-DXd in previously treated HER2-positive breast cancer patients. The first part of the study consisted of 2 cohorts for PK and a dose-finding, respectively.

In the PK stage in part 1 of the study, the dose of T-DXd that was administered was 5.4 mg per kilogram in 22 patients, 6.4 mg/kg in 22 patients, and 7.4 mg/kg in 21 patients. The resulting PK profiles were analyzed in conjunction with those from the phase 1 study, DS8201-A-J101; 5.4 mg/kg and 6.4 mg/kg were chosen for the dose-finding stage.

In the part 1 dose-finding stage, an additional 28 and 26 patients (who were not enrolled during the PK stage) received 5.4 mg/kg and 6.4 mg/kg, respectively. Exposure-efficacy modeling

showed a significant relationship between exposure and response rate and a trend for longer progression-free survival at higher doses. Similarly, exposure-safety modeling showed a significant relationship between exposure and key adverse events (AEs), including interstitial lung disease. On the basis of the balance of safety and efficacy, a dose of 5.4 mg/kg was recommended[13].

1.4.5 Trastuzumab deruxtecan data in HER2-positive Breast Cancer

As of 08 Jun 2023, T-DXd is being evaluated in 34 clinical studies (20 monotherapy studies and 14 combination therapy studies).

Sixteen studies are complete, and 18 studies are ongoing (16 studies in breast cancer; 24 in phase I/II and 10 in phase III).

The 2 most important studies in HER2-positive breast cancer are the DESTINY-Breast01 and DESTINY-Breast03.

As described before, the DESTINY-Breast01 is a phase 2 multicenter trial evaluating T-DXd in previously treated HER2-positive breast cancer patients. The first part of the study established a dose of 5.4 mg/kg. The second part evaluated the safety and efficacy of T-DXd at the recommended dose in patients who had progressive disease while on or after receiving T-DM1 and in patients who discontinued T-DM1 for other reasons. Overall, 184 patients received the recommended dose of 5.4 mg/kg. The observed response rate was 60.9% (112/184), including 6.0% complete response and 54.9% partial response rates. Disease control rate resulted in 97.3% of patients. Median duration of response was 14.8. months, and median PFS was 16.4 months[13,14].

The DESTINY-Breast03 is a phase 3, multicenter, open-label, randomized trial comparing the efficacy and safety of T-DXd and T-DM1 in patients with HER2-positive metastatic breast cancer previously treated with trastuzumab and a taxane. Among the 524 included patients, the proportion who were alive without disease progression at 12 months was 75.8% with T-DXd and 34.1% with T-DM1 (hazard ratio, 0.28; 95% CI, 0.22 to 0.37; P<0.001). The proportion of patients who were alive at 12 months was 94.1% with T-DXd and 85.9% with T-DM1 (hazard ratio, 0.55; 95% CI, 0.36 to 0.86; prespecified P-value significance boundary not reached). The overall response was 79.7% of patients receiving T-DXd and 34.2% of those receiving T-DM1. The incidence of drug-related AEs of any grade was 98.1% with T-DXd and 86.6% with T-DM1, and the incidence of drug related AEs of grade 3 or 4 was 45.1% and 39.8%, respectively. Interstitial lung disease or pneumonitis occurred in 10.5% of patients in the T-DXd group and in 1.9% of those in the T-DM1 group; none of these events were of grade 4 or 5[15,16].

1.5. Study Rationale

The HER2-positive breast cancer subtype (that represents around 20% of all breast cancers) was classically associated with poor prognosis however, new therapeutic advances (particularly

targeted therapies) have significantly increased the cure rate of patients in early stages. In the metastatic setting, anti-HER2 targeted therapies have significantly improved overall survival (OS) with good quality of life, however there is still a substantial group of patients who die, and therefore additional drugs need to be investigated.

In early, locally advanced or inflammatory HER2-positive breast cancer, neoadjuvant/preoperative chemotherapy in combination with trastuzumab and pertuzumab is standard of care in patients with tumors ≥T2 and/or ≥N1, based on the results of several studies [17,18]. For patients who have received neoadjuvant chemotherapy plus HER2-targeted therapy, the risk of disease recurrence or death is higher in patients with residual invasive disease at surgery than the risk in patients with a pCR[19-21].

Recently, T-DM1 has been approved for the adjuvant treatment for patients with HER2-positive early BC who have residual invasive disease following neoadjuvant trastuzumab and chemotherapy based on results of the KATHERINE study[22].

In patients not receiving neoadjuvant chemotherapy, the use of adjuvant trastuzumab in HER2-positive early-stage BC improves patient outcomes as demonstrated in several large, randomized trials[23-25]. In addition the results of the APHINITY study led to the approval of the double blockage with trastuzumab plus pertuzumab in combination with chemotherapy in the adjuvant setting of patients with node positive disease[26].

At disease relapse, the current standard of care for patients with HER2-positive MBC consists of treatment with pertuzumab plus trastuzumab plus a taxane as first-line treatment based on the CLEOPATRA data, followed by trastuzumab-emtansine (T-DM1) in second line, based on the EMILIA data. Recently, results reported in the phase 3 DESTINYBreast-03 trial[15-16] will probably change clinical practice regarding second line setting, with T-DXd showing much better results compared to T-DM1.

The CLEOPATRA study included patients with late recurrences (more than 12 months from the completion of all therapy and the diagnosis of metastatic disease), so no data of the combination of trastuzumab plus pertuzumab plus a taxane in first line in patients recurring earlier are available from this study. For T-DM1, the EMILIA trial included some patients in first line, only those relapsing within the first 6 months after completing adjuvant treatment. Besides, a retrospective real-world cohort of early-relapsing patients demonstrated PFS and OS superiority of trastuzumab pertuzumab and chemotherapy over T-DM1 in patients with relapses between 6 and 12 months and even in the first 6 months[27].

Thus, currently there is not enough data concerning the better strategy in patients with relapse within one year after completing adjuvant treatment, especially in those that have previously received prior trastuzumab and pertuzumab in early stage.

There is an ongoing study exploring T-DXd with or without pertuzumab versus taxane plus trastuzumab plus pertuzumab in HER2-positive MBC, the DESTINY-Breast09 study (NCT04784715), but again, patients with a short disease-free interval will not be included in the study.

Based on all of these it is quite reasonable to study the use of T-DXd in early relapse breast cancer patients that have previously received trastuzumab and pertuzumab and or T-DM1 in early stage.

2. Objectives

2.1. Primary Objective

- To evaluate the antitumor activity of T-DXd in the first-line treatment of HER2-positive breast cancer patients resistant to trastuzumab-pertuzumab based therapy.

2.2. Primary Endpoint

- Objective Response Rate (ORR) is defined as the rate of complete response (CR) plus partial response (PR) based on the investigator's assessment using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1., out of the patients who received at least 1 dose of treatment.

2.3. Secondary Objectives

- To assess other efficacy measures.
- To evaluate safety and tolerability in all patients enrolled in the study.
- To evaluate health-related quality of life (HRQoL).

2.4. Secondary Endpoints

- Other efficacy endpoints:
  - Progression-Free Survival (PFS) is defined as the time from the date of enrollment to the date of disease progression, based on the investigator's assessment using RECIST version 1.1., or death from any cause, whichever occurs first.
  - Overall Survival (OS) is defined as the time from the date of enrollment to the date of death from any cause.
  - Time to treatment response (TTR) is defined as the time from the date of enrollment to the date of first documentation of objective tumor response (CR or PR).
  - Duration of response (DoR) is defined as the time from the date of first documentation of objective tumor response (CR or PR) to the date of first documented progressive disease based on the investigator's assessment using RECIST version 1.1., or death from any cause, whichever occurs first.
- Safety: Incidence and severity of Adverse Events (AEs) and clinical lab abnormalities. AEs grades will be defined by the National Cancer Institute Common Terminology Criteria for AEs (NCI-CTCAE) version 5.0. AE terms will be coded according to the MedDRA dictionary.

- Tolerability: Incidence of T-DXd dose modifications, discontinuations due to AEs, number of administered cycles, dose intensity, etc.
- Changes (mean score) and time to deterioration on EORTC QLQ-C30 Global Health Status/QoL from baseline.

2.5. Exploratory Objectives

- To assess other efficacy measures.
- To assess T-DXd efficacy according to HER2-expression and HER2 spatial distribution.
- To explore T-DXd efficacy based on the intrinsic tumor subtypes, and evaluate if patients with HER2-Enriched intrinsic tumor subtype benefit most from T-DXd.
- To improve the understanding of the mechanism of action and resistance to T-DXd in HER2-positive advanced breast cancer patients. Explore, among others, disease-related inflammatory, immune, or microenvironment-related biomarkers and their correlation with the study treatment, and other potential parameters of clinical utility in blood and tumor samples.
- To explore the possibility of monitoring response to T-DXd by using longitudinal ctDNA assessment.

To improve the understanding of molecular mechanisms of the metastatic breast cancer.

2.6. Exploratory Endpoints

- PFS2 and PFS3 are defined as the time from the date of enrollment to the date of disease progression to the second and third lines of therapy, respectively.
- Investigate the predictive value of HER2 expression levels and HER2 spatial distribution in baseline tumors.
- Intrinsic molecular subtypes of breast cancer.
- Genomic profiles and molecular biomarkers (e.g. PIK3CA mutations, tumor infiltrated lymphocytes, PD-1, PD-L1, gH2AX, CD3, CD4, CD8, CD68, FoxP3, CK/SOX10), analyzed in tumor tissue and blood samples, associated with treatment response or resistance, and the outcome of patients. Biological samples of patients considered as ‘exceptional responders’ or as ‘rapid progressors’ based on the clinical follow-up could be subjected to deeper molecular characterization.
- ctDNA alterations related to response or resistance to treatment.
- Explore tumor subtypes and other biological subgroups, defined by biomarkers involved in immune response, proliferation, cell cycle regulation, DNA-repair, apoptosis, signal transduction pathways and oncogenic dependency, among others.

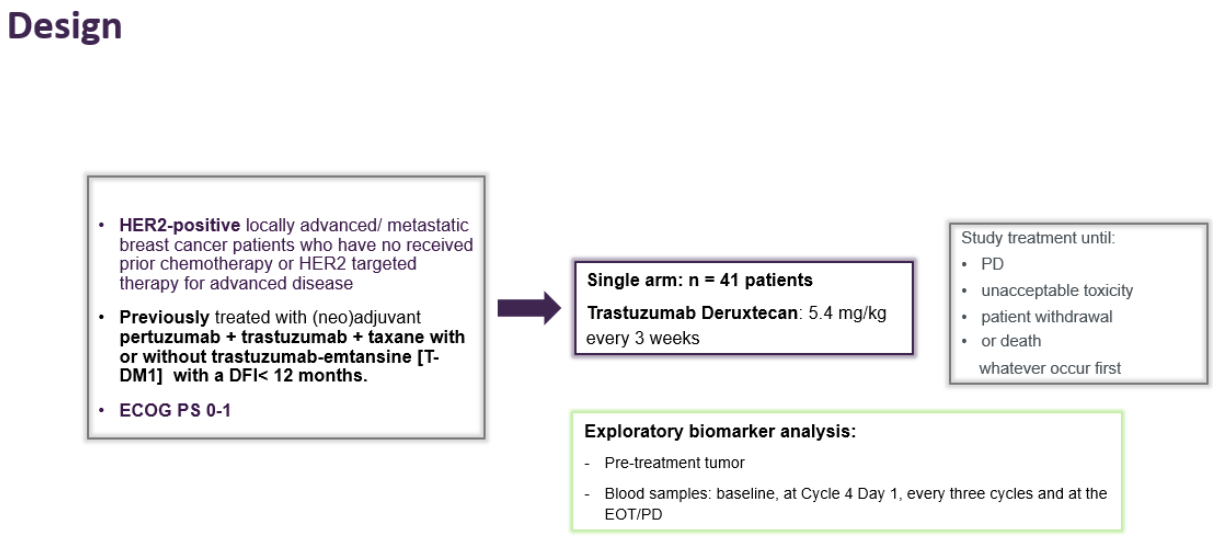
3. Investigational Plan

3.1. Study Design

This is a national, multicenter single arm phase II clinical trial to study the efficacy, safety and tolerability of the administration of T-DXd in HER2-positive locally advanced or metastatic breast cancer (MBC) patients considered resistant to trastuzumab plus pertuzumab plus taxane due to early relapse (< 12 months from last anti-HER2 adjuvant therapy).

Forty-one evaluable patients will be enrolled in the study and treated with T-DXd according to the following study design:

Figure 1. Study Design



Patients will continue to receive their assigned treatment until objective disease progression, symptomatic deterioration, unacceptable toxicity, death or withdrawal of consent, whichever occurs first.

For safety reasons all patients will have a visit 30 (±5) days after finishing treatment with the study treatment. This post-treatment visit must be performed before starting any new anticancer therapy. In case the beginning of a new therapy cannot be delayed as per the investigator's judgment, the safety visit may be performed in advance and always before starting the new anticancer therapy.

After progression, all patients will be followed until death or data cut-off date for secondary objectives or withdrawal of the informed consent.

In order to perform exploratory biomarker analysis, pre-treatment tumor and sequential blood samples (at baseline, every three cycles and at the end of treatment [EOT]/PD) will be obtained.

3.2. Duration of the study

The start date of study is the date of the first site activation.

It is estimated that the accrual will be completed in approximately 24 months.

Patients will be treated till disease progression with an estimated median PFS of 18 months. After progression, all patients will be followed until death or data cut-off date for secondary objectives, assumed to be approximately 36 months, to estimate OS. The end date of study is the date of the last visit of the last patient (LPLV) including follow up.

According to what is outlined above, the duration of the study will be approximately 68 months (5.7 years) from the first patient in.

Performing exploratory objectives will be independent of the date of the end of the study.

4. Study Population

4.1. Inclusion Criteria

Patients are eligible to be enrolled in the study only if they **meet all** of the following criteria:

1. Written and signed informed consent obtained prior to any study-specific procedure.
2. Male or female patients of at least 18 years of age.
3. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 1.
4. Life expectancy ≥ 12 weeks.
5. Recurrent breast cancer that is unresectable locally advanced or metastatic.
6. Pathologically documented HER2-positive status by local laboratory determination, preferably on the most recent available FFPE tumor sample, according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) international guidelines valid at the time of the assay. In case of discordance in HER2 status in different biopsies, the result from the most recent biopsy will be used.
7. Pathologically documented Hormone Receptor (HR)-positive or -negative by local laboratory determination, preferably on the most recent available FFPE tumor sample, and according to ASCO/CAP international guidelines valid at the time of the assay. In case of discordance in HR status in different biopsies, the result from the most recent biopsy will be used.
8. Prior anti-HER2 based therapy (with trastuzumab plus pertuzumab plus taxane with or without trastuzumab-emtansine [T-DM1]) in the (neo)adjuvant setting with a relapse while on therapy or within 12 months from the end of last anti-HER2 therapy.
9. Measurable disease assessed by the investigator based on RECIST version 1.1.
10. Left ventricular ejection fraction (LVEF) ≥ 50% measured by multiple-gated acquisition scan (MUGA) or echocardiogram (ECHO).
11. Adequate organ and marrow function defined as follows:
    a. Absolute Neutrophil Count (ANC) ≥ 1,500/mm^3 ($1.5x10^9$/L).
    b. Platelet count ≥ 100,000/mm^3 ($100x10^9$/L).
    c. Hemoglobin ≥ 9g/dL (90g/L).
    d. Creatinine clearance ≥ 30 mL/min as calculated using the standard method for the institution.
    e. Total serum bilirubin ≤ 1.5 × ULN if no liver metastases or < 3 × ULN in the presence of documented Gilbert's syndrome (unconjugated hyperbilirubinemia) or liver metastases at baseline.

f. Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) ≤ 3.0 x ULN (< 5.0 × ULN in participants with liver metastases).
g. Alkaline phosphatase (ALP) ≤ 2.5 x ULN (≤ 5.0 x ULN if bone or liver metastases are present).
h. Serum albumin ≥ 2.5 g/dL

12. International normalized ratio (INR) and activated partial thromboplastin time (aPTT) ≤ 1.5 x ULN.
13. Willingness and ability to comply with scheduled visits, treatment plan, laboratory tests and other study procedures.
14. Negative serum pregnancy test with a sensitivity of at least 25 mIU/mL (unless permanent previous sterilization procedure such as bilateral salpingectomy, bilateral oophorectomy, or complete hysterectomy) for premenopausal women, and for women who have experienced menopause onset < 12 months prior to first dose of therapy.

4.2. Exclusion Criteria

Patients will be excluded from the study if they **meet any** of the following criteria:

1. Prior chemotherapy or HER2-targeted therapy for locally advanced or MBC (one prior endocrine therapy regimen for MBC without concurrent anti-HER2 therapy or radiotherapy is allowed).
2. Ineligible for treatment with T-DXd.
3. Any substance abuse or other medical conditions that, in the investigator's opinion, may interfere with patient's participation or study results.
4. Patients with spinal cord compression, leptomeningeal disease or clinically active central nervous system (CNS) metastases. Participants with clinically inactive brain metastases or treated brain metastases that are no longer symptomatic, and no needing corticosteroids or anticonvulsants may be enrolled in the study.
5. Active or prior documented interstitial lung disease (ILD)/pneumonitis or suspected ILD/pneumonitis that cannot be ruled out by imaging at screening.
6. Lung criteria:
    a. Lung-specific intercurrent clinically significant illnesses including, but not limited to, any underlying pulmonary disorder (e.g. pulmonary emboli within three months of the study enrollment, severe asthma, severe Chronic obstructive pulmonary disease (COPD), restrictive lung disease, pleural effusion etc.).
    b. Any autoimmune, connective tissue or inflammatory disorders (e.g. Rheumatoid arthritis, Sjogren's, sarcoidosis etc.) where there is documented, or a suspicion of pulmonary involvement at the time of screening. Full details of the disorder

should be recorded in the electronic Case Report Form (eCRF) for patients who are enrolled in the study.

c. Prior pneumonectomy.

7. Medical history of myocardial infarction within 6 months before registration, symptomatic congestive heart failure (CHF), troponin levels consistent with myocardial infarction as defined according to American College of Cardiologists (ACC) guidelines, unstable angina, or serious cardiac arrhythmia requiring treatment. QT interval corrected using Fridericia's formula (QTcF) > 470 msec (females) or > 450 msec (males) based on average of the screening triplicate 12-lead ECG.
8. History of active primary immunodeficiency, known Human Immunodeficiency Virus (HIV), active Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV).
9. Patients who received before treatment starts:

    d. Any investigational agent within 4 weeks.

    e. Chemotherapy within a period of time that is shorter than the cycle duration used for that treatment (e.g. < 3 weeks for fluorouracil, doxorubicine, epirubicine or < 1 week for weekly chemotherapy).

    f. Targeted therapy (e.g., antibodies): up to 4 weeks prior to starting study treatment.

    g. Endocrine therapy: tamoxifen or aromatase inhibitor within 2 weeks prior to starting study treatment.

    h. Radiotherapy within 2 weeks prior to starting study treatment. Patients who received prior radiotherapy to >25% of bone marrow are not eligible regardless of when it was administered.

    i. Major surgery or other anti-cancer therapy not previously specified within 4 weeks prior to starting study treatment.

    In any case, resolution of all acute toxic effects of prior anti-cancer therapy or surgical procedures to NCI-CTCAE version 5.0 grade ≤ 1 (except alopecia or other toxicities not considered a safety risk for the patient at investigator´s discretion) is mandatory.Patients may be enrolled with chronic, stable grade 2 toxicities (defined as no worsening to > grade 2 for at least 3 months prior to enrollment and managed with standard of care treatment) that the investigator deems related to previous anticancer therapy, such as: chemotherapy-induced neuropathy or fatigue and immunotherapy-induced toxicities (e.g. endocrinopathies as hypothyroidism/hyperthyroidism, type 1 diabetes, hypoglycemia, adrenal insufficiency, adrenalitis and skin hypopigmentation [vitiligo]).

10. Have a diagnosis of any other malignancy within 3 years prior to inclusion, except for adequately resected non-melanoma skin cancer, curatively treated in-situ disease, other solid tumors curatively treated and contralateral breast cancer.
11. Receipt of live, attenuated vaccine within 30 days prior to the first dose of T-DXd.

12. Prior treatment with T-DXd or allergic reaction to trastuzumab.
13. Patient is pregnant or breastfeeding or planning to become pregnant within the projected duration of the trial, starting at screening and through 7 months after the last dose of the study treatment. Male patients whose partners plan to become pregnant within the duration of the trial, starting at screening and through 4 months after the last dose of the study treatment.
    - ✓ For premenopausal women it is necessary an agreement to remain complete abstinent or use single or combined non-hormonal contraceptive methods that result in a failure rate of < 1% per year during the treatment period and for at least 7 months after the last dose of study treatment.
        - Examples of non-hormonal contraceptive methods with a failure rate of < 1% per year include bilateral tubal litigation, male sterilization, and certain intrauterine devices (provided coils are copper banded).
        - Alternative, two methods (e.g. two barrier methods such as a condom and a cervical cap or combined with estrogen and progestogen) may be combined to achieve a failure rate of < 1% per year. Barrier methods must always be supplemented with the use of a spermicide.

      Female patients must not donate, or retrieve for their own use, ova from the time of enrollment and throughout the study treatment period, and for at least 7 months after the final study drug administration. They should refrain from breastfeeding throughout this time. Preservation of ova may be considered prior to enrollment in this study.

    - ✓ For men it is necessary an agreement to remain complete abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and to refrain from donating sperm during the same period, as defined below with female partners of childbearing potential or pregnant female partners, men must remain abstinent or use a condom during the treatment period and for at least 4 months after the last dose of study treatment to avoid exposing the embryo.

      Abstinence is only acceptable if it is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

14. Uncontrolled intercurrent illness including uncontrolled infection requiring intravenous (IV) antibiotics, antivirals, or antifungals.

Has substance abuse or any other medical/psychological conditions that may, in the opinion of the investigator, interfere with the patient's participation in the clinical study or evaluation of the clinical study results.

4.3. Discontinuations

4.3.1. Discontinuation of Study Treatment

The criteria for enrollment must be followed explicitly. If a patient who does not meet enrollment criteria is inadvertently enrolled, that patient should be discontinued from the study treatment, but can be allowed to continue in the study in order to provide the follow-up data needed for the analysis of the entire population. An exception may be granted if the patient, in the opinion of the investigator, is having benefit from the study treatment. In these rare cases, the investigator must obtain documented approval from GEICAM to allow the patient to continue to receive the study treatment.

Patients can be discontinued from the study therapy in the following circumstances:

- Patient's own request.
- Unacceptable toxicity as defined in the protocol.
- Tumor progression as defined in the protocol.
- Any clinical AE, laboratory abnormality or inter-current illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the patient.
- Pregnancy:
    - ✓ Instruct to contact the investigator or study staff immediately if they suspect they might be pregnant.
    - ✓ The investigator must immediately notify GEICAM if a study patient or the female partner of a male patient becomes pregnant.
- Termination of the study by GEICAM.
- Physician's decision, including need of other anti-cancer therapy, not specified in the protocol.
- If the patient is non-compliant with study procedures.
- Loss of ability to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (e.g. infectious disease) illness.
- Death

All permanent treatment discontinuation should be recorded by the Investigator in the electronic Case Report Form (eCRF) when considered as confirmed.

4.3.2. Discontinuation of Study Sites

Study Site participation may be discontinued if GEICAM, the investigator or the Ethics Committee/Institutional Review Board (EC/IRB) of the study site judges it necessary for any reason.

4.3.3. Discontinuation of Study

The study may be discontinued by GEICAM if this is medically reasonable and consistent with applicable regulations of Good Clinical Practice (GCP). Stopping the study for medical reasons may be required if patients experienced adverse reactions under the treatment with the study treatment or if new information about the safety or effectiveness of the study treatment justifies it.

4.4. Menopausal status definitions and contraception

For the purpose of this study, a woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormone replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

For the purpose of this study, a man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

Patients of childbearing potential must agree to avoid becoming pregnant while receiving study drug and for 7 months after the last dose of study treatment to avoid exposing the embryo.

If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5. Treatment

5.1. Treatments Administered

All patients enrolled will be treated with the following:

- ✓ Trastuzumab deruxtecan 5.4 mg/kg IV every 3 weeks (± 3 days).

The subject's weight at baseline will be used to calculate the initial dose. If during the course of treatment the subject's weight changes by ± 10% of the baseline weight, the subject's dose will be recalculated based on the subject's updated weight.

Patients will receive T-DXd until unacceptable toxicity, progressive disease (PD), informed consent withdrawal, or other discontinuation criterion is met.

5.2. Materials and Supplies

T-DXd used in this trial will be named as study treatment throughout the protocol.

T-DXd will be provided to the study sites by GEICAM with the appropriate label for clinical trial use for the purpose of this study.

5.2.1. Storage, preparation and administration of trastuzumab deruxtecan

Investigators and site staff are reminded to continuously monitor refrigerator storage temperatures and ensure that thermometers are working correctly as required for proper storage of study treatment. These include thermometers for the refrigerator storage.

Storage area temperature conditions must be monitored and recorded daily. A daily temperature log will also be kept at the study site.

Any issue or deviation that affect the storage conditions or handling of the study treatment must be reported to the Sponsor for its information.

For T-DXd provided by the Sponsor, any temperature excursions must be reported immediately to GEICAM and documented. Once a deviation is identified, the study treatment MUST be quarantined and not used until GEICAM provides documentation of permission to use the study treatment product.

T-DXd should be kept in a secured locked area at the study site in accordance with applicable regulatory requirements. Returned study treatment provided by the Sponsor should be stored separately from study treatment that needs to be dispensed.

5.2.1.1. Packaging and storage

T-DXd will be supplied by AstraZeneca as a 100 mg/vial lyophilized powder for concentrate for solution for infusion in a single-use glass vial. It is supplied in carton containing 1 vial and should

be stored in their original container protected from light at 2°C–8ºC. Storage conditions and the expiry date are indicated on the primary and secondary package label. T-DXd must be stored in its original packaging at controlled refrigerator and protected from moisture. The investigator or designee must confirm that appropriate temperature conditions have been maintained during the transit for the received T-DXd and that any discrepancies are reported and resolved before its use.

Please refer to the current version of the T-DXd IB and/or Pharmacy Manual for further details on the use, handling, and storage of the study drug.

5.2.1.2. Preparation

Dilution for dose solution preparation must occur under appropriate aseptic conditions, as the study drug contains no preservatives.

Reconstitute each 100 mg vial using a sterile syringe to slowly inject 5 mL of water for injections in each vial to obtain a final concentration of 20mg/ml.

Dose solutions should be prepared in a 100 mL IV infusion bag containing sterile 5% aqueous glucose solution. The infusion bag is constructed with product contacting materials of polyvinyl chloride (PVC), polyethylene (PE), or polyolefin (PO) composed of PE and polypropylene (PP). Slowly add the required volume of drug product to the IV bag and gently mix by slowly rotating the bag without shaking.

The solution for infusion should be used immediately to limit product degradation and microbial growth in case of potential accidental contamination. If not used immediately, the dose solution may be stored at 2°C–8°C for 24 hours or at ambient temperature ≤25°C for 4 hours. This time includes storage and time for administration for infusion. If the dose solution is stored at 2°C-8°C, it should be removed from refrigeration and allowed to reach room temperature prior to administration.

5.2.1.3. Administration

In the **first infusion** T-DXd should be infused over 90 minutes.

In the **subsequent infusions** T-DXd should be infused over 30 minutes if the previous infusion was well tolerated without an infusion-related reaction.

5.2.2. Accountability

It is the responsibility of the investigator to ensure that a current record of T-DXd disposition is maintained at each study site where study treatment provided by GEICAM are inventoried and disposed.

A drug dispensing log, including records of T-DXd received from GEICAM and T-DXd administered to the patients, will be provided and kept at the study site. GEICAM will provide forms to facilitate inventory control if the staff at the investigational site does not have an established system that meets these requirements. Records or logs must comply with applicable regulations and guidelines.

T-DXd provided by GEICAM will be preferably destroyed at each participating site. The site must obtain written authorization from GEICAM before it is destroyed, and this destruction must be documented on the appropriate form.

5.3. Method of Assigning a Patient to a Treatment

All patients who meet all criteria for enrollment will be registered to receive T-DXd.

Patients will be screened by one of the investigators prior to study entry. An explanation of the study and discussion of the expected side effects and presentation of the informed consent document (ICD) will take place. Eligible and consented patients will be screened and then enrolled into the study.

All patients screened in the study, will be included in a *Patient Screening Log* maintained at each site and at the GEICAM central office.

No patients can be enrolled and receive study treatment until the patient has been screened in the study. All eligibility criteria must be met at the time of enrollment. There will be no exceptions. Any question should be addressed with GEICAM prior to enrollment. An eligibility checklist must be completed and signed by the Principal Investigator or Sub-Investigator before enrolling each patient to confirm all inclusion/exclusion criteria. This eligibility checklist should be filed with the study documentation. Once the eligibility checklist is completed, the study personnel at the site will enroll the patient through the eCRF and the system will send the unique enrollment number of the patient. This enrollment number should be used on all documentation and correspondence with the GEICAM central office. Only after the confirmation of enrollment by the system, the patient can receive the study treatment. All patients enrolled in the study will be registered in a *Patient Enrollment and Identification Log* that will be only maintained at the site.

Study treatment must be administered within 7 days from enrollment.

5.4. Special Treatment Considerations. Dose Adjustments of Study Treatment

All dose modifications should be based on the worst preceding toxicity.

Every effort should be made to administer study treatment at the planned dose and schedule. However, in the event of significant treatment-related toxicity, administration of study treatment

may need to be adjusted as described in the following sections. Depending on the nature of the toxicity observed, dose adjustments may be required for T-DXd.

All dose modifications/adjustments must be clearly documented in the patient's source notes and the appropriate section of the eCRF.

5.4.1. Dose Interruptions and Reductions

In the event of significant treatment-related toxicity, T-DXd dosing may be interrupted or delayed and/or reduced. Patients are to be instructed to notify Investigators at the first occurrence of any adverse sign or symptom.

T-DXd recommended dose modifications for treatment related toxicities requiring treatment interruption/delay/reduction or persisting despite optimal medical treatment are described in Tables 1 and 2.

Table 1. Dose Reduction Levels of T-DXd

Dose Level	T-DXd
Starting dose	5.4 mg/kg
-1	4.4 mg/kg
-2	3.2 mg/kg

Table 2. Dose Modifications of T-DXd

Worst toxicity NCI-CTCAE v 5.0 Grade (unless otherwise specified)	Management Guidelines for T-DXd
No toxicity	Maintain dose and schedule
Infusion-Related Reaction	
Grade 1 (Mild transient reaction; infusion interruption not indicated; intervention not indicated)	If infusion related reaction (such as fever and chills, with and without nausea/vomiting, pain, headache, dizziness, dyspnea, hypotension) is observed during administration, the infusion rate should be reduced by 50% and patients should be closely monitored. If no other reactions appear, the subsequent infusion rate could be resumed at the initial planned rate.
Grade 2 (Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, nonsteroidal	Administration of T-DXd should be interrupted and symptomatic treatment started (e.g. antihistamines, NSAIDs, narcotics, IV fluids).

anti-inflammatory drugs (NSAIDs), narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hrs)	If the event resolves or improves to grade 1, infusion can be re-started at a 50% reduced infusion rate. Subsequent administrations should be conducted at the reduced rate.
Grade 3 or 4 (Prolonged or life-threatening consequences, urgent intervention indicated)	Administration of T-DXd should be discontinued immediately and permanently. Urgent intervention indicated. Antihistamines, corticosteroids, epinephrine, bronchodilators, vasopressors, intravenous fluid therapy, oxygen inhalation etc., should be administered.
Hematologic Toxicity: if supportive therapy fails (as clinically indicated and according to local practice), consider additional toxicity management guidelines as below. For any grade 4 hematological toxicity with significant clinical symptoms that does not resolve with treatment within 4 weeks, resuming T-DXd may be possible if the toxicity resolves, in consultation with GEICAM.	
Neutrophil Count Decreased and/or White Blood Cell Count Decreased	
Grade 3	Delay dose until resolved to ≤ Grade 2, then maintain dose
Grade 4	Delay dose until resolved to ≤ Grade 2, then reduce dose 1 level
Febrile Neutropenia (absolute neutrophil count < 1 x 10^9/L, fever > 38.3°C or a sustained temperature of ≥ 38 °C for more than one hour)	Delay dose until resolved, then reduce 1 dose level
Lymphocyte Count Decreased	
Grade 1 to Grade 3	No dose modification
Grade 4 (< 0.2 x 10^9/L)	Delay dose until resolved to ≤ Grade 2: - If resolved in ≤ 14 days from day of onset, maintain dose - If resolved in > 14 days from day of onset, reduce dose 1 level
Anemia	
Grade 3 (Hemoglobin [Hb] <8.0 g/dL); transfusion indicated	Delay dose until resolved to ≤ Grade 2, then maintain dose
Grade 4 (Life threatening consequences; urgent intervention indicated)	Delay dose until resolved to ≤ Grade 2, then reduce dose 1 level
Platelet Count Decreased	
Grade 3 (platelets <50 – 25 x 10^9/L)	Delay dose until resolved to ≤ Grade 1: - If resolved in ≤ 7 days from day of onset, maintain dose - If resolved in > 7 days from day of onset, reduce dose 1 level
Grade 4 (platelets < 25 x 10^9/L)	Delay dose until resolved to ≤ Grade 1, then reduce dose 1 level
Cardiac Toxicity	
Symptomatic congestive heart failure (CHF)	Discontinue patient from study treatment

Decrease in Left ventricle ejection fraction (LVEF) 10-20% (absolute value), but LVEF > 45%	Continue treatment with T-DXd
LVEF 40% to ≤ 45% and decrease is < 10% (absolute value) from baseline	Continue treatment with T-DXd Repeat LVEF assessment within 3 weeks
LVEF 40% to ≤ 45% and decrease is 10-20% (absolute value) from baseline	Interrupt T-DXd dosing Repeat LVEF assessment within 3 weeks: - If LVEF has not recovered to within 10% (absolute value) from baseline, discontinue permanently T-DXd - If LVEF recovers to within 10% from baseline, resume treatment with T-DXd at the same dose
LVEF < 40% or > 20% (absolute value) drop from baseline	Interrupt T-DXd dosing Repeat LVEF assessment within 3 weeks: - If LVEF < 40% or > 20% drop from baseline is confirmed, discontinue permanently T-DXd - If not confirmed, follow the rules described above
Electrocardiogram QTc Prolonged	
Grade 3 (average QTc > 500 ms or >60 ms change from baseline)	Delay dose until resolved to ≤ Grade 1 (corrected QT ≤ 480 ms), determine if another medication the patient was taking may be responsible and can be adjusted or if there are any changes in serum electrolytes that can be corrected, then if attributed to T-DXd, reduce dose 1 level
Grade 4 (Torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia)	Discontinue permanently T-DXd

Pulmonary Toxicity	Any evidence of ILD/pneumonitis should be promptly investigated. If a subject develops radiographic changes potentially consistent with ILD/pneumonitis or develops new or worsening pulmonary or other related signs/symptoms such as dyspnea, cough or fever, rule out ILD/pneumonitis.
	If the AE is confirmed to have an etiology other than ILD/pneumonitis, follow the management guidance outlined in other AEs in this table.
	If the AE is suspected to be ILD/pneumonitis, treatment with T-DXd should be interrupted pending further evaluations.
	Evaluations should include:
	- High resolution computer tomography (CT) Scan
	- Pulmonologist consultation (Infectious Disease consultation as clinically indicated)
	- Bronchoscopy and bronchoalveolar lavage if clinically indicated and feasible
	- Pulmonary function tests including forced vital capacity, carbon monoxide diffusing capacity and pulse oximetry (SpO2)
	- Clinical laboratory test
	  - Arterial blood gases if clinically indicated
	  - Blood culture, blood cell count, differential white blood cell count, C-reactive protein, COVID-19 test
	  - One blood sample collection for PK analysis as soon as ILD/pneumonitis is suspected, if feasible
	Other tests could be considered, as needed (e.g., PCP screening, beta-D-glucan test, BAL) in all patients with suspected ILD/pneumonitis as part of the ILD diagnosis of exclusion especially in patients with 1 or more of the following risk factors: lymphopenia, long-term/intermittent steroid use, brain metastases, and chronic lung diseases).
	If the AE is confirmed to be ILD/pneumonitis, follow the ILD/pneumonitis management guidance as outlined below.
	All events of ILD/pneumonitis, regardless of severity or seriousness, will be followed until resolution including after T-DXd discontinuation.

Grade 1	<u>The administration of T-DXd must be interrupted for any ILD/pneumonitis events regardless of grade.</u> - Monitor and closely follow-up in 2 to 7 days for onset of clinical symptoms and pulse oximetry. - Consider follow-up imaging in 1-2 weeks (or as clinically indicated).

	- Consider starting systemic corticosteroids (e.g. at least 0.5 mg/kg/day prednisone or equivalent) until improvement, followed by gradual taper over at least 4 weeks. - If worsening of diagnostic observations despite initiation of corticosteroids, then follow Grade 2 guidelines (unless the patient is asymptomatic) T-DXd can be restarted only if the event is fully resolved to Grade 0: - If resolved in ≤ 28 days from day of onset, maintain dose - If resolved in > 28 days from day of onset, reduce dose 1 level However, if the event grade 1 ILD/pneumonitis has not resolved within 18 weeks (126 days) from the last infusion, T-DXd should be permanently discontinued.
Grade 2	Permanently discontinue T-DXd. - Promptly start and treat with systemic corticosteroids (e.g., at least 1mg/kg/day prednisone or equivalent) for at least 14 days followed by a gradual taper over at least 4 weeks. - Monitor symptoms closely. - Re-image as clinically indicated. - If worsening or no improvement in clinical or diagnostic observations in 5 days: • Consider increasing dose of corticosteroids (e.g., 2 mg/kg/day prednisone or equivalent) and administration may be switched to intravenous (e.g. methylprednisolone). • Re-consider additional work-up for alternative etiologies as described above. • Escalate care as clinically indicated.
Grade 3 or 4	Permanently discontinue T-DXd. - Hospitalization required. - Promptly initiate empiric high-dose methylprednisolone IV treatment (e.g., 500-1000 mg/day for 3 days), followed by at least 1.0 mg/kg/day of prednisone (or equivalent) for at least 14 days followed by a gradual taper over at least 4 weeks. - Re-image as clinically indicated. - If still no improvement within 3 to 5 days, • Re-consider additional work-up for alternative etiologies as described above. • Consider other immuno-suppressants and/or treat per local practice.
Ocular	
Grade 3	Delay T-DXd until resolved to ≤ Grade 1:

	- If resolved in ≤ 7 days from day of onset, maintain dose - If resolved in > 7 days from day of onset, reduce dose 1 level
Grade 4	Permanently discontinue T-DXd
Blood creatinine increased	
Grade 3 (> 3.0 to 6.0 x upper limit of normal [ULN])	Delay dose until resolved to ≤ Grade 2 or baseline, then reduce dose 1 level
Grade 4 (> 6.0 x ULN)	Permanently discontinue T-DXd
Hepatic Toxicity	
Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) with simultaneous total blood bilirubin (TBL) increased (see section 5.4.1.1)	
Aspartate aminotransferase (AST) or alanine aminotransferase (ALT)	
Grade 2 (>3.0 - 5.0 x ULN if baseline was normal; >3.0 - 5.0 x baseline if baseline was abnormal)	No action
Grade 3 (>5.0 - 20.0 x ULN if baseline was normal; >5.0 - 20.0 x baseline if baseline was abnormal) In patients without liver metastases and patients with liver metastases and baseline level ≤ 3 x ULN	Repeat testing within 3 days. Delay dose until resolved to ≤ Grade 1 if baseline was normal, otherwise delay dose until resolved to ≤ baseline, then: - If resolved in ≤ 7 days from day of onset, maintain dose - If resolved in > 7 days from day of onset, reduce dose 1 level
Grade 3: (>8.0 - 20.0 x ULN if baseline was normal; >8.0 - 20.0 x baseline if baseline was abnormal) In patients with liver metastases, if the baseline level was > 3 x ULN	Repeat testing within 3 days. Delay dose until resolved to ≤ baseline level, then: - If resolved in ≤ 7 days from day of onset, maintain dose - If resolved in > 7 days from day of onset, reduce dose 1 level
Grade 4 (>20.0 x ULN if baseline was normal; >20.0 x baseline if baseline was abnormal)	Permanently discontinue T-DXd
Blood bilirubin increased	
Grade 2 (>1.5 - 3.0 x ULN if baseline was normal; >1.5 - 3.0 x baseline if baseline was abnormal)	If no documented Gilbert's syndrome or liver metastases at baseline, repeat testing within 3 days. Delay dose until resolved to ≤ Grade 1: - If resolved in ≤ 7 days from day of onset, maintain dose - If resolved in > 7 days from day of onset, reduce dose 1 level If documented Gilbert's syndrome or liver metastases at baseline, continue study treatment
Grade 3 (>3.0 - 10.0 x ULN if baseline was normal; >3.0 - 10.0 x baseline if baseline was abnormal)	If no documented Gilbert's syndrome or liver metastases at baseline, repeat testing within 3 days. Delay dose until resolved to ≤ Grade 1:

	- If resolved in ≤ 7 days from day of onset, reduce dose 1 level - If resolved in > 7 days from day of onset, permanently discontinue T-DXd If documented Gilbert's syndrome or liver metastases at baseline, repeat testing within 3 days. Delay dose until resolved to ≤ Grade 2: - If resolved in ≤ 7 days from day of onset, reduce dose 1 level - If resolved in > 7 days from day of onset, permanently discontinue T-DXd
Grade 4 (>10.0 x ULN if baseline was normal; >10.0 x baseline if baseline was abnormal)	Permanently discontinue T-DXd
Blood Alkaline Phosphatase Increased	
No modification unless determined by the Investigator to be clinically significant or life-threatening.	
Gastrointestinal	
Nausea	
Grade 3	Delay dose until resolved to ≤ Grade 1 - If resolved in ≤ 7 days from day of onset, maintain dose - If resolved in > 7 days from day of onset, reduce dose 1 level
Diarrhea/Colitis	
Grade 3	Delay dose until resolved to ≤ Grade 1 - If resolved in ≤ 3 days from day of onset, maintain dose - If resolved in > 3 days from day of onset, reduce dose 1 level
Grade 4	Permanently discontinue T-DXd
Other AEs	
Grade 3	Delay dose until resolved to ≤ Grade 1 or baseline: - If resolved in ≤ 7 days from day of onset, maintain dose - If resolved in > 7 days from day of onset, reduce dose 1 level
Grade 4	Permanently discontinue T-DXd

Once the dose of T-DXd has been reduced for a given patient because of toxicity, all subsequent cycles should be administered at that lower dose level unless further dose reduction is required. If toxicity continues after 2 dose reductions, then the patient will be withdrawn from study treatment. T-DXd dose re-escalation is not allowed in the study.

Dose can be interrupted for up to 28 days from the planned date of administration. If a patient is assessed as requiring a dose delay longer than 28 days, the patient will be withdrawn from the study (except for Grade 1 ILD/pneumonitis for which a 126 delay is permitted).

5.4.1.1. Follow-up on potential drug-induced liver injury (DILI) cases

Patients with transaminase increase combined with total bilirubin increase may be indicative of potential DILI and should be considered as clinically important events.

In general, any increase of serum aminotransferases to > 3 x upper limit of normal (ULN) should be followed by repeat testing within 48 to 72 hours.

If total bilirubin is elevated > 2 x ULN, fractionation into direct and indirect bilirubin is required.

The threshold for potential DILI may depend on the patient's baseline AST/ALT and total bilirubin value; patients meeting any of the following criteria will require further follow-up as outlined below:

- For patients with normal ALT and AST and total bilirubin value at baseline: AST or ALT ≥ 3.0 x ULN combined with total bilirubin ≥ 2.0 x ULN.
- For patients with elevated AST or ALT or total bilirubin value at baseline: AST or ALT ≥ 2 x baseline AND > 3.0 x ULN OR AST or ALT ≥ 8.0 x ULN, combined with total bilirubin ≥ 2 x baseline AND ≥ 2.0 x ULN or ≥ 3.0 x ULN in case of Gilbert´s syndrome.

Medical review needs to ensure that liver test elevations are not caused by cholestasis. Cholestasis is defined as ALP elevation > 2.0 x ULN with R value (see note below) < 2 in patients without bone metastasis.

Note: The **R value** is calculated by dividing the ALT by the ALP, using multiples of the ULN for both values. It denotes the relative pattern of ALT and/or ALP elevation is due to cholestatic (R ≤ 2), hepatocellular (R ≥ 5), or mixed (R >2 and < 5) liver injury.

In the absence of cholestasis, these patients should be immediately discontinued from study drug treatment, and repeat liver function testing as soon as possible, preferably within 48 hours from the awareness of the abnormal results. The evaluation should include laboratory tests, detailed history, physical assessment and the possibility of liver metastasis or new liver lesions, obstructions/compressions, etc.

Upon presentation:

- Perform comprehensive medical history including cardiac disease, blood transfusions, i.v. drug abuse, travel, work, alcohol intake, and full clinical examination for evidence of acute or chronic liver disease, cardiac disease, and infection etc.
- History of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, special diets, and chemicals exposed to within one month of the onset of the liver injury.
- Exclude other causes of liver disease.

Patient monitoring:

- Repeat liver chemistry tests within 48-72 hours.
- Retest frequency can decrease to weekly or less if abnormalities stabilize, drug has been discontinued, and the patient is asymptomatic.

As DILI is essentially a diagnosis of exclusion, other causes of abnormal liver tests should be considered, and their role clarified before the diagnosis of DILI is confirmed (see Table 3). Liver biopsy has limited value in the diagnosis of DILI as histopathological findings in DILI can resemble many other liver conditions. However, biopsy can be useful to establish an alternative diagnosis especially if other tests are inconclusive.

If DILI confirmed: permanently discontinue.

If DILI unlikely - interrupt treatment. Consider alternative causes of liver disease (see Table 3) Treat identified cause according to institutional guidelines. If resolved, reduce by one dose level. Re-administration of study treatment should be considered only if the investigator assesses benefit to outweigh the risk. Any decision regarding re-administration of study drug/s and dose regimen should be discussed with the Sponsor.

Table 3. Alternative causes of liver disease

Disease	**Assessment**
Hepatitis A, B, C, E	IgM anti-HAV; HBsAg, IgM anti-HBc, HBV DNA; anti-HCV, HCV RNA, IgM & IgG anti-HEV, HEV RNA
CMV, HSV, EBV infection	IgM & IgG anti-CMV, IgM & IgG anti-HSV; IgM & IgG anti-EBV
Autoimmune hepatitis	ANA & ASMA titers, total IgM, IgG, IgE, IgA
Alcoholic hepatitis	Ethanol history, gammaGT, MCV, CD-transferrin
Nonalcoholic steatohepatitis	Ultrasound or MRI
Hypoxic/ischemic hepatopathy	Medical history: acute or chronic CHF, hypotension, hypoxia, hepatic venous occlusion. Ultrasound or MRI.
Biliary tract disease	Ultrasound or MRI, ERCP as appropriate.
Wilson disease	Caeruloplasmin
Hemochromatosis	Ferritin, transferrin
Alpha-1-antitrypsin deficiency	Alpha-1-antitrypsin

Following appropriate causality assessments, as outlined above, the causality of the drug is estimated as “probable” i.e. > 50% likely, if it appears greater than all other causes combined. The term “drug-induced” indicates probably caused by the drug, not by something else, and only such a case can be considered a DILI case and should be reported as an SAE.

5.5. Medication Errors and Overdose

Medication errors may result in this study from the administration or consumption of the wrong drug, by the wrong patient, at the wrong time or at the wrong dosage strength. Such medication errors occurring to a study participant are to be captured on the AE page of the eCRFs and on the SAE form when appropriate. In the event of medication dosing error, GEICAM should be notified immediately.

Medication errors are reportable irrespective of the presence of an associated AE/SAE, including:

- ✓ Medication errors involving patient exposure to the product;
- ✓ Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating patient.

Whether or not the medication error is accompanied by an AE, as determined by the Investigator, the medication error and, if applicable, any associated AE(s) is captured on the AE eCRF page (refer to Management, Timing and Assessment of Adverse Events section for further details).

5.6. General Concomitant Medication and Supportive Care Guidelines

Patients must be instructed not to take any additional medication (over-the-counter or other products) during the study without prior consultation with the investigator. Any medications including herbal supplements, vitamins, or treatment taken by the patient from 28 days prior to the start of study treatment and up to 30 days following the last dose of study treatment and the reason for their administration must be recorded on the eCRF.

Routine postoperative care, such as dressing changes, suture removal, drain removal, or venous access (central or peripheral), does not need to be recorded. Anesthetics used for any surgical procedures performed during the patient's participation in the study can be recorded as "unspecified anesthesia" on the concomitant treatment records; it is not necessary to list the specific anesthetics. Palliative and supportive care for cancer-related symptoms will be offered to all patients in this study.

5.6.1. Prohibited Medications

The following treatments are prohibited throughout the duration of the active treatment phase:

- ✓ **Anticancer agents:** No additional investigational or commercial anticancer agents such as chemotherapy, immunotherapy, targeted therapy, biological response modifiers, or endocrine therapy will be permitted during the active treatment phase. In general, any drugs containing "for the treatment of breast cancer" on the product insert are not permitted on study.

- ✓ **Hormone replacement therapy,** topical estrogens (including any intra-vaginal preparations), megestrol acetate and selective estrogen-receptor modulators (eg, raloxifene) are prohibited during the active treatment phase.
- ✓ **Any concurrent radiotherapy** (except palliative radiotherapy as specified under Section 5.6.3.) is prohibited throughout the duration of the active treatment phase of the study. Patients requiring this procedure will be discontinued from the active treatment phase and will enter the follow-up phase. This radiotherapy will be considered an alternative cancer therapy.
- ✓ **Chronic immunosuppressive therapies (IV or oral)** withing 14 days prior to first study dose including corticosteroids or other immunosuppressive medications (methotrexate, azathioprine, and tumor necrosis factor- alpha blockers). IV or oral corticosteroids may be used only for: short-term courses (< 2 weeks) of low to moderate dose (<10mg prednisolone per day or equivalent); long-term, alternate-day treatment with short-acting preparations maintenance physiologic doses (replacement therapy). A temporary period of corticosteroid treatment will be allowed for different indications after discussion with the sponsor (e.g., chronic obstructive pulmonary disease, radiation, nausea, etc.). Patient's with bronchopulmonary disorders who require intermittent use of bronchodilators (such as albuterol) will not be excluded from this study.

  The use of immunosuppressive medications for the management of T-DXd related AEs, as antiemetic prophylaxis (see Section 5.6.3) or in patients with hypersensitivity reactions to radiographic contrast agents is acceptable.

  Topical administration (skin or eyes), or by aerosol, or by intra-articular, bursal, or tendon injection prevention is permitted.
- ✓ **Chloroquine or hydroxychloroquine** is not allowed during the study treatment. If treatment with them is absolutely required for SARS-CoV-2 (i.e. COVID-19), T-DXd must be interrupted and a wash-out period of more than 14 days will be required before restarting T-DXd.

5.6.2. Medications Not Recommended

The following treatments are not recommended throughout the duration of the active treatment phase. Alternative therapies should be considered whenever possible. If usage of the following treatments is deemed necessary, consultation and agreement with GEICAM is required prior to treatment initiation.

- ✓ The use of **herbal medicine** is not recommended during the active treatment phase.
- ✓ Patients must avoid using tanning lights and booths and should cover their skin and use sunscreen when in the sun (or exposed to UV radiation) as possible.

5.6.3. Permitted Medications

The following treatments are permitted throughout the duration of the active treatment phase:

- ✓ **Standard therapies** for pre-existing medical conditions, medical and/or surgical complications, and palliation. Any medication intended solely for supportive care (eg, analgesics, antidiarrheals, antidepressants) may also be used at the investigator's discretion. All medications should be recorded.
- ✓ **Bisphosphonates and receptor activator of nuclear factor kappa-B ligand (RANKL)** inhibitors for the treatment of osteoporosis or management of existing bone metastases may be continued for patients who are receiving them. However, the need to initiate or increase the dose of these therapies during the study will be considered as indicative of disease progression leading to the discontinuation of patient from the active treatment phase unless disease progression can be completely ruled out and the exact reason for the use of these therapies clearly documented in the patient's source documentation.
- ✓ **Hematopoietic growth factors** (eg, granulocyte colony stimulating factor [G-CSF], granulocyte macrophage colony stimulating factor [GM-CSF]): Primary prophylactic use of granulocyte-colony stimulating factors is not permitted but they may be used to treat treatment-emergent neutropenia as indicated by the current American Society of Clinical Oncology (ASCO) guideline. If neutropenic complications are observed in a cycle in which primary prophylaxis with CSFs was not received, secondary prophylaxis may be given at the discretion of the investigator, but only if dose reduction or delay are not considered to be a reasonable alternative.
- ✓ **Palliative radiotherapy** is permitted for the treatment of painful bony lesions provided that the lesions were known to be present at the time of study entry and the investigator clearly documents that the need for palliative radiotherapy is not indicative of disease progression. For patients with bone involvement, it is suggested to administer palliative radiotherapy before study initiation if possible and clinically appropriate (eg, lesions at risk for spontaneous micro-factures or painful lesions). The dates on which palliative radiotherapy is administered should be recorded in concomitant medication. It is not required to hold T-DXd during palliative radiotherapy.

  Areas that have received palliative radiotherapy cannot be used to assess response to study treatment.
- ✓ **COVID-19 vaccination.** Regarding the vaccination against COVID-19, national guidelines should be followed.
- ✓ **Hormones for noncancer-related conditions** (e.g., insulin for diabetes and hormone replacement therapy).

- ✓ Antiemetics. Based on the currently available clinical safety data, it is recommended that patients receive prophylactic anti-emetic agents prior to infusion of T-DXd and on subsequent days. Antiemetics such as 5-hydroxytryptamine receptor (5-HT3) antagonists or Neurokinin-1 (NK1) receptor antagonists and/or corticosteroids (e.g. dexamethasone) should be considered and administered in accordance with the prescribing information or institutional guidelines.

5.7. Treatment Compliance

Patients will be treated with T-DXd at the site by the investigator or designee, under medical supervision. The date and start and end time of each dose administered at the site will be recorded in the source documents and in the eCRF.

In case of infusion interruption, the times of infusion stop and restart will be recorded. If an incomplete dose is administered, the amount of drug infused will be calculated and entered in the eCRF.

The dose of study treatment and study patient identification will be confirmed at the time of each dosing by a member of the study site personnel.

6. Efficacy and Safety Evaluations, Sample Collection and Testing (Standard Laboratory Testing) and Appropriateness of Assessments

Study procedures and their timing (including tolerance limits for timing) are summarized in the Study Schedule, Protocol Attachment 1.

6.1. Efficacy Assessments

Tumor response will be assessed using RECIST 1.1. Tumor assessment should be performed during the screening period; however, if a patient has a valid image test performed up to 28 days before the date of enrollment it is not required to repeat it.

Tumor assessment during the screening period should consist of clinical exam and of anatomical imaging consisting of:

- ✓ Contrast enhanced (unless clinically contraindicated) CT scan or MRI of the chest, abdomen and pelvis (CAP). A positron emission tomography (PET)-CT scan will be accepted if the CT scan meets the RECIST version 1.1. requirements for tumor lesion evaluation.
- ✓ Bone scan is mandatory if the patient has bone disease or if there is any suspicion of bone metastases. A PET scan will be also accepted to assess the already known or newly-suspected bone metastatic disease. Any suspicious abnormalities (i.e., hotspots) identified on the bone or PET scan at screening must be confirmed by X-ray, CT scan with bone window or MRI. Additional bone scans will be performed only to confirm a complete response (CR) in patients with bone lesions identified at screening, or whenever clinically or biochemically bone progression or newly diagnosed bone disease is suspected.
- ✓ Brain CT scan with IV contrast or MRI is mandatory if the patient has previously known CNS metastases or if there is any suspicion of CNS metastases.
- ✓ CT scan or MRI of any other sites of disease as clinically indicated.
- ✓ Clinical assessment of superficial disease by callipers which will include colour photographs of all superficial metastatic lesions including a ruler to estimate the size of the lesion. When lesions can be evaluated by both a clinical exam and imaging, imaging evaluation should be undertaken since it is more objective.

All measurable and evaluable lesions should be assessed at the screening visit and re-assessed at each subsequent tumor evaluation and documented in the eCRF. Tumor assessments will be performed at screening, every 9 weeks (±7days) and after the third tumor evaluation every 12 weeks (±7days) until radiographically and/or clinically (i.e., for photographed or palpable lesions) documented PD as per RECIST version 1.1., withdrawal of consent, start of new anticancer treatment, death, or study termination, whichever occurs first.

Tumor assessment will be continued in patients that discontinue treatment without disease progression per RECIST version 1.1. until death, disease progression, start of new anticancer therapy, patient's consent withdrawal, or study termination, whichever occurs first, every 12 weeks (± 7 days) from the last tumor assessment and bone scans (if applicable) as stated above.

The same radiographic procedures and technique must be used throughout the study for each patient.

Each assessment will be performed as scheduled according to the calendar regardless of any dosing delay to prevent the introduction of bias into the assessment of efficacy. Failure to perform any of the required disease assessments will result in the inability to determine disease status for that time point.

Efficacy analyses will be performed using the local radiologist's/investigator's tumor assessments as the primary data source.

Patients discontinuing the tumor assessments for the primary endpoint will enter a follow-up period during which further treatments, date of progressions to the following two additional lines of therapy and survival information will be collected. Data collection will happen every 6 months (± 14 days) from the last tumor assessment. The follow-up period will conclude at the time of the final OS analysis.

6.2. Safety Assessments

Investigators are responsible for monitoring the safety of patients who have been enrolled in this study and for alerting GEICAM of any event that seems unusual.

The investigator is responsible for appropriate medical care of patients during the study.

The investigator remains responsible for following, through an appropriate health-care option, AEs that are serious or that caused the patient to discontinue before completing the study. The patient should be followed until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the investigator.

During the course of the study, all patients enrolled in the trial must be evaluated according to the schedule outlined in the flow charts and described below. The results of the evaluation will be recorded in the eCRF pages until the patients are not followed anymore.

6.2.1. Timing of Assessments

All assessments to be performed at screening and during the study are specified in the Study Schedule, Protocol Attachment 1.

Vital signs assessments will include blood pressure, pulse and body temperature.

Measurement of LVEF by MUGA or ECHO will be performed at screening and at the times specified in the Study Schedule. The same method of LVEF assessment, ECHO or MUGA, must be used throughout the study, and to the extent possible, at the same institution. Triplicate 12-lead ECGs will be obtained at screening (it is recommended to record the triplicate ECGs 2-4 minutes apart) and at the times specified in the Study Schedule. Parameters will be measured, including RR, PR, QT intervals, and QRS duration.

The following safety laboratory assessments will be performed by the local laboratories, at screening and at the times specified in the Study Schedule:

- Hematology: hemoglobin, white blood cells (WBC), absolute neutrophils count (ANC), lymphocytes, platelet count.
- Blood Chemistry: alkaline phosphatase, ALT, AST, total bilirubin, serum creatinine, creatinine clearance (if required), sodium, potassium, total calcium, blood urea nitrogen (BUN) (or urea), albumin.
- Troponin Test.
- Coagulation testing: International Normalized Ratio (INR)] and activated PTT (aPTT).
- Pregnancy test (only applicable to premenopausal patients): serum test (with a sensitivity of at least 25 mIU/mL) prior to enrollment, and urine (or serum) test during the active treatment phase and at the post-treatment visit (30 [±5] days from the last study treatment dose). Any positive urine test must immediately be confirmed by a serum test.
- Ophthalmologic assessments: visual acuity testing, slit lamp examination, and fundoscopy.
- Viral serology (in case of suspicion): HIV, HBV serology [HBsAg, anti-HBsAg] and HCV (anti-HCV). In patients with a positive anti-HCV, HCV RNA detection and quantification by PCR will be additionally performed.

All AEs (and their relatedness to the study treatment) occurring during the study will be documented in the eCRF. AEs will be graded according to NCI-CTCAE version 5.0

6.2.2. Definitions

The safety definitions are described in the Table 4.

Table 4: Safety definitions

Concept	Definition
Adverse Event (AE)	Any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and

	that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product. Laboratory and vital signs abnormalities should be reported as AE only in case they lead to an action on study treatment, need a new concomitant medication/procedure, lead to an action on concomitant medication/procedure or if they are serious.
Special situations (SS) and Overdose	The following events will be considered Special Situations (SS) and they have to be documented as AE in the eCRF: medication error, drug misuse, drug abuse and overdose (whether accidental or intentional). If a SS results in an AE, the AE must be recorded separately in the eCRF and if it fulfills seriousness criteria, it must be reported as a SAE. An overdose (accidental or intentional) of the study treatment is an event suspected by the investigator defined as an administration of an amount of T-DXd that is 10% higher than is normally used for a given cycle.
Adverse Reaction (AR)	A response to a medicinal product which is noxious and unintended. The determination of the possible relation with the study treatment is responsibility of the principal investigator of the site or the person designated by him. Investigators should use their knowledge of the patient, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether an AE is considered related to the study treatment as follows: o Related (probable, possible): there is a temporal relationship between the onset of the AE and the administration of the study treatment, the AE cannot be explained by the patient's clinical state, intercurrent

	illness or concomitant therapies; and/or the AE follows a known pattern of response to the study treatment; and/or the AE abates or resolves upon discontinuation of the study treatment or dose reduction and, if applicable, reappears upon re-challenge. o Not related (unlikely): An AE will be considered as related unless it fulfills the following criterion: the AE has an etiology other than the study treatment (i.e. preexisting medical condition, underlying disease, intercurrent illness, or concomitant medications); and/or has no temporal relationship to administration of the study treatment. All expected ARs of T-DXd are listed in the section 6.9 of the IB. If the nature or the severity of an AR is not consistent with the applicable product information, the AR is defined as unexpected. The basis for the decision is the current version of the corresponding reference document that has been submitted and approved by the competent authority and the ethics committees.
Serious AE (SAE) and Serious AR (SAR)	Any AE or AR that, at any dose: o is fatal (results in death), o initial or prolonged inpatient hospitalization, o a life-threatening experience (that is, immediate risk of dying, defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe), o persistent or significant disability/incapacity, o congenital anomaly/birth defect or o an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (eg. medical, surgical) to prevent one of the other serious outcomes listed above.

	Do not confuse the concept "serious", described before, with "severe" which refers to the intensity of the AE or AR (minor/mild/severe). Second primary malignancy is also considered a SAE and must be reported to GEICAM according to Section 6.2.4. *Management, Timing and Assessment of SAEs/pregnancies.* **Accountability criteria** The causality assessment of a SAE is made by the investigator as described in the AR section. However, the sponsor will perform a causality assessment of SAEs based on the information reported by the investigators. The causality assessment reported by the investigator will not be downgraded by the sponsor. Causality assessment of both the investigator and the sponsor will be recorded in the case file into the GEICAM's safety database. Sponsor will classify the reported SAEs as "Related" or "Not Related" according to the method described in the section "Adverse Reaction (AR)".
Suspected Unexpected SAR (SUSAR)	Any SAR whose nature, intensity or consequences do not correspond with the Reference Safety Information for the investigational product.

6.2.3 Management, Timing and Assessment of Adverse Events

AE Classification	AEs should be classified following version 5.0 of the NCI-CTCAE. A copy can be downloaded in the NCI web site: http://evs.nci.nih.gov/ftp1/CTCAE. The investigators team must have access to the NCI-CTCAE version 5.0. The AE not included in the NCI-CTCAE will be classified as described on Protocol Attachment 3. Wherever possible the reporting Investigator will use the clinical, rather than the laboratory term (e.g., anemia versus low hemoglobin value).
Procedure to notify an AE to GEICAM	The site must notify to GEICAM, through eCRF, the following events: o All AEs that occur after signed ICD.

	○ Preexisting conditions that get worse during the study. ○ The evaluation of the possible relationship of each AE to the study treatment or protocol procedure. ○ The circumstances and data that causes the suspension of the treatment of a patient due to an AE. ○ The signs or symptoms of disease progression will not be recorded as AEs, unless the investigator believes they could have been caused by the study treatment. ○ The events leading to the clinical outcome of death from disease progression will not be recorded as AEs in the eCRF, unless the investigator believes they could have been caused by the study treatment or when the death occurred during the active treatment phase of the study and within 30 days following the last treatment administration, in this case it has to be reported as SAE in the eCRF and a SAE form will be notified to the Pharmacovigilance Department of GEICAM.
Timing and assessment of AE/SAE (see Protocol Attachment 4)	The site personnel will report on the eCRF the information of the AE in the following periods: • **Baseline (after** the patient signs the **ICD and before study treatment** administration): study site personnel will note the occurrence and nature of each patient´s medical condition(s) and preexisting conditions in the appropriate section of the eCRF. If a patient never receives study treatment but experiences an AE/SAE after the ICD is signed, ONLY events the investigator believes may have been caused by a protocol procedure will be reported to GEICAM via eCRF and SAE form (if applicable). • **During treatment with the study treatment:** during the study treatment administration, site personnel will record any change in the condition(s) and the occurrence and nature of any AE/SAE. An NCI-CTCAE grade rating will be assigned to every AE/SAE experienced. • **30-day** (±5 days) **post-treatment follow-up period:** each patient will have a 30-day post-treatment follow-up evaluation approximately 30 days following the

	discontinuation of study treatment. Patients should be closely followed for study treatment AEs in order to detect delayed toxicity. If study treatment-related toxicity is present beyond 30 days post-treatment, patients must be followed until it resolves or improved to baseline, the relationship is reassessed as unrelated, the investigator confirms that no further improvement can be expected, another therapy is initiated, or death. • **Long-Term Follow-up Period (after the 30-day post-discontinuation/post-treatment):** ongoing SAEs, and new SAEs thought to be related to study treatment or protocol procedures should be documented on the eCRF and immediately reported to the GEICAM Pharmacovigilance Department as described in the Section 6.2.4. If the study has been closed, the notification has to be reported only to the GEICAM Pharmacovigilance Department as described in the Section 6.2.4. • **SAES related with study treatment** should be collected and analyzed until they are solved or until the toxicity is considered irreversible.
Adverse Events of Special Interest (AESI)	AESIs for this study are as follows: • **Interstitial lung disease (ILD)/pneumonitis** is considered an important identified risk based on a comprehensive cumulative review of potential interstitial lung disease (ILD)/pneumonitis cases from: the available safety data from the T-DXd clinical development program, available data from recent epidemiology/literature, biological plausibility, and safety information from drugs of similar class. Refer to the current IB for a summary of preliminary clinical study data. • **LVEF decrease** in association with T-DXd is considered an important potential risk based on the available pre-clinical data, literature and available safety information for drugs of similar class. Refer to the current IB for a summary of preliminary clinical trial data.

	• **Hepatic AEs events which meet the potential DILI criteria** defined as: o an elevated ALT or AST ≥ 3.0 x ULN combined with total bilirubin ≥ 2.0 x ULN (in patients with normal ALT, AST and bilirubin at baseline) o an elevated AST or ALT ≥ 2 x baseline AND > 3.0 x ULN OR AST or ALT ≥ 8.0 x ULN, combined with total bilirubin ≥ 2 x baseline AND ≥ 2.0 x ULN or ≥ 3.0 x ULN in case of Gilbert´s syndrome (for patients with elevated AST, ALT or total bilirubin at baseline). AESIs should be documented on the eCRF and immediately reported to the GEICAM Pharmacovigilance Department as described in the Section 6.2.4.

6.2.4. Management, Timing and Assessment of SAEs/AESIs/pregnancies

Timing of SAEs/AESIs (see Protocol Attachment 4)	All SAEs/AESIs (either spontaneously or during the trial visits) will be collected since the patient signs the ICD. All the SAEs/AESIs must be documented in the medical record of the patient and in the eCRF. A follow up of all the SAEs/AESIs should be done until they are solved or until the toxicity is considered irreversible.
Pregnancies	Patients must be instructed to immediately inform the investigator if she or his partner (in case of a male patient) becomes pregnant while the patient is enrolled in this study and up to one month after EOT. If a patient becomes pregnant while enrolled in this study, it must be reported in the Pregnancy Form and sent to the GEICAM Pharmacovigilance Unit within 24 hours of becoming aware of it. If the female partner of a male patient becomes pregnant while he is enrolled in this study, it must be reported as soon as possible to the GEICAM Pharmacovigilance Department in the Pregnancy Form, and always after obtaining the corresponding Informed Consent from the pregnant woman.

	The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications and documented in the Pregnancy Form. All infants born after fetal exposure must be followed for the first 12 months after delivery.
SAEs which do not need to be notified to the Pharmacovigilance Department of GEICAM	The following events are not considered SAEs: o A visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event). o Elective surgery planned before signing consent. o Hospitalization which is due solely to a planned study visit and without prolongation. o Routine health assessment requiring admission for baseline/trending of health status (eg. routine colonoscopy). o Medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases. o Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg. lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative). o SAEs related to progression of the malignancy during the study (including signs and symptoms of progression), should not be reported as SAE, except when the outcome is fatal and death occurs during the active treatment phase of the study or within 30 days after the last treatment administration, in this case it must be reported as SAE to the Pharmacovigilance Department of GEICAM. o An overnight stay in the hospital that is only due to transportation, organization, or accommodation problems and without medical background.

	The rest of SAEs must be notified as described below.
Procedure to notify a SAE or AESI to the Pharmacovigilance Department of GEICAM	The SAEs and AESIs must be notified to the Pharmacovigilance Department of GEICAM. A member of the investigator team must complete and sign the GEICAM SAE notification form which will be sent by fax/mail, immediately and always within the 24 hours of knowledge of the SAE: **Pharmacovigilance Department of GEICAM** Fax: +34 917 371 619 farmacovigilancia@geicam.org GEICAM will review the received form and, if necessary, will ask more information to the investigator. When additional information is obtained about the SAEs, or this is solved or is improbable it will change, a follow-up report must be also completed and sent by fax/mail, immediately and always within the 24 hours of knowledge to the Pharmacovigilance Department of GEICAM. If GEICAM suspects that the SAE could be a SUSAR, the investigator should give the follow up information requested. GEICAM will report all SAEs immediately to the Chief Investigator for assessment. All SAEs will be followed-up by the investigator until satisfactory resolution. Annually all SARs will be reported at the Study Development Safety Update Report (DSUR) to the competent authorities and the leading ethics committee, including all SUSARs.
Death on Study	Any death occurring during the active treatment phase of the study and within 30 days of the last treatment administration must be reported as SAE to GEICAM as the sponsor within 24 hours, regardless of the relationship to study treatment, and must be reported on the death report form and AE sections of the eCRF. The cause of death should be documented (cancer-related, treatment-related, cancer- and treatment-unrelated). Autopsy reports should be collected whenever possible and sent to GEICAM.

	Deaths that occur due to tumor progression do not have to be reported as a SAE unless they occur before EOT (+30 days). Deaths after the end of study which are considered to be related to study treatment have to be reported as SAEs. To the extent feasible sufficient information including relevant laboratory values, ECGs, scans, biopsies or autopsy results must be provided by the investigator in the SAE narrative (even if investigator determines the SAE is not related) so as to permit an independent causality assessment by a Competent Authority.

6.2.5. Management, Timing and Assessment of SUSARs

Expedited Notification of SUSAR to the Competent Authorities	The Pharmacovigilance Department of GEICAM or its designee is responsible to notify to each of the competent authorities of the participating countries, all the SUSARs collected in the study, following the procedures shown in the current legislation.
Timing of notification	The deadline for reporting a SUSAR shall be 15 calendar days from when GEICAM or its designee becomes aware of it. When suspected SUSAR caused the death of the patient or endangered her/his life, GEICAM or its designee will send the information within 7 calendar days from the date on which it becomes aware.
Expedited reporting of other relevant safety information	GEICAM or its designee will also notify, expeditiously, all the information that could modify the balance benefit/risk of the investigational product, or determine changes in its administration pattern or in the study performance, such as: o A qualitative change or an increase in the percentage of occurrence of the SAR expected, which are considered clinically significant. o The SUSAR occurring after completion of the study and reported by the investigator to the sponsor. o New events related to the conduct of a trial or the development of an IMP likely to affect the safety of patients, such as: ✓ SAE that could be related with the study procedure and could modify the conduct of the trial.

	✓ A significant risk to patients such as lack of efficacy in a drug used to treat a life-threatening illness. ✓ A major safety finding from a newly completed animal study (such as carcinogenicity). ✓ A temporary halt of a trial for safety reasons if the trial is conducted with the same investigational medicinal products in another country and if this information is known by GEICAM. ✓ Any recommendation from the chief investigators that is relevant to the safety of patients (if applicable). This relevant information shall be notified as soon as possible and no later than 15 days after GEICAM or its designee becomes aware of it. Additional information will also be notified as quickly as possible.
Development Safety Update Report (DSUR)	The DSUR that includes the SAEs, SAR and SUSARs collected during the study will be sent by GEICAM or its designee to the Competent Authorities and EC/IRB at the time established by the current legislation.
Notification to investigators	GEICAM or its designee will communicate to the investigators any safety information that may affect the safety of trial patients, as soon as possible. Information on SUSAR occurred during the study will be sent to investigators every 6 months, in aggregate, in a list along with a brief analysis of the data provided. They will be also informed, throughout the entire study, of any safety aspect that impacts the performance on the clinical trial or the product development, including the interruption or modification in the development program due to safety-related issues.

6.3. Other Assessments

6.3.1. Biomarker Assessments

Biomarkers will be analyzed centrally in an exploratory fashion to investigate possible associations with resistance/sensitivity to T-DXd in advanced HER2-positive patients.

We will explore the predictive value of HER2 expression by immunohistochemistry (IHC) and in situ hybridization (ISH) analysis in pre-treatment tumors, and the HER2 spatial distribution by artificial intelligence (AI) using weakly supervised and clustering algorithms on HER2-positive slides images at baseline. Additionally, we will explore the correlation of HER2 by IHC/ISH with ERBB2 mRNA levels.

We will evaluate the predictive value of intrinsic molecular breast cancer subtypes in pre-treatment tumors.

We will assess intratumoral and stromal TILs and PD-L1 expression on pre-treatment tumor samples; the immune profiling in tumors (e.g. CD3, CD4, CD8, CD68, FoxP3, CK/SOX10) and in PBMCs (MDSC, Tregs and other immune subpopulations), and the expression pattern of pro- and anti-inflammatory cytokines in sequential blood samples. Protein profiling could be performed in baseline tumors, and in plasma and/or on circulating PBMCs at baseline, on treatment and at PD.

RNA profiling and genomic analysis in tumors and circulating PBMCs may be assessed.

Additionally, we could explore response dynamics, tumor tracking, tumor mutational burden, and clonal diversity during study treatment and at PD, based on genomic and molecular profiling in tumor and blood samples.

Biomarkers may be assessed depending on emerging data.

The following samples will be collected:

- **Tumor Tissue:** Investigators will be encouraged to send the most recent pre-treatment tumor tissue sample, preferably from a metastatic lesion. However, if this is not possible, archived tissue samples either from primary tumor or metastasis will be acceptable.
- **Blood samples:** Whole blood and plasma samples will be collected at the following timepoints:
  - at baseline (within 7 days before treatment initiation),
  - during study treatment, every 3 cycles on Day 1 (pre-dose) until treatment discontinuation for any reason, and
  - at the study treatment discontinuation, collected at the post-treatment visit (30 [±5] days from the last study treatment administration). If a patient ends the study treatment due to a reason other than disease progression, an additional sample must be collected once progression is documented (even if that patient has initiated another treatment).

Detailed instructions for the collection, handling and shipment of samples are outlined in the Sample Management Manual that will be available to the investigator and will be distributed at the time of site activation.

Biological sample leftovers from the study will be included, subject to the patient consent, in the GEICAM Biobank located currently at the Hospital Universitario Fundación Jiménez Díaz in Madrid (Spain), whose owner is GEICAM and is an authorized not for profit institution, which meets all the technical, legal, and ethical requirements for managing, storing and using biological samples.

6.3.2 Patient Reported Outcomes

Patient reported outcomes of health-related quality of life will be assessed using the EORTC QLQ-C30 (see attachment 5).

The **EORTC-QLQ-C30** is a 30-item questionnaire composed of five multi-item functional subscales (physical, role, cognitive emotional, and social functioning), three multi-item symptom scales (fatigue, nausea/vomiting, and pain), a global health/quality of life (QOL) subscale, and six single items assessing other cancer-related symptoms (dyspnea, sleep disturbance, appetite, diarrhea, constipation, and the financial impact of cancer). The questionnaire employs 28 4-point Likert scales with responses from "not at all" to "very much" and two 7-point Likert scales for global health and overall QOL. For functional and global QOL scales, higher scores represent a better level of functioning and are converted to a 0 to 100 scale. For symptom-oriented scales, a higher score represents more severe symptoms.

Patients will complete EORTC-QLQ-C30:

- at baseline (within 7 days before treatment initiation),
- during study treatment, every 3 cycles (- 3 days) until treatment discontinuation, and
- at the post-treatment visit (30 [±5] days from the last study treatment dose) or before if the patient is starting a new treatment.

Completed questionnaires are always considered source document and must be filed accordingly.

Patients must complete these instruments in clinic (cannot be taken home) prior to having any discussion of their progress with healthcare personnel at the site. Interviewer administration in clinic may be used under special circumstances (eg, patient forgot their glasses or feels too ill). The instruments will be given to the patient in the appropriate language for the site.

7. Data Quality Assurance

To ensure accurate, complete and reliable data, GEICAM or its designee will do the following:

- Provide instructional material to the study sites, as appropriate.
- Perform a start-up training session to instruct the investigators and study coordinators. This session will give instructions on the protocol, the completion of the eCRFs, and study procedures.
- Make periodic visits to the study site to review study progress, investigator and patient compliance with the clinical trial protocol requirements and any emergent problems.
- Be available for consultation and stay in contact with the study site personnel by mail, telephone, and/or fax.
- Review and evaluate eCRF data and use standard computer edits to detect errors in data collection. Concurrent manual review of part of the data can be performed and ad hoc queries will be generated within the eCRF and followed-up for resolution.
- Conduct a quality review of the database.
- Verify the quality of the data.

To ensure the safety of participants in the study and to ensure accurate, complete, and reliable data, the investigator will keep records of laboratory tests, clinical notes, and patient medical records in the patient files as original source documents for the study. If requested, the investigator will provide GEICAM, applicable regulatory agencies, and applicable ethical review boards with direct access to original source documents.

7.1. Data Management and Registries file

Data for this study will be recorded in an eCRF with web access, designed, created and maintained by GEICAM using Oracle Clinical®. Data will be transcribed by the site staff from the source documents into the eCRF. The eCRF will never be considered as source data for this trial.

This eCRF meets the FDA's Title 21 Code of Federal Regulations (CFR) Part 11, ensuring the validation of the system, the traceability and the audit trail, the retention, protection, reproducibility and recovery of trial data, control of access to information and electronic signature, among others.

Visit data should be entered into the eCRFs within 7 business days. Each eCRF should be completed by the investigator or delegate as stated in the Site Delegation List.

Electronic queries will be raised if data is unclear or missing. GEICAM will perform a data review and additional requests can be sent through the eCRF, which the investigator is obliged to respond to by modifying or clarifying the data questioned. The requests with their responses will be managed through the eCRF.

If a correction is made, the corrected information will be entered into the eCRF, superseding the initial information. An audit trail allows you to identify the modification.

8. Sample Size and Statistical Methods

8.1. Determination of Sample Size

8.1.1. Sample Size determination

The primary endpoint of the study is to estimate the ORR.

Assuming an ORR of 35% (considering the results of response rates obtained with T-DM1 from published studies)[28] and to achieve a precision of ±15% using two-sided 95% normal asymptotic CI for a single proportion, we will need to include 39 evaluable patients.

With a drop-out rate of 5% we will need 41 patients to be enrolled in the study.

8.2. Statistical and Analytical Plans

8.2.1. General Considerations

Statistical analysis of this study will be the responsibility of GEICAM. The interpretation of study results will be the responsibility of the principal investigator of the study.

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a Statistical Analysis Plan (SAP), which will be dated and maintained by GEICAM. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition and/or its analysis will also be reflected in a protocol amendment.

All analysis will be performed using the SAS Enterprise Guide 7.1 version.

8.2.1.1. Patient Populations

Intent to treat population (ITT): the ITT population will include all patients who are enrolled in the study.

Efficacy population: is a subset of the ITT population that have measurable disease, have received at least one dose of study treatment and completed the study without major protocol deviations which are considered to have a significant impact on results.

The efficacy population will be the primary population for the primary endpoint analysis. A sensitivity analysis will be performed using the ITT population.

Safety population: will include all patients enrolled in the study who received at least one dose of study treatment. This population is for the safety analysis.

QoL population: a subset of the safety population with available QoL questionnaires (the baseline and at least one more).

8.2.2. Patient Disposition

A detailed description of patient disposition will be provided. It will include:

- summary of patients screened and by site
- total number of patients screened
- total number of patients enrolled
- total number of patients treated
- summary of reasons for patients enrolled, but not treated.

A detailed summary of reasons for patient discontinuation from study treatment will be provided.

A summary of all identified important protocol violations will be provided.

8.2.3. Patient Characteristics

Patient characteristics will include a summary of the following:

- patient demographics
- Disease characteristics (at diagnosis and at study entry)
- preexisting conditions/secondary conditions
- prior therapy

Other patient characteristics will be summarized as deemed appropriate.

Standard descriptive statistics, such as the mean, median, range and proportion, will be used to summarize the patient sample and to estimate parameters of interest. Ninety-five percent confidence intervals will be provided for estimates of interest where possible.

8.2.4. Concomitant Therapy

A summary of concomitant therapies will be generated in the safety population.

8.2.5. Treatment Compliance

Treatment information will be collected at each dose administration. The estimate of percent compliance will be given by:

$$\text{Percent Compliance} = \frac{\text{Actual dose administered per week}}{\text{Dose expected to be administered per week}} \times 100$$

No minimal level of compliance will be defined for patient inclusion in efficacy analyses. To be considered compliant patients should have received at least 80% of the planned number of doses. Exploratory analysis of the impact of compliance on selected efficacy endpoints may be performed if deemed necessary.

A summary of the incidence of T-DXd dose modifications and interruptions due to AEs, the number of administered cycles and dose intensity will be generated.

8.2.6. Efficacy Analyses

8.2.6.1. Analyses of Primary Endpoint

The primary endpoint is the Objective response rate (ORR). ORR is defined as the rate of complete response (CR) plus partial response (PR) out of the efficacy population.

A patient will be considered to have achieved an OR if the patient has a CR or PR according to RECIST v.1.1. definitions. Otherwise, the patient will be considered as nonresponder in the ORR analysis. Additionally, patients with inadequate data for tumor assessment (eg, no baseline assessment or no followup assessments) will be considered as nonresponders in the ORR analysis. The best response through the treatment will be recorded.

ORR will be calculated by dividing the number of patients with objective response (CR or PR) by the efficacy population.

$$\text{Objective Response Rate} = \frac{\text{Number of CRs + PRs}}{\text{Efficacy population}}$$

The ORR will be reported, including a 95% CI using the Clopper-Pearson method.

A sensitivity analysis will be performed using the ITT population.

8.2.6.2. Analysis of Secondary Endpoints

The secondary efficacy endpoints are:

Progression-Free survival (PFS): is defined as the time from the date of enrollment to the date of disease progression, based on the investigator's assessment using RECIST version 1.1., or death from any cause, whichever occurs first. PFS data will be censored on the date of the last tumor assessment on study for patients who do not have objective tumor progression and who have not died due to any cause while on study. Additionally, patients who start a new anti-cancer therapy prior to documented PD will be censored at the date of the last tumor assessment prior to the start of the new therapy.

PFS analysis will be performed in the safety population.

PFS will be assessed using the Kaplan-Meier method. The median event time and 95% CI will be estimated if reached.

Overall Survival (OS): is defined as the time from the date of enrollment to the date of death from any cause. OS data will be censored on the last date the patient is known to be alive.

OS analysis will be performed in the safety population.

OS will be assessed using the Kaplan-Meier method. The median event time and 95% CI will be estimated if reached.

Time to Treatment Response (TTR): TTR: is defined as the time from the date of enrollment to the date of first documentation of objective tumor response (CR or PR).

TTR analysis will be performed in the efficacy population in patients with an OR.

TTR will be assessed using the Kaplan-Meier method. The median event time and 95% CI will be estimated.

Duration of Response (DOR): is defined as the time from the date of first documentation of objective tumor response (CR or PR) to the date of first documented progressive disease based on the investigator's assessment using RECIST version 1.1, or death from any cause, whichever occurs first. DOR will be censored on the date of the last tumor assessment on study for patients who do not have objective tumor progression and who have not died due to any cause while on study. Additionally, patients who start a new anti-cancer therapy prior to documented PD will be censored at the date of the last tumor assessment prior to the start of the new therapy.

DOR analysis will be performed in the efficacy population in patients with an OR.

DOR will be estimated using Kaplan-Meier methods. The median event time and 95% CI will be estimated if reached.

Additional sensitivity analyses of all this endpoints will be outlined in the SAP if required.

8.2.6.3. Analysis of Exploratory Endpoints

PFS2: is defined as the time from the date of enrollment to the date of disease progression to the second line of therapy. PFS2 data will be censored on the last date in which the patient was known not to have tumor progression or death due to any cause within the second line of therapy. Additionally, patients who start a third line therapy prior to progression to the second line will be censored at the date of the start of the third line therapy.

PFS2 will be performed in the safety population.

PFS2 will be assessed using the Kaplan-Meier method. The median event time and 95% CI will be estimated if reached.

PFS3: is defined as the time from the date of enrollment to the date of disease progression to the third line of therapy. PFS3 data will be censored on the last date in which the patient was known not to have tumor progression or death due to any cause within the third line of therapy. Additionally, patients who start a fourth line therapy prior to progression to the third line will be censored at the date of the start of the fourth line therapy.

PFS3 will be performed in the safety population.

PFS3 will be assessed using the Kaplan-Meier method. The median event time and 95% CI will be estimated if reached.

Additional sensitivity analyses of these 2 endpoints will be outlined in the SAP if required.

8.2.7. Safety Analyses

The toxicity and tolerability of study treatments will be evaluated in the safety population. Safety analyses will include summaries of the incidence of AEs by maximum NCI-CTCAE grade (v5.0; NCI 2019) and SAEs that occur during the study treatment period or within 30 days of the last dose of study treatment, regardless of causality and according to the relationship to study treatment as assessed by the investigator. Additionally, the following safety-related outcomes will be summarized:

- study treatment discontinuations due to AEs.
- deaths
- hospitalizations and transfusions
- use of key concomitant medications or growth factors.

Analyses for data with discrete dates, for example, deaths, transfusions, and concomitant medications, will be done through 30 days after each patient's last dose of study treatment.

AEs and SAEs will be presented in frequency tables by grade. Hematological and clinical biochemistry toxicities will be assessed from laboratory test parameters. The safety analysis will be performed in the safety population.

8.2.8. Other Analyses

8.2.8.1. Biomarker Analyses

Biomarkers will be analized in an exploratory fashion as described in the Exploratory Statistical Analysis Guide (ESAG) to investigate possible associations with resistance/sensitivity to T-DXd in advanced HER2-positive patients.

The biomarker analysis will make use of descriptive statistical methods. Some of the proposed biomarkers are categorical variables (mutations, tumor subtypes, ...), and others are continuous variables (HER2 expression, gene expression, genetic signatures, ...).

Categorical variables will be summarized by numbers and proportions. In all possible cases, the 95% confidence intervals will be calculated. If appropriate, a chi-squared test will be used to test group differences.

For continuous variables, descriptive statistics including the mean, standard deviation, median, minimum, and maximum values, will be provided. Continuous biomarkers can be categorized

using standard cutoffs, if exist, or the median can be used to establish two groups, high and low expression of genes or signatures, for example.

To analyze the relationship between biomarkers and ORR, we will take into account whether they are continuous or categorical, using the statistical techniques specified below. If applicable, parametric test (analysis of variance or t test) or non-parametric testing, such as Wilcoxon's rank-sum test or Kruskal–Wallis test for continuous variables, and the Pearson $\chi 2$-test or Fisher exact test for categorical variables will be used to test group differences. All test performed will be two-sided and carried out with a 5% α-error rate without correction for multiplicity.

The Kaplan-Meier limit-product method will be used to estimate the secondary and exploratory endpoints: PFS, OS, TTR, DOR, PFS2, PFS3, calculating separate curves for each of the groups of biomarkers defined as categorical. The comparison of those endpoints between groups of biomarkers will be performed using the Log-Rank test. The Kaplan-Meier survival curves will be presented graphically. Median (PFS, OS, TTR, DOR, PFS2, PFS3) with the 95% confidence interval will be reported. Cox regression models will be used to estimate unadjusted and adjusted hazard ratio and its 95% confidence interval, for evaluate the relation between the continuous and categorial biomarkers with the different endpoints. The Wald test will be used to establish the prognostic importance of each variable. Univariate and multivariate analyses could be carried out to explore the influence of the selected variables (include the specific clinical characteristics for each study) in PFS, OS, TTR, DOR, PFS2, PFS3. Any additional sensitivity analyses will be outlined in specific SAPs.

8.2.8.2. Patient Reported Outcomes

Patient reported outcomes of health-related quality of life will be assessed using the QLQ-C30 questionnaire and analized on the QoL population.

Published scoring manuals and guidelines will be used to generate scale scores and handle missing data. Descriptive statistics for actual values will be tabulated at each scheduled time point.

QoL variables and method for analysis:

- **Change from baseline** in the global health status score (GHS) and each scale of the EORTC QLQ-C30 questionnaire. It will be presented at each scheduled time point for the GHS score and each of the functionals and symptoms scales from the QLQ-C30 questionnaires. Longitudinal analysis of scores will be performed using linear mixed models.
- **Time to deterioration (TTD) in QoL**: is defined as the time from the date of enrollment to the date of first detection of a deterioration event. A deterioration event is defined as an increase of ≥ minimally important difference (MID) from baseline for the EORTC QLQ-C30 symptom scales and a decrease of ≥ MID from baseline for the EORTC QLQ-

C30 functional scales and GHS scale. It will be assessed using the Kaplan-Meier. Method. The median event time and 95% CI for the median will be estimated if reached. TTD will be censored at the date of the last QoL assessment prior to the start of a new therapy.

Detailed methodology will be documented in the SAP of the study.

8.2.9. Subgroup Analyses

Exploratory subgroup analysis may be performed if deemed appropriate.

9. Informed Consent, Confidentiality, Responsibility Insurance and Regulatory Considerations

9.1. Informed Consent

The investigator is responsible for ensuring that the patient understands the risks and benefits of participating in the study, including answering any questions the patient may have throughout the study and sharing any new information that may be relevant to the patient's willingness to continue his or her participation in the trial in a timely manner.

The ICD will be used to explain the risks and benefits of study participation to the patient in simple terms before the patient is screened into the study, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study.

The investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the ICD prior to the performance of any protocol procedures and prior to the administration of study treatment.

As used in this protocol, the term "informed consent" includes all consent and assent given by patients or their legal representatives.

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

9.2. Respect of Confidentiality

The investigator will be responsible for preserving the suitable information about each patient (for example, name, address, telephone number, social security number and study identification) so that the competent authorities can have access to said information if necessary. These records must be confidentially preserved for the time indicated by the legislation.

The investigators and GEICAM and its designee will maintain the confidentiality of all patients participating in the study, according to Good Clinical Practice and local legislation.

9.3. Data Protection

This clinical trial will be held in accordance and in compliance with local current legislation. Any treatment of personal data that is held within the clinical trial, for Sponsor, Principal Investigator, Site, and / or any other participant in the clinical trial, especially as far as informed consent, shall conform to the provisions of Organic Law 3/2018, of December 5, Protection of

Personal Data and Guarantee of Digital Rights, Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 (GDPR) and any other rules in the matter.

9.3.1. Personal Data Processed

When a patient participates in the study, his/her personal data is processed. Processing operations includes the collection, storage, analysis, transmission and any other use of the personal data. Personal data includes information that identifies the patient directly (such as his/her name, address, telephone number, or health insurance number, etc) and information that could lead to an indirect identification (such as information relating to the patient's state of health, information about the patient's biological samples and the results of analyses performed on these, the patient's medical treatments and his/her response to medical treatments, etc).

9.3.2. Arrangements to Comply with Data Protection Laws

GEICAM is the data controller of the processing of patient's encoded data collected for the purposes of the Study. The investigators and the sites are also data controllers of the patient's unencoded data collected for their own purposes, if any.

9.3.3. Measures to Protect Personal Data

All personal data are treated in accordance with data protection laws, including the GDPR.

Personal data must be encoded at the study site before any transmission to a third party. The principle of data encoding entails that if a patient participates in the study, a code will be assigned to him/her. The code will replace all directly or indirectly identifying data on documents used for data collection or biological samples collected for the study.

Data unencoded will be kept only in the study sites' medical records.

Individuals accessing the unencoded personal data at the study site are subject to professional secrecy, and/or to confidentiality agreements. In addition, a limited number of the Sponsor's employees, or its contractual partners, ethics committees and regulatory authorities may consult the personal data and these persons are bound by an obligation of confidentiality.

If a transfer of personal data to a third country is needed, the data controller will put in place the appropriate measures equivalent to the measures required by the Spanish and the European data privacy laws to protect patients privacy.

If a patient wishes to exercise his/her rights relating to data about him/her, or if the patient wishes to know more about the measures to protect his/her personal data, he/she can send a request to the investigator or to the data protection officer of the study site. The investigator or the data protection officer (DPO) of the study site can contact the DPO of any of the data controller at incidencia.lopd@geicam.org.

Personal data related to the study is stored on secured servers. These servers protect data against loss, destruction, access, modification or dissemination by unauthorised persons. Only a limited and controlled number of persons are authorized to access the data.

All the personal data transferred over internet are submitted in coded form. Secure Socket Layer (SSL) encryption is used for the transmission of data.

To ensure compliance with GDPR and taking into account the state of the art, the nature, the scope, the context and the purposes of the processing to be carried out, the data controller shall apply appropriate technical and organizational measures to ensure a level of security appropriate to the risk. Some of the measures that may be considered are:

- ✓ Identification, dissemination and documentation of the functions and duties of personnel with access to the data.
- ✓ Definition and implementation of a procedure to identify and authenticate users.
- ✓ Definition and implementation of a data access control procedure.
- ✓ Definition and implementation of a procedure for recording incidents and data breach.
- ✓ Definition and implementation of a system of verification of user accesses to data.
- ✓ Definition and implementation of a backup procedure.
- ✓ Definition of the criteria for filing media and the devices for their storage.
- ✓ Definition and implementation of regular checks on the Company, testing, assessing and evaluating the effectiveness of technical and organizational measures, in order to ensure the security of the processing.
- ✓ Implementation of firewalls, antivirus and spyware in the servers containing personal data.
- ✓ Appointment of a Security Officer(s) or Data Protection Officer, as appropriate.

In case of a data security breach occurs, the data controller will assess the risk for individuals' privacy, the measures to be implemented in order to mitigate the risk, minimizing its consequences and preventing any repeat in the future, as well as they will assess, depending on its severity, the need of notifying the correspondent data protection authority and/or the individuals concerned.

9.4. Insurance and Compensation for Injury

GEICAM shall ensure that the trial patients are compensated for any damage suffered as a result of the trial.

GEICAM has hired an insurance policy covering the damage, as well as any liability that might be incurred by the sponsor, principal investigator and members of the investigator team, including contracted clinical investigators, and the hospital or site where the clinical trial is conducted, according to applicable legislation.

9.5. Regulatory Considerations

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with good clinical practices and the applicable laws and regulations. Each investigator will conduct the study according to applicable local or regional regulatory requirements and align his or her conduct in accordance with the "Responsibilities of the Investigator". The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and investigator responsibilities.

9.5.1. Investigator Information

Physicians who take care of breast cancer patients will participate as investigators in this clinical trial.

If investigators are added after the study has been approved by the Sponsor, an EC/IRB, or a regulatory agency, these additions will not be considered changes to the protocol.

9.5.2. Protocol Signatures

After reading the protocol, each principal investigator will sign the protocol signature page and send a copy of the signed page to GEICAM.

10. Practical Considerations

10.1. Monitoring, Audit and Inspections

Onsite or remote monitoring visits to the study site will be made periodically during the study and according to the Monitoring Plan Document to ensure that all aspects of the protocol are followed. During the visits to the site, the monitor must review the original records of the patients, the records of medication stocks and document preservation according to the Monitoring Plan Document. The monitor must evaluate the study procedures and discuss the possible problems with the investigator. During the course of the study, audit visits can be carried out in the participating sites. The investigator will allow direct access to the source documents/data for the tasks of monitoring, audit, reviewed by the EC/IRB and the inspection by the Competent Authorities, and should inform the site monitor immediately if contacted by a Competent Authority about an inspection at the study site.

10.2 Protocol Deviations

The investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to study patients. Should other unexpected circumstances arise that will require deviation from protocol-specified procedures, the investigator should consult with GEICAM or designee (and IRB or IEC, as required) to determine the appropriate course of action. There will be no exemptions (a prospectively approved deviation) from the inclusion or exclusion criteria.

10.3. Preservation of Study Documentation

The copies of all the relevant information will be preserved by the investigator for a period of at least 25 years after the end of the study, according to current legislation.

10.4. Protocol Modification

Once it has been authorized by the EC/IRB and the competent authority any protocol modification must be documented by writing, in the form of an amendment.

The amendments must be duly identified, by its chronological order number, dated and signed by GEICAM and the Chief investigator.

The protocol amendments considered as substantial must be notified to the EC/IRBs involved in the trial and to the competent authority. The authorization of the involved EC/IRBs and/or the competent authority will be necessary before their application.

After reading the protocol amendment, each principal investigator will sign the protocol amendment signature page and send a copy of the signed page to GEICAM.

10.5. Use of the Information and Publication

All the information concerning the study treatment provided by GEICAM in relation to this study, and not previously published, is considered to be confidential information with property right of GEICAM. This information comprises the basic information about the product, the clinical protocol, the work forms where appropriate, the eCRFs, the assessment methods, the technical methodology and the basic scientific data. This confidential information will be the property of GEICAM, it must not be disclosed to third parties without the prior written consent of GEICAM and it must not be used other than for the purposes of the study.

The information developed during the practice of this clinical study is also considered to be confidential. This information can be disclosed to the extent considered necessary by GEICAM.

To allow the use of the information derived from this study and to ensure the compliance with the current rules, the investigator is obliged to provide GEICAM with all the results of examinations and all the data developed in this study. Except in that required by law, the information obtained during the study can only be provided to the doctors and to the competent authorities by GEICAM.

GEICAM commits to comply with current legislation relating to studies, which establishes the obligation to publish the results, both positive and negative, in conferences and journals, with the reference that EC/IRB approved the study, and its funding source. The list of authors will be developed in accordance with the GEICAM SOPs (standard operating procedures).

10.5.1 Clinical Trial Registration

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable laws, regulations and guidance, GEICAM will, at a minimum register interventional clinical trials sponsored by GEICAM anywhere in the world on ClinicalTrials.gov or other publicly accessible websites (European Union Clinical Trials Register and national clinical trials public website [if applicable]), as required by GEICAM Policy/SOPs and local health authorities.

10.5.2 Clinical Trial Results Disclosure

GEICAM will post the results of clinical trials on ClinicalTrials.gov or other publicly accessible websites, as required by GEICAM Policy/SOPs and applicable local laws and/or regulations.

10.6. Ethics Committees and/or IRBs

GEICAM or its designee or the Investigator will supply relevant documents for submission to the respective EC/IRB for the protocol's review and approval. This protocol, the IB/Summary of Product Characteristics (SmPC), a copy of the ICD, and, if applicable, patient recruitment materials and/or advertisements and other documents required by all applicable laws and

regulations, will be submitted to a central or local EC/IRB for approval. The EC/IRB's written approval of the protocol and patient informed consent must be obtained and submitted to GEICAM or designee before commencement of the study (ie, before shipment of the sponsor-supplied study treatment or study specific screening activity). GEICAM or its designee will notify the site once GEICAM or its designee has confirmed the adequacy of site regulatory documentation and, when applicable, GEICAM or its designee has received permission from competent authority to begin the trial. Until the site receives notification, no protocol activities, including screening, may occur.

As per applicable regulatory requirements, GEICAM or its designee or the Investigator will submit the required reports of the progress of the study to the EC/IRB and will communicate the possible SAE, the life-threatening AEs and deaths. At the end of the study, GEICAM or its designee or the Investigator must inform the EC/IRB of trial closure. Al these notifications will be performed according to the applicable regulatory requirements.

11. References

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Protocol Attachment 1. Study Schedule

Study Schedule of Events and Timelines. GEICAM/2021-08 (TRANSCENDER)			During Study Treatment. All visits ± 3 days of scheduled treatment day			Post-treatment 30 (±5) days from the last study treatment dose	After study treatment termination	
Cycle	**Screening**		**Cycle 1**	**Cycle 2**	**Subsequent Cycles**		**PFS/DOR Follow-up Period (12 weeks ± 7 days)**	**PFS2/PFS 3/OS Follow-up Period (6 months ±14 days)**
Day of cycle		REGISTRATION	**1**	**1**	**1**			
Procedure/Laboratory/ Diagnostic Test	**Within 28 days**							
ICD for Entry (before any study specific tests)[a]	**X**							
Inclusion/Exclusion Criteria	**X**							
Medical and surgical history and demographics[b]	**X**							
Physical examination[c]	**X**		**X**	**X**	**X**	**X**		
ECOG PS	**X**		**X**	**X**	**X**	**X**		
Hematology[d]	**X**		**X**	**X**	**X**	**X**		
Blood Chemistry[e]	**X**		**X**	**X**	**X**	**X**		
Troponin	**X**							
Coagulation[f]	**X**							
Viral serology[g]	**X**							
Pregnancy Test[h]	**X**		**X**	**X**	**X**	**X**		

Ophthalmologic assessment[i]	**X**					**X**		
ECHO or MUGA[j]	**X**				**X**	**X**		
Triplicate 12-lead ECG[k]	**X**			**X**	**X**			
QoL Questionnaires[l]			**X**		Every 3 cycles	**X**		
Concomitant medications	**X**		**X**					
AEs and SAES[m]	**X**		**X**				Only related SAEs	
Study Treatment			T-DXd 5.4 mg/kg IV every 3 weeks**.**					
Tumor Assessment[n]	**X**		Every 9 weeks (±7 days) from the start of treatment.				**X**	
Date of death								**X**[o]
Translational Research								
Blood samples for biomarker analysis[p]			X (Day 1)		Every 3 cycles (Day1)	At study treatment discontinuation and at PD		
Tumor tissue for Biomarker analysis[q]	**X**							

Study Schedule of Events and Timelines. Protocol GEICAM/2021-08 (TRANSCENDER)

a	Signed, written informed consent (approved by the EC/IRB) obtained prior to any study specific procedure.
b	Includes local laboratory ER/PgR/HER2 expression levels and methods used to assess them, previous treatments. Sex, Race and Age
c	Physical examination includes measurements of height (screening only), weight, blood pressure, pulse rate and body temperature. The T-DXd dose will be recalculated in the event that patients experience body weight variations greater than 10% during the treatment period.
d	Hemoglobin, white blood cells (WBC), absolute neutrophils count (ANC), lymphocytes and platelet count. Cycle 1 analysis will not be necessary if the screening analysis is performed within 14 days of the treatment initiation.

e	Alkaline phosphatase, ALT, AST, total bilirubin, serum creatinine, creatinine clearance (if required) sodium, potassium, total calcium, blood urea nitrogen (BUN) (or urea), albumin. Cycle 1 analysis will not be necessary if the screening analysis is performed within 14 days of the treatment initiation.
f	Coagulation includes International Normalized Ratio (INR) and activated PTT (aPTT).
g	Viral serology: HIV, HBV serology [HBsAg, anti-HBsAg] and HCV (anti-HCV). In patients with a positive anti-HCV, HCV RNA detection and quantification by PCR will be additionally performed.
h	Pregnancy test (only applicable to premenopausal patients): serum test within 7 days prior to enrollment. During the active treatment phase, a urine (or serum) test must be performed within 72 hours before each cycle and at the post-treatment visit (30 [±5] days from the last study treatment dose). Any positive urine test must immediately be confirmed by a serum test.
i	Ophthalmologic assessments include visual acuity testing, slit lamp examination, and fundoscopy. This assessment will be performed at screening and at the post-treatment visit (30 [±5] days from the last study treatment dose).
j	Perform an ECHO or MUGA scan assessment (note: the same test must be used for the patient throughout the study) every 4 cycles (±7 days) (Cycle 5, 9, 13…) and at the post-treatment visit (30 [±5] days from the last study treatment dose).
k	Triplicate 12-lead ECGs will be performed at screening and before each cycle.
l	EORTC QLQ-C30 must be completed before other assessments are performed or study drug is administered: - at baseline (within 7 days before treatment initiation), - during study treatment, every 3 cycles (- 3 days) until treatment discontinuation, and - at the post-treatment visit (30 [±5] days from the last study treatment dose) or before if the patient is starting a new treatment.
m	After ICD signature, but prior to initiation of study treatment, only SAEs caused by a protocol-mandated intervention will be collected. AEs to be monitored continuously during the treatment period. All AEs occurring during the study and until the treatment discontinuation visit 30 days after the last study medication to be recorded with grading according to NCI-CTCAE version 5.0, thereafter all study treatment-related SAEs should continue to be collected.
n	Tumor assessment should be performed during the screening period; however, if a patient has a valid image test performed up to 28 days before the date of the study treatment initiation it is not required to repeat it. Disease assessment for all patients will include: ✓ Contrast enhanced (unless clinically contraindicated) CT scan or MRI of the chest, abdomen and pelvis (CAP). A positron emission tomography (PET)-CT scan will be accepted if the CT scan meets the RECIST version 1.1. requirements for tumor lesion evaluation. ✓ Bone scan is mandatory if the patient has bone disease or if there is any suspicion of bone metastases. A PET scan will be also accepted to assess the already known or newly suspected bone metastatic disease. Any suspicious abnormalities (i.e., hotspots) identified on the bone or PET scan at baseline must be confirmed by X-ray, CT scan with bone window or MRI. Additional bone scans will be performed only to confirm a complete response (CR) in patients with bone lesions identified at baseline, or whenever clinically or biochemically bone progression or newly diagnosed bone disease is suspected. ✓ Brain CT scan with IV contrast or MRI is mandatory if the patient has previously known CNS metastases or if there is any suspicion of CNS metastases. ✓ CT scan or MRI scan of any other sites of disease as clinically indicated. ✓ Clinical assessment of superficial disease by calipers which will include color photographs of all superficial metastatic lesions including a ruler to estimate the size of the lesion. When lesions can be evaluated by both a clinical exam and imaging, imaging evaluation should be undertaken since it is more objective.

	All measurable and evaluable lesions should be assessed at the screening visit and re-assessed at each subsequent tumor evaluation and documented in the eCRF. Tumor assessments will be performed at screening, every 9 weeks (±7days) and after the third tumor evaluation every 12 weeks (±7days) until radiographically and/or clinically (i.e., for photographed or palpable lesions) documented PD as per RECIST version 1.1., withdrawal of consent, start of new anticancer treatment, death, or study termination, whichever occurs first. Tumor assessment will be continued in patients that discontinue treatment without disease progression per RECIST version 1.1. until death, disease progression, start of new anticancer therapy, patient's consent withdrawal, or study termination, whichever occurs first, every 12 weeks (± 7 days) from the last tumor assessment and bone scans (if applicable) as stated above.
o	Patients will be followed until death, loss to follow-up, withdrawal of consent or study termination by GEICAM. After progression, tumor assessments will be performed according to the standard medical practice. All treatments received by the patient after progression as well as the dates of disease progression to the second and third lines and death will be collected in the eCRF, to calculate PFS2, PFS3 and OS respectively.
p	Whole blood and plasma samples for biomarker analysis will be collected: - at baseline (within 7 days before treatment initiation), - during study treatment, every 3 cycles on Day 1 (pre-dose) until treatment discontinuation for any reason, and - at the study treatment discontinuation, collected at the post-treatment visit (30 [±5] days from the last study treatment administration). If a patient ends the study treatment due to a reason other than disease progression, an additional sample must be collected once progression is documented (even if that patient has initiated another treatment).
q	Investigators will be encouraged to send the most recent pre-treatment formalin-fixed paraffin embedded (FFPE) tumor tissue sample, preferably from a metastatic lesion, for retrospective biomarker assessments. However, if this is not possible, archived tissue samples from primary tumor will be acceptable. Samples will be sent to the sponsor-designated central laboratories.

Protocol Attachment 2. Eastern Cooperative Oncology Group Performance Status

ECOG Performance Status

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

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. 1982. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5(6):649-65.

Protocol Attachment 3. Adverse event (AE) non defined in NCI-CTCAE

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

Activities of Daily Living (ADL)
*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing, and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Protocol Attachment 4. Adverse Events / Serious Adverse Events Assessment Guide

Time	**After ICD Before Study Treatment**	**During Therapy**	**30-Day** (±5 days) **Post-treatment Follow-up Period**	**Long-Term Follow-up Period**
Events to Collect	AE/SAEs Related to Procedures	New/Ongoing AE/SAEs Regardless of Relatedness to Study Treatment or Procedures		New/Ongoing SAEs Related to Study Treatment or Procedures

Abbreviations: AE = adverse event, ICD = informed consent document, SAE = serious adverse event.

Protocol Attachment 5. QoL Questionnaire

In a separate document