Clinical Study Protocol (CSP)

# A Multicenter, Open-Label, Single-Arm, Multicohort Phase II Clinical Trial of Trastuzumab Deruxtecan (DS-8201a) in Human Epidermal Growth Factor Receptor 2 (HER2)-Positive Advanced Breast Cancer with Brain Metastases and/or Leptomeningeal Carcinomatosis

**DS-8201a for trEatment of aBc, BRain mets, And Her2[+] disease**

**–The DEBBRAH Study–**

**Study Drug:** Trastuzumab deruxtecan (DS-8201a)

**EudraCT#:** 2019-001739-29

**Clinical Trials.gov#**: NCT04420598

**Study Code:** MEDOPP243

**CSP Version and Date**: version 5.0. 18-Jan-2022

**CSP Revision History:**

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| • Initial approved version: | Version 2.0, 28-Apr-2020 |
| • Amendment 1: | Version 3.0, 10-Jul-2020 |
| • Amendment 2: | NA |
| • Amendment 3: | Version 4.0, 30-Dec-2020 |
| • Amendment 4: | Version 5.0, 18-Jan-2022 |
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## I. SPONSOR'S SIGNATURE PAGE

**CSP Title: "**A Multicenter, Open-Label, Single-Arm, Multicohort Phase II Clinical Trial of Trastuzumab Deruxtecan (DS-8201a) in Human Epidermal Growth Factor Receptor 2 (HER2)-Positive Advanced Breast Cancer with Brain Metastases and/or Leptomeningeal Carcinomatosis – The DEBBRAH Study"

**Study Code:** MEDOPP243

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| MEDSIR Medical Scientist | Signature | Signature date (DD-MM-YYYY) |
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## II. KEY CONTACT DETAILS

**Sponsor**

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| Name: | Medica Scientia Innovation Research (MEDSIR) |
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**Scientific Global Coordinator**

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**Clinical Study Coordinator**

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| Name: | [redacted] |
| Position: | |
| Institution: | |
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**Chief Investigator**

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| Name: | [redacted] |
| Position: | |
| Institution: | |
| Email: | |

**Sponsor's Medical Monitor**

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|---|---|
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| E-mail: | |

**Sponsor's Safety Risk Management**

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## III. STEERING COMMITTEE

| Name | Role |
|---|---|
| [redacted] | Clinical Expert |
| [redacted] | Clinical Expert |
| [redacted] | Clinical Expert |
| [redacted] | Sponsor's Medical Monitor |
| [redacted] | Clinical Expert |
| [redacted] | Clinical Expert |
| [redacted] | Clinical Expert |
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## IV. DECLARATION OF INVESTIGATORS

**CSP Title: "**A Multicenter, Open-Label, Single-Arm, Multicohort Phase II Clinical Trial of Trastuzumab Deruxtecan (DS-8201a) in Human Epidermal Growth Factor Receptor 2 (HER2)-Positive Advanced Breast Cancer with Brain Metastases and/or Leptomeningeal Carcinomatosis – The DEBBRAH Study".

**Study Code:** MEDOPP243

**CSP Version and Date**: Version 5.0, 18-Jan-2022

- I have received, reviewed and understood the following:
  1. Clinical Study Protocol (CSP) version 5.0, 18-Jan-2022
  2. Trastuzumab deruxtecan (DS-8201a) Investigator's Brochure (IB) with details of clinical and nonclinical data that are relevant to the study of the product in human subjects.
- I have been adequately informed about the development of the investigational product to date. I will confirm the receipt of updated IB. I have read this CSP and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this CSP.
- I fully understand that any changes instituted by the Investigator(s) without previous agreement with the Sponsor would constitute a violation of the CSP, including any ancillary studies or procedures performed on study patients (other than those procedures necessary for the wellbeing of the patients). I am aware that I cannot deviate from or apply changes to the CSP without prior approval or the favorable opinion of the Institutional Review Board (IRB) or Ethics Committee (EC) and/or before Sponsor's agreement to avoid immediate risk to the trial patients. If this occurs, I agree to inform the Sponsor as to the deviation or changes in writing and their reasons, as soon as possible.
- I will not enroll the first subject in the study until I have received approval from the appropriate IRB/EC and until all legal and regulatory requirements in my country have been fulfilled.
- The study will be conducted in accordance with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and its amendments, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines (ICH E6[R2] GCP) and applicable regulations and laws.
- I agree to obtain, in the manner described in this CSP and in (ICH E6[R2] GCP), written informed consent form (ICF) by the subject or witnessed verbal ICF to participate for all subjects whose participation in this study is proposed to and before any subject's study specific procedure is done.

- I will ensure that the study drug supplied by the Sponsor are being used only as described in this CSP.
- I am aware of the requirements for the correct reporting of serious adverse events, and I commit to document and to report such events as required by the Sponsor and in accordance with Health Authority Regulatory requirements.
- I agree to supply – upon request – the Sponsor or Sponsor's representative with evidence of current laboratory accreditation, the name and address of the laboratory, and a list of normal values and ranges.
- I agree with the use of results of the study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals.
- I agree to keep all source documents and case report forms as specified in the relevant sections of this CSP.
- I will provide all required Regulatory Authority forms, up-to-date curriculum vitae of myself, sub-Investigators and of any member of my study team (if requested) before the study starts, which may be submitted to regulatory authorities.
- I am aware of the possibility of being audited by the Sponsor or its delegate or inspected by regulatory authorities for the performance of this study. I will permit monitoring, auditing and inspection and provide direct access to source data/documents and reports for these purposes.
- Furthermore, I confirm herewith that the Sponsor is allowed to enter and utilize my professional contact details and function in an electronic database for internal purposes and for submission to Health Authorities worldwide.

Name: _____

Signature: _____ Date: _____

## V. CLINICAL STUDY PROTOCOL SYNOPSIS

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| **CSP Title:** | "A Multicenter, Open-Label, Single-Arm, Multicohort Phase II Clinical Trial of Trastuzumab Deruxtecan (DS-8201a) in Human Epidermal Growth Factor Receptor 2 (HER2)-Positive Advanced Breast Cancer with Brain Metastases and/or Leptomeningeal Carcinomatosis – The DEBBRAH Study" |
| **CSP Short Title** | **D**S-8201a for tr**E**atment of a**B**c, **BR**ain mets, **A**nd **H**er2[+] disease – The **DEBBRAH** Study– |
| **Study Code:** | MEDOPP243 |
| **EudraCT Number:** | 2019-001739-29 |
| **Investigational Medicine Product:** | Trastuzumab deruxtecan (DS-8201a) |
| **Trial Design:** | This is a multicenter, international, open-label, single-arm, multicohort, two-stage optimal Simon's design, phase II clinical trial. |
| **Target Disease:** | Pretreated, unresectable locally advanced or metastatic Human Epidermal Growth Factor Receptor 2 (HER2)-positive or HER2-low expressing breast cancer (BC) with untreated or treated brain metastases (BMs) or leptomeningeal carcinomatosis (LMC). |
| **Patients:** | Male and pre- and post-menopausal female patients age ≥ 18 years with pretreated unresectable locally advanced or metastatic HER2-positive or HER2-low expressing BC with stable, treated or untreated BMs, or patients progressing after treatment for BMs or patients with LMC. Evidence of measurable brain disease is required. Patients are not eligible if they are candidates for a local treatment with a radical intention.<br><br>Patients with untreated BMs are those that have not received any local treatment for their central nervous system (CNS) involvement (surgery and/or stereotactic radiotherapy [SRS] and/or whole-brain radiation therapy [WBRT] are considered appropriate).<br><br>The operational definition of patients with stable BM are patients that have been for at least four weeks asymptomatic with or without corticosteroids. The highest dose of corticosteroids acceptable is dexamethasone 8 mg twice daily or any equivalent. |

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| | After confirmed eligibility, patients will be assigned to one of the following five study cohorts:<br>• **Cohort 1:** HER2-positive BC with non-progressing BM (after WBRT and/or SRS and or surgery.);<br>• **Cohort 2:** HER2-positive or HER2-low BC with asymptomatic untreated BM;<br>• **Cohort 3:** HER2-positive BC with progressing BMs after local treatment;<br>• **Cohort 4**: HER2-low expressing BC with progressing BMs after local treatment;<br>• **Cohort 5:** HER2-positive or HER2-low expressing BC with LMC. |
| **Number of Patients:** | 39 patients (N=8 patients in the cohort 1; N=10 in the cohort 2; N=7 in the cohort 3; N=7 in the cohort 4; N=7 in the cohort 5). |
| **Study Objectives:** | **Primary objective:**<br>**Cohort 1**<br>• To assess efficacy –defined as 16 weeks progression-free survival (PFS) per Response Assessment in Neuro-Oncology Brain Metastases (RANO-BM) and Response Evaluation Criteria in Solid Tumors (RECIST) 1.1– of trastuzumab deruxtecan (DS-8201a) in patients with pretreated unresectable locally advanced or metastatic HER2-positive BC with non-progressing BM (after WBRT and/or SRS and or surgery).<br>**Cohorts 2, 3, and 4**<br>• To assess efficacy –defined as intracranial overall response rate (ORR–IC) per RANO-BM– of trastuzumab deruxtecan (DS-8201a) in patients with pretreated unresectable locally advanced or metastatic BC:<br>a) HER2-positive or HER2-low expressing with untreated BMs (Cohort 2);<br>b) HER2-positive with progressing BMs after local treatment (Cohort 3);<br>c) HER2-low expressing with progressing BMs after local treatment (Cohort 4). |

**Cohort 5**

- To assess efficacy –defined as OS– of trastuzumab deruxtecan (DS-8201a) in patients with pretreated unresectable locally advanced or metastatic HER2-positive or HER2-low expressing BC with LMC.

**Secondary objectives:**

**Cohort 1**

- To assess efficacy –defined as PFS, ORR, CBR, time to response (TTR), duration of response (DOR), and best percentage of change in tumor burden per RANO-BM (for IC lesions) and RECIST 1.1 (extracranial lesions and overall lesions)– after treatment with trastuzumab deruxtecan (DS-8201a) in this population.
- To assess OS in this population.
- To assess the safety and tolerability of trastuzumab deruxtecan (DS-8201a) by NCI-CTCAE v.5.0.

**Cohorts 2, 3, and 4**

- To assess efficacy –defined as PFS, CBR, TTR, DOR, and best percentage of change in tumor burden per RANO-BM (for IC lesions) and RECIST 1.1 (for extracranial lesions and overall lesions); and ORR per RECIST 1.1 (for extracranial lesions and overall lesions).
- Time to WBR and/or SRS (only for cohort 2), defined as the time from the treatment initiation to time of CNS disease progression that requires treatment with WBR and/or SRS, and determined locally by the investigator through the use of RANO-BM and RECIST 1.1 criteria.
- To assess the safety and tolerability of trastuzumab deruxtecan (DS-8201a) by NCI-CTCAE v.5.0.
- To assess OS in this population.

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| | **Cohort 5**<br>• To assess efficacy –defined as PFS, ORR, CBR, TTR, DOR, and best percentage of change in tumor burden per RANO-BM (IC lesions) and RECIST 1.1 (extracranial lesions and overall lesions)<br>• To assess the safety and tolerability of trastuzumab deruxtecan (DS-8201a) by NCI-CTCAE v.5.0.<br><br>**Exploratory objectives:**<br><br>**Cohorts 1, 2, 3, 4, and 5**<br>• To assess [redacted] using the [redacted] and its [redacted] in this population.<br>• To evaluate predictive or prognostic biomarkers (plasma and/or tissue and/or CSF) associated with disease activity status or response to treatment in this population.<br>• To identify possible mechanisms of resistance to study treatments through the comparative analysis of potential biomarkers from paired pre-treatment and post-progression tumor and/or blood and/or CSF samples from this population. |
| **Study Endpoints:** | **Primary endpoint:**<br><br>**Cohort 1**<br>○ 16 weeks-PFS, defined as the period of time from treatment initiation to the first occurrence of disease progression or death from any cause, whichever occurs first. Progression will be determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br><br>**Cohorts 2, 3 and 4**<br>○ ORR-IC, defined as a complete response (CR) or partial response (PR), determined locally by the investigator through the use of RANO-BM criteria.<br><br>**Cohort 5** |

- OS defined as the time from treatment initiation to death from any cause. Patients without documented death at the time of the final analysis will be censored at the date of the last follow-up.

**Secondary endpoints:**

**Cohort 1**

- PFS, defined as the period of time from treatment initiation to the first occurrence of disease progression or death from any cause, whichever occurs first. Progression will be determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions
- ORR, defined as a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- CBR, defined as an objective response (CR or PR), or stable disease (SD) for at least 24 weeks, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- TTR, defined as the time from the treatment initiation to time of the first objective tumor response observed for patients who achieved a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- DoR, defined as the time from the first occurrence of a documented objective response to disease progression or death from any cause, whichever occurs first, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- OS, defined as the time from treatment initiation to death from any cause. Patients without documented death at the time of the final analysis will be censored at the date of the last follow-up.
- Best percentage of change from baseline in the size of tumor lesions, defined as the biggest decrease, or smallest increase if

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| | no decrease will be observed, and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ Adverse events (AEs) will be evaluated using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v.5.0.<br><br>**Cohorts 2, 3, and 4**<br>○ PFS, defined as the period of time from treatment initiation to the first occurrence of disease progression or death from any cause, whichever occurs first. Progression will be determined locally per RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ ORR, defined as a CR or PR, determined locally by the investigator through the use of RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ CBR, defined as an objective response (CR or PR), or SD for at least 24 weeks, and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ TTR, defined as the time from the treatment initiation to time of the first objective tumor response (tumor shrinkage of ≥ 30%) observed for patients who achieved a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ DOR, defined as the time from the first occurrence of a documented objective response to disease progression or death from any cause, whichever occurs first, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ OS, defined as the time from treatment initiation to death from any cause. Patients without documented death at the time of the final analysis will be censored at the date of the last follow-up. |

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| | ○ Best percentage of change from baseline in the size of tumor lesions, defined as the biggest decrease, or smallest increase if no decrease will be observed, and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ Time to WBR and/or SRS (only for cohort 2), defined as the time from the treatment initiation to time of CNS disease progression that requires treatment with WBR and/or SRS, and determined locally by the investigator through the use of RANO-BM and RECIST criteria.<br>○ AEs will be evaluated using the NCI CTCAE v.5.0<br><br>**Cohort 5**<br><br>○ ORR, defined as a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ PFS, defined as the period of time from treatment initiation to the first occurrence of disease progression or death from any cause, whichever occurs first. Progression will be determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ CBR, defined as an objective response (CR or PR), or SD for at least 24 weeks, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ TTR, defined as the time from the treatment initiation to time of the first objective tumor response observed for patients who achieved a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ DoR, defined as the time from the first occurrence of a documented objective response to disease progression or death from any cause, whichever occurs first, determined locally by the investigator through the use of RANO-BM criteria for IC lesions |

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| | and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ Best percentage of change from baseline in the size of tumor lesions, defined as the biggest decrease, or smallest increase if no decrease will be observed, and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.<br>○ AEs will be evaluated using the NCI CTCAE v.5.0<br><br>**Exploratory endpoints:**<br><br>**Cohorts 1, 2, 3, 4, and 5**<br><br>○ Overall change from baseline in [redacted]), [redacted] and [redacted]; Time to [redacted] in [redacted]; Time to [redacted] in [redacted]; Time to [redacted].<br><br>○ Relationship between tissue- and/or blood- and/or CSF-based biomarkers and patient clinical characteristics (e.g., baseline features) and outcome (e.g., [redacted]). |
| **Selection Criteria:** | **Inclusion criteria:**<br><br>Patients must meet **ALL** of the following inclusion criteria to be eligible for enrolment into the study:<br><br>1. Signed Informed Consent Form (ICF) prior to participation in any study-related activities.<br><br>2. Male or female patients ≥ 18 years at the time of signing ICF.<br><br>3. Eastern Cooperative Oncology Group (ECOG) performance status of 0-1 for **Cohorts 1 to 4** and 0-2 for **cohort 5**.<br><br>4. Life expectancy ≥ 12 weeks.<br><br>5. Histologically confirmed invasive breast cancer based on local testing on the most recent analyzed biopsy of the following breast cancer (BC) subtypes per 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) criteria: |

- **Cohort 1 and 3**: HER2 positive status
- **Cohort 4**: HER2-low expressing status
- **Cohort 2 and 5**: both HER2 positive and HER2-low expressing status

*Note 1: According to the 2018 ASCO-CAP guidelines, HER2-positive status is defined as HER2 immunohistochemistry (IHC) 3+, in situ hybridization (ISH) ≥ 2.0, or average HER2 copy number ≥ 6.0 signals. HER2-low expressing status defined as IHC 2+ / ISH-negative or IHC 1+ (ISH-negative or untested).*

*Note 2: Central confirmation of HER2 is not required for study entry. However, tissue blocks, or slides, must be submitted to confirm BC subtype by a Sponsor-designated central laboratory retrospectively.*

6. Unresectable locally advanced or metastatic disease documented by computerized tomography (CT) scan or magnetic resonance imaging (MRI) that is not amenable to resection with curative intent.
7. At least one brain lesion needed to be measurable (≥10 mm on T1-weighted, gadolinium-enhanced MRI) (**study cohorts 2 to 4**) or leptomeningeal carcinomatosis (LMC) with positive cerebrospinal fluid (CSF) cytology (**study cohort 5**).
    - **Study cohort 1**: History of BM that are non-progressing after WBRT and/or SRS and or surgery.
    - **Study cohort 2**: Presence of asymptomatic BM without clinical requirement for local intervention (WBRT and/or SRS and/or surgery).
    - **Study cohorts 3 and 4**: Evidence of new and/or progressive BM following previous WBRT and/or SRS and/or surgery.
    - **Study cohort 5**: Evidence of LMC with positive CSF cytology.
8. Previous treatments:

- For HER2-positive patients have been previously treated with a taxane and at least one HER2-targeted therapy in the advanced scenario.
- For HER2-low-expressing patients that also are endocrine receptor negative must have been previously treated with at least one chemotherapy regimen. If endocrine receptor positive, patients must have been previously treated with at least one chemotherapy and one endocrine regimen in the metastatic setting.

9. Patients must agree to collection of blood samples at the time of inclusion, at cycle 2 of treatment, and upon progression or study termination.

   *Note: In **study cohort 5**: Patients must agree to perform spinal taps or must be willing to have an Ommaya reservoir placed for CSF assessment, at baseline, every three weeks for 12 weeks (corresponding to the first 5 cycles of treatment) and every six weeks thereafter.*

10. Willingness and ability to provide tumor biopsy (if feasible) from metastatic lesions or breast primary tumor both at the time of the inclusion and after disease progression in order to perform exploratory studies.

    *Note: If feasible, patients should provide a tissue sample at baseline from metastases amenable to biopsy (at sites of locoregional recurrence [skin, chest wall, breast or lymph nodes], or distant recurrence [bone, liver, lung or abdomen]) or as alternative from breast primary tumor, that will be obtained between progression to the prior regimen and inclusion in the study. Patients for whom tissue sample cannot be obtained (e.g., non-measurable disease, inaccessible tumor or subject safety concern) may submit an archived metastatic tumor specimen only upon agreement from the Sponsor. If feasible, an additional tissue sample should be collected at the end of treatment visit for patients who discontinue treatment due to disease progression.*

11. Patients should have left ventricular ejection fraction (LVEF) ≥ 50% by either an echocardiogram (ECHO) or multigated acquisition (MUGA) scan within 28 days before enrolment.

12. Adequate hematologic and organ function within 14 days before the first study treatment on Day 1 of Cycle 1, defined by the following:

    - Hematological: White blood cell (WBC) count ≥ 3.0 x $10^9$/L, absolute neutrophil count (ANC) ≥ 1.5 x $10^9$/L, platelet count ≥ 100.0 x$10^9$/L, and hemoglobin ≥ 9.0 g/dL. (Platelet, red blood cell transfusion as well as G-CSF administration are not allowed within 1 week of screening assessments).
    - Hepatic: Bilirubin ≤ 1.5 times the upper limit of normal (× ULN) (< 3 x ULN in the case of documented Gilbert's disease and/or liver metastases); aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 3 × ULN (in the case of liver metastases ≤ 5 × ULN); alkaline phosphatase (ALP) ≤ 2 × ULN (≤ 5 × ULN in the case of liver and/or bone metastases ≤ 5 × ULN), serum albumin ≥ 2.5 g/dL
    - Renal: Serum creatinine ≤ 1.5 × ULN or creatinine clearance ≥ 30 mL/min based on Cockcroft−Gault equation (*Cockcroft-Gault equation: CLcr (mL/min)= ([{140 – age in years} × {ACTUAL WEIGHT in kg}] divided by [{72 × serum creatinine in mg/dL} multiplied by 0.85 if female])
    - Coagulation: International Normalized Ratio (INR)/Prothrombin Time (PT) and either partial thromboplastin or activated Partial Thromboplastin Time (aPTT) ≤ 1.5 × ULN (except for patients receiving anticoagulation therapy).

    *Note: Patients receiving heparin treatment should have an aPTT) between 1.5 and 2.5 × ULN (or patient value before starting heparin treatment). Patients receiving coumarin derivatives should have an INR between 2.0 and 3.0 assessed in two consecutive measurements one to four days apart. Patients should be on a stable anticoagulant regimen.*

13. Has adequate treatment washout period before enrollment, as indicated:

a. Major surgery: > 4 weeks;

b. Radiation therapy: > 4 weeks (palliative stereotactic radiation therapy to other areas ≥ 2 weeks);

c. Anticancer systemic treatment (including immunotherapy, retinoid therapy, hormonal therapy): ≥ 3 weeks (≥ 2 weeks or 5 half-lives, whichever is longer, for small-molecule targeted agents such as 5-fluorouracil-based agents, folinate agents, weekly paclitaxel; ≥ 6 weeks for nitrosureas or mitomycin C);

d. Antibody based anti-cancer therapy: ≥ 4 weeks;

e. Chloroquine/Hydroxychloroquine>14 days

14. Resolution of all acute toxic effects of prior anti-cancer therapy to grade ≤ 1 as determined by the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI–CTCAE) v.5.0 (except for alopecia or other toxicities). Subjects with chronic Grade 2 toxicities may be eligible per the discretion of the Investigator after consultation with the Sponsor Medical Monitor or designee (e.g., Grade 2 chemotherapy-induced neuropathy).
15. Male and female subjects of reproductive/childbearing potential must agree to use a highly effective form of contraception or avoid intercourse during and upon completion of the study and for at least 7 months for females and 4 months for males after the last dose of study drug. Methods considered as highly effective methods of contraception include:
    - *Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:*
        - *i. Oral*
        - *ii. Intravaginal*
        - *iii. Transdermal*
    - *Progestogen-only hormonal contraception associated with inhibition of ovulation:*
        - *iv. Oral*
        - *v. Injectable*
        - *vi. Implantable*
    - Intrauterine device (IUD)
    - Intrauterine hormone-releasing system (IUS)

- Bilateral tubal occlusion
- Vasectomized partner
- Complete sexual abstinence defined as refraining from heterosexual intercourse during and upon completion of the study and for at least 7 months after the last dose of study drug. Periodic abstinence (calendar, symptothermal, post-ovulation methods) is not an acceptable method of contraception.

*Note: Non-child-bearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea (in questionable cases, a blood sample with simultaneous follicle-stimulating hormone [FSH] > 40 mIU/mL and estradiol < 40 pg/mL [< 147 pmol/L] is confirmatory). Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods outlined for women of child-bearing potential if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study randomization/enrollment. For most forms of HRT, at least 2-4 weeks will elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT. Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method.*

16. Male subjects must agree to not freeze or donate sperm starting at Screening and throughout the study period, and at least 4 months after the final study drug administration. Preservation of sperm should be considered prior to enrolment in this study.
17. Female subjects must agree to not donate, or retrieve for their own use, ova from the time of Screening and throughout the study treatment period, and for at least 7 months after the final study drug administration.

18. Patients who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, post-treatment follow-up and other study procedures.

**Exclusion criteria:**

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

1. Inability to comply with study and follow-up procedures.
2. Previous treatment with trastuzumab deruxtecan (DS-8201a) or any other antibody drug conjugate (ADC) which consists of an exatecan derivative that is a topoisomerase 1 inhibitor.
3. Medical history of myocardial infarction (MI) within 6 months before enrollment, symptomatic congestive heart failure (New York Heart Association Class II to IV). Subjects with troponin levels above ULN at screening (as defined by the manufacturer), and without any MI related symptoms should have a cardiologic consultation before enrollment to rule out MI.
4. Corrected QT interval (QTc) prolongation to > 470 ms (females) or >450 ms (males) based on average of the screening triplicate12-lead ECG.
5. History of (non-infectious) interstitial lung disease (ILD)/pneumonitis that required steroids, has current ILD/pneumonitis, or where suspected ILD/pneumonitis cannot be ruled out by imaging at screening.
6. Clinically significant corneal disease in the opinion of the Investigator.
7. Spinal cord compression.
8. Multiple primary malignancies within 3 years, except adequately resected non-melanoma skin cancer, curatively treated in-situ disease, other solid tumors curatively treated, or contralateral breast cancer.
9. History of severe hypersensitivity reactions to either the drug substances or inactive ingredients in the drug product.
10. History of severe hypersensitivity reactions to other monoclonal antibodies.
11. Uncontrolled infection requiring IV antibiotics, antivirals, or antifungals.

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| | 12. Patients with substance abuse or any other medical conditions such as clinically significant cardiac or psychological conditions, that may, in the opinion of the investigator, interfere with the subject's participation in the clinical study or evaluation of the clinical study results.<br>13. Known human immunodeficiency virus (HIV) infection, or active hepatitis B or C infection. Patients positive for hepatitis C (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV RNA.<br>14. Female patients who are pregnant or breastfeeding or planning to become pregnant.<br>15. Treatment with other systemic therapy for metastatic disease including chemotherapy, immunotherapy, targeted therapy (small molecules/ monoclonal antibodies), or endocrine therapy.<br>16. Major surgery (defined as requiring general anesthesia) or significant traumatic injury within 4 weeks of start of study drug, or patients who have not recovered from the side effects of any major surgery, or patients who may require major surgery during the study.<br>17. Radiotherapy within 4 weeks or limited-field palliative radiotherapy within 2 weeks prior to study enrolment, or patients who have not recovered from radiotherapy-related toxicities to baseline or grade ≤ 1 and/or from whom ≥ 25% of the bone marrow has been previously irradiated.<br>18. Use of concurrent investigational agents or other concomitant anticancer therapies.<br>19. Use of intrathecal therapy for LMC<br>20. Active bleeding diathesis, previous history of bleeding diathesis, or chronic anti-coagulation treatment (the use of low molecular weight heparin is allowed as soon as it is used as prophylaxis intention).<br>21. Serious concomitant systemic disorder (e.g., active infection including HIV, active hepatitis, liver cirrhosis, end stage chronic renal disease) incompatible with the study (at the discretion of investigator).<br>22. Any of the following within 6 months of enrollment: severe/unstable angina, ongoing cardiac dysrhythmias of NCI–CTCAE v.5.0 grade ≥2, coronary/peripheral artery bypass graft, cerebrovascular |

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| | accident including transient ischemic attack, or symptomatic pulmonary embolism.<br>23. Uncontrolled electrolyte disorders of NCI–CTCAE v.5.0 grade ≥ 2.<br>*24.* 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 randomization<br>25. 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 eCRF for patients who are included in the study.<br>26. Prior pneumonectomy |
| **Treatment:** | After having confirmed eligibility and entered into the clinical trial, patients will be treated with trastuzumab deruxtecan (DS-8201a) at 5.4 mg/Kg administered as an intravenous (IV) infusion on Day 1 of a 21-day cycle (Q3W), initially for at least 90 minutes, then, if there is no infusion-related reaction, for a minimum of 30 minutes.<br><br>In patients with hormone receptor (HR)-positive status (estrogen receptor [ER] and/or progesterone receptor [PgR]) administration of endocrine therapy is not allowed.<br><br>In patients allocated in study cohort 5, administration of intrathecal therapy is not allowed. |
| **Study Procedures, Efficacy and Safety Assessments:** | **Patient visits**:<br><br>Visits are organized in programmed cycles of 21 days (if there are no treatment delays due to the occurrence of AEs). All visits must occur within ± 2 working days from the scheduled date, unless otherwise noted in the schedule of assessments.<br><br>Assessments scheduled for Day 1 (before treatment) of all cycles must be performed within 48 hours prior to study treatment administration, unless otherwise indicated in the schedule of assessments, to confirm to the patient if treatment can be followed up.<br><br>If a mandatory procedure described in the protocol falls on a bank holiday and/or weekend, this procedure should be performed on the day before or after the holiday (i.e. within a period of ± 2 working days). |

End of treatment (EoT) visit will be performed within 40 days (± 7 days) after the last dose of study treatment. Afterwards, follow-up contacts will continue every 12 weeks (± 14 days) up to the end of study (EoS) and survival status and new anticancer therapy information will be collected. Telephone contact is acceptable.

EoS will occur at 12 months after the last patient included in the study, unless premature termination of the study.

**Tumor assessments:**

- **Evaluation of chest, abdomen, and pelvis:**

This assessment should be done preferably by CT scan or MRI in case of contrast allergy, within 28 days prior to the first administration of study medication, every six weeks (± 3 days) from the first dose of study treatment for the first six months and, thereafter, every nine weeks (± 5 days) until the EoS visit.

In the event a positron emission tomography (PET)/CT scan is used for tumor assessments, CT portion of PET/CT is usually of lower quality, and should not be used instead of dedicated diagnostic CT. If the CT scan is of high quality, with oral and intravenous contrast, may be used with caution. Additional information from PET may bias CT assessment.

- **Brain imaging:**

A brain MRI will be performed within 28 days prior to the first administration of study medication, every six weeks (± 3 days) for first six months, then every nine weeks (± 5 days), unless clinically suspected brain progression.

Additionally, for patients included in the study cohort 5, assessment of neuroaxis by MRI together with spinal tap for CSF collection should be obtained every 3 weeks (± 2 days) for the first 12 weeks (corresponding to the first 5 cycles of treatment), every 6 weeks thereafter, and at the time of treatment progression or study termination.

Tumor response in CNS will be assessed for all patients, unless they withdraw from the study for any reason not attributable to disease progression confirmed radiologically or clinically as per RANO-BM

criteria and who have not received an acceptable complete assessment of the disease. The measurable and non-measurable disease must be documented at screening and be re-assessed at every tumor assessment thereafter.

- **Bone scan:**

Bone scan is mandatory at baseline For patients with bone lesions identified at baseline, bone scan will be performed every 24 weeks (± 7 days), unless clinically or biochemically suspected bone progression.

If no bone involvement is demonstrated, then it is no necessary to repeat the bone assessment unless clinically or biochemically suspected bone progression. If an isotope-based bone scan was performed > 28 days but ≤ 60 days prior to start of study treatment, the bone scan does not need to be repeated.

**PROs assessments:**

PRO will be assessed using the EORTC QLQ-C30 and EORTC QLQ BR23 questionnaires. Patients will complete each questionnaire prior to any study or medical procedure: at baseline, Day 1 of cycles 2-4, then on Day 1 of every other subsequent cycle starting with cycle 6 (e.g. cycles 6, 8, 10, etc.), at the end-of-treatment visit and during follow-up period until start of alternative anti-cancer therapy. Completed questionnaires are always considered source document and must be filed accordingly. Patients must complete these instruments in clinic (cannot be taken home) and prior to having any tests and to any discussion of their progress with healthcare personnel at the site. Interviewer administration in clinic may be used under special circumstances (e.g., patient forgot their glasses or feels too ill). The questionnaire will be given to the patient in the appropriate language for the site. Patients discontinuing treatment for any reason including progression disease or any other reason should have a tumor and PROs assessment prior to start alternative anticancer treatment.

**Safety assessments:**

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| | The occurrence and all grade of side effects observed throughout the study will be listed and tabulated according to type and dose level. Any AEs that the investigator reports as unrelated to the drug will also be reported. In this study, side effects will be assessed according to the NCI–CTCAE v.5.0 criteria. |
| **Biological samples:** | **Tumor samples for molecular analysis:**<br><br>If feasible, patients should provide a tissue sample at the time of study entry and at disease progression from metastases amenable to biopsy (at sites of locoregional recurrence [skin, chest wall, breast or lymph nodes], or distant recurrence [bone, liver, lung or abdomen]) or, as alternative, from breast primary tumor.<br><br>Exploratory studies will be performed on tumor biopsies, or formalin-fixed and paraffin-embedded (FFPE) or frozen tumor samples (blocks), or unstained glass slides.<br><br>**Blood samples for molecular analysis:**<br><br>These samples will be collected during the screening period, after two cycles of study treatment, and upon progression or study termination.<br><br>**CSF samples for molecular analysis:**<br><br>In study cohort 5, patients must agree to provide CSF for exploratory analysis, at baseline, every 3 weeks for 12 weeks (corresponding to the first 5 cycles of treatment) and every 6 weeks thereafter, and at the time of treatment progression or study termination. |
| **Statistics:** | **Sample size:**<br><br>A maximum of 39 patients with pretreated unresectable locally advanced or metastatic HER2-positive or HER2-low expressing patients will be recruited in five study cohorts, as follows:<br><br>Cohort 1: 4 patients in the first step, up to a total of 8 patients.<br><br>Cohort 2: A maximum of 10 patients.<br><br>Cohort 3: A maximum of 7 patients. |

Cohort 4: A maximum of 7 patients.

Cohort 5: A maximum of 7 patients.

**Justification of total sample size:**

The primary endpoint for this study in the cohort of patients with stable BMs (cohort 1) is the PFS at 16 weeks. The primary endpoint for this study in the cohorts of patients with untreated or progression BMs (cohorts 2 to 4) is the best ORR-IC. The primary endpoint in the cohort of patients with LMC (cohort 5) is the OS.

The trial uses a two-stage optimal Simon's design for the cohort 1, a one-stage A'Hern design for the cohorts 2 to 4, and a one-stage survival design for the cohort 5. The analysis in the cohort 1 will be one-sided, based on stochastic ordering of uniformly minimum variance unbiased estimator (UMVUE). The analyses in the cohorts 2 to 4 will be one-sided, based on stochastic binomial exact test. The sample size for the LMC cohort was based on a one arm time-to-event design. The analysis will be based on maximum likelihood exponential test.

The local type I and II errors for all designs will be 0.05 and 0.2, respectively.

Futility interim analyses in cohort 1:

A futility interim analysis has been planned in cohort 1. The recruitment will not be stopped during the interim analysis. However, after the interim analysis, if the number of patients achieving the primary endpoint is equal or less than futility boundary in each cohort, the accrual in the cohort must be stopped. The stopping rule is:

Cohort 1: All 4 patients with progressive disease or death at 16 weeks.

Feasibility interim analyses in each cohort:

At the halfway point of the recruitment period, the Steering Committee will evaluate if the accrual objective for the first stage was achieved in each study cohorts (4, 5, 3, 3 and 3 patients for cohorts 1, 2, 3, 4 and 5, respectively). If this accrual objective has not achieved in a cohort,

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| | the Steering Committee will propose a corrective action plan or finalize the study in this cohort. |
| **Study Periods:** | **Recruitment period:** Estimated enrolment period will be at least 18 months.<br><br>**End of study (EoS):** EoS will occur at 12 months after the last patient included in the study, unless premature termination of the study. |

## VI. ABBREVIATIONS

| Abbreviation | Definition |
|---|---|
| **95%CI** | 95% confidence intervals |
| **ABC** | Advanced breast cancer |
| **ADC** | Antibody-drug conjugate |
| **ADL** | Activities of daily living |
| **AE** | Adverse event |
| **AESI** | Adverse event of special interest |
| **ALP** | Alkaline phosphatase |
| **ALT** | Alanine transaminase |
| **ANC** | Absolute neutrophil count |
| **aPTT** | Activated Partial Thromboplastin Time |
| **ASCO/ CAP** | American Society of Clinical Oncology/College of American Pathologists |
| **AST** | Aspartate transaminase |
| **BBB** | Blood-brain barrier |
| **BCRP** | Breast cancer resistance protein |
| **BM** | Brain metastases |
| **BTB** | Blood–tumor barrier |
| **cfDNA** | Cell-free deoxyribonucleic acid |
| **$C_{max}$** | Maximum serum concentration |
| **CNS** | Central nervous system |
| **CR** | Complete response |
| **CRF** | Case report form |
| **CRO** | Clinical Research Organization |
| **CSF** | Cerebrospinal fluid |
| **CSP** | Clinical Study Protocol |
| **CT** | Computerized tomography |
| **CYP** | Cytochrome P450 |
| **DCR** | Disease control rate |
| **DNA** | Deoxyribonucleic acid |
| **DOR** | Duration of response |
| **DSE CSPV** | Daiichi Sankyo Europe Clinical Safety and Pharmacovigilance |
| **DSUR** | Development Safety Update Report |
| **ECHO** | Echocardiogram |
| **ECOG** | Eastern Cooperative Oncology Group |

| Abbreviation | Definition |
|---|---|
| **eCRF** | Electronic Case Report Form |
| **EGFR** | Epidermal Growth Factor Receptor |
| **EMA** | European Medicines Agency |
| **EORTC** | European Organization for Research and Treatment of Cancer |
| **EoS** | End of Study |
| **EoT** | End of Treatment |
| **ER** | Estrogen receptor |
| **ESA** | Erythropoiesis-stimulating agent |
| **FDA** | Food and Drug Administration |
| **FSH** | Follicle-stimulating hormone |
| **G-CSF** | Granulocyte-colony stimulating factor |
| **GDPR** | General Data Protection Regulation |
| **GGT** | *Gamma*-glutamyl transferase |
| **GM-CSF** | Granulocyte-macrophage-colony stimulating factor |
| **HER2** | Human Epidermal Growth Factor Receptor 2 |
| **hERG** | Human ether-a-go-go-related gene |
| **HIV** | Human immunodeficiency virus |
| **HR** | Hormone receptor |
| **HRT** | Hormone replacement therapy |
| **IB** | Investigator's Brochure |
| **IC** | Intracranial |
| **$IC_{50}$** | Inhibitory concentration |
| **ICF** | Informed Consent Form |
| **ICH** | International Conference on Harmonization |
| **IHC** | Immunohistochemistry |
| **ILD** | Interstitial lung disease |
| **IMP** | Investigational medicinal product |
| **INR** | International Normalized Ratio |
| **IRB** | Institutional review board |
| **ISH** | *In situ* hybridization |
| **IT** | Intrathecal |
| **IUD** | Intrauterine device |
| **IUS** | Intrauterine hormone-releasing system |
| **IV** | Intravenous |
| **LDH** | Lactate dehydrogenase |

| Abbreviation | Definition |
|---|---|
| **LMC** | Leptomeningeal carcinomatosis |
| **LVEF** | Left ventricular ejection fraction |
| **MATE** | Multidrug and toxin extrusion |
| **MBC** | Metastatic breast cancer |
| **MedDRA** | Medical Dictionary for Regulatory Activities |
| **MRI** | Magnetic resonance imaging |
| **MRP** | Multidrug resistance-associated protein |
| **MUGA** | Multigated acquisition |
| **NCCN** | National Comprehensive Cancer Network |
| **NCI-CTCAE** | National Cancer Institute-Common Terminology Criteria for Adverse Events |
| **NE** | Non-evaluable |
| **NGS** | Next-generation sequencing |
| **OATP** | Organic anion transporting polypeptide |
| **ORR** | Overall response rate |
| **OS** | Overall survival |
| **P-gp** | P-glycoprotein |
| **PCR** | Polymerase chain reaction |
| **PD-L1** | Programmed Death-ligand 1 |
| **PD1** | Programmed death 1 |
| **PET** | Positron emission tomography |
| **PFS** | Progression Free Survival |
| **PgR** | Progesterone receptor |
| **PP** | Per protocol |
| **PR** | Partial response |
| **PRO** | Patient reported outcome |
| **PT** | Prothrombin Time |
| **Q3W** | Day of 21-day cycle |
| **QLQ** | Quality of Life Questionnaire |
| **QOL** | Quality of life |
| **QTc** | Corrected QT interval |
| **QTcF** | QTc corrected as per Fridericia |
| **RANO-BM** | Response Assessment in Neuro-Oncology Brain Metastases |
| **RECIST** | Response Evaluation Criteria in Solid Tumors |
| **RT** | Radiation therapy |
| **SAE** | Serious adverse event |

| Abbreviation | Definition |
|---|---|
| **SD** | Stable disease |
| **SD** | Stable disease |
| **SOC** | System-organ class |
| **SRS** | stereotactic radiotherapy |
| **TEAE** | Treatment-emergent adverse event |
| **TBL** | Total bilirubin |
| **TNBC** | Triple-negative breast cancer |
| **TQ** | Targeted Questionnaire |
| **T-DXs** | Trastuzumab deruxtecan (DS-8201a) |
| **TTR** | Time to response |
| **ULN** | Upper limit of normal |
| **UMVUE** | Uniformly minimum variance unbiased estimator |
| **UPN** | Unique patient number |
| **$V_{ss}$** | Volume of distribution at steady state |
| **WBC** | White blood cell |
| **WBRT** | Whole-brain radiation therapy |

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# 1 BACKGROUND AND STUDY RATIONALE

## 1.1 Introduction

- **Central Nervous System Metastases in Breast Cancer Patients**

Breast cancer is the second-leading cause of central nervous system (CNS) metastases among solid malignancies (Weil et al. 2005). In cohort studies, the incidence is about 5% and this number increases to 15% in advanced breast cancer (ABC) (Schouten et al. 2002; Barnholtz-Sloan et al. 2004). Moreover, autopsy studies indicate that brain metastases (BM) are found in 30% of patients with ABC (Lee 1983) and some of them asymptomatic and not diagnosed before death. Patients with Human Epidermal Growth Factor Receptor 2 (HER2)-positive or triple-negative breast cancer (TNBC) have a higher risk of developing BMs than patients with luminal-like disease (Kennecke et al. 2010). A previous study analyzing 155 CNS screening images from patients recruited in clinical trials showed that HER2 overexpression in addition to the number of metastatic sites are predictive of CNS involvement by multivariate analysis (Miller et al. 2003).

Treatment for HER2-positive patients with BMS depends on factors such as patient prognosis, symptoms, resectability, number and size of metastases, whether they are diffuse and previous therapy. Patients should receive appropriate local therapy and systemic therapy. Additional options include best supportive care, enrolment in clinical trial and/or palliative care. Current treatment options for BM comprise local approaches such as radiation therapy with or without surgery. Whole-brain radiation therapy (WBRT) is commonly used for patients with multiple metastases, whereas stereotactic radiotherapy (SRS) is principally recommended for those with less than four different metastatic localizations. On the other hand, neurosurgery is reserved for single metastases or in specific cases such as bleeding or mass effect-causing lesions.

Very recently, formal expert consensus-based guideline recommendations on the management of BMs for patients with HER2-positive ABC was released (Ramakrishna et al. 2018). Following revision of 622 publications, the authors stated no additional evidence was identified that would warrant a change to the 2014 recommendations and stressing out the importance of the research in BM in BC patients to find more effective treatments (Ramakrishna et al. 2018).

The landscape of medical treatment for patients with BMs from HER2-positive breast cancer is quite uncertain, traditionally attributed to difficulties in reaching optimal concentrations in the metastatic environment of BM (Fabi et al. 2018). Introduction of anti-HER2 targeted therapy has significantly improved overall survival (OS) among patients with HER2-positive BC. The widespread use of trastuzumab –a humanized anti-HER2 monoclonal antibody that targets the extracellular domain of the receptor– also has allowed to unmask a population in whom CNS progression is a significant source of morbidity and mortality (Lin and Winer 2007). CNS metastases are common among patients receiving trastuzumab-based therapy, including patients responding outside the CNS (Bendell et al. 2003). Interestingly, the CNS metastasis events have

been reported in several of the trastuzumab-based studies in adjuvant setting. A numeric increase in the number of CNS metastases as first event has been observed in the trastuzumab treatment arms from in two North American trials: North Central Cancer Treatment Group NCCTG N9831 trial (NCT00005970) (Combination Chemotherapy With or Without Trastuzumab in Treating Women With HER2-Overexpressing Breast Cancer) (Romond et al. 2005; Perez et al. 2011) and the National Surgical Adjuvant Breast and Bowel Project NSABP B-31 trial (NCT00004067) (Doxorubicin and Cyclophosphamide Plus Paclitaxel With or Without Trastuzumab in Treating Women With Node-Positive Breast Cancer That Overexpresses HER2) (Romond et al. 2012; Perez et al. 2014). This trend was also observed in the HERA trial (NCT00045032), a randomized trial comparing one or two years of trastuzumab given every three weeks in patients with HER2-positive early BC (Piccart-Gebhart et al. 2005).

Besides the biological perspective of more propensity to CNS metastasis, it also seems that trastuzumab has poor penetration in CNS. Although, there are some evidences of better penetration of trastuzumab in cerebrospinal fluid (CSF) under conditions of an impaired blood-brain barrier (BBB), such as in patients with meningeal carcinomatosis or following radiotherapy (Stemmler et al. 2007).

After developing of BMs, OS resulted widely affected. Historical series in patients with from breast cancer who were treated with WBRT report a median overall survival less than six months (Mahmoud-Ahmed et al. 2002). With better disease control outside CNS, mostly conferred by the use of anti-HER2 targeted therapy, OS improved in this group of patients, after diagnoses of BM. Some studies have reported median survival after CNS recurrence of up to 23 months, in patients treated with trastuzumab (Gori et al. 2007; Melisko et al. 2008). Unlike other tumors, like non-small cell lung tumors and acute lymphoblastic leukemia, prophylactic cranial irradiation remains experimental for BC patients.

Few prospective trials have been conducted addressing treatment of BMs in BC and patients with progressive BMs are often excluded from clinical trials, usually because of their poor prognosis, because most of systemic treatments fail to penetrate the BBB and also due to high risk of CNS hemorrhage or toxicity (Gounder and Spriggs 2011).

RegistHER (NCT00105456) was a multicenter, observational, prospective US cohort study of patients with HER2-positive metastatic breast cancer (MBC) (Yardley et al. 2014; Brufsky et al. 2011). In this study, 377 (37,3%) of the 1.012 patients enrolled developed CNS metastases. The role of trastuzumab was prospectively assessed and was associated with a statistically significant improvement in median OS following diagnosis of CNS disease (17,5 vs. 3,8 months) (Brufsky et al. 2011). Some retrospective studies also showed some OS benefit with trastuzumab after development of BMs (Church et al. 2008; Park et al. 2009; Bartsch et al. 2007; Dawood et al. 2008).

- **Meningeal Carcinomatosis in Breast Cancer Patients**

Meningeal carcinomatosis –also known as leptomeningeal carcinomatosis (LMC) or carcinomatous meningitis– is an aseptic inflammatory reaction caused by diffuse spread of malignant cells throughout the subarachnoid space. Definitive diagnosis is made with cytologic identification of malignant cells within the CSF. LMC likely comprises about 11-20% of CNS metastasis (Altundag et al. 2007; H.-J. Kim et al. 2012).

CNS metastasis in BC has been associated with reduced overall survival, with the shortest prognosis generally observed in cases of LMC. Prospective studies have found a median OS of 9-30.3 weeks (Scott and Kesari 2013).

There is currently no generally accepted standard of care for treatment of breast cancer LMC. Like in other BMs treatment, patient performance status, preferences and prognosis must be taken into account. Surgical treatment might be necessary for complications, like hydrocephalus. Radiation therapy (RT) and/or chemotherapy (intravenous [IV]) or intrathecal [IT]) can be used. Targeted agents, like trastuzumab, can also play a role in HER2-positive disease. RT can be administered as WBRT modality, which had shown some benefit when integrated in a multimodality approach in BC patients (J. H. Kim, Jenrow, and Brown 2018) or to palliate symptoms, for example, to relieve pain from nerve root compression. IV high dose methotrexate seems to improve OS over RT alone, but has important systemic toxicity (Boogerd et al. 2004). IT chemotherapy, using methotrexate, lipossomic cytarabine or thiotepa, has the advantage of drug being delivered directly in CNS. However, distribution is dependent on normal CSF circulation and side effects include ventriculitis/ arachnoiditis, leukoencephalopathy and bacterial meningitis associated with the presence of an intraventricular reservoir (Chamberlain 2012). Case reports and small series (less than 10 patients) also showed some benefit of treatment with capecitabine for BC patients who develop LM (Ekenel et al. 2007). There are also some case reports and reporting the use of trastuzumab IT (Oliveira et al. 2011; Figura et al. 2018; Lu et al. 2015; Zagouri et al. 2013).

The dose and schedule of IT trastuzumab was highly variable in literature. A phase I trial (NCT01373710) concluded that maximum tolerated dose and recommended phase II weekly dose of IT trastuzumab in patients with HER2-BC and LMC is 150 mg (Bonneau et al. 2018). Based on these encouraging results, phase II is ongoing.

- **Blood-Tumor Barrier Permeability and Drug Efficacy**

The BBB is often considered a challenge in the treatment of BM in patients with HER2-positive breast cancer, since it may affect drug delivery and penetration (Kabraji et al. 2018). However, abnormal angiogenesis in tumors leads to the formation of blood vessels that lack normal physiological structure, resulting in a compromised BBB, which is referred as the blood–tumor

barrier (BTB). In contrast to the normal BBB, the leaky BTB often allows for the extravasation of larger molecules, including antibodies (Kabraji et al. 2018; Askoxylakis et al. 2016).

Lapatinib (Tykerb®) –a reversible dual exc inhibitor which interrupts the HER2 and Epidermal Growth Factor Receptor (EGFR) pathways by binding to the ATP-binding pocket of their protein kinase domain– was studied in BC patients previously treated with trastuzumab who developed progressive BMs, and at least one measurable metastatic brain lesion. Altogether these studies confirmed the modest CNS antitumor activity of lapatinib, but also that responding patients had an improved time to progression (Lin et al. 2008, 2009).

The combination of lapatinib with cytotoxic agents such as capecitabine (Xeloda®) –an orally fluoropyrimidine carbamate– demonstrated to enhance treatment response in HER2-positive MBC patients with untreated BM. In the LANDSCAPE trial (NCT00967031) –a single-arm phase II, open-label, multicenter study– 45 HER2-positive MBC patients with newly diagnosed BM, not previously treated with WBRT, capecitabine, or lapatinib were enrolled. The CNS overall response rate was 65,9% and median time to whole-brain irradiation was 8,3 months (Bachelot et al. 2013).

The development of antibody-drug conjugates (ADCs) represents a promising strategy for patients with metastatic BC affecting SNC with a compromised BBB. Trastuzumab-emtansine (T-DM1) (Kadcyla®) is an ADC in which emtansine, a specific derivate of mertansine or DM1, is stably bound to trastuzumab. TDM-1 was studied in MBC patients, after trastuzumab- and taxane-based therapy, in the EMILIA trial (Verma et al. 2012). A retrospective analysis of this phase III trial suggested a possible activity of T-DM1 in HER2-positive MBC patients with non-progressing BM previously treated with local therapy with a favorable safety profile and prolonged extracranial disease control (Krop et al. 2015).

A sub-analysis of an Italian retrospective analysis of effectiveness and safety of T-DM1 also showed that T-DM1 is active in BC patients with BMs, with overall response rate of 35,1% and a disease control rate of 53,3% (Fabi et al. 2018).

- **Trastuzumab Deruxtecan (DS-8201a) (T-DXd)**

Trastuzumab deruxtecan (DS-8201a) is a monoclonal antibody targeting HER2 conjugated to a cytotoxic chemotherapeutic agent at a drug-to-antibody ratio of 7-8. MAAL-9001, a humanized IgG1 monoclonal antibody produced in-house with reference to the amino acid sequence of trastuzumab, is used as the antibody component, and MAAA-1181a, a derivative of exatecan, a topoisomerase I inhibitor, is used as the drug component. T-DXd is composed of MAAL-9001 and MAAA-1181a bound together by a maleimide tetrapeptide linker.

#### 1.1.1.1 Non-Clinical Studies

Pharmacology

The results of in vitro cell growth inhibition studies conducted using several cancer cell lines have shown that trastuzumab deruxtecan (DS-8201a) has a more potent growth inhibition effect against HER2-positive cells than MAAL-9001, suggesting that the conjugation of MAAA-1181a enhances the growth inhibition of trastuzumab deruxtecan (DS-8201a). Moreover, no growth inhibition was observed in HER2-negative cells, confirming the HER2 specificity of trastuzumab deruxtecan (DS-8201a). This exhibited antitumor activity in a tumor-bearing mouse model of human breast cancer cell line (KPL-4). In studies conducted in a tumor-bearing mouse model utilizing grafted tumor tissue obtained from breast cancer patients, trastuzumab deruxtecan (DS-8201a) exhibited antitumor activity both in an HER2 high-expressing model with an immunohistochemistry (IHC) score of 2+ and fluorescent in situ hybridization (FISH) positive, and also in an HER2 low-expressing model with an IHC score of 1+ and FISH-negative.

(T-DXd) has an HER2-mediated Akt phosphorylation inhibition effect and an antibody dependent cellular cytotoxic (ADCC) activity and has also been confirmed to cause deoxyribonucleic acid (DNA) damage and induce apoptosis, effects that are assumed to be the result of MAAA-1181a, which has topoisomerase I inhibitory activity. T-DXd (DS-8201a) is considered to exhibit HER2-specific cell growth inhibition and antitumor activity via a novel mechanism of action that combines the pharmacological activities of MAAL-9001, the antibody component, with those of MAAA-1181a, the drug component.

Safety pharmacology

In telemetered male cynomolgus monkeys treated with single intravenous doses of trastuzumab deruxtecan (DS-8201a), no effects on the cardiovascular system, the respiratory system, or the central nervous system were observed at dose levels up to 78.8 mg/kg. In addition, in human ether-a-go-go-related gene (hERG) studies of MAAA-1181a, MAAA-1181a did not inhibit the hERG channel current – responsible for the rapidly activating component of delayed rectifier potassium current - at concentrations of up to 10μmol/L (approximately 5000 ng/mL).

Pharmacokinetics and Drug Metabolism

The volume of distribution at steady state (Vss) was close to the plasma volume. The majority of administered trastuzumab deruxtecan (DS-8201a) circulates in plasma unchanged. The plasma concentrations of MAAA-1181a, the drug that is release from trastuzumab deruxtecan (DS-8201a), were quite low to the decline in the blood concentration, suggesting no accumulation or retention in specific tissues. The plasma protein binding ratios of MAAA-1181a (10ng/mL to

100ng/mL) were 90.3% to 92.5% in mice, 94.2% to 96.7% in rats, 86.5% to 89.1% in monkeys, and 96.8% to 98.0% in humans. Cytochrome P450(CYP) 3A4 was the primary CYP enzyme involved in the metabolism of MAAA-1181a.No human-specific metabolites were detected in hepatocytes in vitro. In rats, the major excretion pathway was the fecal/biliary route. MAAA-1181a was the most abundant component of radioactivity in urine, feces, and bile. In monkeys, radioactivity from administered 14C-trastuzumab deruxtecan (DS-8201a) was predominantly excreted into feces. MAAA-1181a was the most abundant catabolite in urine and feces. MAAA-1181a did not exhibit any potential to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A nor to induce CYP3A4, CYP1A2, or CYP2B6, at concentrations of up to 30 µmol/L.

In vitro, MAAA-1181a was a substrate for organic anion transporting polypeptide (OATP) 1B1, OATP1B3, multidrug and toxin extrusion (MATE) 2-K, P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated protein (MRP) 1.

Toxicology

The common adverse findings with both trastuzumab deruxtecan (DS-8201a) and MAAA-1181a studies were intestinal and lymphatic/hematopoietic system toxicities. For trastuzumab deruxtecan (DS-8201a) treatment, pulmonary, testicular, skin and renal toxicities were observed while heart, liver, and corneal toxicities were found only in the MAAA-1181a study. In intermittent intravenous dose toxicity studies with MAAL-9001in rats and cynomolgus monkeys (every 3 weeks [Q3W], 6 weeks), no MAAL-9001-related toxic changes were observed. In a human cross-reactivity study of trastuzumab deruxtecan (DS-8201a) with a panel of human tissues, trastuzumab deruxtecan (DS-8201a)-related cell membrane staining was found only in the placenta. In a cross-reactivity study of trastuzumab deruxtecan (DS-8201a) with selected cynomolgus monkey tissues (e.g., brain, liver, kidney, lung, heart, intestines, lymphoid organs, testis, and skin), no membranous staining was noted in any tissues. In an in vitro 3T3 NRU phototoxicity study, MAAA-1181a was found to be phototoxic to Balb/c 3T3 mouse fibroblasts. However, in an in vivo single dose phototoxicity study of MAAA-1181a in pigmented rats, no phototoxic reaction was noted at 3mg/kg, the highest dose tested. The genotoxicity studies of MAAA-1181a indicated that MAAA-1181a had no potential to induce gene mutation in bacteria but had the potential to induce structural chromosome aberrations in mammalian cultured cells.

#### 1.1.1.2 Clinical Studies

To date, there are 12 clinical studies investigating trastuzumab deruxtecan (DS-8201a), that are showed in Table 1:

**Table 1. Clinical Trials Investigating Trastuzumab Deruxtecan (DS-8201a)**

| Study Code | Study Phase | Title | Reference |
|---|---|---|---|
| DS8201-A-J101 (NCT02564900) | I | Phase 1, two-part, multicenter, non-randomized, open-label, multiple dose first-in-human study of DS8201a in subjects with advanced solid malignant tumors | (Iwata et al. 2018; S. Modi et al. 2019; Doi et al. 2017) (S. Modi et al. 2019)(S. Modi et al. 2019) |
| DS8201-A-J102 (NCT03366428) | I | Phase 1, multicenter, open-label, multiple-dose study of DS-8201a to assess the effect on the QT interval and pharmacokinetics in subjects with HER2-expressing metastatic and/or unresectable breast cancer | |
| DS8201-A-A103 (NCT03368196) | I | Phase 1, multicenter, open-label study of DS8201a to assess safety and pharmacokinetics in subjects with HER2-positive advanced and/or refractory gastric, gastroesophageal junction adenocarcinoma, or breast cancer | |
| DS8201-A-A104 (NCT03383692): | I | Phase 1, multicenter, open-label, single sequence crossover study to evaluate drug-drug interaction potential of OATP1B/CYP3A inhibitor on the pharmacokinetics of DS8201a in subjects with HER2-expressing advanced solid malignant tumors | |
| DS8201-A-U105 (NCT03523572) | Ib | Phase 1b, multicenter, open-label study of DS-8201a in combination with nivolumab, an anti-PD-1 antibody for subjects with HER2-expressing Advanced Breast and Urothelial Cancer | |
| DS8201-1-U201 (NCT03248492) | II | Phase 2, multicenter, open-label study of DS8201a for unresectable and/or metastatic breast cancer subjects previously treated with T-DM1 - DESTINY-Breast01 | (Baselga et al. 2018) |
| DS8201-A-J202 (NCT03329690) | II | Phase 2, multicenter, open-label study of DS8201a in subjects with HER2-expressing advanced gastric or gastroesophageal junction adenocarcinoma | (Shitara et al. 2019) |
| DS8201-A-J203 (NCT03384940) | II | Phase 2, multicenter, open-label study of DS-8201a in subjects with HER2-expressing advanced colorectal cancer | |
| DS8201-A-U204 (NCT03505710) | II | Phase 2, multicenter, open-label, 2-cohort study of trastuzumab deruxtecan (DS-8201a) for HER2-over-expressing or –mutated, unresectable and/or metastatic non-small cell lung cancer (NSCLC) | |
| DS8201 –A-U301 (NCT03523585) | III | Phase 3 multicenter randomized open-label active-controlled study of DS-8201a versus treatment of investigator's choice for HER2-positive, unresectable and/or metastatic breast cancer subjects pretreated with prior standard of care HER2 Therapies, including T-DM1 | (André et al. 2019) |
| DS8201-A-U302 (NCT03529110) | III | Phase 3 multicenter randomized open-label, active-controlled study of DS-8201a Versus T-DM1 for HER2-Positive, unresectable and/or Metastatic Breast Cancer Subjects Previously Treated With trastuzumab and taxane - DESTINY-Breast03 | |
| DS8201-A-U303 (NCT03734029) | III | Phase 3, Multicenter, Randomized, Open-label, Active Controlled Trial of DS-8201a, an Anti-HER2 | |

| Study Code | Study Phase | Title | Reference |
|---|---|---|---|
| | | ADC, Versus Treatment of Physician's Choice for HER2-low, Unresectable and/or Metastatic Breast Cancer Subjects | |

Efficacy

The majority of referred trials are still ongoing, most of them estimated to be completed between 2019 and 2022.

Preliminary results are available from part I of phase I trial (A-J101), where patients with advanced/unresectable or metastatic breast cancer or gastric or gastroesophageal junction adenocarcinoma that is refractory to or intolerable with standard treatment, or for which no standard treatment is available, received trastuzumab deruxtecan (DS-8201a). In 23 evaluable patients, including 6 with low HER2-expressing tumors, 10 patients achieved an objective response (43%, 95% CI 23,2-65,5) and disease control was achieved in 21 (91%, 95% CI 72,0 – 98,9). In this heavily pretreated study population, trastuzumab deruxtecan (DS-8201a) showed antitumor activity, even in low HER2-expressing tumors (Doi et al. 2017).

In part II of the same trial, cohorts included patients with BC, gastric cancer and other HER2 expressing tumors. In BC patients with a median of 5 prior regimens in metastatic setting (46 patients with HER2 positive, previous treated with TDM-1, and 10 with HER2 low expressing), 37 were evaluable for response at the time of data cutoff. Overall response rate (ORR) was 41%, including 1 complete response (CR). Disease control rate (DCR) was 97%. Median duration of treatment was not reached. For the evaluable patients who had previous received T-DM1, treatment with trastuzumab deruxtecan (DS-8201a) achieved an ORR of 41% (11/27) and DCR of 100% (27/27). In the subset of this patients who also had received previous pertuzumab and T-DM1, the confirmed ORR was 44% (11/25). In patients with low-expressing HER2 BC, the ORR was 40% (4/10), DCR was 90% (9/10) (Shanu Modi et al. 2017).

Clinical Safety

In the said phase I dose escalation trial, no dose-limiting toxic effects, substantial cardiovascular toxic effects or deaths occurred. Most common grade 3 adverse events (AEs) were decreased lymphocyte (n=3) and decreased neutrophil count (n=2); grade 4 anemia was reported by 1 patient. Three serious AEs- febrile neutropenia, intestinal perforation, and cholangitis- were reported by one patient each (Doi et al. 2017). Of the 46 BC patients in part 2, the most common AEs of any grades were nausea, decreased appetite, vomiting, alopecia, and diarrhea. Only 2 patients experienced grade 4 AEs (thrombocytopenia and neutropenia) and 46 % (21/46) experienced grade 3 AEs (most commonly anemia, neutropenia, thrombocytopenia, leukopenia, lymphocytopenia, and vomiting) (Shanu Modi et al. 2017).

## 1.2 STUDY RATIONALE

Currently, there are not specifically approved drugs for MBC patients with BM and/or LMC and consensus guidelines on systemic treatment after BM and/or LMC in MBC patients are lacking. Clinical trials commonly exclude MBC patients with BM and/or LMC from participation. Routine exclusion of such patients limits applicability of trial results and obfuscates potential benefit in this subpopulation. For this reason, it is essential to design specific clinical trials for MBC patients with BM and/or LMC.

Preliminary results from studies on trastuzumab deruxtecan (DS-8201a) –a HER2-targeted ADC with novel topoisomerase I inhibitor payload and linker technology– in BC are very promising, even in heavy pre-treated patients with MBC.

The aim of this two-step, multicenter, international, open-label, single-arm, multicohort phase II trial is to evaluate the efficacy of trastuzumab deruxtecan (DS-8201a) in patients with HER2-positive or HER2-low-expressing MBC with BM, and patients with HER2-positive MBC with LMC.

To our knowledge, it will be the first prospective study to evaluate the role of an ADC in patients with HER2-positive and HER2-low expressing MBC patients with BMs, and patients with HER2-positive MBC with LMC. Because other anti-HER2 drugs will be explored in this setting as well, some data with trastuzumab deruxtecan (DS-8201a) are needed in HER2-positive MBC patients with BM and/or LMC. If this trial is positive, trastuzumab deruxtecan (DS-8201a) will be used in MBC regardless the presence of CNS involvement. It might also represent the first therapeutic option specifically evaluated in HER2-low-expressing MBC patients with BMs.

# 2 STUDY OBJECTIVES AND ENDPOINTS

## 2.1 COHORT 1

- **Study Objectives**

**Primary Objective**

- To assess efficacy –defined as 16 weeks PFS per Response Assessment in Neuro-Oncology Brain Metastases (RANO-BM) and Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 of trastuzumab deruxtecan (DS-8201a) in patients with pretreated unresectable locally advanced or metastatic HER2-positive BC with non-progressing BM (after WBRT and/or SRS and or surgery).

**Secondary Objectives**

Efficacy

- To assess efficacy –defined as PFS, ORR, CBR, time to response (TTR), duration of response (DOR), and best percentage of change in tumor burden per RANO-BM (for IC lesions) and RECIST 1.1 (extracranial lesions and overall lesions)–after treatment with trastuzumab deruxtecan (DS-8201a) in this population.
- To asses OS in this population,

Safety

- To assess the safety and tolerability of trastuzumab deruxtecan (DS-8201a) in this population by NCI-CTCAE v.5.0.

**Exploratory Objectives**

- To assess [redacted] using [redacted] and its [redacted] in this population.
- To evaluate predictive or prognostic biomarkers (plasma and/or tissue and/or CSF) associated with disease activity status or response to treatment in this population.
- To identify possible mechanisms of resistance to study treatments through the comparative analysis of potential biomarkers from paired pre-treatment and post-progression tumor and/or blood and/or CSF samples from this population.

- **Study Endpoints**

**Primary Endpoint**

16 weeks-PFS, defined as the period of time from treatment initiation to the first occurrence of disease progression or death from any cause, whichever occurs first. Progression will be determined locally by the investigator through the use of Response Assessment in Neuro-Oncology Brain Metastases (RANO-BM) criteria (IC lesions) and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.

**Secondary Endpoints**

Efficacy

- PFS defined as the period of time from treatment initiation to the first occurrence of disease progression or death from any cause, whichever occurs first. Progression will be determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- ORR, defined as a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- CBR, defined as an objective response (CR or PR), or stable disease (SD) for at least 24 weeks, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- TTR, defined as the time from the treatment initiation to time of the first objective tumor response observed for patients who achieved a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- DOR, defined as the time from the first occurrence of a documented objective response to disease progression or death from any cause, whichever occurs first, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- OS, defined as the time from treatment initiation to death from any cause. Patients without documented death at the time of the final analysis will be censored at the date of the last follow-up.
- Best percentage of change from baseline in the size of tumors, defined as the biggest decrease, or smallest increase if no decrease will be observed and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.

Safety

- Adverse events (AEs) will be evaluated using the National Cancer Institute (NCI)–Common Terminology Criteria for Adverse Events (CTCAE) v.5.0.

Exploratory Endpoints

- Overall change from baseline i[redacted], [redacted] and [redacted]; Time to [redacted] in [redacted]; Time to [redacted] in [redacted]; Time to [redacted]
- Relationship between tissue- and/or blood- and/or CSF-based biomarkers and patient clinical characteristics (e.g., baseline features) and outcome (e.g., duration of [redacted]).

## 2.2 COHORT 2, 3, and 4

- **Study Objectives**

**Primary Objective**

- To assess efficacy –defined as intracranial overall response rate (ORR–IC) per RANO-BM– of trastuzumab deruxtecan (DS-8201a) in patients with pretreated unresectable locally advanced or metastatic BC:
  a) HER2-positive or HER2-low expressing with untreated BMs (cohort 2);
  b) HER2-positive with progressing BMs after local treatment (cohort 3);
  c) HER2-low expressing with progressing BMs after local treatment (cohort 4).

**Secondary Objectives**

Efficacy

- To assess efficacy –defined as PFS, CBR, TTR, DOR, and best percentage of change in tumor burden per RANO-BM (for IC lesions) and RECIST 1.1 (for extracranial lesions and overall lesions); and ORR per RECIST 1.1 (for extracranial lesions and overall lesions).
- Time to WBR and/or SRS (only for cohort 2), defined as the time from the treatment initiation to time of CNS disease progression that requires treatment with WBR and/or SRS, and determined locally by the investigator through the use of RANO-BM and RECIST 1.1 criteria.
- To assess OS in this population.

Safety

- To assess the safety and tolerability of trastuzumab deruxtecan (DS-8201a) by NCI-CTCAE v.5.0.

**Exploratory Objectives**

- To assess [redacted] using the [redacted] and [redacted], [redacted] in study cohorts 2, 3, and 4.
- To evaluate predictive or prognostic biomarkers (plasma and/or tissue and/or CSF) associated with disease activity status or response to treatment in study cohorts 2, 3, and 4.
- To identify possible mechanisms of resistance to study treatments through the comparative analysis of potential biomarkers from paired pre-treatment and post-progression tumor and/or blood and/or CSF samples from patients of study cohorts 2, 3, and 4.

- **Study Endpoints**

**Primary Endpoint**

- ORR-IC, defined as a complete response (CR) or partial response (PR), and determined locally by the investigator through the use of RANO-BM criteria.

**Secondary Endpoints**

Efficacy

- PFS, defined as the period of time from treatment initiation to the first occurrence of disease progression or death from any cause, whichever occurs first. Progression will be determined locally per RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- ORR, defined as a CR or PR, determined locally by the investigator through the use of RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- CBR, defined as an objective response (CR or PR), or SD for at least 24 weeks, and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- TTR, defined as the time from the treatment initiation to time of the first objective tumor response (tumor shrinkage of ≥ 30%) observed for patients who achieved a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- DOR, defined as the time from the first occurrence of a documented objective response to disease progression or death from any cause, whichever occurs first, determined locally by

the investigator though the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1. for both extracranial lesions and overall (IC and extracranial) lesions.

- OS, defined as the time from treatment initiation to death from any cause. Patients without documented death at the time of the final analysis will be censored at the date of the last follow-up.
- Best percentage of change from baseline in the size of tumor lesions, defined as the biggest decrease, or smallest increase if no decrease will be observed, and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- Time to WBRT and/or SRS (only for cohort 2), defined as the time from the treatment initiation to time of CNS disease progression that requires treatment with WBR and/or SRS, and determined locally by the investigator through the use of RANO-BM and RECIST 1.1 criteria.

Safety

- AEs will be evaluated using the NCI-CTCAE v.5.0.

**Exploratory Endpoints**

- Overall change from baseline in [redacted], [redacted] and [redacted]; Time to [redacted] in [redacted]; Time to [redacted] in [redacted]; Time to [redacted].
- Relationship between tissue- and/or blood- and/or CSF-based biomarkers and patient clinical characteristics (e.g., baseline features) and outcome (e.g., duration of [redacted]).

## 2.3 COHORT 5

- **Study Objectives**

**Primary Objective**

- To assess efficacy –defined as OS– of trastuzumab deruxtecan (DS-8201a) in patients with pretreated unresectable locally advanced or metastatic HER2-positive or HER2-low expressing BC with LMC.

**Secondary Objectives**

Efficacy

- To assess efficacy –defined as PFS, ORR, CBR, TTR, DOR, and best percentage of change in tumor burden per RANO-BM (IC lesions) and RECIST 1.1 (extracranial lesions and overall lesions)

  Safety

- To assess the safety and tolerability of trastuzumab deruxtecan (DS-8201a) by NCI-CTCAE v.5.0.

**Exploratory Objectives**

- To assess [redacted] using the [redacted] and its [redacted], [redacted] in this population.
- To evaluate predictive or prognostic biomarkers (plasma and/or tissue and/or CSF) associated with disease activity status or response to treatment in this population.
- To identify possible mechanisms of resistance to study treatments through the comparative analysis of potential biomarkers from paired pre-treatment and post-progression tumor and/or blood and/or CSF samples from this population.

- **Study Endpoints**

**Primary Endpoint**

- OS defined as the time from treatment initiation to death from any cause. Patients without documented death at the time of the final analysis will be censored at the date of the last follow-up.

**Secondary Endpoints**

Efficacy

- ORR, defined as a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- PFS, defined as the period of time from treatment initiation to the first occurrence of disease progression or death from any cause, whichever occurs first during at least first 12 months. Progression will be determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.

- CBR, defined as an objective response (CR or PR), or SD for at least 24 weeks, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- TTR, defined as the time from the treatment initiation to time of the first objective tumor response observed for patients who achieved a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- DOR, defined as the time from the first occurrence of a documented objective response to disease progression or death from any cause, whichever occurs first, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- Best percentage of change from baseline in the size of tumor lesions, defined as the biggest decrease, or smallest increase if no decrease will be observed, and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.

Safety

- AEs will be evaluated using the NCI-CTCAE v.5.0.

**Exploratory Endpoints**

- Overall change from baseline in [redacted], [redacted] and [redacted]; Time to [redacted] in [redacted]; Time to [redacted] in [redacted]; Time to [redacted].
- Relationship between tissue- and/or blood- and/or CSF-based biomarkers and patient clinical characteristics (e.g., baseline features) and outcome (e.g., duration of [redacted]).

# 3 STUDY OVERVIEW

## 3.1 Study Design

This is a multicenter, open-label, single-arm, multicohort, two-stage optimal Simon's design, phase II clinical trial that is designed to evaluate the safety, tolerability, and efficacy of trastuzumab deruxtecan (DS-8201a) for pretreated patients with unresectable locally advanced or metastatic HER2-positive or HER2-low expressing BC with untreated or treated BMs or LMC.

After signing informed consent form (ICF) and confirmed eligibility, patients will be assigned to one of the following five study cohorts:

- **Cohort 1:** HER2-positive BC with non-progressing BM (after WBRT and/or SRS);(N = 8);
- **Cohort 2:** HER2-positive or HER2-low BC with asymptomatic, untreated BMs (N = 10);
- **Cohort 3:** HER2-positive BC with progressing BMs after local treatment (N = 7);
- **Cohort 4**: HER2-low expressing BC with progressing BMs after local treatment (N = 7);
- **Cohort 5:** HER2-positive or HER2-low expressing BC with LMC (N = 7).

Enrolment will start for all cohorts at the same time.

A futility interim analysis has been planned in cohort 1. The recruitment will not be stopped during the interim analysis. However, after the interim analysis, if the number of patients achieving the primary endpoint is equal or less than futility boundary in each cohort, the accrual in the cohort must be stopped. The stopping rule is:

Cohort 1: All 4 patients with progressive disease or death at 16 weeks.

Additionally a feasibility interim analyses has been planned in each cohort. At the halfway point of the recruitment period, the Steering Committee will evaluate if the accrual objective for the first stage was achieved in each study cohorts (4, 5, 3, 3 and 3 patients for cohorts 1, 2, 3, 4 and 5, respectively). If this accrual objective has not achieved in a cohort, the Steering Committee will propose a corrective action plan or finalize the study in this cohort

For estimation of PFS, TTR, ORR, DOR, CBR, OS, 12-week CNS disease stabilization, and best percentage of change in target tumor lesions, tumor assessment will be based on RANO-BM criteria and on RECIST v.1.1 criteria for estimation in non-target tumor lesions (Brain MRI and chest/pelvis and abdomen CT) (see ***Appendices***

***Appendix*** 1) and will be performed every six weeks (± 3 days) for the first six months and, thereafter, every nine weeks (± 5 days) until disease progression. If patient interrupts treatment without confirmation on disease progression, tumor assessment will continue until event is confirmed unless patient starts new anti-cancer treatment, withdrawal of consent, death, or the end of the study, whichever occurs first. Tumor assessments will be performed on the specified schedule regardless of treatment delays.

In addition, for patient allocated in the study cohort 5, CSF analysis will be performed at base line, every three weeks for 12 weeks (corresponding to the first 5 cycles of treatment) and every six weeks thereafter and at the time of disease progression, treatment discontinuation, the start of new anti-cancer treatment, withdrawal of consent, death, or the end of the study, whichever occurs first.

Bone scans will be only performed every 24 weeks (± 7 days) for patients with bone lesions identified at baseline, unless clinically or biochemically suspected bone progression.

Safety assessments will include the incidence, nature, and severity of AEs and laboratory abnormalities graded per the NCI–CTCAE v.5.0. Laboratory safety assessments will include the regular monitoring of hematology, blood chemistry, coagulation, and pregnancy test (see Appendices

Appendix 1).

To perform exploratory studies, patients must have consented to provide sufficient newly obtained tumor biopsy tissue with the exception of patients for whom tumor biopsies cannot be obtained (e.g., inaccessible tumor or subject safety concern) that may submit an archived metastatic tumor specimen only upon agreement from the Sponsor. Patients will also be given the option of providing a tissue biopsy sample obtained at disease progression for exploratory analyses; this decision will not affect overall study eligibility. Furthermore, patients have agreed to give blood samples (liquid biopsy) at the time of inclusion, after two cycles of study treatment, and upon progression or study termination. Moreover, patient allocated in the study cohort 5, must have consented to provide CSF samples for their analysis at baseline, at base line, every three weeks for 12 weeks (corresponding to the first 5 cycles of treatment) and every six weeks thereafter and at the time of disease progression or study termination.

### 3.2 Study Treatment Management

After having confirmed eligibility and entered into the clinical trial, patients will be treated with trastuzumab deruxtecan (DS-8201a) at 5.4 mg/Kg administered as an IV infusion on Day of 21-day cycle (Q3W), initially for at least 90 minutes, then, if there is no infusion-related reaction, for a minimum of 30 minutes.

In patients with hormone receptor (HR)-positive status (estrogen receptor [ER] and/or progesterone receptor [PgR]) administration of endocrine therapy is not allowed.

In patients allocated in study cohort 5, administration of intrathecal therapy is not allowed.

### 3.3 Duration of Study Treatment

The study treatment period is defined as the time between the study entry and the last dose of trastuzumab deruxtecan (DS-8201a) received within the trial.

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

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

- Radiologically confirmed and documented unequivocal disease progression in the brain as assessed by magnetic resonance imaging (MRI).
- AEs that according to the protocol or in the judgment of the investigator may cause severe or permanent harm or which rule out continuation of study drug.

  *Note: See detailed criteria for study treatment discontinuation due to toxicity in* ***Section*** **7.4**.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- Serious non-compliance with the study protocol.
- Death.
- Lost to follow-up.
- Patient withdraws consent.
- The study site or the Sponsor decides to close the study.

All patients who have not progressed and are still in receipt of study treatment at the End of Study (EoS), as defined in ***Section 3.4***, will continue to receive the drug until trastuzumab deruxtecan (DS-8201a) is available in a reimbursement setting and the Sponsor will provide the study drug satisfying the Ethics Committee (EC)'s requirements. In this case, the patient would be followed appropriately as per standard clinical practice.

### 3.4 EoT and EoS Visits

Patients discontinuing the study treatment period will enter to the End of Treatment (EoT) visit. The first safety assessment (EoT visit) will be scheduled for all patients within 40 days (± 7 days) after the last dose of study treatment. All AEs related to investigational medicinal product (IMP) will be followed up by the investigator until the event or its sequelae resolve or stabilize at the level acceptable to the investigator, and the Sponsor concurs with that assessment.

Afterwards, follow-up contacts will continue every 12 weeks (± 14 days) up to the EoS and survival status and new anticancer therapy information will be collected. Telephone contact is acceptable.

The EoS is defined as the last patient last visit. This will be the last data collection point, which can be a clinic visit or a laboratory sample. EoS will occur at 12 months after the last patient included in the study, unless premature termination of the study.

# 4 PATIENT SELECTION

This study can fulfil its objectives only if appropriate patients are enrolled. The following eligibility criteria are designed to select patients for whom the protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular patient.

Patient eligibility should be reviewed and documented by an appropriate member of the investigator's study team before patients are included in the study.

## 4.1 Target Study Population

Male and pre- and post-menopausal female patients age ≥ 18 years with pretreated unresectable locally advanced or metastatic HER2-positive or HER2-low expressing BC with stable, treated or untreated BMs, or patients progressing after treatment for BMs or patients with LMC. Evidence of measurable brain disease is required. Patients are not eligible if they are candidates for a local treatment with a radical intention.

Patients with untreated BMs are those that have not received any local treatment for their CNS involvement (surgery and/or SRS and/or WBRT are considered appropriate).

The operational definition of patients with stable BM are patients that have been for at least four weeks asymptomatic with or without corticosteroids. The highest dose of corticosteroids acceptable is dexamethasone 8 mg twice daily or any equivalent.

## 4.2 Inclusion Criteria

Patients must meet **ALL** of the following inclusion criteria to be eligible for enrolment into the study:

Patients must meet **ALL** of the following inclusion criteria to be eligible for enrolment into the study:

1. Signed Informed Consent Form (ICF) prior to participation in any study-related activities.
2. Male or female patients ≥ 18 years at the time of signing ICF.
3. Eastern Cooperative Oncology Group (ECOG) performance status of 0-1 for **Cohorts 1 to 4** and 0-2 for **cohort 5**.
4. Life expectancy ≥ 12 weeks.
5. Histologically confirmed invasive breast cancer based on local testing on the most recent analyzed biopsy of the following breast cancer (BC) subtypes per 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) criteria:
    - **Cohort 1 and 3:** HER2 positive status

- **Cohort 4:** HER2-low expressing status
- **Cohort 2 and 5:** both HER2 positive and HER2-low expressing status

*Note 1: According to the 2018 ASCO-CAP guidelines, HER2-positive status is defined as HER2 immunohistochemistry (IHC) 3+, in situ hybridization (ISH) ≥ 2.0, or average HER2 copy number ≥ 6.0 signals. HER2-low expressing status defined as IHC 2+ / ISH-negative or IHC 1+ (ISH-negative or untested).*

*Note 2: Central confirmation of HER2 is not required for study entry. However, tissue blocks, or slides, must be submitted to confirm BC subtype by a Sponsor-designated central laboratory retrospectively.*

6. Unresectable locally advanced or metastatic disease documented by computerized tomography (CT) scan or magnetic resonance imaging (MRI) that is not amenable to resection with curative intent.
7. At least one brain lesion needed to be measurable (≥10 mm on T1-weighted, gadolinium-enhanced MRI) (**study cohorts 2 to 4**) or leptomeningeal carcinomatosis (LMC) with positive cerebrospinal fluid (CSF) cytology (**study cohort 5**).
    a) ***Study cohort 1:*** History of BM that are non-progressing after WBRT and/or SRS and/or surgery.
    b) ***Study cohort 2:*** Presence of asymptomatic BM without clinical requirement for local intervention (WBRT and/or SRS and/or surgery).
    c) ***Study cohorts 3 and 4:*** Evidence of new and/or progressive BM following previous WBRT and/or SRS and/or surgery.
    d) ***Study cohort 5:*** Evidence of LMC with positive CSF cytology.
8. Previous treatments:

a) HER2-positive patients must have been previously treated with a taxane and at least one HER2-targeted therapy in the advanced scenario.
b) For HER2-low-expressing patients that also are endocrine receptor negative must have been previously treated with at least one chemotherapy regimen. If endocrine receptor positive, patients must have been previously treated with at least one chemotherapy and one endocrine regimen in the metastatic setting.

9. Patients must agree to collection of blood samples at the time of inclusion, at cycle 2 of treatment, and upon progression or study termination.

*Note: In **study cohort 5**: Patients must agree to perform spinal taps or must be willing to have an Ommaya reservoir placed for CSF assessment, at base line, every three weeks for 12 weeks (corresponding to the first 5 cycles of treatment) and every six weeks thereafter.*

10. Willingness and ability to provide tumor biopsy (if feasible) from metastatic lesions or breast primary tumor both at the time of the inclusion and after disease progression in order to perform exploratory studies.

*Note: If feasible, patients should provide a tissue sample at baseline from metastases amenable to biopsy (at sites of locoregional recurrence [skin, chest wall, breast or lymph nodes], or distant recurrence [bone, liver, lung or abdomen]) or as alternative from breast primary tumor, that will be obtained between progression to the prior regimen and inclusion in the study. Patients for whom tissue sample cannot be obtained (e.g., non-measurable disease, inaccessible tumor or subject safety concern) may submit an archived metastatic tumor specimen only upon agreement from the Sponsor. If feasible, an additional tissue sample should be collected at the end of treatment visit for patients who discontinue treatment due to disease progression.*

11. Patients should have left ventricular ejection fraction (LVEF) ≥ 50% by either an echocardiogram (ECHO) or multigated acquisition (MUGA) scan within 28 days before enrolment.
12. Adequate hematologic and organ function within 14 days before the first study treatment on Day 1 of Cycle 1, defined by the following:
    - Hematological: White blood cell (WBC) count ≥ 3.0 x $10^9$/L, absolute neutrophil count (ANC) ≥ 1.5 x $10^9$/L, platelet count ≥ 100.0 x$10^9$/L, and hemoglobin ≥ 9.0 g/dL. (Platelet or red blood cell transfusion as well as G-CSF administration are not allowed within 1 week of screening assessments).
    - Hepatic: Bilirubin ≤ 1.5 times the upper limit of normal (× ULN) (< 3 x ULN in the case of documented Gilbert's disease and/or liver metastases); aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 3 × ULN (in the case of liver metastases ≤ 5 × ULN); alkaline phosphatase (ALP) ≤ 2 × ULN (≤ 5 × ULN in the case of liver and/or bone metastases ≤ 5 × ULN), at baseline, serum albumin ≥ 2.5 g/dL.
    - Renal: Serum creatinine ≤ 1.5 × ULN or creatinine clearance ≥ 30 mL/min based on Cockcroft−Gault equation *CLcr (mL/min)* =(*Cockcroft-Gault equation: ([{140 – age in years} × {ACTUAL WEIGHT in kg}] divided by [{72 × serum creatinine in mg/dL} multiplied by 0.85 if female])).
    - Coagulation: International Normalized Ratio (INR)/Prothrombin Time (PT) and either partial thromboplastin or activated Partial Thromboplastin Time (aPTT) ≤ 1.5 × ULN (except for patients receiving anticoagulation therapy).

      *Note: Patients receiving heparin treatment should have an aPTT between 1.5 and 2.5 × ULN (or patient value before starting heparin treatment). Patients receiving coumarin derivatives should have an INR between 2.0 and 3.0 assessed in two consecutive measurements one to four days apart. Patients should be on a stable anticoagulant regimen.*
13. Has adequate treatment washout period before enrollment, as indicated:
    - Major surgery: > 4 weeks;

- Radiation therapy: > 4 weeks (if palliative stereotactic radiation to other areas ≥ 2 weeks);
- Anticancer systemic treatment (including immunotherapy, retinoid therapy, hormonal therapy): ≥ 3 weeks (≥ 2 weeks or 5 half-lives, whichever is longer, for small-molecule targeted agents such as 5-fluorouracil-based agents, folinate agents, weekly paclitaxel; ≥ 6 weeks for nitrosureas or mitomycin C)
- Antibody based anti-cancer therapy: ≥ 4 weeks;
- Chloroquine/Hydroxychloroquine>14 days

14. Resolution of all acute toxic effects of prior anti-cancer therapy to grade ≤ 1 as determined by the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI–CTCAE) v.5.0 (except for alopecia or other toxicities). Subjects with chronic Grade 2 toxicities may be eligible per the discretion of the Investigator after consultation with the Sponsor Medical Monitor or designee (e.g., Grade 2 chemotherapy-induced neuropathy).
15. Male and female subjects of reproductive/childbearing potential must agree to use a highly effective form of contraception or avoid intercourse during and upon completion of the study and for at least 7 months for females and 4 months for males after the last dose of study drug. Methods considered as highly effective methods of contraception include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  i. Oral
  ii. Intravaginal
  iii. Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  i. Oral
  ii. Injectable
  iii. Implantable
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomized partner
  - Complete sexual abstinence defined as refraining from heterosexual intercourse during and upon completion of the study and for at least 7 months after the last dose of study drug. Periodic abstinence (calendar, symptothermal, post-ovulation methods) is not an acceptable method of contraception.

*Note: Non-child-bearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea (in questionable cases, a blood sample with simultaneous follicle-stimulating hormone [FSH] > 40 mIU/mL and estradiol < 40 pg/mL [< 147 pmol/L] is confirmatory). Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods outlined for women of child-bearing potential if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrollment. For most forms of HRT, at least 2-4 weeks will elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT. Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method*

16. Male subjects must agree to not freeze or donate sperm starting at Screening and throughout the study period, and at least 4 months after the final study drug administration. Preservation of sperm should be considered prior to enrolment in this study.
17. Female subjects must agree to not donate, or retrieve for their own use, ova from the time of Screening and throughout the study treatment period, and for at least 7 months after the final study drug administration.
18. Patients who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, post-treatment follow-up and other study procedures.

### 4.3 Exclusion Criteria

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

1. Inability to comply with study and follow-up procedures.
2. Previous treatment with trastuzumab deruxtecan (DS-8201a) or any other antibody drug conjugate (ADC) which consists of an exatecan derivative that is a topoisomerase 1 inhibitor.
3. Medical history of myocardial infarction (MI) within 6 months before enrollment, symptomatic congestive heart failure (New York Heart Association Class II to IV). Subjects with troponin levels above ULN at screening (as defined by the manufacturer) and without any MI related symptoms should have a cardiologic consultation before enrollment to rule out MI
4. Corrected QT interval (QTc) prolongation to > 470 ms (females) or >450 ms (males) based on average of the screening triplicate12-lead ECG.

5. History of (non-infectious) interstitial lung disease (ILD) / pneumonitis that required steroids, has current ILD / pneumonitis, or where suspected ILD / pneumonitis cannot be ruled out by imaging at screening.
6. Clinically significant corneal disease in the opinion of the Investigator.
7. Spinal cord compression.
8. Multiple primary malignancies within 3 years, except adequately resected non-melanoma skin cancer, curatively treated *in-situ* disease, other solid tumors curatively treated, or contralateral breast cancer.
9. History of severe hypersensitivity reactions to either the drug substances or inactive ingredients in the drug product.
10. History of severe hypersensitivity reactions to other monoclonal antibodies.
11. Uncontrolled infection requiring IV antibiotics, antivirals, or antifungals.
12. Patients with substance abuse or any other medical conditions such as clinically significant cardiac or psychological conditions, that may, in the opinion of the investigator, interfere with the subject's participation in the clinical study or evaluation of the clinical study results.
13. Known human immunodeficiency virus (HIV) infection, or active hepatitis B or C infection. Patients positive for hepatitis C (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV RNA.
14. Female patients who are pregnant or breastfeeding or planning to become pregnant.
15. Treatment with other systemic therapy for metastatic disease including chemotherapy, immunotherapy, targeted therapy (small molecules/ monoclonal antibodies), or endocrine therapy.
16. Major surgery (defined as requiring general anesthesia) or significant traumatic injury within 4 weeks of start of study drug, or patients who have not recovered from the side effects of any major surgery, or patients who may require major surgery during the study.
17. Radiotherapy within 4 weeks or limited-field palliative radiotherapy within 2 weeks prior to study enrolment, or patients who have not recovered from radiotherapy-related toxicities to baseline or grade ≤ 1 and/or from whom ≥ 25% of the bone marrow has been previously irradiated.
18. Use of concurrent investigational agents or other concomitant anticancer therapies.
19. Use of intrathecal therapy for LMC.

20. Active bleeding diathesis, previous history of bleeding diathesis, or chronic anti-coagulation treatment (the use of low molecular weight heparin is allowed as soon as it is used as prophylaxis intention).
21. Serious concomitant systemic disorder (e.g., active infection including HIV, active hepatitis, liver cirrhosis, end stage chronic renal disease) incompatible with the study (at the discretion of investigator).

22. Any of the following within 6 months of enrollment: severe/unstable angina, ongoing cardiac dysrhythmias of NCI–CTCAE v.5.0 grade ≥2, coronary/peripheral artery bypass graft, cerebrovascular accident including transient ischemic attack, or symptomatic pulmonary embolism.
23. Uncontrolled electrolyte disorders of NCI–CTCAE v.5.0 grade ≥ 2.
24. 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 randomization
25. 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 eCRF for patients who are included in the study.
26. Prior pneumonectomy.

# 5 STUDY ASSESSMENTS AND PROCEDURES

The schedule of activities to be performed during the study is provided in the *¡Error! No se encuentra el origen de la referencia.*. All activities must be performed and documented for each patient.

Patients will be closely monitored for safety and tolerability throughout the study. Patients should be assessed for toxicity prior to each dose; dosing will occur only if the clinical assessment and local laboratory test values are acceptable.

## 5.1 Patient Entry Procedure

Written ICF from the patient must be (or the patient's legally authorized representative) signed and dated before his or her participation in the study and before performing any study specific procedure.

Before giving their consent, patients will be informed about the nature of the study drug and will receive pertinent information regarding study objectives, study treatment, follow-up procedures, biological samples collection and its legal implications, possible benefits, and potential risk and AEs. Patients will be also informed that they have the right to withdraw from the study at any time and for any reason, without being required to state their reasons for doing so. This decision will not affect any future medical treatment.

After receiving the document, the patient will read it (or receive information verbally in front witnesses) and will sign the previously approved ICF. The patient will receive a signed copy of the ICF and ICFs for enrolled patients and for patients who are not subsequently enrolled will be maintained at the study site.

All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before enrollment. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable. After a failed screening attempt, one re-screening will be allowed to patient who did not meet one or more criteria required for participation in this study. A potential re-screened patient should sign a new ICF before any screening test or study related procedure is performed.

At inclusion, each patient will be given a unique patient number (UPN) for this study, provided by the Sponsor. All data will be recorded in the appropriate case report form (CRF) using this identification number.

Confirmation of patient's eligibility for study participation will be recorded in the CRF. The investigator is responsible for safeguarding patient information (e.g., age, name, address, telephone number, and study identification number), ensuring access to this information by Health

Authorities if necessary. These records will remain confidential for the period of time established by current legislation.

## 5.2 Study Assessments

- **Schedule of Assessments**

Study assessments are outlined in ***Appendices***

***Appendix*** 1.

- **Visit Schedule**

All screening tests and evaluations must be completed within the protocol scheduled time windows and reviewed to confirm that patients meet all eligibility criteria within 28 days prior to the first administration of study medication, unless these procedures have already been conducted during this time period as part of the patient´s routine clinical care. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

Visits are organized in programmed cycles of 21 days (if there are no treatment delays due to the occurrence of AEs). All visits must occur within ± 3 working days from the scheduled date, unless otherwise noted in the schedule of assessments.

Assessments scheduled for Day 1 (before treatment) of all cycles must be performed within 48 hours prior to study treatment administration, unless otherwise indicated in the schedule of assessments, to confirm to the patient if treatment can be followed up.

If a mandatory procedure described in the protocol falls on a bank holiday and/or weekend, this procedure should be performed on the day before or after the holiday (i.e. within a period of ± 2 working days).

EoT visit will be performed within 40 days (± 7 days) after the last dose of study treatment. Afterwards, follow-up contacts will continue every 12 weeks (± 14 days) up to the EoS and survival status and new anticancer therapy information will be collected. Telephone contact is acceptable.

EoS will occur at 12 months after the last patient included in the study, unless premature termination of the study.

- **Demographic Data and Medical History**

Demographic data, general medical history, and prior medical history of breast cancer will be collected during the screening period.

Demographic data includes age, sex, and self-reported race/ethnicity.

General medical history includes clinically significant diseases, surgical interventions, history of smoking, alcoholism, drug addiction, as well as any medications (e.g., prescribed drugs, over-the-counter drugs, medicinal plants, homeopathic remedies, or food supplements) used by the patient for the 4 weeks prior to screening visit.

Previous medical history of breast cancer (including prior antineoplastic treatments and procedures including radiotherapy and surgeries) will be also assessed with further detail. In particular, it will be evaluated the number of chemotherapy regimens for unresectable locally advanced or MBC, including start and stop date and best response, and the prior use of chemotherapeutic and target therapy agents either in the early, locally advanced, or metastatic setting.

Moreover, patients will complete a medication diary each day to assess the actual intake of medication taken outside of the clinic/hospital setting. Patients will receive the diary on the first day of each cycle, with site staff completing information on any prescribed medication, including the recommended dosage and route of administration. Patients should use the diary to record any medication (prescribed or over-the-counter) taken on that cycle of treatment.

- **Vital Signs**

Vital signs will be measured during the screening period (within 14 days before enrollment), and as defined in the Appendix 1. Schedule of Assessments and Study Procedures. On Day 1 of the three initial cycles of treatment vital signs must be assessed prior and after trastuzumab deruxtecan (DS-8201a) infusion, and pre-infusion on Day 1 of subsequent cycles. These will include the measurement of height (only during screening) and weight, respiratory rate, pulse rate, systolic and diastolic blood pressure while the patient is in a seated position after resting for at least five minutes, and oral, axillary, or tympanic temperature. Abnormal or significant changes in vital signs from baseline should be recorded as AEs, if ap propriate. Pulse oximetry will be performed at screening -14 days and on Day 1of every cycle, and until the safety follow-up visit 40 days after last dose. During Cycle 1, pulse oximetry will be assessed on Day 1 prior and after infusion of trastuzumab deruxtecan, on Day 8 and Day 15. On Day 1 pre-dose measurements will be taken thereafter.

- **Physical Examination**

A complete physical examination will be performed during the screening period (within 14days before enrolment) and on Day 1 of each cycle. This examination should include an evaluation of head, eyes, ears, nose, and throat as well as cardiovascular, dermatological, musculoskeletal, respiratory, digestive, genitourinary, and neurological systems.

Moreover, an ophthalmologic assessment that includes visual acuity testing, slit lamp examination and fundoscopy will be performed at screening (within 28 days before enrollment), and EoT and as clinically indicated.

In addition, this physical exam should also include, as part of tumor assessment, evaluation of the breast and regional lymph nodes as well as the presence and degree of increase of other lymph nodes, hepatomegaly, and splenomegaly.

Changes to abnormalities identified during the baseline period should be recorded at all subsequent physical examinations. New or worsening abnormalities should be recorded as AEs, if applicable.

- **Tumor and Response Evaluations**

All known sites of disease must be documented at screening and re-assessed at each subsequent tumor evaluation. Tumor response will be assessed for all patients, unless they withdraw from the study for any reason not attributable to disease progression confirmed radiologically or clinically as per RECIST v.1.1 criteria and who have not received an acceptable complete assessment of the disease. The measurable and non-measurable disease must be documented at screening and be re-assessed at every tumor assessment thereafter.

Tumor assessment during the screening period should include an evaluation of all known and/or suspected lesions/sites of the disease, including color photography of skin lesions, based on the baseline assessment of target and non-target lesions according to RECIST v.1.1 criteria as the reference for comparison at each subsequent tumor assessment. The same radiographic procedure employed at screening should be used throughout the study (e.g., the use of the same contrast protocol for CT scans).

At baseline, all patients should be assessed as follows:

- **Evaluation of chest, abdomen, and pelvis:**
    - This assessment should be evaluated preferably by CT scan or MRI in case of contrast allergy, since these methods are the best currently available and reproducible techniques to measure lesions selected for response assessment, within 28 days prior to the first administration of study medication.

- In the event a positron emission tomography (PET)/CT scan is used for tumor assessments, CT portion of PET/CT is usually of lower quality, and should not be used instead of dedicated diagnostic CT. If the CT scan is of high quality, with oral and intravenous contrast, may be used with caution. Additional information from PET may bias CT assessment.
- After baseline, this evaluation will be performed every 6 weeks (± 3 days) from the first dose of study treatment for the first 6 months and, thereafter, every 9 weeks (± 5 days) until progression or the EoS visit.

- **Bone scan:**
  - Bone scan is mandatory at baseline. For patients with bone lesions identified at baseline, bone scan will be performed every 24 weeks (± 7 days), unless clinically or biochemically suspected bone progression.
  - If no bone involvement is demonstrated, then it is no necessary to repeat the bone assessment unless clinically or biochemically suspected bone progression.
  - If an isotope-based bone scan was performed > 28 days but ≤ 60 days prior to start of study treatment, the bone scan does not need to be repeated.
  - Bone scan should be used only to identify presence of bone lesions and if bone lesions are present, confirmation and accurate measurement must be done with CT scan or MRI. Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT scan or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability. Blastic bone lesions are non-measurable. If a bone scan cannot be performed during the course of the study because of the unavailability of the Tc-99m isotope, the investigator may choose an alternative imaging modality.

- **Brain imaging:**
  - A brain MRI will be performed within 28 days prior to the first administration of study medication.
  - After baseline, this evaluation will be performed every 6 weeks (± 3 days) for first 6 months, then every 9 weeks (± 5 days), unless clinically suspected brain progression.

Additionally, for patients included in the study cohort 5, assessment of neuroaxis by MRI together with spinal tap for CSF collection should be obtained every 3 weeks (± 2 days) for the

first 12 weeks (corresponding to the first 5 cycles of treatment), every 6 weeks thereafter, and at the time of treatment progression or study termination.

Tumor response in CNS will be assessed for all patients, unless they withdraw from the study for any reason not attributable to disease progression confirmed radiologically or clinically as per RANO-BM criteria and who have not received an acceptable complete assessment of the disease. The measurable and non-measurable disease must be documented at screening and be re-assessed at every tumor assessment thereafter.

CNS disease assessment should be evaluated by MRI. Tumor assessment at baseline should include an assessment of all known and/or suspected lesions, based on the baseline evaluation, defined according to the RANO-BM criteria (Lin et al. 2015).

If clinically indicated, CT scan or MRI of other areas of disease as appropriate should be performed. Any potentially measurable lesion that has been previously treated with radiotherapy should be considered as a non-measurable lesion. However, if a lesion previously treated with radiotherapy has clearly progressed since the radiotherapy administration, it can be considered as a measurable lesion.

Patients who discontinue treatment without evidence of disease progression will be followed every nine weeks (± 5 days) for tumor assessments until documented progression, elective withdrawal from the study, the start of new anti-cancer treatment, or study completion or termination.

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.

At the investigator's discretion, CT scans, MRI, and/or bone scans may be obtained at any time when clinically indicated or if progressive disease is suspected. For symptomatic deterioration attributed to disease progression, every effort should be made to document progression through the use of objective criteria per RECIST v.1.1.

- **Laboratory Assessments**

Laboratory tests will be performed at the study site's local laboratory during the screening period and within 48 hours prior to Day 1 of each cycle (including Cycle 1, Day 1). This assessment does not need to be repeated at Cycle 1, Day 1 if it was performed at screening within 48 hours prior to start of study treatment. During cycle 1, laboratory assessments will be performed on Day 8 and Day 15. These tests should include:

- Hematological test: hemoglobin, hematocrit, red blood cell count, platelet count, WBC with differential count [ANC, lymphocytes, monocytes, eosinophils and basophils] Performed at screening (within -14days)
- Coagulation: INR/PT and PTT/aPTT. Performed only at screening (within -14 days) and EoT.
- Blood chemistry: with renal function analysis (serum creatinine, creatinine clearance according to the Cockroft-Gault formula), liver function (AST, ALT, ALP, g*amma*-glutamyl transferase [GGT], total and direct bilirubin), glucose, sodium, potassium, calcium, chloride, magnesium, uric acid, total protein, albumin, and lactate dehydrogenase. Performed at screening (within -14days)

In addition, the following tests are essential assessments at screening, before Cycle 1, Day 1 dosing:

- Urinalysis (within -14 days)
- Screening viral serology (within -14 days): HIV, HBsAg, total HBcAb, HCV antibody; additional tests for HBV DNA or HCV RNA will be required to confirm eligibility in patients with a positive antibody result; Patients positive for hepatitis C (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV RNA

Collect blood sample for Troponin (preferably high-sensitivity troponin-T). Troponin tests will be performed at screening and at the end of treatment (EoT) and as needed based on subject reported cardiac signs or symptoms suggesting congestive heart failure, myocardial infarction, or other causes of cardiac myocyte necrosis. If ECG is abnormal, follow institutional guidelines. If troponin levels are above the upper limit of normal and below the level of myocardial infarction as defined by the manufacturer (CTCAE Grade 1) at baseline, no repeat testing is required if the troponin level is not Grade 3.

- **Pregnancy and Assessment of Fertility**

Only female patients of childbearing potential must undergo serum pregnancy test at screening (within -14 days) to confirm eligibility in the trial and within one week prior to start of study medication (with result available prior to dosing). This assessment does not need to be repeated at Cycle 1 Day 1 if it was performed at screening within 48 hours prior to start of study treatment. Thereafter a urine pregnancy test (with results available prior to dosing) every treatment cycle. A serum test should be performed to confirm any positive urine pregnancy test during the trial.

Post-menopausal status is defined as:

- Age ≥ 60 years,
- OR Age < 60 years and amenorrhea for 12 or more months (in the absence of chemotherapy, tamoxifen, toremifen, or ovarian suppression), and with a documented estradiol level in the postmenopausal range according to local institutional/laboratory standard,
- OR prior bilateral ovariectomy.

Male and female subjects of reproductive/childbearing potential must agree to use a highly effective form of contraception or avoid intercourse during and upon completion of the study and for at least 7 months for females and 4 months for males after the last dose of study drug. Methods *considered as highly effective methods of contraception include:*

1. *Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:*
    - *Oral*
    - *Intravaginal*
    - *Transdermal*
2. *Progestogen-only hormonal contraception associated with inhibition of ovulation:*
    - *Oral*
    - *Injectable*
    - *Implantable*
3. Intrauterine device (IUD)
4. Intrauterine hormone-releasing system (IUS)
5. Bilateral tubal occlusion
6. Vasectomized partner
7. Complete sexual abstinence defined as refraining from heterosexual intercourse during and upon completion of the study and for at least 7 months after the last dose of study drug. Periodic abstinence (calendar, symptothermal, post-ovulation methods) is not an acceptable method of contraception.

Non-child-bearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 months of spontaneous amenorrhea (in questionable cases, a blood sample with simultaneous follicle-stimulating hormone [FSH] > 40 mIU/mL and estradiol < 40 pg/mL [< 147 pmol/L] is confirmatory). Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the contraception methods outlined for women of child-bearing potential if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of post-menopausal status prior to study enrollment. For most forms of HRT, at least 2-4 weeks will elapse between the cessation of therapy and the blood draw; this interval depends on the type

and dosage of HRT. Following confirmation of their post-menopausal status, they can resume use of HRT during the study without use of a contraceptive method

Male subjects must not freeze or donate sperm starting at Screening and throughout the study period, and at least 7 months after the final study drug administration. Preservation of sperm should be considered prior to enrolment in this study.

Female subjects must not donate, or retrieve for their own use, ova from the time of Screening and throughout the study treatment period, and for at least 7 months after the final study drug administration.

In case of pregnancy during study treatment or within 7 months after the last dose of IMP, the patient must permanently stop study treatment immediately, withdraw from the trial, and the pregnancy must be reported on the Clinical Trial Pregnancy Form as specified in ***Section 7.9.***

Male patients will be instructed through the ICF to immediately inform the Investigator if their partner becomes pregnant during the study or within 7 months after the last dose of IMP.

- **Electrocardiograms (ECGs) and Cardiac Function Assessment**

All patients must have a standard **12-lead ECG** and an **LVEF** measurement of at least 50% by ECHO (preferably) or MUGA scan at baseline.

**LVEF** (ECHO or MUGA) assessment must be done within 28 days prior to Cycle 1, Day 1. Afterwards, cardiac function evaluation should be repeated before infusion (48 h) on Cycle 5 Day 1 and every 4 cycles and in the end of treatment visit.

**Triplicate ECGs** will be performed at baseline (within -1 4 days) and every 4$^{th}$ cycle and standard ECG parameters will be measured, including RR, PR, QT intervals, and QRS duration. All ECGs must be evaluated by investigator or delegated physician for the presence of abnormalities. Whether or not measurement is performed, date performed, results, and findings for each parameter will be recorded in the eCRF.

All ECG recordings must be performed using a standard high-quality, high-fidelity digital electrocardiograph machine equipped with computer-based interval measurements. Lead placement should be as consistent as possible. ECG recordings must be performed after the patient has been resting in a supine position for at least ten minutes. All ECGs are to be obtained prior to other procedures scheduled at that same time (e.g., vital sign measurements, blood draws). Circumstances that may induce changes in heart rate, including environmental distractions (e.g., television, radio, conversation) should be avoided during the pre-ECG resting period and during ECG recording.

For safety monitoring purposes, the investigator must review, sign, and date all ECG tracings. Paper copies of ECG tracings will be kept as part of the patient's permanent study file at the site.

After screening (within -14 days), subsequent ECGs will be performed in triplicate in close succession if an abnormality is noted or if clinically indicted. ECGs will be taken at every 4$^{th}$ cycle. If at a particular post-dose timepoint the mean QTcF is > 500 ms and/or > 60 ms longer than the baseline value, another ECG must be recorded, ideally within the next five minutes, and ECG monitoring should continue until QTcF has stabilized on two successive ECGs. The Medical Monitor should be notified and standard-of-care treatment may be instituted per the discretion of the investigator.

- **ECOG performance status**

Performance status will be measured using the ECOG performance status scale (***Table 2***). It is recommended, where possible, that a patient's performance status be assessed by the same person throughout the study. ECOG PS will be assessed at screening (Day-14 to Day -1), Day 1 of each cycle and at the EoT visit.

**Table 2. ECOG Performance Status Scale**

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

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

- **PRO Assessments**

Patient-reported GHS/QoL, functioning and symptoms will be assessed using the EORTC QLQ-C30 and the breast cancer module QLQ-BR23 questionnaires at baseline, Day 1 of cycles 2-4,

then on Day 1 of every other subsequent cycle starting with cycle 6 (e.g. cycles 6, 8, 10, etc.), at the end-of-treatment visit, and every three months thereafter until start of alternative anti-cancer therapy.

Patients will complete each questionnaire prior to any study or medical procedure. Completed questionnaires are always considered source document and must be filed accordingly.

Patients must complete these instruments in clinic (cannot be taken home) and prior to having any tests and to any discussion of their progress with healthcare personnel at the site. Interviewer administration in clinic may be used under special circumstances (e.g., patient forgot their glasses or feels too ill).

The questionnaire will be given to the patient in the appropriate language for the site.

Patients discontinuing treatment for any reason including progression disease or any other reason should have a tumor and PROs assessment prior to start alternative anticancer treatment.

The EORTC-QLQ-C30 is a 30-item questionnaire, composed of five multi-item functional subscales (physical, role, emotional, cognitive, and social functioning), three multi-item symptom scales (fatigue, nausea/vomiting, and pain), a global QOL subscale, and six single item symptom scales assessing other cancer-related symptoms (dyspnea, sleep disturbance, appetite loss, constipation, diarrhea, 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. Responses to all items are then converted to a 0 to 100 scale. For functional and global QOL scales, higher scores represent a better level of functioning/QOL. For symptom-oriented scales, a higher score represents more severe symptoms. A 10-point or higher change in scores from baseline is considered clinically significant. We will evaluate change from baseline between EORTC QLQ-C30 functional and symptom scale scores.

The EORTC-QLQ-BR23 is a 23-item breast cancer-specific companion module to the EORTC-QLQ-C30 and consists of four functional scales (body image, sexual functioning, sexual enjoyment, future perspective) and four symptom scales (systemic side effects, breast symptoms, arm symptoms, upset by hair loss). QLQ-BR23 questionnaire employs 4-point scales with responses from 'not at all' to 'very much'. All scores are converted to a 0 to 100 scale. For functional scales, higher scores represent a better level of functioning. We will evaluate change from baseline between EORTC-QLQ-BR23 functional and symptom scale scores.

Using both questionnaires it will be evaluated:

- Overall change from baseline in patient–reported GHS/QoL, functioning and symptoms.
- Time to Deterioration in global QOL defined as time to 10 point or greater decrease in global QOL scores from baseline with no subsequent increase in scores above that threshold.

- Time to deterioration in pain defined as time between baseline and first occurrence of increase of ≥10 points in pain. Deterioration will be defined increase in score of 10 points or greater from baseline.

- **Biological Samples for Exploratory Analysis**

#### 5.2.1.1 Tumor Samples

If feasible, patients should provide a tissue sample at the time of study entry from metastases amenable to biopsy (at sites of locoregional recurrence [skin, chest wall, breast or lymph nodes], or distant recurrence [bone, liver, lung or abdomen]) that will be obtained between progression to the prior regimen and inclusion in the study. As alternative, patients should provide a tissue sample from breast primary tumor. Patients for whom tissue sample cannot be obtained (e.g., non-measurable disease, inaccessible tumor or subject safety concern) may submit an archived metastatic tumor specimen only upon agreement from the Sponsor.

If feasible, an additional tissue sample should be collected at the end of treatment visit for patients who discontinue treatment due to disease progression.

Exploratory studies will be performed on tumor biopsies, or formalin-fixed and paraffin-embedded (FFPE) or frozen tumor samples (blocks), or unstained glass slides.

Details on tumor tissue samples preparation, processing, storage, and shipment will be provided in a separate study manual.

*Note*: *Tissue blocks, or slides, must be submitted to confirm BC subtype by a Sponsor-designated central laboratory retrospectively*

#### 5.2.1.2 Blood Samples

Blood samples are required for all patients during the screening period, after two cycles of study treatment, and upon progression or study termination.

An aliquot from any of those time-points will be preserved to collect cell-free deoxyribonucleic acid (cfDNA) analysis and compare genomic DNA data from tissue samples and cfDNA data from liquid biopsies.

Details on blood samples processing will be provided in a separate study manual.

#### 5.2.1.3 CSF Samples

In study cohort 5, in addition to CSF samples for LMC clinical monitoring, patients must agree to provide CSF for exploratory analysis, at baseline, every 3 weeks for 12 weeks (corresponding to the first 5 cycles of treatment) and every 6 weeks thereafter, and at the time of treatment progression or study termination.

Centralized analysis of CSF-derived epithelial-cells, lymphocytes, and cfDNA (together with comparison with cfDNA from blood samples) will be performed.

Details on CSF samples processing and cytoblock will be provided in a separate study manual.

#### 5.2.1.4 Molecular Assessments

The multiple assays, described below, may be performed with the material derived from tumor samples and/or the blood samples collected from each patient as part of this study. We will use both a [redacted] and a [redacted] approach. It is likely that not all assays described below will be performed on samples provided by each patient, possibly because of insufficient material or inadequate sample quality.

We anticipate that as molecular and genomic technologies improve, as yet unspecified technologies will be applied.

Biomarker samples (blood, tissue, and CSF) for mandatory exploratory biomarker research include, but not limited to, the following assays and assay platforms:

- [redacted] that may impact exposure or other responses, or [redacted] ([redacted]) results interpretation;
- [redacted] and [redacted] by [redacted] or [redacted] ([redacted])-based methods in tumor tissue and cfDNA;
- Expression analysis of [redacted] related to [redacted], and [redacted] (e.g., [redacted]);
- IHC-based analysis or quantitative digital IHC of markers of [redacted] and [redacted], such as [redacted], [redacted], [redacted] ([redacted]), and [redacted] ([redacted]), among others.

When a patient withdraws from the study, samples collected prior to the date of withdrawal may still be analyzed, unless the patient specifically requests that the samples be destroyed, or local laws require destruction of the samples. However, if samples have been tested prior to withdrawal, results from those tests will remain as part of the overall research data.

Given the complexity and exploratory nature of the analyses, data derived from exploratory studies, including germline mutations, will generally not be provided to study investigators or patients unless required by law. The aggregate results of any conducted research will be available in accordance with the effective Sponsor policy on study data publication.

# 6 STUDY DRUGS INFORMATION

## 6.1 Formulation, Packaging, and Handling

Study drug packaging will be overseen by the Sponsor' and bear a label with the identification required by local law, the protocol number, drug identification, and dosage. The packaging and labelling of the study drug will be in accordance with local regulations. The study drug must be stored according to the details on the Product Information. The drug label indicates the storage temperature. Upon arrival of IMPs at the site, site personnel should check them for damage, verify proper identity, quantity, integrity of seals, and temperature conditions, and report any deviations or product complaints upon discovery.

- **Trastuzumab deruxtecan (DS-8201a)**

After having confirmed eligibility and entered into the clinical trial, patients will be treated with trastuzumab deruxtecan (DS-8201a) at 5.4 mg/Kg administered as an IV infusion Q3W, initially for at least 90 minutes, then, if there is no infusion-related reaction, for a minimum of 30 minutes.

In patients with HR-positive status (ER- and/or PgR-positive) administration of endocrine therapy is not allowed.

In patients allocated in study cohort 5, administration of intrathecal therapy is not allowed.

## 6.2 Dosage and Administration

Trastuzumab deruxtecan (DS-8201a) will be supplied in the dosage form as follows:

- 100 mg in a sterile lyophilized powder dosage form in a single-use vial (Lyo-DP) to be reconstituted with 5 mL of water for injection to 20 mg/mL.

### 6.3 Treatment Modification

Safety and tolerability of all patients will be closely monitored throughout study treatment and the follow-up period. Patients will be assessed in order to detect any AEs before administering new study treatment during each treatment visit.

All dose modifications (interruption, reduction and/or discontinuation) should be based on the worst preceding toxicity (CTCAE v.5.0.). Specific criteria for interruption, re-initiation, dose reduction and/or discontinuation of trastuzumab deruxtecan are listed in table 4, below which is applicable only to TEAEs that are assessed as related to use of trastuzumab deruxtecan by the investigator(s). For non-drug related TEAEs, follow standard clinical practice. Appropriate clinical experts should be consulted as deemed necessary.

- **Dose Reduction Levels**

Dose reductions are allowed from 5.4 mg/kg to 4.4 mg/kg and a second dose reduction to 3.2 mg/kg. Once the dose of trastuzumab deruxtecan (DS-8201a) has been reduced because of toxicity, all subsequent cycles should be administered at that lower dose level unless further dose reduction is required. No more than two dose reductions of trastuzumab deruxtecan (DS-8201a) per patient will be allowed (see ***Table 3***). Any patient requiring a trastuzumab deruxtecan (DS-8201a) dose reduction below 3.2 mg/Kg should be discontinued. Dose re-escalation is not allowed.

Note: The patient's weight at screening (baseline) will be used to initially calculate the dose. If the patient's weight changed by > 10% of the baseline weight, the dose will be recalculated.

**Table 3. Dose Reductions for Trastuzumab Deruxtecan (DS-8201a)**

| Dose Level [1] | Trastuzumab Deruxtecan (DS-8201a) |
|---|---|
| Starting dose | 5.4 mg/kg |
| First dose reduction | 4.4 mg/kg |
| Second dose reduction | 3.2 mg/kg |
| Third dose reduction | Not permitted |
| [1] If the patient continues to experience specified drug-related AEs after the second reduction, the treatment should be discontinued. | |

**Dose Interruption and Modification /Toxicity Management Guidelines:**

A dose can be delayed for up to 28 days (49 days from the last infusion date) from the planned date of administration. If a subject is assessed as requiring a dose delay of longer than 28 days, the subject will be withdrawn from the study.

Treatment cycles for a subject for whom trastuzumab deruxtecan dosing is temporarily withheld for any reason may have future cycles scheduled based on the date of the last trastuzumab deruxtecan dose.
All confirmed or suspected COVID-19 infection events must be recorded in the eCRF. Please refer to Appendix 4 for additional information on dose modification.

**Table 4: Dose Interruption and Modification /Toxicity Management Guidelines**

| **Worst toxicity CTCAE v5.0 Grade (unless otherwise specified)** | **Dose or schedule modification for trastuzumab deruxtecan** |
|---|---|
| **No toxicity** | Maintain dose and schedule |
| **Infusion-Related Reaction** | |
| Grade 1<br>(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 subjects should be closely monitored.<br>If no other reactions appear, the subsequent infusion rate could be resumed at the initial planned rate. |
| Grade 2<br>(Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, nonsteroidal anti-inflammatory drugs (NSAIDs), narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hrs) | Administration of trastuzumab deruxtecan should be interrupted and symptomatic treatment started (e.g. antihistamines, NSAIDs, narcotics, IV fluids).<br>If the event resolves or improves to grade 1, infusion can be re-started at a 50% reduced infusion rate.<br>Subsequent administrations should be conducted at the reduced rate. |
| Grade 3 or 4<br>(Prolonged or life-threatening consequences, urgent intervention indicated) | Administration of trastuzumab deruxtecan should be discontinued immediately and permanently.<br>Urgent intervention indicated. Antihistamines, steroids, epinephrine, bronchodilators, vasopressors, intravenous fluid therapy, oxygen inhalation etc., should be administered. |
| **Hematologic Toxicity** | |
| **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,<br>Reduce dose 1 level |
| Febrile Neutropenia<br>(absolute neutrophil count < 1 x $10^9$/L, fever > 38.3°C or a sustained | Delay dose until resolved,<br>Reduce dose by 1 level |

| Worst toxicity CTCAE v5.0 Grade (unless otherwise specified) | Dose or schedule modification for trastuzumab deruxtecan |
|---|---|
| temperature of ≥ 38 °C for more than one hour) | |
| **Lymphocyte Count Decreased** | |
| Grade 1 to Grade 3<br>lymphopenia | No dose modification |
| Grade 4<br>(< 0.2 x $10^9$/L) | Delay dose until resolved to ≤ Grade 2:<br>- If resolved in ≤ 14 days from day of onset, maintain dose<br>- If resolved in > 14 days from day of onset, reduce dose 1 level |
| **Anaemia** | |
| Grade 3<br>(Hemoglobin (Hb) <8.0 g/dL); transfusion indicated | Delay dose until resolved to ≤ Grade 2, then maintain dose |
| Grade 4<br>Life threatening consequences; urgent intervention indicated | Delay dose until resolved to ≤ Grade 2, then reduce dose 1 level |
| **Platelet Count Decreased** | |
| Grade 3<br>(platelets <50 – 25 x $10^9$/L) | Delay dose until resolved to ≤ Grade 1:<br>- If resolved in ≤ 7 days from day of onset, maintain dose<br>- If resolved in > 7 days from day of onset, reduce dose 1 level |
| Grade 4<br>(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 subject from study treatment |
| Decrease in Left ventricle ejection fraction (LVEF) 10-20% (absolute value), but LVEF > 45% | Continue treatment with trastuzumab deruxtecan |
| LVEF 40% to ≤ 45% and decrease is < 10% (absolute value) from baseline | Continue treatment with trastuzumab deruxtecan<br>Repeat LVEF assessment within 3 weeks |
| LVEF 40% to ≤ 45% and decrease is ≥ 20% (absolute value) from baseline | Interrupt trastuzumab deruxtecan dosing<br>Repeat LVEF assessment within 3 weeks.<br>If LVEF has not recovered to within 10% (absolute value) from baseline, discontinue subject from study treatment.<br>If LVEF recovers to within 10% from baseline, resume study drug treatment |
| LVEF < 40% or > 20% (absolute value) drop from baseline | Interrupt trastuzumab deruxtecan dosing<br>Repeat LVEF assessment within 3 weeks. |

| Worst toxicity CTCAE v5.0 Grade (unless otherwise specified) | Dose or schedule modification for trastuzumab deruxtecan |
|---|---|
| | If LVEF < 40% or > 20% drop from baseline is confirmed, discontinue subject from study treatment |
| **Electrocardiogram QTc Prolonged** | |
| Grade 3<br>(Average QTc> 500 ms or >60 ms change from baseline) | Delay dose until resolved to ≤ Grade 1 (QTc ≤ 480 ms), determine if another medication the subject 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 trastuzumab deruxtecan, reduce dose 1 level |
| Grade 4<br>(Torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia) | Discontinue subject from study treatment |
| **Pulmonary Toxicity** | If a subject develops radiographic changes potentially consistent with ILD/pneumonitis or develops an acute onset of new or worsening pulmonary or other related signs/symptoms such as dyspnea, cough or feve, rule out ILD/pneumonitis.<br>If the AE is confirmed to have an etiology other than ILD/pneumonitis, follow the management guidance outlined in the "Other Non-Laboratory Adverse Events" dose modification section below.<br>If the AE is suspected to be ILD/pneumonitis, treatment with study drug should be interrupted pending further evaluations.<br>Evaluations should include:<br>• high resolution CT<br>• pulmonologist consultation *(infectious disease consultation as clinically indicated),*<br>• *blood culture and CBC (other blood tests could be considered as needed),*<br>• *consider bronchoscopy and bronchoalveolar lavage if clinically indicated and feasible,*<br>• pulmonary function tests and pulse oximetry (SpO2)<br>• arterial blood gases if clinically indicated<br>• One blood sample collection for PKanalysis as soon as ILD/pneumonitis is suspected, if feasible<br>Other tests could be considered, as needed.<br>If the AE is confirmed to be ILD/pneumonitis, follow the management guidance as outlined below.<br>All events of ILD/pneumonitis regardless of severity or seriousness will be followed until resolution including after drug discontinuation. |
| Grade 1 | The administration of trastuzumab deruxtecan must be interrupted for any ILD/pneumonitis events regardless of grade. |

| Worst toxicity CTCAE v5.0 Grade (unless otherwise specified) | Dose or schedule modification for trastuzumab deruxtecan |
|---|---|
| | • *Monitor and closely follow-up in 2 to 7 days for onset of clinical symptoms and pulse oximetry*<br>• *Consider follow-up imaging in 1-2 weeks (or as clinically indicated).*<br>• *Consider starting systemic steroids (e.g. at least 0.5 mg/kg/day prednisone or equivalent) until improvement, followed by gradual taper over at least 4 weeks.*<br>• *If worsening of diagnostic observations despite initiation of corticosteroids, then follow Grade 2 guidelines.**<br><br>For Grade 1 events, trastuzumab deruxtecan can be restarted only if the event is fully resolved to Grade 0:<br>- If resolved in ≤ 28 days from day of onset, maintain dose<br>- If resolved in > 28 days from day of onset, reduce dose 1 level<br><br>However, if the event grade 1 ILD/pneumonitis occurs beyond cycle day 22 and has not resolved within 49 days from the last infusion, the drug should be discontinued.<br>** If patient is asymptomatic, then patient should still be considered as Grade 1 even if steroid treatment is given* |
| Grade 2 | Permanently discontinue subject from study treatment.<br>• Promptly start and treat with systemic steroids (e.g., at least 1mg/kg/day prednisone or equivalent) for at least 14 days or until complete resolution of clinical and chest CT findings, then followed by a gradual taper over at least 4 weeks.<br>• Monitor symptoms closely.<br>• Re-image as clinically indicated.<br>• If worsening or no improvement in clinical or diagnostic observations in 5 days,<br>  • Consider increasing dose of steroids (e.g., 2 mg/kg/day prednisone or equivalent) and administration may be switched to intravenous (e.g. methylprednisolone).<br>  • Re-consider additional work-up for alternative etiologies as described above.<br>  • Escalate care as clinically indicated. |

| Worst toxicity CTCAE v5.0 Grade (unless otherwise specified) | Dose or schedule modification for trastuzumab deruxtecan |
|---|---|
| *Grade 3 and 4* | Permanently discontinue subject from study treatment.<br>• Hospitalization required.<br>• 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 or until complete resolution of clinical and chest CT findings, then followed by a gradual taper over at least 4 weeks<br>• Re-image as clinically indicated.<br>• If still no improvement within 3 to 5 days,<br>  • Re-consider additional work-up for alternative etiologies as described above.<br>Consider other immuno-suppressants and/or treat per local practice. |
| **Ocular** | |
| Grade 3 | Delay dose until resolved to ≤ Grade 1:<br>If resolved in ≤ 7 days from day of onset, maintain dose<br>If resolved in > 7 days from day of onset, reduce dose 1 level |
| Grade 4 | Discontinue subject from study treatment |
| **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) | Discontinue subject from study treatment |
| **Hepatic Toxicity** | |
| **Aspartate aminostransferase (AST) or alanine aminotransferase (ALT) with simultaneous Total bilirubin (TBL)** | |
| AST/ALT ≥ 3.0 x ULN with simultaneous TBL > 2.0 x ULN | Delay study medication until drug-induced liver injury can be ruled out.<br>If drug-induced liver injury is ruled out, the subject should be treated accordingly, and resumption of study drug may occur after discussion between the Investigator and Sponsor.<br>If drug-induced liver injury cannot be ruled out from diagnostic workup, permanently discontinue study treatment.<br>Monitor AST/ALT and TBL twice weekly until resolution or return to baseline. |
| **Aspartate aminotransaminase (AST) or alanine aminotransaminase (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 for Grade 2 AST/ALT |

| Worst toxicity CTCAE v5.0 Grade<br>(unless otherwise specified) | Dose or schedule modification for trastuzumab deruxtecan |
|---|---|
| Grade 3 (>5.0 - 20.0 x ULN if baseline was normal; >5.0 - 20.0 x baseline if baseline was abnormal)<br>In subjects without liver metastases and subjects with liver metastases and baseline level ≤ 3 x ULN | Repeat testing within 3 days. Delay dose until resolved to ≤ Grade 1*if* baseline ≤ 3 x ULN, otherwise delay dose until resolved to ≤ baseline then:<br>If resolved in ≤ 7 days from day of onset, maintain dose<br>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)<br>In subjects with liver metastases, if the baseline level was > 3 x ULN | Repeat testing within 3 days. Delay dose until resolved to ≤ baseline level, then:<br>If resolved in ≤ 7 days from day of onset, maintain dose<br>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) | Discontinue subject from study treatment |
| **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, delay dose until resolved to ≤ Grade 1:<br>- If resolved in ≤ 7 days from day of onset, maintain dose<br>- If resolved in > 7 days from day of onset, reduce dose 1 level<br>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:<br>- If resolved in ≤ 7 days from day of onset, reduce dose 1 level<br>- If resolved in > 7 days from day of onset, discontinue trastuzumab deruxtecan<br>If documented Gilbert's syndrome or liver metastases at baseline, repeat testing within 3 days. Delay dose until resolved to ≤ Grade 2:<br>- If resolved in ≤ 7 days from day of onset, reduce dose 1 level<br>-If resolved in > 7 days from day of onset, discontinue trastuzumab deruxtecan |
| Grade 4 (>10.0 x ULN if baseline was normal; >10.0 x baseline if baseline was abnormal) | Discontinue subject from study treatment |
| **Blood Alkaline Phosphatase Increased** | |

| Worst toxicity CTCAE v5.0 Grade (unless otherwise specified) | Dose or schedule modification for trastuzumab deruxtecan |
|---|---|
| Grade 3 (>5.0 - 20.0 x ULN if baseline was normal; >5.0 - 20.0 x baseline if baseline was abnormal)<br>or<br>Grade 4 (>20.0 x ULN if baseline was normal; >20.0 x baseline if baseline was abnormal) | 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<br>If resolved in ≤ 7 days from day of onset, maintain dose<br>If resolved in > 7 days from day of onset, reduce dose 1 level |
| **Diarrhea/Colitis** | |
| Grade 3 | Delay dose until resolved to ≤ Grade 1<br>If resolved in ≤ 3 days from day of onset, maintain dose<br>If resolved in > 3 days from day of onset, reduce dose 1 level |
| Grade 4 | Discontinue subject from study treatment |
| **Other Laboratory Adverse Events** | |
| Grade 3 | Delay dose until resolved to ≤ Grade 1 or baseline level:<br>If resolved in ≤ 7 days from day of onset, maintain dose<br>If resolved in > 7 days from day of onset, reduce dose 1 level |
| Grade 4 | Discontinue subject from study treatment |
| **Other Non-Laboratory Adverse Events** | |
| Grade 3 | Delay dose until resolved to ≤ Grade 1 or baseline:<br>If resolved in ≤ 7 days from day of onset, maintain dose<br>If resolved in > 7 days from day of onset, reduce dose 1 level |
| Grade 4 | Discontinue subject from study treatment |

In addition, Investigators may consider dose reductions or discontinuations of the study drug according to the subject's condition and after discussion with Sponsor's Medical Monitor or designee.

### 6.4 Potential Drug-Drug Interaction

Nonclinical pharmacokinetic studies have indicated that MAAA-1181a –one of the components of trastuzumab deruxtecan (DS-8201a)– is primarily metabolized by CYP3A4 and is a substrate for transporters OATP-1B1 and -1B3, MATE2-K, P-gp, BCRP, and MRP1. Concomitant use of strong CYP3A4 inhibitors (e.g., boceprevir, clarithromycin, itraconazole, ketoconazole,

lopinavir/ritonavir, nefazodone, telaprevir, telithromycin, voriconazole) or OATP inhibitors (e.g., lopinavir/ritonavir, cyclosporine, rifampicin) should be avoided. On a second level, patients must also avoid concomitant use of strong inhibitors of MATE2-K, P-gp, BCRP, or MRP1.

MAAA-1181a did not show any potential to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A (inhibitory concentration [IC50] >50 µmol/L). MAAA-1181a did not show any potential to induce CYP3A4, CYP1A2, and CYP2B6 up to 30 µmol/L. MAAA-1181a did not inhibit OAT3, OCT1, OCT2, OATP1B3, MATE1, MATE2-K, P-gp, BCRP, and BSEP (IC50 >30 µmol/L). MAAA-1181a inhibited OAT1 and OATP1B1 with IC50 values of 12.7 and 14.4 µmol/L, respectively, although the values were much higher than the maximum serum concentration (Cmax) of MAAA-1181a in humans (9.25 ng/mL [0.019 µmol/L]) at 8.0 mg/kg of DS-8201a.

### 6.5 General Concomitant Medication and Supportive Care Guidelines

Concomitant treatment and prior medication are defined as non-IMP. Concomitant treatment includes any prescribed medication or herbal medicines between the 21 days prior to the administration of the first treatment dose and the last safety follow-up visit. All concomitant treatments will be recorded. After study discontinuation, information will only be collected on any anti-cancer drugs taken by the patient until EoS.

Information on concomitant medication will include start date, end date, brand or generic name, route of administration, dose, and treatment indication.

Information on prior medication will include details on prior anti-cancer therapies.

No pre-medication for study drug administration is required; however, pre-medication is allowed at the investigator's discretion.

The following concomitant treatments are permitted during the study:

- Corticosteroids are allowed as long as patients have been on a stable dose for at least four weeks. The highest allowed dose is dexamethasone 8 mg twice daily or an equivalent
- Erythropoiesis-stimulating agents (ESA) are allowed (such as Procrit®, Aranesp®, Epogen®) for the supportive treatment of anemia. Blood transfusions are permitted during the study as well as iron administration.
- The prophylactic use of granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage-colony stimulating factor (GM-CSF) is not allowed during the first treatment cycle but can be used for cases of neutropenia arising during treatment, in accordance with the National Comprehensive Cancer Network (NCCN) guidelines.
- The use of medication for the treatment of diarrhea, nausea, or vomiting is permitted.

- The use of bisphosphonates or denosumab is allowed if treatment starts within 2 weeks of the active study treatment for the control of bone pain, prevention and/or treatment of bone metastases, and treatment of osteoporosis.
- Palliative radiotherapy is permitted to treat painful bone metastases.
- Strong inhibitors of CYP3A4, OATP, MATE2-K, P-gp, BCRP or MRP1 should be avoided
- 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 steroids (e.g. dexamethasone) should be considered and administered in accordance with the prescribing information or institutional guidelines
- Any medications deemed necessary to ensure patient safety and well-being may be administered at the discretion of the investigator with the exception of prohibited therapies described in below Section.

### 6.6 Prohibited Therapies

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

Any therapies intended for the treatment of cancer, other than trastuzumab deruxtecan (DS-8201a) whether they are approved by national Health Authorities or experimental, including cytotoxic chemotherapy, immunotherapy, endocrine therapy, biologic or targeted agents and other investigational therapeutic agents. Patients are not allowed to participate in other clinical trials while they are participating in the DEBBRAH trial. The Sponsor must be notified if a patient receives any of these during the study.

- Other anticancer therapy, including cytotoxic, targeted agents, immunotherapy, antibody, retinoid, or anti-cancer hormonal treatment [concurrent use of hormones for noncancer-related conditions (e.g. insulin for diabetes and hormone replacement therapy) is acceptable].
- Any concurrent cancer-related surgery is prohibited throughout the duration of the active treatment phase of the study unless it is life-saving.
- Radiotherapy (except for palliative radiation to known metastatic sites as long as it does not affect assessment of response or interrupt treatment for more than the maximum time specified in dose modification section).
- Radiotherapy to the thorax.

- Concomitant use of chronic systemic (IV or oral) corticosteroids or other immunosuppressive medications except for managing adverse events; (Inhaled steroids or intra articular steroid injections are permitted in this study.)
- Subjects with bronchopulmonary disorders who require intermittent use of bronchodilators (such as albuterol) will not be excluded from this study.
- Concomitant treatment with chloroquine or hydroxychloroquine is not allowed during the study treatment. Refer to appendix 4 for further details

In general, the following treatments are not prohibited but they are not recommended during the treatment phase:

- Chronic daily immunosuppressive therapies including systemic corticosteroids are allowed with a daily dose of at least 16 mg/day of dexamethasone or equivalent.
- Herbal medicines.
- Use of tobacco products, e-cigarettes and vaping is strongly discouraged but not prohibited.
- Hematopoietic growth factors may be used for prophylaxis or treatment based on the clinical judgment of the Investigator.
- Concomitant use of dietary supplements, medications not prescribed by the Investigator, and alternative/complementary treatments is discouraged, but not prohibited.
- Prophylactic or supportive treatment of study-drug induced AEs will be otherwise as per investigator's discretion and institutional guidelines.

Moreover, CSF administration, platelet and red blood cell transfusions is not allowed within 1 week prior to screening assessment.

### 6.7 Drug Storage and Drug Accountability

Storage conditions stated in the Study Reference Safety Document (e.g., Investigator's Brochure [IB], or Local Product Document) may be superseded by the label storage information.

Investigators and site staff are reminded to continuously monitor room storage temperatures and ensure that thermometers are working correctly as required for proper storage of IMP. These include thermometers for both the room storage and refrigerator storage. Any temperature excursions must be reported immediately to the Sponsor and documented. Once a deviation is identified, the IMP must be quarantined and not used until the Sponsor provides documentation of permission to use the IMP.

At the end of the trial, the Sponsor will provide instructions as to disposition of any unused IMP. If the Sponsor authorizes destruction at the trial site, the investigator must ensure that the materials are destroyed in compliance with applicable environmental regulations, institutional policy, and any special instructions provided by the Sponsor. Destruction must be adequately documented.

Trastuzumab deruxtecan (DS-8201a) must be stored in their original container according to labelled storage conditions: under refrigeration at 2-8°C (protected from light).

Medication should be kept in a secured locked area at the study site in accordance with applicable regulatory requirements. Returned medication should be stored separately from medication that needs to be dispensed.

Accurate records of all IMPs received at, dispensed from, returned to, and disposed of by the study site should be recorded on the Drug Inventory Log. In particular, to ensure adequate records, trastuzumab deruxtecan (DS-8201a) will be accounted for as instructed by the Sponsor.

# 7 SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

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

The Sponsor or its designee is responsible for reporting relevant SAEs to competent authorities, other applicable regulatory authorities, and participating investigators, in accordance with International Conference on Harmonization (ICH) guidelines, European Clinical Trials Regulation (Regulation (EU) No 536/2014), and/or local regulatory requirements.

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

All SAEs must be reported to DSE CSPV team address: debbrah.SAE@medsir.org

## 7.1 AEs Definitions

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

An abnormal test finding should only be reported as an AE if meets any of the following criteria:

- Is associated with accompanying symptoms and a general diagnostic term, including the symptoms and the abnormal test finding, cannot be defined.
- Requires additional diagnostic testing or medical/surgical intervention, leads to a change in study drug(s) dosing or discontinuation from the study.
- Needs additional concomitant drug treatment.
- Is considered to be an AE by the investigator or by the Sponsor.

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as the AE, and the resulting appendectomy should be recorded as treatment of the AE.

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

All AEs must be recorded in the CRF

The causal relationship between an AE and the IMP will be defined as follows:

- Unrelated: The temporal association between the AE and the administration of the IMP makes a causal relationship unlikely, or the patient's clinical state or the study procedure/conditions provide a sufficient explanation for the AE.
- Related: The temporal association between the AE and the administration of the IMP makes a causal relationship possible, and the patient's clinical state or the study procedure/conditions do not provide a sufficient explanation for the AE.

Each AE must be assessed by the investigator as to whether or not there is a reasonable possibility of causal relationship to the IMP. If the investigator does not know whether or not the IMP caused the event, then the event will be handled as "related to IMP" for reporting purposes.

The descriptions and grading scales found in the revised NCI–CTCAE v.5.0 will be utilized for all toxicity reporting. A copy of the NCI–CTCAE v.5.0 can be downloaded from the CTEP website: (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf).

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
- Grade 4 Life-threatening consequences; urgent intervention indicated.
- Grade 5 Death related to AE.

* 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.

Note the distinction between the severity and the seriousness of an AE. A severe event is not necessarily a serious event. For example, a headache may be severe (interferes significantly with patient's usual function) but would not be classified as serious unless it meets one of the criteria for SAEs, listed below. Seriousness rather than severity serves as a guide for defining regulatory reporting obligations.

All confirmed or suspected COVID-19 infection events must be recorded in the eCRF.

### 7.2 SAEs

Per definition, a SAE is defined as any AE that either:

- Results in death (e.g., the AE actually causes or leads to death).
- Is life-threatening (e.g., the AE, in the view of the investigator, places the patient at immediate risk of death when it occurs).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person ability to conduct normal life functions).
- Constitutes a congenital anomaly/birth defect (in a neonate/infant born to a mother exposed to the IMP.

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

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

Hospitalization for elective surgery or routine clinical procedures, which are not the result of an AE, need not to be notified according to immediate reporting criteria. If anything untoward is reported during any procedure, this must be reported as an AE and either 'serious' or 'non-serious' attributed according to the usual criteria.

Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical AE not to be notified according to immediate reporting criteria. Examples include:

- Admission for treatment of a pre-existing condition not associated with the development of a new AE or with a worsening of the pre-existing condition (e.g., for work-up of persistent pre-treatment lab abnormality).
- Social admission (e.g., patient has no place to sleep).
- Administrative admission (e.g., for yearly physical examination).
- Protocol-specified admission during a study (e.g., for a procedure required by the study protocol).
- Optional admission not associated with a precipitating clinical AE (e.g., for elective cosmetic surgery).
- Hospitalization for observation without a medical AE.
- Pre-planned treatments or surgical procedures should be noted in the baseline documentation and/or for the individual patient.
- Admission exclusively for the administration of blood products.

Definition of clinically/medically significant event: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. Clinically/medically significant events MUST be reported as SAEs.

Overdose: defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. An "excessive and medically important" overdose includes any overdose in which either a serious adverse event, a non-serious adverse event, or no adverse event occurs and is considered by the Investigator as clinically relevant, i.e. poses an actual or potential risk to the subject.

Overdose is always serious. By definition an overdose is medically important, which meets the seriousness criterion of important medical event. Details of the overdose including trastuzumab deruxtecan dosage, clinical course, associated AEs, and outcome must be captured in the Narrative form of the CRF.

### 7.3 AESIs to Report immediately

AESIs must be reported by the investigator to the Sponsor expeditiously (see ***Section 7.6***), regardless of their seriousness (e.g. no more than 24 hours after learning of the event). AESIs for this study include:

- **Hepatic events** (both serious and non-serious) which meet the potential drug-induced liver injury as assessed by laboratory criteria for Hy's law defined as an elevated (ALT or AST). The following laboratory abnormalities are indicative of possible Hy's law cases and must be reported as an AESI:
    - AST and/or ALT elevations >3 times the ULN with concurrent elevation of total bilirubin >2 times the ULN (or clinical jaundice if total bilirubin measures are not available), except in patients with documented Gilbert's syndrome. For patients with Gilbert's syndrome, elevation of direct bilirubin >2 times the ULN should be used instead.

In addition, based on the available pre-clinical data, review of the cumulative literature, reported toxicities for the same class of agents and biological plausibility, the following events are considered to be adverse events of special interest (AESI): Interstitial lung disease/pneumonitis, QT prolongation, LVEF decrease, and Infusion related reactions. Management Guidance for these AESIs are detailed on section 6.3 Treatment Modification.

- **Interstitial Lung Disease (IDL)**

Interstitial lung disease/pneumonitis is considered an important identified risk based on a comprehensive cumulative review of the available safety data from the clinical development program, literature and available safety information for drugs of similar class. Refer to the current IB for a summary of preliminary clinical study data

- **Left Ventricular Dysfunction**

LVEF decrease in association with T-Dxd is considered to be 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.

Subjects receiving T-Dxd should be monitored for signs and symptoms of any of the toxicities observed in nonclinical studies and clinical studies with other drugs of similar class.

#### 7.3.1.1 Other non-product specific AESI requiring expeditiously reporting

- Suspected transmission of an infectious agent by a medication, whereby any organism, virus, or infectious particle (e.g. prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. Transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings indicating an infection in a patient exposed to a medicinal product. This term only applies when contamination of a medication is suspected and does not apply to infections supported by the mode of action, e.g. immunosuppression.

### 7.4 Treatment-emergent adverse event (TEAE)

A Treatment-emergent adverse event (TEAE) is defined as an AE that occurs, having been absent before the first dose of study drug, or has worsened in severity or seriousness after the initiating the study drug until 47 days after last dose of the study drug. SAEs with an onset or worsening 48 days or more after the last dose of study drug, if considered related to the study treatment, are also TEAEs

### 7.5 AEs Reporting and Other Safety Related Issues Reporting

For serious and non-serious AEs, the reporting period to the Sponsor (or its designated representative) begins from the time that the patient provides ICF.

Disease progression/worsening of breast cancer will not be recorded as an AE on the Adverse Event eCRF. However, events associated with disease progression, such as [as thrombocytopenia, may be recorded as AEs.

Death due to disease progression should be recorded on the Death eCRF.

Reporting period for SAEs/AESIs that are NOT related with the study IMP and also all non-serious AEs is as follows:

- If patient discontinues treatment, until 40 calendar days after the last administration of the study IMP.
- If patient still on treatment at the time of the EoS visit (see definition on ***Section 3.4***) until the EoS visit.

All study patients will be carefully monitored for the occurrence of AEs (including SAEs and AESIs) during the above specified AE reporting period.

If the investigator becomes aware of a SAE/AESI at any time after the end of administration of study treatment and believes that it is possibly related to trastuzumab deruxtecan (DS-8201a) (a serious adverse reaction to trastuzumab deruxtecan (DS-8201a)), the investigator should

notify the serious adverse reaction to the Sponsor immediately irrespective of the time elapsed since last administration of the study IMP.

For all grade ≥ 3 AEs with causal relationship to the IMP, follow-up by the Investigator may be required until the event or its sequelae resolve or stabilize at the level acceptable to the Investigator, and the Sponsor concurs with that assessment.

All confirmed or suspected COVID-19 infection events must be recorded in the eCRF. Please refer to Appendix 4 for additional information on dose modifications

### 7.6 SAE Reporting and Timeframe

Reporting requirements will comply with all EU safety reporting requirements as detailed in Clinical Trials Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use, repealing Directive 2001/20/EC and all applicable local regulations for safety reporting.

The investigator or investigator's team will report all protocol defined SAEs and AESIs to the Sponsor (MEDSIR) no later than 24 hours of any site study team staff becoming aware of the event as follows:

- The full details of the SAE and/or AESI should be collected and fully documented using the SAE form and sent to the Sponsor (MEDSIR).
- Follow-up information, copies of any relevant test results, event outcome and the opinion of the investigator as to the relationship between trastuzumab deruxtecan (DS-8201a) and the SAE and AESI, accompanied by other applicable documentation when it is requested, will be sent along with the SAE form, if available on the day the event is reported or as soon as possible if it is not.
- The original SAE reporting form and the confirmation from the Sponsor must be kept with the CRF documentation at the study site(s).

All SAE forms will be sent by the investigator or investigator's team to the Sponsor (MEDSIR) according to the reporting instructions provided by MEDSIR at the site initiation visit and filed in the Investigator's File.

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

As sponsor, MEDSIR will be responsible for ensuring that events are reported within the mandated timeframe to the European Medicines Agency (EMA), and other competent authorities, IRBs/ECs, and investigator(s), as necessary and in accordance with all applicable guidelines, approved directives and regulations. All safety reporting local regulatory requirements will be followed.

### 7.7 Expedited Reporting to Health Authorities, Investigators, IRBs, and ECs

To determine reporting requirements for single SAE cases, MEDSIR (as Sponsor) or its designee will assess the expectedness of these events using the following reference documents:

- IB for trastuzumab deruxtecan (DS-8201a).

MEDSIR (as Sponsor) or its designee will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

Within seven calendar days after being notified of the event, MEDSIR (as Sponsor) or its designee will report unexpected fatal or life-threatening events associated with the use of the study drug to the regulatory agencies and competent authorities, to the investigators and IRBs/ECs. MEDSIR (as Sponsor) or its designee will report other unexpected SAEs associated with the use of the study medication to the appropriate competent authorities (according to local guidelines), investigators, and central IRBs/ECs by a written safety report within 15 calendar days of notification. All safety expedited reports will be reported in accordance to all regulatory reporting obligations (including timelines) and local regulatory requirements.

### 7.8 Other Safety-Related Reports

As Sponsor, MEDSIR will assess constantly the benefit/risk profile of the trial, that means a continuous evaluation of the safety profile of the drugs under investigation will be done using all available information. MEDSIR will provide the regulatory agencies and competent authorities and the investigators with any relevant information that may affect the benefit/risk profile of the trial. An annual Development Safety Update Report (DSUR) safety report for trastuzumab deruxtecan (DS-8201a) will be prepared and distributed by MEDSIR or its designee in accordance to all regulatory reporting obligations and local regulatory requirements.

MEDSIR or its designee will report any finding of noncompliance (as failure to follow any applicable regulation or institutional policies that govern human subjects' research) and/or serious noncompliance (as noncompliance that materially increases risks that result in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants) according to any reporting obligation and local regulatory requirements.

### 7.9 Pregnancy Reporting

Irrespective of the treatment received by the patient, any patient's or patient's partner pregnancy occurring during study treatment or within 30 days after completing therapy must be reported within 24 hours of investigator's knowledge of the event.

Pregnancies will be treated as SAEs and the investigator will complete a pregnancy form and forward it to the Sponsor according to the reporting instructions provided by MEDSIR at the site initiation visit and filed in the investigator's File.

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

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

Additional follow-up information on any trastuzumab deruxtecan (DS-8201a)-exposed pregnancy and infant will be requested at specific time points (e.g., after having received the initial report, at the end of the second trimester, two weeks after the expected date of delivery, and at three, six, and 12 months of the infant's life).

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

# 8 STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

## 8.1 Determination of Sample Size

A maximum of 39 patients with pretreated unresectable locally advanced or metastatic HER2-positive or HER2-low expressing patients will be recruited following the five study cohorts:

- **Cohort 1**: Patients with HER2-positive BC and non-progressing BM (after WBRT and/or SRS);(N = 8);
- **Cohort 2**: Patients with HER2-positive or HER2-low expressing BC with untreated BMs (N = 10);
- **Cohort 3**: Patients with HER2-positive BC and progressing BMs after local treatment (N = 7);
- **Cohort 4:** Patients with HER2-low expressing and progressing BMs after local treatment (N = 7);
- **Cohort 5:** Patients with HER2-positive BC and LMC (N = 7).

## 8.2 Justification of Sample Size

The primary endpoint for this study, in the cohort of patients with stable BMs (cohort 1) is the PFS at 16 weeks. The primary endpoint for this study, in the cohorts of patients with untreated or progression BMs (cohorts 2 to 4) is the best ORR-IC. The primary endpoint for this study, in the cohort of patients with LMC (cohort 5) is the OS.

The trial uses a two-stage optimal Simon's design for the cohort 1, a one-stage A'Hern design for the cohorts 2 to 4, and a one-stage survival design for the cohort 5. The analysis in the cohort 1 will be one-sided, based on stochastic ordering of uniformly minimum variance unbiased estimator (UMVUE). The analyses in the cohorts 2 to 4 will be one-sided, based on stochastic binomial exact test. The sample size for the LMC cohort was based on a one arm time-to-event design. The analysis will be based on maximum likelihood exponential test.

The local type I and II errors for all designs will be 0.05 and 0.2, respectively.

- **Cohort 1**

We hypothesized that excluding a rate of patients without PFS events ≤5% while targeting an improvement of patients without PFS events to ≥40% would be an optimal approach to evaluation of the study strategy. Non evaluable patients enrolled in the initial stage can be replaced. We will accrual 4 patients in the first stage. We will stop this cohort if all 4 patients progressed at 16 weeks. Otherwise, 4 additional patients will be accrued for a total of 8. At least 2 patients without progressive disease at 6 months among 8 patients enrolled will be adequate to justify the investigation of this strategy in further clinical trials.

- **Cohort 2**

We hypothesized that excluding an ORR-IC ≤5% while targeting an improvement of the ORR-IC to ≥40% would be an optimal approach to evaluation of the study strategy. At least 3 patients with overall response among 10 patients will be adequate to justify the investigation of this strategy in further clinical trials.

- **Cohort 3**

We hypothesized that excluding an ORR-IC ≤5% while targeting an improvement of the ORR-IC to ≥40% would be an optimal approach to evaluation of the study strategy. At least 2 patients with overall response among 7 patients will be adequate to justify the investigation of this strategy in further clinical trials.

- **Cohort 4**

We hypothesized that excluding an ORR-IC ≤5% while targeting an improvement of the ORR-IC to ≥40% would be an optimal approach to evaluation of the study strategy. At least 2 patients with overall response among 7 patients will be adequate to justify the investigation of this strategy in further clinical trials.

- **Cohort 5**

The one-sided maximum likelihood exponential test had an 80% power to detect a 4-month increase in median OS over a 2-month median OS. We scheduled an 18-month accrual period and 6-month of follow-up period. At least 3 patients will be accrued in the first stage to evaluate the feasibility of this cohort. A total of 7 petiens will be accrued in this cohort.

<u>Stopping boundary based on accrual</u>

At the halfway point of the recruitment period, the steering committee will evaluate if the accrual objective for the first stage was achieved in every study cohort (4, 5, 3, 3 and 3 patients for cohorts 1, 2, 3, 4 and 5, respectively). If this accrual objective has not achieved in a cohort, the Steering Committee will propose a corrective action plan or finalize the study in this cohort.

## 8.3 Analysis Sets

- **Full Analysis and Safety Set**

The full analysis and safety set includes patients who received at least one dose of study treatment.

- **Per-Protocol Set**

All patients who meet selection criteria, receive at least one drug exposure, and receive the per protocol (PP) required study drug exposure and processing. Criteria for determining the PP group assignment would be established by the Steering Committee before the statistical analysis begins. This analysis will only occur if this set differs by ≥ 10% from the full analysis set.

- **Exploratory Evaluable Set**

Exploratory analyses will be performed on those patients in the safety set who consented to participate in the exploratory research program and were evaluable for exploratory endpoints.

- **Analysis Set Schedule**

Primary and secondary endpoints will be analyzed on the full analysis set, and per-protocol sets. Full analysis set will be considered the primary population for the analysis. Exploratory analysis will be performed on [redacted]. Safety analysis will be performed on the safety set.

## 8.4 Response Assessment

Lesions will be classified as target and non-target at baseline, in accordance with RANO-BM criteria (for CNS lesions) and RECIST criteria v.1.1 (in case of non-CNS lesions).

- **RECIST v.1.1 Criteria**

The response assessment of the tumor is defined as best response, in terms of complete CR, PR, SD, PD and non-evaluable (NE) according the following Table 4:

**Table 4. Time Point Response in Patients with Target (+/–Non-Target) Disease**

| Target lesions | Non-target lesions | New lesions | Overall response |
|---|---|---|---|
| CR | CR | No | CR |
| CR | Non-CR/non-PD | No | PR |
| CR | Not evaluated | No | PR |
| PR | Non-PD or not all evaluated | No | PR |
| SD | Non-PD or not all evaluated | No | SD |
| Not all evaluated | Non-PD | No | NE |
| PD | Any | Yes or No | PD |
| Any | PD | Yes or No | PD |
| Any | Any | Yes | PD |

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

The objective tumor response for target lesions will be defined as follows:

- CR: Complete disappearance of all target lesions. Any pathological lymph nodes (target or non-target) must have reduction in short axis to < 10 millimeter (mm).
- PR: At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- PD: At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum diameters while on study. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The development of new, previously undetected lesions is also considered progression.
- SD: Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

The tumor response for non-target lesions will be defined as follows:

- CR: Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).
- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
- PD: Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

- **RANO-BM criteria**

**Target lesions**

- CR: Disappearance of all CNS target lesions sustained for at least 4 weeks; with no new lesions, no use of corticosteroids, and patient is stable or improved clinically.
- PR: At least a 30% decrease in the sum longest diameter of CNS target lesions, taking as reference the baseline sum longest diameter sustained for at least 4 weeks; no new lesions; stable to decreased corticosteroid dose; stable or improved clinically.
- PD: At least a 20% increase in the sum longest diameter of CNS target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, at least one lesion must increase by an absolute value of 5 mm or more to be considered progression.
- SD: Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter while on study.

**Non-target lesions**

Non-target lesions should be assessed qualitatively at each of the timepoints specified in

the protocol.

- CR: Requires all of the following: disappearance of all enhancing CNS non-target lesions, no new CNS lesions.
- Non-CR or non-PD: Persistence of one or more non-target CNS lesion or lesions.
- PD: Any of the following: unequivocal progression of existing enhancing non-target CNS lesions, new lesion(s) (except while on immunotherapy-based treatment), or unequivocal progression of existing tumor-related non-enhancing (T2/FLAIR) CNS lesions. In the case of immunotherapy-based treatment, new lesions alone may not constitute progressive disease.

### 8.5 Primary Efficacy Endpoints

The primary endpoint for this study, in the cohort of patients with stable BMs (cohort 1) is the PFS at 16 weeks. PFS is defined as the number of patients without PD or death at 16 weeks (non-PFS event) divided by the number of patients in the analysis set. Progression will be assessed based on local Investigator's assessment according RANO-BM criteria (for IC lesions) and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.. We will consider a patient as PFS event if accomplish any of both criteria for progression or die from

any cause in absence of documented progressive disease. The schedule of tumor assessments has been defined in AAppendices

Appendix 1.

The primary endpoint for this study, in the cohorts of patients with untreated or progression BMs (cohorts 2 to 4) is the best ORR-IC. The ORR-IC is defined as the number of patients with CR or PR divided by the number of patients in the analysis set. Best overall response will be assessed based on local Investigator's assessment according RANO-BM criteria. We will consider a patient as responder if accomplish the criteria at the same tumor evaluation. Patients without tumor response evaluation will be considered as no responder.

The primary endpoint for this study, in the cohorts of patients with LMC (cohort 5) is the OS. OS is defined as the time from treatment initiation to death from any cause. Patients without documented death at the time of the final analysis will be censored at the date of the last follow-up.

### 8.6 Secondary Efficacy Endpoints

The secondary efficacy variables are PFS, ORR, DOR, TTR, OS and Maximum Tumor reduction:

- ORR in cohort 1 to 4, as defined in secondary endpoint section.
- OS in cohorts 1 to 4, as defined in secondary endpoint section.
- PFS in cohorts 2 to 4, as defined in secondary endpoint section.
- PFS in cohorts 2 to 4, defined as time to event outcome. PFS is defined as the period of time from treatment initiation to the first occurrence of disease progression or death from any cause, whichever occurs first. Progression will be determined locally per RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.. We will consider a patient as PFS event if accomplish any of both criteria for progression or die from any cause in absence of documented progressive disease.
- CBR in cohorts 1 to 4, CBR is defined as an objective response (CR or PR), or SD for at least 24 weeks, and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions..
- TTR in Study cohort 1. TTR, defined as the time from the treatment initiation to time of the first objective tumor response observed for patients who achieved a CR or PR, determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions
- TTR in study cohorts 2 to 4. TTR defined as the time from the treatment initiation to time of the first objective tumor response (tumor shrinkage of ≥ 30%) observed for patients who achieved a CR or PR, determined locally by the investigator through the use of RANO-BM

criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.

- DOR in study cohorts 1 to 4, DOR is defined as the time from the first occurrence of a documented objective response to disease progression or death from any cause, whichever occurs first, as determined locally by the Investigator through the use of RANO-BM criteria for IC lesiosn and RECIST v.1.1 criteria, for both extracranial lesions and overall (IC and extracranial) lesions. In case of the patient first reaching PR and later CR, the duration of CR will be measured and reported separately, starting from the date when first documented, and ending when a progressive disease is diagnosed, or the patient dies.
- Best percentage of change from baseline in the size of tumor lesions, defined as the biggest decrease, or smallest increase if no decrease will be observed, and determined locally by the investigator through the use of RANO-BM criteria for IC lesions and RECIST criteria v.1.1 for both extracranial lesions and overall (IC and extracranial) lesions.
- Maximum tumor reduction in study cohorts 1 to 4. Maximum tumor reduction is defined as the biggest decrease, or smallest increase if no decrease will be observed, as determined locally by the Investigator through the use of RANO-BM criteria.

### 8.7 Exploratory Endpoints

The objective of the statistical analyses of biomarkers is the identification of those markers or combinations of markers which show a positive (predictors of benefit) or negative (predictors of resistances) association with the clinical outcome of treatment. We will measure the clinical efficacy of those analysis in terms of [redacted]. The biomarker analyses will be explorative. According to experience many biomarkers show [redacted] across subjects and within subject. Frequently there is also some biochemical background of this [redacted], in that the [redacted] process has a [redacted]. Biomarkers with [redacted] may cause problems when [redacted] are to be used. When used as [redacted] in [redacted], these biomarkers as well can [redacted]. Therefore, s[redacted] or [redacted] need to be found which [redacted] these measurements into [redacted] with an [redacted]. Typical choices in the biomarker area are [redacted] of the [redacted]. These [redacted] do not [redacted] the order of the [redacted], such [redacted] based on [redacted] remain [redacted] by the [redacted]. The basic statistics and interdependencies of the different markers will be descriptively investigated.

- **Analysis of Baseline and Demographic Variables**

The demographics and baseline characteristics including disease history and prior therapy are summarized using descriptive statistics.

- **Decision Rules and Adjustment of Alpha in the Study**

The study would be defined as positive at final analysis in the study cohort 1, if the rate of patients without PFS events in the trastuzumab deruxtecan (DS-8201a) arm is statistically significantly better ($p<0.05$) than expected under the null hypothesis (H0: % of pts without PFS events ≤ 5%). The analysis will be one-sided, based on stochastic ordering of UMVUE.

The study would be defined as positive at final analysis in the study cohort 2, if the ORR in the trastuzumab deruxtecan (DS-8201a) arm is statistically significantly better ($p<0.05$) than expected under the null hypothesis (H0: ORR ≤ 5%). The analysis will be one-sided, based on binomial exact test.

The study would be defined as positive at final analysis in the study cohort 3, if the ORR in the trastuzumab deruxtecan (DS-8201a) arm is statistically significantly better ($p<0.05$) than expected under the null hypothesis (H0: ORR ≤ 5%). The analysis will be one-sided, based on binomial exact test.

The study would be defined as positive at final analysis in the study cohort 4, if the ORR in the trastuzumab deruxtecan (DS-8201a) arm is statistically significantly better ($p<0.05$) than expected under the null hypothesis (H0: ORR ≤ 5%). The analysis will be one-sided, based on binomial exact test.

The study would be defined as positive at final analysis in the study cohort 5, if the median overall survival in the DS-8310a arm is statistically significantly better ($p<0.05$) than expected under the null hypothesis (H0: median OS ≤ 2 months). The analysis will be based on the one-sided maximum likelihood exponential test.

- **Primary Efficacy Analysis**

We will describe number and proportion of patients without PFS, alive, or with overall response, at interim and final analyses. We will estimate these rates with the 95% confidence intervals (95%CI) based on stochastic ordering of UMVUE and Clopper-Pearson methods. The p-values will be calculated based on stochastic ordering of UMVUE methods and exact binomial test methods.

We will describe for time to event endpoints (OS) number and proportion of events, median survival time and survival rates, with corresponding 95% CI. We will use the Kaplan-Meier method.

Analysis will be performed on the Full analysis and PP sets. ITT will be considered the primary set for the analysis.

- **Secondary Efficacy Analysis**

We will describe number and proportion of patients with overall response, clinical benefit and without PFS. We will estimate the proportion with the 95% Pearson-Clopper confidence intervals. For time to event endpoints (PFS, ORR, CBR, TTR, DOR, and OS) we will use the Kaplan-Meier method. Number and proportion of events, median survival time and survival rates, with corresponding 95%CI, will be calculated in study arm. For continuous outcome (Maximum Tumor reduction) we will use statistics of central tendency (median with 95%CI) and dispersion [range and interquartile range]. We provide waterfall plots of maximum tumor shrinkage from baseline.

Analysis will be performed on the ITT, and PP sets. ITT will be considered the primary population for the analysis. For all tests, we will use two-sided p-values with alpha ≤ 0.05 level of significance.

- **Exploratory Analysis**

Changes in biomarkers will be evaluated on a [redacted] regarding their association with treatment. Baseline and post-treatment values of [redacted] biomarkers will be described with [redacted] and [redacted] in [redacted] (copy numbers, immunoscore…). Baseline and post-treatment values of [redacted] ([redacted] and [redacted]…) will be described with [redacted] and [redacted].

Change from baseline between values of quantitative biomarkers will be analyzed with [redacted] and [redacted] differences. The corresponding 95% confidence intervals, applicable test statistics and p-values will be presented. P-values and 95%CI for mean differences will be based on paired [redacted]. P-values for ranks will be based on [redacted]. The 95%CI for median difference will be based on bootstrap percentile method. Change from baseline between percentage of categorical biomarkers will be analyzed with [redacted].

Markers will be evaluated on a univariate level regarding their change over potential for prediction of the clinical endpoints [redacted]. Biomarker and response correlations with clinical [redacted] will be investigated. It will be checked whether [redacted] can improve the prediction and whether there is an interaction with the biomarkers. Further multivariate techniques (e.g.

[redacted] will be evaluated in order to study combinations of markers. Techniques to control [redacted] will be also considered. Analysis will be performed on [redacted].

### 8.8 Safety Analysis

Patient safety and AEs will be assessed using the NCI–CTCAE v.5.We will summarize AEs, AEs grade ≥ 3, SAEs (as described in previous section), premature withdrawal from study medication, laboratory parameters, exposure to study medication, concomitant medications, vital signs, ECOG performance status, and physical examination.

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

Laboratory parameters, hematology, and biochemistry, will be presented in shift tables of NCI–CTCAE grade at baseline versus worst grade during treatment.

Other safety variables, such as exposure to study medication, concomitant medications, vital signs, and physical examination, ECOG performance status will be summarized over time and the percentage of patients in different categories will be presented by bar charts at different time points.

### 8.9 Interim Analyses

*Futility interim analyses in cohort 1.*

A futility interim analysis has been planned in cohort 1. The recruitment will not be stopped during the interim analysis. However, after the interim analysis, if the number of patients achieving the primary endpoint is equal or less than futility boundary the cohort may be stopped. Otherwise, the stage II recruitment will be open when the criteria to study continuation was achieved. However, the decision to stop or continue the trial should be endorsed by the steering committee after reviewing the interim safety and efficacy data.

*Feasibility interim analyses at each cohort*

At the halfway point of the recruitment period, the steering committee will evaluate if the accrual objective for the first stage was achieved in each study cohorts (4, 5, 3, 3 and 3 patients for cohorts 1, 2, 3, 4 and 5, respectively). If this accrual objective has not achieved in a cohort, the Steering Committee will propose a corrective action plan or finalize the study in this cohort.

### 8.10 Missing Data Management

Study variables could be missing for patients who withdrawn from the trial or for specific visits. We will report reasons for withdrawal. Patient with missing values in tumor response outcomes (ORR, CBR or without PFS events at 6 months) will considered as no responders, or last observation will be carried forward (maximum tumor reduction). Other outcomes will be managed with simple imputations methods. The effect that any missing data might have on results will be assessed via sensitivity analysis of study data sets.

For PFS, TTR and DOR, patients without a date of disease progression or death will be analyzed as censored observations on the date of last tumor assessment. If no post-baseline tumor assessment is available, patients will be censored at the date of enrollment + 1 day. Data for patients with an event who missed two or more scheduled assessments immediately prior to the event will be censored at the last tumor assessment prior to the missed visits.

For OS, patients who are not reported as having died will be analyzed as censored observations on the date they were last known to be alive. If no post-baseline data are available, OS will be censored at the date of enrollment + 1 day.

### 8.11 Steering Committee Review

A Steering Committee will be established for this study. It will be composed by the study site Investigators, the Sponsor's Medical Monitor, the Scientific Global Coordinator, and additional physicians with experience in experimental therapy management.

The Steering Committee will meet on demand to review, discuss, and evaluate all of the gathered safety data. In case of any arising safety concern, these meetings can also be called at any time at request of a participating investigator. At these meetings, the Sponsor and the participating Investigators must reach a consensus on safety data. The Sponsor will prepare minutes from these meetings and circulate them to each Investigator for comment prior to finalization.

Study site Investigators and the Sponsor will review patient data at least every six months. Each study site Investigator will monitor patient's data for serious toxicities on an ongoing basis.

# 9 ETHICAL CONSIDERATIONS

## 9.1 Regulatory and Ethics Compliance

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

## 9.2 IRBs/IECs

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

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

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

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

## 9.3 Informed Consent

For each study subject, written ICF will be obtained prior to any protocol related activities. As part of this procedure, the study site Investigator or designee must explain orally and in writing the nature, duration, and purpose of the study, and the action of the drug in such a manner that the study subject is aware of the potential risks, inconveniences, or adverse effects that may occur. The study subject should be informed that he is free to withdraw from the study at any time. The subject will receive all information that is required by local regulations and ICH guidelines.

The ICF must be signed and dated by the patient before his participation in the study. The case history or clinical records for each patient shall document the informed consent process and that written ICF was obtained prior to participation in the study.

A copy of each signed Consent Form must be provided to the patient.

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

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

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

### 9.4 Data Protection

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

The Sponsor as Data Controller according to the EU Data Protection Directive (95/46/EC) and the General Data Protection Regulation (2016/679) (GDPR) on the protection of individuals with regard to the processing of personal data and on the free movement of such data confirms herewith compliance to Directive 95/46/EC and GDPR in all stages of Data Management.

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

# 10 SOURCE DOCUMENTATION, STUDY MONITORING, AND QUALITY ASSURANCE

## 10.1 Source Data Documentation

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

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

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

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

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

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

The source documents must also be available for inspection, verification, and copying, as required by regulations, officials of the regulatory health authorities (i.e., Food and Drug Administration [FDA], EMA, and others), and/or ECs/IRBs. The Investigator and study site staff must comply with applicable privacy, data protection, and medical confidentiality laws for use and disclosure of information related to the study and enrolled subjects.

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

### 10.2 Study Monitoring and Source Data Verification

Study progress will be monitored by MEDSIR or its representative (i.e., a Clinical Research Organization [CRO]) as frequently as necessary to ensure:

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

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

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

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

### 10.3 Retention of Records

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

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. “Essential documents” are defined as documents that individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents should be retained for a longer period, however, if required by the applicable e regulatory requirements or by an agreement with the Sponsor. The CHMP requires retention for the maximum period of time permitted by the institution, but not less than 15 years (ICH E6[R2], 4.9.5). It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained (ICH E6[R2], 5.5.12).

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

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

### 10.4 Data Quality Assurance

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

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

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

In accordance with ICH E6[R2] GCP and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor's (or designee's) Quality Assurance Department. Inspection of site facilities (i.e., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the study conduct and compliance with the protocol, ICH GCP (ICH E6 [R2]), and applicable country regulatory requirements.

# 11 DATA MANAGEMENT

## 11.1 Data Entry and Management

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

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

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

## 11.2 Data Clarification

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

## 11.3 Data Coding Procedures

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

# 12 STUDY MANAGEMENT

## 12.1 Discontinuation of the Study

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

## 12.2 Protocol amendments

Any change or addition to this protocol requires a written protocol amendment or administrative letter that must be approved by MEDSIR, the Scientific Global Coordinator, the study site Investigator, and the IRB/IE/CA before implementation. This requirement for approval should in no way prevent any immediate action from being taken by the study site Investigator or MEDSIR in the interests of preserving the safety of all subjects included in the trial. If an immediate change to the protocol is felt to be necessary by the study site Investigator and is implemented for safety reasons, MEDSIR should be notified as soon as possible (within 24 hours if possible) and the IRB/IEC/CA should be informed as necessary.

## 12.3 Protocol Deviations

The investigator should document and explain any protocol deviations. The investigator should promptly report any deviations that might have an impact on patient safety and data integrity to the Sponsor and to the IRB/EC in accordance with established IRB/EC policies and procedures. The Sponsor will review all protocol deviations and assess whether any represent a serious breach of Good Clinical Practice guidelines and require reporting to health authorities. As per the Sponsor's standard operating procedures, prospective requests to deviate from the protocol,including requests to waive protocol eligibility criteria, are not allowed.

## 12.4 Publication Policy Protection of Trade Secrets

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

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

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

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# 14 Appendices

## 14.1 Appendix 1. Schedule of Assessments and Study Procedures

| *Study Period* | **Screening Period** | | **Treatment Period*** | | | **Post-Treatment Follow-Up Period** |
|---|---|---|---|---|---|---|
| *Day* | **-28 to -1** | **-14 to -1** | **Cycle 1 Day 1** | **Cycle X Day 1** | **Within 40 days (± 7 days) after last dose of study treatment (EoT)** | **Follow-up every 12 weeks (± 14 days)[19]** |
| Informed Consent Form[1] | X | | | | | |
| HER2 status[2] | X | | | | | |
| Medical history[3] | X | | | | | |
| Physical examination | | X | X | X | X | |
| ECOG performance status | | X | X | X | X | |
| Weight and vital signs[4] | | X | X | X | X | |
| Concomitant medication reporting[5] | | X | X | X | X | |
| AE reporting[6] | | X | X | X | X | |
| 12-lead ECG[7] | | X | X[7] | X[7] | X[7] | |
| ECHO or MUGA scan[8] | X | | | X[8] | X | |
| Troponin[9] | | X | | | X | |
| Ophthalmologic assessments[10] | X | | | | X | |
| $pSO_2$[11] | | X | X | X | X | |
| Tumor assessments[12] | X | | | X[12] | X[12] | |
| Tumor samples (for exploratory study)[13] | X (if feasible) | | | | At the time of progression (if feasible) | |
| Blood samples (for exploratory study)[14] | X | | | X | At the time of progression | |
| Hematology[15] | | X | X | X | X | |
| Chemistry[15] | | X | X | X | X | |

| *Study Period* | **Screening Period** | | **Treatment Period*** | | | **Post-Treatment Follow-Up Period** |
|---|---|---|---|---|---|---|
| *Day* | **-28 to -1** | **-14 to -1** | **Cycle 1 Day 1** | **Cycle X Day 1** | **Within 40 days (± 7 days) after last dose of study treatment (EoT)** | **Follow-up every 12 weeks (± 14 days)[19]** |
| INR/PT and aPTT [15] | | X | | | X | |
| Urinalysis | | X | | | | |
| Pregnancy test[16] | | X | X | X | X[16] | |
| Viral serology[17] | | X | | | | |
| Trastuzumab deruxtecan (DS-8201a) administration | | | X | X | | |
| Review patient diary | | | X | X | | |
| PRO assessments[18] | | X | X | X | X | X |
| Survival status | | | | | X | X |
| Post-study anticancer therapy | | | | | X | X |

AEs = Adverse events; aPTT = Activated partial thromboplastin time; ECG = Electrocardiogram; ECHO = Echocardiogram; ECOG = Eastern Cooperative Oncology Group; EoT = End of Treatment; HER2 = Human Epidermal Growth Factor Receptor 2; INR = International normalized ratio; MUGA = Multiple-Gated Acquisition; PRO QOL: Patient reported outcome-Quality of life; PT = Prothrombin time.
* All visits must occur within ± 2 working days. Assessments scheduled for Days 1 each cycle must be performed within 48 hours prior to study treatment administration, respectively, unless otherwise indicated in the schedule of assessments, in order to confirm to the patient if treatment can be followed up.

1. **Informed Consent Form:** Signed written Informed Consent Form obtained prior to any trial-specific procedure.
2. **HER2 status:** Local confirmation of HER2-positive status.
3. **Medical history:** Complete medical history and demographics (including age, gender, and ethnic origin). All medications taken in the last 28 days prior to enrolment will be collected.
4. **Weight and vital signs**: Weight, height (only at screening), respiratory rate, blood pressure measurements (systolic and diastolic), pulse rate, and body temperature (oral, axillary, or tympanic temperature). Vital signs should be measured at screening (within 14 days), before and after the end of the infusion on Day 1 of cycle 1 to 3 and only before infusion on Day 1 of subsequent cycles.

5. **Concomitant medication reporting:** Relevant concomitant medication will be recorded at screening and on an ongoing basis.
6. **AE reporting:** All AEs occurring during the trial and until 30 days after treatment discontinuation visit (EoT visit) have to be recorded with grading according to the NCI–CTCAE v.5.0 criteria.
7. **12-lead ECG:** ECGs (will be taken in triplicate, in close succession, while in a supine/semi-recumbent position) to be collected at screening (within 14 days before enrollment). Subsequent ECGs will be performed in triplicate in close succession. If an abnormality is noted, ECG will be taken. ECGs will be taken at every 4$^{th}$ cycle while in a supine/semirecumbent position.
8. **ECHO or MUGA scan:** LVEF assessment will be performed at screening (within 28 days before enrollment), Cycle 5 Day 1, and on Day 1 of every four cycles (±7 days) thereafter (e.g., Cycles 9, 13, 17, etc.) while on treatment, and at the EoT visit *Note: the same test must be used for the subject throughout the study*
9. **Troponin**:Collect blood samples for troponin (preferably high-sensitivity troponin-T) at screening, EOT, and if at any time a subject reports signs or symptoms suggesting congestive heart failure, myocardial infarction, or other causes of myocyte necrosis. Subjects may also have local troponin testing as clinically indicated during the treatment phase based on subject reported cardiac symptoms . If ECG is abnormal, follow institutional guidelines. If troponin levels are above the upper limit of normal and below the level of myocardial infarction as defined by the manufacturer (CTCAE Grade 1) at baseline, no repeat testing is required if the troponin level is not Grade 3.
10. **Ophthalmologic assessments**: They will include visual acuity testing, slit lamp examination and fundoscopy will be performed at screening (within 28 days before enrollment), at EoT and if patient present sign or symptoms of ophthalmologic problems.
11. **$pSO_2$:** pulse oximetry will be performed at screening (within 14 days) and on Day 1, of every cycle, and until the safety follow-up visit 40 days after last dose. During Cycle 1, pulse oximetry will be assessed on Day 1 prior and after infusion of trastuzumab deruxtecan, on Day 8 and Day 15. On Day 1 pre-dose measurements will be taken thereafter.
12. **Tumor assessments:** Baseline assessments of the chest, abdomen, and pelvis (preferably CT or MRI in case of contrast allergy) must be performed no more than 28 days before the first dose of study treatment. Post-baseline assessments will be performed every 6 weeks (± 3 working days) from the first dose of study treatment for the first 6 months of treatment and every 9 weeks (± 5 working days) thereafter using the same imaging method and where

possible obtained at the same institution for an individual patient as used during screening until progression disease or EoS. Bone scans will be only performed at baseline and every 24 weeks (± 7 working days) for patients with bone lesions identified at baseline, unless clinically or biochemically suspected bone progression. If a bone scan was performed > 28 days but ≤ 60 days prior to start of study treatment, the bone scan does not need to be repeated. Brain imaging (MRI) will be performed within 28 days prior to the first administration of study medication and during the trial should be performed every 6 weeks [± 3 working days] from the first dose of study treatment for the first 6 months, and, thereafter, every 9 weeks [± 5 working days]) unless clinically suspected brain progression. Patients who discontinue treatment without evidence of disease progression will be followed every nine weeks (± 5 days) for tumor assessments until documented progression, elective withdrawal from the study, the start of new anti-cancer treatment, or study completion or termination.*Note: For patients included in the study cohort 5, axis MRI and spinal tap for CSF collection must also be performed, at baseline, every 3 weeks for 12 weeks (corresponding to the first 5 cycles of treatment), every 6 weeks thereafter, and at the time of treatment progression or study termination.*

13. **Tumor samples (for exploratory study):** A tissue sample should be provided at baseline (if feasible) from breast primary tumor or metastases amenable to biopsy (at sites of locoregional recurrence [skin, chest wall, breast or lymph nodes], or distant recurrence [bone, liver, lung or abdomen]) that will be obtained between progression to the prior regimen and inclusion in the study. Patients for whom tissue sample cannot be obtained (e.g., non-measurable disease, inaccessible tumor or subject safety concern) may submit an archived metastatic tumor specimen only upon agreement from the Sponsor. If feasible, an additional tissue sample should be collected at the end of treatment visit for patients who discontinue treatment due to disease progression
14. **Blood samples (for exploratory study):** Blood samples are required for all patients at the time of inclusion, after two cycles of study treatment, and upon progression or study termination.
15. **Hematology/chemistry:** Cycle 1 Day 1 hematology and chemistry panel assessments are not required if the screening hematology and chemistry panel was performed at screening within 48 hours prior to start of study treatment. On cycle 1, hematology, chemistry assessment must be repeated on day 8 and day 15.Coagulation assessment INR/PT and PTT/aPTT only in screening (within 14 days) and EoT.
16. **Pregnancy test:** A serum pregnancy test at screening to confirm eligibility in the trial and within one week prior to start of study medication (with result available prior to dosing). This assessment does not need to be repeated at Cycle 1 Day 1 if it was performed at screening within 48 hours prior to start of

study treatment. Thereafter, repeat pregnancy tests (urine or serum test per institutional guideline) before infusion of each cycle and at end of treatment. A serum test should be performed to confirm any positive urine pregnancy test during the trial.

17. **Viral serology:** Human Immunodeficiency Virus, Hepatitis B surface Antigen (HBsAg), total Hepatitis B core Antibody (HBcAb), Hepatitis C Virus antibody; additional tests for Hepatitis B Virus DNA or Hepatitis C Virus RNA will be required within 14 days before enrollment to confirm eligibility. Patients positive for hepatitis C (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV RNA
18. **PRO Assessments:** The EORTC-QLQ-C30 and the EORTC-QLQ-BR23 questionnaires will be completed at baseline, Day 1 of cycles 2-4, then on Day 1 of every other subsequent cycle starting with cycle 6 (e.g. cycles 6, 8, 10, etc.), End of Treatment visit and during follow-up period until start of anticancer therapy.
19. **Follow-up every 12 weeks:** After study treatment discontinuation, post-treatment follow-up (including survival status and post-study anticancer therapy evaluation) will be collected every 12 weeks (± 14 days) from the last dose of study treatment up to the end of study visit. Telephone contact is acceptable.

## 14.2 Appendix 2. Response Evaluation Criteria According to RANO-BM Criteria (Lin et al. 2015)

Measurable disease is defined as a contrast-enhancing lesion that can be accurately measured in at least one dimension, with a minimum size of 10 mm, and is visible on two or more axial slices that are preferably 5 mm or less apart with 0 mm skip (and ideally ≤1·5 mm apart with 0 mm skip).

Additionally, although the longest diameter in the plane of measurement is to be recorded, the diameter perpendicular to the longest diameter in the plane of measurement should be at least 5 mm for the lesion to be considered measurable.

Measurement of a tumor around a cyst or surgical cavity is a particularly difficult challenge. Generally, such lesions should be considered non-measurable unless there is a nodular component that measures 10 mm or more in longest diameter and 5 mm or more in the perpendicular plane.

Non-measurable disease includes all other lesions, including lesions with longest dimension less than 10 mm, lesions with borders that cannot be reproducibly measured, dural metastases, bony skull metastases, cystic-only lesions, and leptomeningeal disease.

**Response assessment**

Target lesions

- Complete response: Disappearance of all central nervous system (CNS) target lesions sustained for at least 4 weeks; with no new lesions, no use of corticosteroids, and patient is stable or improved clinically.
- Partial response: At least a 30% decrease in the sum longest diameter of CNS target lesions, taking as reference the baseline sum longest diameter sustained for at least 4 weeks; no new lesions; stable to decreased corticosteroid dose; stable or improved clinically.
- Progressive disease: At least a 20% increase in the sum longest diameter of CNS target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, at least one lesion must increase by an absolute value of 5 mm or more to be considered progression.
- Stable disease: Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter while on study.

Non-target lesions

Non-target lesions should be assessed qualitatively at each of the timepoints specified in the protocol.

- Complete response: Requires all of the following: disappearance of all enhancing CNS non-target lesions, no new CNS lesions.
- Non-complete response or non-progressive disease: Persistence of one or more non-target CNS lesion or lesions.
- Progressive disease: Any of the following: unequivocal progression of existing enhancing non-target CNS lesions, new lesion(s) (except while on immunotherapy-based treatment), or unequivocal progression of existing tumor-related non-enhancing (T2/FLAIR) CNS lesions.

## 14.3 Appendix 3. Actions Required in Cases of Increases in Liver Biochemistry and Evaluation of Hy's Law.

**Introduction**

This Appendix describes the process to be followed in order to identify and appropriately report cases of Hy's Law (HL). It is not intended to be a comprehensive guide to the management of elevated liver biochemistries.

During the course of the study the Investigator will remain vigilant for increases in liver biochemistry. The investigator is responsible for determining whether a patient meets potential Hy's Law (PHL) criteria at any point during the study.

The Investigator participates in reviewing and assessing of cases meeting PHL criteria to agree whether HL criteria are met. HL criteria are met if there is no alternative explanation for the elevations in liver biochemistry other than Drug Induced Liver Injury (DILI) caused by the IMP.

The Investigator is responsible for recording data pertaining to PHL/HL cases and for reporting AE and SAE according to the outcome of the review and assessment in line with standard safety reporting processes.

**Definition**

**Potential Hy's Law (PHL)**

Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) ≥ 3x Upper Limit of Normal (ULN) together with Total Bilirubin (TBL) ≥ 2xULN at any point during the study following the start of study medication irrespective of an increase in Alkaline Phosphatase (ALP).

**Hy's Law (HL)**

AST or ALT ≥ 3x ULN together with TBL ≥ 2xULN, where no other reason, other than the IMP, can be found to explain the combination of increases (e.g., elevated ALP indicating cholestasis, viral hepatitis, another drug). Therefore, if increases are reasonably explainable as due to cholestasis other than drug liver injury, then reporting is not necessary

For PHL and HL the elevation in transaminases must precede or be coincident with (i.e. within one cycle) the elevation in TBL, but there is no specified timeframe within which the elevations in transaminases and TBL must occur.

**Identification of Potential HY'S law cases**

In order to identify cases of PHL it is important to perform a comprehensive review of laboratory data for any patient who meets any of the following identification criteria in isolation or in combination:

• ALT ≥ 3xULN;

• AST ≥ 3xULN;

• TBL ≥ 2xULN.

Note: There are different processes for the identification of potential Hy's law cases depending on whether central or local laboratories are being used.

When a patient meets any of the identification criteria, in isolation or in combination, the Investigator will immediately:

• Notify the Sponsor's representative (or designee's);

• Request a repeat of the test (new blood draw) by the local laboratory;

• Complete the appropriate unscheduled laboratory CRF module(s) with the original local laboratory test result.

When the identification criteria are met from local laboratory results the Investigator will without delay:

• Determine whether the patient meets PHL criteria (see 2. Definition within this Appendix) by reviewing laboratory reports from all previous visits.

The Investigator will without delay review each new laboratory report and if the identification criteria are met will:

• Notify the Sponsor's representative (or designee's);

• Promptly enter the laboratory data into the laboratory CRF.

References

FDA Guidance for Industry (issued July 2009) 'Drug-induced liver injury: Premarketing clinical evaluation' (66):

https://www.fda.gov/media/116737/download

## 14.4 14.4 Appendix 4. Instructions related to Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2)

**Inclusion criteria**

1. *Has adequate treatment washout period before randomization/enrollment, defined as:*
    - *Chloroquine/Hydroxychloroquine: > 14 days*

**Prior and Concomitant Medications**

The following core safety protocol restrictions (*italicized* below) are applicable to all Phase 1-3 clinical study protocols developed on or after the PSR effective date, regardless of indication. Contact your GCL and CSPV representatives for protocol-specific safety restrictions that may differ from the core minimum restrictions presented hereafter.

Concomitant treatment with chloroquine or hydroxychloroquine is not allowed during the study treatment. If treatment with chloroquine or hydroxychloroquine treatment is absolutely required for SARS-CoV-2 (ie COVID-19), study treatment must be interrupted. If chloroquine or hydroxychloroquine is administered, then a wash-out period of no less than >14 days is required before restarting study treatment.

**Dose modification criteria for suspected or confirmed SARS-CoV-2**

All confirmed or suspected SARS-CoV-2 infection events must be recorded in the eCRF. Dose modifications will be based on the worst CTCAE grade. All interruptions or modifications must be recorded on the AE and drug administration eCRFs. **Please use CTCAE v5.0 general grading criteria to evaluate COVID-19.**

**Dose modification criteria**

If SARS-CoV-2 infection is suspected, interrupt trastuzumab deruxtecan and rule out SARS-CoV-2 per local guidance.

- If SARS-CoV-2 is ruled out, follow dose modification and management guidelines as outlined in the CE protocol template.
- If SARS-CoV-2 is confirmed or diagnosis is suspected after evaluation, follow dose modification as outlined below and manage SARS-CoV-2 per local guidance until recovery of SARS-CoV-2. SARS-CoV-2 recovery is defined as no signs/symptoms

SARS-CoV-2, at least 1 negative RT-PCR test result*,and nearly or completely resolved chest CT findings. Then follow below dose modifications:

**SARS-CoV-2 Dose Modification Criteria**

| **SARS-CoV-2 Worst Toxicity NCI-CTCAE Version 5.0 Grade (unless otherwise specified)** | **Schedule Modification for trastuzumab deruxtecan** |
|---|---|
| Grade 1 | Resume study drug at the same dose[a] |
| Grade 2 | Resume study drug at the same dose if chest CT findings are completely resolved[a]<br>Reduce by 1 dose level if chest CT findings are nearly resolved |
| Grade 3 | Reduce by 1 dose level if chest CT findings are completely resolved<br>Discontinue study drug if chest CT findings are **not** completely resolved |
| Grade 4 | Discontinue study drug |

SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); CT = computed tomography

[a] Closely monitor signs/symptoms after resuming study treatment, initially with a phone call every 3 days for the first week, and then with a weekly phone call thereafter, for a total of 6 weeks.

- In addition to the recommendations outlined in the table above, Investigators may consider dose modifications of the study drug according to the subject's condition and after discussion with the study Medical Monitor or designee.
- If an event is suspected to be drug related ILD/pneumonitis, manage per protocol ILD/pneumonitis management guideline.

* If PCR testing is not available, the subject must not have any sign/symptoms for at least 2 weeks, in addition to meeting the requirement for chest CT imaging.