Statistical Analysis Plan (SAP)

Final analysis of DAISY

DAISY

Protocol Version n°2.0 – January 31, 2020

Phase 2, Open label Study of DS-8201a, an Anti-HER2-AntIbody Drug Conjugate (ADC) for advanced BreaSt Cancer patients, with biomarkers analysis to characterize response/resistance to therapY

EudraCT N°: 2018-004868-57
Sponsor N°: UC-0105/1815

Version n°1.0 – May 28, 2021

Sponsor: UNICANCER. 101 rue de Tolbiac 75654 Paris Cedex 13

Principal Investigator: Dr Véronique DIERAS, Centre Eugène Marquis, Rennes

Co-Coordinator: Dr Elise DELUCHE, Hôpital DUPUYTREN – CHU de Limoges, Limoges

Ancillary/translational coordinator: Prof Fabrice ANDRE, Gustave Roussy, Villejuif

Methodologist: Thomas FILLERON, Institut Claudius Regaud, IUCT-O, Toulouse

Biostatistician: Amélie LUSQUE, Institut Claudius Regaud, IUCT-O, Toulouse

APPROVAL AND SIGNATORIES OF THE STATISTICAL ANALYSIS PLAN

	Name	Date (dd/mm/yyyy)	Signature
Principal Investigator	Dr Véronique DIERAS	Fait le 08/09/2021	Véronique DIERAS Signé par Véronique DIERAS Signé et certifié par yousign
Sponsor	Céline MAHIER AIT OUKHATAR	Fait le 18/06/2021	Céline Mahier Ait-Oukhatar Signé par Céline Mahier Ait-Oukhatar Signé et certifié par yousign
Biostatistician	Thomas FILLERON	Fait le 18/06/2021	Thomas FILLERON Signé par Thomas FILLERON Signé et certifié par yousign

LIST OF ABBREVIATIONS

AE	Adverse event
CBR	Clinical benefit rate
CR	Complete response
DOR	Duration of response
FAS	Full analysis set
HER2	Human epidermal growth factor receptor 2
IDMC	Independent data monitoring committee
IHC	Immunohistochemistry
INN	International non-proprietary name
ISH	In situ hybridization
NCI	National cancer institute
OS	Overall survival
PD	Progressive disease
PR	Partial response
PFS	Progression-free survival
RECIST	Response evaluation criteria in solid tumors
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Stable disease

TABLE OF CONTENTS

1. INTRODUCTION

This document describes the statistical analyses planned for the DAISY clinical trial (EudraCT N°: 2018-004868-57). The statistical analyses will be carried out using SAS 9.4 and STATA 16 softwares at the Institut Claudius Regaud IUCT-Oncopole (Service de Biostatistique, Bureau des Essais Cliniques) under supervision of Thomas Filleron.

This document contents a brief description of the clinical trial (design, sample size, objectives and endpoints) and the statistical methodology that will be used to analyze the primary and secondary objectives of the DAISY clinical trial.

1.1 STUDY DESIGN

This is a multicenter, open-label phase II trial assessing the efficacy of DS-8201a monotherapy in patients with metastatic breast cancer. The design of the trial is shown in the figure below.

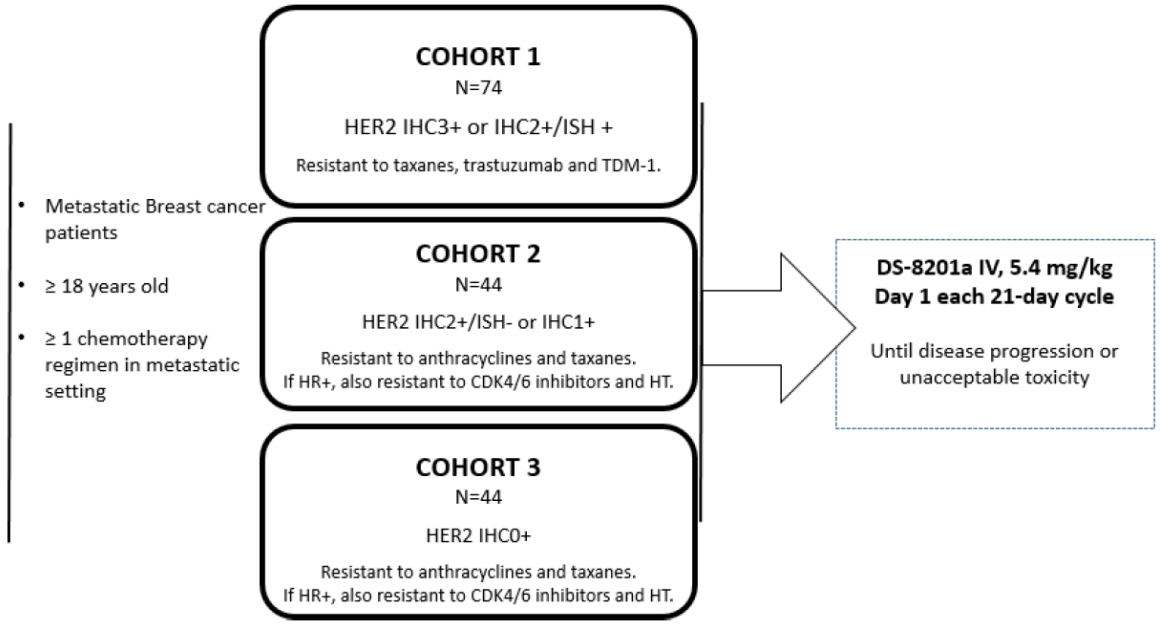

Figure: study scheme from the protocol Version n°2.0 – January 31, 2020 and Version 3.0 – January 25, 2021.

DS-8201a will be administrated as an intra-venous injection of 5.4 mg/kg every three weeks (1 cycle = 21-day treatment). DS8201a will be given until disease progression and/or unacceptable toxicity. Table below gives the description of the posology and the method of administration.

Drug Name (INN)	**Registered Name**	**Pharmaceutical Form**	**Administration Route**	**Posology**
DS-8201a (Trastuzumab deruxtecan)	NA	Lyophilized powder	Intra-venous	5.4 mg/kg every 21 days

Two dose reductions will be permitted as shown in table below.

Starting Dose	**Dose Level -1**	**Dose Level -2**
5.4 mg/kg	4.4 mg/kg	3.2 mg/kg

1.2 SAMPLE SIZE

One hundred and sixty-two patients have to be included in three cohorts to answer the main question of this study:

- Cohort 1 : 74 patients HER2 IHC3+ or IHC2+/ISH+
- Cohort 2 : 44 patients HER2 IHC1+ or IHC2+/ISH-
- Cohort 3 : 44 patients HER2 IHC0+

However, since the HER2 status is confirmed after inclusion, some switches may occur after inclusion from the initially allocated cohort to the confirmed HER2 status cohort.

Considering that cohort 2 will be the first to be terminated due to the higher frequency of HER2 IHC1+ or 2+/ISH- scores, we thus estimate that there will be around 25% of switches from cohort 1 and 3 to cohort 2, until they will be completed.

In order to reach the expected cohorts sample size, we estimate that around 30 additional patients may be necessary at the most to achieve the expected recruitment in cohort 1 and 3.

1.2.1 Cohort 1: HER2 IHC3+ or IHC2+/ISH+

The primary endpoint of the phase II is the rate of patients presenting an objective response. The following hypotheses are used for cohort 1:

- p0 = 30%, maximal unacceptable rate of patient presenting an objective response for whom the experimental treatment will be considered as insufficiently active.
- p1 = 45%, minimal acceptable rate of patients presenting an objective response for whom the experimental treatment will be considered as sufficiently active.

Using an A'Hern design (alpha = 5%, 1-beta = 80%) and (p0 = 30%; p1 = 45%), 67 evaluable patients need to be included in the study. The decision rules are summarized in the table below:

Nb of patients	Insufficiently active	Sufficiently active
67 evaluable / 74 included	<27 successes	≥27 successes

Assuming a rate of around 10% of non-evaluable patients for the first assessment, to reach 67 evaluable patients, 67/90% = 74 patients HER2 IHC3+ or IHC2+/ISH+ have to be included in the cohort 1.

1.2.2 Cohort 2 HER2 IHC1+ or IHC2+/ISH-

The primary endpoint of the trial is the rate of patients presenting an objective response. The following hypotheses are used for cohort 2:

- p0 = 20%, maximal unacceptable rate of patients presenting an objective response for whom the experimental treatment will be considered as insufficiently active.
- p1 = 40%, minimal acceptable rate of patients presenting an objective response for whom the experimental treatment will be considered as sufficiently active.

Using an A'Hern design (alpha = 5%, 1-beta = 85%) and (p0 = 20%; p1 = 40%), 40 evaluable patients need to be included in the study. The decision rules are summarized in table below:

Nb of patients	Insufficiently active	Sufficiently active
40 evaluable / 44 included	<13 successes	≥13 successes

Assuming a rate of around 10% of non-evaluable patients for the first assessment, to reach 40 evaluable patients, 40/90% = 44 patients HER2 IHC1+ or IHC2+/ISH- have to be included in the cohort 2.

1.2.3 Cohort 3: HER2 IHC0+

The primary endpoint is the rate of patients presenting a success. A success is defined as a patient presenting an objective response.
The short-term endpoint is the rate of patient without progression at 3 months.

The following hypotheses are used for cohort 3:

- Primary endpoint:
  - p10 = 20%, maximal unacceptable rate of patients presenting a success for whom the experimental treatment will be considered as insufficiently active.
  - p11 = 40%, minimal acceptable rate of patients presenting a success for whom the experimental treatment will be considered as sufficiently active.
- Short-term endpoint:
  - p20 = 30%, maximal unacceptable rate of patients without progression at 3 months for whom the experimental treatment will be considered as insufficiently active.
  - p21 = 50%, minimal acceptable rate of patients without progression at 3 months for whom the experimental treatment will be considered as sufficiently active.

Using a Kunz design (Kunz et al., SMMR 2017) with (alpha=5%, 1-beta=85%) and (p10=20%, p11=40%, p20=30% and p21=50%), 40 patients need to be included in the study and an interim analysis will be performed after inclusion of 16 patients. The decision rules are summarized in the table below:

Step	Nb of patients	Insufficiently active	Sufficiently active
1	16	≤ 4 non-progression	
2	40 evaluable / 44 included	≤ 12 successes	≥13 successes

Assuming a rate of around 10% of non-evaluable patients for the first assessment, to reach 40 evaluable patients, 40/90% = 44 patients HER2 IHC0+ have to be included in the cohort 3.

2. OBJECTIVES

Primary objective:
The main objective is to evaluate the anti-tumor activity of DS-8201a in the three cohorts of advanced breast cancer patients

- a cohort of HER2 over-expressing (HER2 IHC3+ or HER2 IHC2+/ISH+)
- a cohort HER2 low-expressing (IHC1+ or IHC2+/ISH-)
- a cohort HER2 non-expressing (IHC0+)

Secondary objectives:

- To evaluate the efficacy in each cohort, in term of:
    - Best objective response as assessed by central review
    - Progression Free Survival (PFS)
    - Duration of response (DOR)
    - Clinical benefit rate (CBR)
    - Overall survival (OS)
- To evaluate the safety of DS-8201a overall and per cohort by NCI-CTCAE v5.0

Translational objectives:

- Exploration of bystander effect of DS-8201a in human samples
- Description of immune effects of DS-8201a
- Develop predictors of primary resistance or outcome
- Identify mechanisms of secondary resistance

3. ENDPOINTS

3.1 PRIMARY ENDPOINT

The primary endpoint is anti-tumor activity of DS-8201a, carried out by the determination of the confirmed best objective response (BOR) rate in each cohort. The BOR will be defined as the presence of a confirmed partial or complete response assessed by investigators. The investigator will evaluate the objective response using RECIST v1.1 criteria (Eisenhauer 2009).

For the cohort 3 (IHC0+), a short term primary endpoint is used for the interim analysis. The short term primary endpoint is the rate of patient without progression at 3 months. The investigator will evaluate the progression using RECIST v1.1 criteria (Eisenhauer 2009).

Tumor assessments should be obtained at least every 6 weeks (+/-7 days) during the first 12 months of treatment phase, and every 12 weeks (+/- 7 days) thereafter. Imaging disease assessments should be repeated at least 4 weeks after assessment of a PR or CR as per RECIST v1.1 guidelines.

For patients dropped-out for other reasons than disease progression, tumor evaluation has to be continued during the post-treatment period and documented every 6 weeks (or every 12 weeks after the first 12 months of treatment phase) until disease progression or initiation of an antineoplastic treatment.

3.2 SECONDARY ENDPOINTS

3.2.1 Efficacy

The efficacy endpoints will be evaluated using RECIST v1.1 criteria (Eisenhauer 2009) with the following parameters.

Best objective response (BOR) by central review
The BOR assessed by central review will be defined as the presence of a confirmed partial or complete response assessed by central review. This central review will evaluate the objective response using RECIST v1.1 every 6 weeks.

Progression Free Survival (PFS)
PFS will be defined as the time from inclusion to the first documented progression of disease (assessed via RECIST v1.1 or clinical progression) or death due to any cause. At the time of analysis, a patient alive and without progression (including lost to follow-up) will be censored at the date of the last tumor assessment (TA).

If the patient progresses or dies after two or more missed visits, the patient will be censored at the last tumor assessment date before missed visits. Given the scheduled visit assessment scheme, two missing visits will equate to more than 12 weeks since the previous tumor assessment in the period from baseline to 12 months or to more than 24 weeks after the first 12 months.

Progression and censoring scheme are summarized in the following table:

Situation	Date of progression or Censoring	Outcome
Progression documented between scheduled visits	Date of progression assessment	Progressed
No progression	Date of last TA with no documented progression	Censored
Death before first TA	Date of death	Progressed
Death between adequate assessment visit	Date of death	Progressed
Death or progression after more than two missed visit	Date of last TA with documented non-progression before missed visit	Censored

Duration of response (DOR)
DOR will be applicable to subject with the best overall response (BOR), either complete response (CR) or partial response (PR), and will be defined as the time from the first documented CR or PR to the date of disease progression or death.

Clinical benefit rate (CBR)
CBR will be defined as the presence of at least a complete response (CR) or partial response (PR), or a stable disease (SD) >6 months.

A sensitivity analysis will be performed to evaluate PFS, DOR and CBR using tumor assessments assessed by central review. This central review will evaluate using RECIST v1.1 every 6 weeks.

Overall Survival (OS)
OS will be defined as the time from inclusion to death due to any cause. Patients still alive at the time of analysis (including lost to follow-up) will be censored at the date of last follow-up.

3.2.2 Safety

Safety will be evaluated continuously using NCI-CTCAE v5.0 criteria.

3.3 TRANSLATIONAL ENDPOINTS

- Concentration of trastuzumab and deruxtecan in HER2 expressing and HER2 non expressing tumor cells at 24h after treatment initiation
- Percentage of stained cells and intensity of staining for immune response markers detected by IHC on tumor samples at baseline and 42 days after treatment initiation
- Percentage of stained cells and intensity of staining for biomarkers detected by IF and IHC on tumor samples at baseline
- Identification of genomic alterations of interest through whole exome sequencing and RNA sequencing on tumor and ctDNA samples collected after resistance to DS-8201a
- Describe the variation of CTC levels and evaluate its predictive value on the objective response, the clinical benefit, and survival
- Explore changes over time in tumor CTC phenotype induced by ADC

4. STATISTICAL CONSIDERATION

4.1 ANALYSIS POPULATIONS

Full Analysis Set (FAS) population: All patients who received at least one dose of study drug and who had a valid first post-baseline assessment of disease status or who had progressive disease (assessed via RECIST v1.1 or clinical progression).

Safety population: All patients who received the study drug at least once.

4.2 PATIENT DISPOSITION

Patient disposition will be summarized by cohort and on the overall population as follows:

- Number of patients by site
- Patients discontinuing treatment and the reason for treatment discontinuation
- Patients discontinuing study and the reason for study end
- Patients in the FAS population. Patients excluded will be listed with the reason for exclusion
- Patients in the Safety population. Patients excluded will be listed with the reason for exclusion

4.3 PROTOCOL DEVIATIONS

Major protocol deviations will be identified and documented before the database lock for the primary analysis.

4.4 BASELINE DATA

Characteristics will be summarized by cohort and on the overall population using descriptive statistics. Continuous variables will be summarized using median, minimum, maximum, number of available observations and number of missing observations. Qualitative variables will be summarized using counts, percentages and number of missing observations.

4.5 FOLLOW-UP DURATION

Median of follow-up with its 95% confidence interval (95%CI) will be described by cohort and on the overall population according to the reverse Kaplan-Meier method.

4.6 STUDY DRUG (DS-8201a)

Each of the following will be described by cohort and on the overall population:

- Duration of treatment
- Number of cycles
- Number of cycles by dose level of DS-8201a
- Reason for treatment discontinuation

4.7 PRIMARY ENDPOINT

The primary endpoint will be analyzed on the FAS population (see section “4.1 Analysis populations”). The primary endpoint of the phase II is the rate of patients presenting a best objective response as assessed by the investigator. It will be assessed and described by cohort using frequency, percentage and 95% confidence intervals (Binomial exact).

Short term endpoint for cohort 3 (IHC0+) will be analyzed on the FAS population. The short term endpoint of the cohort IHC0+ is the rate of patients presenting a non-progressive disease at 3 months according to RECIST v1.1 (local evaluation). It will be assessed and described using frequency, percentage and 95% confidence intervals (Binomial exact).

The recruitment will be interrupted in cohort 3 according to the methodology after the inclusion of 16 patients to perform the pre-planned interim analysis. The recruitment in the cohort will be resumed or definitely interrupted upon IDMC decision.

4.8 SECONDARY ENDPOINTS

4.8.1 Efficacy analysis

Secondary efficacy endpoints will be analyzed on the FAS population (see section “4.1 Analysis populations”):

- BOR by central review will be analyzed with the same methods as the primary endpoint (see section “4.7 Primary endpoint”).
- For each cohort and on the overall population, survival rates (PFS, OS, and duration of response) will be estimated at different time points using the Kaplan-Meier method (with their respective confidence interval). Median survival times will be estimated by cohort with corresponding 95% confidence interval.
- Clinical benefit rates will be assessed and described by cohort and on the overall population using frequency, percentage, and 95% confidence intervals (Binomial exact).

Sensitivity analyses of PFS, DOR and CBR assessed by central review will be analyzed with the same methods as described above.

4.8.2 Safety analysis

Safety analysis will be performed on the safety population (see section “4.1 Analysis populations”) by cohort and on the overall population. Each of the following will be assessed:

- AEs by maximum toxicity grade
- SAEs
- AEs and SAEs related to study drug
- AEs leading to DS8201a dose reduction or interruption
- AE with toxicity grade >2

Frequency and percentage will be computed for each event.

4.9 CONVENTIONS

The following conventions will be used in all analyses:

- 1 year = 365.25 days
- 1 month = 30.4375 days
- Duration = (last date – first date + 1)
- Interval = (last date – first date)

4.10 HANDLING OF MISSING DATA

The primary method for handling missing time-to-event data will be censoring.

The following conventions will be applied to impute partial date:

- If the day is missing, the 15th of the month will be used to replace the missing day.
- If the day and month are missing, June 15 will be used to replace the missing day and month.
- If the day, month, and year are all missing, the date will not be imputed.

Other missing data will not be imputed.