PROTOCOL SUMMARY

A) TRIAL IDENTIFICATION	
Sponsor – protocol code number: UC-BCG-2312	
EU-CT number: 2023-509811-98-00	
Version (Number & date): v1.3 – October 14, 2024	
Trial title: De-escalation of medical therapies in HER2-positive metastatic breast cancer in long-term persistent response and minimal residual disease undetectable in circulating tumor DNA **Phase (for trials on medicinal products):** Phase II	
Trial title for lay people: Decreasing treatment for metastatic HER2-Positive Breast Cancer with undetectable cancer levels in blood tests	
Abbreviated title: HEROES	
Coordinating investigator: Dr Thibault DE LA MOTTE ROUGE (Centre Eugène Marquis)	
Vice-Coordinating investigator: Dr Benjamin VERRET (Gustave Roussy)	
Number of centres: up to 35	**Number of patients:** 170

B) SPONSOR IDENTIFICATION	
Name:	**UNICANCER** 101, rue de Tolbiac 75654 Paris Cedex 13 France
Contact person:	Clara GUYONNEAU Project Manager **R&D UNICANCER** Tel: +33 (0)1 44 23 55 60 Email: c-guyonneau@unicancer.fr

C) TRIAL GENERAL INFORMATION

Indication:

Patients with HER2-positive locally advanced inoperable or metastatic breast cancer with disease controlled after 2 years of maintenance treatment with anti-HER2 targeted therapy.

Rational:

Maintenance therapy with Trastuzumab/Pertuzumab until progression after induction therapy with taxanes + Trastuzumab/Pertuzumab was established as the gold standard for HER2-positive metastatic breast cancer (MBC) based on results from the Cleopatra trial. Although this strategy is recommended by all major international guidelines, little is known about the optimal duration of maintenance therapy. New tools could help triage patients for whom maintenance of anti-HER2 targeted therapy can be spared. Signatera™ is a highly sensitive custom minimal residual disease (MRD) test using circulating tumor DNA (ctDNA).

The current HEROES trial will establish whether anti-HER2 targeted therapy maintenance treatment can be safely interrupted among selected patients with metastatic HER2-positive breast cancer and negative ctDNA. Because ctDNA is a very sensitive tool, it is anticipated that in most patients with progressive disease, progression will be detected solely on the basis of ctDNA positivity, before radiological or clinical progression. This may allow treatment to be resumed without impacting quality of life due to tumor progression. Furthermore, it is possible that restarting treatment after detection of minimal residual disease may be more effective than when progression is detected. This strategy has been shown to be useful in hematological cancers such as chronic myeloid leukemia.

As a consequence, this study has the potential to change the management of patients with MBC HER2+. This protocol could identify patients for whom temporary or permanent discontinuation of treatment is possible without impacting prognosis, thanks to the monitoring of MRD. It could also show that some patients could be cured in a metastatic situation. From an individual perspective, this strategy may reduce treatment-related toxicities without affecting disease outcome. This proof-of-concept study is a first step to optimize the benefit/toxicity/cost ratio of maintenance in this situation**.**

Trial description/design:

This is a multicentre, national, non-randomized, open-label, phase 2 study

Primary objective:

To evaluate the feasibility of therapeutic de-escalation in HER2-positive metastatic breast cancer with disease controlled after 2 years of maintenance treatment with anti-HER2 targeted therapy AND ctDNA negative testing.

Secondary objectives:

❖ **Efficacy:**

- To assess in the ctDNA negative cohort at baseline:
  - The investigator-assessed progression-free survival (PFS),
  - The ctDNA dynamics and positivity rate,
  - The overall survival (OS).
  - The molecular response
  - In patients with radiological progression and anti-HER2 treatment has been reintroduced:
    - The Overall Response Rate (ORR),
    - The Duration of Response (DoR)
- To investigate prognostic effect on PFS and OS of ctDNA values at specific landmark times (3 months, 6 months, 9 months 12 months, 15 months and 18 months)
- To assess among ctDNA positive cohort at baseline:
  - The PFS
  - A patern of progression and treatment in the subsequent line
  - The OS

❖ **Quality of life:**

- To assess the quality of life (QoL) and other patient reported outcomes (PROs) (anxiety and decision regret) in the ctDNA negative cohort.

Inclusion criteria:

1. Patient must have signed a written informed consent prior to any trial specific procedures. When the patient is physically unable to give their written consent, a trusted person of their choice, independent from the investigator or the sponsor, can confirm in signing the patient's consent;

   Note: A first consent will be obtained during the screening phase in order to collect and allow the shipment of the archived tumor sample of the patient and the first blood draw.

   After receipt of the ctDNA results, a second consent will only be collected from patients with a negative ctDNA test.

2. Men or women ≥ 18 years of age;
3. Documented diagnosis of locally advanced inoperable or metastatic histologically-proven HER2-positive breast cancer (HER2-positive is defined as HER2 3+ immunohistochemical overexpression, or the presence of HER2 amplification, according to ASCO-CAP guidelines);
4. Must have an adequate archival tumor tissue sample available for NGS analysis by central laboratory, in order to design the ctDNA test (based on most recent available tumor tissue sample, metastatic biopsy (bone tissue excluded) and primary tumor authorized);
5. Patient with ECOG Performance Status (PS) ≤1;
6. Patient must have received continuous anti-HER2 targeted therapy (including Trastuzumab, Trastuzumab/Pertuzumab, Trastuzumab-Deruxtecan or T-DM1) treatment for at least 2 years in any line setting, for their locally advanced inoperable or metastatic HER2 + breast cancer (prior treatment interruption of 3 months maximum is allowed), with complete response or partial response at last radiological assessment;

   Note: The number of patients who received anti-HER2 targeted therapy in second line setting or more will be capped to 50% of the overall population

7. In case of bone disease only, complete metabolic response in 18-FDG pet-scanner is required;
8. Patient with treated (surgery and/or radiation therapy) and controlled primary tumor;
9. Patients with ER-positive disease may or may not have received concomitant endocrine therapy (which must be continued if present). Concomitant ovarian blockade using LHRH agonists is authorised as well;
10. Adequate cardiac, renal, haematological and hepatic functions according to guidelines hospital;
11. Women of childbearing potential must have a negative serum or urine pregnancy test done within 28 days before inclusion;

12. Non post-menopausal women and fertile men must agree to use adequate contraception methods during the study. Hormonal contraceptives such as birth control pills, patches, implants, or injections are not allowed in patients who are hormone receptor positive

13. Patients must be willing and able to comply with the protocol for the duration of the study including scheduled visits, treatment plan and other study procedures including follow-up;

14. Patients must be affiliated to a Social Security System (or equivalent).

Exclusion criteria:

1. Any breast cancer progression over the past 2 years or at study entry;
2. Patient concurrently using other approved or investigational antineoplastic agents than trastuzumab, pertuzumab, Trastuzumab-Deruxtecan, TDM-1 +/- endocrine therapy;
3. Had an history of tumoral meningitis or clinically active central nervous system metastases, defined as untreated or symptomatic, or requiring therapy with corticosteroids or anticonvulsants to control associated symptoms;
    a. Subjects with curatively treated brain metastases (i.e., complete removal surgery or stereotactic radiotherapy) who are no longer symptomatic and do not require treatment with corticosteroids or anticonvulsants may be included in the study provided they have recovered from the acute toxicity of radiotherapy and there has been no progression of the brain metastases within the past 24 months.
    b. Subjects with brain metastases only or treated with whole brain radiotherapy will be excluded of the study
4. Major concurrent disease affecting cardiovascular system, liver, kidneys, haematopoietic system or else considered as clinically important by the investigator and that could be incompatible with patient's participation in this trial or would likely interfere with study procedures or results;
5. History of any prior ipsi or contralateral breast cancer (except in case of DCIS) unless if both primary tumors were confirmed to be HER2-positive
6. Prior history of other malignancies other than study disease (except for basal cell or squamous cell carcinoma of the skin or carcinoma in situ of the cervix) unless the patient has been free of the disease and treatment for at least 3 years;
7. Major surgery within 2 weeks prior to study entry.
8. Pregnant women or women who are breast-feeding;
9. Patients unwilling or unable to comply with the medical follow-up required by the trial because of geographic, familial, social, or psychological reasons;
10. Participation in another clinical study whose procedures interfere with those of the study (within 28 days prior to patient enrolment and for the duration of the study);
11. Persons deprived of their liberty or under protective custody or guardianship.

Primary endpoint:

The progression-free rate (PFR) defined by radiological, and/or molecular progression. The PFR is defined as the time from the date of registration to the date of the first documented event among radiological progressions (RECIST 1.1), molecular progressions defined by ctDNA+, or death due to any cause, whichever occurs first. Patients alive without any of these events will be censored.

Secondary endpoint(s):

Efficacy:

- PFS will be defined as the time from the date of registration to the date of the first documented cancer progression measured by RECIST 1.1 or death due to any cause, whichever occurs first. Patients alive without progression will be censored.
- The ctDNA dynamics is defined as changes in the level of ctDNA (from negative to positive) during the surveillance phase. Positivity rate is defined as the proportion of patients with positive ctDNA.
- OS will be defined as the time from the date of registration to the date of death due to any cause.
- Molecular response is defined as the percentage of patients who reached molecular remission (ctDNA clearance) 3 months after reintroduction of anti-HER2 treatment among patients with ctDNA positive without RECIST progression.
- Objective Response Rate (ORR), defined as the number of patients with at least a confirmed complete response (CR) or partial response (PR) to reintroduction of anti-HER2 treatment, among patients with RECIST progression during the surveillance phase
- Duration of Response (Dor) is defined, among patients with RECIST progression during the surveillance phase, as the time from the onset of response after reintroduction of anti-HER2 treatment, to progression or death due to any cause, whichever occurs first.

Quality of life:

- QoL will be assessed using the EORTC QLQ-C30 and QLQ-BR45 questionnaire
- Anxiety will be assessed by the STAI-state questionnaire
- Decision regret will be assessed by the decision regret scale

D) INVESTIGATIONAL MEDICINAL PRODUCTS

Therapeutic regimens:

Heroes is a de-escalation study.

Patient must have received continuous anti-HER2 targeted therapy (including Trastuzumab, Trastuzumab/Pertuzumab, Trastuzumab-Deruxtecan, T-DM1) for at least 2 years in any line setting for their HER2-positive metastatic breast cancer with complete response or partial response at last radiological assessment. Prior treatment interruption of 3 months maximum is allowed.

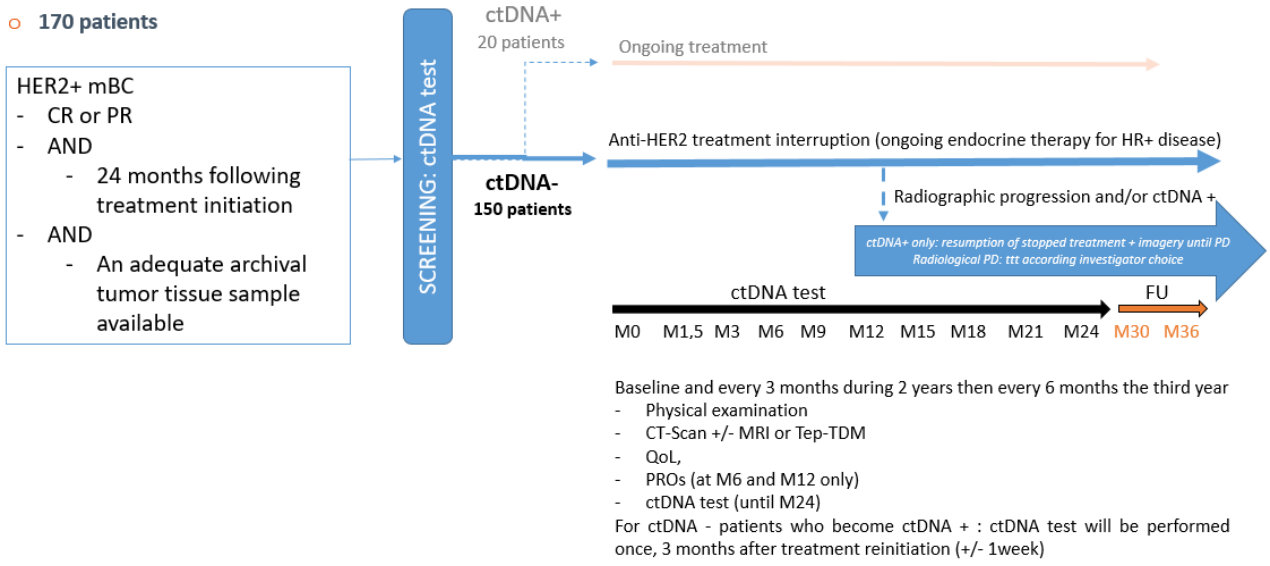
- Patients with ctDNA negative test at baseline will stop their anti-HER2 targeted therapy during the study
  - In case of radiological progression with or without ctDNA positive test, prior treatment will be restarted or new anti-HER2 treatment will be initiated according investigator choice
  - In case of ctDNA positive test without radiological progression, prior treatment will be restarted.
- Patients with ctDNA positive test at baseline will continue anti-HER2 maintenance therapy as indicated in standard care and will be followed up during the study.

Authorized continuous anti-HER2 targeted therapy before starting the study:

Drug name (INN)	Registered name (1)	Pharmaceutical form	Administration route	Posology (2)
Trastuzumab	Herceptin	Powder for concentrate for solution for infusion	Intravenous or Subcutaneous	According to SmPc
Trastuzumab/Pertuzumab	Phesgo	Solution for injection.	Subcutaneous	According to SmPc
Trastuzumab-Deruxtecan	Enhertu	Powder for concentrate for solution for infusion.	Intravenous	According to SmPc
T-DM1	Kadcyla	Powder for concentrate for solution for infusion	Intravenous	According to SmPc

1) *When any generic drug can be is used indicate only the INN name. The choice of the registered name or brand name at the decision of the investigational center's discretion.*
2) *In a maintenance treatment context*

Trial Flowchart:



E) STATISTICAL ANALYSIS PLAN

Required number of patients to be screened/included:

170 patients will be enrolled in the study for ctDNA testing, expecting a low ctDNA positivity rate of 12%. An expected number of 150 ctDNA negative patients (including a third treated with Trastuzumab-Deruxtecan) will be enrolled in the ctDNA negative cohort where anti-HER2 targeted therapy will be interrupted.

The primary endpoint PFR will be evaluated in the ctDNA negative cohort where anti-HER2 targeted therapy will be interrupted.

We utilized reconstructed individual patient data from the Cleopatra trial (Swain SM, Baselga J, Kim S-B, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med 2015; 372: 724–34) available in the R package kmdata (https://github.com/raredd/kmdata), to fit an exponential survival function to the overall survival data. This resulted in an estimated 1-year mortality rate of 12%. Consequently, since Heroes aims at identifying excellent prognosis patients with undetectable minimal residual disease (ctDNA-negative at baseline) in which therapy can be deescalated, we selected this rate as a conservative target 1-year PFR under the alternative hypothesis for the power calculation. Recent long-term follow up results from destiny breast 03 trial (Cortes & al Nature medicine 2024) suggest that patient with long response to Trastuzumab-Deruxtecan have a low progression rate beyond 2 years of treatment.

The null hypothesis that, in the ctDNA negative cohort at baseline, 1-year PFR, is lower or equal to 80% - the lower limit considered as acceptable by UCBG breast cancer experts panel, which also includes two patient representatives, will be tested against the alternative - that it is above 80%. For an expected 1-year PFR of 88% under the alternative hypothesis, corresponding to a hazard ratio of 0.57, an estimated 2-years inclusion and time of analysis 1-year after recruitment of the last patient, 135 ctDNA-negative patients are required to be included to obtain 80% using a one-sample logrank test at 0.05 one-sided significance level and an interim analysis for futility in an optimal two-stage design (Wu at al, Pharm Stat 2020). The interim analysis will be conducted when 88 patients are included, 16 months after start of inclusion. If the one-sample logrank z-statistic at the interim is below -0.2044 the study will be stopped for futility. At the final analysis stage, the z-statistic will be compared to the critical value 1.9315.

Assuming a ctDNA positive rate at baseline of 12% and a drop-out rate of 10%, a total of 170 patients will be tested for ctDNA and an expected number of 150 patients with ctDNA negative tests at baseline.

Statistical analysis:

The primary PFR hypothesis will be tested using a logrank test in the ctDNA- cohort at baseline. Survival rates will be estimated at different time points using the Kaplan-Meier method (with their respective 95% confidence interval). CtDNA dynamics will be analysed using linear mixed models and landmark survival models or joint modelling techniques for their association on survival. Quality of life scales will be analysed descriptively over time and mean values will be reported with 95% confidence intervals.

F) SAMPLES COLLECTED FOR TRANSLATIONAL RESEARCH

Sample types and quantities:

Mandatory for all the patients:

- **Tumor sample**

  The most recent tumor sample, preferably from metastasis, has to be available

  - one FFPE block with 25mm^2 surface area **OR;**
  - 6-10 unstained slides (charged and unbaked) at 10-microns each (or 12-20 unstained slides at 5-microns) PLUS one H&E slide),
    - at screening: the tumor sample will be sent to NATERA to build the personalized ctDNA test (Signatera™ test)

- **Whole blood samples**
  - at screening: one 6mL EDTA tube and two 10mL Streck tubes
  - at month 1.5 (M1.5): two 10mL Streck tubes
  - every 3 months during 2 years (M3, 6, 9, 12, 15, 18, 21, 24) (if applicable, for ctDNA- patients): two 10mL Streck tubes

  Note: For the ctDNA negative patients who become ctDNA positive: ctDNA test will be performed once, 3 months after restarting treatment (+/-1 week).

Optional:

- **A tumor biopsy of progressive metastases for future ancillary studies**

G) TRIAL DURATION

Inclusion period: 2 years

Trial treatment period: NA (de-escalation of treatment)

Follow-up: 3 years

Duration until primary endpoint evaluation: 3 years

Overall trial duration (including follow-up): 5 years

SCHEDULE OF VISITS AND ACTIVITIE

VISITS	**Screening**	**Baseline**	***Follow-up*** **For ctDNA negative patients at baseline**[1]	
VISITS N°	**S**	**V0**	**V1 to V8**	**V9 & V10**
Visit Dates	At least 3 months before Baseline	D0 (after receipt of ctDNA results)	Every 3 months during 2 years (+/-15days)	Every 6 months the third year (+/-15days)
Inclusion/non-inclusion criteria	**x**	**x**		
Written informed consent n°1	**x**			
eCRF registration	**x**			
Written informed consent n°2 ***For ctDNA negative patients only**		**x***		
eCRF inclusion (after receipt of ctDNA results)[1]		**x**		
PHYSICAL EXAMINATION[2]				
PS status (ECOG)	**x**	**x**	**x**	**x**
Vital signs, weight [3]	**x**	**x**	**x**	**x**
Examination of all major body systems		**x**	**x**	**x**
Medical history	**x**			
Concomitant treatments	**x**	**x**	**x**	**x**
Adverse Event Reporting [4]	**x**	**x**	**x**	**x**

[1] Patients with ctDNA positive test at screening are not eligible for de-escalation, but until the end of the study, the investigating physician will continue to collect data from their medical records, concerning their state of health and any further treatment they may receive.

[2] At Baseline, specific physical examination for research purposes is not required for patients with ctDNA positive results

[3] Vital signs must include heart rate, respiratory rate, oxygen saturation, systolic and diastolic blood pressure, body temperature and body weight. Height will be required only at screening.

[4] Adverse events to be reported from the date of registration until the end of treatment follow-up period according to CTCAE V5.0. Ongoing toxicities or adverse event must be monitored until resolution or returned to baseline level.

Pregnancy test (serum or urine (highly sensitive))		**x** Within 28 days before D0	**X**[5]	**X**[5]
PARACLINICAL EXAMINATION				
Scanner (CT-scan) or TEP-TDM		**x** Within 28 days before D0	**x**	**x**
Cerebral MRI		**x** Within 28 days before D0	**x**	**x**
ctDNA TEST (Signatera™ test)				
Sending of the most recent tumor sample (FFPE block or slides), preferably from a metastasis & the first blood draw [6]	**x**			
Blood sample collection [7]			**x** Additional Timepoint at Month 1.5	
QUALITY of LIFE QUESTIONNARY: For ctDNA negative patients at baseline				
EORTC QLQ-C30		**x**	**x**	**x**
EORTC QLQ-BR45		**x**	**x**	**x**
Patient reported outcomes (anxiety and decision regret)			**x** At M6 and Year 1 only	
TREATMENT				
Patients with a ctDNA **positive** test at screening	Anti-HER2 maintenance therapy			
Patients with ctDNA **negative** test at screening	Anti-HER2 maintenance therapy	Discontinuation of the anti-HER2 maintenance therapy[8]		
TRANSLATIONAL RESEARCH: For ctDNA negative patients at baseline				
Biopsy of progressive metastases		**x** In case of radiological progression (optional)		

[5] *Women of child bearing potential who resume treatment must realize a pregnancy test every month and therefore at least until 7 months after the end of each treatment*

[6] *At screening, 26mL of whole blood will be collected (one 6mL EDTA tube AND two 10mL DNA Streck tubes).*

[7] *During follow-up 20mL of whole blood will be collected (two 10mL DNA Streck tubes) for patients with ctDNA negative test at screening (at month 1.5, month 3, then, every 3 months during 2 years). Blood samples will be sent to Natera for ctDNA test. For ctDNA- patients who become ctDNA+ patients during study, blood collection will reset once at M3 after restarting treatment (+/-1 week)*

[8] *In the event of detection of ctDNA by the Signatera™test or radiological tumor progression, treatment will be restarted. In case of radiological progression with or without ctDNA positive test, prior treatment will be restarted or new treatment will be initiated according investigator choice. In case of ctDNA positive test without radiological progression, prior treatment will be restarted*