PEPSI PROTOCOL

Pronostic impact of the Neutrophil/Lymphocyte ratio (NLR) in the treatment of metastatic or locally advanced first-line breast cancer treated with CDK4/6 inhibitor.

N°ID-RCB: 2021-A02664-37

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TRIAL CLASSIFICATION: Category 2 RIPH

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REGULATORY AUTHORIZATIONS	CPP East III	Notice date: 08/12/2021

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PEOPLE INVOLVED IN THE PREPARATION AND CONDUCT OF RESEARCH

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1 SYNOPSIS

TITLE	Pronostic impact of the Neutrophil/Lymphocyte ratio (NLR) in the treatment of Breast cancer in 1^{era} metastatic or locally advanced line treated with CDK4/6 inhibitor.
ACRONYM	**PEPSI**
Coordinator	**Dr Angélique DA SILVA**
Indication	Breast cancer in women with hormone receptor positive, HER2 negative, on the front line of a metastatic or locally advanced situation
Methodology	Prospective cohort, multicenter study
Goals	**Objective main** To study the prognostic impact of the pre-therapeutic Neutrophil/Lymphocyte (NLR) ratio on progression-free survival in patients receiving CDK4/6 inhibitor therapy for locally advanced or first-line metastatic breast cancer. **Secondary objectives** - To evaluate the association between progression-free survival on CDK4/6 inhibitor therapy and the following pre-therapeutic blood parameters: o platelet to Lymphocyte Ratio (PLR) o lymphocyte to Monocyte (LMR) ratio o lymphocyte count (total, CD4 and CD8 ratio) o CRP rate o vitamin D and albumin levels o LDH rate - Evaluate overall survival across the entire cohort, then its association with the pre-therapeutic blood parameters listed above. - Evaluate the association between the evolution of the lymphocyte profile and the response to treatment with a CDK4/6 inhibitor measured on the radiological assessment (thoraco-abdomino-pelvic scan and bone scintigraphy or PET scan) - To evaluate the benefit of CDK4/6 inhibitor treatment in terms of early response (at 3 months) and better response, according to the pre-therapeutic NLR rate. - To evaluate tolerance to CDK4/6 inhibitor treatment.
Judgment criteria	**Main criterion** Progression-free survival rate at 12 months, measured from the start of treatment with CDK4/6 inhibitor, as a function of pre-therapeutic NLR (dichotomized according to an NLR cut-off set at 2.53). **Secondary criteria** - Median progression-free survival time (if achieved), and progression-free survival rate at 12 months, based on PLR, MRL and pre-therapeutic lymphopenia measured by standard biology before treatment initiation. - Median overall survival time (if achieved) and 12-month overall survival rate, based on NLR, PLR, MRL of pre-therapeutic lymphopenia - Treatment responses, defined by the proportion of patients alive and presenting an objective response (complete or partial) 3, 6 and 12 months after the start of treatment with CDK4/6 inhibitor, according to standard radiological results (TAP scan +/- bone scintigraphy or PET scan). - Treatment responses at 3, 6 and 12 months based on lymphocyte profile measured at the third, sixth and twelfth months of CDK4/6 inhibitor treatment. - Better response (complete, partial, stable, progression) to treatment with CDK4/6 inhibitor at 12 months according to standard radiological results (TAP scan +/- bone scintigraphy or PET scan). - Safety profile of the treatment evaluated according to CTCAE grade version 5 and collection of possible dosage adjustments.

Inclusion criteria	- Patient with RH positive, Her2 negative, locally advanced or metastatic breast cancer - Patient requiring treatment of 1^{era} metastatic line by CDK4/6 inhibitor associated with hormonal therapy in accordance with Marketing Authorization - Patient who has not received previous anti-neoplastic therapies for metastatic or advanced disease (chemotherapy, targeted therapy or hormonal therapy). It will nevertheless be possible to have initiated hormone therapy by 1^{era} line within 4 to 6 weeks prior to inclusion. - Prior radiotherapy authorized even in metastatic situations. In case of treatment with radiotherapy, side effects attributable to the treatment should be resolved. - Postmenopausal patient or with suppression of ovarian function - Patient with measurable or non-measurable disease (according to RECIST v1.1 criteria) - Adequate function of organs and marrow allowing the prescription of CDK inhibitor treatment 4/6 - Patient ≥ 18 years old - Desire and ability to adhere to planned visits, treatment plan, biological tests and other trial procedures including assessments requested for inclusion - Patient affiliated to a social security system - Signature informed consent before any specific procedure related to the study
Non-inclusion criteria	- Men (no marketing authorization for CDK4/6 inhibitors in men in France) - Previous systemic treatment for metastatic disease (chemotherapy, hormone therapy...) - Previous treatment with a CDK 4/6 inhibitor (as an adjuvant or for metastatic disease) - Locally advanced breast cancer or presenting locoregional relapse for which curative treatment would be considered - Her2-positive tumor status either on the primary tumor or on relapse, defined by the ASCO criteria - Patient with advanced visceral extension, symptomatic who may be at risk of potentially fatal complications in the short term ("visceral crisis") and who requires treatment with chemotherapy - Patient deprived of liberty, under guardianship, or subject to a legal protection measure or unable to express their consent - Patient unable to undergo trial monitoring for geographical, social or psychopathological reasons
Description of the protocol/experimental plan	The study does not modify the oncological care of patients in any way: the methods of prescription and administration of treatments (CDK4/6 inhibitor and hormonal therapy) will be carried out according to standard care. Thus, the treatment will include: - one of the 3 CDK4/6 inhibitors currently available in France for this indication and at the doctor's choice: • palbociclib • ribociclib • or abemaciclib - administered in combination with hormonal therapy by: • A aromatase inhibitor or fulvestrant • In premenopausal women, treatment with an LHRH analogue will be combined.

	The study will be based on carrying out biological assessments and collecting data on the progress of the treatment (tolerance and effectiveness). **In total, 4 specific blood tests for the study will be carried out during the patient's care:** - After inclusion, before initiation of treatment with CDK4/6 inhibitor: ✓ CBC, liver test, complete ionogram (usual pre-therapeutic test according to the SPC) ✓ to which we will add a CRP, an albumin, a vitamin D, LDH as well as phenotyping of CD4+ and CD8+ lymphocytes (lymphocyte levels (total, CD4 and CD8 ratio)) - At 3 months: lymphocyte count (total, CD4 and CD8 ratio) - At 6 months: lymphocyte count (total, CD4 and CD8 ratio) - At 12 months or at the time of premature study discharge: lymphocyte count (total, CD4 and CD8 ratio) Data collection will concern the period of the first 12 months of processing, and will be based on collection: - data on response to treatment (complete, partial response, stability or progression). Radiological monitoring will be carried out every 3 months according to usual practices (TAP scan +/- bone scintigraphy +/- PET scan) - treatment tolerance data evaluated by the oncologist throughout treatment according to CTCAE grade version 5. - survival data (progression, death), as well as causes of death and treatment discontinuation.
Number of patients needed	In this prospective observational study, few validated hypotheses have been documented in the literature. Therefore, we plan in this study to include a staff of 150 evaluable patients. With this number, we hope to be able to put forward results on the prognostic nature of pre-therapeutic NLR on progression-free survival with statistical power that we will estimate *a posteriori*. We plan to include a total of 165 patients (10% increase) to compensate for possible non-evaluable patients.
Participating centers	François Baclesse Center (Caen), Henri Becquerel Center (Rouen).
Duration of the study	39 months: duration of inclusion (27 months) + follow-up time of patients in the study (12 months)

2 SUMMARY OF EXAMINATIONS TO BE CARRIED OUT

<table>
<tr><th></th><th>Baseline
within 28 days before the start of treatment</th><th colspan="3">During treatment
(1 cycle = 28 days)</th><th>If Premature end of treatment (before 12 months)</th><th>Monitoring of survival at the end of 12 months of follow-up</th></tr>
<tr><th></th><th></th><th>At 3 months
or cycle 4</th><th>At 6 months
or cycle 7</th><th>At 12 months
or cycle 13</th><th></th><th></th></tr>
<tr><td>Signature of informed consent</td><td>✓</td><td></td><td></td><td></td><td></td><td rowspan="5">Collection of the patient's vital status and the status of the disease</td></tr>
<tr><td>Biological assessment:</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Research-specific assessment [1]:
- Lymphocyte typing [2]
- LDH, CRP, albumin, vitamin D*</td><td>✓
✓</td><td>✓</td><td>✓</td><td>✓</td><td>✓</td></tr>
<tr><td>Usual therapeutic assessment according to the SmPC:
CBC, liver test, complete ionogram [3], CA15-3, ACE</td><td>✓</td><td colspan="3" rowspan="2">Examinations (biological, radiological, ECG...) carried out as part of usual monitoring
Collection of this data for the entire duration of processing up to 12 months</td><td rowspan="2"></td></tr>
<tr><td>Collection of medical data:
✓ on the history of the disease
✓ on monitoring treatment tolerance
✓ on monitoring the effectiveness of the treatment (radiological examinations left to the free choice of the investigator)</td><td>✓
✓
✓</td></tr>
</table>

[1] Additional and study-specific examinations/activities (would not usually be carried out as part of treatment)
[2] Lymphocyte typing on 5 mL peripheral whole blood on EDTA tube (total lymphocytes, CD4 and CD8 ratio)
[3] *PAL, ALT, AST, GGT, total and conjugated bilirubin, sodium, potassium, chlorine, bicarbonates, proteins, calcium, magnesium, serum creatinine and calculated creatinine clearance*

3 SCIENTIFIC JUSTIFICATION OF STUDY

3.1 CURRENT STATE OF KNOWLEDGE

Breast cancer remains the most common cancer in women, with nearly 58,459 new cases in 2018 in France, or nearly a third of all incident cases of women's cancer. It is the leading cause of cancer death among women with more than 13,300 deaths in 2018 (santepubliquefrance.fr). Standardized net survival is 87% at 5 years and 76% at 10 years, with an average age at diagnosis of 63 years. However, survival in breast cancer is strongly linked to the carcinological stage: it exceeds 95% at 5 years in patients with localized breast cancer, but drops to 30% in the case of metastatic cancer.

The presence of metastases at diagnosis occurs only in approximately 5% of cases, and the progression of breast cancer to a metastatic stage represents 20 to 30% of cases within 2 to 10 years of carcinological history.

The prognosis of patients depends on several parameters including the characteristics of the patient (Performans Status (PS) and age), tumor characteristics (tumor size, grade and histological type, lymph node and metastatic status, expression of hormonal receptors (HR) and receptors HER2) and response to systemic and locoregional treatments.

3.2 CURRENT MANAGEMENT OF METASTATIC BREAST CANCER /LOCALLY ADVANCES RH positive/HER2 negative

Approximately 75% of breast cancers express hormone receptors, that is, at least 10% of tumor cells express estrogen (OR) and/or progesterone (RP) receptors on their surface.

The arrival of cyclin-dependent kinase inhibitors CDK4/6 has modified the management of metastatic and locally advanced RH positive (RH-pos) HER2 negative (HER2-neg) breast cancers by providing a benefit on progression-free survival of several months, with a gain of 10.3 months for example for Palbociclib (Finn, N Engl J Med, 2016).

The FDA and EMA recently authorized the marketing of three CDK4/6 inhibitors: palbociclib, ribociclib and abemaciclib. Thus, apart from the visceral crisis which requires chemotherapy to be carried out, the first-line metastatic treatment of RH-pos /HER2-neg breast cancers is based on hormonal therapy associated with a CDK4/6 inhibitor (Cardoso, Ann Oncol. 2018). To date, no predictive or prognostic factors for a better response to CDK4/6 inhibitors have been demonstrated.

Because the systemic inflammatory host response has a demonstrated role in tumor growth, invasion, angiogenesis, and tumor progression, it has also been recognized as an independent prognostic factor for recurrence and survival in many solid cancers (Guthrie, Crit Rev Oncol Hematol, 2013; Clarke, Clin Pharmacol Ther, 2011; Templeton, J Natl Cancer Inst, 2014). This inflammation of the tumor microenvironment can be indirectly assessed by a routine peripheral blood test to estimate the lymphocyte level and determine the NLR (neutrophil – lymphocyte ratio) as well as the PLR (platelet – lymphocyte ratio).

Ethier et al. (Breast Cancer Res, 2017) recently demonstrated, through a metanalytic, that high pre-treatment NLR was an independent factor in poor prognosis of overall survival and progression-free survival in breast cancer all stages combined, and that this association was stronger for RH-pos and HER2-neg cancers.

Furthermore, it has been demonstrated, in esophageal cancer, that patients with a high NLR (≥ 2.2) had a lower pathological response rate compared to those with an NLR < 2.2 (21% vs 56%, p=0.001), but also a delayed response time to chemotherapy (Sato, World J Surg, 2012).

In this context, a single-center retrospective pilot study on 126 patients carried out at the François Baclesse center, from July to September 2020, showed that a pre-therapeutic NLR greater than 2.53 (cut-off determined by the analysis of ROC curves) was significantly associated with unfavorable progression-free survival time (Hazard Ratio 1.93 [1.07-3.50], p=0.030) (Rottier et al., Front. Oncol. 2023).

3.3 RESEARCH HYPOTHESIS AND EXPECTED RESULTS

To our knowledge, the impact of the inflammatory microenvironment in predicting the benefit of a CDK4/6 inhibitor in the treatment of locally advanced or first-line metastatic breast cancer has not been studied until now.

In this context, we propose a multicenter study aimed at prospectively confirming the prognostic interest of pre-therapeutic NLR on the progression-free survival of patients initiating treatment with a CDK4/6 inhibitor combined with hormonal therapy for RH breast cancer -pos /HER2-neg locally advanced or metastatic.

It will also be necessary to evaluate other markers of inflammation and their prognostic and predictive interest of a better response to treatment with a CDK4/6 inhibitor, in combination with hormone therapy in these patients.

4 OBJECTIVES OF THE STUDY

4.1 MAIN OBJECTIVE

The main objective is to evaluate the prognostic impact of the pre-therapeutic Neutrophil to Lymphocyte (NLR) ratio on progression-free survival in patients receiving treatment with CDK4/6 inhibitor in combination with hormonal therapy for breast cancer RH-pos /HER2-neg locally advanced or first-line metastatic.

4.2 SECONDARY OBJECTIVES

The secondary objectives are:

- To evaluate the association between progression-free survival on CDK4/6 inhibitor therapy and the following pre-therapeutic blood parameters:
    - platelet to Lymphocyte Ratio (PLR)
    - ratio of Lymphocytes to Monocytes (LMR)
    - lymphocyte count (total, CD4 and CD8 ratio)
    - CRP (C-reactive protein) levels
    - vitamin D and albumin levels
    - LDH rate
- Evaluate overall survival across the entire cohort, then its association with the pre-therapeutic blood parameters listed above.
- Evaluate the association between the evolution of the lymphocyte profile and the tumor response to treatment with CDK4/6 inhibitor measured on the standard radiological assessment (thoraco-abdomino-pelvic scan +/- bone scintigraphy or PET scan)
- To evaluate the benefit of CDK4/6 inhibitor treatment in terms of early response (at 3 months) and better response, according to the pre-therapeutic NLR.
- To evaluate tolerance to CDK4/6 inhibitor treatment.

5 JUDGMENT CRITERIA

5.1 MAIN CRITERION

The main criterion of the study is the progression-free survival rate at 12 months, measured from the start of treatment with CDK4/6 inhibitor, as a function of pre-therapeutic NLR.

- The progression-free survival rate is the proportion of patients without progression, according to the usual tumor evaluation criteria, 12 months after initiation of treatment with CDK4/6 inhibitor.
- Pre-therapeutic NLR is the ratio of blood neutrophil to lymphocyte levels measured before starting treatment with a CDK4/6 inhibitor.

5.2 SECONDARY CRITERIA

The secondary criteria are:

- Median progression-free survival time (if achieved), and progression-free survival rate at 12 months, based on PLR, MRL and pre-therapeutic lymphopenia measured by standard biology before initiation of CDK4 inhibitor treatment/6
- Median overall survival time (if achieved) and 12-month overall survival rate, based on NLR, PLR, MRL of pre-therapeutic lymphopenia
- Treatment responses at 3, 6 and 12 months based on lymphocyte profile measured at the third, sixth and twelfth months of CDK4/6 inhibitor treatment.
- Better response (complete, partial, stable or progressing) to treatment with CDK4/6 inhibitor at 12 months according to standard radiological examinations (TAP scan +/- bone scintigraphy or PET scan).
- Safety profile of CDK4/6 inhibitor treatment evaluated according to CTCAE version 5 criteria and collection of possible dosage adjustments.

6 STUDY PLAN

6.1 METHODOLOGY

This is a prospective, multicenter cohort study.

6.2 DURATION OF STUDY

The estimated duration of the study is estimated at 39 months including:

- an inclusion period of 27 months
- and a participation period of 12 months per patient.

6.3 PATIENT SELECTION

6.3.1 Inclusion criteria

- Patient with RH positive, Her2 negative, locally advanced or metastatic breast cancer
- Patient requiring first-line metastatic treatment with a CDK4/6 cyclin inhibitor in accordance with marketing authorization combined with hormonal therapy.
- Patient who has not received previous antineoplastic therapies for metastatic or advanced disease (chemotherapy, targeted therapy or hormonal therapy). It will nevertheless be possible to have initiated first-line hormone therapy in the 4 to 6 weeks preceding inclusion.
- Prior radiotherapy authorized even in metastatic situations. In case of treatment with radiotherapy, side effects attributable to the treatment should be resolved.
- Postmenopausal patient or with suppression of ovarian function
- Patient with measurable or non-measurable disease (according to RECIST v1.1 criteria)
- Adequate function of organs and marrow allowing the prescription of CDK inhibitor treatment 4/6
- Patient ≥ 18 years old
- Desire and ability to adhere to planned visits, treatment plan, biological tests and other trial procedures including assessments requested for inclusion
- Patient affiliated to a social security system
- Signing informed consent before any specific procedure related to the study

6.3.2 Non-inclusion criteria

- Men (no marketing authorization for CDK4/6 inhibitors in men in France)
- Previous systemic treatment for metastatic disease (chemotherapy, hormone therapy...)
- Previous treatment with a CDK 4/6 inhibitor (as an adjuvant or for metastatic disease)

- .
- Locally advanced breast cancer or presenting locoregional relapse for which curative treatment would be considered
- Her2-positive tumor status either on the primary tumor or on relapse, defined by the ASCO criteria
- Patient with advanced visceral extension, symptomatic who may be at risk of potentially fatal complications in the short term ("visceral crisis") and who requires treatment with chemotherapy
- Patient deprived of liberty, under guardianship, or subject to a legal protection measure or unable to express their consent
- Patient unable to undergo trial monitoring for geographical, social or psychopathological reasons

6.4 PROGRESS OF THE STUDY

6.4.1 Signing of study consent

The study will be offered by oncologists to patients meeting the eligibility criteria. They will be given an information note and an informed consent form. Patients will have a reflection period of the duration of their choice.

After obtaining the patient's agreement by signing the study consent, the selection criteria will be verified before inclusion in the trial.

Study-specific examinations requested before inclusion (study-specific blood samples) will be carried out after signing consent.

6.4.2 Inclusion procedure

After obtaining the patient's agreement by signing the study consent, verifying the selection criteria and carrying out the inclusion assessment, inclusion will be carried out before starting treatment with a CDK4/6 inhibitor. It will nevertheless be possible to have initiated hormone therapy in the 4 to 6 weeks preceding inclusion.

The inclusion will be recorded on the software dedicated to the study via an internet portal.

An identification number will be assigned to the patient and will be used throughout the study.

6.4.3 Processing progress

The study does not modify the oncological care of patients in any way: the methods of prescription and administration of treatments (CDK4/6 inhibitor and hormonal therapy) will be carried out according to standard care.

Thus, treatment will be based on the administration of a CDK4/6 inhibitor in combination with hormonal therapy.

Hormone therapy and cyclin-dependent kinase inhibitors should be prescribed and administered according to summaries of product characteristics. Toxicity management and dose adjustment are carried out according to the usual RCPs and local practices.

Patients will thus be able to receive one of the **3 CdK4/6 inhibitors** currently available in France for this indication, at the discretion of the prescribing oncologist:

- **Palbociclib** : The recommended dose is 125 mg Palbociclib once daily for 21 consecutive days, followed by 7 days without treatment (scheme 3/1), constituting a complete 28-day cycle. Palbociclib is taken orally, one capsule at a fixed time per 24 hours, during a meal. Dosage modification is recommended based on safety and individual tolerance.
- **Ribociclib** : Concerning Ribociclib, the recommended dose dosage is 600 mg/day or 3 tablets of 200mg to be taken in one dose, for 21 consecutive days, followed by 7 days without treatment (diagram 3/1), constituting a complete cycle of 28 days.

- **Abemaciclib** : the recommended initial dose is 150 mg twice a day (morning and evening), continuously for 28 days, and without interruption between two cycles.

Combined with hormonal therapy:

- A aromatase inhibitor in postmenopausal women: **letrozole** (2.5 mg tablet orally continuously, once daily) or **anastrozole** (1 mg per day once, continuously) or **fulvestrant** (500 mg intramuscularly on D1, D15 and D29 the first month then once a month)
- in pre- or peri-menopausal women, addition of treatment with LHRH analogue.

6.5 EVALUATIONS OF THE STUDY

A summary table of the requested investigations is present at the start of the protocol.

The study is based on:

- carrying out biological assessments (in total 4 research-specific blood tests)
- and the collection of data on the progress of the treatment (tolerance and effectiveness).

6.5.1 Inclusion report

After inclusion and before initiation of treatment with a CDK4/6 inhibitor, a biological assessment will be carried out and will include:

- ✓ A usual pre-therapeutic assessment which includes CBC, liver assessment, complete ionogram (*PAL, ALT, AST, GGT, total and conjugated bilirubin, sodium, potassium, chlorine, bicarbonates, proteins, calcium, magnesium, serum creatinine and calculated creatinine clearance)* **,** ACE and CA15-3
- ✓ A specific assessment under study:
    - a CRP, an albumin, a vitamin D, LDH
    - as well as a lymphocyte typing (5 mL peripheral whole blood on EDTA tube for lymphocyte typing (totals, CD4 and CD8 ratio))

The initial pre-therapeutic assessment, particularly radiological, is left to the free choice of the investigator (thoraco-abdomino-pelvic CT scan +/- bone scintigraphy +/- or, PET scan +/- CT scan or brain MRI).

Data from these assessments will be collected in the study.

6.5.2 Follow-up assessment during treatment

It is planned to carry out 3 additional specific blood tests during treatment:

- At 3 months, 6 months and 12 months

and will understand

- a lymphocyte typing (5 mL peripheral whole blood on EDTA tube for lymphocyte typing (totals, CD4 and CD8 ratio)).

Data collection will be carried out in parallel over the period of the first 12 months of processing, and will be based on collection:

- data on response to treatment (complete, partial response, stability or progression). Radiological monitoring will be carried out every 3 months according to usual practices (TAP scan +/- bone scintigraphy +/- PET scan)
- treatment tolerance data evaluated by the oncologist throughout treatment (collection of side effects according to CTCAE grade version 5 and possible adaptations of CDK4/6 inhibitor dosages)
- survival data (progression, death), as well as causes of death and treatment discontinuation.

6.5.3 Study exit report

If treatment with a CDK4/6 inhibitor is stopped before 12 months of treatment, the specific biological assessment for the study:

- lymphocyte count (total, CD4 and CD8 ratio)

must be carried out within 3 weeks of the end of treatment.

6.5.4 Follow-up after the end of participation (at the end of 12 months of follow-up)

At the end of 12 months of treatment, protocol monitoring will be completed.

Survival data (vital status and disease status) will nevertheless be collected approximately once a year.

6.6 PREMATURE CESSATION OF STUDY

The study will be interrupted at any time under the following circumstances:

- Patient's decision (data already collected during research may be kept and used unless the patient objects''to it), withdrawal of consent.

  The patient can leave the study at any time, at her request. She is not obliged to justify her withdrawal of consent. Withdrawal can be done orally or in writing. However, the investigator is required to become aware of the reason for the withdrawal, without questioning the patient's wishes, and must document it, as well as the date of withdrawal.

- Intercurrent illness or other reason that requires stopping study follow-up
- Patient lost to follow-up
- Investigator's decision
- Stopping treatment (for progression, toxicities or any other reason)

7 REGULATORY HEALTH VIGILANCE

In accordance with the new regulations for research involving humans, there will be no collection of serious adverse events organized by the sponsor as part of the research.

However, as with all research involving humans, the promoter will transmit to the investigators concerned any information likely to affect the safety of people (Art. R1123-52 CSP) and will immediately inform the competent authority and the personal protection committee of new facts* and, where applicable, of the measures taken (Art. R1123-59 of the CSP).

However, health professionals are reminded that **health vigilance applies**, therefore any incident or adverse effect suspected of being due to a medication or another health product defined in article L5311-1 must be reported by the health professional to the vigilance networks which, after analysis, report them to the ANSM.

* New fact: any new data that could lead to a reassessment of the ratio of benefits and risks of the research or the product subject to the research, to modifications in the use of this product, in the conduct of the research, or of the documents relating to research, or to suspend or interrupt or modify the research protocol or similar research.

8 STATISTICAL CONSIDERATIONS

8.1 NUMBER OF SUBJECTS NEEDED

In this prospective observational study, few validated hypotheses have been documented in the literature. Therefore, we plan in this study to include a staff of 150 evaluable patients. With this number, we hope to be able to put forward results on the prognostic nature of pre-therapeutic NLR on progression-free survival with statistical power that we will estimate *a posteriori*.

We plan to include a total of 165 patients (10% increase) to compensate for possible non-evaluable patients.

8.2 STATISTICAL ANALYSIS

All statistical analyzes will be described in a detailed Statistical Analysis Plan (SAP). Any modification made after the launch of the study will appear, and will be justified, in the final analysis report.

General considerations:

Qualitative variables will be described using numbers and percentages, quantitative variables using mean (+/- standard deviation) or median and extent if the assumption of normality is not not verified.

Study population:

Patients who have received at least one cycle of treatment with a CDK4/6 inhibitor and for whom baseline biological assessment data will be available will be considered for the analysis of the primary endpoint.

Definition of criteria:

Survival times will be measured from the start date of CDK4/6 inhibitor treatment until:

- Progression or death, whatever the cause, for progression-free survival
- Death whatever the cause, for overall survival.

Patients not presenting an event during the follow-up period will be censored on the date of the last news. The occurrence of progression will be documented by radiological monitoring assessments, according to RECIST criterion 1.1.

Main objective: Survival data

Progression-free and overall survival curves will be estimated using the Kaplan-Meier method. The median survival (if reached), as well as the 12-month survival rates, will be estimated with their 95% confidence interval, over the general population and based on the factors studied. The prognostic nature of NLR on progression-free survival will be evaluated by a log-rank test, considering the dichotomization of NLR according to an optimal cut-off estimated by ROC curves and sensitivity /specificity analysis. In addition, progression-free survival will be evaluated based on the NLR according to a cut-off at 2.53, as observed in the retrospective pilot study that we conducted (Rottier et al., Front. Oncol. 2023).

The Hazard Ratio will also be provided with confidence interval. In addition, a Cox model will measure the prognostic nature of NLR on progression-free survival as a continuous variable.

Secondary objectives: Response to treatment

The association between treatment response (objective and best response) and biological parameters will be measured by the chi2 test (or Fisher's exact if necessary) for categorical variables (notably biological measurements dichotomized by a cut-off), and by the Student test (or non-parametric Wilcoxon Mann-Whitney test if necessary) for quantitative variables.

Security profile

All toxicities linked to CDK4/6 inhibitor treatment occurring during the duration of the study will be described by type, frequency, grade and time to onset, collected according to CTCAE 5.0 standards.

Processing missing data

As a lot of biological data is collected at different times, some missing data can be expected. In this case, a biological value missing at a fixed time can be attributed by the average value observed at the population level at the same time.

Risk of the first kind

A bilateral risk of 5% will be retained for each statistical test.

Analysis software

The statistical analysis will be conducted on the R software (version 4.0.2). Survival graphics will be made from the package `ggplot2`.

9 TEST MONITORING

In order to guarantee the authenticity and credibility of the data in accordance with the GCPs, the promoter will put in place a quality assurance system which includes:

- management of the trial according to the procedures of the Clinical Research Unit,
- quality control of data from the investigating site by the monitor whose role is to verify the concordance and consistency of the data in the observation notebook in relation to the source documents,
- the provision, if funding provides, of dedicated staff in the department to help the investigator with the logistics of the study and the collection of data in the observation notebooks.

10 ETHICAL AND REGULATORY CONSIDERATIONS

The research will be conducted in compliance with current French regulations, in particular the provisions relating to research involving biomedical humans of the Public Health Code, articles L1121-1 et seq. (law n° 2012-300 of 03/05/2012 as amended by Ordinance No. ° 2016-800 of June 16, 2016), bioethics laws, the Data Protection Act, the Declaration of Helsinki, and Good Clinical Practices.

10.1 REGULATORY AUTHORIZATIONS

A request for authorization will be sent by the Promoter before the start of the study to the Personal Protection Committee (CPP).

Information will be given to the Competent Authority (ANSM), with transmission of the summary of the study and the favorable opinion of the CPP.

This study falls within the framework of the "Reference Methodology" (MR-001) in application of the provisions of article 54 paragraph 5 of the amended law of January 6, 1978 relating to data processing, files and freedoms. This change was approved by decision of January 5, 2006.

The François Baclesse Center complies with current regulations, in particular the rights of persons subject to processing within the meaning of EU Regulation n°2016/679 relating to data protection ("GDPR").

Any substantial modification of the protocol, concerning the objectives of the study, its plan, the population, the examinations or significant administrative aspects, will require the approval of the coordinating investigator, the sponsor, the favorable opinion of the CPP and the information from the competent authority before any implementation.

10.2 PATIENT INFORMATION AND WRITTEN INFORMED CONSENT FORM

Patients will be informed completely and fairly, in understandable terms, of the objectives and constraints of the study, the possible risks involved, the necessary monitoring and safety measures, their rights to refuse to participate in the study or the possibility of withdrawing at any time.

All this information appears on an information and consent form given to the patient. The patient's free, informed and written consent will be obtained by the investigator, or a doctor who represents him before final inclusion in the study. A copy of the information and consent form signed by both parties will be given to the patient, the other copy will be kept by the investigator. For any substantial modification of the protocol, concerning the objectives of the study, its plan, the population, the examinations or significant administrative aspects, new consent from the people participating in the research will be obtained if necessary.

10.3 CONDUCT OF THE STUDY AND RESPONSIBILITIES OF INVESTIGATORS

The principal investigator of each establishment concerned undertakes to conduct the clinical trial in accordance with the protocol which has been approved by the CPP. The investigator must not make any modification to the protocol without the authorization of the sponsor and without the CPP having given a favorable opinion on the proposed modifications.

It is the responsibility of the principal investigator:

- ✓ to provide the promoter with his curriculum vitae as well as those of the co-investigators,
- ✓ to identify the members of your team who participate in the trial and to define their responsibilities,
- ✓ to start patient recruitment after authorization from the sponsor.
- ✓ to do its utmost to include the required number of patients within the established recruitment period.

It is the responsibility of each investigator:

- ✓ to obtain informed consent dated and personally signed by the patient before any trial-specific selection procedure,
- ✓ to regularly complete the observation notebooks (CRF) for each of the patients included in the trial and to give the Clinical Research Assistant (ARC) direct access to the source documents so that the latter can validate the CRF data,
- ✓ to date, correct and sign the corrections of the CRFs for each of the patients included in the study,
- ✓ to accept regular visits from the ARC and possibly those from auditors mandated by the promoter or inspectors from the supervisory authorities.

All documentation relating to the study (protocol, consents, observation notebooks, investigator file, etc....), as well as original documents (laboratory results, radiologies, consultation reports, clinical examination reports carried out, etc.) must be held in a secure location and considered confidential material.

The archiving of data will be the responsibility of the investigator and according to current legislation. The documents that must be archived are the protocol and annexes including possible amendments, original information forms and consents signed, questionnaires, FIUs as well as a patient identification list. All of these documents will be kept for a minimum period of 15 years after the end of the study.

10.4 DATA OWNERSHIP AND CONFIDENTIALITY

The investigator undertakes, for himself and for all persons required to monitor the progress of the trial, to guarantee the confidentiality of all information relating to the project until the publication of the results of the trial. This obligation of confidentiality will not apply to the information that the investigator will be required to communicate to patients as part of their participation in the trial nor to information already published.

The investigator undertakes not to publish, disclose or use, in any way, directly or indirectly, the scientific or technical information in relation to the trial.
The test cannot be the subject of any written or oral comment without the agreement of the promoter; all information communicated or obtained during the carrying out of the test belongs automatically to the promoter who may freely dispose of it.

11 DATA PROCESSING AND STORAGE

11.1 COLLECTION AND PROCESSING OF DATA

Data management will be carried out by the Data Processing Center (CTD) of the Cancéropôle Nord-Ouest. The CTD provides a database management software package dedicated to clinical research: Ennov Clinical (version 7.5.10, ENNOV /CLINSIGHT, 33155 Cenon, France).

This software package, which is based on an Oracleä database architecture, is designed for the overall management of clinical and epidemiological studies, meets the regulatory requirements linked to this type of study. The CTD Ennov Clinical instance is validated in its IT environment. A data validation plan will be developed jointly by the Clinical Research Unit and the Data Processing Center and will describe in detail the checks to be carried out for each variable.

A study-specific database will be created, tested and validated before entry begins. All information required by the protocol must be recorded in the paper observation notebooks - or in the electronic observation notebook - under the responsibility of the principal investigator and an explanation must be provided for each missing data. The data must be entered in these notebooks as they are obtained, and the promoter will take charge of monitoring.

The data will then be monitored by the CTD in accordance with the data validation plan.

The database will be frozen after final quality control, then exported in the format suitable for statistical analysis according to an automated and validated procedure.

11.2 ARCHIVING

The promoter must ensure the archiving of essential documents on the conduct of the study under conditions ensuring their security, for the minimum period provided for by the BPCs, i.e. 15 years after the end of the research.
These documents are the protocol and annexes including any amendments, original information forms and consents signed, questionnaires, FIUs, monitoring documents, statistical analyses, the final report of the study.

11.3 DATA PROPERTIES AND PUBLICATION RULES

The results of this study, owned by the promoter (Centre François Baclesse), will be published in the form of scientific articles. Publications concerning or resulting from this research will be communicated and submitted for rereading by the study coordinators to all investigators.
The authors will include the investigators who included the most patients, the biostatistician who carried out the data analysis, the project manager, as well as participants who have made a substantial contribution to the development of the study, the analysis and interpretation of the results and/or the writing of the manuscript. No publication will be made without the agreement without the agreement of the coordinator and the promoter. The organization that contributed to funding the study will be mentioned in the publication.

Publication rank will be defined based on the investment provided in developing and conducting the study.
This work will be the property of all authors and will be made available to them for the production of communications and transversal publications.
Publications relating to the results of possible additional studies will be subject to the prior agreement of the coordinating investigator and the methodologist; they will be subsequent to the publication of the main study, which must be cited as a reference.

12 FINANCING AND INSURANCE

12.1 STUDY BUDGET

Any additional costs referred to in the Public Health Code are the subject of an agreement negotiated between the CFB and the representative of the establishment, taking into account the financial means available to the CFB as part of its public promotion activity.

However, the CFB ensures the organization of the study and takes charge of providing the following equipment (protocol, observation notebook, investigator file) necessary for conducting the study.

In the event that materials or treatments are provided by other partners, the conditions must be specified in the study agreement.

12.2 INSURANCE

The Promoter has taken out insurance for the entire duration of the study guaranteeing its own civil liability as well as that of any doctor involved in carrying out the study. It will also ensure full compensation for the harmful consequences of the research for the person who takes part in it and their beneficiaries, unless proven by them that the damage is not attributable to their fault or that of any participant, without that the act of a third party or the voluntary withdrawal of the person who had initially agreed to take part in the research can be opposed (see. article L 1121-10).

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