SAFIR-TOR

IDENTIFICATION OF THE MOLECULAR ALTERATIONS ASSOCIATED WITH RESISTANCE TO ENDOCRINE THERAPY AND IMPACTING TREATMENT WITH MTOR INHIBITOR OF HR+ METASTATIC BREAST CANCER IN POST-MENOPAUSAL WOMEN

Statistical Analysis Plan

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2 Introduction

This statistical analysis plan (SAP) describes the analysis sets and the derived variables, as well as the statistical analyses that will be produced for the "*SAFIR-TOR*" study.

2.1 Study design

The SAFIR-TOR trial is a prospective biomarker study that aims to show that p4EBP1 staining predicts clinical benefit from treatment with the mTOR inhibitor everolimus in patients with locally advanced or metastatic breast adenocarcinoma which cannot be treated with surgery and/or radiation therapy.

2.1.1 Planned study duration

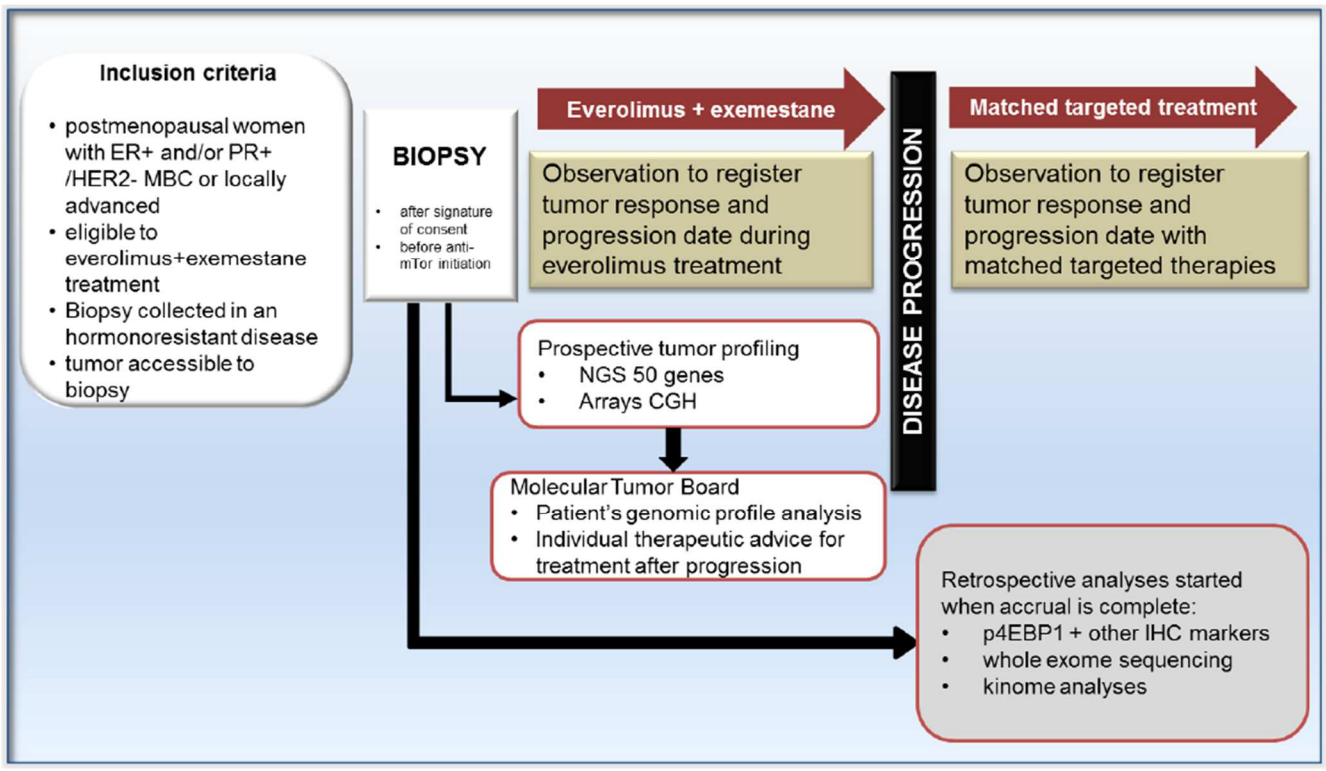
- A 36-month period of accrual is planned for a sample size of 150 patients.
- The expected treatment duration with the combination everolimus (EVE) and exemestane (EXE) is 10 months.
- Post-treatment duration follow-up period: 36 months.
- Estimated duration of the study: 64 months.

2.1.2 Trial early termination criteria

The trial can be suspended or stopped by the sponsor in agreement with the coordinating investigator and the Steering Committee and/or the competent authority and/or the ethics committee (EC) for the following reasons:

- Unexpected occurrence or severity of toxicity linked to the biopsy procedure.
- Insufficient patient recruitment.
- Poor quality of data collection.

Figure 1. Study design



2.2 Study endpoints

2.2.1 Primary endpoint

The primary endpoint of the trial is the predictive value of p4EBP1 in determining mTOR inhibitor efficacy, measured by the association between expression level of the biomarker (high vs low expression) and clinical benefit rate (CBR) 6 months after initiating EVE-EXE treatment. Clinical benefit at 6 months is defined as a complete or partial response or stable disease at 6 months after treatment initiation.

2.2.2 Secondary endpoints

- Predictive value of genomic events in determining mTOR inhibitor efficacy where genomic events are defined as mutations, amplifications, gains, and losses.
- Predictive value of protein biomarkers (pTEN, AKT, pSRB, LKB1) in determining mTOR inhibitor efficacy.
- Molecular landscape of endocrine-resistant disease, in particular correlations between genomic events observed

3 General statistical methods and analysis population definition

3.1 Sample size

The primary endpoint of the study is the association between expression levels of p4EBP1 (high vs low expression) and clinical benefit after 6 months of EVE-EXE treatment (CBR-6m).

We plan to enroll 150 patients in the study in order to have a minimum of 120 patients evaluable for the primary endpoint (some patients may not be evaluable: their tumor samples may not being analyzable, patients may not have been treated with the treatment combination [EVE-EXE]…).

With a sample size of 120 patients (60 patients in each subgroup defined by low vs high expression), a two-group chi-square test with a 0.05 two-sided significance level will have 80% power to detect an increase in CBR at 6 months corresponding to an odds ratio of 2.8 (from 30-40% in p4EBP1- group to 55-65% in p4EBP1+ group). We estimate that this benefit, compared with those observed in the TAMRAD study (Bachelot, 2012), will reflect a clinically meaningful predictive value of the studied biomarker.

3.2 General statistical methods

Quantitative data will be described using the number of observations, mean, standard deviation, median, minimum and maximum values.

Qualitative variables will be described using frequency and percentage distributions. The number of missing data will be given, but will not be considered for the calculation of proportions.

All confidence intervals and statistical tests will be 2-sided and performed at a 5% level. P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as “<0.001”.

Statistical analyses will be performed using SAS® software version 9.4.

3.3 Analysis population definition

Global population: all patients included.

Efficacy analysis population: A patient will be considered evaluable if:

1) no major violation of eligibility criteria, 2) the patient has received at least one dose of everolimus, 3) the patient has at least one tumor evaluation performed before and another 6 months after treatment initiation and 4) an expression levels of p4EBP1 is available for the patient.

Safety population: all patients having received at least one administration of treatment, either everolimus or exemestane.

4 Statistical Analysis

4.1 Descriptive analysis

4.1.1 Inclusion

The total number of:

- included patients,
- patients included in the safety analysis,
- patients included in the efficacy analysis

We also describe deviations regarding eligibility criteria. A listing of patients with major protocol deviations including subject ID and type of deviation.

4.1.2 Baseline characteristics

The following data will be presented for the global population and for the efficacy population.

Baseline evaluation

- Demographic data: sex, age
- Vital signs : height, weight, ECOG performance status, pulse, O_2 saturation, body temperature, and dystolic/systolic blood pressure
- Genetic mutations: BRCA1, BRCA2

History of disease

- Delay between date of diagnosis and date of inclusion
- Delay between date of disease diagnosis and date of diagnosis of the metastatic disease (or advanced disease if not metastatic)
- Delay between date of the last disease progression before inclusion and date of inclusion
- Clinical characteristics: tumor form, laterality, clinical size, TNM staging, and SBR
- Pathological examinations: pathological tumor size, histological tumor size, number of nodes involved and removed,
- Hormonal receptors status: estrogen, progesterone, androgen, IHC, amplification (if IHC 2+)
- Metastatic site at inclusion

History of treatment

- Neoadjuvant treatment (hormone therapy, chemotherapy)
- Adjuvant treatment (hormone therapy, chemotherapy)
- Radiotherapy
- Surgery
- Other

Significant medical and surgical history

The symptoms present at inclusion will be described in terms of:

- Number of patients with at least one symptom

- Number of patients with at least one grade ≥ 2 symptom

Protein expression study: immunohistochemistry

Immunohistochemical staining will be performed on FFPE biopsy samples. The frequencies and other characteristics of positive tumor markers (P4EBP1, PTEN, pAKT, pS6RB, LKB1) will be presented.

The ALLRED score will be calculated as follows:

- < 10% : score 0 + intensity value (0 to 3)
- 10-30%: score 1 + intensity value (1 to 3)
- 30-50%: score 2 + intensity value (1 to 3)
- 50-70%: score 3 + intensity value (1 to 3)
- 70%-100%: score 4 + intensity value (1 to 3)

The ALLRED score is between 0 and 7.

Laboratory tests

- Complete blood count (CBC): hemoglobin, platelets, leucocytes, and neutrophils
- Coagulation: prothrombin time, partial thromboplastin time, and international normalized ratio
- Blood electrolytes: calcium and phosphorus
- Kidney function: creatinine and creatinine clearance
- Liver and pancreas function: AST, ALT, total bilirubin

4.1.3 Study treatment administration

The following data will be summarized for each treatment (exemestane and everolimus):

- Number of patients who received at least one dose of treatment
- Treatment duration
- Dose received at treatment initiation
- Number of patients with at least one dose modification (Yes/No) and reason
- Number of patients with definitive discontinuation of study treatment (Yes/No) and reason

Note that the end of treatment is defined as the date at which everolimus was discontinued (for whatever reason), or when a new antineoplastic treatment is initiated. A definitive discontinuation of exemestane is not considered as a discontinuation of study treatment.

4.1.4 End of study

Number of patients having terminated/withdrawn from the study and associated reason.

4.2 Efficacy endpoint

4.2.1 Primary endpoint

For the primary endpoint, the analysis will be performed on the efficacy population and will include all treated and evaluable patients. A patient will be considered as evaluable if 1) no major violation of eligibility criteria, 2) the patient has received at least one dose of everolimus, 3) the patient has at least one tumor evaluation performed before and another 6 months after treatment initiation and 4) an expression levels of p4EBP1 is available for the patient.

The clinical benefit rate at 6 months (CBR-6m) will be defined as the proportion of patients with a complete response, a partial response, or a stable disease 6 months after initiation of treatment. The CBR-6m and its associated 95% confidence interval (CI) will be described globally and according to high vs low p4EBP1 expression, and will be compared between the 2 subgroups using a chi-square test. In the absence of assumptions, the choice of cut-off for dichotomized the population depending on p4EBP1 values will be done using the median value of the biomarker distribution, the aim being to consolidate data into subgroups of comparable strengths to maximize the power. A second cut-off will be determined using the X-tile software, proposing a division of the population for an optimal separation of data seeing the responses. This second analysis will be considered as a sensitivity analysis.

Logistic regression analyses will be performed to assess the predictive potential effect of biomarker level on clinical benefit rate at 6 months (Yes/No). Clinical factors known to be predictive of clinical benefit will be added to the model to verify the independence of the marker. A step-by-step descending selection of variables will be used to retain factors that were independently linked to clinical benefit. All variables sufficiently informed (with <10% missing value) and significant (at 15% level) in the univariate approach will be introduced in the initial multivariate model. The final multivariate model will be include only variables which will be statistically significant at a 5% threshold. Odd ratio (OR) will be presented with 95% confidence interval (CI). A p-value <0.05 will be considered statistically significant.

For each patient, the variable “clinical benefit rate at 6 months Yes/No” will be determined as follows:

- (1) if a progression is recorded in the tumor evaluation form and if the duration between treatment initiation (everolimus) and the first documented progression is ≤7 months, then the variable “clinical benefit rate at 6 months” will be set to “no”
- (2) if condition (1) is not fulfilled and a complete response, a partial response or a stable disease is recorded in the tumor evaluation form, and if the duration between treatment initiation (everolimus) and the tumor evaluation form is ≥5 months, then the variable “clinical benefit rate at 6 months” will be set to “yes”
- (3) if condition (2) is not fulfilled and if we have no tumor evaluation between 5 and 7 months after treatment initiation and patient is non progressive or without tumor evaluation before 5 months but progressive after 7 months, or non-progressive in last evaluation occurring within 5 months then the primary endpoint “clinical benefit rate at 6 months” will be defined patient by patient during blind review but blinded to p4EBP1 level.
- (4) if condition (3) is not fulfilled and if the patient died before 7 months from other cause than clinical progression, then the variable “clinical benefit rate at 6 months” will be set to “Not Available”.

4.2.2 Secondary endpoints

- **Progression-free survival (PFS)** will be measured from the date of treatment initiation to the date of event defined as the first documented disease progression or death from any cause. Patients with no event at the time of analysis will be censored at the date of last adequate tumor assessment. The date of first progression will be determined using data from the tumor evaluation form.

- **Overall survival (OS)** will be measured from the date of treatment initiation to the date of death from any cause. Patients who are alive at the time of analysis will be censored at the date of last contact. Number of patients deceased and cause of death will be reported.

PFS and OS will be estimated using the Kaplan-Meier method. Survival curves will be plotting according to p4EBP1 (high vs low) and compared using a logrank test.

A Cox proportional hazards model will be performed to explain the progression-free survival time. Variables sufficiently informed (less than 10% missing values) and significant at a 15% level in a univariate approach will be included in a backward selection procedure to keep factors significant at a 5% level in the final multivariate model. Hazard ratios (HRs) will be presented with their 95% confidence intervals (CI).

- **The clinical benefit rate at 6 months** will be described with its 95% confidence interval according to:
    - the presence/absence of each studied genomic event
    - the expression levels of each studied proteomic biomarker (cutoff will be defined in the same way as mentioned for the primary endpoint)

- Descriptive statistics will be used to **characterize the molecular landscape** of endocrine-resistant disease in terms of genomic events observed. Correlations between the different types of genomic events will be studied.

4.3 Description of safety data

All patients who have received the study treatment (exemestane or everolimus) will be included in the safety analysis.

Number of patients (count, %) with:

- At least one grade >= 3 AE
- At least one related grade >= 3 AE
- At least one SAE
- At least one SAE related to study treatment