IBCSG

International Breast Cancer Study Group Statistical Center

TRIAL 53-14 / BIG 14-04 (PYTHIA)

Statistical Analysis Plan for TK1

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Introduction:
The purpose of this analysis is to explore the prognostic value of TK1 activity levels in serum samples of patients enrolled in the PYTHIA trial.

Serum samples for TK1 analyses are taken at the following time points:
1) Baseline
2) D15C1 – day 15 of cycle 1
3) D1C2 – day 1 of cycle 2
4) EoT – End of treatment

The baseline and end of treatment samples were collected via AURORA; the D15 C1 and D1 C2 samples were collected specifically for this TK1 analysis.

Timepoints from 1) to 3) are defined "early timepoints" in the present document.

TK1 activity is measured in DU/L (Divitum Unit/Liter) and the dynamic range is 20 DU/L to >10.000 DU/L. Coefficient of variation of the test is about 10%.

Baseline TK1 activity is prognostic in patients treated with endocrine therapy alone (data from our group): patients with High TK1 baseline have an adverse outcome. This may not be true for patients treated with endocrine therapy + Palbociclib as proliferation markers do not seem to predict response to CDK4/6 inhibitors. Indeed, our data on the TREnd trial (McCartney et al, Clin Cancer Res 2020) show that baseline TK1 levels are not prognostic in patients treated with Palbociclib single agent.

TK1 activity is expected to be reduced during treatment with Palbociclib and Fulvestrant as a pharmacodynamic effect of treatment, compared to baseline.
Preliminary data from our group suggest that some patients (10-15%) do not experience a drop in TK1 during treatment , measured at day 1 cycle 2 (no data available for day 15 cycle 1 in our cohort). These patients show primary resistance to Palbociclib single agent (TREnd trial, submitted for AACR meeting 2019). These data may be confirmed in the PYTHIA cohort.

As Palbociclib is administered with a 3 weeks on/one week off schedule, D15 C1 is "on treatment" while D1 C2 is "off treatment" (blood draw taken after one week rest). Based on published data in the neoadjuvant setting, it may be expected that TK1 levels have some rebound after the week off due to the absence of palbociclib treatment. The frequency and prognostic value of the "rebound" is non currently known.

Our data suggest that TK1 levels rise significantly at time of disease progression. Patients with higher TK1 levels (based on the median value at progression) have an adverse outcome on subsequent treatment (post-palbociclib) compared with patients with lower TK1 levels.

Hypotheses:

- Baseline TK1 values are not prognostic

- Patients who do not experience a drop in TK1 during treatment (measured either at D15 C1 or at D1 C2) have an adverse outcome (it may be hypothesized that the drug does not reach its target in such de novo resistant patients)

- Patients who experience a "rebound" between D15 C1 and D1 C2 have an adverse outcome as compared to those who have a drop at D15 C1 and stay low at D1 C2)
(it may be hypothesized that those pts with a rebound have an incomplete suppression of cell cycle under treatment and have therefore a reduced benefit from treatment)

- TK1 levels at time of disease progression to Palbociclib and Fulvestrant may inform prognosis on subsequent therapy post-study.

- Dynamics of TK1 changes across the whole treatment history of the patient (from start to end of treatment) may identify predictive patterns.

PRIMARY ANALYSIS

The primary outcome measure for analysis is Progression Free Survival.

Protocol definition*: the date of progression is the date that objective progression was first documented. Progression-free survival (PFS) is defined as time from treatment initiation until documented disease progression according to RECIST 1.1 criteria or death, whichever occurs first. For patients without progression, follow-up will be censored at the date of last disease assessment without progression, unless death occurs within a short period of time (12 weeks, corresponding to the interval of tumor re-evaluation) following the date last known progression-free, in which case the death will be counted as a PFS event. Patients who discontinue treatment prior to documented disease progression, including those who initiate non-protocol therapy prior to progression, will be followed for disease progression, for a maximum of 12 months.*

TK1 values at different timepoints, as well as differences between each timepoint and baseline will be modelled as continuous variables. If appropriate, explanatory and TK1 variables will be log-transformed.

Note: EoT TK1 measurements will be done in a separate batch from early timepoints. According to Biovica DiviTum assay manual *"Standard samples included in the kit are used to generate a standard curve by which the optical density readings from the patient samples are converted to TK activity expressed as DiviTum Units per liter (Du/L)."* Therefore, we do not expect issues with the batch effects when analyzing TK1 measurements from different timepoints together (for e.g. when taking difference between BASELINE and EoT).

We will use Cox proportional hazards model to test if TK1 values have statistically significant effect on PFS. We will report Hazard Ratios (HRs) relative to a one unit change in TK1 values (or differences from baseline) and their 95% CIs. We will fit univariable models with TK1 values only, as well as multivariable models that include other prognostic factors, such as: (i) visceral vs non visceral disease; (ii) number of disease sites (categorical: 1-2 vs 3+, or continuous); bone-only disease vs all other; (iv) PYTHIA therapy as first line treatment for MBC vs second line; (v) DFI; (vi) duration of prior ET from MBC; (vii) primary vs secondary ET resistance; (viii) ER+/PR+ vs ER+/PR-; (ix) de novo MBC vs not; (x) IHC LumA vs LumB.
Models will also be assessed using c-index.

For visualization we will consider discretization of TK1 values as described below. Survival curves will be estimated using Kaplan-Mayer method. If appropriate, we will also fit Cox models with TK1 groups as categorical predictors. HRs with 95% CIs will be reported, and c-index will be used to further evaluate prognostic potential of the groups. Multivariate models with TK1 variable and known prognostic factors will be fitted as well.

Categories of interest for visualization using KM-curves:

1) BASELINE time point: patients will be dichotomized in "HIGH TK1" or "LOW TK1" based on the median TK1 value
2) Difference of BASELINE AND D15C1 time points: patients will be divided in three groups: "TK1 drop" (i.e. patients who have a significant decrease of TK1 levels at D15 compared to baseline); "TK1 increase" (i.e. patients who have a significant increase of TK1 levels at D15 compared to baseline); "TK1 no change" (i.e. patients who have no significant changes in TK1). Statistical significance of a change in a sample can be established by using a z-statistic defined in following way:

$$z = \frac{(TK1_{Baseline} - TK1_{D15C1}) - \delta}{0.1\sqrt{TK1^2_{Baseline} + TK1^2_{D15C1}}}$$

where δ is the desired threshold (for e.g. $\delta = 0.1 * TK1_{Baseline}$ for a 10% change), and variances for TK1 values are derived from the coefficient of variation of the assay.

3) Difference of BASELINE AND D1C2 – similarly to Difference between BASELINE and D15C1, as described in (2)
4) Difference of BASELINE AND EoT – similarly to Difference between BASELINE and D15C1, as described in (2)
5) Patterns across three early timepoints: the subgroup of patients with "TK1 drop" or "TK1 no change" based on difference of BASELINE AND D15 C1 will be divided in two groups: "rebound" if TK1 levels at D1 C2 are > D15 C1 (by 10%), "no rebound" if TK1 levels at D1 C2 are < or = D15 C1.
6) As an exploratory option we will consider unsupervised clustering of Baseline-C1D15-C2D1-EoT trends using partitioning around medoids to identify data-driven patterns, similar to (4) but without thresholding.

Note: if data prompts log transformation of TK1 values differences between time points of transformed variables will be interpretable as log fold changes.

SECONDARY ANALYSES

Exploratory analysis of TK1 distributions:

We will perform exploratory data analysis (EDA) for TK1 values and examine distributions of TK1 values at various time points between each other and with respect to other prognostic clinico-pathologic variables. We will examine boxplots and/or density plots by categorical variables, and scatterplots for continuous ones. Distributions will described with 5-points summaries. If EDA suggests strong associations between clinico-pathologic variables and TK1, we will estimate variance explained in TK1 measurements by clinico-pathologic factors using linear regression. Explanatory and response variables might be transformed if appropriate. TK1 values for individual patients across all time points might be visualized using spaghetti plots.

Analysis of TK1 relationship with secondary outcome measures:

We will compare TK1 values at baseline as well as differences between baseline and C1D14 and C2D1 between "early progressors" (i.e. those patients who have disease progression at first

imaging) (expected 5-8% of all study population) and remaining patients. Parametric (t-test) or non-parametric (Wilcoxon) tests will be used as appropriate.

HANDLING OF MISSING DATA

If up to 10% of patients do not have TK1 Values for all timepoints – we will exclude patients with missing data from all of the analyses. If more than 10% of patients have incomplete data we will exclude patients only from the analyses corresponding to missing timepoints.

TIMELINES:

Serum samples will be analyzed in batches.

- First batch analysis will be done after the collection of all "early time points" – projected in Q2 of 2020.
- Second batch analysis will be done when EoT samples assay data becomes available.