European Journal of Cancer 186 (2023) 1–11

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Serum thymidine kinase activity in patients with HR-positive/HER2-negative advanced breast cancer treated with ribociclib plus letrozole: Results from the prospective BioItaLEE trial

Luca Malorni [a,*,1], Giampaolo Bianchini [b,1], Roberta Caputo [c], Alberto Zambelli [d], Fabio Puglisi [e,f], Giulia V. Bianchi [g], Lucia Del Mastro [h], Ida Paris [i], Filippo Montemurro [j], Giacomo Allegrini [k], Marco Colleoni [l], Stefano Tamberi [m], Claudio Zamagni [n], Marina E. Cazzaniga [o], Michele Orditura [p], Valentina Guarneri [q,r], Daniela Castelletti [s,2], Matteo Benelli [t], Mariacristina Di Marino [s], Grazia Arpino [u,3], Michelino De Laurentiis [c,3]

[a] *Department of Oncology and Translational Research Unit "Sandro Pitigliani", Ospedale di Prato, Azienda USL Toscana Centro, Italy*
[b] *Department of Oncology, Ospedale San Raffaele, Milano, Italy*
[c] *Department of Breast and Thoracic Oncology, IRCCS Istituto Nazionale dei Tumori Fondazione G Pascale, Napoli, Italy*
[d] *Medical Oncology Unit, IRCCS Humanitas Research Hospital and Department of Biomedical Sciences - Humanitas University, Milano, Italy*
[e] *Department of Medical Oncology, IRCCS, Centro di Riferimento Oncologico,Aviano, Italy*
[f] *Department of Medicine, University of Udine, Italy*
[g] *SC Oncologia Medica 1, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy*
[h] *U.O.S.D. Breast Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy*
[i] *Department of Woman and Child Sciences, Fondazione Policlinico Universitario Agostino Gemelli, IRCCS, Rome, Italy*
[j] *Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Torino, Italy*
[k] *U.O.C. Oncologia Medica, Presidio Ospedaliero Livorno, Italy*
[l] *Division of Medical Senology, Istituto Europeo di Oncologia (IEO), IRCCS, Milano, Italy*
[m] *U.O. Oncologia, P.O. Ospedale degli Infermi – AUSL, Ravenna, Italy*
[n] *IRCCS Azienda ospedaliero-universitaria di Bologna, Bologna, Italy*
[o] *Phase 1 Research Unit & Oncology Unit, Azienda Socio Sanitaria Territoriale Monza & Milano Bicocca School of Medicine and Surgery, Monza, Italy*
[p] *U.O.C. Oncologia Medica e Ematologia, A.O.U. Università degli Studi L. Vanvitelli, Napoli, Italy*
[q] *Deparment of Surgery, Oncology and Gastroenterology, University of Padova, Italy*

[*] *Corresponding author*: "Sandro Pitigliani" Department of Oncology and Translational Research Unit, Hospital of Prato, Azienda USL Toscana Centro, 59100 Prato, Italy.
E-mail address: Luca.malorni@uslcentro.toscana.it (L. Malorni).
[1] These authors contributed equally.
[2] Current affiliation: AstraZeneca, Milan, Italy.
[3] These authors contributed equally.

https://doi.org/10.1016/j.ejca.2023.03.001

[r] Oncologia 2, Istituto Oncologico Veneto (IOV) IRCCS, Padova, Italy
[s] Oncology, Novartis Farma SpA, Origgio, Italy
[t] Department of Oncology and Bioinformatics Unit, Ospedale di Prato, Azienda USL Toscana Centro, Italy
[u] Department of Medical Clinics and Surgery, Università Federico II, Napoli, Italy

Received 2 December 2022; Received in revised form 28 February 2023; Accepted 1 March 2023
Available online 8 March 2023

KEYWORDS
Advanced breast cancer;
Ribociclib;
Letrozole;
Thymidine kinase;
Biomarker

Abstract ***Background:*** Thymidine kinase 1 (TK1) is an enzyme downstream of the CDK4/6 pathway, with a critical role in DNA synthesis; serum TK1 activity (sTKa) is a novel liquid biopsy biomarker of tumour cell proliferation.
Methods: The phase IIIb, BioItaLEE trial (NCT03439046) collected sera from postmenopausal patients with hormone receptor–positive (HR+), HER2-negative (HER2–) advanced breast cancer (ABC) treated with first-line ribociclib plus letrozole at baseline, day 15 of cycle 1 (C1D15), day 1 of cycle 2 (C2D1), and at first imaging. Associations between sTKa assessed at different time points or sTKa dynamic patterns, and progression-free survival (PFS) were evaluated using multivariate Cox models.
Results: Overall, 287 patients were enroled. Median follow-up was 26.9 months. High sTKa (> median) at baseline was associated with higher risk of progression (hazard ratio [HR], 2.21; 95% confidence interval [95% CI], 1.45, 3.37; $P = 0.0002$); similar results were observed for patients with high sTKa levels at C1D15 and C2D1. Early sTKa dynamic patterns were strongly predictive of PFS. The pattern with high sTKa levels at C2D1 following initial decrease at C1D15 was associated with higher risk of progression versus the pattern with low sTKa levels at both time points (HR, 2.89; 95% CI, 1.57, 5.31; $P = 0.0006$), while the pattern with high sTKa levels at C1D15 was associated with the shortest PFS (HR, 5.65; CI: 2.84, 11.2; $P < 0.0001$). Baseline and dynamic sTKa changes provided independent information.
Conclusions: sTKa appears to be a new promising prognostic and pharmacodynamic biomarker in patients with HR+/HER2– ABC treated with ribociclib plus letrozole as first-line therapy.

1. Introduction

Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) in combination with endocrine therapy are currently the cornerstone of treatment for advanced hormone receptor–positive (HR+), human epidermal growth factor receptor 2-negative (HER2–) advanced breast cancer (ABC); however, approximately 10–30% of patients display primary resistance to treatment [1–3]. Upfront identification of discrete subsets of patients with different risk of disease progression during treatment with a CDK4/6i may help tailor clinical management and disease monitoring.

Previous studies showed that CDK4/6i can modulate serum thymidine kinase 1 activity (sTKa), a marker of cell proliferation, both in cellular models and in patients with early or metastatic breast cancer [4–8]. In an early disease setting, sTKa was strongly inhibited at day 15 of neoadjuvant treatment with the CDK4/6i palbociclib plus anastrozole [4], with a significant rebound at the time of surgery, after palbociclib wash-out. In the metastatic setting, similar sTKa dynamics were also observed, and absence of reduction of sTKa below the limit of detection (LOD) at day 15 of therapy with palbociclib and fulvestrant was associated with an adverse outcome in patients with endocrine-resistant disease [8]. Collectively, these data suggest that sTKa may represent a non-invasive marker of tumour proliferation and of a luminal B-like phenotype in HR+/HER2– tumours; however, the role of sTKa as a prognostic and monitoring biomarker in patients with endocrine-sensitive ABC remains unexplored.

BioItaLEE is a phase IIIb study evaluating multiple potential biomarkers of response, their evolution during treatment, and their association with clinical outcomes in postmenopausal women with HR+, HER2– ABC treated with ribociclib plus letrozole as first-line therapy. Here, we attempt to define the role of baseline and on-treatment sTKa levels as prognostic and predictive biomarkers of response to first-line treatment with ribociclib plus letrozole in patients enroled in the BioItaLEE study (CLEE011AIT01; ClinicalTrials.gov NCT03439046).

2. Materials and methods

2.1. Study design and patients

BioItaLEE is a phase IIIb, multicentre, open-label, single-arm trial conducted in postmenopausal women with HR+, HER2– ABC enroled across 47 Italian centres and treated upfront with ribociclib plus letrozole (Supplementary Fig. 1). Patients were enroled from 2nd February 2018 to 28th November 2018. The results presented here are relative to the core phase of the trial, with a cut-off date of 15th October 2020.

Eligible patients were postmenopausal women with a histologically and/or cytologically confirmed diagnosis of locoregionally recurrent, not amenable to surgery or metastatic HR+, HER2– ABC, who were treatment naïve for the advanced setting and had adequate bone marrow and organ function, as well as an Eastern Cooperative Oncology Group performance status ≤2. Patients who received (neo)adjuvant therapy for breast cancer were eligible, as were those who received prior (neo)adjuvant therapy with letrozole or anastrozole if the treatment-free interval was > 12 months from the completion of treatment until study entry. Patients who received ≤28 days of letrozole or anastrozole for advanced disease prior to inclusion in this trial were also eligible.

The full analysis set comprised all eligible patients who received at least one dose of either ribociclib or letrozole. The biomarker analysis set comprised patients who had at least one valid baseline serum sample (Supplementary Fig. 2A).

2.2. Objectives and end-points

The primary objective of the BioItaLEE study was to identify ctDNA alterations, their changes during treatment and possible association with clinical outcomes [9]. Evaluation of sTKa levels over time during treatment with ribociclib plus letrozole and their association with clinical outcomes was a key, pre-specified secondary objective of the study; time to progression and progression-free survival (PFS) were secondary end-points.

2.3. Treatment, assessments, and samples

Patients received ribociclib (600 mg per day, orally) on a 3 weeks on/1 week off schedule, plus letrozole (2.5 mg orally) once a day. Treatment was continued until disease progression, unacceptable toxicity, or patient decision to withdraw from the study. Tumour measurements according to Response Evaluation Criteria in Solid Tumours 1.1 criteria were performed locally with a recommended frequency of every 12 weeks from start of study treatment. Serum samples were collected at baseline (D0, before treatment start), on day 15 of cycle 1 (C1D15 ± 3 days), on day 1 of cycle 2 (C2D1 ± 3 days), and at the time of first imaging (FI) tumour evaluation (foreseen at approximately 12 weeks after treatment start). Investigators were recommended to collect C2D1 samples approximately 4 weeks after day 1 of cycle 1, even when the beginning of cycle 2 was delayed due to toxicity.

2.4. Thymidine kinase activity

Three-hundred microlitre serum aliquots were shipped to the central laboratory at Hospital of Prato (Italy), labelled with an anonymised code. The central laboratory had no access to clinical data. sTKa was determined by the DiviTum® assay, a commercial ELISA-based method (Biovica, Uppsala, Sweden) [10]. Briefly, the DiviTum® assay measures bromo-deoxyuridine (BrdU) incorporation into an immobilised synthetic DNA strand that is further revealed via an anti-BrdU monoclonal antibody. The resulting signal in the sample was expressed as DiviTum units per litre (Du/L); the LOD of the assay was 20 Du/L. The median coefficient of variation of all samples was 4.6% at baseline. For all analyses, a conventional value of 19 Du/L was given to all samples with values below the LOD.

2.5. Statistical methods

Descriptive statistics of sTKa over time were provided, together with an assessment of the potential association between sTKa levels and clinical outcomes. The study was descriptive in nature, and no pre-specified sample size considerations were applied. The results were intended to be hypothesis-generating. The association between sTKa levels at different time points and PFS was assessed by Kaplan-Meier method and multivariate Cox models. For the pre-specified analysis, sTKa at baseline and C2D1 was dichotomised (high versus low) relative to the median value at that time point. For baseline and C2D1 data, an exploratory analysis was also performed using a cut-off of 200 Du/L, which has been recently proposed as clinically informative [11]. For C1D15, sTKa was dichotomised relative to the LOD. Patient status (i.e. recurrent versus *de novo*), tumour subtype (i.e. luminal A versus luminal B), visceral metastases (presence versus absence) and number of organs involved by metastases (< 3 versus ≥3) were the clinical variables included in the models to adjust for possible confounding factors.

2.6. Ethics

The study was designed, implemented, and reported in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations, and with the ethical principles laid

down in the Declaration of Helsinki. The protocol and informed consent form were reviewed and approved by a properly constituted Institutional Review Board/ Independent Ethics Committee/Research Ethics Board before study commencement. Written informed consent was obtained from all patients. A steering committee oversaw the conduct of the trial as per the approved protocol.

3. Results

3.1. Patients and treatment

A total of 287 patients were enroled, of whom 280 were eligible (e.g. had no major deviation from inclusion/exclusion criteria). A valid baseline sample was available for 263 patients, representing the biomarker population.

Among all enroled patients, with a median follow-up of 26.9 months (range, 22.3–32.3), 64.1% (n = 184) had discontinued treatment whereas 35.9% (n = 103) were still on ribociclib plus letrozole (Supplementary Fig. 2B). Median PFS was 23.4 months (95% confidence interval [95% CI], 20.8–not estimable).

In the biomarker population, patients had a median age of 66 years (interquartile range [IQR], 60.0–72.0), 35.4% were aged ≥70 years and 39.9% had a diagnosis of *de novo* metastatic disease (Table 1). Nearly half (43%) of patients presented with visceral disease, with one or two organs involved in the majority of cases.

Of the 166 patients with measurable disease at baseline, 149 patients performed at least one post-baseline imaging evaluation up to the cut-off date. The overall response rate was 53.0% (52.3% of patients experienced a partial response and 0.7% a complete response); 35.6% of patients experienced stable disease and progressive disease was observed in 10.7% of patients, while the best overall response was unknown in 0.7% of patients. In patients achieving a response, the median duration of response was not reached (95% CI, 22.3–not estimable).

3.2. sTKa levels across study timepoints

sTKa was assessed at baseline in all patients from the biomarker population (n = 263), in 245 patients at C1D15 (93.2%), 241 patients at C2D1 (91.6%), and 208 patients at FI (79.1%). A total of 232 patients had sTKa levels assessed in matched pre-treatment, C1D15 and C2D1 samples (Supplementary Fig. 2A). At baseline, median sTKa was 74.8 Du/L (IQR, 34.8–243.5), and 31 patients (11.8%) had sTKa below the LOD (20 Du/L) (Fig. 1). When using a cut-off of 200 Du/L, 74 (28.1%) patients had high and 189 (71.9%) had low sTKa levels at baseline.

Median sTKa was below LOD at C1D15, 48.1 Du/L (IQR, 19.0–121.7) at C2D1, and 31.5 Du/L (IQR, 19.0–99.2) at FI. The proportion of patients displaying sTKa levels below the LOD were 84.9%, 28.6%, and 40.4% at C1D15, C2D1, and FI, respectively (Fig. 1). At FI, we performed an exploratory analysis taking into account whether patients were on or off ribociclib at the time of sTKa sampling; patients who were off ribociclib

Table 1
Patient demographics and disease characteristics.

Patient characteristic	Enrolled patients N = 287	Biomarker population N = 263
Age (years), median (IQR)	65.5 (59.0–71.0)	65.7 (60.0–72.0)
≥70 years, n (%)	98 (34.2)	93 (35.4)
ECOG PS, n (%)		
0	205 (71.4)	191 (72.6)
1	77 (26.8)	68 (25.9)
2	5 (1.7)	4 (1.5)
Disease characteristics, n (%)		
Tumour subtype		
Luminal A[a]	83 (28.9)	74 (28.1)
Luminal B	185 (64.5)	173 (65.8)
Unknown	19 (6.6)	16 (6.1)
Disease status		
De novo metastatic[b]	114 (39.7)	105 (39.9)
Recurrent	173 (60.3)	158 (60.1)
Disease-free interval period, n (%)[c]		
≤2 years	19 (11.0)	18 (11.4)
>2 years and ≤5 years	10 (5.8)	8 (5.1)
>5 years and ≤7 years	18 (10.4)	14 (8.9)
>7 years	118 (68.2)	111 (70.3)
Missing	8 (4.6)	7 (4.4)
Metastatic sites, n (%)[d]		
Bone	206 (71.8)	193 (73.4)
Bone only	64 (22.3)	62 (23.6)
Visceral	127 (44.3)	114 (43.3)
Liver	41 (14.3)	36 (13.7)
Lung	96 (33.5)	87 (33.1)
Other visceral	18 (6.3)	17 (6.5)
CNS	0	0
Lymph nodes	159 (55.4)	142 (54.0)
Skin	8 (2.8)	8 (3.0)
Breast	21 (7.3)	21 (8.0)
Other	28 (9.8)	26 (9.9)
Number of organs of interest involved, n (%)		
0	2 (0.7)	1 (0.4)
1	107 (37.3)	99 (37.6)
2	124 (43.2)	113 (43.0)
≥3	54 (18.8)	50 (19.0)

CNS, central nervous system; ECOG PS, Eastern Cooperative Oncology Group performance status; eCRF, electronic case report form; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; IQR, interquartile range; PgR, progesterone receptor.

[a] Luminal A: Ki67 <20%, ER-positive, PgR ≥20%, HER2-negative; or Ki67 <20%, ER-negative, PgR ≥20%, HER2-negative. Luminal B: Ki67 ≥20% or PgR <20%.

[b] *De novo* patients were defined as patients with the 'date of first recurrence/progression' information blank in the 'Diagnosis and extent of cancer' eCRF page.

[c] Percentages were computed on non *de novo* patients.

[d] Patients could report more than one metastatic site within the same macro-category or in different macro-categories.

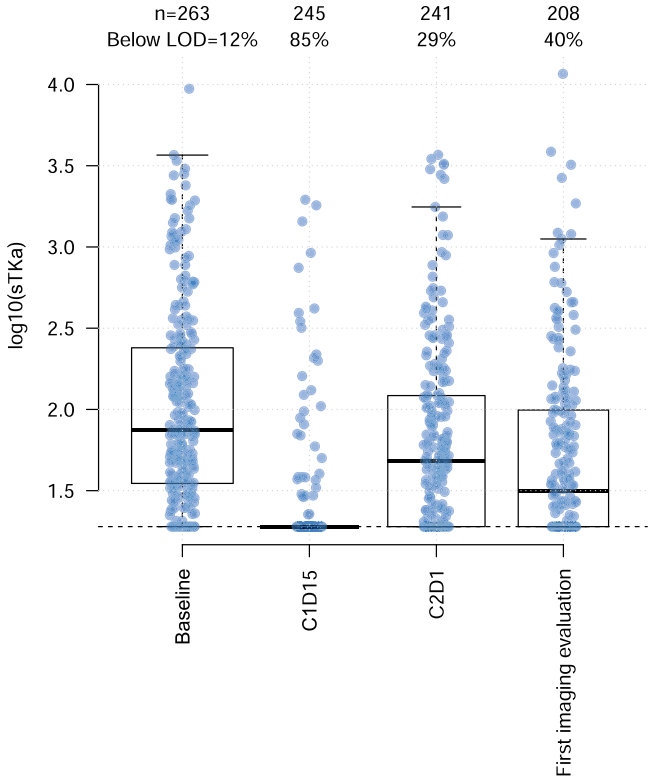


Fig. 1. sTKa levels across study time points. Thick black line represents median value; white boxes represent the interquartile range. Dots represent individual data; dots outside upper and lower fences are considered outliers (higher or lower than 1.5*Q1–Q3 range). sTKa levels recorded as < 20 Du/L have been considered as 19 Du/L to calculate median values. C, cycle; D, day; sTKa, serum thymidine kinase activity.

at the time of sampling were split according to the last day of ribociclib treatment. For patients with ongoing treatment with ribociclib (n = 113; 54.3%), median sTKa was 26.9 Du/L (IQR, 19.0–95.1); for patients off-treatment ≤7 days (n = 67; 32.2%) it was 19.0 Du/L (IQR, 19.0–56.9), and for patients off-treatment > 7 days (n = 28; 13.5%) it was 116.0 Du/L (IQR, 63.8–429.1).

At baseline, high sTKa levels (> median) were significantly associated with younger mean age ($P = 0.0214$) and higher tumour proliferation as assessed by Ki67-positive tumour cells (stratified according to Ki67 staining < 20%, 20–35%, and > 35%; $P = 0.0278$), suggesting a difference in tumour type distribution in patients with high versus low sTKa at baseline (Supplementary Table 1).

3.3. sTKa levels and clinical outcomes

Patients with high sTKa levels (> median) at baseline had a significantly higher risk of progression compared to patients with low sTKa levels (hazard ratio [HR] for disease progression 2.21; 95% CI, 1.45, 3.37; $P = 0.0002$) (Fig. 2A and Table 2). Similar results were obtained when using the 200 Du/L cut-off (> 200 Du/L versus ≤200 Du/L: HR, 2.53; 95% CI, 1.68, 3.81; $P < 0.0001$) (Supplementary Fig. 3A).

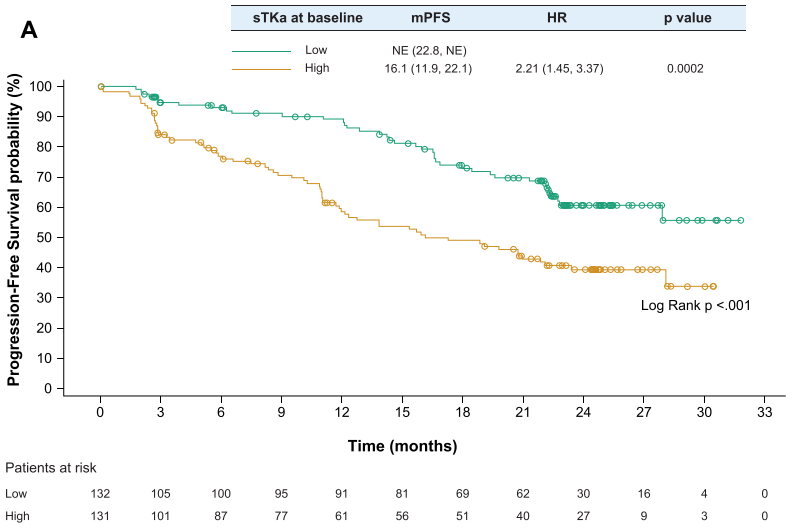
Risk of disease progression for the group of patients with sTKa > LOD at C1D15 was significantly higher than for patients with sTKa < LOD at that time point (HR, 2.62; 95% CI, 1.64, 4.20; $P < 0.0001$) (Fig. 2B and Table 2). Similarly, patients with high sTKa levels (> median) at C2D1 had a significantly higher risk of disease progression compared to those with low sTKa levels (HR, 3.05; 95% CI, 1.98, 4.69; $P < 0.0001$) (Fig. 2C and Table 2). Similar results were obtained when using the 200 Du/L cut-off (Supplementary Fig. 3B).

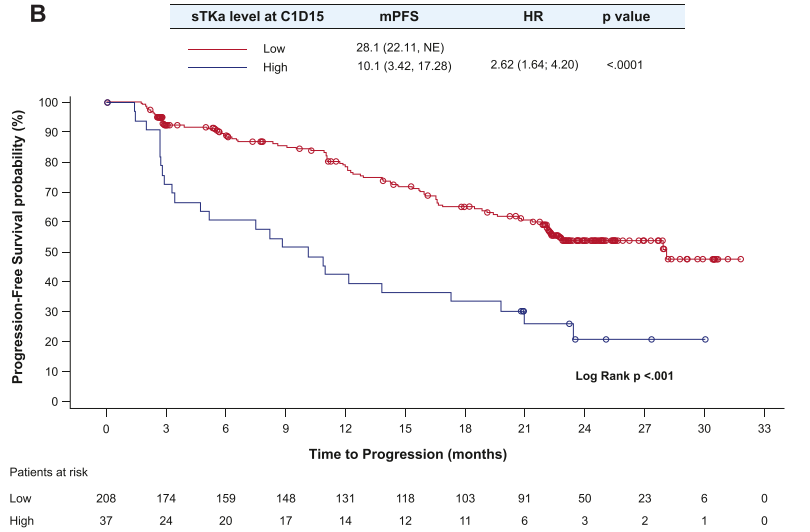
3.4. sTKa early dynamic changes and clinical outcomes

Based on the changes in sTKa levels across the first treatment cycle, patients were divided into three major groups: Group 1 ($n = 62$) with sustained inhibition (sTKa < LOD at C1D15 and at C2D1); Group 2 ($n = 135$) with sTKa rebound at C2D1 after initial inhibition at C1D15 (sTKa < LOD at C1D15 but > LOD at C2D1) and Group 3 ($n = 37$) with insufficient inhibition at C1D15 (sTKa > LOD at C1D15) independently of their sTKa levels at C2D1 (Fig. 3A). This analysis was based on 232 (88.2%) patients with matched sTKa values at baseline, C1D15 and C2D1, and included also two patients with sTKa above the LOD at C1D15 and sTKa missing at C2D1 (categorised in Group 3).

The three groups showed distinct prognosis, with Group 1 having the best outcomes (mPFS, not estimable [95% CI, 28.1, not estimable]), and Group 3 having the worst prognosis (mPFS, 10.1 months [95% CI, 3.4, 17.3]) (Fig. 3B), whereas Group 2 had an intermediate outcome which was similar to that of the overall population (mPFS, 22.1 months [95% CI, 16.8, not estimable]). These differences were statistically significant (HR for disease progression Group 2 versus Group 1, 2.89 [95% CI, 1.57, 5.31; $P = 0.0006$]; Group 3 versus Group 1, 5.65 [95% CI, 2.84, 11.23; $P < 0.0001$]) at multivariate analysis.

Median (IQR) sTKa levels at baseline were significantly different among these three groups: 39.8 Du/L (19.0–80.6), 83.8 Du/L (43.5–178.1) and 1163.0 Du/L (606.8–1950.8) in Groups 1, 2 and 3, respectively ($P < 0.0001$). This prompted an exploratory analysis of whether the prognostic information provided by the three groups was affected by baseline sTKa levels. In


A
sTKa at baseline
mPFS
HR
p value
Low
NE (22.8, NE)
High
16.1 (11.9, 22.1)
2.21 (1.45, 3.37)
0.0002
Progression-Free Survival probability (%)
Log Rank p <.001
Time (months)
Patients at risk
Low 132 105 100 95 91 81 69 62 30 16 4 0
High 131 101 87 77 61 56 51 40 27 9 3 0


B
sTKa level at C1D15
mPFS
HR
p value
Low
28.1 (22.11, NE)
High
10.1 (3.42, 17.28)
2.62 (1.64; 4.20)
<.0001
Progression-Free Survival probability (%)
Log Rank p <.001
Time to Progression (months)
Patients at risk
Low 208 174 159 148 131 118 103 91 50 23 6 0
High 37 24 20 17 14 12 11 6 3 2 1 0

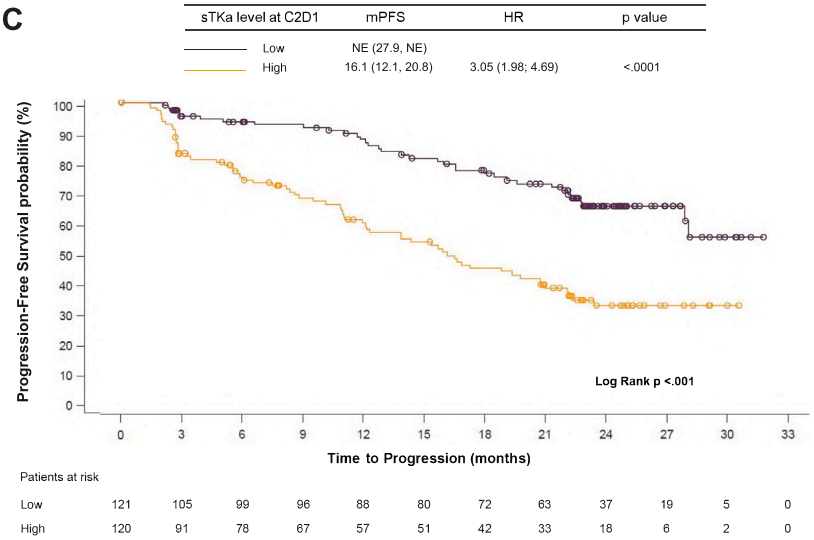

C
sTKa level at C2D1
mPFS
HR
p value
Low
NE (27.9, NE)
High
16.1 (12.1, 20.8)
3.05 (1.98; 4.69)
<.0001
Progression-Free Survival probability (%)
Log Rank p <.001
Time to Progression (months)
Patients at risk
Low 121 105 99 96 88 80 72 63 37 19 5 0
High 120 91 78 67 57 51 42 33 18 6 2 0

Fig. 2. Correlation between PFS and sTKa levels at different time points in the study: (A) baseline; (B) C1D15; (C) C2D1. Curves represent Kaplan-Meier estimates of PFS. Dots represent censored events. Subjects at risk are patients who have no censored observations and have not experienced a PFS event at the evaluated time point. For (A) and (C), 'low' means sTKa levels were equal or lower than the median value (74.8 Du/L at baseline; 48.1 Du/L at C2D1), while 'high' means sTKa levels were higher than the median; for (B), 'low' means sTKa levels were equal or lower than the LOD (20 Du/L), while 'high' means sTKa levels were higher than the LOD. C, cycle; D, day; HR, hazard ratio; LOD, limit of detection; mPFS, median progression-free survival; NE, not estimable; PFS, progression-free survival; sTKa, serum thymidine kinase activity.

patients with low baseline sTKa levels (< median) (n = 114), we did not observe a significantly different outcome among the three groups ($P = 0.099$) (Supplementary Fig. 4A). It should be noted that there were only three patients in Group 3 with sTKa levels < median (two of which stopped therapy within the first three months of treatment without disease progression), limiting the power of this analysis. On the other hand, among patients with high baseline sTKa levels (> median) (n = 120), the three groups showed a significantly different outcome ($P < 0.001$), with patients in Group 2 (n = 70) and Group 3 (n = 34) displaying a significantly higher risk of progression compared to patients in Group 1 (n = 16) (HR for disease progression, 3.33 [95% CI, 1.17, 9.51]; $P = 0.0243$ and 6.18 [95% CI, 2.09, 18.22]; $P = 0.001$, respectively) (Supplementary Fig. 4B).

3.5. sTKa levels at FI and clinical outcomes

Median sTKa at FI was 31.5 Du/L (IQR, 19.0–99.2). Out of 208 patients with evaluable disease at FI and a valid sTKa value, 20 (9.6%) had progressive disease as assessed by imaging and clinical evaluation at this time point (early progressors). Notably, sTKa levels in early progressors were significantly higher than in patients without progression at all time points assessed in the study (baseline, C1D15 and C2D1; $P < 0.001$) (Fig. 4). Of note, only 4 out of 101 (4.0%) patients with sTKa < median at FI had progressive disease at this time point.

4. Discussion

Our study showed that pre-treatment and dynamic assessment of sTKa levels is a novel informative biomarker of progression risk in patients with HR+, HER2– ABC treated with ribociclib plus letrozole as first-line therapy, independent of standard clinico-pathological features and outperforming other markers, such as luminal phenotype classification. sTKa dynamic changes were also informative of patient outcomes and provided independent information compared to baseline sTKa levels. Persistent sTKa decrease < LOD at C1D15 and C2D1 may identify patients with sustained CDK4/6 inhibition and excellent prognosis. Interestingly, patients in this group had the lowest sTKa levels at baseline. However, patients with high sTKa levels at baseline (n = 16) with persistent sTKa decrease below LOD at C1D15 and C2D1 also had excellent outcomes, indicating that positive response to treatment can revert poor prognosis associated with intrinsic tumour biology. Conversely, lack of sTKa decrease at C1D15, which was observed in a minority of patients (15.1%), may identify patients with primary resistance to treatment, resulting in poor prognosis. The majority of these patients (91.9%) had high sTKa levels at baseline, suggesting that primary resistant tumours are enriched with factors associated with aggressive clinical behaviour and poor response to treatment [12]. Patients with a rebound in sTKa levels at C2D1 had an intermediate outcome, which may indicate early tumour adaptation to ribociclib plus letrozole treatment, linked to a specific tumour biology; however, it should be noted that these patients still had a good prognosis and benefited from treatment.

At FI, patients with sTKa levels < median had a low likelihood of experiencing progressive disease. This finding warrants independent confirmation, but it might suggest an additional role for sTKa in monitoring disease progression, with the potential to reduce the use of radiological assessments in patients with low or non-increasing sTKa levels, or to adapt and optimise radiological tumour re-evaluation schedules.

A major strength of our study is that this is a prospective phase IIIb biomarker discovery study with a large sample size. In addition, sTKa was analysed via a commercially available ELISA-based assay using serum samples, which has analytical and practical advantages compared to other approaches, including ctDNA analysis. This study is limited by the lack of a control arm to explore the predictive value of sTKa dynamic patterns. Another limitation is that we did not collect serum samples at regular intervals beyond FI, and therefore could not ascertain the potential additional value of sTKa measurement during the entire duration of first-line treatment.

Overall, our findings suggest that sTKa may be a novel tool to stratify risk of progression based on

Table 2
Multivariate analysis of PFS for sTKa levels at different time points in the study.

Characteristic	Baseline HR (95% CI)	*P* value	C1D15 HR (95% CI)	*P* value	C2D1 HR (95% CI)	*P* value
sTKa levels at time point (high versus low)	2.21 (1.45, 3.37)	0.0002	2.62 (1.64, 4.20)	<0.0001	3.05 (1.98, 4.69)	<0.0001
Recurrent versus *de novo* disease	0.98 (0.66, 1.45)	0.9147	0.98 (0.65, 1.46)	0.9037	1.06 (0.70, 1.59)	0.7877
Tumour type (luminal B versus luminal A)	1.38 (0.83, 2.27)	0.2097	1.69 (1.02, 2.80)	0.0431	1.63 (0.97, 2.74)	0.0631
No visceral metastases versus visceral metastases	0.65 (0.42, 1.02)	0.0612	0.75 (0.48, 1.17)	0.2096	0.66 (0.41, 1.05)	0.0783
≥3 organs involved by metastases versus <3 organs involved by metastases	0.9 (0.53, 1.51)	0.6860	0.94 (0.55, 1.59)	0.8085	1.05 (0.61, 1.81)	0.8713

C, cycle; CI, confidence interval; D, day; HR, hazard ratio; PFS, progression-free survival; sTKa, serum thymidine kinase activity.

assessment of this biomarker at baseline and during the first treatment cycle; this may represent a new strategy for patient stratification for clinical trials. Further studies are needed to ascertain the clinical utility of sTKa as a biomarker to guide treatment tailoring.

Conflict of interest statement

L.M. has received institutional research grants from Pfizer; has received consultancy fees from Novartis and Seagen; has received honoraria from Novartis; has participated in advisory board meetings for Novartis. GBianchini has received consulting fees from AstraZeneca, Daiichi Sankyo, Gilead, MSD, Roche, Sanofi, and Seagen; has received honoraria from AstraZeneca, Chugai, Daiichi Sankyo, Eisai, Gilead, Lilly, MSD, Roche, and Seagen; has received travel support from AstraZeneca, Chugai, MSD, Novartis, Pfizer, and Roche; has participated in advisory board meetings for Agendia, Amgen, AstraZeneca, Chugai, Daiichi Sankyo, Eisai, Exact Sciences, Gilead, Lilly, MSD, Novartis, Pfizer, Roche, and Seagen. R.C. has received consulting fees from Pierre Fabre; has received honoraria from Daiichi Sankyo, Gilead, Lilly, MSD, Novartis, and Roche; has received travel support from Lilly, MSD, and Novartis; has participated in advisory board meetings for Daiichi Sankyo, Gilead, Lilly, MSD, Novartis, and Roche. A.Z. has received consultancy fees from AstraZeneca, Daiichi Sankyo, Gentili, Gilead, Lilly, MSD, Novartis, Pfizer, Roche, and Seagen; has participated in advisory board meetings for AstraZeneca, Daiichi Sankyo, Gentili, Gilead, Lilly, MSD, Novartis, Pfizer, Roche, and Seagen. F.P. has received research funding from AstraZeneca, Eisai, and Roche; has received honoraria, research grants, travel support from and has participated in advisory board meetings for Amgen, AstraZeneca, Celgene, Daiichi Sankyo, Eisai, Eli Lilly, Gilead, Ipsen, MSD, Novartis, Pierre Fabre, Pfizer, Roche, Seagen, Takeda, and Viatris. G.V.B. has participated in advisory board meetings for AstraZeneca/Daiichi, Eli Lilly, Novartis, Roche, and Seagen. L.D.M. has received consultancy fees from AstraZeneca, Daiichi Sankyo, Eisai, Eli Lilly, Exact Sciences, Gilead, GSK, MSD, Novartis, Pierre Fabre, Roche, and Seagen; has received honoraria from AstraZeneca, Daiichi Sankyo, Eisai, Eli Lilly, Exact Sciences, Gilead, GSK, MSD, Novartis, Pierre Fabre, Roche, Seagen; has participated in advisory board meetings for AstraZeneca, Daiichi Sankyo, Eisai, Eli Lilly, Exact Sciences, Gilead, GSK, MSD, Novartis, Pierre Fabre, Roche, and Seagen. I.P. has received honoraria from AstraZeneca, Genetic Pharma, Gilead, Lilly, Novartis, Pfizer, and Seagen. F.M. has received consultancy fees from AstraZeneca, Daiichi Sankyo, Eli Lilly, MSD, Novartis, Pierre Fabre, Pfizer, Roche, and Seagen; has received honoraria from AstraZeneca, Daiichi Sankyo, Eli Lilly, MSD, Novartis, Pierre Fabre,

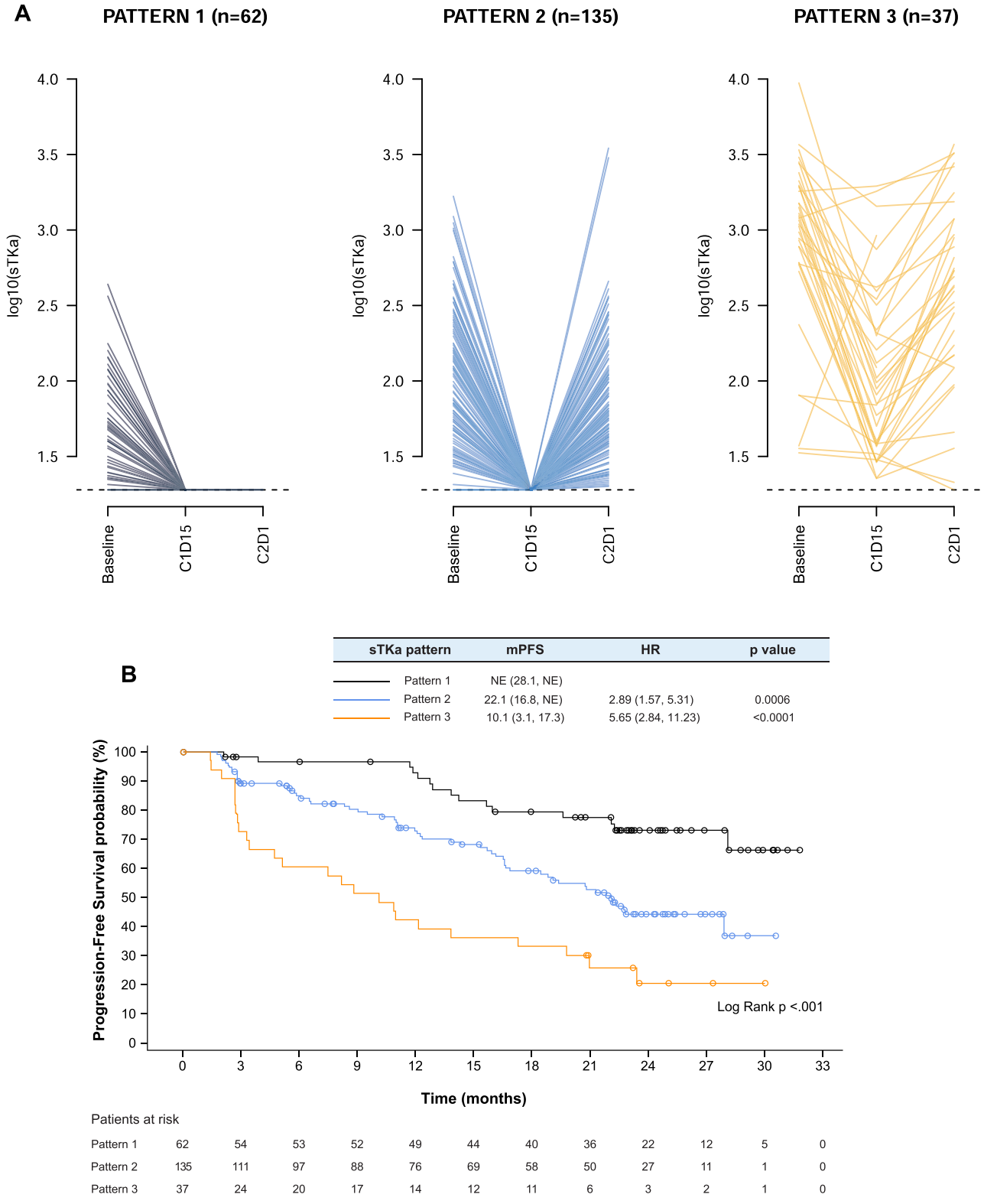


Fig. 3. Correlation between PFS and sTKa dynamic patterns. (A) sTKa levels at different time points by group/pattern. (B) Kaplan-Meier estimates of PFS by pattern. Group/Pattern 1: sustained inhibition during the first treatment cycle (sTKa < LOD at C1D15 and at C2D1); Group/Pattern 2: sTKa rebound at C2D1 after initial inhibition at C1D15 (sTKa < LOD at C1D15 but > LOD at C2D1); Group/Pattern 3: insufficient inhibition at C1D15 (sTKa > LOD at C1D15) independently of sTKa levels at C2D1. Dots represent censored events. Subjects at risk are patients who have no censored observations and have not experienced a PFS event at the evaluated time point. C, cycle; D, day; HR, hazard ratio; LOD, limit of detection; mPFS, median progression-free survival; NE, not estimable; PFS, progression-free survival; sTKa, serum thymidine kinase activity.

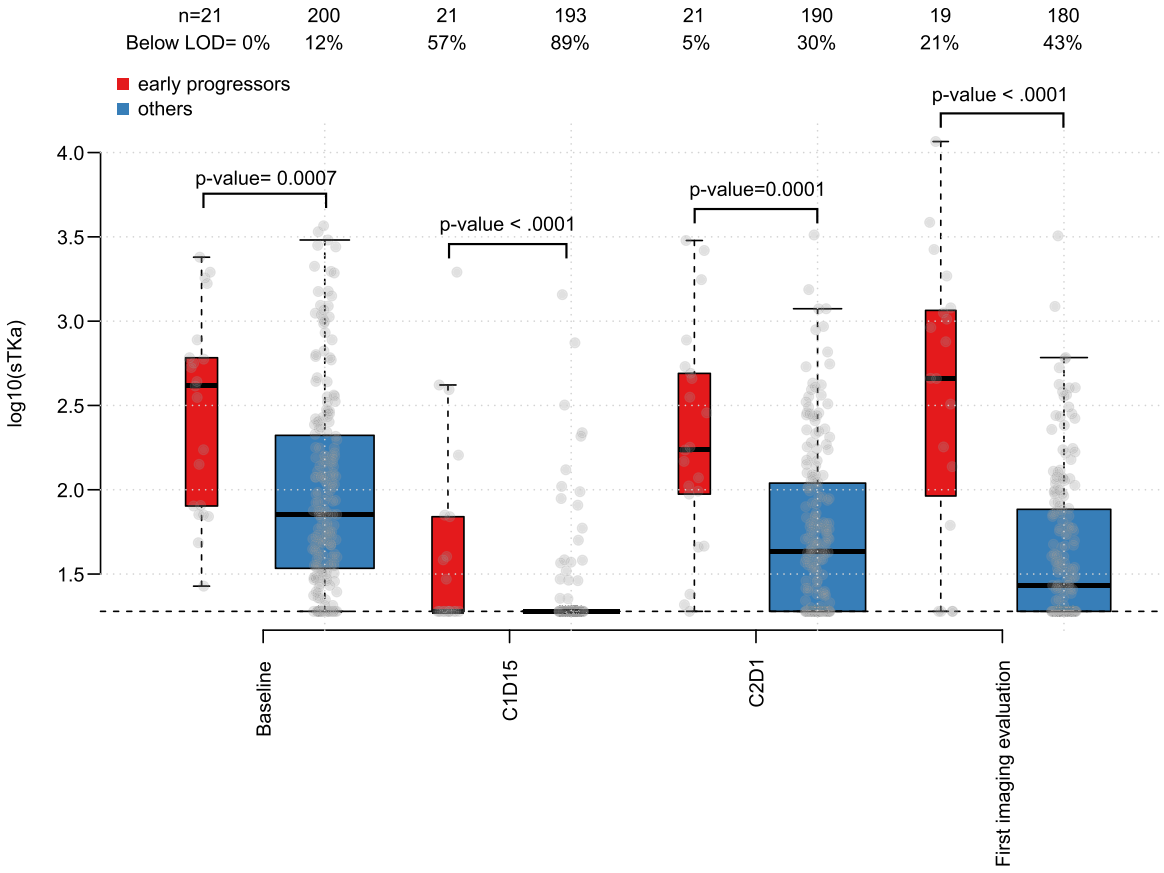


Fig. 4. sTKa levels over scheduled time points for early progressors versus other patients. Early progressors were patients who experienced disease progression within 112 days after treatment start. Dots represent individual data; dots outside upper and lower fences are considered outliers (higher or lower than 1.5*Q1–Q3 range). sTKa levels recorded as < 20 Du/L have been considered as 19 Du/L to calculate median values. C, cycle; D, day; Du, DiviTum units; LOD, limit of detection; sTKa, serum thymidine kinase activity.

Pfizer, Roche, and Seagen; has received travel support from Roche; has participated in advisory board meetings for AstraZeneca, Daiichi Sankyo, Eli Lilly, MSD, Novartis, Pierre Fabre, Pfizer, Roche, and Seagen. M.C. has received a research grant from Roche and is co-chair of the IBCSG scientific committee. C.Z. has participated in advisory board meetings for Amgen, AstraZeneca, Celgene, Eisai, Lilly, Novartis, Pfizer, PharmaMar, QuintilesIMS, Roche, and Tesaro; has received honoraria from Istituto Gentili and Pierre Fabre; has received institutional research grants from AbbVie, Array BioPharma, AstraZeneca, Istituto Gentili, Medivation, Morphotek, Novartis, Pierre Fabre, Pfizer, Roche, Seattle Genetics, Synthon, Takeda, Tesaro, and TEVA. M.E.C. has received institutional consultancy fees from Eisai and Eli Lilly; has received honoraria from Eisai, Eli Lilly, Novartis, Pierre Fabre, Roche, and Seagen; has participated in advisory board meetings for Unicancer; is president of the scientific committee 'Qui donna si cura onlus.' M.O. has received travel support from Eisai and Roche; has participated in advisory board meetings for Amgen, AstraZeneca, Eisai, Eli Lilly, MSD, Novartis, Roche, Seagen, and Tesaro. V.G. has participated in advisory board meetings for Amgen, Eli Lilly, Exact Sciences, Gilead, Merck Serono, MSD, Novartis, Pfizer, and Sanofi; has participated in speaker's bureaus for Amgen, Eli Lilly, Gilead, GSK, and Novartis; has received travel support from Gilead. D.C. and M.D.M. are employees of Novartis. M.B. has received consultancy fees from Novartis. GArpino has received research grants from AstraZeneca, Lilly, MSD, Novartis, Pfizer, and Roche; has received consulting fees from AstraZeneca, Lilly, MSD, Novartis, Pfizer, and Roche; has received honoraria from AstraZeneca, Lilly, MSD, Novartis, Pfizer, and Roche; has received travel support from AstraZeneca, Lilly, MSD, Novartis, Pfizer, and Roche; has participated in advisory board meetings for AstraZeneca, Lilly, MSD, Novartis, Pfizer, and Roche. M.D.L. has received honoraria from Amgen, AstraZeneca, Celgene, Daiichi Sankyo, Eisai, Eli Lilly, Exact Sciences, Gilead, MSD, Novartis, Pfizer, Pierre Fabre, Roche, and Seagen; has received travel support from AstraZeneca; has participated in advisory board meetings for AstraZeneca, Daiichi Sankyo, Eisai,

Eli Lilly, Gilead, MSD, Novartis, Pfizer, Pierre Fabre, Roche, and Seagen. GAllegrini and S.T. have no conflicts of interest to disclose.

Funding

This work was supported by Novartis Farma SpA, Italy.

Role of the funding source

The funding source was involved in the study design, the collection, analysis, and interpretation of data, and the writing of the manuscript.

Authors' contributions

L.M., G.B., G. Arpino, M.D.L.: Conceptualisation, Supervision; L.M., Writing – original draft; All authors: Data curation, Formal analysis, Writing – review & editing.

Data sharing statement

Novartis is committed to sharing with qualified external researchers, access to patient-level data and supporting clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data provided is anonymised to respect the privacy of patients who have participated in the trial in line with applicable laws and regulations. This trial data availability is according to the criteria and process described on www.clinicalstudydatarequest.com.

Acknowledgements

The authors would like to thank the patients enroled in this study and their families, as well as all the participating investigators and their site teams. They would also like to thank Paola Amore, BSc, of Novartis Farma SpA for support and assistance in the review process; Michela Magnoli, Senior Statistician at OPIS, Italy, for statistical review; and Vanesa Martinez, PhD, of Novartis Ireland Ltd, for providing medical writing support/editorial support, which was funded by Novartis Farma SpA, Italy in accordance with Good Publication Practice (GPP 2022) guidelines (https://www.ismpp.org/gpp-2022).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.03.001.

References

[1] McCartney A, Migliaccio I, Bonechi M, et al. Mechanisms of resistance to CDK4/6 inhibitors: potential implications and biomarkers for clinical practice. Front Oncol 2019;9:666.

[2] Hui R, de Boer R, Lim E, et al. CDK4/6 inhibitor plus endocrine therapy for hormone receptor-positive, HER2-negative metastatic breast cancer: the new standard of care. Asia Pac J Clin Oncol 2021;17(Suppl. 1):3–14.

[3] Migliaccio I, Bonechi M, McCartney A, et al. CDK4/6 inhibitors: a focus on biomarkers of response and post-treatment therapeutic strategies in hormone receptor-positive HER2-negative breast cancer. Cancer Treat Rev 2021;93:102136.

[4] Bagegni N, Thomas S, Liu N, et al. Serum thymidine kinase 1 activity as a pharmacodynamic marker of cyclin-dependent kinase 4/6 inhibition in patients with early-stage breast cancer receiving neoadjuvant palbociclib. Breast Cancer Res 2017;19(1):123.

[5] McCartney A, Malorni L. Thymidine kinase-1 as a biomarker in breast cancer: estimating prognosis and early recognition of treatment resistance. Biomark Med 2020;14(7):495–8.

[6] Krishnamurthy J, Luo J, Suresh R, et al. A phase II trial of an alternative schedule of palbociclib and embedded serum TK1 analysis. NPJ Breast Cancer 2022;8(1):35.

[7] Cabel L, Rosenblum D, Lerebours F, et al. Plasma thymidine kinase 1 activity and outcome of ER+ HER2- metastatic breast cancer patients treated with palbociclib and endocrine therapy. Breast Cancer Res 2020;22(1):98.

[8] Malorni L, Tyekucheva S, Hilbers FS, et al. Serum thymidine kinase activity in patients with hormone receptor-positive and HER2-negative metastatic breast cancer treated with palbociclib and fulvestrant. Eur J Cancer 2022;164:39–51.

[9] Arpino G, Bianchini G, Malorni L, et al. Circulating tumor DNA (ctDNA) and serum thymidine kinase 1 activity (TKa) matched dynamics in patients (pts) with hormone receptor–positive (HR+), human epidermal growth factor 2–negative (HER2-) advanced breast cancer (ABC) treated in first-line (1L) with ribociclib (RIB) and letrozole (LET) in the BioItaLEE trial. J Clin Oncol 2022;40(Suppl. 16). 1012–1012.

[10] McCartney A, Bonechi M, De Luca F, et al. Plasma thymidine kinase activity as a biomarker in patients with luminal metastatic breast cancer treated with palbociclib within the TREnd trial. Clin Cancer Res 2020;26(9):2131–9.

[11] Paoletti C, Barlow WE, Cobain EF, et al. Evaluating serum thymidine kinase 1 in patients with hormone receptor-positive metastatic breast cancer receiving first-line endocrine therapy in the SWOG S0226 trial. Clin Cancer Res 2021;27(22):6115–23.

[12] Bianchini G, Pusztai L, Karn T, et al. Proliferation and estrogen signaling can distinguish patients at risk for early versus late relapse among estrogen receptor positive breast cancers. Breast Cancer Res 2013;15(5):R86.