European Journal of Cancer 114 (2019) 55–66

Available online at www.sciencedirect.com

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Original Research

Prognostic role of serum thymidine kinase 1 activity in patients with hormone receptor–positive metastatic breast cancer: Analysis of the randomised phase III Evaluation of Faslodex versus Exemestane Clinical Trial (EFECT)

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Received 12 December 2018; received in revised form 28 March 2019; accepted 2 April 2019
Available online 3 May 2019

KEYWORDS
Breast cancer;
Biomarker;

Abstract ***Background:*** Thymidine kinase 1 (TK1) plays a critical role in DNA synthesis and cell proliferation. Recent studies have shown potential for serum TK1 activity (sTKa) as a prognostic marker and indicator of early response to endocrine therapy in advanced breast

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https://doi.org/10.1016/j.ejca.2019.04.002

Prognosis;
Treatment response;
Endocrine resistance;
EFECT

cancer. The aim of this study is to assess the correlation between sTKa and patient outcome.
Patients and methods: The Evaluation of Faslodex versus Exemestane Clinical Trial (EFECT) was a double-blind, double-dummy, randomised trial of fulvestrant versus exemestane after progression on non-steroidal aromatase inhibitor therapy, in postmenopausal women with advanced breast cancer. Retrospective analyses of serum archived from EFECT were conducted. sTKa was assessed using the DiviTum® assay on samples collected at baseline, after three and six months of endocrine therapy, and at disease progression.
Results: The median time to progression (mTTP) for patients with low baseline sTKa levels was 5.03 months (95% confidence interval [CI]: 3.91–5.89) versus 2.57 months (95% CI: 2.04–3.52) in patients with high sTKa baseline levels ($P < 0.0001$). On treatment, patients whose sTKa increased from baseline had a significantly shorter mTTP (3.39 months, 95% CI: 2.14–4.11) than those without an sTKa increase (5.39 months, 95% CI: 4.01–6.68) ($P = 0.0045$). Similar results were observed in the separate EFECT treatment arms. After adjusting for major prognostic factors, sTKa remained an independent marker.
Conclusion: sTKa is a potential circulating prognostic marker in patients with advanced breast cancer treated with endocrine therapy. It may also represent a tool for upfront identification of endocrine therapy resistance and early positive response to therapy. Independent validation of these results is warranted.

1. Introduction

Thymidine kinase (TK) is an enzyme that has long-held interest as a potential biomarker in breast cancer (BC) [1–3]. Essential to DNA synthesis and as a marker of cell proliferation [4], TK catalyses the phosphorylation of thymidine into thymidine monophosphate, synthesising deoxythymidine triphosphate via the nucleotide DNA salvage pathway [5]. In normal cell cycling, TK activity increases after G1-S transition, with a subsequent decrease in early G2 phase, after DNA replication is complete. TK exists in both mitochondrial (TK2) and cell cycle–dependent cytosolic (TK1) forms [6]. Elevated levels of TK1 can be detected in peripheral blood from patients with BC [1,7], and the level is comparatively low in healthy volunteers [8]. High baseline levels of serum TK1 have been shown to predict risk of locoregional or distant recurrence of disease in early BC [9]. High expression of serum TK1 following neoadjuvant chemotherapy and surgery for locally advanced BC has been correlated with a higher incidence of recurrence and cancer death [10]. High baseline levels and on-treatment TK1 activity have both been correlated with high-risk clinicopathological features and poor prognosis [7,11–13] and a diminished response to tamoxifen [14].

Only recently has technology evolved to the point of accessible and reliable quantification of TK1. Previous methods were largely radioimmunoassay techniques which thereby limited widespread application. Multiple previous studies have used DiviTum™ (DiviTum® technology, Biovica International, Sweden), a refined ELISA assay, to assess serum TK1 activity (sTKa) in patients with early and advanced BC. These studies have collectively demonstrated that sTKa, measured at baseline and serially on treatment, yields accurate prognostic information [15–17]. Recently, our group utilised DiviTum™ in a pilot study involving plasma collected at baseline, after one month of treatment, and at disease progression from women receiving endocrine therapy for metastatic hormone receptor–positive (HR+), HER2-negative BC [18]. Low baseline TK1 levels were associated with significantly longer progression-free survival (PFS) than high baseline levels. After one month of endocrine therapy, patients whose TK1 level decreased had longer median PFS than those whose levels increased.

To validate and expand upon the results of this pilot study, a retrospective analysis of serum collected as a part of the Evaluation of Faslodex versus Exemestane Clinical Trial (EFECT) [19] was conducted, evaluating the correlation between patient outcome and both baseline and on-treatment sTKa.

2. Patients and methods

2.1. Patients

EFECT (NCT00065325) was a randomised, double-blind, double-dummy, placebo-controlled phase III trial which enrolled postmenopausal women with endocrine receptor–positive advanced BC to receive either exemestane 25 mg daily or fulvestrant (500 mg intramuscular (IM) loading dose, followed by 250 mg monthly), following previous progression or recurrence of disease after treatment with a non-steroidal aromatase inhibitor. The present substudy assessed serum samples archived for the purposes of future exploratory research, for which patients optionally provided

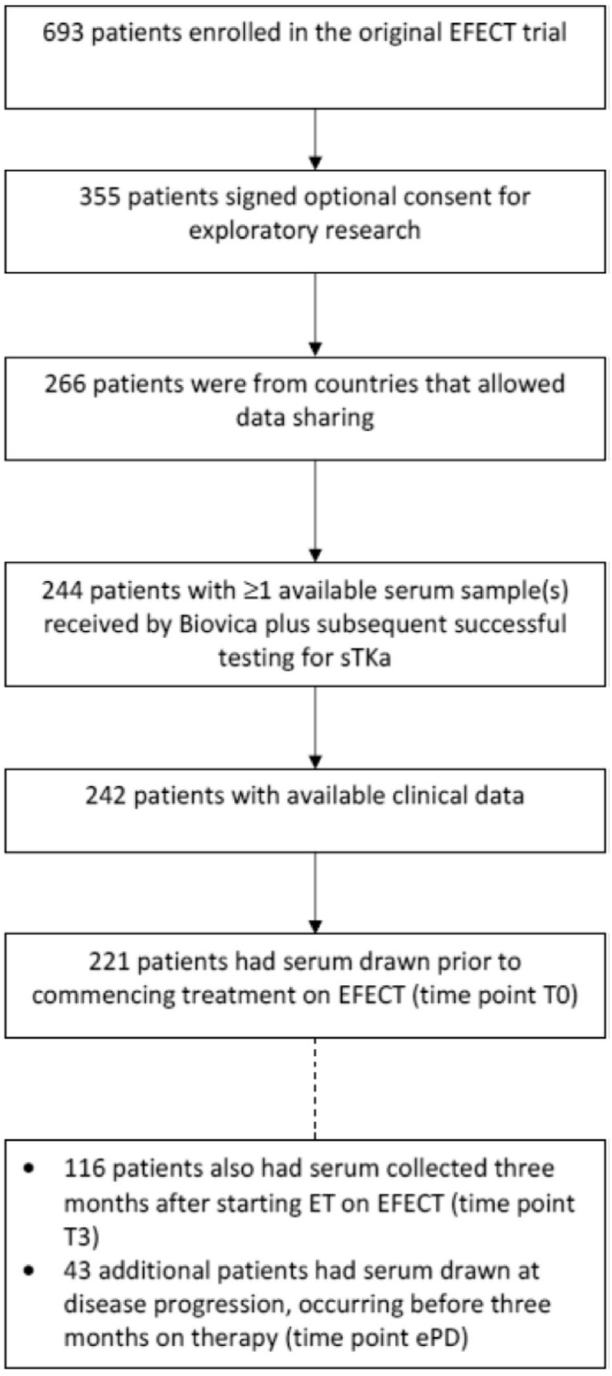

Fig. 1. CONSORT diagram for the EFECT substudy. EFECT, Evaluation of Faslodex versus Exemestane Clinical Trial; ePD, early disease progression; sTKa, serum thymidine kinase 1 activity. ET, endocrine therapy.

prospective consent (see CONSORT diagram, Fig. 1). Available serum samples for this study (including duplicate samples derived from the same patient) were drawn at the following time points: baseline (T0) (N = 227); after three months of study treatment (T3) (N = 135); after six months of study treatment (T6) (N = 80) and at disease progression (PD) (N = 137). Among the patients with an available serum sample at PD, 43 experienced early disease progression (ePD) within 13 weeks from randomisation.

Overall, the original EFECT study enrolled a relatively heavily pre-treated patient population with significant disease burden: approximately 60% of those randomised had received at least two prior lines of endocrine therapy for advanced disease, 23% had received chemotherapy for advanced disease and 57% had visceral metastatic involvement. An amendment to the EFECT study protocol allowed the inclusion of patients with assessable but non-measurable-by-RECIST (Response Evaluation Criteria in Solid Tumours) bone-only (lytic or mixed) metastatic lesions. Resistance to aromatase inhibitor therapy was identified in 37% of the overall population. Sensitivity to aromatase inhibition was determined by the investigator and defined by the presence of a complete response, partial response or stable disease for at least six months while receiving an aromatase inhibitor for advanced disease. Clinical benefit as described in this study followed the same definition set by the EFECT investigators, that being a patient having a best overall response of a complete response, partial response or stable disease for at least 24 weeks. Furthermore, stable disease was defined as a patient having achieved neither a partial response nor progressive disease at week 24 or later [19].

3. Laboratory analysis

Serum samples were retrieved from the biorepository of AstraZeneca. Aliquots of 1 mL from eligible patients were directly shipped to the central laboratory of Biovica in Uppsala (Sweden), labelled with an anonymised code. BIOVICA had no access to clinical data. TK1 activity was determined by a refined enzyme-linked immunosorbent assay (ELISA)–based method, the DiviTum™ assay (Biovica, Instructions for Use, www.biovica.com).

For this assay, an aliquot of 100 μL of serum was diluted 1/10 in a dilution buffer. The diluted sample was transferred to a well with the reaction mixture on the assay plate. During incubation, the thymidine analogue bromodeoxyuridine (BrdU) was phosphorylated to BrdU-monophosphate by TK in the serum sample, then further phosphorylated and incorporated in an immobilised DNA strand in the well. After washing, BrdU incorporation was detected using an ELISA technique using an anti-BrdU monoclonal antibody conjugated to the enzyme alkaline phosphatase and a chromogenic substrate. The absorbance of the product of the reaction was measured at 405 nm with the reference wavelength of 630 nm after 30 and 60 min of incubation. The measured signal was proportional to the TK activity of the tested sample and given as DiviTum™ units per litre (Du/L), calculated from a standard curve based on calibrators of

known activity. The DiviTum™ assay has a working range from 204000 Du/L, and the median coefficient of variation of all samples analysed was 6%. Two control samples included with the kit were run in parallel.

4. Statistical analysis

A value of 20 Du/L was used to impute the measurements of sTKa under the detection limit (20 Du/L) for statistical analysis. DiviTum currently has no established formal 'cut-off' value, and as such, sTKa low/high delineations were defined according to the median sTKa value in the entire baseline population, in accordance with the approach adopted in previous studies using DiviTum [15,18]. On-treatment sTKa changes were calculated from T0 to the next available time point within 13 weeks from randomisation ('T3' or 'ePD'). Patients with ePD were included in the analysis of on-treatment sTKa activity to account in part for the significant proportion of patients whose disease progressed within three months. In accordance with previous work [17,18], we accounted for a coefficient of variation of 10% in estimating sTKa measure uncertainty. Baseline sTKa levels were considered not equal to on-treatment levels if the absolute difference between the two values was more than one standard deviation. Differences in distribution of major clinicopathological factors across different sTKa classes were evaluated through a two-sided Fisher exact test at the 5% significance level. Distribution of time to progression (TTP) was estimated using the Kaplan–Meier method and compared using the log-rank test. Univariate and multivariate Cox proportion hazard models were fitted to evaluate the independent effect of each covariate on TTP, estimating hazard ratio (HR) with 95% confidence interval (CI). Clinical benefit rate was evaluated separately according to baseline and on-treatment sTKa.

In evaluating the overall median sTKa levels observed at different time points, all available serum samples were included in analysis, including all duplicate samples drawn from a single patient at a single time point. In evaluating baseline and on-treatment levels of sTKa in relation to clinical outcome, when duplicate samples from a single patient at any one time point were available, the median value of these samples was taken.

As these were exploratory analyses, the *P*-values and supportive analyses should be considered descriptive only. All statistical analyses were performed using R version 3.4.3 (http://www.R-project.org/).

5. Results

5.1. Clinicopathological characteristics of the studied groups

The clinical and pathological characteristics of the patients included in the analysis of sTKa T0 baseline levels divided by high/low sTKa values (median) are described in Table 1 ($N = 221$). There were no significant differences among patients with high and low baseline sTKa levels, with the notable exception of a higher percentage of patients with only 0–1 RECIST-measurable metastatic sites found in the low baseline sTKa group (64% versus 36%, $P = 0.002$). The characteristics of the patients with both available T0 and on-treatment sTKa (T3 or ePD) levels ($N = 159$) were well balanced across both categories, with no significant differences identified. Among these patients, those in the ePD group had a

Table 1
Clinical and pathological characteristics of patients categorised according to baseline (T0) sTKa (low versus high) and according to on-treatment (at three months or point of progressive disease occurring before 13 weeks) sTKa changes (increase versus no increase).

Clinical characteristic	Low baseline sTKa, *N* (%)	High baseline sTKa, *N* (%)	Total (*N*)	*P*-value	sTKa increase, *N* (%)	sTKa no increase, *N* (%)	Total (*N*)	*P*-value
Age								
<65 years	55 (45)	67 (55)	122	0.105	37 (42)	51 (58)	88	0.87
≥65 years	56 (57)	43 (43)	99		31 (44)	40 (56)	71	
Sensitivity to prior AI								
AI resistant	42 (45)	51 (55)	93	0.221	24 (37)	40 (63)	64	0.33
AI sensitive	69 (54)	59 (46)	128		44 (46)	51 (54)	95	
Visceral involvement								
No	54 (53)	47 (47)	101	0.419	29 (38)	47 (62)	76	0.27
Yes	57 (47)	63 (53)	120		39 (47)	44 (53)	83	
Number of metastatic sites								
0–1	53 (64)	30 (36)	83	0.002	28 (43)	37 (57)	65	1.0
2–5	58 (42)	80 (58)	138		40 (43)	54 (57)	94	
Receptor status								
ER+/PR+	66 (50)	65 (50)	131	1.0	42 (45)	52 (55)	94	0.63
Not ER+/PR+	45 (50)	45 (50)	90		26 (40)	39 (60)	65	
Total	111 (50)	110 (50)	221		68 (43)	91 (57)	159	

Values expressed in *N* (%) unless otherwise indicated.
sTKa, serum thymidine kinase 1 activity; AI, aromatase inhibitor; ER+, oestrogen receptor–positive; PR+, progesterone receptor–positive.

greater frequency of visceral disease and a higher number of metastatic sites than the patients in the T3 group (supplementary Table 1). The characteristics of both the baseline and on-treatment substudy cohorts closely resembled those of the original EFECT population [19].

5.2. Median sTKa readings in all analysed serum samples at different time points

Overall, in all successfully evaluated serum samples collected at assigned time points, the median baseline (T0) sTKa reading was 97 Du/L (range: 5–13,980) (serum sample $N = 227$). In progression-free patients with available serum collected at three months after treatment commencement (T3) (sample $N = 135$), the median value dropped to 57 Du/L (range: 4–4185), thought to be generally reflective of disease response in the absence of progression. In the smaller subset of patients without progressive disease (T6) (sample $N = 80$) with available serum collected at six months, the median value remained stable (median, 57.5 Du/L; range, 8–7010), suggestive of sustained disease response. In patients with available serum collected at disease progression occurring at any point on trial (sample $N = 137$), the median sTKa rose to 132 Du/L (range: 10–36,990). Interestingly, the median sTKa level at disease progression in patients within the ePD group was 663 Du/L (range, 20–36,990), with a median baseline sTKa at T0 of 408 Du/L (range, 20–5690)—notably higher than both the median sTKa values at PD and at baseline for all other patients.

5.3. Baseline cohort (T0 samples)

To test the prognostic role of sTKa measured at baseline (T0), we analysed the clinical outcome of patients divided in two groups (high vs low sTKa) according to the median value. Of the proportion of patients who derived clinical benefit on trial, the majority had low baseline sTKa. Concordantly, the majority of patients who did not derive clinical benefit on trial had high baseline readings ($P = 0.001$) (Table 2). The median time to progression (mTTP) was 3.68 months (95% CI: 3.42–4.11) for the entire baseline cohort ($N = 221$). In those with low levels of sTKa at baseline, mTTP was significantly longer than those with high levels (5.03 versus 2.57 months, respectively; $P < 0.0001$) (Fig. 2a). Similar results were observed in the two separate treatment arms. In those who received exemestane on trial, mTTP was 5.26 months (95% CI: 3.91–6.68) in patients with low sTKa, versus 1.88 months (95% CI: 1.74–3.52) in patients with high sTKa at baseline ($P = 0.002$) (Fig. 3a). In patients who received fulvestrant, those with low baseline sTKa had an mTTP of 4.14 months (95% CI: 3.72–8.45) versus 3.19 months (95% CI: 2.17–4.01) in those with high baseline ($P = 0.001$) (Fig. 3b). A multivariate analysis, adjusting for major prognostic factors including treatment received, presence of visceral disease, number of metastatic sites and presence of previous resistance to aromatase inhibitors, was performed. After adjustment, sTKa levels at baseline remained an independent marker of outcome (Table 3).

Table 2
Clinical benefit observed in patients according to baseline sTKa (low versus high).

Clinical benefit	Low baseline sTKa, *N* (%)	High baseline sTKa	Total (*N*)	*P*-value
Yes	36 (71)	15 (29)	51	0.001
No	75 (44)	95 (56)	170	
Total	111 (50)	110 (50)	221	

Values expressed in *N* (%) unless otherwise indicated.
sTKa, serum thymidine kinase 1 activity.

5.4. On-treatment cohort (T0 compared with T3 or ePD samples)

To test the prognostic role of sTKa changes between the baseline (T0) and the next available time point within 13 weeks from randomisation (T3 or ePD), we analysed the clinical outcome of patients divided into two groups (increase vs no increase). Of the proportion of patients who derived clinical benefit on trial, the majority had no increase in sTKa on treatment. Conversely, patients who did not derive clinical benefit were equally represented in the 'sTKa increase' and 'sTKa no increase' groups ($P = 0.013$) (Table 4). The mTTP was 4.08 months (95% CI: 3.68–5.49) for the overall on-treatment population ($N = 159$). Patients who demonstrated no increase in sTKa while on treatment had an mTTP of 5.39 months (95% CI: 4.01–6.68), versus 3.39 months (95% CI: 2.14–4.11) in those with increased levels ($P = 0.017$) (Fig. 2b). The same analysis performed separately in the T3 ($N = 116$; 33.6% with sTKa increase) and ePD ($N = 43$; 67.5% with sTKa increase) groups did not show any statistically significant difference. In patients who received exemestane, the mTTP was 4.57 months in those with no increase in sTKa versus 3.45 months in those with increase ($P = 0.02$) (Fig. 4a). In the fulvestrant group, a statistically insignificant difference in mTTP was found in patients with no increase in sTKa, compared with those with an increase (5.79 versus 2.89 months, respectively; $P = 0.11$) (Fig. 4b). After a multivariate analysis, adjusting for prognostic factors, on-treatment sTKa remained an independent marker of outcome (Table 5).

6. Discussion

This substudy provides positive evidence in favour of baseline sTKa levels as a potential prognostic marker.

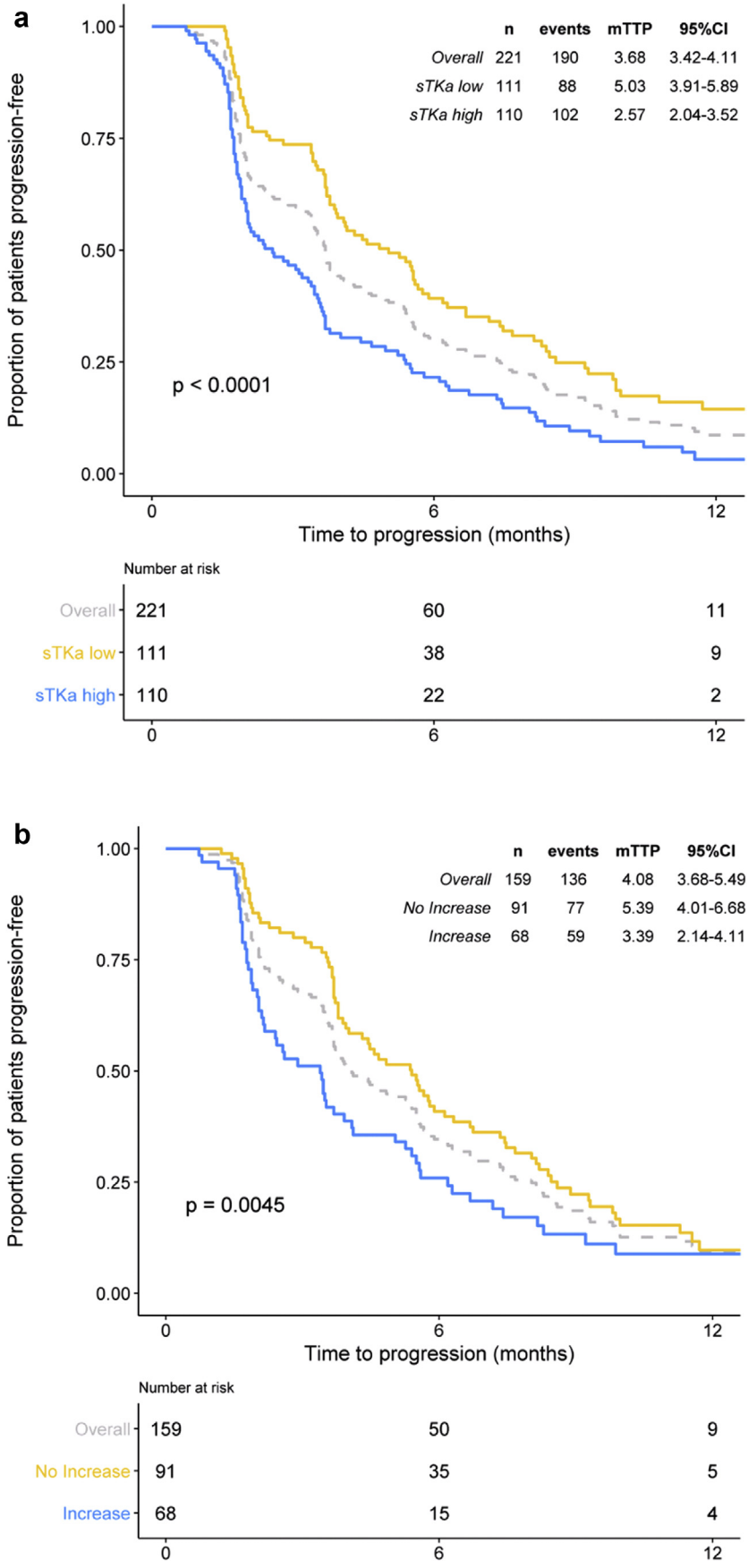

Fig. 2. Median time to progression according to baseline sTKa in the overall population (Fig. 2a) and according to on-treatment sTKa in the overall population (Fig. 2b). CI, confidence interval; mTTP, median time to progression; sTKa, serum thymidine kinase 1 activity.

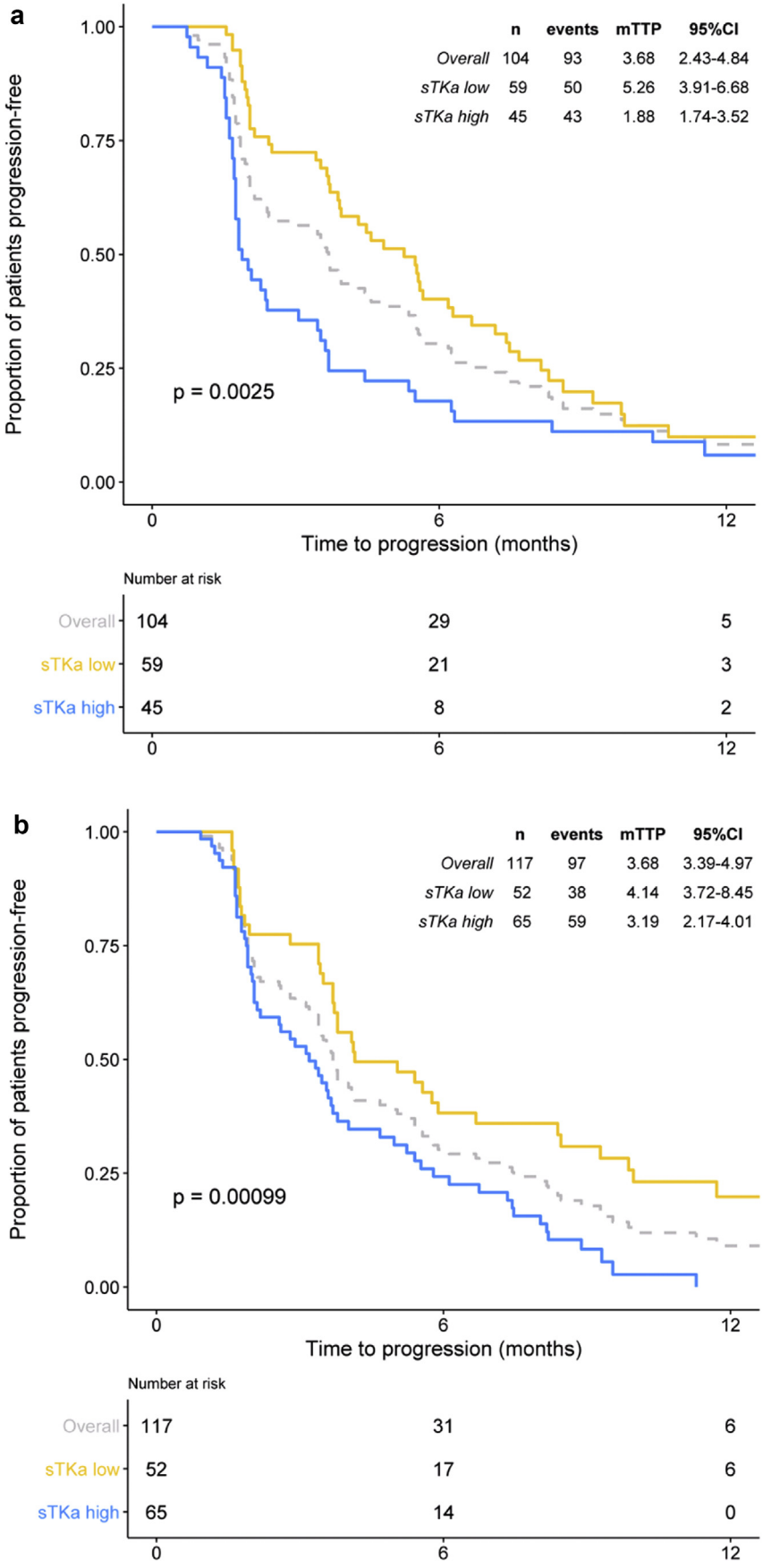

Fig. 3. Median time to progression according to baseline sTKa, observed in the two separate treatment arms—exemestane (Fig. 3a) and fulvestrant (Fig. 3b). CI, confidence interval; mTTP, median time to progression; sTKa, serum thymidine kinase 1 activity.

Table 3
Univariate and multivariate analyses of time to progression according to baseline sTKa.

Analyses variables	HR	95% CI	*P*-value
Univariate analysis			
Baseline sTKa (high vs low)	1.85	1.39–2.50	<0.001
Multivariate analysis			
Study treatment (FUL vs EXE)	0.84	0.63–1.10	0.25
Visceral involvement (Y vs N)	1.52	1.10–2.10	0.01
AI sensitivity (Y vs N)	1.06	0.79–1.40	0.71
Number of metastatic sites (2–5 vs 0–1)	1.09	0.78–1.50	0.62
Baseline sTKa adjusted for above prognostic factors	1.88	1.40–2.50	<0.001

sTKa, serum thymidine kinase 1 activity; HR, hazard ratio; CI, confidence interval; EXE, exemestane; FUL, fulvestrant; AI, aromatase inhibitor.

Table 4
Clinical benefit observed in patients according to on-treatment (at three months or at point of early progressive disease occurring before 13 weeks) sTKa changes (increase versus no increase).

Clinical benefit	sTKa no increase, *N* (%)	sTKa increase, *N* (%)	Total (*N*)	*P*-value
Yes	33 (73)	12 (27)	45	0.013
No	58 (51)	56 (49)	114	
Total	91 (57)	68 (43)	159	

Values expressed in *N* (%) unless otherwise indicated.
sTKa, serum thymidine kinase 1 activity.

The mTTP was significantly longer in patients with low pre-treatment sTKa than those with a high baseline reading (5.03 months versus 2.57 months, respectively; $P < 0.0001$). Furthermore, variation of sTKa on treatment, as reflected by an elevation of sTKa after starting therapy, may represent a tool for early identification of patients with resistance to endocrine therapy. This provides further validation of previous data in support of TK1 as a prognostic and monitoring marker of luminal advanced BC treated with endocrine therapy [18]. Eventual resistance to endocrine therapy is considered inevitable in all patients with HR + disease, and similarly, primary resistance is present from the start of treatment in a significant proportion of patients with advanced BC. As such, early identification via a biomarker of resistance is of considerable clinical importance—potentially enabling clinicians to direct an early switch to a more efficacious regimen, rather than waiting for treatment failure to be confirmed by tangible evidence of progressive disease. This study demonstrated that only 18% (12/68) of patients whose sTKa rose on treatment derived clinical benefit from endocrine therapy. Conversely, of those with no change or a decrease in sTKa after starting endocrine therapy, 36% (33/91) had clinical benefit, thus demonstrating the potential for this biomarker to provide early reassurance of the likelihood of therapeutic efficacy (Table 4). sTKa has also previously been shown to outperform CA15.3 in predicting outcome in patients with metastatic disease [15]. Beyond endocrine therapy, sTKa may prove to have further utility as a marker in other treatment modalities. Recently, sTKa has demonstrated potential utility as a marker of cyclin-dependent kinase 4/6 inhibition in patients receiving neoadjuvant endocrine therapy plus palbociclib [20].

The present study conducted multivariate analyses of baseline and on-treatment sTKa, adjusting for major prognostic factors including presence of visceral involvement, number of metastatic sites, sensitivity to aromatase inhibitor therapy and treatment received. sTKa was upheld as an independent marker of both prognosis and early treatment response. This finding is intuitively sound, given that sTKa is seen to reflect tumour biology and commensurate proliferation rate, rather than disease burden alone. In this study of those patients with only 0–1 metastatic sites at enrolment, a greater proportion was found to have low baseline sTKa, which may be reflective of the biology and proliferation seen in oligometastatic disease, compared with that of heavy visceral involvement. Furthermore, patients whose disease progressed within three months (ePD) (overall mTTP: 1.74 months, 95% CI: 1.68–1.88) were more likely to have 2–5 sites of metastatic disease and visceral disease than patients free from progression at three months (overall mTTP: 5.76 months, 95% CI: 5.39–7.34). This adds to previous data that suggest TKa values may differ according to specific metastatic sites, with locoregional/skin and bone metastases reflecting lower median levels of sTKa than hepatic metastases [15]. As a marker of proliferation, TK1 may be regarded as similar to Ki67, with the latter shown to possess some potential as a prognostic marker in early BC [21,22]. However, clinical utilisation of Ki67 as a clinical marker is impeded by factors such as a high degree of discordance between pathology reports [23] and variability in results secondary to intratumoural heterogeneity [24]. In contrast, sTKa may represent an attractive proliferation marker in BC with the advantage of not requiring tumour tissue for evaluation, with peripheral blood draws easily analysed via an ELISA-based assay.

This study was limited by several factors. The studied population, not unlike the overall cohort of EFECT, was generally heavily pre-treated with advanced disease, and therefore, a significant number of patients progressed on trial in less than 13 weeks from entry. As was the case with the original EFECT study, which reported an overall response rate of approximately 7% for both treatment arms and a clinical benefit rate of approximately 31% [19], the majority of those in this substudy did not derive clinical benefit from study treatment. To account for the significant proportion of patients with ePD in the face of treatment failure, this study analysed the on-treatment effect of sTKa by combining those with early progression with patients who had available sera

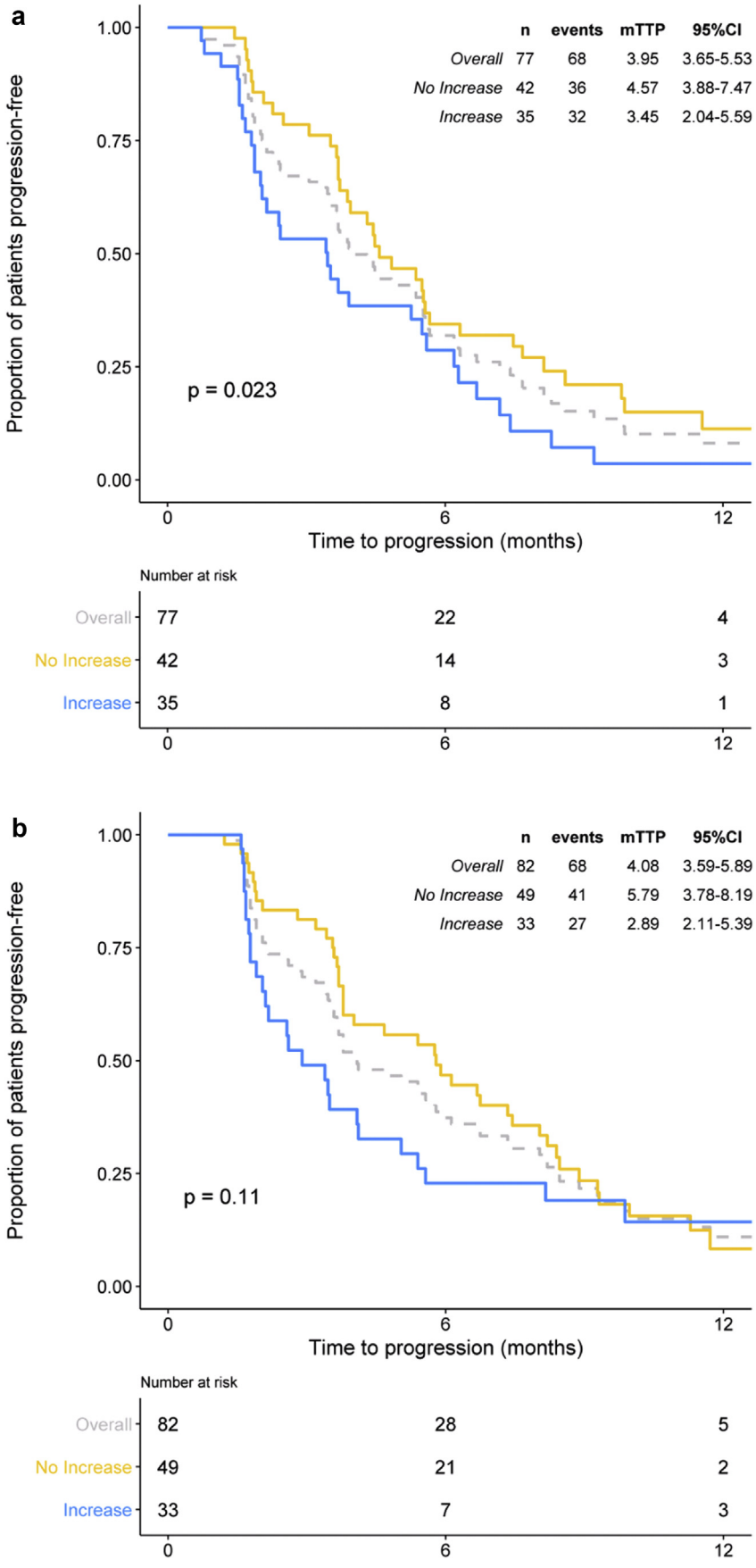

Fig. 4. Median time to progression according to on-treatment sTKa observed in the two separate treatment arms—exemestane (Fig. 4a) and a statistically non-significant difference in the fulvestrant arm (Fig. 4b). CI, confidence interval; mTTP, median time to progression; sTKa, serum thymidine kinase 1 activity.

Table 5
Univariate and multivariate analysis of time to progression according to on-treatment sTKa changes.

Analyses variables	HR	95% CI	*P*-value
Univariate analysis			
sTKa change (increase vs no increase)	1.64	1.16–2.30	0.004
Multivariate analysis			
Study treatment (FUL vs EXE)	0.82	0.58–1.20	0.27
Visceral involvement (Y vs N)	1.53	1.06–2.20	0.02
AI sensitivity (Y vs N)	1.21	0.86–1.70	0.27
Number of metastatic sites (2–5 vs 0–1)	1.45	0.99–2.10	0.06
sTKa change adjusted for above prognostic factors	1.83	1.28–2.60	<0.001

sTKa, serum thymidine kinase 1 activity; HR, hazard ratio; CI, confidence interval; EXE, exemestane; FUL, fulvestrant; AI, aromatase inhibitor.

drawn at three months in the absence of progressive disease. As such, the smaller ePD subgroup may have introduced bias, given that sTKa measured at the point of disease progression represents a direct signal of that progression, rather than a prediction of the event. Separate analyses of on-treatment sTKa in the T3 and ePD cohorts failed to reach statistical significance, possibly due to the reduced number of patients in the two subgroups and the consequent limited statistical power to detect a prognostic effect among subgroups with such a homogeneous prognosis. However, we considered it important to include the ePD population, given they represent a subgroup with an especially poor prognosis existing within a population which had a relatively short TTP overall. Indeed, the notably high median baseline and on-progression sTKa levels observed in the ePD group may reflect a biologically more proliferative, more aggressive clinical phenotype with a relative endocrine resistance at the point of study entry.

The retrospective nature of the analysis is acknowledged, as is the ongoing uncertainty regarding the ideal time point(s) at which serum should be collected to compare on-treatment sTKa with baseline. Owing to the limitations imposed by precollected sera, this study established that time point at three months following treatment commencement or at the point of disease progression within that period. Consequently, this study was not able to conclude that sTKa on treatment reliably anticipated progressive disease. In the clinical setting, radiological assessment would commonly occur after approximately three months of treatment, and thus, a biomarker should reflect response at an earlier juncture. Our earlier pilot study collected repeat samples one month after commencing endocrine therapy, with similar positive findings to those observed in the on-treatment EFECT cohort. We also demonstrated that endocrine-sensitive BC cell lines showed diminished levels of TK1 activity after only two days of endocrine therapy exposure, anticipating the detection of reduced cellular proliferation seen at later time points with a standard proliferation assay [18]. Cumulatively, these data suggest that sTKa measured at early time points may be clinically feasible, supporting its potential use as an early biomarker of response to endocrine therapy. In addition, the general dynamics of median sTKa levels measured at different time points provided noteworthy data which may be considered in future studies. In the ePD group, representative of patients with a particularly poor prognosis whose disease progressed quickly on trial within three months of study enrolment, the median sTKa at baseline was considerably higher than that of the entire T0 cohort (408 Du/L versus 97 Du/L, respectively). Similarly, at the point of progression on trial, ePD patients had much higher median sTKa levels (663 Du/L) than patients whose disease progressed at a later juncture (95 Du/L), far exceeding the median level at baseline. In contrast, the median sTKa in patients with later progression was 95 Du/L, similar to the T0 median overall, likely preceded by an initial drop noted at T3 and/or T6 (57 Du/L). This preliminary data suggest that not only may baseline sTKa be prognostic but the amplitude and magnitude of baseline activity and succeeding dynamic change might also be a significant factor.

The overall heavily pre-treated population recruited to the EFECT study had demonstrated resistance to prior endocrine therapy, which resulted in generally rapid times to disease progression and poor clinical benefit rates alike. Despite this, sTKa was capable of discerning a prognostically more favourable subgroup of patients within a group with an overall poor prognosis. This may reflect the presence of ongoing sensitivity to endocrine therapy in this particular subgroup. The mTTP in the original EFECT study was 3.70 months in both treatment arms [19], whereas this substudy demonstrated an mTTP of 5.03 months in patients with low baseline sTKa, and similarly, this study demonstrated an mTTP of 5.39 months in patients with no increase of sTKa on treatment. Conversely, our prior pilot study was performed in a less heavily pre-treated population, showing the potential of sTKa to identify a population with extreme endocrine sensitivity in an earlier setting, with markedly better median progression-free survival (mPFS) seen in patients with low sTKa levels at baseline or a subsequent drop in levels in response to the introduction of endocrine therapy [18], suggesting that sTKa has potential value as a biomarker throughout the time course of managing metastatic disease. Furthermore, given its prognostic utility as a proliferative marker, sTKa has future capacity to be evaluated in the adjuvant setting.

Additional validation of sTKa as a biomarker of prognosis and early treatment resistance in BC is warranted, with such future studies providing further data regarding the most effective time point at which to assess

TK1 activity. Furthermore, based on these results, it would be timely to consider a prospective trial testing the clinical utility of sTKa, particularly in clinical settings wherein clinical uncertainty exists regarding the addition of targeted therapies to first-line endocrine therapy in the treatment of metastatic disease.

Funding

This work was funded in part by a research grant from Associazione Italiana per la Ricerca sul Cancro (MFAG grant number 18880 to LM).

Acknowledgements

The authors would like to thank the ongoing support provided by the Sandro Pitigliani Foundation (Prato, Italy).

Conflict of interest statement

G.S. is an employee of AstraZeneca and holds ownership interests (including patents) in the company. M.Ber. and K.M. are employees of Biovica International and hold stock/stock options in the company. L.B. reports an advisory role and lecture fees/honoraria from Novartis, AstraZeneca and Pfizer. M.Pic. declares honoraria from Pfizer and AstraZeneca. A.D.L. declares an advisory role and lecture fees/honoraria from AstraZeneca, Novartis and Pfizer and holds research grants from Pfizer and Novartis. L.M. is a consultant for AstraZeneca, Pfizer and Novartis. A.M., C.B., I.M., M.Ben., D.R., M.Bon., G.B., M.Pes., F.G., F.D.L., W.J.G. and S.C. declare no competing conflicts of interest for the work under consideration.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.04.002.

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