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Dynamic thymidine kinase activity independently captures treatment-specific biological response and is independently associated with outcomes in endocrine-resistant HR+ /HER2 − metastatic breast cancer: A translational analysis of the GEICAM/2013–02 PEARL trial

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ABSTRACT

Background: Treatment selection in endocrine-resistant HR+ /HER2 − metastatic breast cancer (MBC) remains challenging, and early predictive biomarkers are lacking. Circulating thymidine kinase 1 activity (TKa) is a blood-based proliferation marker suitable for dynamic monitoring.

Methods: This translational study was conducted within the phase III GEICAM/2013–02 PEARL trial comparing endocrine therapy (ET) plus palbociclib versus capecitabine in HR+ /HER2 − MBC. Plasma from 555 patients was analyzed using a standardized FDA-cleared assay. TKa was assessed at baseline using a prespecified cutoff of 250 DuA to define low versus high proliferative activity, and additional thresholds were explored for treatment interaction and on-treatment monitoring. Associations with progression-free survival (PFS) and overall survival (OS) were evaluated using multivariable Cox models.

Results: Low baseline TKa (≤250 DuA) was independently associated with longer PFS (11.4 vs 4.0 months) and OS (38.5 vs 17.3 months), in multivariate analysis and irrespective of treatment. However, baseline TKa did not discriminate benefit between arms. Early on-treatment TKa dynamics provided treatment-specific information. At cycle 1 day 15, median TKa was higher with capecitabine than with ET plus palbociclib (448 vs 28 DuA), consistent with distinct mechanisms. In the capecitabine arm, a > 2-fold early increase in TKa was independently associated with improved PFS and OS. Conversely, persistently elevated TKa (>50 DuA) during ET plus palbociclib identified patients with poorer outcomes, regardless of baseline levels.

Conclusions: Baseline TKa is strongly prognostic, while early dynamic changes provide additional, treatment-specific information, reflecting differences in mechanism of action between therapies, that support response monitoring.

1. Introduction

Hormone receptor–positive, HER2-negative (HR+/HER2 −) metastatic breast cancer (MBC) is the most prevalent subtype of advanced breast cancer and exhibits substantial biological and clinical heterogeneity [1]. The incorporation of cyclin-dependent kinase 4/6 (CDK4/6) inhibitors into endocrine therapy (ET) has significantly improved outcomes; however, primary and acquired resistance are common, and optimal treatment selection following ET resistance remains a major clinical challenge. In this setting, cytotoxic chemotherapy is an important therapeutic option, yet biomarkers guiding the choice between endocrine-based strategies and chemotherapy are lacking [2–4].

Current decision-making relies largely on static clinicopathological features and baseline biomarkers that provide prognostic but limited predictive information. Biomarkers capable of dynamically capturing early biological response to therapy could improve patient stratification and enable treatment selection based on mechanism of action rather than solely on baseline risk [5–7]. Markers of tumor proliferation are of particular interest, given the central role of cell-cycle dysregulation in HR+ breast cancer [8,9].

Thymidine kinase 1 (TK1) is a proliferation-associated enzyme required for DNA synthesis through the thymidine salvage pathway [10]. Circulating TK1 activity (TKa) can be quantified using the DiviTum™ assay, enabling non-invasive monitoring of proliferative dynamics during treatment [11]. Elevated baseline TKa has consistently been associated with poor prognosis in MBC; however, baseline measurements do not capture treatment-induced biological changes, and the clinical relevance of dynamic TKa assessment remains incompletely defined [12,13].

The phase III GEICAM/2013–02 PEARL trial compared ET (aromatase inhibitor or fulvestrant) plus palbociclib with capecitabine in aromatase inhibitor–resistant HR+ /HER2 − MBC and reported no significant difference in survival outcomes between arms [14]. We hypothesized that dynamic assessment of TKa, integrated with multivariable clinical modeling, could reveal biologically and clinically meaningful heterogeneity within PEARL that was not apparent in the primary analysis.

2. Materials and Methods

2.1. Study design and patients

This was a translational analysis of the randomized, open-label, phase III GEICAM/2013–02 PEARL trial (NCT02028507). Eligible patients were postmenopausal women with HR+ /HER2 − metastatic breast cancer who had relapsed or progressed during or after aromatase inhibitor therapy. Patients were randomized to receive ET (Exemestane or Fulvestrant) plus palbociclib or single-agent capecitabine according to the trial protocol. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and all patients provided written informed consent for biomarker analyses. TKa analyses were performed retrospectively by Biovica International AB using blinded samples identified by coded IDs, without access to clinical data. The study sponsor (GEICAM) designed the analysis, conducted the statistical analyses independently, and integrated biomarker results with clinical data; Biovica contributed to assay-related methodology and reviewed the manuscript.

2.2. Plasma sampling and TKa measurement

Peripheral blood samples were collected at baseline (C1D1), pre-dose or within 7 days prior to treatment initiation, and early during treatment at cycle 1 day 15 (C1D15) and cycle 2 day 15 (C2D15), both pre-dose (±3 days). Plasma was isolated using standardized procedures and stored at −80 °C. TKa was measured using the FDA-cleared and CE-marked DiviTum® TKa assay (Biovica International AB), which quantifies TK1 enzymatic activity. Laboratory personnel were blinded to clinical data and outcomes.

2.3. Molecular Subtype Classification

The breast cancer intrinsic subtypes were assigned using the single sample predictor algorithm Absolute Intrinsic Molecular Subtyping (AIMS) classifier [15], via EdgeSeq Oncology BM Panel (HTG Molecular Diagnostics, Tucson, AZ) for genomic expression mRNA profiling.

2.4. ESR1 mutation status

ESR1 mutations were analyzed in plasma samples collected at study inclusion using commercially available multiplex droplet digital PCR (ddPCR) assays (Bio-Rad) targeting the seven most common *ESR1* mutations: multiplex 1 (E380Q, L536R, Y537C, D538G) and multiplex 2 (S463P, Y537N, Y537S). ddPCR was performed on the QX-200 system (Bio-Rad) as previously described in a central laboratory [14]. *ESR1* mutation status was available for 553 patients of the 555 included in the biomarker analysis.

2.5. Cut-offs and endpoints

TKa clinical reference values of 250 and 50 DiviTum units of activity (DuA) were used for baseline and on-treatment analyses, respectively. The 250 DuA threshold corresponds to the 95th percentile of TKa levels in a healthy donor population and represents the FDA-cleared cutoff for elevated TKa [11,13], while 50 DuA reflects the lower limit of quantification of the assay (Biovica data on file) [16]. Additional prespecified baseline exploratory thresholds of 50 DuA [17,18] (to assess proliferative suppression), 150 DuA (rounded from the cohort median 153 DuA), quartiles (85, 153 and 320.5 DuA), and 400 DuA were also evaluated. The 400 DuA prespecified cutoff was selected to explore higher levels of proliferation, based on the hypothesis that highly proliferative disease may be more responsive to cytotoxic chemotherapy than ET, while maintaining an adequate sample size for analysis. Primary endpoints for this translational analysis were PFS and OS.

2.6. Statistical analysis

Time-to-event endpoints were estimated using the Kaplan–Meier method and compared using log-rank tests. Cox proportional hazards regression models were used to estimate unadjusted and adjusted hazard ratios (HRs and aHRs) with 95% confidence intervals (CIs). Multivariable models adjusted for treatment arm, baseline TKa, number and site of metastatic lesions, prior chemotherapy for metastatic disease, prior endocrine sensitivity, and ECOG performance status. Interaction terms were included to explore treatment-specific associations. All tests were two-sided.

3. Results

3.1. Patient population and sample availability

Plasma samples suitable for TKa analysis were available from 555 patients, representing 92% of the full study cohort, with a total of 1129 plasma samples analyzed across all time points (plasma samples were available from 489 patients (81%) at C1D15 and from 85 patients (14%) at C2D15). Baseline clinicopathological characteristics were well balanced between treatment arms (not shown) and were similar between the biomarker cohort and the full PEARL study cohort [Table 1_supplemental]. Progression-free survival (PFS) and overall survival (OS) outcomes by treatment were consistent with those reported in the primary PEARL trial, showing no significant differences between treatment arms for PFS (aHR 1.11; 95% CI 0.92–1.35) or OS (aHR 0.97; 95% CI 0.75–1.26). [Table 1_supplemental]

3.2. Baseline TKa is an independent prognostic biomarker

Baseline TKa showed a wide distribution across the study population (median: 153 DuA, min-max:18–34400 DuA). Baseline TKa was

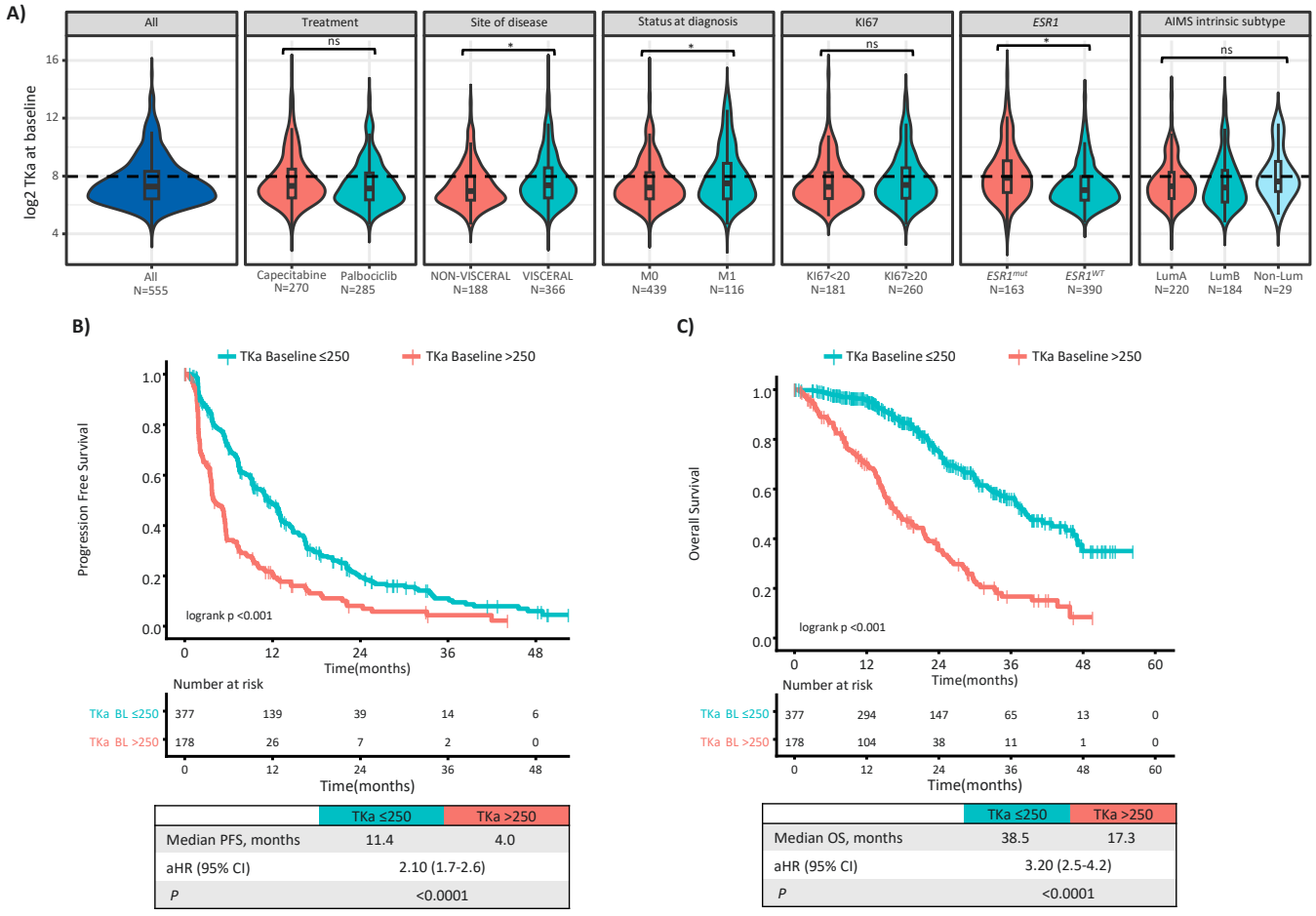


Fig. 1. Baseline TKa in DiviTum units of activity (DuA) and clinical outcomes.(A) Distribution of baseline thymidine kinase activity (TKa) by clinical and biological characteristics; dashed line indicates the prespecified cutoff at log2 250 DuA. (B) Kaplan–Meier curves for progression-free survival (PFS) stratified by baseline TKa (≤250 vs >250 DuA), with adjusted hazard ratios (aHRs) and p-values from multivariable Cox models; log-rank test p-values are shown (univariable data not shown). (C) Kaplan–Meier curves for overall survival (OS) stratified by baseline TKa (≤250 vs >250 DuA), with aHRs and corresponding p-values; log-rank test p-values are shown (univariable data not shown).

significantly higher in patients with visceral versus non-visceral disease (median: 163 DuA vs 123 DuA, p = 0.006) and in patients with de novo metastatic disease compared to those who were not metastatic at initial diagnosis (median: 180 DuA vs 146 DuA, p = 0.04). Baseline TKa was also significantly higher in patients with *ESR1*-mutated tumors than in those with *ESR1* wild-type tumors (median: 233 DuA vs 129 DuA, p < 0.0001). No differences were observed according to treatment arm, Ki67 levels (<20% vs ≥20%) nor AIMS-intrinsic subtype in the whole cohort (Luminal A, Luminal B, Non-Luminal); however, it is important to note that a metastatic tissue sample was not mandatory at study entry, and patients could enter the study using an archival tumor sample derived either from the primary tumor (73% of included patients) or from metastatic sites (27%), however TKa levels did not differ significantly across AIMS intrinsic subtypes irrespective of tumor sample origin. [Figure 1a and Figure 1 supplemental]

Patients with low baseline TKa (≤250 DuA) experienced significantly longer median PFS compared with those with high TKa (>250 DuA) (11.4 vs 4.0 months, p < 0.0001). Importantly, after multivariable adjustment for treatment arm and established clinical prognostic factors, baseline TKa remained independently associated with PFS (aHR 2.10, 95% CI 1.70–2.60; p < 0.0001). [Figure 1b]

Similarly, low baseline TKa was associated with substantially longer median OS (38.5 vs 17.3 months, p < 0,0001) and remained independently associated with OS after multivariable adjustment (aHR 3.20, 95% CI 2.45–4.19; p < 0.0001). [Figure 1c]

ESR1 mutations were detected in plasma in 163 patients (29%) of the study population. Univariable analyses showed that patients without *ESR1* mutations had better outcomes in terms of PFS (HR 0.81; 95% CI 0.66–1.00; p < 0.05) and OS (HR 0.63; 95% CI 0.48–0.83; p < 0.01). Baseline TKa remained independently associated with both PFS and OS after adjustment for *ESR1* mutational status and other relevant clinical covariables in multivariable models. The results were similar in the analysis of baseline TKa by treatment arm, showing that TKa can stratify the prognosis of patients both in the *ESR1* and in the WT group independently of the treatment received. [Figure 2 supplemental]

3.3. Limited Ability of Baseline TKa to Identify Subgroups with Differential Treatment Benefit

Exploratory interaction analyses across multiple baseline TKa cutoffs (quartiles, 50, 150, 250 and 400 DuA) identified a significant interaction between baseline TKa and treatment arm only at the highest TKa levels. Among patients with baseline TKa (>400 DuA), capecitabine was associated with longer median PFS compared with ET plus palbociclib (4.04 vs 2.01 months; aHR 1.72, 95% CI 1.14–2.59; interaction p = 0.0096), This interaction was modest in magnitude, and not observed for OS (15.4 vs 14.7 months, aHR; 1.29, 95% CI 0.84–1.99; interaction p = 0.2428). No significant differences in treatment effect were observed among patients with baseline TKa ≤ 400 DuA. [Figure 3 supplemental]

3.4. Early TKa dynamics differ by treatment mechanism

Following treatment initiation, marked differences in early TKa dynamics were observed. At C1D15, median TKa was significantly higher in patients receiving capecitabine compared with those treated with ET plus palbociclib (448 vs 28 DuA; p < 0.0001). These opposing effects on TKa levels persisted at C2D15. [Figure 2]

3.5. Independent, treatment-specific prognostic value of on-treatment TKa

In the capecitabine arm, 35% of patients exhibited an early increase in TKa between baseline and C1D15 (fold change C1D15/baseline >2). In univariable analyses, this early TKa increase was associated with longer median PFS (13.0 vs 6.3 months, p = 0.0003) and median OS (39.3 vs 23.2 months, p < 0.0001). In multivariable analysis, early TKa increase remained independently associated with improved PFS (aHR 0.59, 95% CI 0.43–0.81; p = 0.0013) and OS (aHR 0.31, 95% CI 0.20–0.50; p < 0.0001) [Figure 3a,b]. When evaluating the interaction between baseline TKa levels (≤250 vs >250 DuA) and on-treatment TKa dynamics (fold change at C1D15/baseline >2), on-treatment assessment retained prognostic value for both PFS and OS among patients with low baseline TKa (≤250 DuA). In contrast, among patients with high baseline TKa (>250 DuA), the adverse prognosis associated with elevated baseline TKa was not modified by on-treatment TKa assessment, indicating limited additional prognostic discrimination in this subgroup [Figure 3c,d]. There were 12 (2.5%) patients with the C1D15 sample extracted out of the window and 40 (8.2%) patients with Capecitabine dose reductions on cycle 1. Sensitivity analyses removing those patients yielded similar PFS/OS results to those shown above (data not shown).

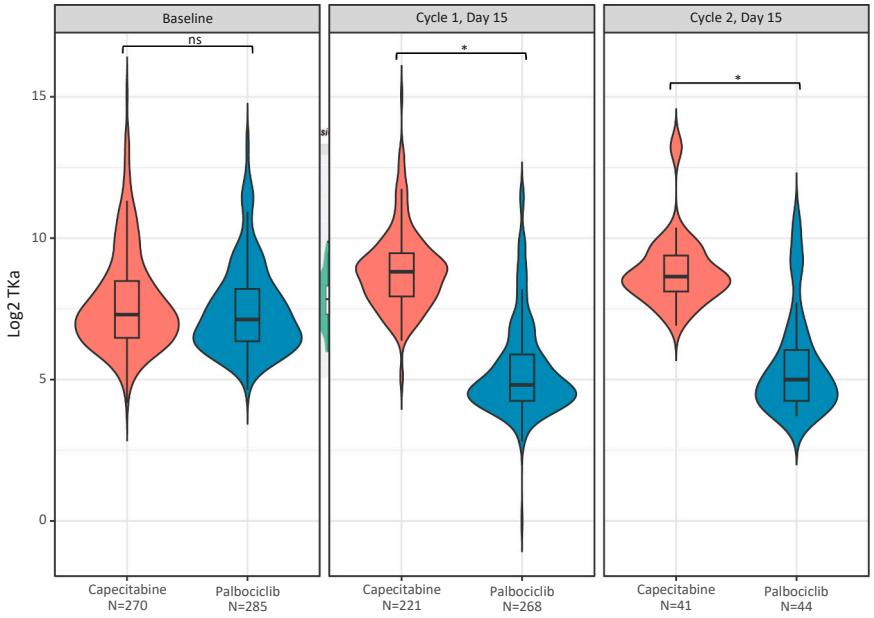
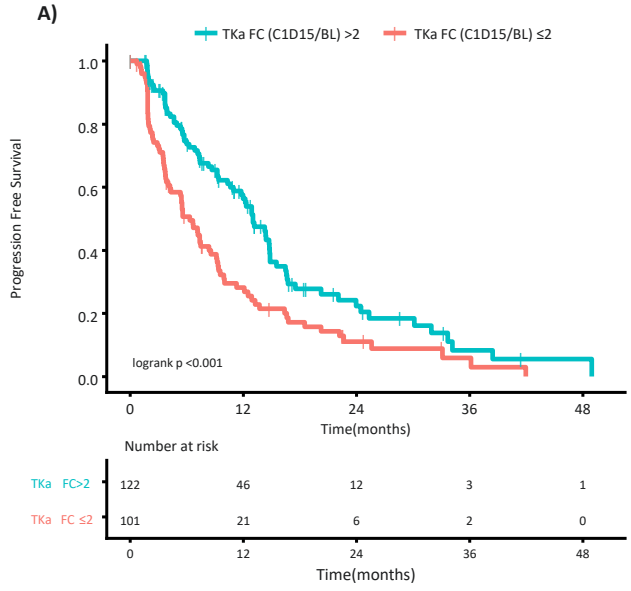
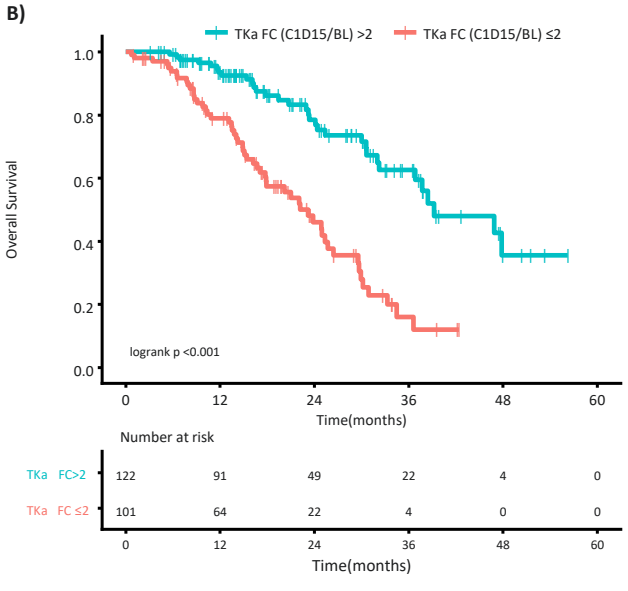


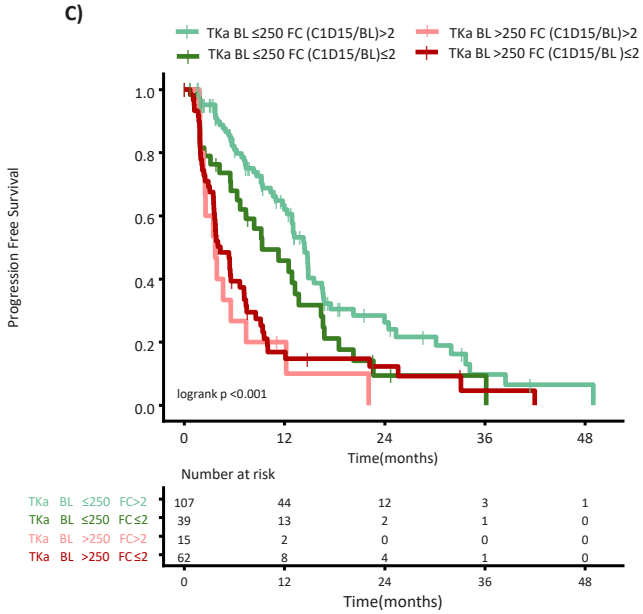
Fig. 2. Thymidine kinase activity (TKa) dynamics in DiviTum units of activity (DuA) differ by treatment. Distribution of TKa levels by treatment at Baseline, at Cycle 1, Day 15 and at Cycle 2, Day 15.



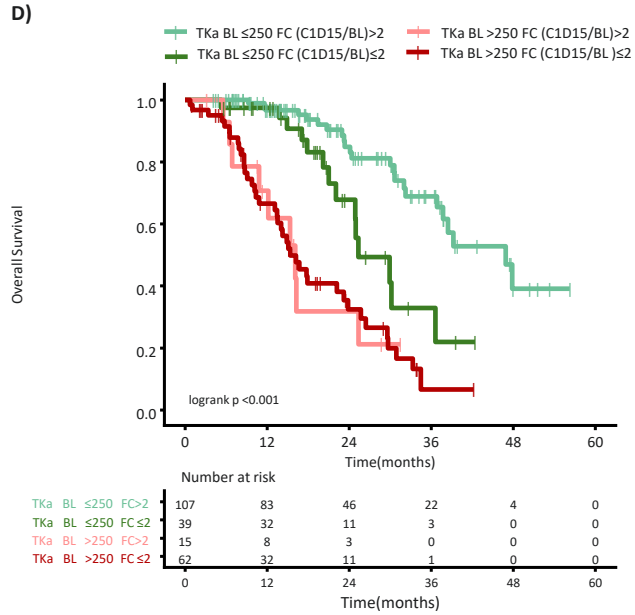
	TKa FC>2	TKa FC≤2
Median PFS, months	13.0	6.3
aHR (95% CI)	0.6 (0.4-0.8)	
P	0.0013	



	TKa FC>2	TKa FC≤2
Median OS, months	39.3	23.2
aHR (95% CI)	0.3 (0.2-0.5)	
P	<0.0001	



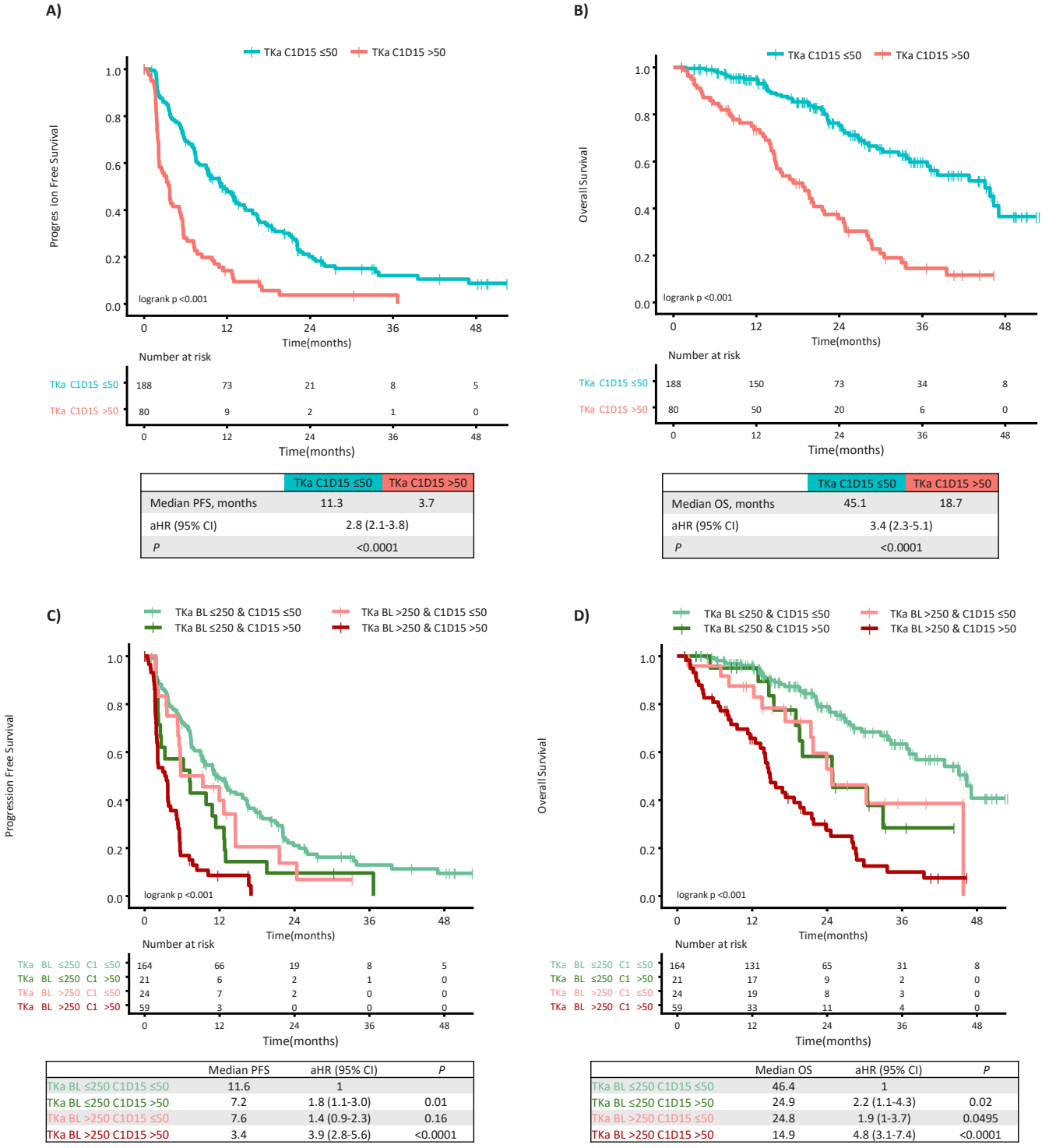
	Median PFS	aHR (95% CI)	*P*
TKa BL ≤250 FC>2	14.4	1	
TKa BL ≤250 FC≤2	9.3	1.5 (0.9-2.3)	0.08
TKa BL >250 FC>2	3.7	2.8 (1.5-5.2)	0.001
TKa BL >250 FC≤2	4.3	2.4 (1.6-3.5)	<0.0001



	Median OS	aHR (95% CI)	*P*
TKa BL ≤250 FC>2	46.9	1	
TKa BL ≤250 FC≤2	25.4	2.5 (1.3-5.0)	0.009
TKa BL >250 FC>2	16.1	6.7 (2.9-15.3)	<0.0001
TKa BL >250 FC≤2	15.4	6.6 (3.7-11.5)	<0.0001

Fig. 3. TKa dynamics in DiviTum units of activity (DuA) at Cycle 1 Day 15 (C1D15) in patients treated with capecitabine and their association with clinical outcomes. (A) Baseline (BL) TKa and C1D15 fold change (FC) in relation to progression-free survival (PFS). (B) BL TKa and C1D15 FC in relation to overall survival (OS). (C) Interaction between BL TKa and TKa dynamics and their effect on PFS. (D) Interaction between BL TKa and TKa dynamics and their effect on OS. Adjusted hazard ratios (aHRs) and corresponding p-values were derived from multivariable Cox proportional hazards models (univariable data not shown); between-group comparisons were assessed using the log-rank test, and treatment interaction effects were evaluated using interaction terms in adjusted Cox models (treatment interaction p-values were not significant (A) p = 0.53; (B) p = 0.52; (C) p = 0.19; (D) p = 0.53).

Among patients treated with endocrine therapy plus palbociclib, most patients experienced a decrease in TKa between baseline and C1D15, either onefold (97% of patients) or twofold (99% of patients). Given this consistent suppression, we explored on-treatment TKa (<50 DuA) as a biologically relevant surrogate of cell-cycle suppression. Persistently elevated C1D15 TKa (>50 DuA) identified a subgroup of patients with particularly poor outcomes. Elevated on-treatment TKa was associated with significantly shorter median PFS (3.7 vs 11.3 months; $p < 0.0001$) and median OS (18.7 vs 45.1 months; $p < 0.0001$) in univariable analysis. These associations remained statistically



	TKa C1D15 ≤50	TKa C1D15 >50
Median PFS, months	11.3	3.7
aHR (95% CI)	2.8 (2.1-3.8)	
P	<0.0001	

	TKa C1D15 ≤50	TKa C1D15 >50
Median OS, months	45.1	18.7
aHR (95% CI)	3.4 (2.3-5.1)	
P	<0.0001	

	Median PFS	aHR (95% CI)	*P*
TKa BL ≤250 C1D15 ≤50	11.6	1	
TKa BL ≤250 C1D15 >50	7.2	1.8 (1.1-3.0)	0.01
TKa BL >250 C1D15 ≤50	7.6	1.4 (0.9-2.3)	0.16
TKa BL >250 C1D15 >50	3.4	3.9 (2.8-5.6)	<0.0001

	Median OS	aHR (95% CI)	*P*
TKa BL ≤250 C1D15 ≤50	46.4	1	
TKa BL ≤250 C1D15 >50	24.9	2.2 (1.1-4.3)	0.02
TKa BL >250 C1D15 ≤50	24.8	1.9 (1-3.7)	0.0495
TKa BL >250 C1D15 >50	14.9	4.8 (3.1-7.4)	<0.0001

Fig. 4. TKa dynamics in DiviTum units of activity (DuA) at Cycle 1 Day 15 (C1D15) in patients treated with ET + palbociclib and their association with clinical outcomes. (A) TKa dynamics in relation to progression-free survival (PFS). (B) TKa dynamics in relation to overall survival (OS). (C) Interaction between baseline (BL) TKa levels and TKa dynamics and their effect on PFS. (D) Interaction between BL TKa levels and TKa dynamics and their effect on OS. Adjusted hazard ratios (aHRs) and corresponding p-values were derived from multivariable Cox proportional hazards models (univariable data not shown); between-group comparisons were assessed using the log-rank test, and treatment interaction effects were evaluated using interaction terms in adjusted Cox models (treatment interaction p-values were all significant (A) $p = 0.004$; (B) $p = 0.021$; (C) $p = 0.019$; (D) $p = 0.025$).

significant after multivariable adjustment for other prognostic covariables (PFS aHR 2.81, 95% CI 2.08–3.80; OS aHR 3.44, 95% CI 2.34–5.06; both $p < 0.0001$) [Figure 4a,b]. When assessing the interaction between baseline TKa levels (≤250 vs >250 DuA) and on-palbociclib TKa dynamics (TKa <50 DuA at C1D15), on-treatment TKa assessment further stratified outcomes for both PFS and OS independent of patient and disease characteristics, demonstrating that dynamic TKa evaluation provides prognostic information beyond baseline proliferative activity for patients treated with ET + palbociclib [Figure 4c,d]. The GEICAM PEARL trial ET arm was composed of two cohorts that differed only for the ET component of the palbociclib arm (exemestane or fulvestrant). We analyzed these two cohorts separately and found that baseline and C1D15 median TKa values were similar in the two groups (baseline mTKa=138 DuA and C1D15 mTKa=29 DuA in patients treated with exemestane, and baseline mTKa=141 DuA and C1D15 mTKa=29 DuA in patients treated with fulvestrant; $p = 0.13$). In addition, the prognostic value of early on-treatment TKa suppression was consistent across both exemestane and fulvestrant, supporting the relevance of TKa assessment across ET with different mechanisms of action [Figure 4 supplemental].

4. Discussion

In this large translational analysis of a randomized phase III trial, we demonstrate that TK1 activity (TKa) provides prognostic information that is independent of established clinical variables and highly dependent on treatment mechanism. While the primary PEARL analysis showed no overall survival advantage between capecitabine and ET plus palbociclib, multivariable modeling of dynamic TKa measurements uncovered biologically distinct patterns of treatment response and resistance.

Baseline TKa at the prespecified cutoff value of 250 DuA emerged as a strong and independent prognostic biomarker, confirming and extending prior observations in metastatic breast cancer [13,17–22]. Importantly, the prognostic value of baseline TKa persisted after adjustment for treatment arm, disease burden, prior therapies, *ESR1* mutation status and performance status, underscoring that circulating proliferative activity captures biological information not fully reflected by conventional clinical factors. Importantly, baseline TKa provided predominantly prognostic rather than predictive information, as differential treatment benefit was observed only at high proliferative levels (>400 DuA). Nevertheless, the modest magnitude of this interaction (2 months absolute benefit in PFS for capecitabine over palbociclib) and the lack of an overall survival signal indicate that baseline TKa alone is insufficient to support treatment selection based on proliferative activity.

Beyond baseline proliferative activity stratification, the most clinically informative finding was the divergence in early TKa dynamics between capecitabine and ET. The increase in TKa observed with capecitabine is biologically plausible in the context of thymidylate synthase inhibition and compensatory activation of the thymidine salvage pathway, a phenomenon supported by prior FLT-PET and serum TK1 studies [23–25]. Conversely, suppression of TKa during palbociclib-based therapy reflects effective CDK4/6-mediated cell-cycle arrest, and failure to suppress TKa independently identifies primary resistance to CDK4/6 inhibition [19].

Importantly, early on-treatment TKa dynamics were independently associated with clinical outcomes in a treatment-specific manner. Among patients receiving capecitabine, an early increase in TKa was associated with significantly improved PFS and OS, even after multivariable adjustment. This finding suggests that activation of the thymidine salvage pathway following thymidylate synthase inhibition by capecitabine reflects effective cytotoxic engagement. In contrast, among patients treated with ET plus palbociclib, failure to suppress TKa early during treatment identified a subgroup with particularly poor outcomes. Persistently elevated TKa independently predicted shorter PFS and OS, even after accounting for baseline proliferative activity and other clinical covariables. These results suggest that early lack of proliferative suppression reflects primary resistance to CDK4/6 inhibition and has profound clinical consequences. Notably, dynamic TKa assessment provided prognostic information beyond baseline TKa in this setting, which is consistent with the broader concept that biomarkers capturing treatment-induced biological changes may provide more clinically relevant information than static baseline measurements alone [26].

The present findings should also be considered in the context of other circulating biomarkers, particularly circulating tumor DNA (ctDNA), which has emerged as a powerful tool in metastatic breast cancer. Baseline ctDNA levels and early changes in ctDNA burden have consistently been associated with prognosis and treatment response, and ctDNA analysis enables identification of actionable genomic alterations and mechanisms of resistance, such as *ESR1* mutations [5]. However, ctDNA primarily reflects tumor-derived genomic material and clonal composition, and its detectability depends on tumor shedding and disease burden [23]. In contrast, TKa represents a functional, mutation-agnostic biomarker of systemic proliferative activity. It does not rely on detectable tumor-specific genomic alterations and can be dynamically assessed even in settings of low ctDNA abundance. Moreover, TKa directly reflects biological processes targeted by key therapies in HR+ breast cancer, particularly CDK4/6 inhibitors. As such, TKa and ctDNA capture complementary dimensions of tumor biology: ctDNA informs on "who is there" at the genomic level, whereas TKa informs on "what the tumor is doing" in functional, real-time terms. Integration of genomic biomarkers such as ctDNA with functional proliferative readouts like TKa may therefore enable a more complete and clinically actionable characterization of treatment response. In the evolving treatment landscape of endocrine-refractory HR+ /HER2 − metastatic breast cancer, antibody–drug conjugates such as trastuzumab deruxtecan and sacituzumab govitecan are increasingly incorporated into routine practice in earlier lines of therapies [27,28]. Nevertheless, capecitabine remains an important therapeutic option with good toxicity profile and available in healthcare settings, where access to newer agents is limited. In this context, our findings suggest that TKa dynamics may help identify a subset of patients who derive meaningful benefit from capecitabine, supporting a more selective and biologically informed use of this agent rather than uniform escalation to ADC-based therapy. Prospective validation of a TKa-guided approach may therefore refine treatment sequencing and enable the biomarker to transition from a purely prognostic indicator to a clinically actionable tool for response-adaptive management.

Strengths of this study include the large sample size, randomized trial context, prospective collection of plasma samples, and use of a standardized, FDA-cleared assay. One important limitation of the manuscript is the use of predominantly archival primary tumor samples for intrinsic subtype classification and Ki67 assessment, which may have limited our ability to detect associations with baseline TKa. Additional limitations include the retrospective nature of the translational analysis which highlight the need for prospective validation of the proposed cutoffs and dynamic patterns.

5. Conclusions

In endocrine-resistant HR+ /HER2 − MBC, a low baseline TKa was independently associated with a significantly longer PFS (11.4 vs 4.0 months) and OS (38.5 vs 17.3 months), regardless of treatment, indicating strong prognostic value. Early dynamic TKa assessment complements baseline proliferative risk and reflects treatment-specific biological responses that differ between capecitabine and endocrine therapy. TKa is a robust biomarker for response monitoring, but its clinical utility requires prospective validation.

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: **Ángel Guerrero Zotano** has received Consulting or advisory role fees from: Pierre Fabre, Exact sciences, Novartis, Roche. Speakers Bureau: Lilly, Menarini, AstraZeneca, Novartis. Expert Testimony: AstraZeneca, Pierre Fabre, Menarini, Novartis, Lilly, Pfizer. Travel, accommodation, expenses: Menarini, Roche, Gilead Sciences, Novartis. **Miguel Gil-Gil** has received Honoraria fees from: Lilly; Novartis; AstraZeneca; Daiichi-Sankyo; Pfizer; Roche. Advisory: Menarini; Daiichi-Sankyo. Travel expenses: Daiichi-Sankyo; Pfizer; Puma. **Amy Williams** is an Employee of Biovica Inc. **Hanna Ritzén** is an Employee of Biovica Inc. and shareholder. **Eva M[a] Ciruelos** has received Advisory role fees from: AstraZeneca, Avenzo, Beigene, Daiichi Sankyo, Lilly, MSD, Novartis, Pfizer, Reveal Genomics, Roche, Instituto de Salud Carlos III. Speakers Bureau / Invited Speaker: Gilead, Lilly (Symposia and Education), Pfizer (Educational activities), Roche (Speakers Bureau, Educational activities), Roche (Symposia and Educational activities). Travel, accommodation, expenses: AstraZeneca. Research funding / Institutional support: Daiichi Sankyo (Research Grant), Roche (Funding / Research proposal), Seagen (Research Grant). Non-financial interests: Instituto de Salud Carlos III (Scientific evaluator), SOLTI Cooperative Group (Member of Board of Directors). **Mireia Margelí** has received Honoraria fees from Novartis, Pfizer, Lilly, Gilead, Piere Fabre and MSD as advisor role or consulting, research funding from Pfizer and travel expenses from Pfizer and Gilead. Research funding from Pfizer to her Institution. Travel expenses from Pfizer and Gilead. **Emilio Alba** has received Expert/advisor role fees from: Exact Sciences, Bristol Myers Squibb, Novartis, Pfizer, Eli Lilly. Research Grants: Pfizer, AstraZeneca. **Pablo Álvarez** has received Consulting/Speaker fees: MSD, Merck, Pierre-Fabre, Ipsen, Brystol Myers, Roche, Novartis, Pfizer. Travel accommodation: MSD, Merck, Pierre Fabre, Ipsen, Gilead, Astellas. Advisory Board: Pierre Fabre. **Isabel Álvarez** is an employee of OSAKIDETZA (Servicio Vasco de Salud) and has received Consultant or Advisory Role fees from: AstraZeneca, Pfizer, Roche, Gilead, MSD, Novartis. Research Funding: AstraZeneca, Pfizer, Novartis, Roche, Lilly. Speaking: AstraZeneca, Pfizer, Novartis, Roche, Eisai, MSD, Lilly. Other: Meeting inscription & travel expenses: AstraZeneca, Pfizer, Roche, Eisai, Lilly, Daichii-Sankyo, MSD. **Helle Fisker** is an Employee of Biovica Inc. **Federico Rojo** has received Speaker's Role fees from: Roche, Astra Zeneca, BMS, MSD, Merck, Abbvie, Novartis, Agilent, Sophia Genetics, Exact Sciences, GSK, Pfizer, Johnson, Menarini-Stemline, Boehringer, Astellas, Amgen, Lilly, Bio-Rad, Sysmex. Expert/advisor role: Roche, Astra Zeneca, BMS, MSD, Merck, Abbvie, Novartis, Agilent, Sophia Genetics, Pfizer, Menarini-Stemline, Boehringer, Astellas, Amgen, Lilly, Sysmex. Research Grants: Astra Zeneca, Pfizer, Menarini-Stemline, Abbvie, Astellas, Sysmex. **Miguel Martín** has received Consulting or advisory role fees from: AstraZeneca, Daiichi Sankyo, Lilly, Menarini–Stemline, Roche/Genentech. Speakers Bureau/Invited Speaker: AstraZeneca, Lilly, Novartis, Pfizer, Roche/Genentech. Research funding/Institutional support: Novartis (Research Grant), Puma (Research Grant), Roche (Research Grant). **All remaining authors have declared no conflicts of interest.**

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2026.116817.

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