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Authors

Paoletti, Costanza
Barlow, William E
Cobain, Erin F
et al.

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Evaluating Serum Thymidine Kinase 1 in Hormone Receptor Positive Metastatic Breast Cancer Patients Receiving First Line Endocrine Therapy in the SWOG S0226 Trial

Costanza Paoletti[1], **William E. Barlow**[2], **Erin F. Cobain**[1], **Mattias Bergqvist**[3], **Rita S. Mehta**[4], **Julie R. Gralow**[5], **Gabriel N. Hortobagyi**[6], **Kathy S. Albain**[7], **Lajos Pusztai**[8], **Priyanka Sharma**[9], **Andrew K. Godwin**[10], **Alastair M. Thompson**[11], **Daniel F. Hayes**[1], **James M. Rae**[1,§]

[1]University of Michigan Rogel Cancer Center, Ann Arbor, MI, USA, 48109

[2]SWOG Statistical Center, Seattle, WA, USA

[3]Biovica International, Uppsala, Sweden

[4]Chao Family Comprehensive Cancer Center, University of California Irvine Medical Center, 101 The City Drive South, Bldg. 23, Orange CA, USA, 92868

[5]Seattle Cancer Care Alliance and University of Washington Medical Center, 825 Eastlake Ave E G3-630 Seattle, WA, USA 98109-1023

[6]Department of Breast Medical Oncology, University of Texas M.D. Anderson Cancer Center, Houston, 1515 Holcombe Blvd, Houston, TX, USA, 77030

[7]Loyola University Chicago Stritch School of Medicine, Maywood, IL, 2160 S 1st Ave, Maywood, IL, USA, 60153

[8]Medical Oncology, PO Box 208028, New Haven, CT, USA 06520-8028

[9]Division of Medical Oncology, Department of Internal Medicine, University of Kansas Medical Center, Kansas City, USA.

[10]Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, USA, 66160

[11]Baylor College of Medicine Dan L Duncan Cancer Center, 7200 Cambridge St, 7th floor, Houston, Texas, USA 77030

Abstract

[§]**To whom correspondence should be addressed:** James M Rae, PhD, Department of Internal Medicine, Division of Hemetology & Oncology, University of Michigan Medical School, 6310 University of Michigan Rogel Cancer Center, 1500 E. Medical Drive, Ann Arbor, MI 48109, USA, Telephone: 734-764-1460, jimmyrae@umich.edu.

Author Contribution:

Conception and design: C.P., W.E.B., M.B., D.F.H., J.M.R., A.K.G., G.N.H. P.S. R.S.M., J.R.G., K.S.A., L.P., A.M.T.

Collection and assembly of data: C.P., W.E.B., M.B., D.F.H., J.M.R.

Data analysis and interpretation: C.P., W.E.B., M.B., A.K.G., D.F.H., J.M.R.

Manuscript writing: C.P., E.F.C., W.E.B., M.B., D.F.H., J.M.R., A.K.G., G.N.H. P.S. R.S.M., J.R.G., K.S.A., L.P., A.M.T.

Final approval of manuscript: C.P., E.F.C., W.E.B., M.B., D.F.H., J.M.R., A.K.G., G.N.H. P.S. R.S.M., J.R.G., K.S.A., L.P., A.M.T.

Agree to be accountable for all aspects of the work, which includes ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved:

Disclaimers: none

Purpose: Serum Thymidine Kinase 1 (sTK1) activity is associated with poor prognosis in metastatic breast cancer (MBC). We assessed the prognostic effect of sTK1 in patients with hormone receptor-positive MBC treated on a prospective randomized trial of anastrozole (A) vs. A plus fulvestrant (A+F).

Experimental Design: sTK1 was assessed in 1,726 serums [baseline (BL), cycles 2, 3, 4, and 7] using the DiviTum® assay. A pre-specified cutoff of ≥200 Du/L was considered high. Progression-free survival (PFS) and overall survival (OS) were analyzed by Kaplan-Meier, log-rank tests, and Cox regression.

Results: BL sTK1 was elevated in 171 (40%) of 432 patients. Patients with high vs. low BL sTK1 had significantly worse PFS [median 11.2 versus 17.3 months, hazard ratio (HR)= 1.76; 95% CI (1.43–2.16); p<0.0001] and OS (median 30 versus 58 months, HR=2.38; 95% CI (1.91–2.98); p<0.0001). OS was significantly better for patients with high sTK1 who did not have prior adjuvant tamoxifen and who received A+F vs. A alone [median 46 versus 21 months, HR=0.58; 95% CI (0.38–0.87); p=0.0087]. Patients with low sTK1 had no difference in outcomes by therapy (p=0.44). At serial time-points, high vs. low sTK1 had significantly worse subsequent PFS and OS [at cycle 2: PFS HR=1.70, 95% CI (1.34–2.17); p<0.0001, OS HR=2.51, 95% CI (1.93–3.26); p<0.0001].

Conclusions: High sTK1 at BL and subsequent time-points is associated with worse prognosis in MBC patients starting 1st line endocrine therapy (ET). Patients with low sTK1 at BL have comparable outcomes on single agent or combination ET.

Keywords

Thymidine kinase 1 (TK1); DiviTum® assay; overall survival (OS); progression free survival (PFS); metastatic breast cancer (MBC); hormone receptor-positive; circulating biomarker; serum marker; S0226

INTRODUCTION

Endocrine therapy (ET), including the aromatase inhibitor anastrozole (A) and the selective estrogen receptor (ER) degrader fulvestrant (F), is effective for the treatment of hormone receptor-positive metastatic breast cancer (MBC), but resistance is a major clinical problem[1]. We have previously reported that in the SWOG prospective randomized clinical trial S0226, the combination of A+F improves progression-free and overall survival (PFS, OS) in selected patients with MBC, specifically, in patients without prior adjuvant tamoxifen[2,3]. Moreover, targeted agents such as CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib), mTOR inhibitors (everolimus), and a PIK3CA inhibitor (alpelisib) improve progression-free survival (PFS) when combined with standard ET as first- and second-line therapy for hormone receptor-positive MBC[4–7].

Combination ET or ET plus other targeted agents is associated with increased toxicities and costs compared to single agent ET. Thus, identification of patients who may not need combination therapy would serve to spare them these adverse events. Currently, there is no tool available to help clinicians tailor treatment for each patient, underlining the compelling need for identification of biomarkers of resistance and response to ET agents [8,9].

Thymidine Kinase (TK) is a fundamental enzyme in DNA synthesis and cellular proliferation[10]. High versus low levels of cellular TK are associated with poorer prognosis in many cancer types, including breast[11,12]. TK has both cytosolic (TK1) and mitochondrial (TK2) forms[13]. TK1 activity can be measured in serum (sTK1) and is frequently elevated in patients with cancer[11,14]. Preliminary studies have suggested that high sTK1 is associated with poor prognosis in MBC treated with chemotherapy or ET [15–17]. However, data from the latter are limited.

We conducted this prospective retrospective study of sTK1 in specimens collected from participants in S0226 to test the hypothesis that baseline and serial levels would be prognostic in patients with hormone receptor-positive MBC treated with first line ET.

MATERIALS AND METHODS

S0226 Conduct/Study design

This was a prospectively designed retrospective translational medicine study of S0226 (ClinicalTrials.gov number NCT00075764), for which the conduct and final survival outcomes have been previously reported [2,3]. All patients who were eligible and evaluable for the primary analysis of S0226 (N=694) were considered for inclusion in this study. The study was approved by an institutional review board at each participating site, and all participants (or participant's guardian) provided written informed consent before enrollment. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and provisions of the Declaration of Helsinki. Submission of blood for future research was voluntary and all patients who contributed blood gave written informed consent for use of the serum samples.

Briefly, S0226 was a phase III, randomized clinical trial in which postmenopausal women with previously untreated hormone receptor-positive (estrogen receptor-positive, progesterone receptor-positive, or both) MBC were randomly assigned to receive either 1 mg A orally daily or to the combination of A+F (F: 500 mg intramuscular as loading dose, followed by 250 mg on days 14, 28 and 250 mg maintenance monthly thereafter). Randomization was stratified by prior adjuvant tamoxifen use[3]. A prospectively written study plan for this correlative study was approved by the NCTN Core Correlative Science Committee.

Patient staging and follow-up

Details regarding patient eligibility, accrual, and overall conduct of the trial have been reported[2]. Estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor 2 (HER2) status were determined locally by routine pathology at the treating institutions.

Assessing TK1 activity by DiviTum® assay

Serum samples from the designated collection timepoints of baseline and cycles 2, 3, 4, and 7 were included, if available. Cycle length was 28 days. Serum samples were stored at the SWOG Specimen Repository. After removing identifying information, aliquots of

approximately 1 ml for each time-point were shipped to Biovica Inc. (Uppsala, Sweden) for assessing the enzymatic activity of the sTK by the ELISA-based DiviTum® assay (Biovica, Inc., Uppsala, Sweden), according to the manufacturer's instructions and as previously reported[15]. Biovica was blinded to treatment assignment and outcome. The DiviTum® assay has a lower limit of quantitation of 20 DiviTum Units per liter (Du/L). Recommended working range is from 20 Du/L up to 4,000 Du/L or to the plate reader's asserted upper limit of detection if this limit corresponds to a TK activity lower than 4,000 Du/L. At 100 Du/L, the assay has a coefficient of variation lower than 20%. The TK activity unit was originally determined with a reference sample of recombinant TK. 1,000 Du/L corresponds to the activity that was obtained with 1000 ng TK/L. Clinical samples have been observed to have activities ranging from less than 10 Du/L to greater than 100,000 Du/L. All specimens were assayed in duplicate and the final result is the mean of both results. The overall coefficient of variation (CV) of the specimens assayed in this study was ≤ 10%. If an individual sample had a larger CV between the duplicates, the sample was re-analyzed.

Selection of cutoff for sTK.—For this study, we chose a pre-determined cut point of 200 Du/L which was an approximate median from prior studies for the hormone receptor positive MBC population included in S0226[18]. As secondary analysis, we also explored the median sTK1 level and divided the sTK1 values into quartiles (Supplementary Figure 1).

Statistical analysis

Assay results were returned to SWOG Statistical Center for analysis to determine the association between sTK levels and clinical outcomes overall and then specifically for patients who had not received adjuvant tamoxifen. The assigned treatment arms of A and A+F were compared using Intent-to-Treat analyses. The primary outcomes were PFS (defined as time from randomization to progression or death due to any cause) and OS (defined as time from randomization to death from any cause), as prescribed in the parent clinical trial protocol. Patients who did not meet the outcome definition were censored at last follow-up. Survival curves were estimated using the Kaplan-Meier method; 95% confidence intervals (CI) for median (m)PFS and mOS were calculated using the method of Brookmeyer and Crowley. Testing was by log-rank test and Cox regression with stratification on prior adjuvant tamoxifen therapy when both strata are used. The analysis of prognosis from Cycle 2 values used a landmarked analysis starting at 56 days. A time-dependent Cox model was also used to include baseline sTK1 and current value of sTK1 with stratification by prior adjuvant tamoxifen use. In this analysis, the most recent measurement of sTK1 was used as a time-dependent covariate with adjustment for baseline sTK1.

All results are reported according to REMARK criteria[19].

RESULTS

Patient characteristics

A total of 707 patients were enrolled in S0226, but 13 were ineligible, and therefore 694 were included in the original analysis[3] (Figure 1). sTK1 was evaluated in 1,726 specimens

which were evaluable from baseline or later time-points [Baseline n=432 (62% of the 694 patients eligible for S0226); and at cycle 2, 3, 4, 7: n= 365, 342, 324, 263, respectively]. Not all patients had consecutive specimen collection (for example, specimens might have been available at baseline and cycles 2 & 4, but not cycles 3 & 7).

The patients enrolled in this correlative sTK1 study sub-cohort had similar characteristics to the original S0226 cohort (Table 1). Survival outcomes did not differ between those patients that had baseline sTK1 values (n=432) and those that did not (n=262) (p=0.57 for PFS and p=0.86 for OS in stratified log-rank tests). Treatment effects comparing single agent A vs. combination A+F were also similar in this subset compared to the full cohort. Prior analyses [2,3] had shown that the benefit of the combination was only seen in the subset without prior adjuvant tamoxifen treatment (N=282/432; 65.3%), so all prediction analyses were restricted to that subset.

Further, most characteristics were not significantly different between the cohorts with high vs. low sTK1, with a few exceptions. Compared to low sTK1, patients with high sTK1 were more likely to have measurable disease (64% vs. 53%, P=0.019) and more likely to be diagnosed as *de novo* MBC (48% vs. 36%, p=0.012), but less likely to have bone-only disease (13% vs. 25%, p=0.005).

Incidence of high sTK1 and median sTK1 at baseline and follow-up time points

All the samples available were assessed using DiviTum® assay with an 100% evaluation rate. Serum TK1 was successfully analyzed in all 1,726 specimens and was elevated in 40% (171/432) of patients at baseline. Median pre-treatment level of sTK1 was 135 Du/L (range: 19–35,340) [Supplementary Table1]. In serial specimens, sTK1 was elevated in 28% (102/365), 21% (73/342), 17% (55/324), and 15% (40/263) of patients at Cycles 2, 3, 4, and 7, respectively. Median sTK1 for the follow-up time points was 73 (19–28,470), 65 (19–20,140), 57 (19–55,250), and 50 Du/L (19–27,380) at Cycles 2, 3, 4, and 7, respectively.

Clinical outcomes according to sTK1 level at baseline using the pre-specified cut-off

PFS.—Overall, patients with high baseline sTK1 had significantly worse PFS than those with low sTK1 [HR=1.76; 95% CI (1.43–2.16); P<0.0001; median PFS 11.2 vs.17.3 months] (Figure 2A; Table 2). This strong prognostic effect was not altered by inclusion of treatment, age, and disease characteristics in a multivariable analysis (Supplementary Table 2). In patients with no prior adjuvant tamoxifen and high sTK1, PFS was significantly better for those treated with A+F vs. A alone [HR=0.64; 95% CI (0.43–0.95); p=0.027; median PFS 13.6 vs. 8.7 months] (Figure 2B; Table 2). Conversely, in patients with low sTK1, we observed no difference in PFS between the A+F vs. A arms [HR=0.85; 95% CI (0.61–1.19); p=0.34] (Figure 2C) with no prior adjuvant tamoxifen. The interaction of treatment arm and sTK1 was not statistically significant, though power was low to test the interaction (p=0.26). Patients with previous tamoxifen did not show a treatment difference overall[2] or separately by sTK1 (Supplementary Figures 2A and 2B)

OS.—Differences in OS for high vs. low sTKI at baseline were more pronounced than for PFS. Patients with high sTK1 had significantly worse OS than those with low sTK1

[HR=2.38; 95% CI (1.91–2.98); p<0.0001; median OS 30 vs. 58 months] (Figure 3A; Table 2). Even after adjustment for treatment arm, age, and disease characteristics, sTK1 remained highly prognostic (any other variables prognostic in MV analysis). In patients with high sTK1 and no prior adjuvant tamoxifen, OS was significantly better for those treated with combinational therapy (A+F) vs. A alone (Figure 3B; Table 2) [HR=0.58; 95% CI (0.38–0.87); p=0.0087; 46 vs. 21 months], whereas for low sTK1, we observed no difference in OS between the A+F vs. A arms (Figure 3C; Table 2) [HR=0.85; 95% CI (0.56–1.29); p=0.44]. Despite this apparent difference, there was not a statistically significant interaction (p=0.16) between randomized treatment and sTK1 for women with no prior tamoxifen.

In addition to the pre-set cutpoints, we performed an exploratory analysis of PFS and OS by quartiles (Q: Q1 <58 Du/L; Q2: 58–134; Q3: 135–491; Q4: >491) of sTK1, and observed a statistically significant association for trend of increasing marker levels (Q4 to Q1 gives a HR=2.09; 95% CI 1.57–2.77; p<0.0001 for PFS and HR=2.97; 95% CI 2.16–4.07; p<0.0001for OS) Supplementary Figure 1A **and** 1B).

Clinical outcomes according to sTK1 quartiles at baseline

sTK1 levels were also split into 4 different quartiles (Q) based on pre-treatment level (135 Du/L): Q1 <58 Du/L; Q2: 58–134; Q3: 135–491; Q4: >491. PFS decreased over the four quartiles (trend p<0.0001) with medians 17.9, 15.0, 13.8, 11.0 months, respectively. Comparison of Q4 to Q1 gives a HR=2.09; 95% CI 1.57–2.77; p<0.0001 (Supplementary Figure 1A). Likewise, OS also decreased over the four quartiles (trend p<0.0001) with medians 62.1, 52.6, 41.6, 29.3 months, respectively. Comparison of Q4 to Q1 gives a HR=2.97; 95% CI 2.16–4.07; p<0.0001. (Supplementary Figure 1B).

Clinical outcomes according to sTK1 level at follow-up time points during therapy

The prognostic role of sTK1 was also tested in subsequent time points in landmarked analyses. At cycle 2, patients with high sTK1 had significantly worse subsequent PFS than those with low values (HR=1.70, P<0.0001) (Figure 4A). Likewise, serial monitoring of sTK1 levels during therapy at cycle 3, 4, and 7 was also significantly associated with subsequent PFS (Figure 4B–D), respectively. Patients that had sTK1 measurements at both baseline and Cycle 2 were classified by baseline and Cycle 2: (1) pos-pos (n=72); (2) pos-neg (n=56); (3) neg-pos (n=24); (4); neg-neg (n=187). In a joint model baseline positive sTK1 remained significant [HR=1.63; 95% CI (1.24–2.14); p=0.0004] while Cycle 2 sTK1 was only marginal [HR=1.33; 95% CI (1.00–1.77); p=0.053]. In a time-dependent Cox model both baseline sTK1 and most current value of sTK1 were included in the same model and both were statistically significant: Baseline [HR=1.57; 95% CI (1.25–1.97); p=0.0001; most current [HR=1.40; 95% CI (1.08–1.81); p=0.010].

Similar results were observed for OS. At cycle 2, high values had significantly worse subsequent PFS than those with low values (OS HR=2.51, P<0.0001) (Figure 4E). Likewise, serial monitoring of sTK1 levels during therapy at cycle 3, 4, and 7 was significantly associated with subsequent OS (Figure 4 F–H), respectively. Patients that had sTK1 measurements at both baseline and Cycle 2 were modeled using both values. Baseline positive sTK1 was significant [HR=2.02; 95% CI (1.51–2.70); p<0.0001] as was the Cycle 2

sTK1 [HR=1.75; 95% CI (1.29–2.37); p=0.0004]. In a time-dependent Cox model with both baseline sTK1 and most current value of sTK1 both were statistically significant: Baseline [HR=1.92; 95% CI (1.51– 2.43); p<0.0001; Most current [HR=1.96; 95% CI (1.47–2.61); p<0.0001].

DISCUSSION

In this prospective-retrospective translational medicine study, we observed that high sTK1 using the DiviTum® assay at BL is associated with worse prognosis in hormone receptor-positive MBC patients starting 1st line ET. In addition, high sTK1 at subsequent time points was also associated with worse PFS and OS. Furthermore, in patients with high BL sTK1 and no prior adjuvant tamoxifen, PFS and OS were significantly better for those treated with A+F vs. A alone (PFS: p=0.027; OS: p<0.0001), whereas PFS and OS did not differ between A+F vs. A alone for those with low BL sTK1 levels (PFS: p=0.34; OS: p=0.44).

The results of this study are consistent with previous reports in which BL sTK1 was demonstrated to be prognostic in patients with MBC on chemotherapy or ET alone or in combination with other agents[15–18,20,21]. Our results are also concordant with those reported in the TREnd trial, where it was noted that increases in plasma TK1 levels at four weeks were associated with worse PFS compared to those with stable or decreasing plasma TK1 levels in patients with hormone receptor-positive MBC treated with CDK 4/6 inhibitors with or without ET[21]. Similarly, in S0226, patients with high sTK1 at Cycle 2 had worse PFS and OS compared to those patients with low sTK1. However, our study was exclusively conducted in patients with MBC starting first-line ET in patients treated either with A alone or A+F and TK1 was investigated in serum instead of plasma. Our results are partially dissimilar from those of other investigators, who have found that changes from 3 months to 6 months during therapy are significantly correlated to PFS and OS, whereas early changes were not correlated[18].

Taken together with results from previous studies, our results suggest that there are several potential practical applications for sTK1 in the care of patients with MBC. For example, since patients treated in S0226 with low BL sTK1 had nearly identical PFS and OS regardless of whether they received mono- or combination ET, baseline sTK1 could potentially be used to select patients best suited for ET monotherapy as upfront treatment, whereas those with elevated sTK1 appeared to benefit from combination A + F.

Further, these results suggest that perhaps sTK1 might be used to identify patients who might not need the addition of CDK4/6 inhibitors to first-line ET. It is possible that patients with low baseline sTK1 have indolent disease and may have excellent clinical outcomes with first-line ET monotherapy. At present, the current standard of care for patients with hormone receptor-positive MBC is to treat all patients with this combination upfront, exposing them to the additional toxicity and cost of the CDK4/6 inhibitors. Our data suggest that patients with low baseline sTK1 have indolent disease and may do well with single agent first-line endocrine therapy. We speculate that identification of this fraction of patients with ER-positive MBC who are very highly likely to benefit from ET monotherapy would help these patients to avoid, or at least delay, these adverse complications. By contrast,

our data, consistent with those by McCartney *et al.*[17], also suggest that high sTK1 levels following initiation of a systemic therapy may identify patients who are resistant to the chosen treatment and might benefit from an alternative strategy. Each of these hypotheses would need to be validated in prospective clinical trials.

Strengths of this study include that samples were obtained from a prospective, randomized clinical trial (S0226) with robust clinical outcomes data. In addition, serum samples at BL and at subsequent timepoints were available from a large number of trial participants, with very similar characteristics between the cohort analyzed in this study and the overall patient population of the S0226 study. The patients included in this cohort were also well-balanced across treatment arms. Weaknesses of this study include that patients enrolled in S0226 are not necessarily representative of our current population of patients with hormone receptor-positive MBC initiating 1st line ET. For example, many patients in S0226 had *de novo* metastatic disease, and were therefore ET naïve at study entry. In current practice, most patients who present with hormone receptor-positive MBC have previously received adjuvant tamoxifen or aromatase inhibition in the early stage setting. Furthermore, in the S0226 study, the dose of fulvestrant (250 mg administered intramuscularly) administered was lower than that currently used (500 mg intramuscularly) and there was no single agent F arm. It is unknown if the superior clinical outcomes comparing the combination of A + F vs. A alone would have been even greater if the higher dose of F had been administered to all patients, or if single agent higher dose F alone would give similar results.

In conclusion, the results of this translational medicine study suggest that BL sTK1 identified patients with hormone receptor-positive MBC with a very favorable prognosis. We speculate that low BL sTK1 might identify patients who have indolent disease and may do well for a long time with 1st line ET alone and could safely be treated with ET monotherapy as upfront treatment for their metastatic disease. Delaying CDK4/6 or mTOR inhibitors might be warranted, decreasing toxicity and cost of therapy. We further speculate that serial monitoring of sTK1 in MBC may allow early identification of patients who are refractory to the chosen treatment and who might benefit from switching to an alternative therapeutic strategy prior to detecting evidence of radiographic disease progression. Further evaluation of the predictive potential of sTK1 in hormone receptor-positive MBC is warranted in prospective clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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for clinical and laboratory research and is the inventor named on a patent held by the University of Michigan and licensed to MSB. Related to this work, he has received honoraria for services rendered from Biovica, the manufacturer of the DiviTum® assay. Unrelated to this work, he also receives research funding on his behalf from Merrimack, Eli Lilly, Puma Biotechnology, Pfizer, AstraZeneca, and holds stock options from InBiomotion, and serves on advisory boards for Cepheid, Freenome, CellWorks, Agendia, Salutogenic, EPIC Sciences and L-Nutra and UM. W.E.B. received institutional research support from AstraZeneca and Merck. A.K.G. is co-founder of Sinochips Diagnostics and related to this work, he has received research support from Biovica.

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Statement of Translational Relevance

Results of this study suggest that hormone receptor – positive metastatic breast cancer (HR+ MBC) patients with low serum thymidine kinase 1 (sTK1) have a very favorable prognosis. We speculate that low baseline sTK1 might identify patients who have indolent disease and who will do well on 1st line endocrine therapy and could safely be treated with endocrine monotherapy as upfront treatment for their metastatic disease. We further speculate that serial monitoring of sTK1 in MBC may allow early identification of patients who are refractory to the chosen treatment and who might benefit from switching to an alternative therapeutic strategy prior to detecting evidence of radiographic disease progression. Further evaluation of the predictive potential of sTK1 in HR+ MBC is warranted in prospective clinical trials.

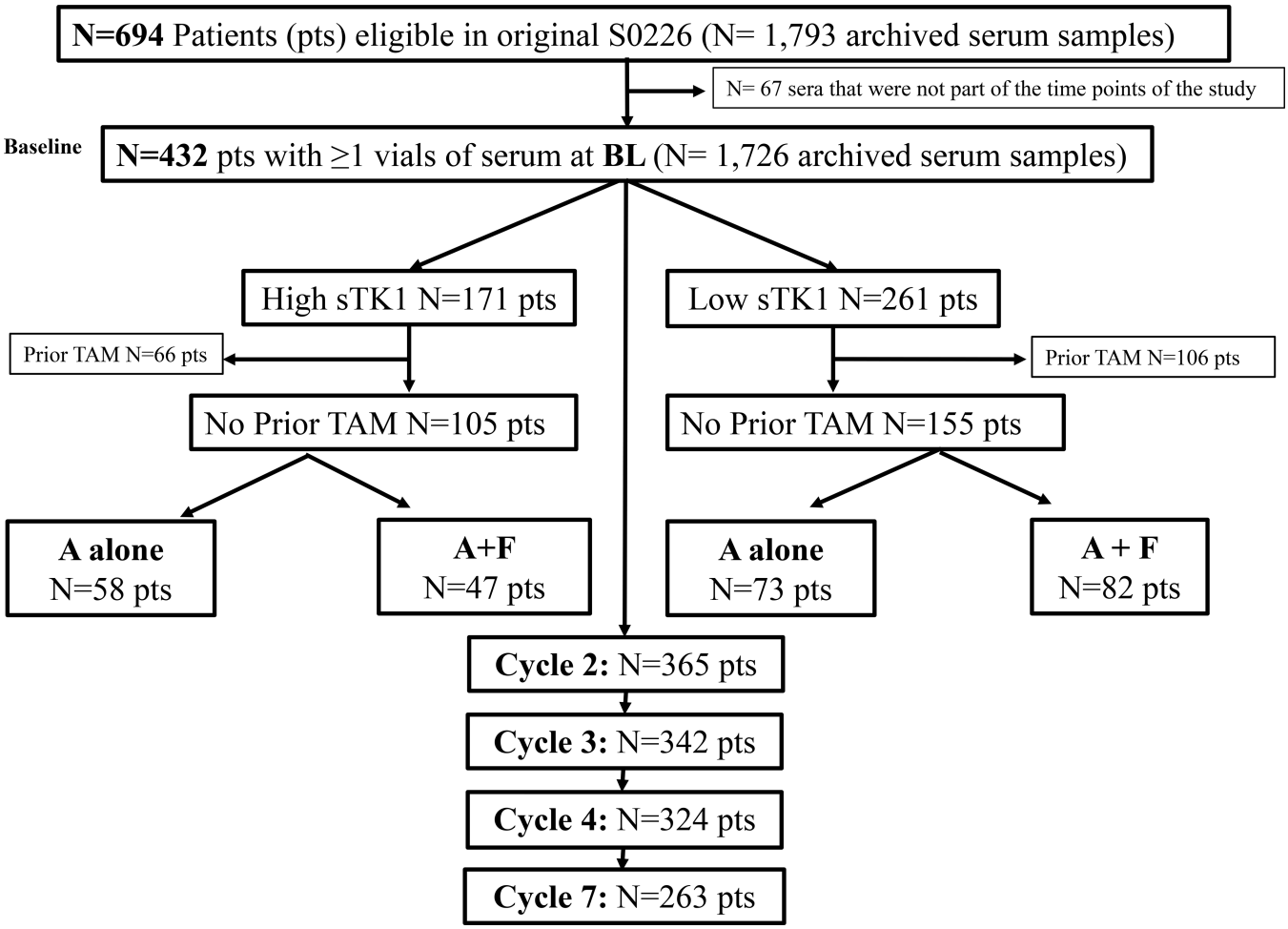
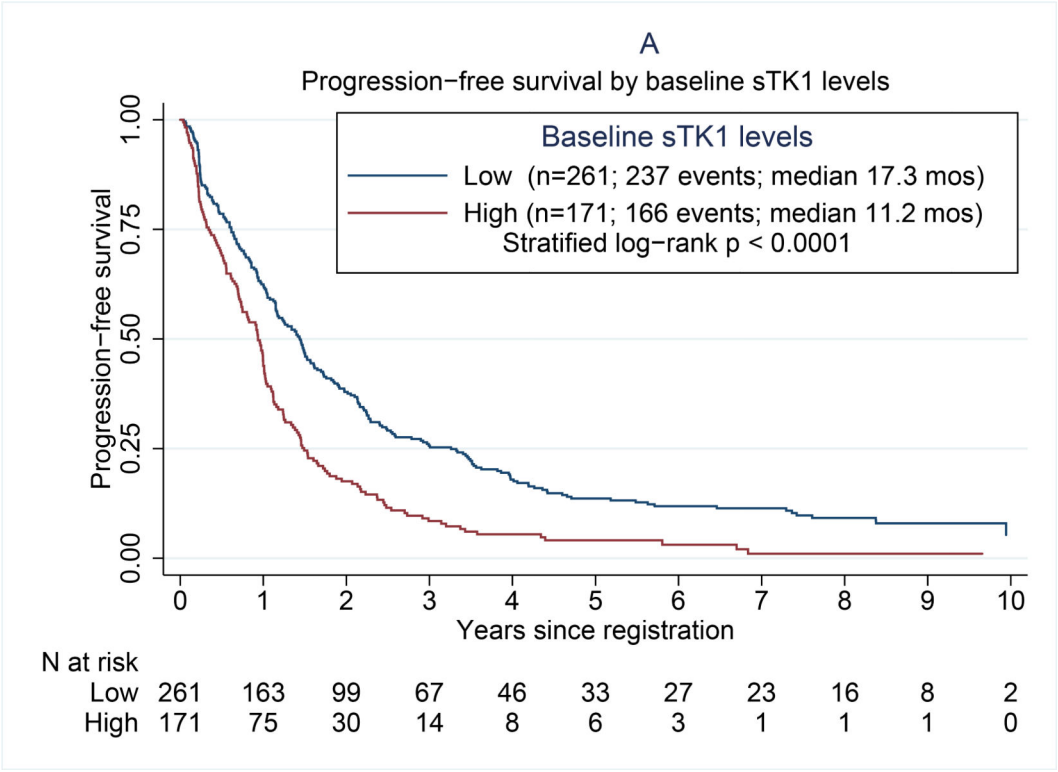
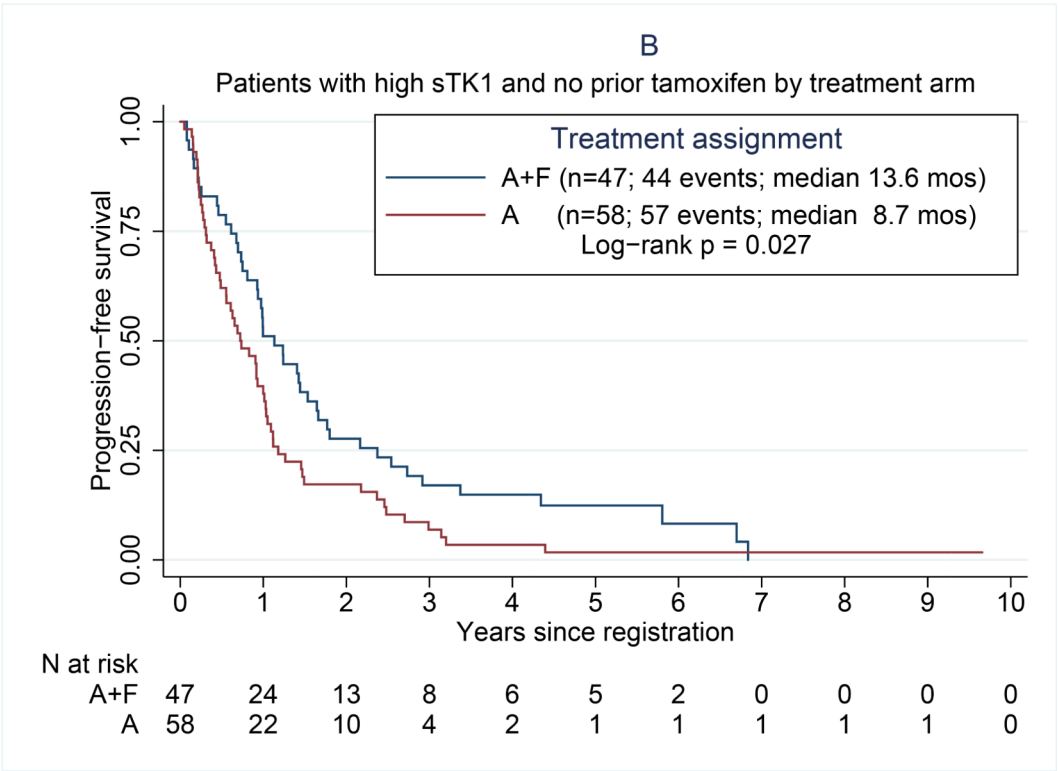


Figure 1. REMARK diagram for sTK1 analysis of S0226.

Of the 694 patients originally eligible for S0226, 432 had ≥1 vial serum stored at baseline and assessable for TK1 level analysis.





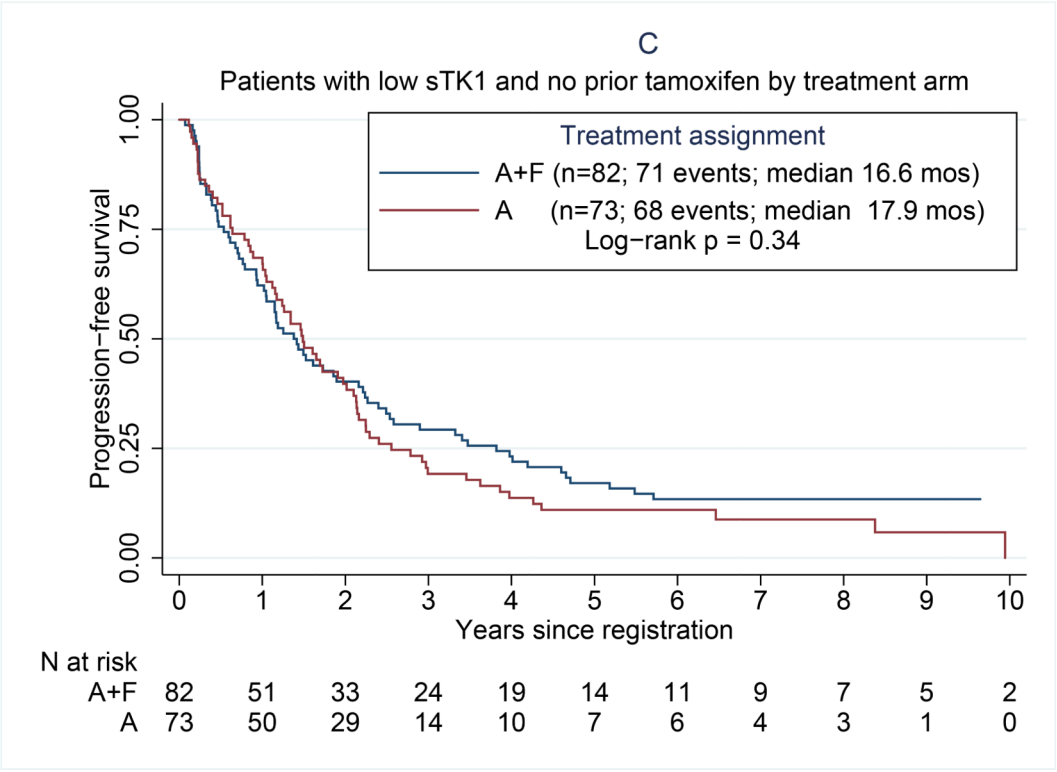
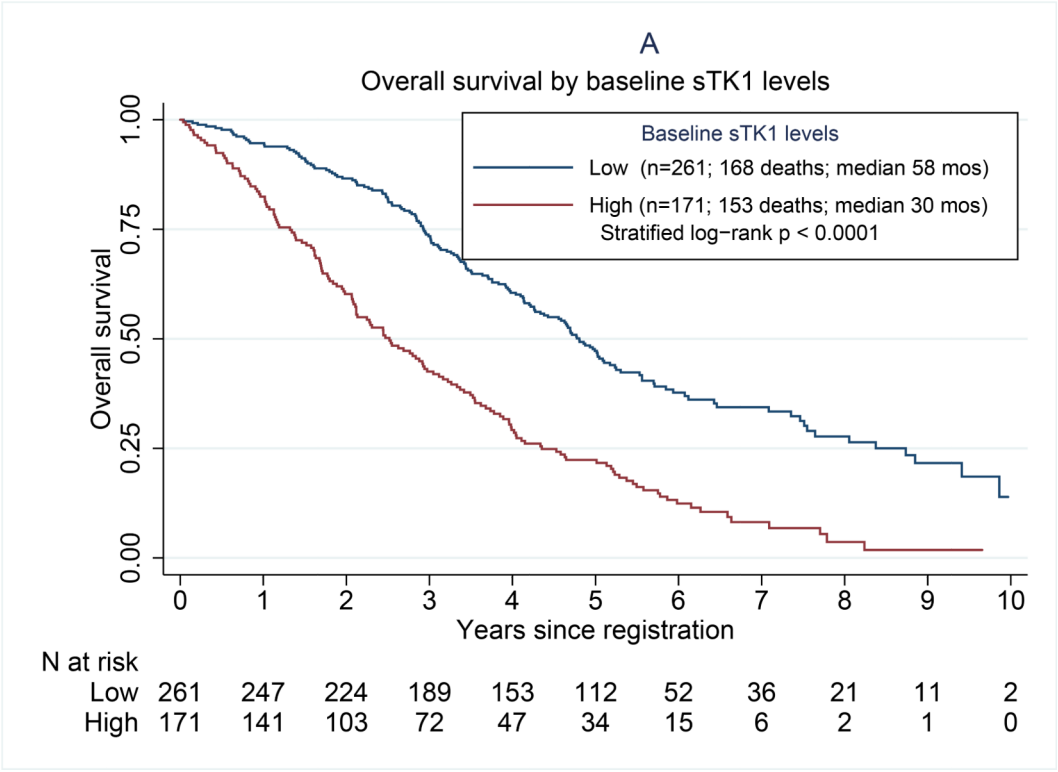
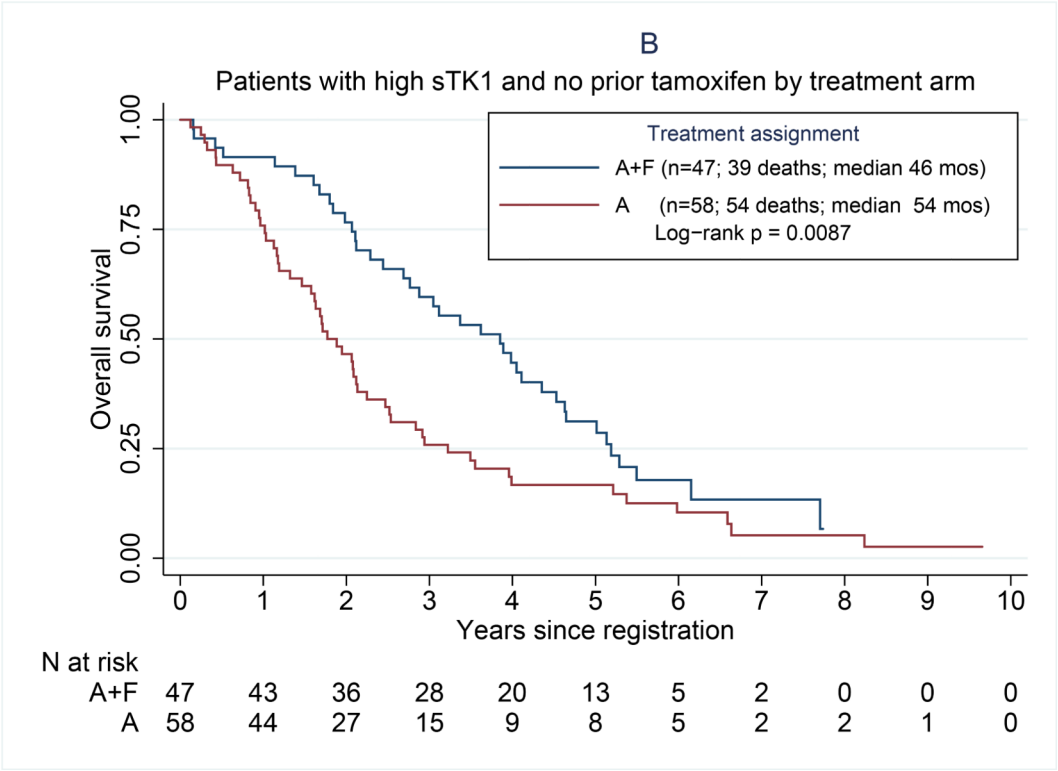


Figure 2.

Kaplan-Meier Curves for Progression free survival (PFS) at baseline; **2A.** All patients in cohort by sTK1 level (blue line, low sTK1; red line, high sTK1). **2B.** Patients with high sTK1 and no prior tamoxifen by treatment arm (blue line, Anastrozole + Fulvestrant; red line, Anastrozole). **2C.** Patients with low sTK1 and no prior tamoxifen and according to treatment arm (blue line, Anastrozole + Fulvestrant; red line, Anastrozole).





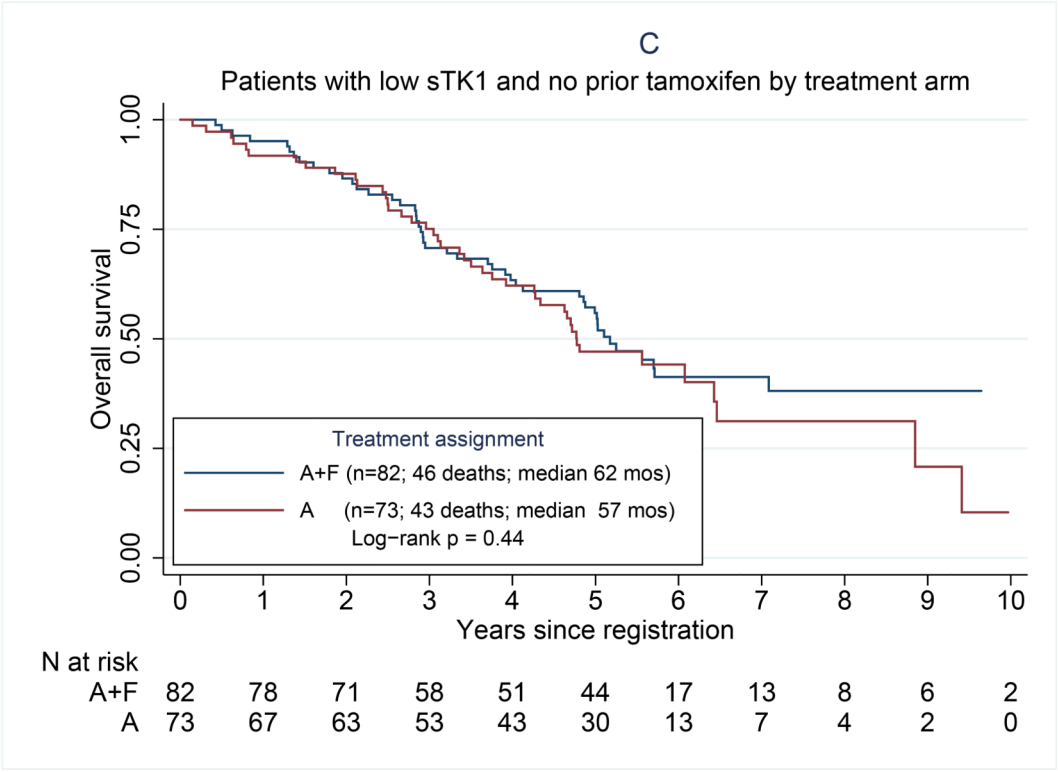
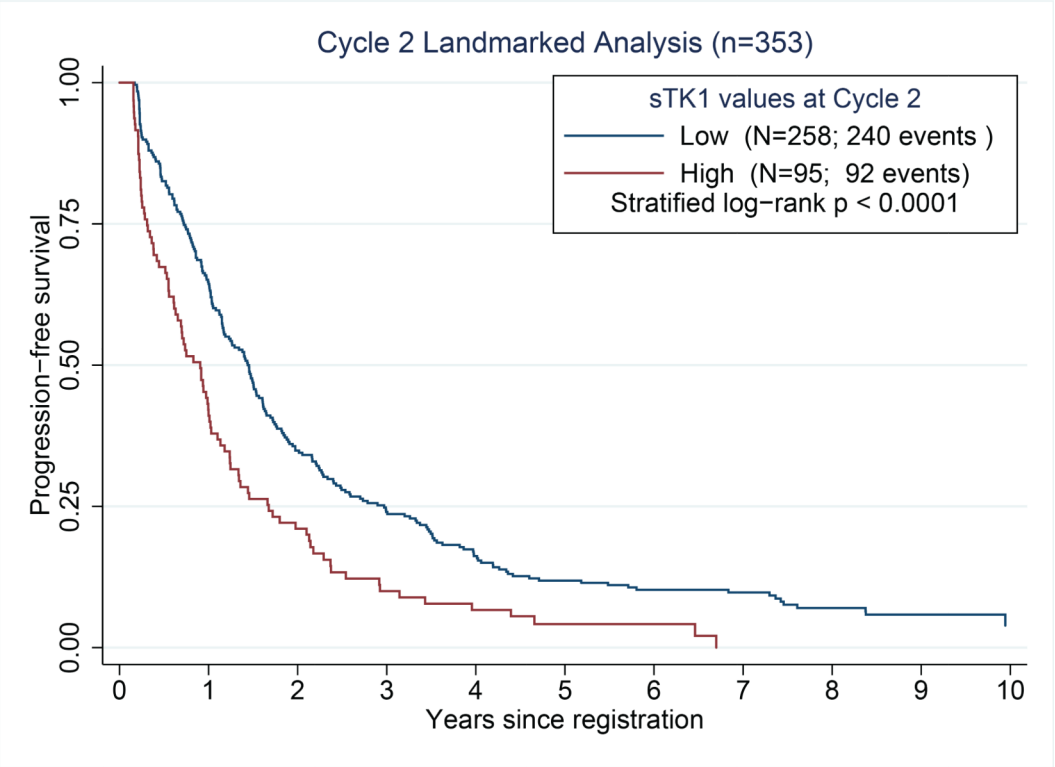


Figure 3.

Kaplan-Meier Curves for overall survival (OS) at baseline; **3A.** All patients in cohort by sTK1 level (blue line, low sTK1; red line, high sTK1). **3B.** Patients with high sTK1 and no prior tamoxifen by treatment arm (blue line, Anastrozole + Fulvestrant; red line, Anastrozole). **3C.** Patients with low sTK1 and no prior tamoxifen by treatment arm (blue line, Anastrozole + Fulvestrant; red line, Anastrozole).

A



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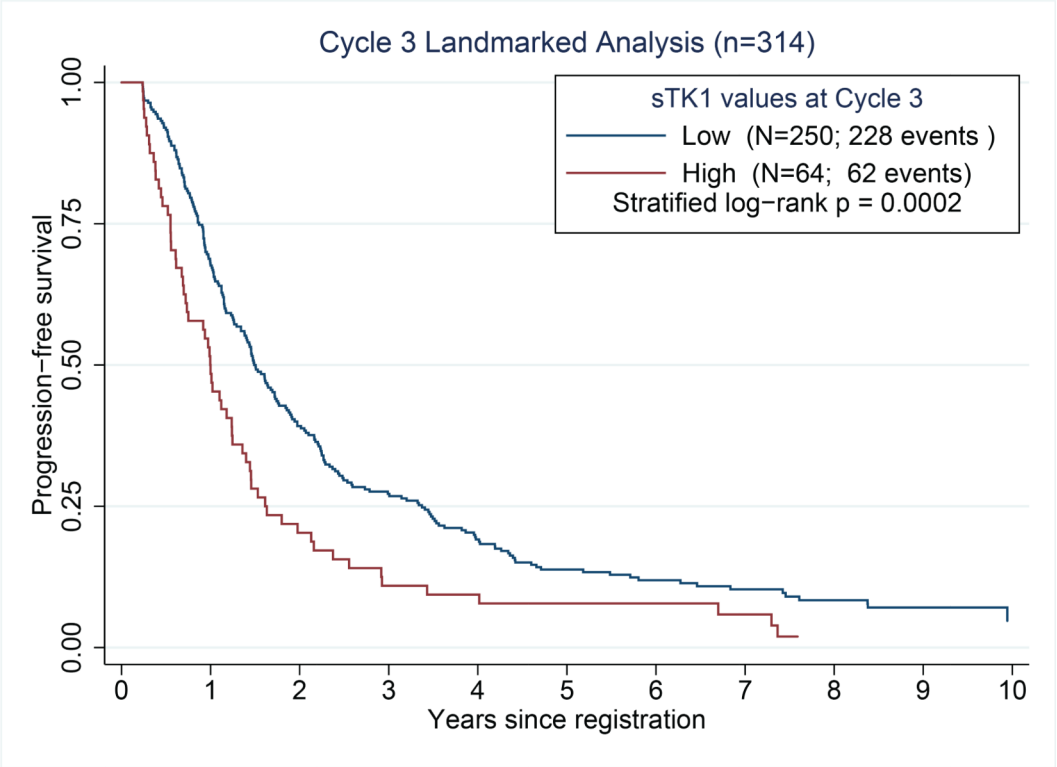

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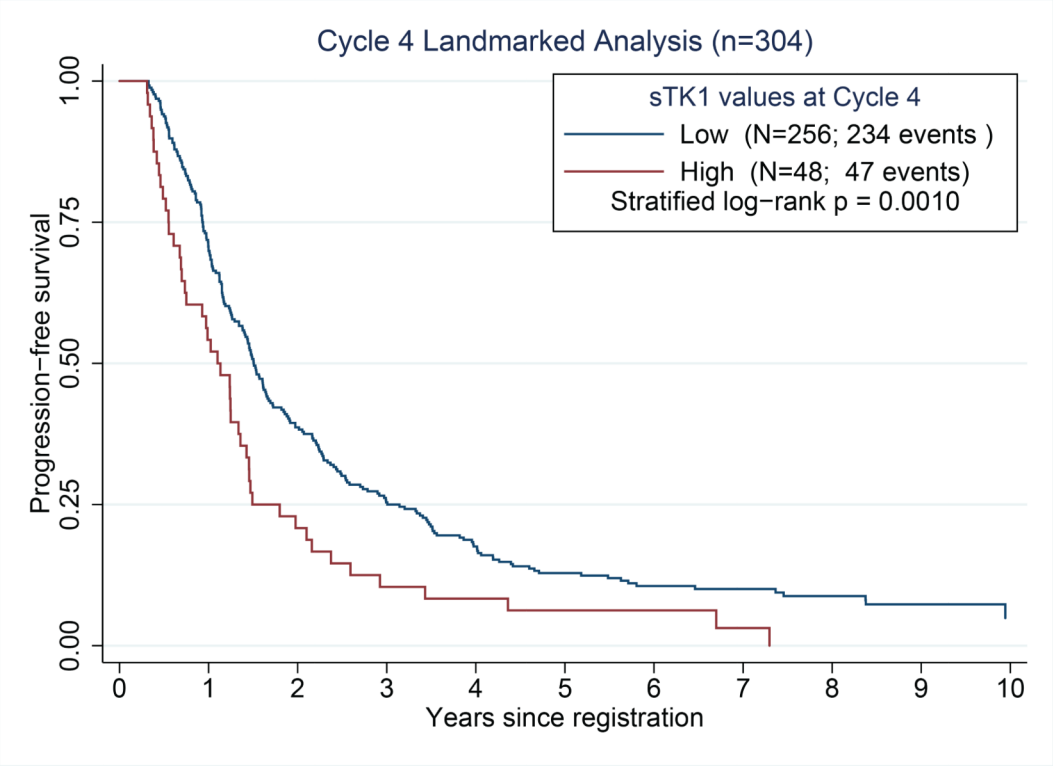

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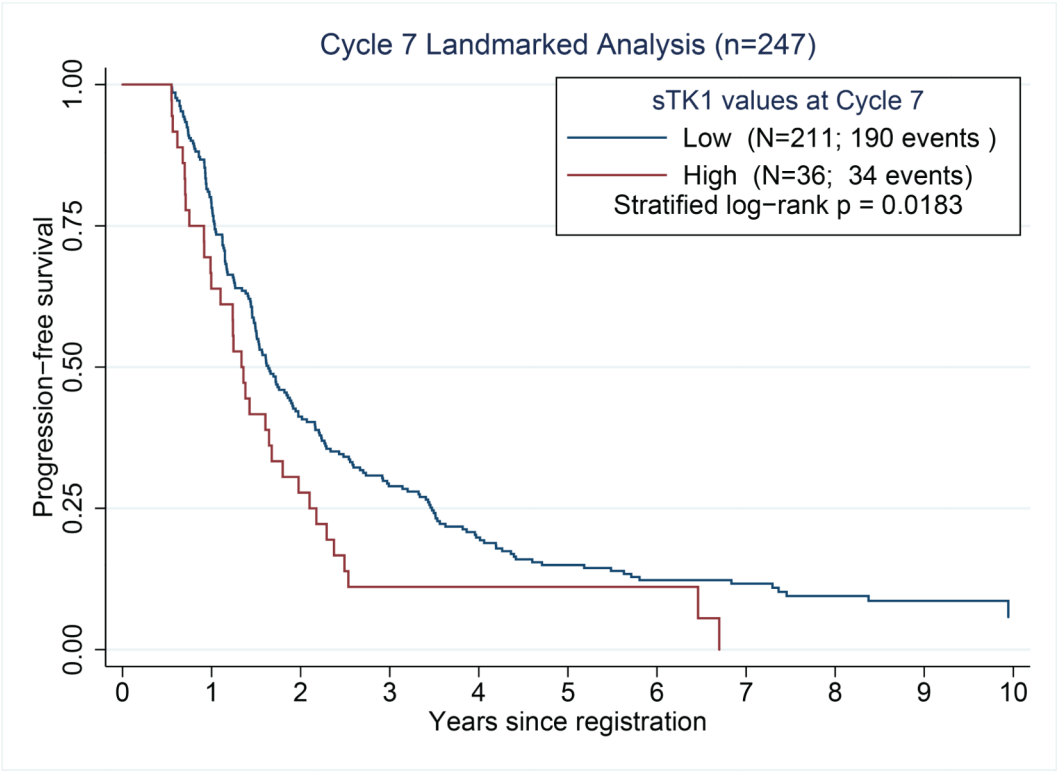

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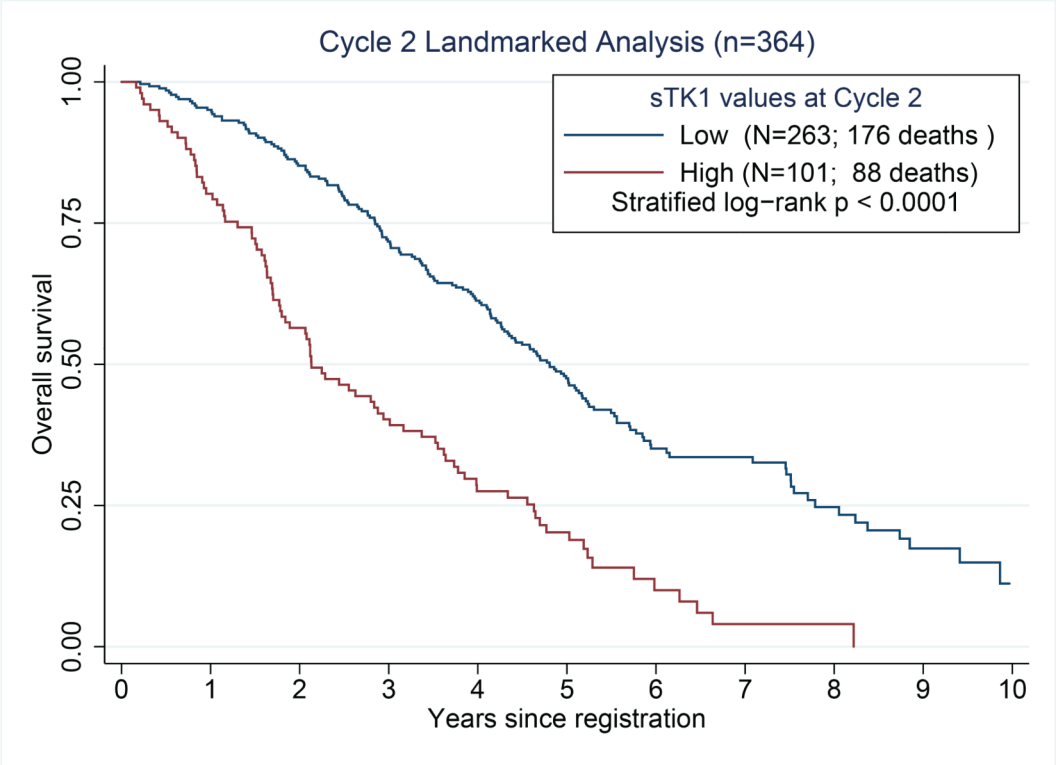

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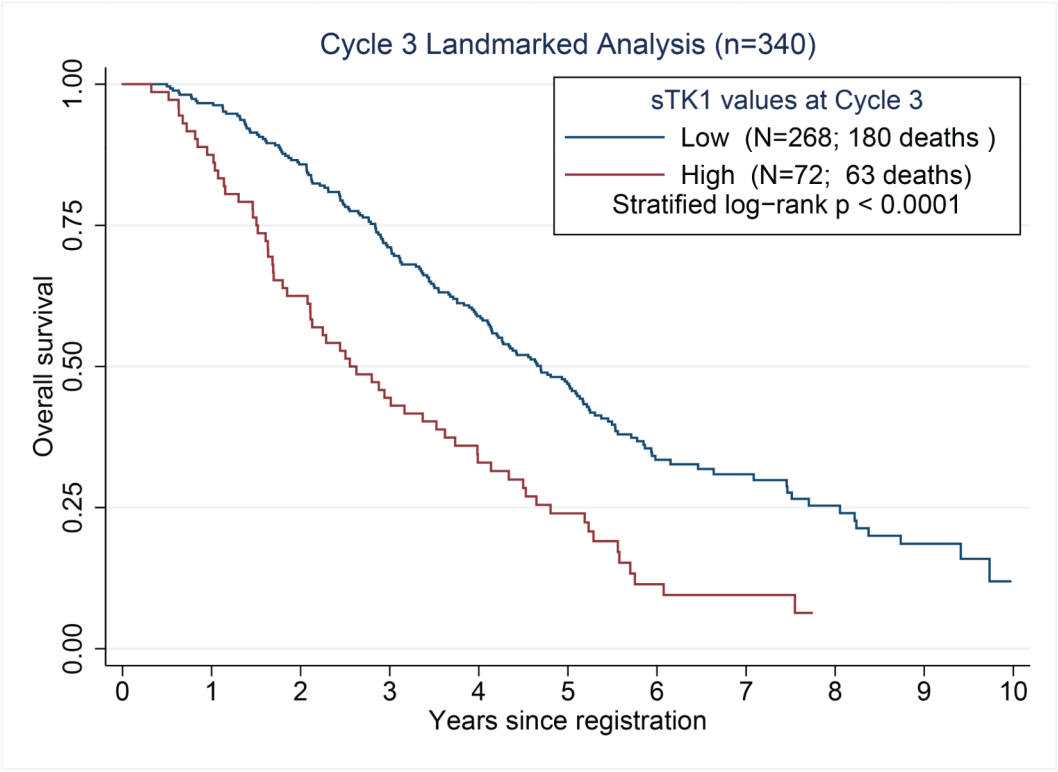

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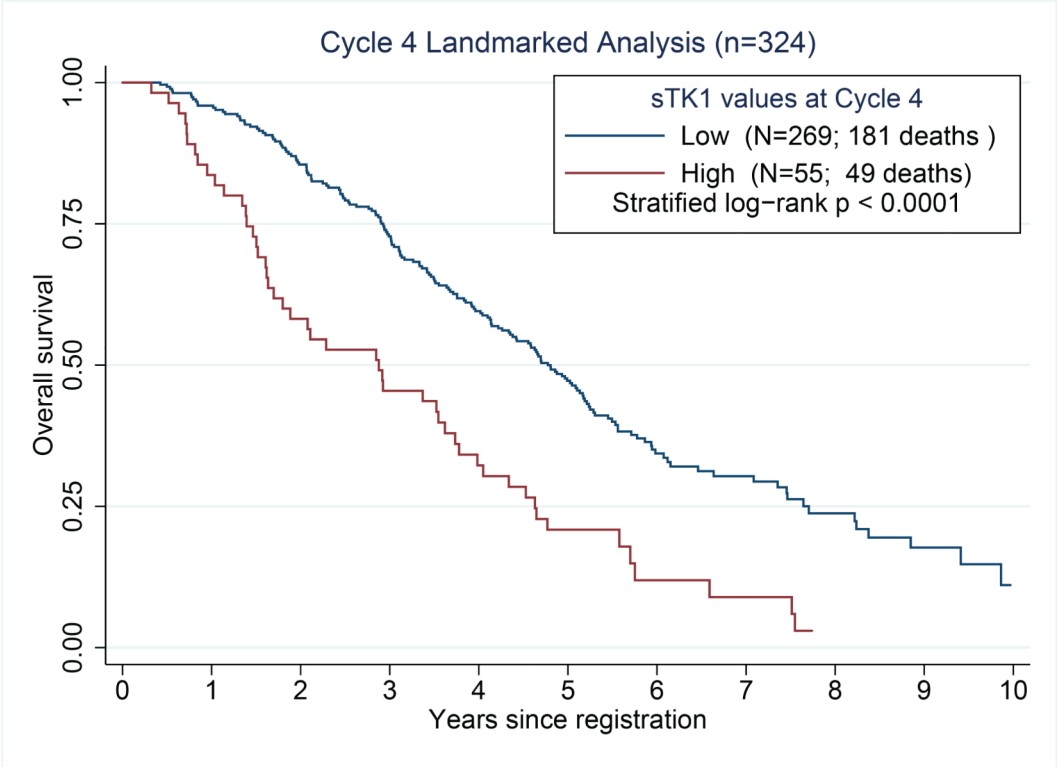

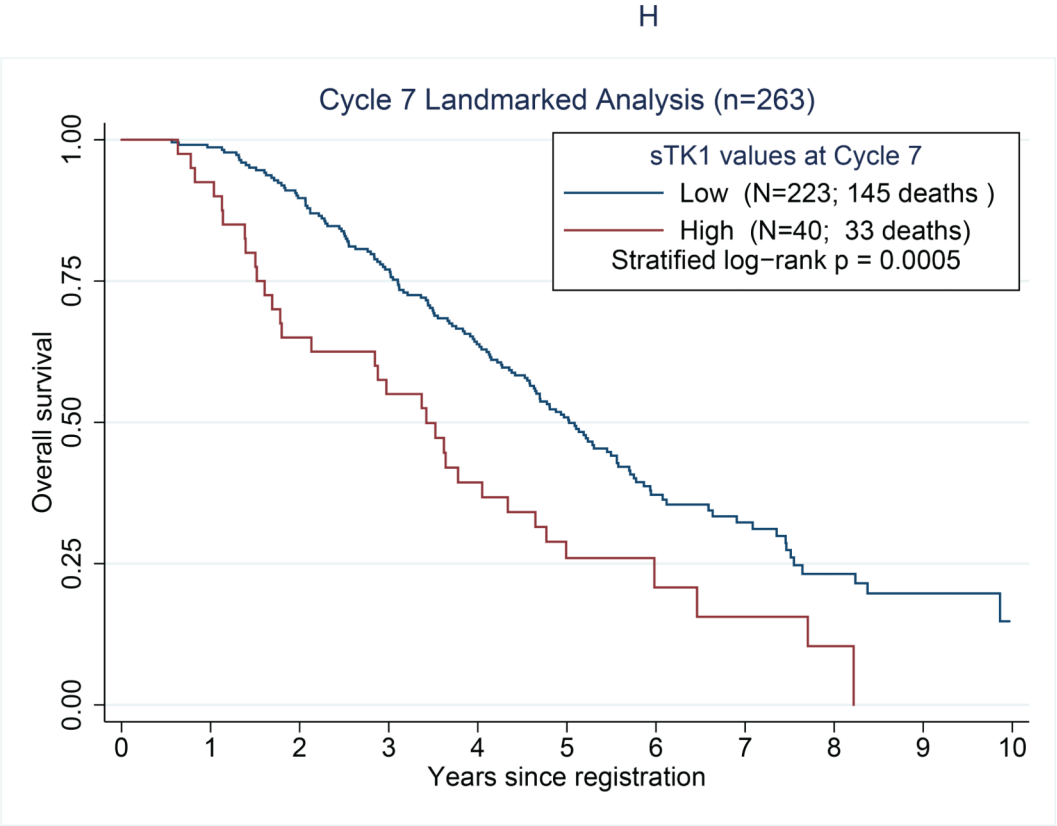

Figure 4.

Kaplan-Meier Curves at Cycles 2, Cycle 3, Cycle 4, and Cycle 7 for Progression free survival (PFS) (**Figures A-D**) and Overall survival (OS) (**Figures E-H**) according to sTK1 level, respectively (blue line, low sTK1; red line, high sTK1).

Table 1.

Comparison of clinical characteristics of full cohort vs. sTK1 study cohorts.

	Total S0226 trial	sTK1 Cohort		
		Entire sTK1^	High sTK1*	Low sTK1
Total N	694#	432	171	261
Age median (range)	65 (27–92)	64 (27–92)	64 (27–91)	64 (40–92)
	N (%)	**N (%)**	**N (%)**	**N (%)**
Treatment assignment				
Anastrozole alone (A)	345 (49.7%)	215 (49.8%)	90 (52.6%)	125 (47.9%)
Anastrozole + fulvestrant (A+F)	349 (50.3%)	217 (50.2%)	81 (47.4%)	136 (52.1%)
Prior adjuvant tamoxifen				
Yes (Stratum 1)	280 (40.4%)	170 (39.8%)	66 (38.6%)	106 (40.6%)
No (Stratum 2)	414 (59.6%)	262 (60.2%)	105 (61.4%)	155 (59.4%)
Prior adjuvant chemotherapy				
Yes	232 (33.4%)	150 (34.7%)	52 (30.4%)	98 (37.6%)
No	462 (66.6%)	282 (65.3%)	119 (69.6%)	163 (62.4%)
Disease type				
Measurable	376 (54.2%)	248 (57.4%)	110 (64.3%)	138 (52.9%)
Non-measurable	318 (45.8%)	184 (42.6%)	61 (35.7%)	123 (47.1%)
Disease site				
Bone only	149 (21.5%)	87 (20.1%)	23 (13.5%)	64 (24.5%)
Visceral	348 (50.1%)	217 (50.2%)	89 (52.0%)	128 (49.0%)
Non-visceral	197 (28.4%)	128 (29.6%)	59 (34.5%)	69 (26.4%)
Time between primary dx and metastatic disease (n=11 missing)				
De novo	268 (39.2%)	176 (41.2%)	82 (48.8%)	94 (36.3%)
3 months – 5 years	88 (12.9%)	53 (12.4%)	20 (11.9%)	33 12.7%)
5 – 10 years	135 (19.8%)	82 (19.2%)	29 (17.3%)	53 (20.5%)
> 10 years	192 (28.1%)	88 (27.2%)	37 (22.0%)	79 (30.5%)
Number of events				
PFS events	647 (93.2%)	403 (93.3%)	166 (97.1%)	237 (90.8%)
OS events	508 (73.2%)	321 (74.3%)	153 (89.5%)	168 (64.4%)

#Patients eligible and evaluable for primary analysis of parent trial

^2None of the characteristics was statistically significant between the total trial and the sTK1 cohort

*the differences between the high and low sTK1 cohorts were statistically significant for having measurable disease (64% vs. 53%, P=0.019), less likely to have bone-only disease (13.5% vs. 24.5%, p=0.005), and more likely to be diagnosed as de novo MBC (49% vs. 36%, p=0.012).

Table 2.

Hazard Ratios for High sTK1 vs. Low sTK1 at Baseline for PFS and OS

	HR for high sTK1 vs. low sTK1 (95% CI)	
	PFS	**OS**
Overall	1.76 (1.43–2.16)	2.38 (1.91–2.98)
Treatment assignment	***Interaction p = 0.64***	***Interaction p = 0.28***
Anastrozole alone (A)	1.85 (1.38–2.46)	2.62 (1.91–3.57)
Anastrozole + Fulvestrant (A+F)	1.65 (1.23–2.22)	2.12 (1.54–2.93)
Prior adjuvant tamoxifen	***Interaction p = 0.20***	***Interaction p = 0.47***
Yes (Stratum 1)	2.10 (1.50–2.93)	2.17 (1.53–3.06)
No (Stratum 2)	1.59 (1.23–2.06)	2.56 (1.91–3.43)
Prior adjuvant chemotherapy	***Interaction p = 0.59***	***Interaction p = 0.021***
Yes	1.88 (1.31–2.70)	1.67 (1.14–2.46)
No	1.63 (1.27–2.09)	2.82 (2.12–3.75)
Disease type	Interaction p = 0.24	Interaction p = 0.98
Measurable	1.62 (1.24–2.12)	2.41 (1.79–3.25)
Non-measurable	1.99 (1.44–2.76)	2.28 (1.62–3.24)
Disease site	Interaction p = 1.00	Interaction p = 0.33
Bone only	1.84 (1.10–3.08)	1.96 (1.13–3.40)
Visceral	1.78 (1.33–2.37)	2.21 (1.61–3.04)
Non-visceral	1.68 (1.15–2.45)	2.97 (1.96–4.49)
Time between primary dx and metastatic disease (n=11 missing)	***Interaction p = 0.39***	***Interaction p = 0.86***
De novo	1.45 (1.06–1.98)	2.52 (1.77–3.59)
3 months – 5 years	1.38 (0.76–2.48)	2.54 (1.35–4.78)
5 – 10 years	2.26 (1.34–3.81)	2.14 (1.26–3.63)
> 10 years	2.38 (1.55–3.67)	2.19 (1.38–3.49)