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Clinical significance of circulating tumor cells in hormone receptor-positive metastatic breast cancer patients who received letrozole with or without bevacizumab

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Abstract

Purpose: We evaluated the prognostic and predictive value of circulating tumor cells (CTCs) hormone-receptor positive (HR+) metastatic breast cancer (MBC) patients randomized to letrozole (Let) alone or letrozole plus bevacizumab (Let+Bev) in the first-line setting (CALGB 40503).

Methods: Blood samples were collected at pretreatment and three additional time points during therapy. The presence of ≥5 CTCs per 7.5 mLs of blood was considered CTC-positive. Association of CTCs with progression-free survival (PFS) and overall survival (OS) was assessed using Cox regression models.

Results: Of 343 patients treated, 294 had CTC data and were included in this analysis. Median follow-up was 39 months. In multivariable analysis, CTC-positive patients at baseline (31%) had significantly reduced PFS (HR=1.49; 95% CI: 1.12-1.97) and OS (HR=2.08; 95% CI: 1.49-2.93) compared to CTC-negative. Failure to clear CTCs during treatment was associated with significantly increased risk of progression (HR=2.2; 95% CI: 1.58-3.07) and death (HR=3.4; 95% CI: 2.36-4.88). CTC-positive patients who received only Let had the worse PFS (HR=2.3; 95% CI: 1.54-3.47) and OS (HR=2.6; 95% CI: 1.59-4.40). Median PFS in CTC-positive patients was significantly longer (18.0 versus 7.0 months) in Let+Bev versus Let arm (p=0.0009). Restricted

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mean survival time analysis further revealed that addition of Bev was associated with PFS benefit in both CTC-positive and CTC-negative patients, but OS benefit was only observed in CTC-positive patients.

Conclusions: CTCs were highly prognostic for the addition of Bev to first-line Let in patients with HR+ MBC in CALGB 40503. Further research to determine the potential predictive value of CTCs in this setting is warranted.

Keywords

circulating tumor cells; hormone receptor-positive; metastatic breast cancer; bevacizumab; letrozole

INTRODUCTION

Hormone-receptor positive (HR+) breast cancer represents approximately 70% of all breast cancers (1,2). The standard of care for metastatic breast cancer (MBC) includes sequential endocrine therapy (ET) alone or in combination with targeted agents (1,2). In the Cancer and Leukemia Group B (CALGB, now part of Alliance for Clinical Trials in Oncology) 40503 trial, the addition of bevacizumab (Bev, an antibody to VEGF-A) to letrozole (Let, an aromatase inhibitor) prolonged progression-free survival (PFS) but not overall survival (OS) in postmenopausal women with HR+ MBC (3). The mechanisms involved in resistance to ET are not fully understood and remain an active area of research (4). Biomarkers that can identify patients whose tumors are more likely to respond or develop resistance to ET and ET combinations are an unmet need.

Blood-based biomarkers, e.g., circulating tumor cells (CTCs), offer a minimally invasive approach for assessing prognosis and monitoring of disease burden in MBC (5,6). Increased levels of CTCs prior to treatment is highly prognostic for disease progression and death (6-8). Moreover, failure to clear CTCs early in treatment is associated with poor response to therapy (6,9-13). In principle, CTCs can facilitate monitoring of disease status and tumor response, and thus enable the potential use of more effective therapy earlier in the disease course.

Efforts to demonstrate the clinical utility of CTCs have been actively pursued (8). For example, the STIC CTC trial recently examined the potential role of CTCs in early treatment modification in HR+HER2-negative MBC (14). Investigators found that switching to chemotherapy in patients with high levels of CTCs prior to treatment (≥5 CTC/7.5 mL of blood) resulted in significant improvements in PFS compared to patients who received standard hormone therapy (14). Smerage and colleagues conducted a single, prospective, randomized study (SWOG0500 trial) to examine whether serial monitoring of CTCs could guide treatment decisions in MBC (15). While the study failed to demonstrate that treatment modification based on CTC response at first follow-up could improve outcomes, it did confirm previous observations showing that patients who have high CTC counts at baseline (≥5 CTC/7.5 mL of blood) had worse outcomes—regardless of treatment—compared to those with <5 CTCs (15).

In this study, we hypothesized that CTCs could serve as a prognostic and predictive marker in HR+ MBC treated with Let or Let+Bev in the first-line setting. To address this hypothesis, we performed an ancillary study in the CALGB 40503 trial to evaluate whether baseline and changes in serial CTC levels were associated with PFS and OS, and whether baseline CTCs could predict benefit from the addition of Bev to Let (16).

METHODS

Patients.

This is a pre-planned study to examine the clinical significance of CTCs in the CALGB 40503 trial (NCT00601900) (3). This trial was a multicenter randomized phase III study that compared the efficacy of Let alone with Let given in combination with Bev (antibody against vascular endothelial growth factor-A or VEGF-A) as first-line endocrine-based therapy in postmenopausal women with HR+ advanced breast cancers. Patients who received more than one prior chemotherapy for MBC were not eligible. Prior adjuvant or neoadjuvant chemotherapy was allowed. The study design and efficacy results have been previously reported (3). Patients were enrolled between December 2008 and December 2011. The institutional review boards at the National Cancer Institute and at each site approved the study. All participants provided a written informed consent that included the use of collected specimens. The study was performed in accordance with the Declaration of Helsinki.

Enumeration of CTCs.

Blood was collected at 4 time points: (baseline and before every third Bev cycle (3-week cycles): 2 (T1), 3 (T2) and 4 (T3) or approximately 21-day intervals in the Let only arm. Samples were drawn into CellSave preservative tubes (Menarini Silicon Biosystems, LLC) at each participating site and shipped to the University of California San Francisco (John W. Park Laboratory) for analysis. CTC enumeration was performed by investigator (JS) who was blinded to the clinical data.

CTCs were enumerated within 96 hours using the CellSearch system (Menarini Silicon Biosystems, LLC) following manufacturer's instructions without modification (17). Briefly, 7.5 mL of blood was subjected to immunomagnetic enrichment to capture EPCAM-positive cells using the CellSearch Circulating Tumor Cell Kit. This was followed by immunofluorescence microscopy to enumerate CTCs, which were defined as nucleated (DAPI-positive) cells of epithelial origin (cytokeratin-positive and CD45-negative). Samples with ≥5 CTCs per 7.5 mL of blood were considered CTC-positive.

Study design: clinical data and endpoints.

The primary endpoint of the study was PFS, defined as the interval between study entry and first documented disease progression or death without progression. A secondary end point was OS, defined as time from study entry to death from any cause. Event-free patients were censored at their last clinical evaluation. Stratification factors (disease measurability and disease-free interval), age and HER2 status were included as covariables in the multivariable models. Survival analysis was performed on follow-up data available as of July 31, 2019.

Statistical analysis.

Patient and tumor characteristics were summarized according to CTC status and the proportions between groups (CTC-positive versus CTC-negative) were compared using Pearson's chi-squared test.

Survival curves were estimated by the Kaplan-Meier method and compared using the log-rank test (18). Multivariable Cox regression models adjusted for known prognostic factors were used to estimate hazard ratios (HR) and 95% confidence intervals (CI). Restricted mean survival times and differences were calculated for different time points (19). The prognostic effect of changes in CTC status between baseline and other time points was tested using a time-dependent Cox model. Data collection and statistical analyses were conducted by the Alliance Statistics and Data Center. Statistical analysis was performed using R (version 3.6.0) and SAS software (version 9.4).

RESULTS

Patient characteristics

Of the 391 patients randomized in the CALGB 40503 (3), 48 were excluded due to missing adverse events, treatment or disease evaluation data (Figure 1A). Of the remaining 343, 3 had no stratification data and 46 did not have pretreatment CTC data and were excluded from the present analysis. The baseline analysis cohort consisted of 294 patients, of whom, 154 received Let+Bev and 140 received Let only. No significant differences in characteristics were observed between patients in the original study cohort and those in the present study (Supplementary Table 1). The proportion of patients with impaired functioning (Eastern Cooperative Oncology Group performance status 1) was higher in the group that was excluded from this study.

A summary of the patient and tumor characteristics of the study cohort by baseline CTC status is shown in Table 1. The median age was 58 years old; 52% received both Let and Bev; 99% were ER-positive and 19% were HER2-positive; 63% had measurable disease; and 49% received prior hormone therapy. The treatment arms were balanced within the CTC-positive and CTC-negative patient groups.

CTCs-positivity and clinicopathologic variables

Evaluation for CTCs was performed in 7.5 mL of blood at 4 time points using CellSearch (Figure 1B). Of the 294 patients, 92 (31.3%) were CTC-positive at baseline. CTC-positivity was significantly associated with bone only metastasis ($p<0.01$) (Table 1). The overall CTC-positive rates decreased over time (T1: 23% of 233; T2: 20% of 172, T3: 15% of 196; Figure 1C).

Prognostic value of CTCs

We examined the association of CTC levels at baseline and serial CTC measurements with clinical outcomes. The median follow-up time for the patients in this study was 39 months. The first three analyses below were performed irrespective of arm assignment.

CTCs at baseline.—CTC-positive patients had a significantly shorter median PFS (13.6 months, 95% CI: 8.4-16.9; Figure 2A) and OS (32.5 months, 95% CI: 26.6-36.1; Figure 2B) compared with CTC-negative patients (PFS: 16.9 months, 95% CI: 14.1-19.3; OS: 47.5 months, 95% CI: 42.9-50.2). In a multivariable Cox regression analysis that included other prognostic variables, CTCs remained a significant negative prognostic factor for PFS (HR=1.79; 95% CI: 1.35-2.36; Figure 2C and Supplementary Table 2) and OS (HR=2.72; 95% CI: 1.98-3.73; Figure 2D and Supplementary Table 2).

Changes in CTC status from baseline to T1.—We assessed whether change in CTC status from baseline to the first time point (T1) during therapy was associated with patient outcome. We identified 4 groups according to serial CTC status: patients who were positive at baseline and remained (1) positive (42 of 219, 19%) or (2) became negative (37 of 219, 17%) at the T1 measurement, and patients who were negative at baseline and (3) became positive at T1 (11 of 219, 5%); or (4) remained negative at T1 (129 of 219, 59%). There were significant differences in the PFS (p=0.02) (Figure 2D) and OS among the four groups (p <0.01) (Figure 2E). Multivariable Cox regression analysis revealed that patients who remained CTC-positive at T1 had a significant increased risk of progression (HR=2.15; 95% CI: 1.43-3.23) and death (HR=2.7; 95% CI: 1.66-4.38) compared to those who remained CTC-negative at T1 (Supplementary Table 3). Similarly, patients who became CTC-positive at T1 had a significant increased risk of death compared to those who remained CTC-negative (HR=3.2; 95% CI: 1.57-6.51).

Changes in CTC status over follow-up.—We assessed whether change in CTC status throughout therapy was associated with patient outcome. In this analysis, the CTC status was treated as a time-dependent variable. At baseline, patients were classified as CTC-positive or CTC-negative. At each time point, patients were re-assigned to CTC-positive or CTC-negative if their status changed. Multivariable Cox regression analysis revealed that patients with CTC-positive status at any time (baseline or at a follow-up time point) had significant increased risk of progression (HR=2.2; 95% CI: 1.58-3.07) and death (HR=3.4; 95% CI: 2.36-4.88) compared to patients who were CTC-negative (Supplementary Table 4).

Predictive value of CTCs

CTC status at baseline by arm.—Next, we stratified patients into 4 groups according to CTC status (at baseline) and treatment arm (Table 2). Patients who were CTC-positive and received Let only had the worse PFS (adjusted likelihood-ratio p<0.01) and OS (adjusted likelihood-ratio p<0.01) (Figure 3A).

For CTC-negative patients, there was no significant difference in median PFS (Let: 14.7 months versus Let+Bev: 18.4 months, adjusted likelihood-ratio p=0.18) and OS (Let: 45 months versus Let+Bev: 49.1 months, adjusted likelihood-ratio p=0.2) between treatment arms (Figure 3B).

For CTC-positive patients, there was no significant difference in the median OS between arms (Let: 27.1 months versus Let+Bev: 33.6 months, adjusted likelihood-ratio p=0.5). Interestingly, median PFS was significantly longer in the Let+Bev (18.0 months) versus Let (7.0 months; adjusted likelihood-ratio p=0.009).

We evaluated whether CTCs at baseline were predictive of treatment efficacy. The tests for interaction between baseline CTCs (positive versus negative) and Bev (yes versus no) were not statistically significant for PFS (p=0.87) or OS (p=0.99).

Changes in CTC status from baseline to T1.—We assessed whether change in CTC status from baseline to the first time point (T1) during therapy was associated with patient outcome in each of the treatment arms. We observed that patients in the letrozole arm who failed to clear CTCs (CTC+CTC+) had the worse PFS (adjusted likelihood-ratio p=0.05, Figure 3C) and OS (adjusted likelihood-ratio p<0.01, Figure 3D). This observation was less apparent in the Let+Bev arm. Patients who remained CTC-negative (CTC-CTC-) had the most favorable PFS (adjusted likelihood-ratio p<0.01, Figure 3E). Patients who were CTC-negative at baseline and became CTC-positive at T1 (CTC-CTC+) had the worse OS (adjusted likelihood-ratio p<0.01, Figure 3F). Interestingly, OS of patients who were initially CTC-positive and either became negative (CTC+CTC-) or remained positive (CTC+CTC+) was better to compared to CTC-CTC+ group.

PFS and OS benefit.—We calculated the restricted mean survival time differences at 6, 12, 18 and 24 months between patients who received Let+Bev versus Let (Table 3). Results revealed significant PFS benefit with the addition of Bev for both CTC-positive and CTC-negative patients. For example, at 24 months, disease progression was, on average, delayed by 5.9 months (95% CI: 2.4-9.4) and 2.5 months (95% CI: 0.1-5) in CTC-positive and CTC-negative patients, respectively.

Significant differences in mean OS of 2.1 months (95% CI: 0.3-3.8) and 3.0 months (95% CI: 0.4-5.6) were observed at 18 and 24 months, respectively, in CTC-positive patients who received Let+Bev versus those who received Let alone. (Table 3). No significant differences were observed in earlier time points. Among the CTC-negative patients, there was no significant difference in mean OS between arms at all time points examined.

DISCUSSION

The CALGB 40503 trial was conducted to examine the efficacy of Bev in extending PFS and OS when added to first-line Let in HR+ MBC (3). The study was activated in 2008 soon after the US Food and Drug Administration granted accelerated approval of Bev (in combination with first-line chemotherapy) for treatment of HER2-negative MBC. This approval was revoked in 2011 because of lack of evidence in prolonged OS, and while improvement in PFS was observed, the toxicities associated with Bev remained significant (20). Subsequently, CALBG 40503 reported results that were consistent with this assessment (3). In contrast, a neoadjuvant study in early stage breast cancer (NSABP B40: chemotherapy with or without bevacizumab in treating women with stage I, stage II, or stage IIIA breast cancer that can be removed by surgery) showed improvement in OS (21). These findings suggest that there may be subgroup(s) of patients who may benefit from Bev treatment, and a biomarker which can identify this subset is clearly warranted.

We performed a prospective CTC study in patients enrolled in CALGB 40503. We enumerated CTCs in serially collected blood samples and evaluated the prognostic impact of

these cells. Results of our study showed that baseline levels of CTCs were highly prognostic for both PFS and OS. Furthermore, we found that changes in CTC status between baseline and other time points were prognostic, i.e., failure to clear CTCs (being consistently CTC-positive) or a switch from CTC-negative to CTC-positive were associated with poor outcomes. These findings are consistent with results from previous studies (6-13).

In CALGB 40503, serial CTC information was not used to guide therapy. A clinical study in the first-line setting for treatment of HR+ MBC did show that changing to chemotherapy in patients with persistent increase in CTCs led to improvements in patient outcomes (15). In contrast, a previous study in unselected patients with MBC (all comers) failed to demonstrate that early change of chemotherapy regimen can improve survival in patients who failed to clear CTCs during first follow-up.

We examined the predictive value of CTCs by evaluating differences in survival of patients according to CTC status (i.e., CTC-positive or -negative at baseline) and treatment received. Comparison of the median PFS and OS revealed longer PFS and OS among CTC-positive patients who received Bev compared to CTC-positive patients who only received Let. Furthermore, our exploratory analysis revealed significantly longer mean PFS (at all time points) and OS (at 18 and 20 months) in CTC-positive patients in the Let+Bev versus the Let only arm. In contrast, there was no significant difference in mean OS in CTC-negative patients between the two arms.

A large study by Cristofanilli and colleagues showed that that MBC patients with ≥5 CTCs per 7.5 mL blood (Stage $IV_{aggressive}$) have significantly worse OS compared to those with <5 (Stage $IV_{indolent}$) (8). In our study, baseline CTCs in postmenopausal women with HR-positive MBC were highly prognostic not only for OS but also PFS. Taken together, these studies confirm CTCs as a strong negative prognostic factor in MBC regardless of breast cancer subtype.

Interesting but counterintuitive observations have been made regarding the clinical impact of CTC levels during treatment with Bev (22-24). For example, Bidard and colleagues found that only baseline CTCs—but not on-treatment levels—were associated with progression in MBC patients treated with Bev and chemotherapy (24). More interestingly, Gazzaniga and colleagues noted that more than half of metastatic colorectal cancer patients who progressed on Bev had undetectable CTCs (23). The investigators speculated that treatment with Bev facilitated epithelia-to-mesenchymal transition in CTCs, and thus became undetectable by CellSearch, an epithelial-based assay (23). And since Bev affects vessel endothelium (25), impairment of CTC intravasation was proposed to explain why CTC counts considerably decreased during treatment (24) even in patients who do not respond to Bev (23).

In contrast to the observations made in previous studies (22-24), our study showed significant differences in PFS and OS of patients based on baseline CTCs and at first follow-up. Interestingly, patients who were CTC-positive at baseline regardless of CTC status at T1 had comparable OS with those who were consistently CTC-negative especially in the first 1.5 years of follow-up (Figure 3F). In addition, these patients (CTC+CTC+ and CTC+CTC-) had significantly longer OS compared to those who were initially CTC-negative and became

positive (CTC-CTC+). The latter group was, however, very small (n=5). Collectively, these observations suggest that serial analysis of CTCs may help identify groups of patients who could potentially benefit most from Bev treatment.

Clinical studies have also examined circulating endothelial cells (CEC) as potential biomarkers of Bev efficacy. Recent report by Vasseur and colleagues showed that high levels of CECs at baseline, but not during treatment, were associated with reduced PFS in patient with HER2-negative MBC treated with chemotherapy and Bev (26). Contradictory results from previous studies from the same group (24,27) as well as others (28)—particularly on the direction of prognostic significance of CECs—have highlighted the need to further examine the clinical impact of these cells.

Our findings demonstrate the potential application of CTCs for patient stratification in clinical studies that investigate benefit from Bev treatment—and potentially the benefit of adding other targeted agents to endocrine therapy in the metastatic setting. Understanding CTC and treatment interactions could eventually help guide patient randomization and risk stratification to facilitate accurate testing of efficacy of novel agents for treatment of MBC. For example, inclusion of CTCs as a stratification factor can facilitate the enrichment of patients with specific risks and could in turn better identify patients most likely to benefit or not benefit from added therapy.

Our results suggest that elevated levels of CTCs at baseline may be predictive of benefit from Bev treatment. Highly vascularized tumors may have the potential to better respond to Bev (29). Moreover, it is hypothesized that extensive vascularization may promote the shedding of CTCs into the blood (30). We therefore speculate that increased CTC levels in the blood due to high vascularity may explain the associated benefit of Bev in CTC-positive patients.

A limitation of the study was the modest sample size particularly in the CTC-positive subset (92/294=31%), and thus, validation in a larger cohort is warranted.

In summary, our findings demonstrate that CTCs are robust prognostic markers in postmenopausal women with HR+ MBC patients treated with Let or Let+Bev in the first-line setting. Our results also suggest a potential OS benefit from adding Bev to Let in patients with poor prognosis MBC as defined by CTC-positivity at baseline. If confirmed, CTCs may be useful as predictive markers for treatment benefit from Bev and may aid in patient selection for future clinical trials investigating the efficacy of Bev and, importantly, other targeted agents in HR+ MBC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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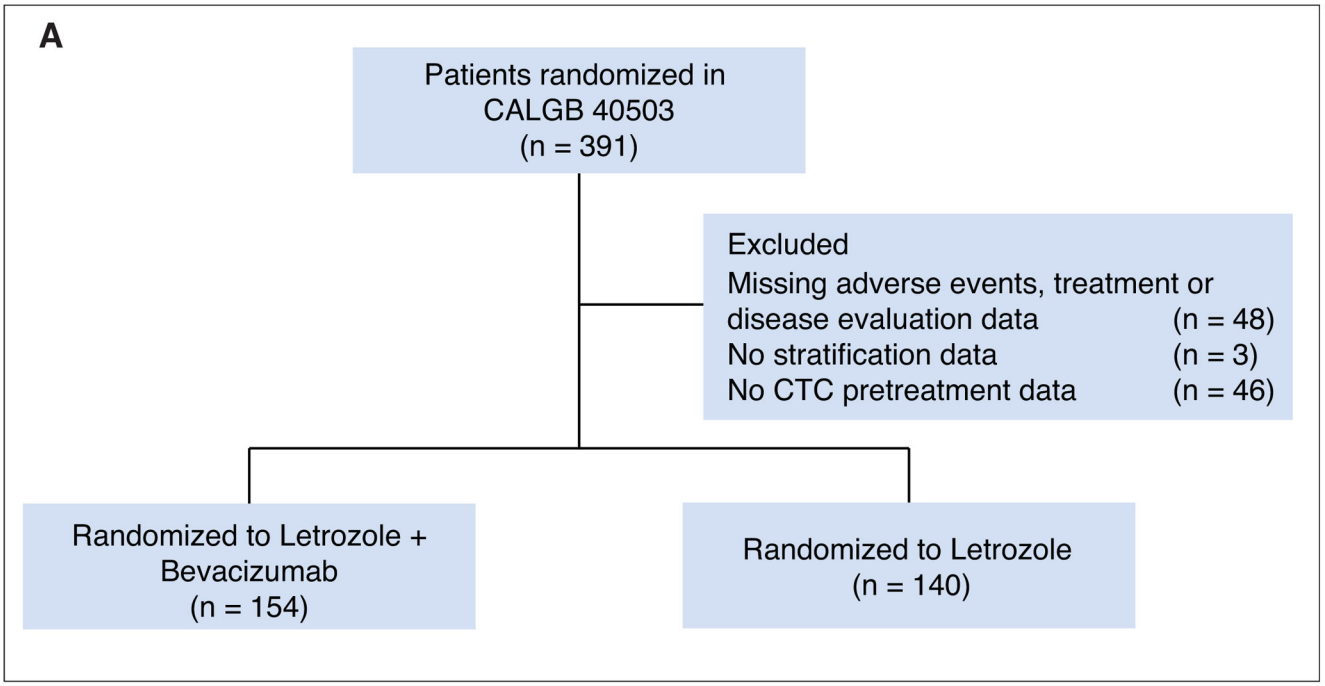
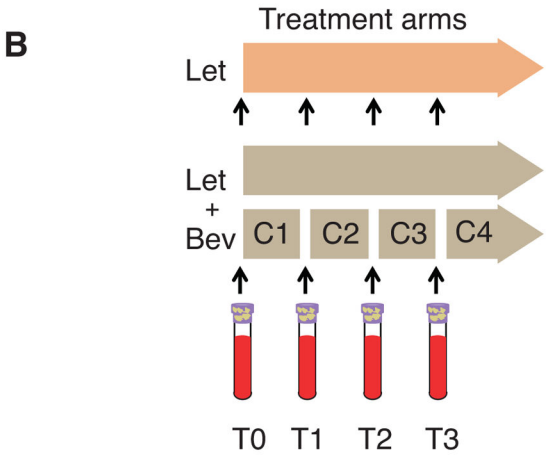
STATEMENT OF TRANSLATIONAL RELEVANCE

Prognostic and predictive biomarkers are needed for robust estimation of risk of progression and death in hormone-receptor positive (HR+) metastatic breast cancer (MBC). Blood-based biomarkers, e.g., circulating tumor cells (CTCs), offer a minimally invasive approach for assessing prognosis and monitoring of disease burden and therapeutic response. Our findings demonstrate that CTCs are robust prognostic markers in postmenopausal women with HR+ MBC who received letrozole (an aromatase inhibitor) with or without bevacizumab (an antibody to VEGF-A). Results of exploratory analysis suggest a potential survival benefit from adding bevacizumab to letrozole in poor prognosis patients as defined by CTC-positivity at baseline. Pending validation, CTCs may serve as predictive markers of benefit from bevacizumab treatment and may aid in the selection of patients in future clinical trials that investigate the efficacy of bevacizumab in MBC.





C

CTC enumeration

	T0	T1	T2	T3
Total N	294	233	172	196
CTC+ (N)	92	53	35	30
% CTC+	31	23	20	15

Figure 1. Circulating tumor cell (CTC) analysis in CALGB 40503.

A. CONSORT flow chart showing the number of patients included and excluded from the study. B. Study schema and sample collection. Arrows indicate time points for blood collection. C. Number of patients with CTC data and percentages of CTC-positive samples at each time point.

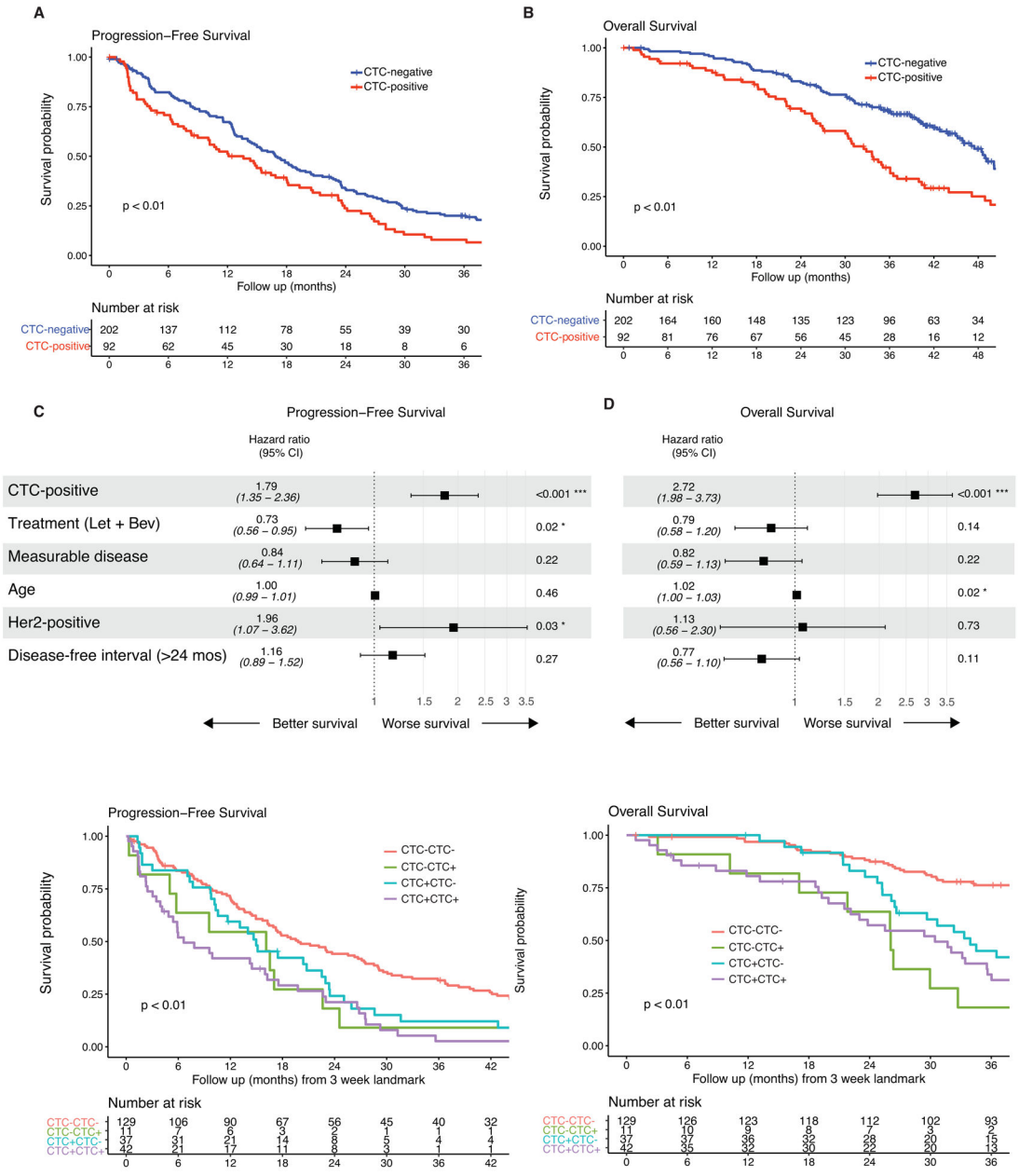


Figure 2. Prognostic impact of baseline CTCs and changes in CTC status from baseline and first follow-up.

Kaplan-Meier curves for: A. progression-free survival and B. overall survival in CTC-positive and CTC-negative patients at baseline (T0). Forest plot of PFS and OS. The dashed vertical line represents a hazard ratio of 1.0 (i.e., no difference in survival between the group shown vs. its reference group). Kaplan-Meier curves for: C. progression-free survival and D. overall survival of patients according to CTC status at baseline (T0) and at 3 weeks after initiation of therapy (T1).

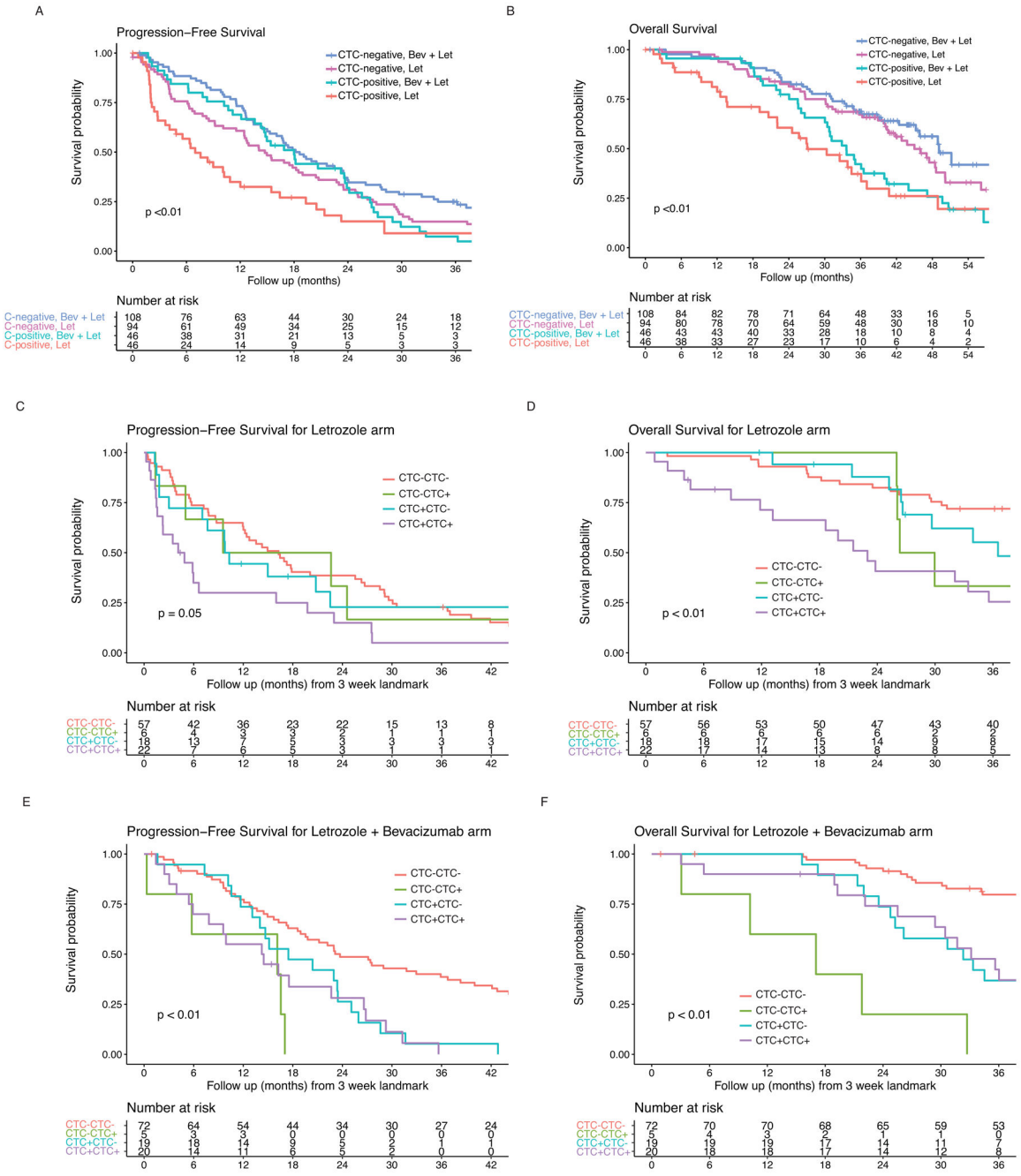


Figure 3. Patient survival according to CTC status at baseline and changes in CTC status from baseline and first follow-up stratified according to treatment arm.

Kaplan-Meier curves for: A. progression-free survival and B. overall survival in CTC-positive and CTC-negative patients at baseline (T0) grouped according to treatment arm. Kaplan-Meier curves showing: C and E. progression-free survival and; D and F. overall survival for patients stratified based on CTC status at baseline (T0) and at 3 weeks after initiation of therapy (T1) randomized to treatment arms: C and D. letrozole and E and F. letrozole + bevacizumab.

Table 1.

Patient and tumor characteristics according to circulating tumor cells (CTC) status at baseline.

	CTC-negative (N=202)	CTC-positive (N=92)	Total (N=294)	Chi-square p-value
Treatment				0.58
Letrozole/Bevacizumab	108 (53.5%)	46 (50.0%)	154 (52.4%)	
Letrozole	94 (46.5%)	46 (50.0%)	140 (47.6%)	
Measurable disease				0.84
No	75 (37.1%)	33 (35.9%)	108 (36.7%)	
Yes	127 (62.9%)	59 (64.1%)	186 (63.3%)	
Age				0.48
Median	57.9	55.9	57.7	
Range	(24.7-85.3)	(31.6-82.1)	(24.7-85.3)	
ECOG performance status				0.96
Missing	2	0	2	
0	131 (65.5%)	60 (65.2%)	191 (65.4%)	
1	69 (34.5%)	32 (34.8%)	101 (34.6%)	
Disease-free interval				0.17
≤ 24 months	99 (49.0%)	53 (57.6%)	152 (51.7%)	
>24 months	103 (51.0%)	39 (42.4%)	142 (48.3%)	
Estrogen receptor				0.50
Missing	2	0	2	
Negative	1 (0.5%)	0 (0.0%)	1 (0.3%)	
Positive	199 (99.5%)	92 (100.0%)	291 (99.7%)	
Progesterone receptor				0.69
Missing	2	0	2	
Negative	35 (17.5%)	20 (21.7%)	55 (18.8%)	
Positive	163 (81.5%)	71 (77.2%)	234 (80.1%)	
Unknown	2 (1.0%)	1 (1.1%)	3 (1.0%)	
HER2				0.69
Missing	2	0	2	
Positive	35 (17.5%)	20 (21.7%)	55 (18.8%)	

	CTC-negative (N=202)	CTC-positive (N=92)	Total (N=294)	Chi-square p-value
Negative	163 (81.5%)	71 (77.2%)	234 (80.1%)	
Unknown	2 (1.0%)	1 (1.1%)	3 (1.0%)	
Prior chemotherapy				0.65
No	122 (60.4%)	53 (57.6%)	175 (59.5%)	
Yes	80 (39.6%)	39 (42.4%)	119 (40.5%)	
Any prior endocrine therapy				0.66
No	102 (50.5%)	49 (53.3%)	151 (51.4%)	
Yes	100 (49.5%)	43 (46.7%)	143 (48.6%)	
Prior aromatase inhibitor				0.60
No	155 (76.7%)	68 (73.9%)	223 (75.9%)	
Yes	47 (23.3%)	24 (26.1%)	71 (24.1%)	
Prior tamoxifen				0.85
No	134 (66.3%)	60 (65.2%)	194 (66.0%)	
Yes	68 (33.7%)	32 (34.8%)	100 (34.0%)	
Metastatic site				<0.01
Missing	2	0	2	
Bone only	87 (43.5%)	56 (60.9%)	143 (49.0%)	
Visceral only	53 (25.5%)	22 (23.9%)	75 (25.7%)	
Bone + Visceral	60 (30.0%)	14 (15.2%)	74 (25.3%)	
No. of metastatic sites				0.39
Missing	3	0	3	
1-2	146 (73.3%)	63 (68.5%)	209 (71.8%)	
≥3	53 (26.6%)	29 (31.5%)	82 (28.2%)	

Table 2.

Survival of patients according to circulating tumor cells (CTC) status and treatment arm.

Endpoint	CTC status at baseline	Treatment arm	Total	Number of events	Median survival in months (95% CI)	Adjusted hazard ratio (95% CI)	Adjusted Likelihood-ratio p-value
PFS							**<0.01**
	CTC-negative	Let+Bev	108	70	18.4 (15.0-23.5)	1	
	CTC-negative	Let	94	74	14.7 (11.4-18.9)	1.44 (1.02-2.02)	
	CTC-positive	Let+Bev	46	42	18.0 (13.6-23.7)	1.44 (0.98-2.13)	
	CTC-positive	Let	46	38	7.0 (2.8-10.9)	2.31(1.54-3.47)	
OS							**<0.01**
	CTC-negative	Let+Bev	108	35	49.1 (42.4-NE)	1	
	CTC-negative	Let	94	44	45.0 (40.1-50.1)	1.29 (0.82-2.03)	
	CTC-positive	Let+Bev	46	34	33.6 (26.6-40.0)	2.20 (1.37-3.55)	
	CTC-positive	Let	46	28	27.1 (20.6-36.1)	2.64 (1.59-4.40)	

Table 3.

Restricted mean survival time (RMST) difference between patients in different arms (Let+Bev minus Let) at specified time points in CTC-positive and CTC-negative patients. CTC status were determined at baseline.

End point	Follow-up time point (months)		RSMT difference: Let+Bev minus Let (months)	Lower 95%	Upper 95%	p value		RMST difference: Let+Bev minus Let (months)	Lower 95%	Upper 95%	p value
			CTC-positive at baseline					CTC-negative at baseline			
PFS											
	6		1.0	0.4	1.7	**<0.01**		0.4	0.0	0.8	**0.04**
	12		3.0	1.3	4.6	**<0.01**		1.4	0.3	2.4	**0.01**
	18		4.7	2.1	7.3	**<0.01**		2.1	0.3	3.8	**0.02**
	24		5.9	2.4	9.4	**<0.01**		2.5	0.1	5.0	**0.04**
OS											
	6		0.2	−0.2	0.5	0.32		0.0	−0.1	0.1	0.74
	12		0.8	−0.2	1.8	0.14		−0.1	−0.5	0.3	0.58
	18		2.1	0.3	3.8	**0.02**		0.1	−0.6	0.8	0.80
	24		3.0	0.4	5.6	**0.02**		0.3	−0.9	1.6	0.59