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Efficacy of Lapatinib in Patients with HER2-Negative Metastatic Breast Cancer and HER2-Positive Circulating Tumor Cells—The DETECT III Clinical Trial

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BACKGROUND: The phenotypes of tumor cells change during disease progression, but invasive rebiopsies of metastatic lesions are not always feasible. Here we aimed to determine whether initially HER2-negative metastatic breast cancer (MBC) patients with HER2-positive circulating tumor cells (CTCs) benefit from a HER2-targeted therapy.

METHODS: The open-label, interventional randomized phase III clinical trial (EudraCT Number 2010-024238-46, ClinicalTrials.gov Identifier: NCT01619111) recruited from March 2012 until September 2019 with a follow-up duration of 19.5 months. It was a multicenter clinical trial with 94 participating German study centers. A total of 2137 patients with HER2-negative MBC were screened for HER2-positive CTCs with a final modified intention-to-treat population of 101 patients. Eligible patients were randomized to standard therapy with or without lapatinib. Primary study endpoints included CTC clearance (no CTCs at the end of treatment) and secondary endpoints were progression-free survival, overall survival (OS), and safety.

RESULTS: In both treatment arms CTC clearance at first follow-up visit—although not being significantly different for both arms at any time point—was significantly associated with improved OS (42.4 vs 14.1 months; $P = 0.002$). Patients treated additionally with lapatinib had a significantly improved OS over patients receiving standard treatment (20.5 vs 9.1 months, $P = 0.009$).

CONCLUSIONS: DETECT III is the first clinical study indicating that phenotyping of CTCs might have clinical utility for stratification of MBC cancer patients to HER2-targeting therapies. The OS benefit could be related to lapatinib, but further studies are required to prove this clinical observation.

ClinicalTrials.gov Registration Number: NCT01619111.

Introduction

The HER2 status of breast cancer tumors is an important biomarker for predicting the response to HER2-targeted

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therapy. The expression of the HER2 receptor can differ between primary tumors and metastatic disease or vary between different metastatic lesions (1–3). Thus, the HER2 status should be reassessed in metastatic lesions and in cases of progression (4). However, invasive tissue biopsies of metastatic lesions are not always feasible due to the location of the metastatic site.

In this regard, circulating tumor cells (CTCs) have the potential to contribute to current personalized treatment approaches as noninvasive surrogates for tissue-based biomarkers. Many studies have shown the prognostic and predictive clinical value of CTCs for primary and metastatic breast cancer (MBC) (5–7). Additional characterization of CTCs, including determination of their HER2 status, might be a clinical strategy to provide further valuable information for tailoring therapies. The HER2 status can be assessed for CTCs as for tissue samples by immunostaining or fluorescence/chromogenic in situ hybridization (8–10). Some studies have demonstrated heterogeneity of the HER2 status of primary breast tumors and patient-matched CTCs during the course of metastatic disease (8, 10–21). To date, it is still unclear whether a therapy based on the CTCs' HER2 status offers any clinical benefit.

Several HER2-targeting agents have demonstrated their efficacy for HER2-positive breast cancer, including the small-molecule dual tyrosine kinase inhibitor lapatinib (22, 23), which targets both HER2 and the epidermal growth factor receptor. Lapatinib is approved for the treatment of HER2-positive MBC in combination with capecitabine or, in case of a positive hormone receptor status, with endocrine therapy.

In this context, the multicenter phase III DETECT III trial was designed with the purpose of evaluating the efficacy of HER2-targeted therapy in MBC patients with initially HER2-negative primary breast cancer but HER2-positive CTCs at the time of metastatic relapse.

Materials and Methods

STUDY DESIGN AND SETTING

The DETECT III trial is a randomized multicenter open-label phase III trial design conducted in 94 participating German study centers from March 2012 to September 2019 (last patient randomized). The data cutoff for the analysis presented here was April 2021. The trial protocol is available in Supplemental Material 1. All enrolled patients provided written informed consent. The study concept was in accordance with the Declaration of Helsinki, adhered to good clinical practice and German pharmaceutical law, and was approved by the local ethics committees of the participating centers (525/2011AMG1).

PARTICIPANTS

Patients eligible for the initial screening phase were MBC patients who could not be treated by surgery or radiotherapy alone and with a history of at most 3 chemotherapy lines for metastatic disease. The primary tumor and/or investigated lesions had to show HER2 negativity, and at least one lesion had to be evaluated according to RECIST 1.1 criteria. Overall, a total of 2137 patients with HER2-negative MBC were screened for CTCs (Fig. 1). The CTC enumeration and HER2 detection methods are described in Supplemental Methods 1 in Supplemental Material 2 and have been published in detail elsewhere (24). Only patients with at least one detected HER2-positive CTC in the initial CTC screening phase were eligible for randomization into the clinical DETECT III trial. Further details regarding the definition for HER2 positivity of CTCs in the screening phase are provided in Supplemental Methods 2 in Supplemental Material 2.

PROCEDURES

The originally planned primary objective was to estimate the hazard ratio (HR) of progression-free survival (PFS) for standard therapy alone vs standard therapy plus lapatinib in patients with initially HER2-negative MBC and HER2-positive CTCs. However, based on the unexpected low rate of screened patients with HER2 positivity of CTCs (as required for randomization) and the associated slow recruitment, it was decided that the number of patients to be enrolled had to be reduced. As PFS was not a suitable primary endpoint for a study with such a reduced sample size, the CTC clearance rate (defined as the proportion of patients with at least one CTC detected in 7.5 mL of peripheral blood drawn before treatment that show no evidence of CTCs in the blood after treatment) was chosen as new primary endpoint of the study. The secondary endpoints included PFS, overall survival (OS) and safety.

Safety and tolerability were assessed by evaluation of adverse event and serious adverse event reports using the international Common Terminology Criteria for Adverse Events, version 4.0.

Randomization was performed using a randomized permuted block design with the number of CTCs (<5 vs ≥ 5) and line of metastatic treatment (first vs at least second) as stratification factors. More details regarding randomization, as well as information regarding the study treatment and assessment phases, are provided in Supplemental Methods 3 in Supplemental Material 2.

STATISTICAL ANALYSIS

The original sample size calculation for the DETECT III study with the original primary endpoint (PFS) is described in Supplemental Methods 4 in Supplemental Material 2. According to the new primary endpoint

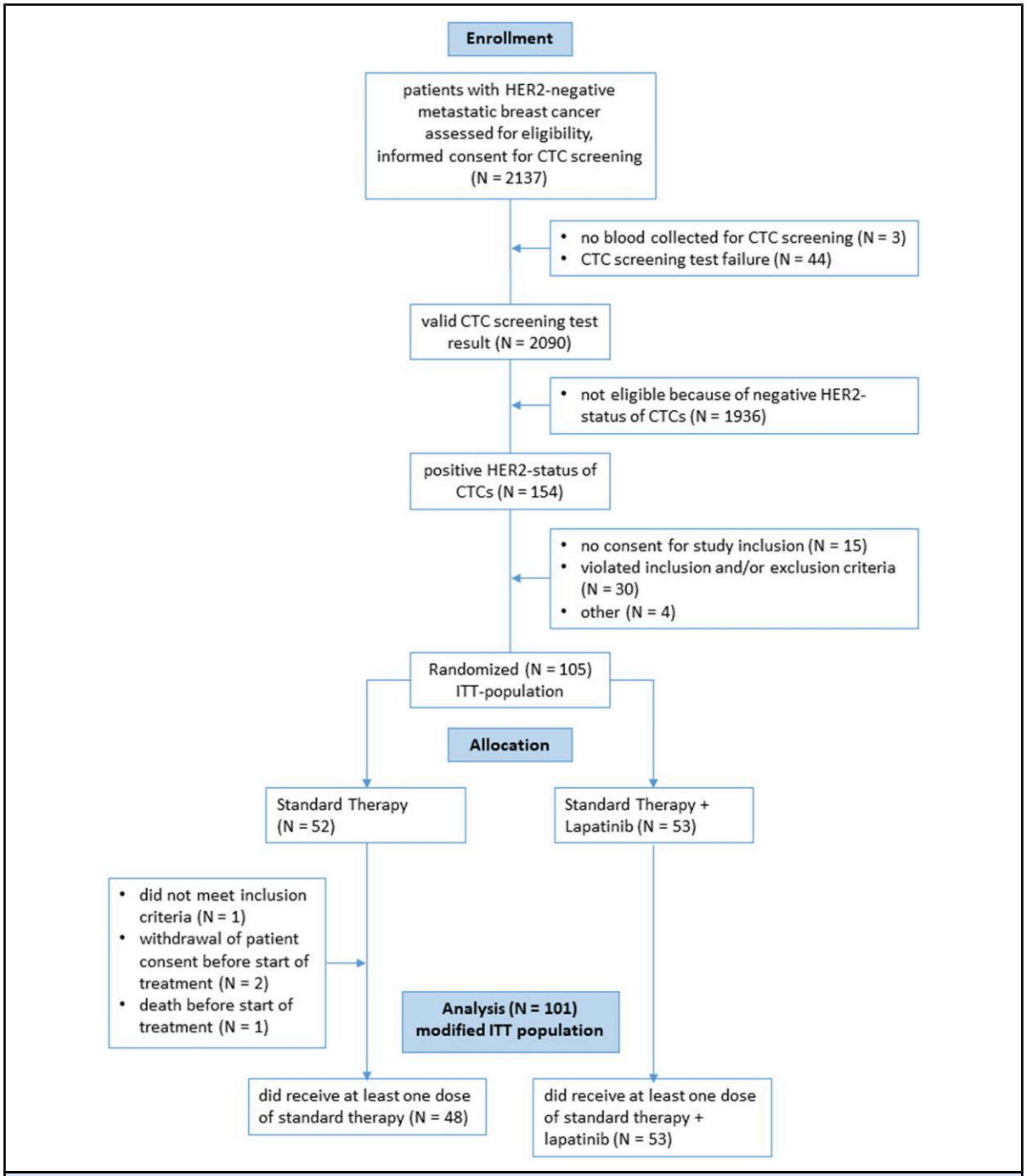

Fig. 1. CONSORT flow chart of the DETECT III study. A total of 2137 patients with HER2-negative MBC were screened for CTCs. A total of 105 patients were prospectively randomized to receive standard therapy alone or standard therapy with lapatinib. Four patients in the standard arm did not receive any study treatment after randomization and were excluded. A total of 101 patients in the prespecified modified intention-to-treat population were available for the final analysis.

(CTC clearance rate) and the new adjusted sample size calculation, 102 subjects (51 per treatment arm) were required to show an increase in the proportion of patients with CTC clearance after treatment from 54% in the standard chemotherapy or endocrine therapy arm to 77% in the standard chemotherapy or endocrine therapy plus lapatinib arm using a one-sided test with a type I error rate of 5% and a power of 80%. Assuming a loss to follow-up of approximately 15%, 120 subjects (60 per treatment arm) had to be randomized.

Clinicopathological characteristics of the study cohort were described as absolute and relative frequencies for categorical and ordinal variables and as medians and ranges for continuous variables. Differences between groups were analyzed by chi-square tests for categorical variables (the Fisher exact test in cases of expected cell frequencies ≤ 5 in 2×2 contingency tables) and by Mann–Whitney *U*-tests for continuous (not normally distributed) variables, including CTC numbers.

The primary endpoint, i.e., the CTC clearance rate after study treatment, was calculated as the proportion of patients with at least one CTC detected in 7.5 mL of peripheral blood drawn before treatment who showed no evidence of CTCs in the blood after study treatment (i.e., at the time of the closure visit, which took place either after a patient had received 1 year of study treatment or at the time the study treatment had to be stopped because of progression, toxicity, or patient wish). To evaluate the role of CTCs as an early response marker, the CTC clearance rate at the time of the first CTC assessment (at least 3 weeks after randomization) was also calculated. In addition to the CTC clearance rates, we specifically investigated the clearance rates of HER2-positive CTCs, with separate analyses regarding the clearance rate of CTCs with strong [immuno fluorescence (IF) 3+] HER2 expression for patients with at least one CTC with strong HER2 expression at baseline ($n = 78$ patients) and the clearance rate of CTCs with any (IF 1+, 2+, or 3+) HER2 expression for the full set of patients ($n = 101$). Details on the calculation of the secondary survival endpoints PFS and OS are provided in Supplemental Methods 5 in Supplemental Material 2. Statistical analyses were performed using SPSS (version 24); all statistical tests were two-sided, and *P* values smaller than 0.05 were defined as significant. There was no adjustment of the significance level for multiple testing.

Results

BASELINE CHARACTERISTICS OF THE PATIENTS AND TERMINATION OF STUDY TREATMENT

A total of 105 patients were randomized to receive standard therapy alone or standard therapy with lapatinib. Four patients in the standard arm did not receive any study treatment after randomization and were excluded, resulting in a total of 101 patients in the modified intention-to-treat population (48 patients in the standard arm, 53 patients in the lapatinib arm) available for the final analysis (see CONSORT flow diagram, Fig. 1). The median age of the enrolled patients was 59 years, and 95% of patients presented with HR-positive HER2-negative MBC. Overall, 50 patients had a negative HER2 status of the primary tumor confirmed with a negative HER2 status of the metastatic lesion (30 and 20 patients in the lapatinib and standard arm, respectively), while for 42 patients with a negative HER2 status of the primary tumor a confirmatory HER2 assessment of metastatic biopsies was not available (18 and 24 patients in the lapatinib and standard arm, respectively). For 8 patients (5 and 3 patients in the lapatinib and standard arm, respectively), a negative HER2 status was determined solely based on a metastatic lesion, as the HER2 status of the primary tumor was unknown. For one patient in the standard arm with an unknown HER2 status of the primary tumor, there was also no information on the HER2 status of metastatic lesions available; following the definitions and criteria of HER2 status as laid out in the study protocol, this patient was assigned a negative HER2 status.

The 2 treatment arms were balanced with regard to most baseline patient and tumor characteristics (Table 1). However, some parameters with a potential impact on the patients' survival, such as disease subtype, the presence of bone metastases only, and the number of previous chemotherapy lines for metastatic disease, were not well balanced between standard and experimental arms. CTC characteristics at baseline are provided in Supplemental Results 1 in Supplemental Material 2.

The study treatment had to be discontinued in 86 (85.1%) patients, 40 (83.3%) of whom were in the standard arm and 46 (86.8%) of whom were in the lapatinib arm ($P = 0.625$). The most frequent reason for discontinuation in both randomization arms was progression or death (25 and 26 patients in the standard and lapatinib arms, respectively), followed by toxicity (8 and 7 patients in the standard and lapatinib arms, respectively), and patient wish (6 and 8 patients in the standard and lapatinib arms, respectively). The safety and tolerability characteristics are summarized in Supplemental Tables 1 and 2 in Supplemental Material 2.

CTC CLEARANCE RATES AT THE TIME OF THE FIRST CTC ASSESSMENT AND SURVIVAL

A first follow-up CTC assessment could be performed in 69 patients (30 and 39 patients in the standard and lapatinib arms, respectively) after a median time of 73

Table 1. Clinical data of the patients at baseline.

Variable	Total (n = 101)	Standard therapy (n = 48)	Standard therapy + lapatinib (n = 53)	*P* value[a]
Age (median, range)	59 (26–80)	59.5 (29–80)	58 (26–79)	0.799
Menopausal status (%)				0.624
Postmenopausal	84 (83.2)	39 (81.3)	45 (84.9%)	
Premenopausal	17 (16.8)	9 (18.8)	8 (15.1)	
IHC tumor type (%)				0.188
TNBC	5 (5.0)	4 (8.3)	1 (1.9)	
Luminal[b]	96 (95.0)	44 (91.7)	52 (98.1)	
Grading (%)				0.216
G I	6 (5.9)	1 (2.1)	5 (9.4)	
G II	66 (65.3)	32 (66.7)	34 (64.2)	
G III	21 (20.8)	12 (25.0)	9 (17.0)	
Missing	8 (7.9)	3 (6.3)	5 (9.4)	
Histology (%)				0.370
Ductal	45 (44.6)	19 (39.6)	26 (49.1)	
Lobular	35 (34.7)	20 (41.7)	15 (28.3)	
Other	21 (20.8)	9 (18.8)	12 (22.6)	
Metastatic site (%)				0.220
Visceral	29 (28.7)	11 (22.9)	18 (34.0)	
Nonvisceral[c]	72 (71.3)	37 (77.1)	35 (66.0)	
Metastasis-free interval (%)				0.159
≥12 months	66 (65.3)	28 (58.3)	38 (71.7)	
<12 months[d]	35 (34.7)	20 (41.7)	15 (28.3)	
(Neo)adjuvant chemotherapy (%)				0.295
No	45 (44.6)	24 (50.0)	21 (39.6)	
Yes	56 (55.4)	24 (50.0)	32 (60.4)	
Therapeutic setting (number of chemotherapy lines for metastatic disease) (%)				0.254
0	59 (58.4)	24 (50.0)	35 (66.0)	
1	20 (19.8)	11 (22.9)	9 (17.0)	
≥2	22 (21.8)	13 (27.1)	9 (17.0)	
Standard therapy within the trial (%)				0.164
Chemotherapy	78 (77.2)	40 (83.3)	38 (71.7)	
Endocrine-based	23 (22.8)	8 (16.7)	15 (28.3)	
Docetaxel	3 (3.0)	2 (4.2)	1 (1.9)	
Paclitaxel	30 (29.7)	12 (25.0)	18 (34.0)	
Capecitabine	23 (22.8)	13 (27.1)	10 (18.9)	
Vinorelbine	17 (16.8)	10 (20.8)	7 (13.2)	
NPLD	5 (5.0)	3 (6.3)	2 (3.8)	
Exemestane	9 (8.9)	4 (8.3)	5 (9.4)	
Letrozole	11 (10.9)	3 (6.3)	8 (15.1)	

Continued

Table 1. (continued)

Variable	Total (n = 101)	Standard therapy (n = 48)	Standard therapy + lapatinib (n = 53)	*P* value[a]
Anastrozole	3 (3.0)	1 (2.1)	2 (3.8)	

IHC, invasive ductal carcinoma; TNBC, triple negative breast cancer; NPLD, nonpegylated liposomal doxorubicin.
[a]Mann–Whitney *U*-test for age; chi-square test for categorical variables (Fisher exact test in cases of expected cell frequencies ≤5 in 2 × 2 contingency tables).
[b]Including one patient with unknown hormone receptor status for the primary tumor but positive estrogen receptor status of the metastatic lesion in each of the treatment arms.
[c]Including 32 and 24 patients with bone-only metastatic disease in the standard and experimental arms, respectively.
[d]Including 15 and 12 patients with de novo metastatic disease in the standard and experimental arms, respectively.

days (interquartile range 64–87 days, range 22–215 days). CTC clearance was observed in 8 [26.7%, 95% confidence interval (CI), 9.9–43.5] patients in the standard arm and in 12 (30.8%, 95% CI, 15.6–45.9) patients in the lapatinib arm; the difference was not statistically significant ($P = 0.710$; Fig. 2A). The median number of CTCs detected at the first CTC assessment was 4.5 CTCs in the standard arm and 3 CTCs in the lapatinib arm ($P = 0.806$).

Patients with no evidence of CTCs at the first follow-up CTC assessment (i.e., patients with CTC clearance at the first follow-up) showed better OS than patients with CTCs (CTC clearance: median OS 42.4 months; no CTC clearance: median OS 14.1 months; HR 0.33; 95% CI, 0.16–0.68; $P = 0.002$; Fig. 3). Patients with a CTC clearance at the first follow-up CTC assessment also had numerically longer PFS, but the difference was not significant (CTC clearance: median PFS 9.2 months; no CTC clearance: median PFS 6.4 months; HR 0.66; 95% CI, 0.35–1.25; $P = 0.198$).

No significant differences between the standard and lapatinib arms were found with regard to the clearance of only HER2 3+ CTCs for patients with at least one HER2 3+ CTC at baseline (24 and 28 patients in the standard and lapatinib arm, respectively; clearance rate 58.3%, 14 patients vs 67.9%, 19 patients; $P = 0.477$; Fig. 2B). Likewise, there were no significant differences between the standard and lapatinib arms with regard to the clearance rate of CTCs with any (IF 1+, 2+, or 3+) HER2 expression (30 and 39 patients in the standard and lapatinib arm, respectively; clearance rate 43.3%, 13 patients vs 35.9%, 14 patients; $P = 0.530$; Fig. 2C).

CTC CLEARANCE RATES AFTER STUDY TREATMENT AND SURVIVAL

Overall, 47 patients (21 and 26 patients in the standard and lapatinib arms, respectively) had a CTC assessment at the end of study treatment. The CTC clearance rate at the end of study treatment, which was the primary endpoint of the study, did not differ significantly between the standard arm and the lapatinib arm (33.3%, 7 patients vs 15.4%, 4 patients; $P = 0.181$) (Fig. 2A). CTC clearance at the end of study treatment was not significantly associated with OS (HR 0.94; 95% CI, 0.42–2.08; $P = 0.871$) or PFS (HR 0.70; 95% CI, 0.29–1.69; $P = 0.421$).

No significant differences between the standard and lapatinib arms were found with regard to the clearance of only HER2 3+ CTCs for patients with at least 1 HER2 3+ CTC at baseline (16 and 18 patients in the standard and lapatinib arms, respectively; clearance rate 68.8%, 11 patients vs 50.0%, 9 patients; $P = 0.268$; Fig. 2B). Likewise, there were no significant differences between the standard and lapatinib arms with regard to the clearance rate of CTCs with any (IF 1+, 2+, or 3+) HER2 expression (21 and 26 patients in the standard and lapatinib arm, respectively; clearance rate 38.1%, 8 patients vs 23.1%, 6 patients; $P = 0.263$; Fig. 2C).

SURVIVAL ACCORDING TO RANDOMIZATION ARM

Death was documented for 40 (83%) out of 48 patients in the standard arm and 34 (64%) out of 53 patients in the lapatinib arm. The median OS follow-up time was 19.5 months. Patients in the lapatinib arm had a significantly improved OS with a median survival time of 20.5 months (95% CI, 12.7–28.4 months) compared to a median survival time of 9.1 months (95% CI, 8.3–9.9 months) for patients in the standard arm (Fig. 4A). This finding was evident based on univariable Cox regression analysis (HR 0.54; 95% CI, 0.34–0.86; $P = 0.008$) and multivariable Cox regression analysis adjusted for the metastasis-free interval (<12 months or >12 months), type of metastases (visceral or nonvisceral), hormone receptor status (positive or negative), (neo)adjuvant chemotherapy (yes, no), and metastatic treatment line (first, second, third, or higher) (HR 0.53; 95% CI, 0.33–0.86; $P = 0.010$). We repeated

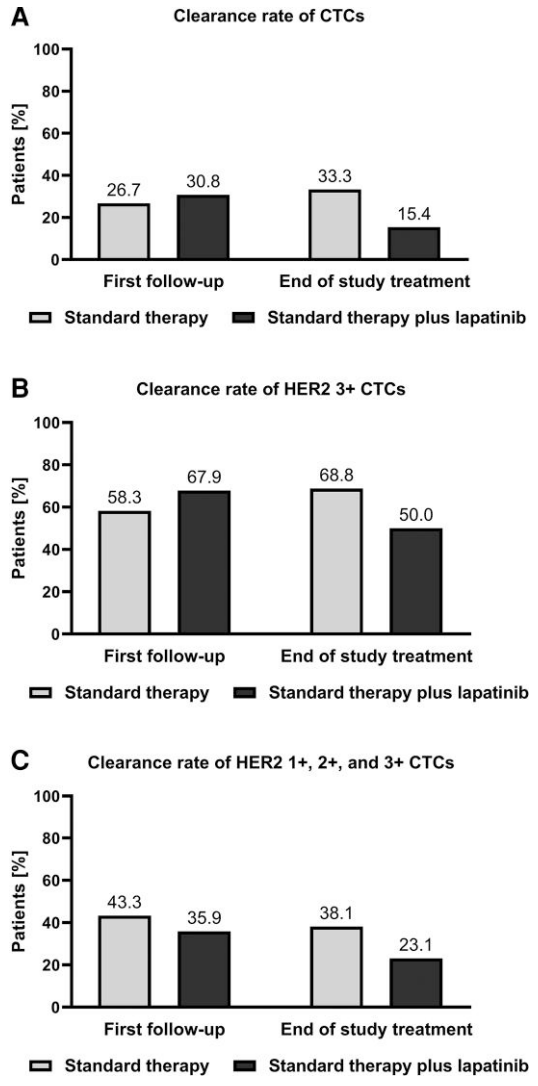

Fig. 2. CTC clearance rate of HER2-positive CTCs at the time of first follow-up of CTC assessment and at the end of study treatment. The CTC clearance rate was defined as the proportion of patients with at least one CTC detected in 7.5 mL of peripheral blood drawn before treatment who showed no evidence of CTCs in the blood at the time of first follow-up CTC assessment and at the end of study treatment. (A), CTC clearance rate of all CTCs; (B), CTC clearance rate of CTCs with strong (IF 3+) HER2 expression; (C), CTC clearance rate of CTCs with weak (IF 1+), moderate (IF 2+), or strong (IF 3+) HER2 expression. There were no significant differences between the standard and lapatinib arms with regard to the CTC clearance rate in (A), (B), or (C) at baseline or at the end of treatment.

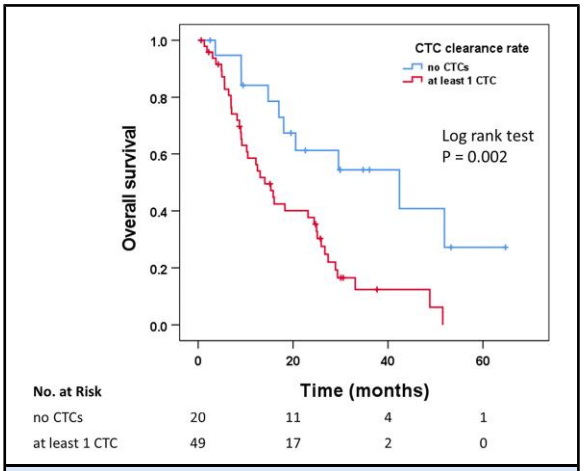

Fig. 3. Kaplan–Meier plot of OS according to CTC clearance at first follow-up CTC assessment.

the multivariable Cox regression analysis for patients with a first follow-up CTC assessment (n = 69) and included CTC clearance (yes or no) as an additional factor. Here, both treatment with lapatinib (HR 0.51; 95% CI, 0.28–0.95; $P = 0.034$) and CTC clearance (HR 0.37; 95% CI, 0.17–0.79; $P = 0.010$) were significantly and independently associated with improved OS.

PFS data were available for 84 patients. The median PFS follow-up time for patients without progression was 5.2 months. Progressive disease occurred in 32 (84%) of 38 patients in the standard arm and in 34 (74%) of 46 patients in the lapatinib arm. Patients in the lapatinib arm showed a numerically but not significantly better PFS than patients in the standard arm (lapatinib arm: median PFS 6.7 months, 95% CI, 4.7–8.8 months; standard arm: median PFS 4.1 months, 95% CI, 2.3–5.9 months; HR 0.71; 95% CI, 0.44–1.15; $P = 0.16$; Fig. 4B). The significant OS benefit for patients in the lapatinib arm was also evident when only the patients with valid PFS data were included (n = 84, HR 0.55; 95% CI, 0.33–0.91; $P = 0.019$).

Discussion

Changes in tumor biology due to natural and therapy-induced evolution, such as switches of HER2 status during the course of disease, have implications for the efficacy of targeted therapies. The analysis of CTCs, as a noninvasive liquid biopsy, may reveal information on such tumor biological changes. This raises the question of whether metastatic patients may benefit from therapy decisions based on the phenotype of CTCs if it differs from that of the primary tumor. The DETECT III trial is the largest randomized trial addressing this question by investigating the efficacy of lapatinib and standard

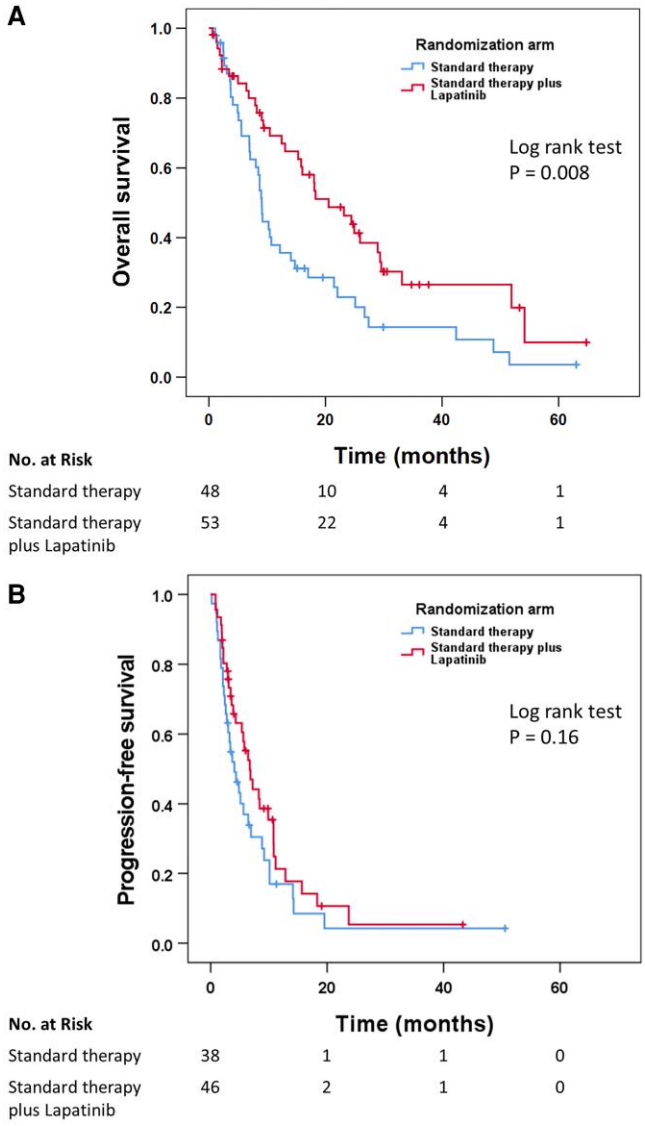

Fig. 4. Kaplan–Meier plot of OS (A) and PFS (B) according to randomization arm.

treatment in MBC patients with HER2-negative primary tumors but HER2-positive CTCs.

The primary endpoint of the study was the CTC clearance rate at the end of study treatment. A significant difference in CTC clearance rates was not observed between the 2 treatment arms at the time of first CTC assessment to evaluate early treatment response or at the end of study treatment. This result was independent from the CTCs' HER2-positivity levels. A potential trend to a lower CTC clearance rate at the end of treatment, as compared to the standard treatment not only for total CTCs but also for HER2 3+ CTCs and for CTCs showing any HER2 expression, which may be interpreted from the data, may result from inadequate selection of this time point for CTC measurement since due to progression of disease the CTC numbers may have increased. Similar observations were made by the randomized TREAT CTC study in the post(neo)adjuvant setting. Patients with HER2-negative tumors but persisting CTCs after (neo)adjuvant chemotherapy were randomized to trastuzumab vs observation. Since a preplanned interim analysis showed no significant CTC reduction, the trial was prematurely stopped by the recommendations of the independent data monitoring committee (25).

We observed a significant and independent OS benefit of additional lapatinib in MBC patients with

HER2-positive CTCs. The lack of a significant PFS benefit may be because the date of progression—in contrast to the date of death—was not available for all patients.

The results presented by the DETECT III study suggest a significant clinical benefit that considered the phenotype of CTCs in treatment decisions in MBC patients with HER2-negative primary tumors but HER2-positive CTCs.

Similar results concerning the clinical benefit of CTC-guided therapy have been reported by Wang et al. (26). A small subset (6 out of 15) of high-risk patients (≥2 HER2+ CTCs) received different HER2-targeted treatments and had a significantly better PFS than high-risk patients who did not receive these treatments. This PFS benefit was not observed in the low-risk cohort (<2 HER2+ CTCs).

A key question regarding our results is why we observed a survival benefit by adding lapatinib to standard treatment without demonstrating a significant difference in the CTC clearance rates. Several trials have clearly demonstrated that patients who were initially CTC positive and switched to CTC negative (<5 CTCs) have an improved OS compared to those who remained CTC positive (cutoff ≥ 5 CTCs) (5). In our trial, patients with early CTC clearance at the first follow-up blood evaluation also had a significantly better clinical outcome based on the multivariate analysis, while CTC clearance measured at a later time point showed no significant correlation with PFS and OS. Thus, the CTC clearance data were inconclusive and depended on the time point of blood analysis, which might be at least in part explained by the low concentration of CTCs and the rather low number of patients amenable to a second or third blood draw in our study. In future studies, the analysis of larger blood volumes might be required to measure numerical changes more reliably (27).

The key observation of our study was that patients with HER2-positive CTCs benefitted from additional therapy with lapatinib independent of the clearance of CTCs. Although one explanation for this result may be that it may not be attributed to lapatinib at all, it may also result from lapatinib targeting only a specific but very small subgroup of CTCs with high tumor-initiating capacity and stem cell features. Studies have shown that their elimination has a major impact on clinical outcome in MBC patients (28, 29). Ithimakin et al. (30) demonstrated that stem cells are regulated by HER2 not only in HER2-positive but also in HER2-negative breast cancer. This finding might explain our results wherein the addition of lapatinib had a significant benefit on survival independent of the extent of HER2 expression in CTCs—and may add up to the discussion here.

Other nonrandomized trials have also investigated the role of HER2-targeted therapies in patients with HER2-negative breast cancer but with HER2-positive CTCs, but the numbers of patients enrolled in these trials were much smaller. In the phase II "CirCe T-DM1" trial (31), 11 of 14 patients with HER2-amplified CTCs received trastuzumab-emtansine as HER2-targeted treatment. One patient achieved partial remission, while stable disease was observed in 4 patients. Agelaki et al. (32) investigated the efficacy of lapatinib as a maintenance treatment option in MBC patients with HER2-positive CTCs regardless of the HER2 status of the primary tumor and observed a decrease in HER2-positive CTCs in 76.2% of patients, all of whom had stable disease but no objective therapy response. A lack of effect of lapatinib monotherapy in patients with HER2-positive CTCs was shown by Pestrin et al. (33) and Stebbing et al. (34). In contrast, patients in our study did not receive lapatinib monotherapy based on current treatment recommendations for HER2-positive MBC.

The limitations of the DETECT III trial include the challenges associated with patient recruitment, which are also seen in many other CTC trials and should be considered in future CTC studies evaluating CTC clearance after study treatment as an endpoint. A major limitation is that data regarding the primary endpoint CTC clearance after study treatment were available only for 47 patients of the 101 patients, considerably reducing the statistical power for the analysis of this endpoint. The main reason for this apparent noncompliance is that the study treatment had to be discontinued prematurely in 86 patients due to disease progression, death, toxicity, or patient wish and that the adherence of blood sampling at this time point was impaired by the patients' advanced disease stages.

Further limitations are that it is not unlikely that subsequent treatment lines affect OS since the frequency of endocrine therapy in subsequent treatment lines may be imbalanced between the 2 arms. Although most of the patients recruited in the trial were chemotherapy-naïve, a difference already exists between the 2 arms regarding the percentage of endocrine therapy as standard therapy within the trial; therefore, it is likely that this could also happen in subsequent lines of treatment.

Another limitation is a possible discordance of HER2 status between primary tumor and metastatic lesions in cases without re-evaluation/confirmation of HER2 status on metastatic lesions. Since the discordance rates are not available for 18 and 24 patients in the lapatinib and standard arm, respectively, a difference in discordance rates between the 2 arms may be likely and would considerably impact the outcome of patients, especially of those with HER2-positive metastatic disease who did not receive a HER2-targeted therapy. However, this issue rather reflects the strength of a CTC-based therapy selection in cases where information of the tumor or metastasis is missing, as the HER2 status of CTCs measured in real time before the start of a new treatment line might better represent

current tumor biology and its evolution over the course of previous treatment lines than the HER2 status determined from a biopsy of a single metastatic lesion.

A major consequence of these limitations is that the final patient cohort represents a highly selected population, in which a bias cannot be excluded. We acknowledge that some parameters with a potential impact on the patients' survival are not well balanced between standard and experimental arms (as even a randomized permuted block design can produce imbalances between treatment arms by chance, in particular if sample sizes are as low as in this study), suggesting that the lapatinib group might comprise patients with on average better survival prospects. We also acknowledge that the OS benefit shown in the lapatinib arm is only an observation that requires further investigation. The OS benefit could be related to lapatinib; however, due to the considerable limitations of the current trial, the results presented here can only provide the basis for future randomized clinical trials using OS as a primary endpoint.

Despite the limitations described in detail here, our results indicate that CTC-guided therapy may be useful to optimize treatment strategies and should be further investigated in randomized clinical trials, including newly developed HER2-targeted therapies (e.g., tucatinib and trastuzumab deruxtecan), to optimize the treatment of metastatic patients with HER2-positive CTCs.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: CTC, circulating tumor cell; MBC, metastatic breast cancer; HR, hazard ratio; PFS, progression-free survival; OS, overall survival; IF, immuno fluorescence; CI, confidence interval.

Human Genes: HER2: *ERBB2*, erb-b2 receptor tyrosine kinase 2.

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Data Sharing Statement: Deidentified data will be made available to other researchers, subject to approval of a formal data access request that includes a detailed description of the purpose/scientific rationale of the proposed project. Requests are reviewed by the DETECT III study group Steering Committee and will be approved if the proposed projects have a sound scientific or patient benefit rationale. Data recipients are required to sign a formal data sharing agreement that describes the conditions for release and requirements for data transfer, storage, archiving, and publication and intellectual property. Data will be available beginning 9 months and ending 5 years following article publication. Additional documents (e.g., full study protocol and the informed consent form) are available from the clinical trial website of the DETECT III study group at https://www.detect-studien.de/detect3.html.

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