ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN CANCER HORIZONS

ORIGINAL RESEARCH

Prognostic relevance of the HER2 status of circulating tumor cells in metastatic breast cancer patients screened for participation in the DETECT study program☆

V. Müller[1*†], M. Banys-Paluchowski[2,3†], T. W. P. Friedl[4], P. A. Fasching[5], A. Schneeweiss[6], A. Hartkopf[7], D. Wallwiener[7], B. Rack[4], F. Meier-Stiegen[8], J. Huober[4], M. Rübner[5], O. Hoffmann[9], L. Müller[10], W. Janni[4], P. Wimberger[11,12], B. Jäger[8], K. Pantel[13], S. Riethdorf[13], N. Harbeck[14] & T. Fehm[8], on behalf of DETECT study group

[1]Department of Gynecology, University Medical Centre Hamburg-Eppendorf, Hamburg; [2]Gynecology and Obstetrics Department, University of Schleswig-Holstein, Campus Lübeck, Lübeck; [3]Medical Faculty, Heinrich-Heine-University of Düsseldorf, Düsseldorf; [4]Department of Gynecology and Obstetrics, University Hospital Ulm, Ulm; [5]Department of Gynecology and Obstetrics, University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen; [6]Division Gynecologic Oncology, National Center for Tumor Diseases (NCT), Heidelberg University Hospital and German Cancer Research Center, Heidelberg; [7]Department of Gynecology and Obstetrics, University Hospital Tübingen, Tübingen; [8]Department of Gynecology and Obstetrics, University Hospital Düsseldorf, Düsseldorf; [9]Department of Gynecology and Obstetrics, University Hospital Essen, Essen; [10]OnkologieUnterEms, Leer; [11]Department of Gynecology and Obstetrics, University Hospital Carl Gustav Carus Dresden, TU Dresden; [12]National Center for Tumor Diseases (NCT), Dresden; [13]Institute of Tumor Biology, University Medical Centre Hamburg-Eppendorf, Hamburg; [14]Breast Center, Department of Gynecology and Obstetrics and CCC Munich, LMU University Hospital, Munich, Germany

Available online xxx

Background: Circulating tumor cells (CTCs) have been reported to predict clinical outcome in metastatic breast cancer (MBC). Biology of CTCs may differ from that of the primary tumor and HER2-positive CTCs are found in some patients with HER2-negative tumors.

Patients and methods: Patients with HER2-negative MBC were screened for participation in DETECT III and IV trials before the initiation of a new line of therapy. Blood samples were analyzed using CELLSEARCH. CTCs were labeled with an anti-HER2 antibody and classified according to staining intensity (negative, weak, moderate, or strong staining).

Results: Screening blood samples were analyzed in 1933 patients with HER2-negative MBC. As many as 1217 out of the 1933 screened patients (63.0%) had $\geq$1 CTC per 7.5 ml blood; $\geq$5 CTCs were detected in 735 patients (38.0%; range 1-35 078 CTCs, median 8 CTCs). HER2 status of CTCs was assessed in 1159 CTC-positive patients; $\geq$1 CTC with strong HER2 staining was found in 174 (15.0%) patients. The proportion of CTCs with strong HER2 staining among all CTCs of an individual patient ranged between 0.06% and 100% (mean 15.8%). Patients with estrogen receptor (ER)- and progesterone receptor (PR)-positive tumors were more likely to harbor $\geq$1 CTC with strong HER2 staining. CTC status was significantly associated with overall survival (OS). Detection of $\geq$1 CTC with strong HER2 staining was associated with shorter OS [9.7 (7.1-12.3) versus 16.5 (14.9-18.1) months in patients with CTCs with negative-to-moderate HER2 staining only, $P = 0.013$]. In multivariate analysis, age, ER status, PR status, Eastern Cooperative Oncology Group performance status, therapy line, and CTC status independently predicted OS.

Conclusion: CTC detection in patients with HER2-negative disease is a strong prognostic factor. Presence of $\geq$1 CTC with strong HER2 staining was associated with shorter OS, supporting a biological role of HER2 expression on CTCs.

Key words: breast cancer, circulating tumor cell, liquid biopsy, HER2 status, survival

INTRODUCTION

Circulating tumor cells (CTCs) are considered a strong independent prognostic factor in metastatic breast cancer (MBC).[1] Despite the high prognostic relevance of CTC counts demonstrated in numerous studies, their clinical utility in guiding therapy decisions remains unclear.[2]

Beyond enumeration of CTCs, CTC phenotype has been proposed as another important predictor for clinical

*Correspondence to: Prof. Dr Volkmar Müller, University Medical Centre Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany
E-mail: v.mueller@uke.de (V. Müller).

†These authors contributed equally to this work.

☆Note: Parts of this work have been presented at the *San Antonio Breast Cancer Symposium* 2020 (PS2-02).

outcome and treatment responsiveness. In this context, several studies showed a discordance in the expression of predictive markers such as the HER2 status between primary tumor, metastatic tissue, and CTCs.[3-6] Although current guidelines recommend re-evaluation of the receptor status using metastatic tissue,[7,8] treatment decisions in the metastatic setting are often based on the receptor status of the primary tumor. Another aspect that needs to be considered is the well-described heterogeneity between various metastatic sites and the ability to acquire or loose targetable features during disease progression.[9] Which of these cell populations should be taken into account when choosing systemic therapy for an MBC patient remains to be clarified.

The DETECT trials, a large study program on CTC-based therapy interventions, were initiated to address these issues. Blood samples from MBC patients with HER2-negative primary tumor and/or metastasis, who are potential candidates for the DETECT III or IV trials, are screened for the presence of CTCs.[10] Depending on the presence of CTCs and their HER2 status, patients can be enrolled in different randomized and nonrandomized studies within the DETECT program. This analysis aimed at evaluating the HER2 status of CTCs in nearly 2000 patients screened for study participation and at examining its clinical significance.

MATERIAL AND METHODS

The DETECT study concept has been previously described in detail.[10] Briefly, this prospective, multicenter, open-label clinical trial program consists of two phase III studies (DETECT III, NCT01619111 and DETECT V/CHEVENDO, NCT02344472) and one phase II study (DETECT IV, NCT02035813; Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2021.100299). Patients with MBC were screened for presence of CTCs in peripheral blood before the start of first- or later-line therapy in one of the participating study sites (>100 in Germany). The trials were conducted in accordance with the Declaration of Helsinki and approved by the responsible ethical committees (DETECT III: 525/2011AMG1; DETECT IV: MC-LKP-668; DETECT V: 113/15) and all local ethical committees. In this analysis, only patients with HER2-negative MBC screened for participation in DETECT III and IV were included. Survival results of the interventional part of this cohort, that is, patients receiving HER2-targeted treatment with lapatinib, were excluded from this analysis and will be reported elsewhere. First-line therapy was defined as therapy initiated within 6 weeks after the first diagnosis of metastatic disease.

Detection of CTCs and evaluation of their HER2 status

Peripheral blood samples (7.5 ml) were collected into CellSave tubes and examined using the standardized semiautomatic CELLSEARCH assay (Menarini Silicon Biosystems; Bologna, Italy). This Food and Drug Administration-approved method is based on immunomagnetic enrichment for selection of CTCs by ferrofluid nanoparticles bound to anti-EpCAM (epithelial cell adhesion molecule) antibodies. After enrichment, cells were stained with fluorescent anti-cytokeratin (epithelial cells) and anti-CD45 antibodies (leukocyte marker) and then with 4′,6-diamidino-2-phenylindole (DAPI) to indicate the presence of a nucleus.[11] Enriched and stained CTCs were placed into the CELLTRACKS ANALYZER for imaging and final check ($CD45^-$, cytokeratins 8, 18, and/or 19+ and DAPI+). Patients with at least one CTC were considered CTC positive. Assessment of the HER2 status of CTCs was conducted using an anti-HER2 antibody.[12] *In situ* hybridization was not used. HER2 expression was categorized as negative [immunohistochemistry score (IHC) 0], weak (IHC 1+), moderate (IHC 2+), or strong (IHC 3+) according to Riethdorf et al.[12] based on the comparison of HER2-specific immunofluorescence of cell lines with known expression of HER2 (Figure 1). All experiments were performed at four reference laboratories with trained scientists and peer-reviewed systems in an established laboratory network.[13] In each laboratory, two independent readers evaluated each sample regarding the CTC status and HER2 expression of these CTCs. HER2 expression of CTCs had to be confirmed by all analyzing laboratories using an online evaluation tool. The status of predictive markers, such as estrogen, progesterone, and HER2, assessed in primary tumor and/or metastatic tissue was documented for each patient. In most patients the receptor analysis was available from primary tumor tissue only.

Statistical analysis

Chi-square test and Fisher's exact test were used to evaluate the relationship between CTC detection and HER2 status and clinical–pathological factors. *P*-values <0.05 were considered statistically significant. Data cut-off for this analysis was 16 September 2020. Survival data were analyzed using log-rank tests, univariable, and adjusted multivariable cox regressions.

RESULTS

Screening blood samples were analyzed in 1933 patients with HER2-negative MBC with HER2 status determined in tumor tissue. The HER2 status of the primary tumor was available in 1660 patients and of metastatic tissue in 1061 patients. Median age was 62 years (range 25-89 years). Of these, 102 were enrolled in the DETECT III and 213 in the DETECT IVa/b trials, respectively (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2021.100299).

Detection of circulating tumor cells

A total of 1217 out of the 1933 screened patients (63.0%) had ≥1 CTC per 7.5 ml blood; ≥5 CTCs were detected in 735 patients (38.0%; range 1-35 078 CTCs, median 8 CTCs, mean 1489). CTC positivity was associated with estrogen receptor (ER)-positive tumors and worse Eastern Cooperative Oncology Group (ECOG) status (Table 1).

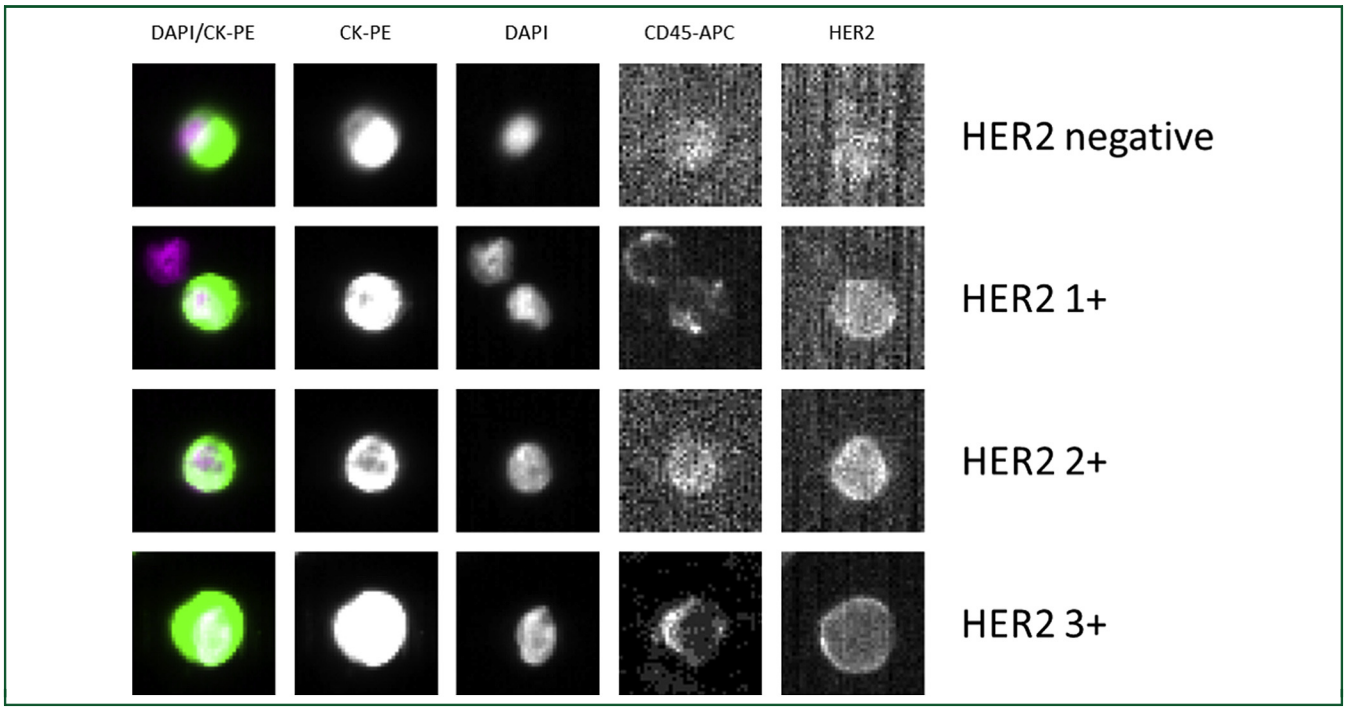

Figure 1. HER2 status of circulating tumor cells analyzed by immunocytochemistry.
APC, allophycocyanin; CK, cytokeratin; DAPI, 4′,6-diamidino-2-phenylindole; PE, phycoerythrin.

HER2 status of the circulating tumor cells

HER2 status of CTCs was assessed in 1159 CTC-positive patients. Altogether, 179 205 CTCs were analyzed with respect to their HER2 status; 2% showed strong, 6% intermediate, and 15% weak staining intensity, whereas 77% were HER2 negative. At least one CTC with strong HER2 staining was found in 174 (15.0%) patients, and 408 patients (35.2%) had at least one CTC with moderate or strong staining (Table 2, Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2021.100299). The proportion of CTCs with strong HER2 staining among all CTCs of an individual patient ranged between 0.06% and 100% (mean 15.8%). Patients with positive hormone receptor status were more likely to present with HER2-positive CTCs (Table 2). Most patients with strong CTC-HER2 staining also had CTCs with negative to moderate staining, possibly reflecting tumor heterogeneity. Patients with higher CTC counts were more likely to have at least one CTC with strong HER2 staining (1-10 CTCs: 5.0%; 11-50 CTCs: 20.4%; 51-100 CTCs: 25.7%; and >100 CTCs: 42.9%, $P < 0.001$).

Table 1. CTC status and patients' characteristics (significant *P* values are shown in bold)

Characteristics	*N*	≥1 *n* (%)	*P* value	≥5 CTCs, *n* (%)	*P* value
Total	1933	1217 (63.0)		735 (38.0)	
Age, years			0.556		0.824
≥50	1588	995 (62.7)		602 (37.9)	
<50	345	222 (64.3)		133 (38.6)	
Hormone receptor status[a]			**0.010**		**<0.001**
ER and/or PR positive	1556	1005 (64.6)		634 (40.7)	
Triple negative[b]	266	150 (56.4)		74 (27.8)	
ER status[a]			**0.011**		**<0.001**
Positive	1510	977 (64.7)		616 (40.8)	
Negative	312	178 (57.1)		92 (29.5)	
PR status[a]			0.777		0.218
Positive	1313	834 (63.5)		522 (39.8)	
Negative	500	314 (62.8)		183 (36.6)	
Menopausal status			0.388		0.950
Premenopausal	263	163 (62.0)		102 (38.8)	
Postmenopausal	1444	935 (64.8)		563 (39.0)	
ECOG			**0.020**		**<0.001**
0	929	587 (63.2)		337 (36.3)	
1-3[c]	581	401 (69.0)		274 (47.2)	
Therapy line after blood sampling			0.698		0.182
First line	603	383 (63.5)		242 (40.1)	
Further line	1318	825 (62.6)		487 (36.9)	

CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; PR, progesterone receptor.
[a] Assessed in the primary tumor.
[b] Defined as ER and PR negative.
[c] Including 73 patients with ECOG 2-3.

Survival analysis

In survival analysis, we focused on patients with HER2-negative tumors who did not receive anti-HER2 therapy to evaluate the prognostic impact of HER2-positive CTCs in HER2-negative MBC. Therefore, 52 patients receiving experimental HER2-targeted therapy with lapatinib in the interventional arm of the DETECT III trial were excluded from this survival analysis.

Median follow-up was 38 months; follow-up data were available for 1435 patients with 873 deaths. The following causes of death were reported: due to disease progression ($n = 695$), accident ($n = 1$), suicide ($n = 1$), not related ($n = 7$), other ($n = 2$), and unknown ($n = 185$). Progression of disease was reported in 865 patients (local progression in 113 cases and/or distant progression in 836 patients).

Table 2. Patients' characteristics and correlation with HER2 status of the CTCs

Characteristics	N	≥1 CTC with strong staining, n (%)	P value	≥1 CTC with moderate or strong staining, n (%)	P value
Total	1159	174 (15.0)		408 (35.2)	
Age, years			0.363		0.271
≥50	954	139 (14.6)		329 (34.5)	
<50	205	35 (17.1)		79 (38.5)	
Hormone receptor status[a]			**0.001**		**<0.001**
ER and/or PR positive	964	161 (16.7)		369 (38.3)	
Triple-negative[b]	137	8 (5.8)		27 (19.7)	
ER status[a]			**0.011**		**0.001**
Positive	940	155 (16.5)		356 (37.9)	
Negative	161	14 (8.7)		40 (24.8)	
PR status[a]			**0.002**		**<0.001**
Positive	798	138 (17.3)		318 (39.8)	
Negative	296	29 (9.8)		76 (25.7)	
Menopausal status			0.506		0.210
Premenopausal	152	26 (17.1)		62 (40.8)	
Postmenopausal	893	134 (15.0)		317 (35.5)	
ECOG			0.574		0.117
0	551	88 (16.0)		188 (34.1)	
1-3[c]	386	67 (17.4)		151 (39.1)	
Therapy line			0.988		0.443
First line	371	56 (15.1)		137 (36.9)	
Further line	780	118 (15.1)		270 (34.6)	

CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; PR, progesterone receptor.
[a] Assessed in the primary tumor.
[b] Defined as ER and PR negative.
[c] Including 60 patients with ECOG 2-3.

Overall survival

CTC status was significantly associated with overall survival (OS), independently of the cut-off value (1 CTC or 5 CTCs) used (Figure 2A). Median OS in patients with ≥1 CTC was 15.5 months [95% confidence interval (CI) 14.2-16.8 months] compared with 37.2 months without CTCs (95% CI 32.7-41.7 months) and 12.0 months with ≥5 CTCs (95% CI 10.0-14.0 months) compared with 28.6 months with <5 CTCs (95% CI 25.5-31.6 months). Patients with ≥1 CTC with strong HER2 staining had shorter OS than those with CTCs with negative-to-moderate staining only [median OS 9.7 (95% CI 7.1-12.3) versus 16.5 (14.9-18.1) months, respectively, $P = 0.013$, hazard ratio 1.360; Figure 3A]. When a different cut-off for HER2 positivity of CTCs was considered (i.e. presence of ≥1 CTC with moderate or strong HER2 staining), OS was not significantly different between the two groups [median OS in patients with ≥1 CTC with moderate or strong HER2 staining: 14.9 (95% CI 10.6-19.2) versus 15.7 (14.4-17.1) months in patients with CTCs with HER2 negative/weak staining only, respectively, $P = 0.917$, Supplementary Figure S4A, available at https://doi.org/10.1016/j.esmoop.2021.100299]. In multivariate analysis, only age, ER status, progesterone receptor (PR) status, ECOG performance status, therapy line, and CTC counts but not the HER2 status of CTCs predicted OS (Table 3).

Progression-free survival

CTC status significantly predicted shorter progression-free survival (PFS; Figure 2B). Median PFS in patients with ≥1 CTC was 7.1 months (95% CI 6.1-8.2 months) compared with 8.8 months without CTCs (95% CI 7.7-9.9 months) and 6.0 months in those with ≥5 CTCs (95% CI 4.8-7.3 months) compared with 8.5 months in patients with <5 CTCs (95% CI 7.6-9.5 months). No significant association was found between the HER2 status of CTCs defined as presence of ≥1 CTC with strong HER2 staining and PFS in univariate analysis ($P = 0.498$, hazard ratio 0.877; Figure 3B). Patients with ≥1 CTC with moderate or strong HER2 staining had similar PFS as patients with CTCs with HER2-negative/weak staining only [median PFS: 6.9 months (95% CI 5.2-8.5 months) versus 7.1 (95% CI 5.7-8.4 months), $P = 0.240$; Supplementary Figure S4B, available at https://doi.org/10.1016/j.esmoop.2021.100299]. In multivariate analysis, only therapy line and hormone receptor status, but not other factors significantly predicted PFS (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2021.100299).

DISCUSSION

In this large multicenter analysis with nearly 2000 patients, we observed a prognostic impact of CTC detection in patients with HER2-negative disease. To our knowledge, this is the largest patient cohort in this context.

CTCs are accepted as a strong prognostic factor in MBC. Recently, a large, pooled analysis confirmed that high CTC counts, defined as the presence of at least five CTCs per 7.5 ml peripheral blood, significantly predict poor clinical outcome.[1] Our study confirms the feasibility of CTC determination in a multicenter setting. Despite a prognostic value shown in numerous studies, the clinical utility of CTC characterization is not yet well defined.[2] While CTC dynamics during systemic therapy for MBC reflect clinical response to treatment, switching to an alternative chemotherapy regimen in case of persisting high CTC levels did not improve outcome in the randomized SWOG 0500 trial.[14] To date, STIC CTC remains the only study showing that CTC counts can guide treatment decisions in MBC (i.e. the choice between first-line chemotherapy and endocrine therapy), but its clinical relevance is somewhat limited due to introduction of CDK4/6 inhibitors which were not used in either arm of the trial.[15]

Beyond simple enumeration of CTCs, characterization of detected cells may provide insights into targetable features of metastatic disease. In clinical practice, while current guidelines recommend re-evaluating the receptor status before starting a new line of therapy,[7,8] conducting a biopsy of a metastatic site is omitted in many patients due to invasiveness of the procedure and concerns for patient's well-being, as well as technical and logistical challenges. In such cases, systemic treatment is usually based on phenotypic features of the primary tumor tissue, frequently

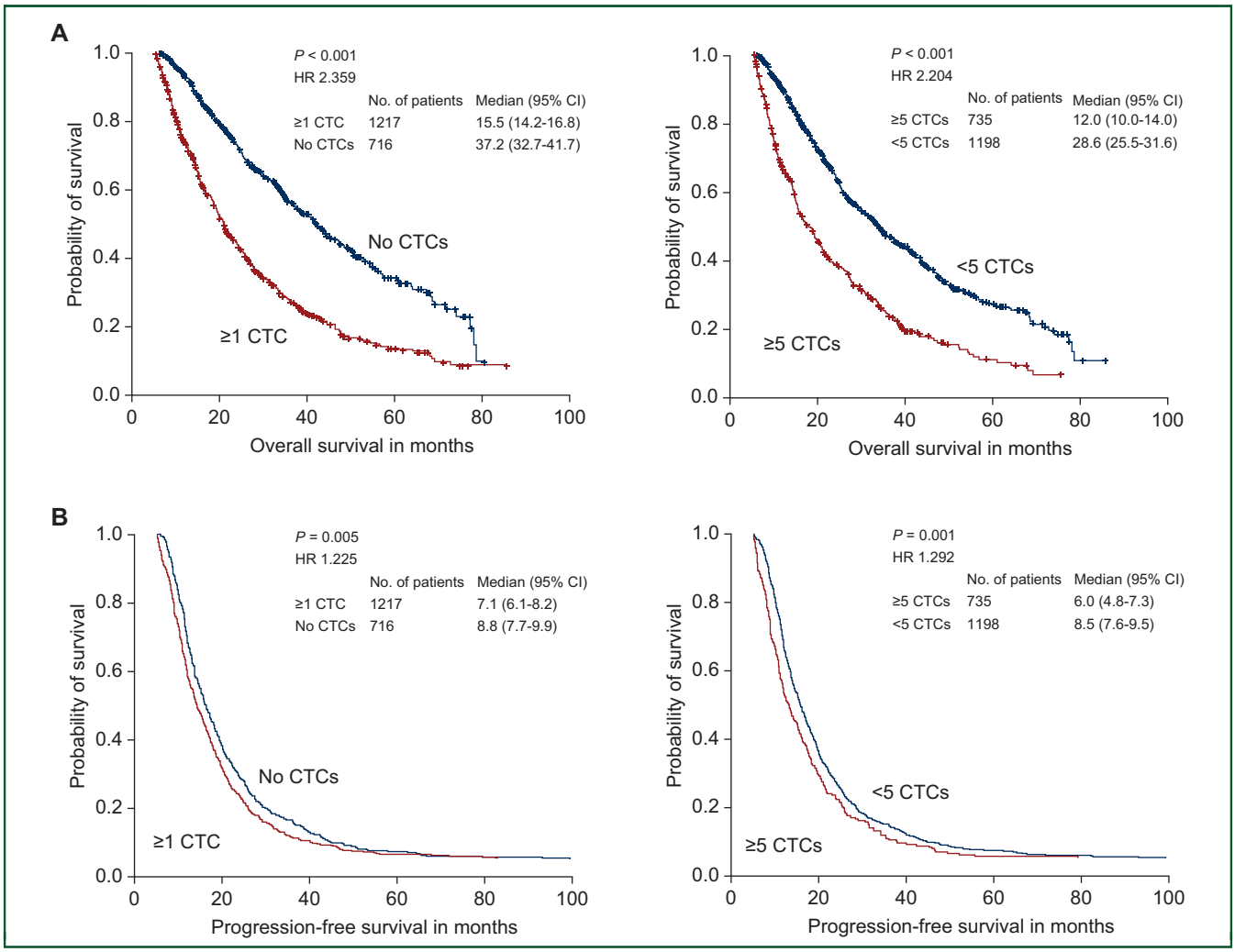

Figure 2. **Kaplan–Meier plot of (A) overall survival and (B) progression-free survival stratified by circulating tumor cell (CTC) counts.**

obtained several years earlier. However, multiple studies have shown that expression profiles may differ between primary tumor, metastatic tissue, and CTCs, and the reasons for this discordance are assumed to relate to tumor heterogeneity, change in tumor biology over time, and effects of prior treatment on clonal subsets.[3,5,16-20] According to a

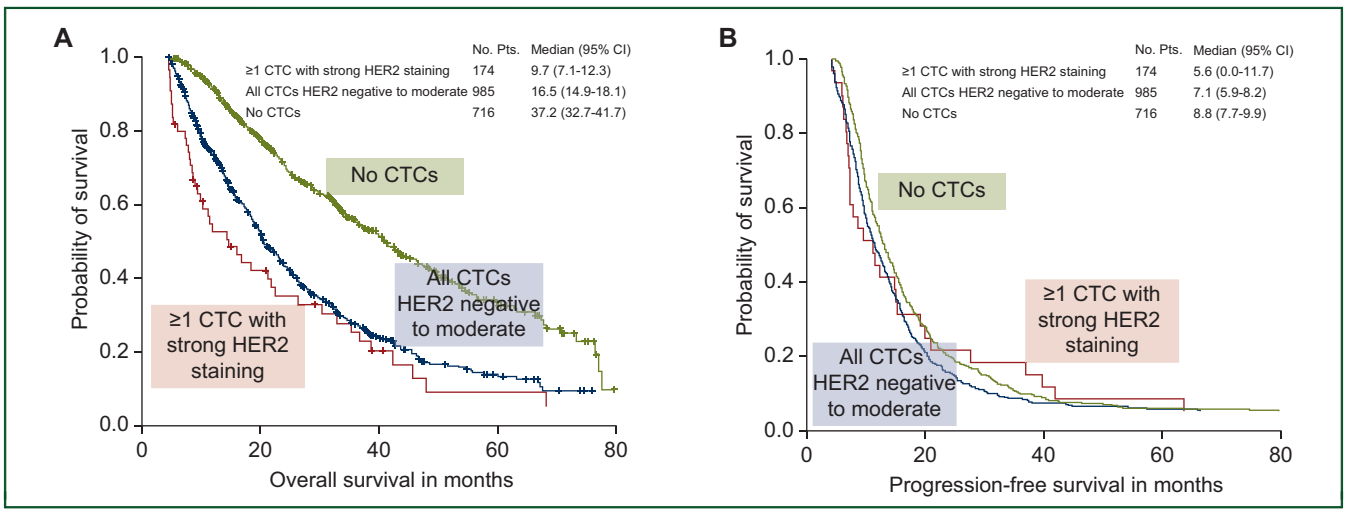

Figure 3. **Kaplan–Meier plot of (A) overall survival and (B) progression-free survival stratified by HER2 status of circulating tumor cells (CTCs); CTC-HER2-positive status defined as the presence of ≥1 CTC with strong HER2 staining.**

Table 3. Multivariate analysis of overall survival

Variables	*P* value	Odds ratio (95% CI)
Therapy line	<0.001	0.610 (0.496-0.750)
Age	0.031	0.770 (0.607-0.976)
CTC counts (≥5 versus <5)	<0.001	1.478 (1.203-1.815)
HER2 status of CTCs	0.275	1.166 (0.885-1.535)
Hormone receptor status[a]	<0.001	0.401 (0.310-0.518)
ECOG	<0.001	1.476 (1.223-1.781)

CI, confidence interval; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group.
[a] Positive status defined as ER and/or PR positive.

recent meta-analysis, up to 9.5% of patients with HER2-negative primary tumor acquire a positive HER2 status in metastatic tissue and, conversely, 21% of patients initially HER2 positive become HER2 negative in the metastatic setting.[5]

We have evaluated the methods for HER2 determination used here in a multicenter setting of an independent pilot study.[13] In our cohort, the HER2 status of CTCs was significantly associated with OS. These results confirm data previously reported by Wang et al.[21] In this study, the HER2 status of CTCs from 105 advanced-stage breast cancer patients with HER2-negative tumors was analyzed. In contrast to our study, the optimal cut-off value for HER2-positive CTCs was two cells, that is, patients with ≥2 HER2-positive CTCs had a shorter survival time and an increased risk for disease progression, compared with those with ≤1 HER2-positive CTCs (hazard ratio 2.16, 95% CI 1.20-3.88, $P = 0.010$).

In our study, the impact of HER2-positive CTCs on prognosis was observed even though the proportion of HER2-positive CTCs among all CTCs of an individual patient ranged widely (0.06%-100%, mean 15.8%) and in the majority of patients most CTCs had negative-to-weak HER2 staining. Further, we did not observe an association between the proportion of CTCs with positive HER2 status among all CTCs and clinical outcome (data not shown). Although HER2 expression was not prognostic in multivariate analysis, our findings support a biologic impact of HER2 expression on CTCs. As only a proportion of HER2-positive tumor cells are sufficient to define a primary tumor or a metastatic site as HER2 positive,[22] it seems possible that these cells have distinct biological behavior and their detection in the circulation reflects this. This hypothesis is supported by the findings of Jordan et al.[23] indicating that HER2 status of CTCs may change, and this might contribute to progression of breast cancer and acquisition of drug resistance.

One major question is the potential targetability of HER2-positive status on CTCs in patients in whom the primary tumor and/or metastatic tissue were HER2 negative. In the survival analysis, we did not include patients receiving HER2-directed treatment based on the detection of HER2-postive CTCs because this CTC-guided intervention might have altered the clinical course of disease and improved patient's outcome. These patients were treated within our pivotal DETECT III trial that examined the addition of lapatinib to standard treatment in this cohort. Preliminary results indicate that patients may benefit from HER2-targeted therapy in this setting.[24] The final results are still awaited. A possible clinical benefit from HER2-targeted therapies in patients with HER2-negative tumors but HER2-positive CTCs was also reported by Wang et al.[21] By contrast, Pestrin et al.[25] reported disappointing results from their proof-of-concept phase II trial on lapatinib in HER2-negative MBC with HER2-positive CTCs. In this study, patients were defined as CTC-HER2 positive if ≥50% of CTCs were HER2 positive. Altogether, seven patients with HER2-positive CTC status received experimental anti-HER2 treatment. None achieved objective response and only one remained stable for 8.5 months. Another trial investigating this issue was the Circe-T-DM1 trial.[26] In this single-arm study, patients with HER2-negative tumors but HER2-amplified CTCs received the anti-HER2 antibody—drug-conjugate T-DM1. A total of 155 patients who were partially heavily pretreated were screened; in 9.2%, at least one HER2-positive CTC was detected, and 11 patients were treated with T-DM1. Among these, only one achieved partial remission. Therefore, the authors concluded that the therapeutic approach is not promising. The reason for this disappointing result might be the very low prevalence of HER2-amplified CTCs among all detected CTCs (median 1.6%). In contrast to our study, analysis of the HER2 status was based on *in situ* hybridization and not on immunocytochemistry.

In the past decade, evaluation of targetable features using circulating tumor DNA (ctDNA) has become widely available due to development and implementation of validated genomic profiling. Recently, Guan et al.[27] reported on the feasibility of serial blood-based monitoring of HER2 copy numbers in ctDNA. In 15% of patients, the HER2 status assessed in the ctDNA and tissue was discordant. While high-level amplification in HER2 copy numbers of ctDNA predicted better response to anti-HER2 therapy in patients that were histologically HER2 positive, data on HER2 copy numbers in HER2-negative patients have not been reported in detail. By contrast, HER2 copy number increases in patients with HER2-negative primary tumor and/or metastasis were rare in the plasmaMATCH study.[28] However, a small subset (1.7%) of patients with HR-positive HER2-negative and triple-negative disease, assessed in prior tissue, had HER2 amplification detected in ctDNA, probably reflecting acquisition of HER2 amplification. Whether HER2 status assessed in ctDNA rather than on CTCs can predict clinical benefit from anti-HER2 treatment in patients with histologically HER2-negative disease remains unclear.

Conclusions

We could show in our analysis that the presence of CTCs with strong HER2 staining has a significant impact on prognosis and might potentially be a useful tool to guide therapy in metastatic disease. While the utility of phenotyping CTCs is obviously limited to patients with detectable CTCs, the described approach offers the possibility to assess

the HER2 status in tumor cells other than those of the primary tumor and metastatic site(s). Therefore patients in whom an invasive tissue biopsy to reassess predictive features of the disease is not possible might benefit from a noninvasive blood sampling. In case a targetable marker, such as the HER2 receptor, is detected using liquid biopsy, this information might potentially guide therapy decisions. Therefore, study concepts evaluating clinical outcomes of patients whose treatment is based on a result of blood examination, either using CTCs or ctDNA, are becoming a major focus of oncological research.[24,29,30] In this context, our results should contribute to designing future clinical trials.

FUNDING

This work was supported by GlaxoSmithKline (now Novartis), Amgen, Eisai, Pierre Fabre, Teva, and Menarini Silicon Biosystems without any influence on design and analysis of the study. This research project was financially supported by the German Cancer Aid, Priority Program 'Translational Oncology' grant number 70112504.

The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

DISCLOSURE

VM received speaker honoraria from Amgen, Astra Zeneca, Daiichi-Sankyo, Eisai, GSK, Pfizer, MSD, Novartis, Roche, Teva, and Seagen; consultancy honoraria from Genomic Health, Hexal, Roche, Pierre Fabre, Amgen, Clin Sol, Novartis, MSD, Daiichi-Sankyo, Eisai, Lilly, and Seagen; institutional research support from Novartis, Roche, Seagen, and Genentech; travel grants from Roche, Pfizer, and Daiichi Sankyo. MBP received honoraria for lectures and advisory role from Novartis, Roche, Pfizer, Eli Lilly, Eisai, Daiichi Sankyo, GSK, AstraZeneca, pfm, and Amgen; and study support from Endomag, Mammotome and Merit Medical. PW has received research funding from Amgen, AstraZeneca, MSD, GlaxoSmithKline, Novartis, Pfizer, PharmaMar, Roche Pharma, Clovis, and Lilly; honoraria from Amgen, AstraZeneca, MSD, GlaxoSmithKline, Novartis, Pfizer, Roche Pharma, Clovis, TEVA, Eisai, and Lilly; serves on the advisory board of Amgen, AstraZeneca, MSD, GlaxoSmithKline, Novartis, Pfizer, Roche Pharma, Clovis, TEVA, Eisai, and Lilly. NH reports honoraria for consulting and/or lectures from Astra Zeneca, Daiichi-Sankyo, Exact Sciences, Lilly, MSD, Novartis, Pierre Fabre, Pfizer, Roche, Sandoz, and Seagen. JH received research funding from Celgene, Novartis, and Hexal; honoraria from Lilly, Novartis, Roche, Pfizer, AstraZeneca, MSD, Celgene, Eisai, and AbbVie; honoraria for consulting/advisory relationship from Lilly, Novartis, Roche, Pfizer, Hexal, AstraZeneca, MSD, Celgene, and AbbVie; and travel expenses from Roche, Pfizer, Novartis, Celgene, and Daiichi. TF reports honoraria from Novartis, unrelated to this work. OH received honoraria for advisory boards, lectures, and congress and travel expense support from Roche, Novartis, MSD, Pfizer, Astra Zeneca, Daiichi Sankyo, Riemser Pharma, Celgene, and Seagen. FMS reports consulting fees from Amgen. TF received consulting fees (e.g. advisory boards) from Amgen, Daiichi Sankyo, Roche, AstraZeneca, MSD Oncology, Novartis, Pierre Fabre, and Pfizer Teva, and fees for non-CME services received directly from commercial interest or their agents (e.g. speakers' bureaus): Daiichi Sankyo, Roche, and Novartis. All other authors have declared no conflicts of interest.

REFERENCES

1. Cristofanilli M, Pierga JY, Reuben J, et al. The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): international expert consensus paper. *Crit Rev Oncol Hematol*. 2019;134:39-45.
2. Banys-Paluchowski M, Reinhard F, Fehm T. Circulating tumor cells in metastatic breast cancer: clinical applications and future possibilities. *Appl Sci*. 2020;10(9):3311.
3. Santinelli A, Pisa E, Stramazzotti D, Fabris G. HER-2 status discrepancy between primary breast cancer and metastatic sites. Impact on target therapy. *Int J Cancer*. 2008;122(5):999-1004.
4. Ligthart ST, Bidard FC, Decraene C, et al. Unbiased quantitative assessment of Her-2 expression of circulating tumor cells in patients with metastatic and non-metastatic breast cancer. *Ann Oncol*. 2013;24(5):1231-1238.
5. Schrijver W, Suijkerbuijk KPM, van Gils CH, van der Wall E, Moelans CB, van Diest PJ. Receptor conversion in distant breast cancer metastases: a systematic review and meta-analysis. *J Natl Cancer Inst*. 2018;110(6):568-580.
6. Krawczyk N, Banys M, Neubauer H, et al. HER2 status on persistent disseminated tumor cells after adjuvant therapy may differ from initial HER2 status on primary tumor. *Anticancer Res*. 2009;29(10):4019-4024.
7. Recommendations of the AGO Breast Committee. Diagnosis and Treatment of Patients with early and advanced Breast Cancer. 2021. Available at www.ago-online.de. Accessed October 29, 2021.
8. NCCN Clinical Practice Guidelines in Oncology, Breast Cancer, Version 2.2021. 2021. Available at NCCN.org. Accessed October 29, 2021.
9. Meng S, Tripathy D, Shete S, et al. HER-2 gene amplification can be acquired as breast cancer progresses. *Proc Natl Acad Sci U S A*. 2004;101(25):9393-9398.
10. Schramm A, Friedl TW, Schochter F, et al. Therapeutic intervention based on circulating tumor cell phenotype in metastatic breast cancer: concept of the DETECT study program. *Arch Gynecol Obstet*. 2016;293(2):271-281.
11. Riethdorf S, Fritsche H, Müller V, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CELLSEARCH system. *Clin Cancer Res*. 2007;13(3):920-928.
12. Riethdorf S, Muller V, Zhang L, et al. Detection and HER2 expression of circulating tumor cells: prospective monitoring in breast cancer patients treated in the neoadjuvant GeparQuattro trial. *Clin Cancer Res*. 2010;16(9):2634-2645.
13. Muller V, Riethdorf S, Rack B, et al. Prognostic impact of circulating tumor cells assessed with the CellSearch System and AdnaTest Breast in metastatic breast cancer patients: the DETECT study. *Breast Cancer Res*. 2012;14(4):R118.
14. Smerage JB, Barlow WE, Hortobagyi GN, et al. Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500. *J Clin Oncol*. 2014;32(31):3483-3489.
15. Bidard FC, Jacot W, Kiavue N, et al. Efficacy of circulating tumor cell count-driven vs clinician-driven first-line therapy choice in hormone receptor-positive, ERBB2-negative metastatic breast cancer: the STIC CTC Randomized Clinical Trial. *JAMA Oncol*. 2021;7(1):34-41.
16. Fehm T, Muller V, Aktas B, et al. HER2 status of circulating tumor cells in patients with metastatic breast cancer: a prospective, multicenter trial. *Breast Cancer Res Treat*. 2010;124(2):403-412.

17. Simmons C, Miller N, Geddie W, et al. Does confirmatory tumor biopsy alter the management of breast cancer patients with distant metastases? *Ann Oncol*. 2009;20(9):1499-1504.
18. Lindstrom LS, Karlsson E, Wilking UM, et al. Clinically used breast cancer markers such as estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 are unstable throughout tumor progression. *J Clin Oncol*. 2012;30(21):2601-2608.
19. Pusztai L, Viale G, Kelly CM, Hudis CA. Estrogen and HER-2 receptor discordance between primary breast cancer and metastasis. *Oncologist*. 2010;15(11):1164-1168.
20. Gregorio AD, Friedl TWP, Huober J, et al. Discordance in human epidermal growth factor receptor 2 (HER2) phenotype between primary tumor and circulating tumor cells in women with HER2-negative metastatic breast cancer. *JCO Precision Oncol*. 2017;1:1-12.
21. Wang C, Mu Z, Ye Z, et al. Prognostic value of HER2 status on circulating tumor cells in advanced-stage breast cancer patients with HER2-negative tumors. *Breast Cancer Res Treat*. 2020;181(3):679-689.
22. McLemore LE, Albarracin CT, Gruschkus SK, et al. HER2 testing in breast cancers: comparison of assays and interpretation using ASCO/CAP 2013 and 2018 guidelines. *Breast Cancer Res Treat*. 2021.
23. Jordan NV, Bardia A, Wittner BS, et al. HER2 expression identifies dynamic functional states within circulating breast cancer cells. *Nature*. 2016;537(7618):102-106.
24. Fehm T, Müller V, Banys-Paluchowski M, et al. Efficacy of the tyrosine kinase inhibitor lapatinib in the treatment of patients with HER2-negative metastatic breast cancer and HER2-positive circulating tumor cells — results from the randomized phase III DETECT III trial (San Antonio Breast Cancer Symposium 2020; PD3-12). 2020.
25. Pestrin M, Bessi S, Puglisi F, et al. Final results of a multicenter phase II clinical trial evaluating the activity of single-agent lapatinib in patients with HER2-negative metastatic breast cancer and HER2-positive circulating tumor cells. A proof-of-concept study. *Breast Cancer Res Treat*. 2012;134(1):283-289.
26. Jacot W, Cottu P, Berger F, et al. Actionability of HER2-amplified circulating tumor cells in HER2-negative metastatic breast cancer: the CirCe T-DM1 trial. *Breast Cancer Res*. 2019;21(1):121.
27. Guan X, Liu B, Niu Y, et al. Longitudinal HER2 amplification tracked in circulating tumor DNA for therapeutic effect monitoring and prognostic evaluation in patients with breast cancer. *Breast*. 2020;49:261-266.
28. Kingston B, Cutts RJ, Bye H, et al. Genomic profile of advanced breast cancer in circulating tumour DNA. *Nat Commun*. 2021;12(1):2423.
29. Andre F, Ciruelos EM, Juric D, et al. Alpelisib plus fulvestrant for PIK3CA-mutated, hormone receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: final overall survival results from SOLAR-1. *Ann Oncol*. 2021;32(2):208-217.
30. Turner NC, Kingston B, Kilburn LS, et al. Circulating tumour DNA analysis to direct therapy in advanced breast cancer (plasmaMATCH): a multicentre, multicohort, phase 2a, platform trial. *Lancet Oncol*. 2020;21(10):1296-1308.