Circulating tumor cells predict progression-free and overall survival in Chinese patients with metastatic breast cancer, HER2-positive or triple-negative (CBCSG004): a multicenter, double-blind, prospective trial†


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Background: The aim of this multicenter, double-blind, prospective study was to evaluate the potential utility of circulating tumor cell (CTC) measurements in predicting responses to anticancer therapies, including response to human
epidermal growth factor receptor-2 (HER-2)-targeted agents, progression-free survival (PFS), and overall survival (OS) in Chinese women with metastatic breast cancer (MBC).

**Patients and methods:** Three hundred MBC patients planned to complete three CTC blood draws and two imaging studies.

**Results:** A total of 294 of the 300 MBC patients enrolled from six leading Chinese cancer centers were assessable. In multivariate Cox regression analyses, the baseline CTC number remained an independent prognostic factor for PFS (hazard ratio (HR) = 1.93; 95% confidence interval (CI) = 1.39–2.69; P < 0.001) and OS (HR = 3.76; 95% CI = 2.35–6.01; P < 0.001). Similar results were observed for CTC counts at the first follow-up visit for both PFS (P = 0.049) and OS (P < 0.001).

**Conclusions:** Enumeration of CTCs in Chinese MBC patients provides substantial prognostic information and is an independent factor associated with PFS and OS. Moreover, we demonstrated the prognostic value in the various disease subtypes, including HER-2-positive disease irrespective of therapy.

**Key words:** metastatic breast cancer, circulating tumor cells, overall survival, progression-free survival

**introduction**

Circulating tumor cells (CTCs) have been proven to be a strong, independent predictor of overall survival (OS) and progression-free survival (PFS) in metastatic breast cancer (MBC) [1–4]. However, retrospective data seem to indicate a limited prognostic value of CTCs in breast cancer patients with newly diagnosed MBC and human epidermal growth factor receptor-2 (HER-2) overexpression/amplification [5–9]. Moreover, studies have shown controversial reports on the incidence of CTC detection and a predictive value in disease with enriched triple-negative breast cancers (TNBCs) [10, 11]. Prospective studies evaluating the prognostic and predictive value for these disease subsets are currently lacking [12].

Based on the above information, our study was designed to evaluate the potential utility of CTC measurements in predicting responses to anticancer therapy, PFS, and OS in Chinese women with MBC, including newly diagnosed MBC patients with particular focus on patients with HER2-positive disease, and TNBCs.

**materials and methods**

**study design**

This was a prospective multicenter, double-blind study in 300 measurable MBC Chinese patients with an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 starting a new line of systemic therapy, including any form of endocrine manipulation, cytotoxic chemotherapy, or immunotherapy, alone or in combination. The control population comprised of 99 healthy women with no known medical illness and no history of cancer (81 premenopausal and 18 postmenopausal women), and 101 women with documented benign breast diseases (95 premenopausal and 6 postmenopausal).

The primary objective of the study was to evaluate whether a five CTC cut point is predictive of PFS and OS in Chinese MBC patients. Secondary objectives included assessment of the correlation between CTC values and radiographic response to systemic therapy, and assessment of the prognostic value of CTCs in various subgroups of MBC patients (i.e. HER2-positive, TNBCs, and newly diagnosed MBC). Prior adjuvant treatment and/or treatment of metastatic disease were permitted. The protocol mandated a blinded, centralized review of all imaging studies to confirm objective response rates. The Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.0) for responsiveness to therapy, disease stability, or disease progression were used to determine patients’ response to treatment [13]. The institutional review board at each center approved the study protocol. Women in control groups and all patients provided written informed consent. Approximately 10 ml of blood from each patient was drawn into a 10-ml CellSave™ Preservative Tube as for standard specifications. The CellSearch® system (Veridex, LLC, Raritan, NJ, USA) was used to detect the number of CTCs in 7.5 ml of whole peripheral blood, as has been done in previous studies [3, 5].

**statistical analysis**

We used χ² or Fisher’s exact tests to assess associations between the presence of CTCs at the blood draw time points of baseline, first follow-up visit, and second follow-up visit and primary tumor characteristics. We used Fisher’s exact test when any one of the observed frequencies in the two-by-two contingency table was <5. Kaplan–Meier plots for PFS and OS were constructed for the favorable and unfavorable CTC patient groups. The log-rank test was used to test for statistical significance between the curves for the two groups. Multivariate Cox regression analysis was used to determine the hazards ratio of CTCs for predicting PFS and OS. All statistical tests were two-sided, and P-values of <0.05 were considered statistically significant. All statistical analyses were carried out with the SAS software version 9.2 (SAS Institute, Inc., Cary, NC, USA).

**results**

**patient characteristics**

A total of 294 of the 300 patients enrolled between March 2010 and January 2011 from six leading Chinese cancer centers were assessable. The average (±SD) age of the assessable patients was 49.4 ± 9.4 years (median, 50) (supplementary Table S1, available at Annals of Oncology online). Blood samples for CTC analysis were obtained from 227 and 233 patients at the first and second CTC follow-ups, respectively. Of the 67 (22.3%) patients with no CTC sample collected at the first CTC follow-up, 11 died before any further follow-up.

**prevalence of CTC**

CTCs were rare in healthy women (mean, 0.05 ± 0.26 per 7.5 ml of whole blood; median 0, range = 0–2) and in patients with benign breast disease (mean, 0.09 ± 0.35 per 7.5 ml of whole blood; median 0, range = 0–2). None of the control subjects had ≥2 CTC (Fisher’s P < 0.001, supplementary Table S2, available at Annals of Oncology online). One hundred and fifteen (39.1%) patients had ≥5 CTCs. CTC levels correlated with the sites of
metastatic disease, hormone receptor status, and HER-2 status, and ECOG performance status (supplementary Table S1, available at Annals of Oncology online).

CTC and imaging to assess response to therapy
Thirteen of the 294 assessable patients died before the first follow-up imaging visit, while 233 of these patients had CTC analysis during the course of the study. At the first CTC follow-up, CTC number decreased in 123 (54.9%) patients, increased in 34 (15.2%), and remained unchanged in 67 (29.9%) patients, compared with the baseline. At the follow-up imaging visit, 79 (35.0%) of the 226 patients, who had both a CTC determination and an imaging evaluation at 6–8 weeks, were classified as having either a complete response or partial response (PR), with 33 of these patients (41.8%) having ≥ 5 CTCs before the initiation of therapy but only 7 (8.9%) patient having ≥ 5 CTCs at the follow-up imaging visit. In contrast, of the 30 (13.3%) patients classified as having progressive disease, 15 (50.0%) had ≥ 5 CTCs before the initiation of therapy, with no change observed at the first CTC follow-up (51.9%) and imaging visit (46.7%).

prognostic significance of CTC number at baseline
For all 294 patients, the median PFS and OS were 7.9 (95% CI = 6.8–8.9) months and 20.9 (95% CI = 17.3 to >24.8) months, respectively. At baseline, 115 (39.1%) had ≥ 5 CTCs. These patients had a significantly shorter median PFS (6.7 months; 95% CI = 4.7–7.9) and OS (13.2 months; 95% CI = 10.6–15.9) compared with those who had <5 CTCs (median PFS = 9.0 months, 95% CI = 7.3–11.3, P < 0.001; median OS >24.6 months, P < 0.001; supplementary Table S1, available at Annals of Oncology online and supplementary Figure S1, available at Annals of Oncology online).

Among the subset of 119 newly diagnosed MBC patients, 45 (37.8%) had ≥ 5 CTCs at baseline. These 45 patients had a shorter median PFS (8.7 versus 12.3 months; P = 0.081) and OS (14.6 versus >24.6 months; P = 0.001), compared with the 74 newly diagnosed MBC patients with <5 CTCs (supplementary Table S3, available at Annals of Oncology online and supplementary Figure S2, available at Annals of Oncology online).

In the subset of 104 patients with HER2-positive cancers, 29 (27.9%) had ≥ 5 CTCs at baseline. These 29 patients had a shorter median PFS (6.7 months) and OS (11.3 months), compared with the 75 HER2-negative patients with <5 CTCs (median PFS = 7.9 months; median OS > 24.5 months; supplementary Figure S3, available at Annals of Oncology online and supplementary Table S4, available at Annals of Oncology online). Both CTC groups of HER2-negative patients who received anti-HER2-targeted therapy had similar median PFS (P = 0.139; supplementary Table S4, available at Annals of Oncology online and supplementary Figure S4, available at Annals of Oncology online). Among the 36 HER2-positive patients who did not receive anti-HER2-targeted therapy because of economic issues, 12 (33.3%) had ≥ 5 CTCs at baseline and had a shorter median OS than did the 24 patients with <5 CTCs (8.9 versus >24.4 months; P = 0.001). However, there was no difference in median PFS (P = 0.471; supplementary Table S4, available at Annals of Oncology online and supplementary Figure S5, available at Annals of Oncology online). There were no differences in PFS (P = 0.554) and OS (P = 0.170) between those HER2-positive patients whether or not they received anti-HER2 treatment (Figure 1). The results suggest that the prognostic value of CTCs in this subset is independent of the type of therapy (HER-2-directed therapy or chemotherapy alone).

In the subset of 39 TNBC patients, 18 (46.2%) had ≥ 5 CTCs at baseline. These 18 patients had a shorter median PFS (3.9 months) and OS (10.6 months), compared with the 21 patients with <5 CTCs (median PFS 7.7 months and median OS 14.0 months). However, the difference did not achieve statistical significance, most likely due to the small sample size (P = 0.247 and 0.086, Figure 2).

prognostic significance of CTC number after initiation of therapy
Patients with <5 CTCs (n = 178) at the first CTC follow-up exhibited a significantly longer median PFS (8.2 versus 5.9 months; P = 0.012) and OS (20.1 versus 12.4 months; P < 0.001), compared with the 49 patients with ≥ 5 CTCs. At the second CTC follow-up, the 39 (16.7%) patients with ≥ 5 CTCs had a significantly shorter median PFS (2.0 months) and OS (9.5 months) than did the 194 patients with <5 CTCs (median PFS = 7.6 months, P < 0.001 and median OS >23.2 months, P < 0.001; supplementary Table S5, available at Annals of Oncology online and supplementary Figure S1, available at Annals of Oncology online). There were no differences in PFS between those HER2-positive patients whether or not they received anti-HER2 treatment at the first follow-up (P = 0.622) as well as the second follow-up (P = 0.479) (Figure 1). In the subset of TNBC patients, at both the first and second CTC follow-up visits, those with <5 CTCs had significantly longer median PFS (P < 0.001 and 0.002) and OS (P = 0.003 and <0.001) times, compared with those with ≥ 5 CTCs (Figure 2).

multivariate Cox proportional hazards regression analyses
In multivariate Cox regression analyses, adjusting for clinically significant univariate factors, the CTC number at baseline remained an independent prognostic factor for PFS (HR = 1.93; 95% CI = 1.39–2.69; P < 0.001) and OS (HR = 3.76; 95% CI = 2.35–6.01; P < 0.001). Similar results were observed for CTC at the first CTC follow-up visit both for PFS (P = 0.049) and OS (P < 0.001) as well as at the second CTC follow-up visit, both for PFS (P < 0.001) and OS (P < 0.001; Table 1).

Additionally, multivariate Cox regression analyses showed that the CTC counts at baseline, first CTC follow-up visit, and second CTC follow-up visit were significant independent prognostic factors for both PFS and OS in newly diagnosed MBC patients (supplementary Table S6, available at Annals of Oncology online) as well as in MBC patients with HER2-positive tumors (supplementary Table S7, available at Annals of Oncology online). In the subset of TNBC patients, the baseline CTC counts were not statistically significant independent prognostic factors for either PFS or OS; however, the CTC counts at the first and second CTC follow-up visits were
Figure 1. Outcome in HER-2-positive disease. Kaplan–Meier curves for (A, C, E) progression-free survival (PFS) and (B, D, F) overall survival (OS) of metastatic breast cancer (MBC) patients with HER2-positive according to whether or not receive anti-HER2 treatment at baseline (A, B), first CTC follow-up (C, D), and second CTC follow-up visits (E, F).
Figure 2. Outcome in triple-negative breast cancer (TNBC). Kaplan–Meier curves for (A, C, E) progression-free survival (PFS) and (B, D, F) overall survival (OS) of metastatic breast cancer (MBC) patients with TNBC according to the levels of circulating tumor cells (CTCs) in 7.5 ml of blood (<5 and ≥5 CTCs) at baseline (A, B), first CTC follow-up (C, D), and second CTC follow-up visits (E, F).
Table 1. Multivariate analysis for the prediction of progression-free survival and overall survival

<table>
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<tr>
<th>Variable</th>
<th>Progression-free survival</th>
<th>Overall survival</th>
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<tr>
<td></td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
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<tr>
<td>Consider single CTC count at baseline</td>
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<td></td>
</tr>
<tr>
<td>≥5 versus &lt;5 CTCs</td>
<td>1.93 (1.39–2.69)</td>
<td>3.76 (2.35–6.01)</td>
</tr>
<tr>
<td>Positive ER/PR versus negative</td>
<td>0.66 (0.46–0.93)</td>
<td>0.51 (0.32–0.82)</td>
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<td>Second or subsequent line of therapy versus first</td>
<td>1.42 (1.17–1.73)</td>
<td>1.61 (1.20–2.16)</td>
</tr>
<tr>
<td>Others versus hormone therapy</td>
<td>0.64 (0.43–0.97)</td>
<td>NS</td>
</tr>
<tr>
<td>ECOG 1/2 versus 0</td>
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<td>NS</td>
</tr>
<tr>
<td>Consider single CTC count at the first follow-up visit</td>
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<td></td>
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<tr>
<td>≥5 versus &lt;5 CTCs</td>
<td>1.54 (1.00–2.36)</td>
<td>2.96 (1.67–5.25)</td>
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<tr>
<td>Second or subsequent line of therapy versus first</td>
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<td>ECOG 1/2 versus 0</td>
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<td>NS</td>
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<tr>
<td>Positive ER/PR versus negative</td>
<td>NS</td>
<td>2.22 (1.25–3.97)</td>
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<td>Analysis with CTC count at the second follow-up visit</td>
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<tr>
<td>≥5 versus &lt;5 CTCs</td>
<td>3.24 (2.00–5.25)</td>
<td>5.14 (2.64–10.01)</td>
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<tr>
<td>Second or subsequent line of therapy versus first</td>
<td>1.34 (1.08–1.66)</td>
<td>1.44 (1.04–2.01)</td>
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<tr>
<td>Positive ER/PR versus negative</td>
<td>NS</td>
<td>0.45 (0.25–0.80)</td>
</tr>
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</table>

ECOG, Eastern Cooperative Oncology Group; NS, not significant; ER, estrogen receptor; PR, progesterone receptor.

Statistically significant independent prognostic factors for PFS (P = 0.014 and 0.015; supplementary Table S8, available at Annals of Oncology online).

Discussion

This study is the first prospective, multicenter study to demonstrate that CTC enumeration before and after the initiation of a new line of therapy are useful predictors of PFS and OS in Chinese MBC patients treated with standard therapies. This population of breast cancer patients has unique characteristics compared with MBC patients in Western countries included in similar studies [1, 3]. Chinese women with MBC are younger, rarely obese, still with predominant hormone receptor-positive disease (65% of patients), but with higher incidence of HER-2 positive disease. Moreover, these patients received endocrine therapy in a much lower percentage of cases (19%) compared with the similar population in Western countries [3, 6]. In spite of such dramatic differences in clinical and pathological features, it is remarkable to note that CTC enumeration maintain a strong prognostic significance. The study also confirms the superiority of CTC enumeration for therapeutic monitoring in patients with MBC.

Cristofanilli et al. [3] showed for the first time in 2004 that CTC counts before starting a new line of treatment were an independent predictor of PFS and OS in MBC patients. This prognostic utility was independently confirmed by numerous prospective studies [6, 14–17], and now again in our study. Our study provided the unique possibility to explore the interaction between HER-2-targeted therapies and CTCs, considering that a fraction of patients eligible for those treatments could not receive it because of economical issues. We reported on the longitudinal monitoring of the largest group of HER-2-positive MBC with prospectively planned evaluation of CTCs during the course of their disease. We clearly demonstrated that a baseline detection of ≥5 CTCs predicts for survival in all patients, irrespective of their treatment (HER-2-targeted therapies versus chemotherapy). Interestingly, the baseline assessment was not associated with substantial prediction of PFS irrespective of the treatment. Furthermore, we confirmed that the subsequent follow-up evaluations increase the prognostic and predictive discrimination, arguing in favor of using CTC detection as a tool to evaluate the potential benefit of using combined HER-2 blockage versus single modality [18]. Munzone et al. [11] recently demonstrated the prognostic significance of baseline CTC enumeration in all molecular subtypes, including luminal B (HER-2-positive) and HER-2 non-luminal. On the contrary, Giordano et al. [9] published a retrospective study from the University of Texas, MD Anderson Cancer Center and showed that baseline detection of HER2+ MBC patients (50 patients) with ≥5 CTCs had a similar PFS and OS, compared with those with <5 CTCs. Our prospective, longitudinal study with defined time of disease assessment by imaging, including a rigorous central review process, demonstrated that there were no differences in PFS and OS between those HER2-positive patients with <5 CTCs whether or not they received anti-HER2 treatment at the baseline, first follow-up, and second follow-up. Most recently, Liu et al. [19] carried out a large pooled analysis included 841 patients with MBC with CTC evaluation at baseline and follow-up. The results indicated that CTC detection predicted for worse OS independently of disease subtype (included HER-2 positive), but the authors were unable to assess the impact of treatment. This study offers the unique opportunity for investigating the interaction between CTCs and therapy in this specific subset. Moreover, we conclude that the results urge the opportunity to determine whether the HER-2 status of CTCs using molecular characterization of those cells can further explore increase our capacity to provide appropriate targeted therapy and better to evaluate trastuzumab-resistance in some cases related to the heterogeneous level of HER-2 expression [20].

TNBC is a heterogeneous disease; thus, identification of specific clinical/biological parameters to better tailor treatment and novel therapeutic targets is needed to best approach the
treatment of TNBCs. Although our study had a small subset of TNBC patients, we did observe that TNBC patients with ≥5 CTCs at the first or second CTC follow-up visits exhibited shorter PFS and OS times, compared with TNBC patients with <5 CTCs. We also confirmed the prognostic significance of CTCs in patients with newly diagnosed MBC starting first-line therapy, consistent with the previously published results [5]. Furthermore, the data continue to support the potential application of CTC enumeration for a baseline staging stratification at the time of initial diagnosis and radiological assessment in order to advance the use of more sensitive and cost-effective staging and monitoring modalities.

In summary, we report for the first time the prognostic utility of CTC, as detected by CellSearch™, in Chinese patients with MBC. This analysis demonstrates that the detection of CTC in Chinese patients with MBC is associated with substantial prognostic information. Additionally, in women with newly diagnosed MBC starting first-line therapy, MBC patients with HER2-positive tumors, and TNBC patients, CTC enumeration is associated with substantial prognostic information.

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disclosure

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references