Circulating tumor cells and brain metastasis outcome in patients with HER2-positive breast cancer: the LANDSCAPE trial


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Background: Decrease of circulating tumor cells (CTC) during treatment is an independent prognostic factor in metastatic breast cancer (MBC). We specifically evaluated the impact of CTC on brain metastasis outcome.

Methods: HER2-positive MBC with brain metastasis not previously treated with whole-brain radiotherapy received first-line combination of lapatinib and capecitabine in a phase II study. CTC were detected at baseline and day 21 (CellSearch).

Results: Median follow-up of the 44 analyzed patients was 21.2 months. The central nervous system objective response (CNS-OR) rate was 66%. At baseline, 20 of 41 assessable patients for CTC (49%) had ≥1 CTC (range 1–301, median 3) and 9 (22%) had ≥5 CTC. At day 21, 7 of 38 patients (18%) had ≥1 CTC (P = 0.006, versus baseline), and CTC had disappeared in 11 patients. CNS-OR rate was significantly higher in patients with no CTC at day 21 [25 of 31 (80%) versus 2 of 7 (29%), P = 0.01]. The 1-year overall survival rate was 83.9% in patients with no CTC at day 21 versus 42.9% in patients with ≥1 CTC (P = 0.02).

Conclusions: This is the first report showing a correlation between CNS metastasis response, outcome and early CTC clearance under targeted treatment of HER2+ MBC.

Clinical Trials number: NCT00967031.

Key words: circulating tumor cells, brain metastasis, HER2, breast cancer, lapatinib, capecitabine

introduction

Decrease of circulating tumor cells (CTC) level during treatment in metastatic breast cancer (MBC) is an independent prognostic and predictive factor of patients’ outcome [1, 2]. Monitoring CTC in addition to clinical response criteria is currently evaluated in clinical trials in various cancers and in particular in breast cancer (BC) [3, 4]. Brain metastases are increasingly frequent in the course of MBC, likely due to longer life expectancy and improvements in diagnosis methods [5]. HER2-positive metastatic disease has a natural propensity to develop more often brain metastases, in up to 30%–50% of cases [6]. That effect cannot be fully overcome by trastuzumab, as the antibody, due to its large molecular size, cannot pass through an intact blood–brain barrier [7]. Lapatinib is a dual tyrosine kinase inhibitor of erbB1 and HER2, approved in association with capecitabine for the treatment of metastatic HER2-positive BC progressing after trastuzumab [8]. Lapatinib as a small molecule may penetrate the blood–brain barrier and could exhibit prophylactic as well as therapeutic effects [9]. As single agent, it was associated with modest responses in 242 patients pretreated with trastuzumab and with progressive central nervous system (CNS) metastases after radiotherapy (RR in brain 6%) [10]. However, an enhanced efficacy of lapatinib reaching a 20% response rate, by the addition of capecitabine, has been reported [10].

The aim of the LANDSCAPE study (NCT00967031) was to investigate the efficacy of upfront systemic treatment with the lapatinib and capecitabine combination for newly diagnosed brain metastasis nonpretreated with whole-brain radiotherapy. Its main results have been reported recently [11]: the objective
response (OR) rate of brain metastasis was high (66%) and the median time to CNS progression was 5.5 months. We sought to evaluate the clinical interest of peripheral blood CTC for patients included in the LANDSCAPE study. Correlations of CTC variations under systemic treatment with brain metastasis outcome have never been previously described.

patients and methods
This analysis is a preplanned secondary objective of the LANDSCAPE study. Efficacy and safety results of the study have been previously reported [11].

patients
Patients over 18 years were eligible if they had histologically confirmed MBC overexpressing HER2, defined as 3+ by immunohistochemistry or 2+ and evidence of gene amplification by fluorescence in situ hybridization; at least one measurable CNS lesion ≥10 mm on magnetic resonance imaging (MRI); ECOG performance status of 0–2 and life expectancy of at least 3 months; adequate hematologic, renal and hepatic function. Any previous systemic treatment of BC was allowed, except lapatinib or capecitabine.

Exclusion criteria included patients with single-brain metastases amenable to surgical resection, previous WBRT or SRS, current radiation therapy or current systemic treatment of BC. Written informed consent was obtained from all patients. The study was approved by a Central National Ethic Committee and the French National Drug Agency and registered as NCT00967031. It was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

This phase II, open-label, multicenter study was carried out in 11 centers in France. Patients received oral lapatinib at the approved dose of 1250 mg once daily [8] in combination with oral capecitabine 2000 mg/m2 per day from day 1 to day 14, every 21 days. Radiological response was evaluated every 6 weeks by CT scan and MRI for CNS lesions. CNS and non-CNS objective response was first assessed by the investigators using RECIST 1.0 criteria, and all CNS MRI scan were secondarily evaluated by a single investigator for centrally CNS volumetric response. Clinical assessment, including neurological examination for neurologic signs and symptoms (NSS) and toxic effect evaluation, was carried out every 3 weeks.

The primary end point was the CNS centrally assessed OR rate (CNS-OR rate), a composite criterion already used by Lin et al. [10]. Other end points included time to progression (TTP), time to radiotherapy, overall response rate for CNS and extra-CNS disease and toxic effect, prognostic and predictive value of CTC at baseline and day 21.

CTCs’ detection
CTCs were detected in 7.5 ml of blood using the CellSearch® system (Veridex, NJ), combining EpCAM immunomagnetic selection followed by anticytokeratin (C11 and A53-B/A2) fluorescently staining for CTC at baseline and at day (D) 21, before cycle 2. Samples were maintained at room temperature and processed within 96 h in a blinded fashion in an experienced laboratory (Institut Curie, Paris). Technical details of the CellSearch® technique have been described elsewhere [1]. The main objective was to test whether changes in CTC counts between inclusion and cycle 2 are associated with brain metastases response, and OS.

statistical analysis
Using a Simon’s optimal two-stage design with a minimum CNS-OR rate considered of interest of 20% and an uninteresting rate of 5%, 41 assessable patients were required. The study size was determined according its primary clinical end point, and no statistical power was calculated for the secondary end point, such as the ancillary CTC study reported here.

Correlations between patients’ characteristics and baseline CTC values (0 versus ≥1) were studied using the Wilcoxon test for characteristics expressed as quantitative variables and the Fisher exact test for characteristics expressed as qualitative variables. Evolution of CTC values (0 versus ≥1) between baseline and day 21 was analyzed using a McNemar test for matched pair data.

The CNS-OR rate was calculated with its 95% exact confidence interval (CI), and was compared between patients with and without CTC at baseline and D21 using a Fisher exact test. TTP was defined as the time from inclusion to the date of first documented progression (according to the definition of progressive disease detailed above) or death due to underlying cancer. Overall survival (OS) was defined as the time from inclusion to death. Survival data were estimated by the Kaplan–Meier method. Patients who did not experience an event were censored at the date of last tumor evaluation for TTP, and the date of last contact for OS analysis. Survival curves were compared between subgroups using the log-rank test. All expressed P-values and CIs are two sided. Subgroup analyses are purely descriptive and explorative.

results
patients’ characteristics
From April 2009 to August 2010, 45 patients were included in the study. Median age was 56 years (range 35–79 years). Most
patients had PS of 0 (38.6%) or 1 (56.8%) at inclusion. Median disease-free interval was 34.2 months (0–205 months). Forty-four patients were assessable for efficacy, with a median follow-up of 21.2 months (range 2.2–27.6 months). Forty-four patients were assessable for efficacy, with a median follow-up of 21.2 months (range 2.2–27.6 months). Among the efficacy population (N = 44), 41 patients were assessable for CTC at baseline (three blood draws missing), 38 at day 21 (four blood draws missing and two study withdraws) and 36 at both baseline and day 21.

At baseline, 20 of 41 (48.8%) patients had ≥1 CTC (range 1–301, median 3) and 9 of 41 (22%) ≥5 CTC. CTC were detected in patients with (18 of 35) or without disease outside CNS (2 of 6) (P = 0.66). Baseline CTC were not found to be correlated with other patients’ characteristics (Table 1). After 21 days of treatment, a disappearance of CTC was observed in 11 of 36 patients (31%). At day 21, only seven (18.4%) patients had ≥1 CTC (P = 0.006, D21 versus baseline) (Figure 1) (supplementary Table S1, available at Annals of Oncology online) and three (8%) had ≥5 CTC (median 3, range 1–50 among patients with ≥1 CTC).

**efficacy results**

Results of the primary end point analysis on the efficacy population showed 29 CNS objective responses in 44 assessable patients, corresponding to a CNS-OR rate of 66% (95% CI 50%–80%). A total of 29 partial responses and no complete response were observed. Thirty-seven patients (84%) exhibited a reduction in tumor volume. Median TTP was 5.5 months (95% CI 4.3–6).

**correlation between efficacy end points and baseline CTC**

There was a trend for a higher CNS-OR rate in patients with no CTC at baseline when compared with patients with ≥1 CTC at baseline [17 of 21 (81%) versus 11 of 20 (55%), P = 0.1] (Table 2). The median TTP was 6.0 months (95% CI 4.9–7.4) versus 4.3 months (95% CI 2.8–5.9) in patients without and with CTC at baseline, respectively (P = 0.11) (supplementary Figure S1a, available at Annals of Oncology online). The 1-year OS rate was 85.7% (95% CI 62.0–95.2) in patients with no CTC at baseline versus 55.0% (95% CI 31.3–73.5) in patients with ≥1 CTC at baseline (P = 0.006) (Figure 2A).

![Figure 1](image1.png)

**Figure 1.** CTC number at D1 and D21 and changes under treatment (A) in patients with CNS objective response (N = 28); (B) in patients without CNS objective response (N = 13). Thirty-six patients had CTC detection at both D1 and D21, 26 in responders and 10 in nonresponders.

<table>
<thead>
<tr>
<th>CTC status</th>
<th>CNS nonresponders, N = 15 (%)</th>
<th>CNS responders, N = 29 (%)</th>
<th>Fisher’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTC at baseline, N = 41</td>
<td>CTC = 0 at baseline</td>
<td>4 (30.8%)</td>
<td>17 (60.7%)</td>
</tr>
<tr>
<td>CTC ≥ 1 at baseline</td>
<td>9 (69.2%)</td>
<td>11 (39.3%)</td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CTC &lt; 5 at baseline</td>
<td>9 (69.2%)</td>
<td>23 (82.1%)</td>
<td>P = 0.429</td>
</tr>
<tr>
<td>CTC ≥ 5 at baseline</td>
<td>4 (30.8%)</td>
<td>5 (17.9%)</td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CTC at D21, N = 38</td>
<td>CTC = 0 at day 21</td>
<td>6 (54.5%)</td>
<td>25 (92.6%)</td>
</tr>
<tr>
<td>CTC ≥ 1 at day 21</td>
<td>5 (45.5%)</td>
<td>2 (7.4%)</td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Positive predictive value of negative CTC count at D21 was 80.6% and negative predictive value was 71.4%.
correlation between efficacy end points and CTC at day 21
CNS-OR rate was significantly higher in patients with no CTC at day 21 [25 of 31 (80.6%) versus 2 of 7 (28.6%) in patients with ≥1 CTC, \( P = 0.01 \)] (Table 2). Response outside CNS was not correlated with CTC at D21 (supplementary Table S2, available at *Annals of Oncology* online). The median TTP was 5.6 months [4.5; 6.04] versus 2.8 months [1.4; 8.7] in patients without and with CTC at day 21, respectively (\( P = 0.25 \)) (supplementary Figure S1b, available at *Annals of Oncology* online). The 1-year OS rate was 83.9% (95% CI 65.5%–92.9%) in patients without CTC at day 21 versus 42.9% (95% CI 9.8%–73.4%) in patients with ≥1 CTC at day 21 (\( P = 0.02 \)) (Figure 2B).

discussion
The US Food and Drug Administration has approved a semiautomated immunomagnetic method, the CellSearch System (Veridex, LLC, Warren, NJ) as the number of CTCs at baseline is an independent prognostic factor of progression-free survival (PFS) and OS in MBC patients [3]. A recent pooled analysis on published data of 49 eligible studies has confirmed that the presence of CTC was significantly associated with shorter survival in the total population [12]. This prognostic marker seems so strong that even with a small number of patients (\( N = 44 \)), we were able to report the same observation with a threshold of one CTC at baseline for OS (\( P = 0.006 \)) and with a trend for PFS (\( P = 0.11 \)). A report by Giordano et al. [13] suggested that the number of baseline CTCs was not predictive.
of OS in the HER2-positive population treated with anti-HER2 therapies. We did not confirm in our study this observation here, in line with recently published series that showed the independence of CTC from any BC histological subgroup [1, 2, 14]. Several observational studies support the fact that CTC changes during the weeks following the first cycle of chemotherapy are associated with PFS and OS [1, 15, 16]. Soluble protein markers often rise before they decline—the so-called tumor marker spike. This phenomenon makes assessment of protein biomarkers much less accurate in the early phases of a new treatment, whereas CTC spikes have not been observed [4]. Therefore, CTC levels, early in the course of a new therapeutic regimen for metastatic disease, appear to reflect response, whereas lack of reduction of CTC levels may reflect futility of the respective treatment, thus making early CTC levels a short-term ‘predictive’ factor. CTC count to evaluate the efficacy of any chemotherapy after only one cycle and then to switch nonresponding patients to another chemotherapy before the disease progression is the main objective of two interventional clinical trials: SWOG 500 and CIRCE 01 [3]. However, the clinical benefit of such management—compared with the standard radiological evaluation—is still unknown. In the patient’s population of the LANDSCAPE trial, absence of early CTC decrease may be a tool to decide which patients could not benefit of postponed irradiation. However, it is of interest that even among patients with ≥1 CTC at D21, the OR rate was 28.6%, which is still respectable. It does raise the question of whether the test is sufficiently predictive to direct therapy.

Central nervous system (CNS) or brain metastasis is an emerging area of interest in organ-specific metastasis research. This disease complication contributes significantly to the morbidity and mortality of HER2-positive BC; as such, brain metastasis is designated an unmet medical need [17]. Here, we report the first observation of a correlation of CTC level and response of brain metastasis to a systemic treatment. The brain is considered a ‘sanctuary site’ as the blood–tumor barrier limits the ability of drugs to enter and kill tumor cells. Detection of CTC in two of six (33%) patients with CNS as the only metastatic site is also of particular interest, raising the question of the capacity of tumor cells to circulate from brain to general circulation. Traditionally, metastatic models describe metastasis as a unidirectional process, whereby cancer cells leave a primary tumor and unidirectionally seed metastasis in regional lymph nodes or distant sites. By contrast, recent data indicate that metastasis is a multidirectional process whereby cancer cells can seed distant sites as well as the primary tumor itself. This later process, known as ‘self-seeding,’ has been validated in diverse experimental models [18]. Here, the self-seeding model may explain the observation of this correlation between CTC level and brain metastasis response to a systemic treatment.

Progress in treating brain metastases has been hampered by a lack of model systems, a lack of human tissue samples, and the exclusion of brain metastatic patients from many clinical trials [19]. CTC can provide the basis for a ‘liquid biopsy’ and may guide the use of targeted therapies. HER-2 expression can be heterogeneous among CTC within each patient [20]. Patients with a HER2-negative primary tumor can develop HER2-positive CTC during disease progression [21]. The question if these patients benefit from a therapy targeted against HER2 is of particular importance. This question is raised in the DETECT III clinical trial currently running in Germany and another forthcoming in France (CirceTDM1) [3].

In conclusion, in an ancillary study of the Landscape trial, we were able to demonstrate a correlation between early clearance of CTC detected with the CellSearch technique and brain metastasis response to a targeted therapy in HER2-positive MBC patients. This suggests the possibility of using CTC detection for early diagnosis of treatment failure. In this trial evaluating the opportunity of postponing whole-brain irradiation, this could be a tool to decide which patients could be a candidate for early irradiation in case of absence of CTC drop at day 21.

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disclosure

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references

Dual HER2 inhibition in combination with anti-VEGF treatment is active in heavily pretreated HER2-positive breast cancer

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Background: Preclinical data indicate that dual HER2 inhibition overcomes trastuzumab resistance and that use of an HER2 inhibitor with an anti-angiogenic agent may augment responses.

Patients and methods: We conducted a dose-escalation, phase I study of a combination of trastuzumab, lapatinib and bevacizumab. The subset of patients with metastatic breast cancer was analyzed for safety and response.

Results: Twenty-six patients with metastatic breast cancer (median = 7 prior systemic therapies) (all with prior trastuzumab; 23 with prior lapatinib; one with prior bevacizumab) received treatment on a range of dose levels. The most common treatment-related grade 2 or higher toxicities were diarrhea (n = 11, 42%) and skin rash (n = 2, 8%). The recommended phase 2 dose was determined to be the full Food and Drug Administration (FDA) approved doses for all the three agents (trastuzumab 8 mg/kg loading dose, 6 mg/kg maintenance dose, intravenously every 3 weeks; lapatinib 1250 mg daily, bevacizumab 15 mg/kg intravenously every 3 weeks). The overall rate of stable disease (SD) ≥ 6 months and partial or complete remission (PR/CR) was 50% (five patients with SD ≥ 6 months; seven PRs (including one unconfirmed); one CR). The rate of SD ≥ 6 months/PR/CR was not compromised in patients who had previously received study drugs, those with brain metastases, and patients treated at lower dose levels.

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