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CLINICAL TRIAL

Correlation of HER2 status between primary tumors and corresponding circulating tumor cells in advanced breast cancer patients

Marta Pestrin · Silvia Bessi · Francesca Galardi · Mara Truglia · Annibale Biggeri · Chiara Biagioni · Silvia Cappadona · Laura Biganzoli · Augusto Giannini · Angelo Di Leo

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Abstract Biocharacterization of circulating tumor cells (CTCs) in the peripheral blood of advanced breast cancer (ABC) patients may represent a real-time tumor biopsy. We assessed HER2 status on CTCs from blood samples of ABC patients. CTCs were separated and stained using the CellSearch System®. HER2 status was assessed by immunofluorescence and, when technically feasible, by fluorescence in situ hybridization. Blood samples were obtained from 66 ABC patients. Forty patients had a positive CTC sample (61%) and of these, 15 (37%) had HER2 + CTCs. We found non-concordant results in 32% of cases: 29% (8/28) of HER2-negative primary tumors had HER2-positive CTCs and 42% (5/12) of HER2-positive primary tumors had HER2-negative CTCs ($k = 0.278$). Our study suggests that a subset of patients with HER2-negative primary tumors develops HER2-positive CTCs during disease progression.

Keywords Breast cancer · HER2 status · Circulating tumor cells · Predictive markers · Anti-HER2 therapies

Marta Pestrin and Silvia Bessi have equally contributed to this work.

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M. Pestrin · S. Bessi · F. Galardi · M. Truglia · C. Biagioni · S. Cappadona · L. Biganzoli · A. Giannini · A. Di Leo
Translational Research Unit c/o Department of Oncology, Hospital of Prato, Istituto Toscano Tumori, Piazza dell'Ospedale 2, 59100 Prato, Italy

A. Biggeri
Department of Statistics "G. Parenti", University of Florence and Biostatistics Unit ISPO (Institute for Cancer Prevention and Research), Viale Morgagni 59, 50134 Florence, Italy

A. Di Leo (✉)
"Sandro Pitigliani" Medical Oncology Unit, Hospital of Prato, Istituto Toscano Tumori, Piazza dell'Ospedale 2, 59100 Prato, Italy
e-mail: adileo@usl4.toscana.it

Introduction

Metastatic disease, the most common cause of death in cancer patients, is a multistep process during which tumor cells disseminate from the primary tumor site and establish secondary tumors in remote sites [1]. The detection and biocharacterization of circulating tumor cells in cancer patients may provide relevant information on the progression of metastatic events and may have important implications for disease prognosis and treatment choices [2].

Circulating tumor cells (CTCs) can be detected from the peripheral blood of early or metastatic breast cancer patients [3–5]. A semiautomated system, the CellSearch™ system (Immunicon Corp., Huntington valley, PA, USA), has been developed for CTCs isolation using an EpCAM antibody-based immunomagnetic enrichment and an automated staining methodology [6]. This assay has been shown to have high specificity and reproducibility [7]. In landmark papers, Cristofanilli et al. have used this assay for CTCs isolation from blood samples of metastatic breast cancer patients. A major finding of these studies, carried out according to the REMARK Criteria [8], was that CTCs quantification, performed before and during treatment, predicted a response to medical therapies as early as 3–4 weeks after initiation of treatment. In addition, CTCs baseline quantification was a predictor of overall survival [9, 10].

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Current use of targeted therapies is based on the concept that metastatic cells are linear descendants of primary tumor cells with the same biologic features as the primary tumor. However, a hallmark of breast cancer is its genetic instability [11]. CTCs may express contrasting biological features to the corresponding primary tumor cells. Hence, CTCs biocharacterization may lead to identification of specific targets and subsequently direct therapy in advanced breast cancer patients.

The HER2 gene encodes for a 185-kDa tyrosine kinase glycoprotein [12]. Trastuzumab, a humanized monoclonal antibody directed against the extracellular domain of the HER2 protein, has significantly improved the clinical outcome of HER2-positive disease [13–15]. Several studies have compared HER2 status between primary tumors and matched metastatic site samples [16–19]. All of these studies have suggested an acceptable level of concordance between HER2 status of the primary and the metastatic site samples (concordance rate: 80–94%) [16–19]. Notably, in these studies, most of the metastatic site samples were drawn at the time of first relapse. In this context, it is impossible to account for selective pressure on tumor clones progression played by different lines of treatment for metastatic disease [16–19].

In an exploratory study, Meng et al. [20] reported on nine of 24 advanced breast cancer patients, whose primary tumor was HER2 negative by FISH, carrying HER2 gene amplified CTCs. Interestingly, in this study, patients had advanced disease previously treated with multiple lines of systemic therapy. These preliminary results support the hypothesis that in approximately 30% of advanced breast cancer patients, pretreated with multiple lines of systemic therapies, a shift in HER2 status might occur when a primary tumor sample is compared to circulating tumor cells isolated from the same patient.

Accordingly, we decided to prospectively evaluate the level of concordance in HER2 status between primary tumor samples and their corresponding circulating tumor cells. We collected CTCs from two different series of breast cancer patients, the first with HER2-negative and the second with HER2-positive primary tumors. The results of this study are reported in the present manuscript.

Materials and methods

Study population

Patients with locally advanced or metastatic breast cancer, with known HER2 status of their primary tumor, were eligible for this study. All patients were informed about the experimental design of the study and gave written informed consent. The study was previously approved by the local ethical board. Other eligibility criteria were ECOG performance status 0–3, previous systemic treatment(s) for early or advanced disease were allowed, an interval > 7 days between last day of systemic treatment and day of blood sample withdrawal for CTCs evaluation (http://immunicon.com/CellSearch/CellSearch HCP.aspx). A previous diagnosis of secondary malignancy was an exclusion criterion.

A twenty milliliter sample of peripheral blood was withdrawn at the time of study entry and collected in a Cellsave^{TM} tube (Immunicon, Huntingdon Valley, PA) that contains a specific cell preservative. Under these conditions the sample was stable at room temperature for 72 h. All samples were processed within 72 h from the withdrawal time (http://immunicon.com/CellSearch/CellSearch HCP.aspx).

Ten milliliter of blood was used for CTCs enumeration and HER2 evaluation by immunofluorescence (IF). The remaining 10 ml was used for CTCs immunomagnetic enrichment. Morphological and molecular CTC characterization was carried out on the enriched samples.

From the primary tumor samples, histological type, nodal status, estrogen receptor (ER), progesterone receptor (PgR), Ki-67, and HER2 status were retrieved from the pathology report. All of these biomarkers were evaluated by immunohistochemistry (IHC). The primary tumor was defined as HER2+ if IHC 3+ (Pathway® HER2−clone CB11- by Ventana Medical System, Inc., Tucson, Arizona–USA) or if IHC 2+ with evidence of gene amplification by fluorescence in situ hybridization (FISH) (ratio HER2/CEP 17 > 2.2) or chromogenic in situ hybridization (CISH) (HER2 copy number > 6) [21]. Both FISH and CISH were performed according to standard procedures (PathVysion kit for FISH by Abbott Laboratories, Downers Grove, IL-USA; Spot Light Kit for CISH by Zymed Laboratories, Inc., San Francisco, CA–dUSA).

CTCs immunomagnetic isolation and HER2 immunofluorescent staining

The methodology for automated CTCs immunomagnetic isolation and for HER2 immunofluorescent staining has been described elsewhere [6–11]. Technical details of the CellSearch® and CellSpotter® systems pertaining to accuracy, precision, linearity, and reproducibility have previously been reported [6, 7].

Briefly, ferro fluid particles conjugated to anti-EpCAM antibodies are used for isolation of EpCAM-positive cells using a magnetic field without centrifugation. Thereafter, the supernatant, containing unbound cells, is removed and the enriched sample is processed for fluorescent staining: nucleic acids are stained with 4, 6-diamidino-2-phenylindole (DAPI); epithelial cells are stained with anti-cytokeratin

(CK)-phycoerythrin, and leukocytes are stained with an allophycocyanin-conjugated anti-CD45 antibody. At this point, the CellSearch™ HER2 Tumor Phenotyping Reagent (Immunicon®) is added to identify HER2 overexpressing CTCs.

Stained cells are analyzed on Cell Track Analyzer II™ (Immunicon®), a fluorescence microscope that scans the reaction cartridge. Cells with a size of at least 4 μm presenting the composite CK+/DAPI+/CD45−/HER-2+ phenotype are classified as HER2-positive CTCs.

In this study, a case was defined as positive for CTCs when ≥ 2 cell/7.5 ml were isolated [6]. A CTCs-positive case was defined as HER2-positive when at least 50% of CTCs were HER2 positive by IF. The arbitrary selected 50% cut-off could potentially identify true HER2-positive cases on CTCs.

Quality control was maintained via the CellSearch™ Circulating Tumor Cell Control Kit used to check reagents, instruments, and operator technique. Moreover, to test the reliability of the HER2 Tumor Phenotyping Reagent (Immunicon®), blood samples from healthy donors were spiked with HER2-positive tumor cells (SBRK3 cell line).

Slide preparation for CTC morphological and molecular analysis

Ten milliliter of blood was used for standard cytology and molecular analysis. The CellSearch Profile kit® (Immunicon®) was used for automated immunomagnetic isolation of cells of interest without the staining procedures. The cell suspension obtained was subsequently cytocentrifugated and the slide was fixed in Carnoy's fixative (3:1 methanol: glacial acetic acid) for 10 min. Papanicolaou staining was done by standard protocol, and stained cells were examined by a cytopathologist.

The PathVysion HER2/neu probe kit® (Abbott laboratories) was used for the FISH analysis applied on a stained or white slide. Cases were interpreted as amplified when the ratio of HER2/CEP17 signals was greater than 2.2 [21].

Statistical analysis

Data are presented in tables of frequencies (see Tables 3 and 4). To assess the diagnostic performance of IF for HER2 status, we calculated sensitivity, specificity, and likelihood ratios, taking the FISH method as the gold standard. Crude percentage of correct classification and Cohen's Kappa as chance-adjusted index of agreement are reported. Inference on Kappa values is done using standard error under the alternative hypothesis following Fleiss et al. [22].

To assess agreement on HER2 status between primary tumor and CTCs, we calculated Cohen's Kappa and 95% confidence intervals as mentioned earlier.

Considering that HER2 status on CTCs was evaluated by IF (i.e., a nonstandardized method), we also calculated corrected Kappa values taking into account a non-differential misclassification [23].

Results

CTCs count and cytopathology examination

Between June 2006 and December 2007, 66 patients with locally advanced (14 patients) or metastatic (52 patients) breast cancer were considered potentially eligible for the study. Of the metastatic patients, the vast majority had progressive disease at the time of study entry.

Among the 66 patients, 40 (61%) were CTCs positive. The median and the corrected mean of CTCs number were 5 and 85 cells/7.5 ml of blood, respectively (range: 0–60.000). Thirty-three patients (50%) had at least 5 cells/7.5 ml of blood.

Clinical and biological characteristics of the study population by CTCs count are shown in Table 1.

Table 2 reports main clinical characteristics of metastatic breast cancer patients by CTCs count. No clinically relevant correlations were found.

Twenty-nine CTCs-positive cases were characterized morphologically by Papanicolaou staining. It was observed that CTCs differ from cells of the corresponding primary tumor tissue. In our study, two cell types were observed: the most common was characterized by small, rounded cells with a high nucleus/cytoplasmic ratio, either isolated (similar to blood cells) or arranged in clusters; the less common was characterized by larger and sometimes elongated cells (Fig. 1a–b).

HER2 protein expression and gene amplification on CTCs

Among the 40 CTCs-positive patients, 25 (63%) were classified as HER2 negative and 15 (37%) as HER2 positive by IF analysis (Fig. 1c–d). The mean percentage of immunofluorescence stained cells was 3 (range: 0–27%) in the HER2-negative group and 92.5 (range: 50–100%) in the HER2-positive group.

Thirty-four CTCs-positive cases were evaluated for HER2 gene amplification by FISH. Of these, nine were non-interpretable due to technical issues, five were HER2–amplified, and twenty were HER2-non-amplified (Fig. 1e–f).

Table 3 summarizes the concordance of HER2 status on CTCs between IF and FISH. Taking FISH as the gold standard to define the HER2 status, IF had a sensitivity and a specificity of 80 and 95%, respectively, and a likelihood ratio of a positive and negative test equal to 16 and 0.21, respectively.

Table 1 Patient and primary tumor characteristics by CTC count

	Patients' characteristics by CTC count		
	CTC ≥ 2	CTC < 2	Overall
Age (years)			
≥50	32 (80%)	16 (62%)	48
<50	8 (20%)	10 (38%)	18
Histology			
Ductal	31 (71%)	19 (73%)	50
Lobular	6 (15%)	5 (19%)	11
Other	3 (8%)	2 (8%)	5
Nodal status			
N+	19 (47%)	18 (69%)	37
N−	11 (28%)	3 (12%)	14
Nx	10 (25%)	5 (19%)	15
ER status			
Pos	26 (65%)	22 (85%)	48
Neg	13 (33%)	4 (15%)	17
NA	1 (2%)	0	1
PgR status			
Pos	16 (40%)	17 (65%)	33
Neg	23 (58%)	9 (35%)	32
NA	1 (2%)	0	1
HER2			
Pos	12 (30%)	5 (19%)	17
Neg	28 (70%)	21 (81%)	49
Overall	40	26	66

CTC circulating tumor cells, *NA* not available

Table 2 Main clinical characteristics of MBC patients by CTC count

	CTC ≥ 2	CTC < 2	Total patients ($N = 52$)
Number of metastatic sites			
≥3	19 (53%)	7 (44%)	26
<3	17 (47%)	9 (56%)	26
Type of metastatic sites (visceral/non-visceral)			
±	3 (8%)	0	3
∓	9 (25%)	7 (44%)	16
+/+	24 (67%)	9 (56%)	33
DFI			
>12 months	21 (58%)	13 (81%)	34
≤12 months	15 (42%)	3 (19%)	18

+, Presence; −, absence

MBC metastatic breast cancer, *DFI* disease free interval calculated from date of breast cancer diagnosis to date of first relapse

Considering that CTCs were defined as HER2 positive by IF if ≥ 50% of cells had HER2 staining, we found that the two techniques had a 92% agreement rate, $k = 0.75$, $P = 0.033$ (95% CI: 0.422–1.000).

HER2 protein expression on CTCs and corresponding primary tumors

Table 4 summarizes the results of the HER2 status comparison between CTCs, evaluated by IF, and corresponding primary tumor, evaluated by IHC and in situ hybridization.

In our series, we found 13 discordant cases (32%). Among the HER2-positive primary tumors, 42% (5/12) developed HER2-negative CTCs. These five patients had previously been treated with several lines of therapy and three of them had received trastuzumab before CTCs analysis. In three of the five HER2-negative cases on CTCs, FISH evaluation on CTCs was technically feasible confirming the HER2-negative status.

Among the HER2-negative primary tumors, 29% (8/28) developed HER2-positive CTCs. In two of the eight cases, FISH on CTCs was feasible and confirmed the HER2 positivity.

In our series, a weak concordance was found between HER2 status evaluated on primary tumor and on corresponding CTCs, $k = 0{,}278$ (95% CI: −0.028 to 0.584).

As HER2 status on CTCs was defined by IF (i.e., a nonstandardized method), we applied a correction for non-differential misclassification of HER2 status on CTCs using the IF sensitivity and specificity from Table 3. According to this analysis, corrected kappa, using integer values, ranges from 0.309 to 0.375; thus, confirming a weak concordance.

Table 5 reports main patient characteristics for the 13 cases with HER2 discordant results.

HER2 by FISH was reassessed on the primary tumor for all discordant cases, and in all, the originally assigned HER2 status was confirmed.

Discussion

In clinical practice, we tend to assume that the expression of biological markers of metastatic tumors mirrors the primary tumor biological profile.

However, tumors are biologically and clinically heterogeneous, and systemic therapies may play a role in selective tumor clones progression, explaining the prevalence of a given tumor clone over the others [24].

In the past, studies have explored the concept of biological heterogeneity between the primary tumor and its metastatic sites by comparing the expression of a given marker, most frequently HER2 or hormone receptors [16–19].

All of the reported studies had a retrospective design and, more importantly, in the vast majority of cases expression of biomarkers was evaluated on a metastatic site biopsied at the time of the first disease relapse. Of note, patients experiencing first relapse had not yet received

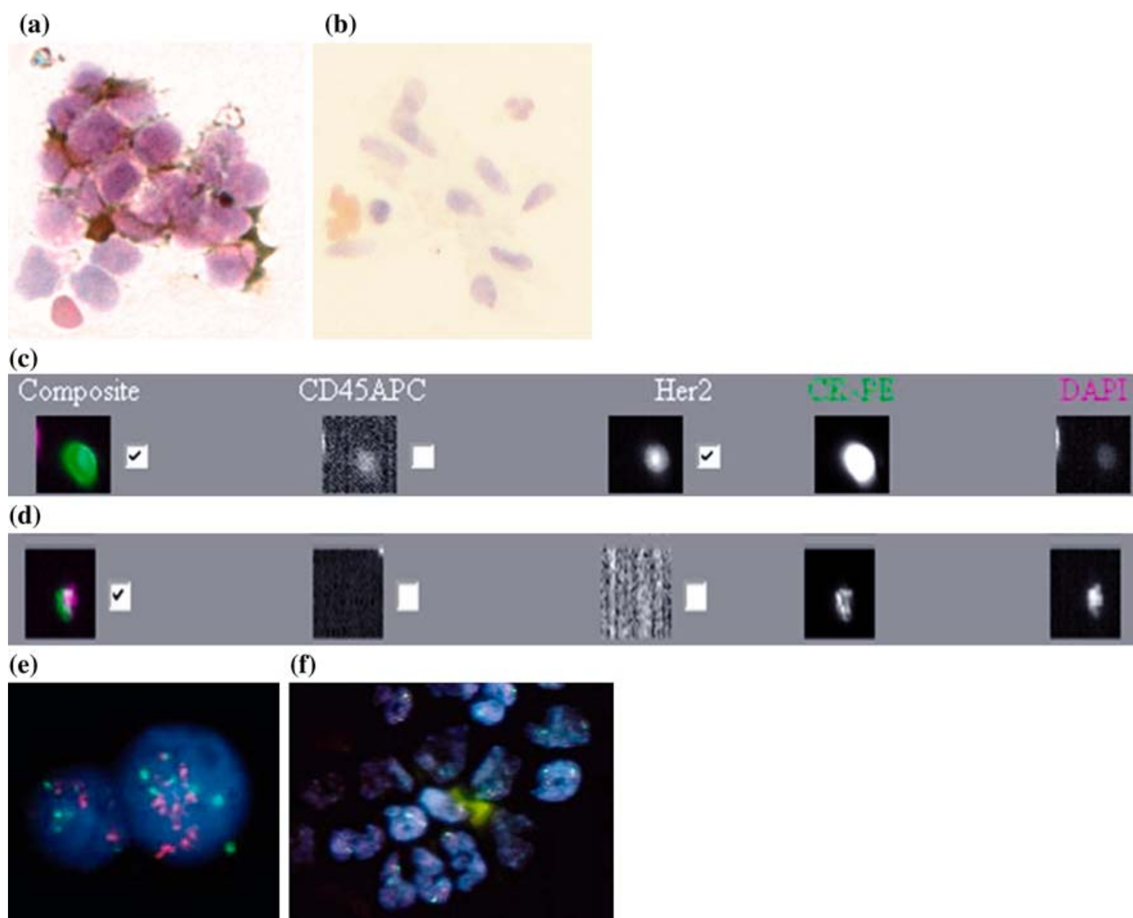

Fig. 1 Morphological analysis: *panel* **a** rounded cells with a high nucleus/cytoplasmic ratio, arranged in clusters; *panel* **b** elongated cells. Immunofluorescence analysis: *panels c* and *d* HER2, CK-PE, and DAPI windows reproduce a computer-elaborated image of the membrane, cytoplasm, and nucleus, respectively. *Panel* **c** positive circulating tumor cell (DAPI+/CK-PE+/CD45APC−/HER2+); *panel* **d** HER2-negative circulating tumor cell (DAPI+/CK-PE+/CD45APC−/HER2−). FISH analysis; *panel* **e** HER2-amplified circulating tumor cells; *panel* **f** HER2-non-amplified circulating tumor cells

Table 3 Concordance of HER2 status between IF and FISH evaluated on CTC

	IF positive	IF negative	Total
FISH positive	4	1	5
FISH negative	1	19	20
Total	5	20	25

CTC circulating tumor cells, *IF* immunofluorescence, *FISH* fluorescence in situ hybridization

Kappa (95% CI): 0.750 (0.422–1.000)

Table 4 HER2 status comparison between primary tumors and corresponding CTC

Primary tumor	CTC		
	HER2+ by IF	HER2− by IF	Total
HER2+	7	5	12
HER2−	8	20	28
Total	15	25	40

CTC circulating tumor cells, *IF* immunofluorescence

Kappa (95% CI): 0.278 (−0.028 to 0.584)

treatment for metastatic disease. In this circumstance, the potential selective pressure played by systemic therapies on tumor clone progression cannot occur. In addition, in these studies, biomarkers expression was most frequently evaluated on a single metastatic site, assuming that this site would be representative of the bulk of metastatic disease.

The use of modern technologies allowing for the isolation of CTCs from the peripheral blood of cancer patients has enabled the undertaking of studies aiming to biologically characterize metastatic tumors [25–33]. Theoretically, CTCs isolation and biocharacterization may become a real time and minimally invasive biopsy of the metastatic tumor and could provide the clinician with relevant information in terms of prognosis and prediction of treatment activity.

Table 5 Main patient characteristics of the discordant cases

Patient number	HER2 assessment on primary tumor		Δ time	HER2 assessment by IF on CTC		Previous treatments[a]	
	Method	Result (score)	Months	#CTC	% HER2 + CTC	CT	HT
CTC011	FISH	NA (0.95)	0	3	67	0	0
CTC012	FISH	NA (1.36)	36	5	100	3	2
CTC018	FISH	NA (1.17)	52	6	67	1	2
CTC022	FISH	NA (1.02)	1	5	100	0	0
CTC023	FISH	NA (1.19)	68	2	100	0	2
CTC046	FISH	NA (1.01)	45	5	100	1	1
CTC048	FISH	NA (0.93)	3	5	100	0	1
CTC064	FISH	NA (1.07)	1	21	100	0	0
CTC010m	FISH	A (2.30)	12	11	27	2	1
CTC049	FISH	A (2.34)	21	101	2	1	1
CTC055	FISH	A (2.58)	52	18	0	0	1
CTC078	FISH	A (>2.2)	96	12	8	4	2
CTC079	FISH	A (>2.2)	78	37	3	1	3

CTC circulating tumor cells, # number, *CT* chemotherapy, *HT* hormonotherapy, *NA* non-amplified, *A* amplified, *IF* immunofluorescence, *FISH* fluorescence in situ hybridization

Δ time: time interval between date of metastatic disease diagnosis and date of CTC evaluation

[a] For advanced disease

In the present study, we have used an immunomagnetic-based, semiautomated technology namely CellSearch®. In a clinical setting, CTCs counting by CellSearch® has been shown to have a prognostic value for advanced breast cancer patients [9, 10]. This suggests that CTCs isolated by this technology are biologically relevant.

In this study, CTCs were isolated in only 61% of the study population. In addition, only half of the 66 locally advanced or metastatic cases reported in the present manuscript had at least 5 cells/7.5 ml of peripheral blood. The limited number of CTCs isolated by CellSearch® may be poor representative of the tumor bulk of any single patient, and it may generate technical difficulties in evaluating biomarkers.

Conversely, a technology based on microchips and interaction between CTCs and anti-EpCAM-coated microposts has shown to be highly sensitive in detecting relevant numbers of CTCs from peripheral blood samples of up to 99% of advanced solid tumor patients, although the clinical significance of isolated CTCs is still unclear [33].

In the present study, we have focused our efforts on the comparison of HER2 status between primary tumor and corresponding CTCs isolated from advanced breast cancer patients. HER2 is recognized as a clinically important treatment target [13–15]. The demonstration that some HER2-negative primary tumors might develop, during the course of disease progression, HER2-positive CTCs could clinically be relevant.

Our results seem to support the concept of HER2 status shifting between the primary tumor and its corresponding CTCs. The presented results are in line with previously reported data (Table 6) [34–39]. Nevertheless, it is important to emphasize that the present study had as primary end point the evaluation of the HER2 status shift between primary tumors and CTCs in view of a clinical trial testing anti-HER2 treatments in patients with HER2-negative primary tumors and HER2-positive CTCs. Shifting seems to occur in both directions. In five of twelve HER2-positive primary tumors, CTCs were defined as HER2-negative. Interestingly, in these five discordant cases the HER2/CEP17 copy numbers ratio evaluated on the primary tumor by FISH was close to the cut-off. In addition, in the eight cases with HER2-negative primary tumor and HER2-positive CTCs, the number of isolated CTCs was <10/7.5 ml of blood in all but one cases.

These considerations suggest that the present results have to be taken with caution.

A further caveat of the present study is the fact that HER2 status was evaluated by IF on CTCs and by standard IHC and in situ hybridization on primary tumors. Nevertheless, it is important to emphasize that in 23 of 25 cases in which both IF and FISH were technically feasible on peripheral blood samples drawn at the same time from the same patient, a concordance between the two techniques in defining the HER2 status on CTCs was found (Table 3). This suggests that evaluation of HER2 by IF on CTCs may be accurate enough.

Table 6 Previous studies correlating HER2 status between primary tumor and corresponding CTC

Author	Disease setting	No. of patients	Method of HER2 evaluation		Discordance rate (no) (%)	Comments
			Primary	CTC		
Meng et al. [34]	MBC	52	FISH	IF/FISH	13.5 (7/52)	Analysis of individual tumor cells from primary tumor samples
Fehm et al. [35]	MBC	15	NS	IF/FISH or RT-PCR	40 (6/15)	No homogeneous HER2 testing in CTCs
Tewes et al. [36]	MBC	22	IHC	RT-PCR	36 (8/22)	No CTC morphology analysis
Apolostolaki et al. [37]	EBC	52	IHC	RT-PCR	NA	Number of patients with HER2-negative CTCs was not reported
Ignatiadis et al. [38]	EBC	49	IHC	RT-PCR	NA	Number of patients with HER2-negative CTCs was not reported
Wulfing et al. [39]	EBC	27	IHC on TMA	ICC	48 (13/27)	% of staining in HER2-positive CTC was not reported

MBC Metastatic breast cancer, *EBC* early breast cancer, *FISH* fluorescent in situ hybridization, *IF* immunofluorescence, *NS* not specified, *RT-PCR* reverse transcriptase-polymerase chain reaction, *IHC* immunohistochemistry, *TMA* tissue microarray, *ICC* immunocytochemistry, *NA* not applicable

The results of this study generate a new question: is the evaluation of HER2 status on CTCs representative of the metastatic tumor HER2 status? This critical issue must be addressed because of its clinical relevance. Only a properly designed clinical trial can address this question. Meng et al. [20] reported that three of four heavily pretreated advanced breast cancer patients with HER2-negative primary tumors and HER2-positive CTCs had an objective tumor response to trastuzumab combined with chemotherapy. This report, although hypothesis-generating, is not strong enough to promote this strategy in the clinical practice. It was not a prospectively designed clinical trial and patients received trastuzumab in combination with chemotherapy.

Based on the results of the presently reported study, our group has recently initiated a prospectively designed phase II clinical trial, whose primary aim is the evaluation of single-agent lapatinib in patients with HER2-negative primary tumors by standard criteria and HER2-positive CTCs. Whenever feasible, a biopsy from a metastatic site will be collected in parallel with CTCs biocharacterization. This will allow for correlation in HER2 status between a metastatic tumor sample and CTCs from the same patient. We believe that the results of this ongoing trial might pragmatically address the question raised by the present study in relation to the clinical significance of HER2 status determination on CTCs.

In conclusion, the present study shows that in 32% of patients with advanced breast cancer a shift in HER2 status between the primary tumor and corresponding CTCs occurred. The shift was seen to be bidirectional. The next step is the clinical evaluation of anti-HER2 therapies in patients with HER2-negative primary tumors and HER2-positive CTCs. An ongoing Phase II trial is addressing this clinically relevant question.

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References

1. Steeg PS (2006) Tumor metastasis: mechanistic insights and clinical challenges. Nat Med 12:895–904
2. Ring A, Smith IE, Dowsett M (2004) Circulating tumour cells in breast cancer. Lancet Oncol 5:79–88
3. Terstappen LW, Rao C, Gross S et al (2000) Peripheral blood tumor cell load reflects the clinical activity of the disease in patients with carcinoma of the breast. Int J Oncol 17:573–578
4. Pachmann K, Camara O, Kavallaris A et al (2008) Monitoring the response of circulating epithelial tumor cells to adjuvant chemotherapy in breast cancer allows detection of patients at risk of early relapse. J Clin Oncol 10:1208–1215
5. Paterlini-Brechot P, Benali NL (2007) Circulating tumor cells (CTC) detection: clinical impact and future directions. Cancer Lett 253:180–204
6. Allard WJ, Matera J, Miller MC et al (2004) Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 10:6897–6904
7. Riethdorf S, Fritsche H, Müller V et al (2007) Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res 13:920–928
8. McShane LM, Altman DG, Sauerbrei W et al (2005) Reporting recommendations for tumor marker prognostic studies. J Clin Oncol 23(36):9067–9072
9. Cristofanilli M, Budd GT, Ellis MJ et al (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351:781–791
10. Cristofanilli M, Hayes DF, Budd GT et al (2005) Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 23:1420–1430

11. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100:57–70
12. Yarden Y, Sliwkowski MX (2001) Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2:127–137
13. Slamon DJ, Clark GM, Wong SG et al (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER2/neu oncogene. Science 235:177–182
14. Baselga J, Perez EA, Pienkowski T et al (2006) Adjuvant trastuzumab: a milestone in the treatment of HER2-positive early breast cancer. Oncologist 11:4–12
15. Gonzalez-Angulo AM, Hortobágyi GN, Esteva FJ (2006) Adjuvant therapy with trastuzumab for HER2/neu-positive breast Cancer. The Oncologist 11:857–867
16. Gancberg D, Di Leo A, Cardoso F et al (2002) Comparison of HER2 status between primary breast cancer and corresponding distant metastatic sites. Ann Oncol 13:1036–1043
17. Edgerton SM, Moore D, Merkel D et al (2003) erbB-2 (HER2) and breast cancer progression. Appl Immunohistochem Mol Morphol 11:214–221
18. Zidan J, Dashkovsky I, Stayerman C et al (2005) Comparison of HER2 overexpression in primary breast cancer and metastatic sites and its effect on biological targeting therapy of metastatic disease. Br J Cancer 93:552–556
19. Tanner M, Järvinen P, Isola J (2001) Amplification of HER2/neu and topoisomerase IIalpha in primary and metastatic breast cancer. Cancer Res 61:5345–5348
20. Meng S, Tripathy D, Shete S et al (2004) HER2 gene amplification can be acquired as breast cancer progresses. Proc Natl Acad Sci USA 101:9393–9398
21. Wolff AC, Hammond ME, Schwartz JN et al (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 25:118–145
22. Fleiss JL, Levin B, Paik MC (2003) Statistical methods for rates and proportions, 3rd edn. Wiley, New York, pp 606–607
23. Kleinbaum DG, Kupper LL, Morgenstern H (1982) Epidemiologic research. LL publication, Belmont, CA, p 234
24. Vecchi M, Confalonieri S, Nuciforo P et al (2008) Breast cancer metastases are molecularly distinct from their primary tumors. Oncogene 27:2148–2158
25. Hayes DF, Smerage J (2008) Is there a role for circulating tumor cells in the management of breast cancer? Clin Cancer Res 14:3646–3650
26. Pantel K, Brakenhoff RH, Brandt B (2008) Detection, clinical relevance and specific biological properties of disseminating tumour cells. Nat Rev Cancer 8:329–340
27. Kagan M, Howard D, Bendele T (2002) Circulating tumor cells as cancer markers, a sample preparation and analysis system. In: Diamandis EP, Fritsche HA, Lilja H et al (eds) Tumor markers: physiology. pathobiology, technology, and clinical applications. AACC Press, Washington (DC), pp 495–498
28. Vona G, Sabile A, Louha M et al (2000) Isolation by size of epithelial tumor cells: a new method for the immunomorphological and molecular characterization of circulating tumor cells. Am J Pathol 156:57–63
29. Rosenberg R, Gertler R, Friederichs J et al (2002) Comparison of two density gradient centrifugation systems for the enrichment of disseminated tumor cells in blood. Cytometry 49:150–158
30. Kahn HJ, Presta A, Yang LY et al (2004) Enumeration of circulating tumor cells in the blood of breast cancer patients after filtration enrichment: correlation with disease stage. Breast Cancer Res Treat 86:237–247
31. Balic M, Dandachi N, Hofmann G et al (2005) Comparison of two methods for enumerating circulating tumor cells in carcinoma patients. Cytometry B Clin Cytom 68:25–30
32. Benoy IH, Elst H, Philips M et al (2006) Prognostic significance of disseminated tumor cells as detected by quantitative real-time reverse-transcriptase polymerase chain reaction in patients with breast cancer. Clin Breast Cancer 7:146–152
33. Nagrath S, Sequist LV, Maheswaran S et al (2007) Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 450:1235–1239
34. Meng S, Tripathy D, Shete S et al (2006) uPAR and HER-2 gene status in individual breast cancer cells from blood and tissues. Proc Natl Acad Sci U S A 103:17073–17074
35. Fehm T, Becker S, Duerr-Stoerzer S et al (2007) Determination of HER2 status using both serum HER2 levels and circulating tumor cells in patients with recurrent breast cancer whose primary tumor was HER2 negative or of unknown HER2 status. Breast Cancer Res 9:R74
36. Tewes M, Aktas B, Welt A, Mueller et al (2008) Molecular profiling and predictive value of circulating tumor cells in patients with metastatic breast cancer: an option for monitoring response to breast cancer related therapies. Breast Cancer Res Treat 115(3):581–590
37. Apostolaki S, Perraki M, Kallergi G et al. (2008) Detection of occult HER2 mRNA-positive tumor cells in the peripheral blood of patients with operable breast cancer: evaluation of their prognostic relevance. Breast Cancer Res Treat. Nov 19
38. Ignatiadis M, Kallergi G, Ntoulia M et al (2008) Prognostic value of the molecular detection of circulating tumor cells using a multimarker reverse transcription-PCR assay for cytokeratin 19, mammaglobin A, and HER2 in early breast cancer. Clin Cancer Res 14:2593–2600
39. Wülfing P, Borchard J, Buerger H et al (2006) HER2-positive circulating tumor cells indicate poor clinical outcome in stage I to III breast cancer patients. Clin Cancer Res 12:1715–1720