Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data

François-Clément Bidard, Dieter J Peeters, Tanja Fehm, Franco Nolé, Rafael Gisbert-Criado, Dimitrios Mavroudis, Salvatore Grisanti, Daniele Generali, Jose A Garcia-Saenz, Justin Stebbing, Carlos Caldas, Paola Gazzaniga, Luis Manso, Rita Zamarchi, Angela Fernandez de Lascoiti, Leticia De Mattos-Arruda, Michail Ignatiadis, Ronald Lebofsky, Steven J van Laere, Franziska Meier-Stiegen, Maria-Teresa Sandri, Jose Vidal-Martinez, Eleni Politaki, Francesca Consoli, Alberto Bottini, Eduardo Diaz-Rubio, Jonathan Krell, Sarah-Jane Dawson, Cristina Raimondi, Annemie Rutten, Wolfgang Janni, Elisabetta Munzone, Vicente Carañana, Sofia Agelaki, Camillo Almici, Luc Dirix, Erich-Franz Solomayer, Laura Zorzino, Helene Johannes, Jorge S Reis-Filho, Klaus Pantel, Jean-Yves Pierga*, Stefan Michiels**

Summary

Background We aimed to assess the clinical validity of circulating tumour cell (CTC) quantification for prognostication of patients with metastatic breast cancer by undertaking a pooled analysis of individual patient data.

Methods We contacted 51 European centres and asked them to provide reported and unreported anonymised data for individual patients with metastatic breast cancer who participated in studies between January, 2003, and July, 2012. Eligible studies had participants starting a new line of therapy, data for progression-free survival or overall survival, or both, and CTC quantification by the CellSearch method at baseline (before start of new treatment). We used Cox regression models, stratified by study, to establish the association between CTC count and progression-free survival and overall survival. We used the landmark method to assess the prognostic value of CTC and serum marker changes during treatment. We assessed the added value of CTCs or serum markers to prognostic clinicopathological models in a resampling procedure using likelihood ratio (LR) χ^2 statistics.

Findings 17 centres provided data for 1944 eligible patients from 20 studies. 911 patients (46·9%) had a CTC count of 5 per 7·5 mL or higher at baseline, which was associated with decreased progression-free survival (hazard ratio [HR] 1·92, 95% CI 1·73–2·14, p<0·0001) and overall survival (HR 2·78, 95% CI 2·42–3·19, p<0·0001) compared with patients with a CTC count of less than 5 per 7·5 mL at baseline. Increased CTC counts 3–5 weeks after start of treatment, adjusted for CTC count at baseline, were associated with shortened progression-free survival (HR 1·85, 95% CI 1·48–2·32, p<0·0001) and overall survival (HR 2·26, 95% CI 1·68–3·03) as were increased CTC counts after 6–8 weeks (progression-free survival HR 2·20, 95% CI 1·66–2·90, p<0·0001; overall survival HR 2·91, 95% CI 2·01–4·23, p<0·0001). Survival prediction was significantly improved by addition of baseline CTC count to the clinicopathological models (progression-free survival LR 38·4, 95% CI 21·9–60·3, p<0·0001; overall survival LR 64·9, 95% CI 41·3–93·4, p<0·0001). This model was further improved by addition of CTC change at 3–5 weeks (progression-free survival LR 8·2, 95% CI 0·78–20·4, p=0·004; overall survival LR 11·5, 95% CI 2·6–25·1, p=0·0007) and at 6–8 weeks (progression-free survival LR 15·3, 95% CI 5·2–28·3; overall survival LR 14·6, 95% CI 4·0–30·6; both p<0·0001). Carcinoembryonic antigen and cancer antigen 15-3 concentrations at baseline and during therapy did not add significant information to the best baseline model.

Interpretation These data confirm the independent prognostic effect of CTC count on progression-free survival and overall survival. CTC count also improves the prognostication of metastatic breast cancer when added to full clinicopathological predictive models, whereas serum tumour markers do not.

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Introduction

Over the past two decades, many systems for circulating tumour cell (CTC) detection have been developed.[1] In 2004, CTC enumeration using the CellSearch system (Janssen Diagnostics, Raritan, NJ, USA) was shown to be significantly associated with progression-free survival and overall survival in 177 patients with metastatic breast cancer.[2] The hazard ratio (HR) for the difference between late and early progression of disease reached a plateau at 5 CTC per 7·5 mL or higher. In the same cohort, changes in CTC count after the initiation of a new course of therapy were also shown to correlate with progression-free survival and overall survival.[3] These results prompted the US Food and Drug Administration (FDA) to approve this CTC detection technique as a method to "monitor breast cancer treatment and indicate its effectiveness".[4]

Since 2004, several other observational studies of patients with metastatic breast cancer, mainly done in European countries, have been reported using the CellSearch system.[5–14] Most of these studies, however,

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*Joint senior authors

Department of Medical Oncology and SIRIC, Institut Curie, Paris, France (F-C Bidard MD, R Lebofsky PhD, Prof J-Y Pierga MD); **Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA** (F-C Bidard, Prof J S Reis-Filho MD); **Translational Cancer Research Unit, GZA Hospitals Sint-Augustinus, Antwerp, Belgium** (D J Peeters MD, Prof S J van Laere PhD, A Rutten MD, L Dirix MD); **University of Antwerp, Antwerp, Belgium** (D J Peeters, Prof S J van Laere, L Dirix); **Department of Gynecology and Obstetrics, Heinrich Heine University Düsseldorf, Düsseldorf, Germany** (Prof T Fehm MD, F Meier-Stiegen PhD); **Division of Medical Senology, European Institute of Oncology, Milan, Italy** (F Nolé MD, E Munzone MD); **Clinical Laboratory, Hospital Arnau de Vilanova, Valencia, Spain** (R Gisbert-Criado MD, J Vidal-Martinez MD); **University Hospital of Heraklion, Heraklion, Greece** (Prof D Mavroudis MD, E Politaki MSc, S Agelaki MD); **Department of Transfusion Medicine, Laboratory for Stem Cells Manipulation and Cryopreservation, AO Spedali Civili di Brescia, Brescia, Italy** (S Grisanti MD, F Consoli MD, C Almici MD); **AZ Istituti**

Ospitalieri di Cremona, Cremona, Italy (D Generali MD, A Bottini MD); **Department of Oncology, Hospital Clinico San Carlos, Department of Medicine, University Complutense of Madrid, Spain** (J A Garcia-Saenz MD, Prof E Diaz-Rubio MD); **Imperial College and Imperial College Healthcare NHS Trust, London, UK** (Prof J Stebbing MD, J Krell MD); **Cancer Research UK Cambridge Institute and Department of Oncology, Li Ka Shing Centre, University of Cambridge, Cambridge, UK** (Prof C Caldas MD, S-J Dawson MD); **Cambridge Experimental Cancer Medicine Centre** (Prof C Caldas), **and Cambridge Breast Unit** (Prof C Caldas), **Addenbrooke's Hospital, Cambridge University Hospital NHS Foundation Trust, and NIHR Cambridge Biomedical Research Centre, Cambridge, UK; Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy** (P Gazzaniga MD, C Raimondi MD); **Hospital 12 de Octubre, Madrid, Spain** (L Manso MD); **IOV-IRCCS, Padova, Italy** (R Zamarchi MD); **Hospital de Navarra, Pamplona, Spain** (A F de Lascoiti MD); **Val d'Hebron Institute of Oncology, Val d'Hebron University Hospital, and Universitat Autònoma de Barcelona, Barcelona, Spain** (L De Mattos-Arruda MD); **Department of Medical Oncology and Breast Cancer Translational Research Laboratory, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium** (M Ignatiadis MD); **Department of Oncology, KU-Leuven, Leuven, Belgium** (Prof S J van Laere); **Division of Laboratory Medicine, European Institute of Oncology, Milan, Italy** (M-T Sandri MD, L Zorzino MSc); **Frauenklinik, University of Ulm, Ulm, Germany** (Prof W Janni MD); **Clinical oncology, Hospital Arnau de Vilanova, Valencia, Spain** (V Carañana MD); **Saarland University, Homburg, Germany** (Prof E-F Solomayer MD); **International Drug Development Institute, Louvain-La-Neuve, Belgium** (H Johannes MSc); **Department of Tumor Biology, Center of**

reported either progression-free survival[5–7] or overall survival,[8] but not both endpoints, and contradictory results have been reported.[7,12] None of the reported studies had sufficient statistical power to ascertain the contribution of CTC count to prognostication above and beyond that provided by a full clinicopathological prognostication model and serum markers, or to assess prognostic effects across subgroups.[15] To answer these questions, we did a pooled analysis to investigate the clinical validity of CTCs as detected by the CellSearch platform in patients with metastatic breast cancer.

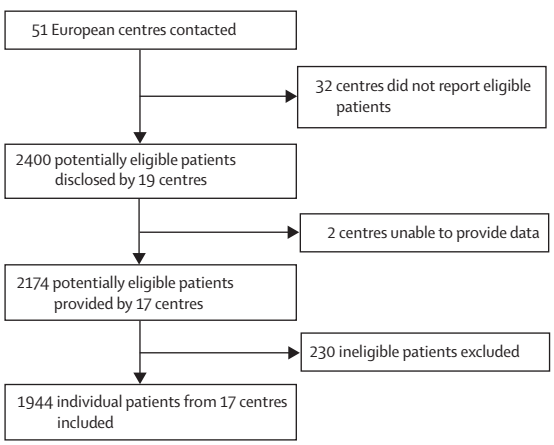

Figure 1: **Study flow**

	Patients	Baseline CTC ≥5 per 7·5 mL	p value from Fisher's exact test	p value from Kruskal-Wallis test
Age				
<65 years	1233 (63·4%)	583 (47·3%)	0·88	0·47
≥65 years	676 (34·8%)	316 (46·7%)	..	..
Unknown	35 (1·8%)	..	..	..
Performance status				
0	617 (31·7%)	247 (40·0%)	<0·0001	<0·0001
1	743 (38·2%)	373 (50·2%)	..	..
2	171 (8·8%)	107 (62·6%)	..	..
3	47 (2·4%)	31 (66·0%)	..	..
4	9 (0·5%)	8 (88·9%)	..	..
Unknown	357 (18·4%)	..	..	..
Tumour subtype				
HR+ HER2–	1166 (60·0%)	596 (51·1%)	<0·0001	<0·0001
HER2+	474 (24·4%)	182 (38·4%)	..	
HR– HER2–	240 (12·3%)	106 (44·2%)	..	..
Unknown	64 (3·3%)	..	..	..
Histological grade				
I	116 (6·0%)	47 (40·5%)	0·30	0·68
II	668 (34·4%)	322 (48·2%)	..	..
III	746 (38·4%)	346 (46·4%)	..	..
Unknown	414 (21·3%)	..		
Metastasis-free interval				
0–3 years	1028 (52·9%)	503 (48·9%)	0·088	0·059
>3 years	867 (44·6%)	390 (45·0%)	..	..
Unknown	49 (2·5%)	..	..	..

(Table 1 continues on next page)

Methods

Study design and population

The study protocol was set up by the study secretariat (F-CB, MI, KP, J-YP, SM), and discussed with investigators (appendix pp 16–22). We contacted each of the 51 European centres running a CellSearch platform between Sept 6, and Dec 31, 2012, and invited them to participate. We also searched Medline and major oncology congress abstracts to identify relevant studies (appendix pp 16–22).

Inclusion criteria were: reported and unreported studies done in European centres in patients with metastatic breast cancer starting a new line of therapy; data for progression-free survival or overall survival, or both; approval by relevant institutional review board or ethics committee; CTC quantification by the CellSearch method[16] at baseline (before start of new treatment); and studies with accrual initiated in or after January, 2003, and closed in or before July, 2012. We excluded studies in which CTC count was disclosed to clinicians and affected management of patients. Treatments were chosen by clinicians.

Procedures

Each local investigator was responsible for collecting and sharing individual anonymised data, which were centralised until Feb 28, 2013. We monitored data files manually for eligibility and sent queries to centres whenever needed. Subsequently, data were merged into a centralised repository accessible only to the lead investigators and statisticians (F-CB, HJ, J-YP, SM).

The mandatory de-identified individual data to be provided by single centres were centre and anonymised patient identification, CTC count (per 7·5 mL) at baseline, date of CTC count, and date of tumour progression or death (censored whenever appropriate). Optional individual data are detailed in the full study protocol (appendix pp 16–22), and included breast cancer histological subtype and grade, metastasis-free interval (when null, metastases were deemed synchronous), type of treatment initiated, location of metastatic sites, serum marker concentrations at baseline, and date and results of any further CTC count or serum marker assessment during the treatment. We normalised serum markers using their upper limit of normal value (ULNV). No financial compensation was offered to individuals or participating centres.

Statistical analysis

We defined progression-free survival as the time from baseline CTC assessment to disease progression or death from any cause, whichever came first. We defined overall survival as time from baseline CTC assessment to death

	Patients	Baseline CTC ≥5 per 7·5 mL	p value from Fisher's exact test	p value Kruskal-Wallis test
(Continued from previous page)				
Metastatic sites				
Bone	1240 (63·8%)	697 (56·2%)	<0·0001	<0·0001
Liver	825 (42·4%)	470 (57·0%)	<0·0001	<0·0001
Lung or pleura	774 (39·8%)	343 (44·3%)	0·035	0·0048
Soft tissue	609 (31·3%)	269 (44·2%)	0·068	0·073
Locoregional	384 (19·8%)	172 (44·8%)	0·30	0·60
CNS	194 (10·0%)	88 (45·4%)	0·60	0·81
Other	205 (10·5%)	118 (57·6%)	0·0018	0·0016
Unknown	47 (2·4%)	..	..	..
Number of metastatic sites				
<3 sites	684 (35·2%)	334 (48·8%)	0·29	0·39
≥3 sites	1200 (61·7%)	554 (46·2%)	..	..
Unknown	60 (3·1%)	..	..	..
Number of previous hormone therapy lines				
0	1083 (55·7%)	498 (46·0%)	0·014	0·0076
1	358 (18·4%)	173 (48·3%)	..	..
≥2	272 (14·0%)	152 (55·9%)	..	..
Unknown	231 (11·9%)	..	..	..
Number of previous chemotherapy lines				
0	1110 (57·1%)	494 (44·5%)	0·0063	0·0003
1	338 (17·4%)	174 (51·5%)	..	..
≥2	372 (19·1%)	196 (52·7%)	..	..
Unknown	124 (6·4%)	..	..	..
Baseline carcinoembryonic antigen				
Normal	410 (21·1%)	137 (33·4%)	<0·0001	<0·0001
Elevated	483 (24·8%)	287 (59·4%)	..	..
Unknown	1051 (54·1%)	..	..	..
Baseline cancer antigen 15-3				
Normal	406 (20·9%)	134 (33·0%)	<0·0001	<0·0001
Elevated	892 (45·9%)	513 (57·5%)	..	..
Unknown	646 (33·2%)	..	..	..
Treatment initiated				
Including chemotherapy	1555 (80·0%)	742 (47·7%)	0·043	0·017
Including hormone therapy	274 (14·1%)	111 (40·5%)	0·030	0·0054
Including anti-HER2 targeted therapy	379 (19·5%)	137 (36·1%)	<0·0001	<0·0001
Including bevacizumab	400 (20·6%)	180 (45·0%)	0·46	0·37
Including other targeted therapy	42 (2·2%)	21 (50·0%)	0·75	0·93
Unknown	88 (4·5%)	..	..	..

Data are n (%). Fisher's exact test and Kruskal-Wallis test were used for CTC considered as a binary (<5 or ≥5) or a continuous variable, respectively. CTC=circulating tumour cell. HR=hormone receptor. Soft tissue metastasis=lymph nodes, skin, gynaecological tract, and peritoneum. Treatment initiated=new line of treatment initiated after the baseline CTC count.

Table 1: **Patients baseline characteristics and CTC detection**

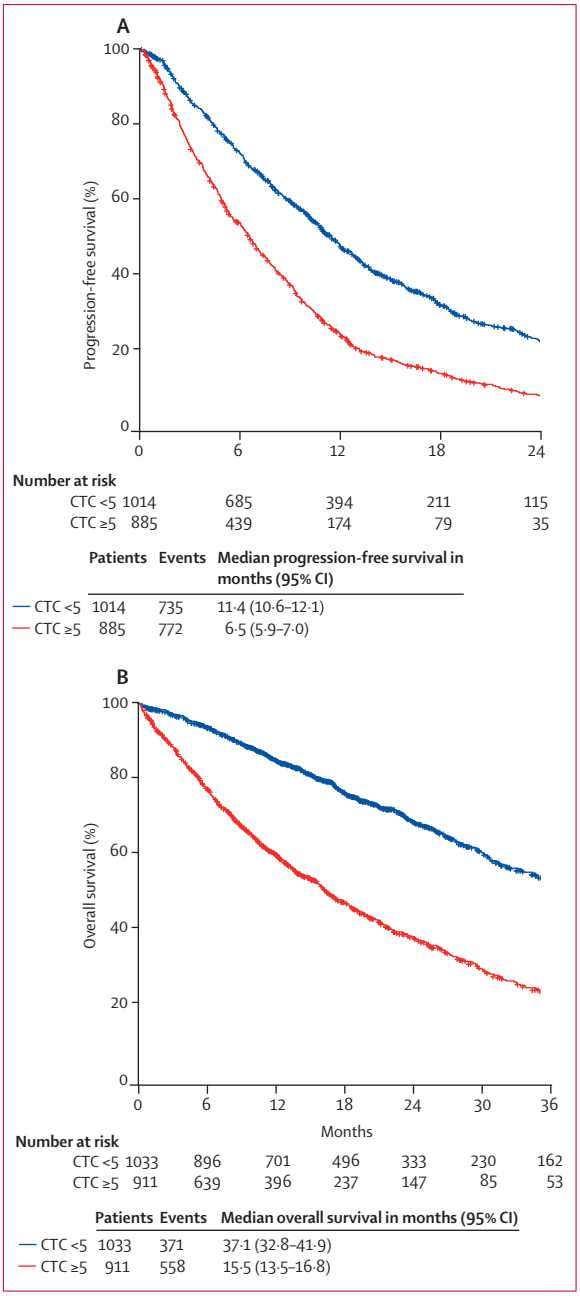

Figure 2: **Kaplan-Meier analysis of progression-free survival and overall survival, by baseline CTC count**
(A) Progression-free survival. (B) Overall survival. CTC=circulating tumour cell.

from any cause. Patients without documented evidence of an event were censored at the date of last follow-up. We used Fisher's exact tests and Kruskal-Wallis tests to investigate associations of population characteristics with CTC count. The primary prespecified statistical analysis consisted of likelihood ratio (LR) statistics in Cox regression models stratified by study, to estimate the added value of CTCs or serum markers to a clinicopathological model. The clinicopathological model

Experimental Medicine, University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany (Prof K Pantel MD)**; Université Paris Descartes, Paris, France** (Prof J-Y Pierga)**; and Service de Biostatistique et d'Epidémiologie, Gustave Roussy, Université Paris-Sud, Villejuif, France** (S Michiels PhD)

Correspondence to:
Prof Jean-Yves Pierga, Department of Medical Oncology, Institut Curie, 75005 Paris, France
jean-yves.pierga@curie.fr

See **Online** for appendix

was established on the basis of the baseline characteristics, except CTCs and serum markers, using a forward selection strategy (p<0·05). To control for overfitting of the clinicopathological model and to estimate the added value of CTCs or serum markers in an unbiased fashion, we randomly divided the dataset 500 times into a training and validation series using the method described by Cuzick and colleagues,[17] with minor modifications. We fitted the clinicopathological model on the training series, and we calculated the average increases in LR statistic (χ^2 LR value and associated p value) and in c-index[18] on the validation series with 95% CIs based on the percentiles of the 500 resamples. We used cubic splines with two degrees of freedom to investigate non-linear associations in the Cox models; for example we compared whether CTCs added more prognostic value as a binary variable (≥5 *vs* <5 CTCs) or as a spline function. We used the landmark method to assess the prognostic effects of CTC and serum marker changes during treatment.[19] We used the Kaplan-Meier method to estimate survival curves. p values were two tailed. We used SAS (versions 9.2 and 9.3) and R (version 3.0) for statistical analyses.

As per French law, we reported this in-silico study of fully anonymised data to the French National Committee on Computing and Liberty (CNIL number 1659562v0).

Role of the funding source

Janssen Diagnostics, the Nuovo-Soldati foundation for cancer research, and the funding bodies of each of the studies included in this pooled analysis had no role in study design, data collection, data analysis, data interpretation, or writing of the report. F-CB, HJ, J-YP, and SM had access to the raw data. The corresponding author (J-YP) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

2400 potentially eligible patients were disclosed by 19 of the 51 centres we contacted. Two centres did not provide further data. The remaining 17 centres provided data for 2174 potentially eligible patients, of which we excluded 230 ineligible patients, leaving 1944 eligible patients from 20 studies and 17 centres (figure 1; appendix p 1–2, p 12).

We identified a high baseline CTC count (≥5 CTC per 7·5 mL) in 911 of the 1944 patients (46·9%, 95% CI 44·7–49·1). Median CTC count was 3 CTC per 7·5 mL

	Model 1 average c-index	Model 2	Model 2 average c-index	Average c-index increase model 2–model 1 (95% CI)	Average increase χ^2 (95% CI)	Likelihood ratio test p value
Progression-free survival (N=1196 patients)						
Model 1: CP	0·668	CP+CTC_{BL} (< or ≥5 CTC)	0·684	0·016 (0 to 0·029)	38·4 (21·9 to 60·3)	<0·0001
Model 1: CP	0·668	CP+CTC_{BL} (splines)	0·673	0·005 (–0·001 to 0·010)	18·7 (9·1 to 35·4)	<0·0001
Overall survival (N=1501 patients)						
Model 1: CP	0·714	CP+CTC_{BL} (< or ≥5 CTC)	0·745	0·031 (0·013 to 0·047)	64·9 (41·3 to 93·4)	<0·0001
Model 1: CP	0·714	CP+CTC_{BL} (splines)	0·721	0·007 (0·001 to 0·014)	21·2 (10·2 to 37·3)	<0·0001
Progression-free survival, CTC count at weeks 3–5 (N=436 patients)						
Model 1: CP+CTC_{BL}	0·652	CP+CTC_{BL}+CTC_{3-5} (< or ≥5 CTC)	0·659	0·008 (–0·009 to 0·021)	8·2 (0·78 to 20·4)	0·004
Model 1: CP+CTC_{BL}	0·652	CP+CTC_{BL}+CTC_{3-5} (splines)	0·655	0·004 (–0·009 to 0·017)	7·4 (2·3 to 16·7)	0·02
Overall survival, CTC count at weeks 3–5 (N=568 patients)						
Model 1: CP+CTC_{BL}	0·720	CP+CTC_{BL}+CTC_{3-5} (< or ≥5 CTC)	0·732	0·011 (–0·008 to 0·027)	11·5 (2·6 to 25·1)	0·0007
Model 1: CP+CTC_{BL}	0·721	CP+CTC_{BL}+CTC_{3-5} (splines)	0·725	0·004 (–0·01 to 0·018)	8·2 (3·4 to 23·7)	0·02
Progression-free survival, CTC count at weeks 6–8 (N=279 patients)						
Model 1: CP+CTC_{BL}	0·602	CP+CTC_{BL}+CTC_{6-8} (< or ≥5 CTC)	0·628	0·026 (0 to 0·053)	15·3 (5·2 to 28·3)	<0·0001
Model 1: CP+CTC_{BL}	0·601	CP+CTC_{BL}+CTC_{6-8} (splines)	0·613	0·012 (–0·01 to 0·036)	10·2 (3·7 to 18·6)	0·006
Overall survival, CTC count at weeks 6–8 (N=380 patients)						
Model 1: CP+CTC_{BL}	0·671	CP+CTC_{BL}+CTC_{6-8} (< or ≥ 5 CTC)	0·686	0·016 (–0·015 to 0·041)	14·6 (4·0 to 30·6)	0·0001
Model 1: CP+CTC_{BL}	0·670	CP+CTC_{BL}+CTC_{6-8} (splines)	0·680	0·010 (–0·028 to 0·051)	10·6 (3·4 to 22·1)	0·005
Progression-free Survival, CTC count available both at weeks 3–5 and 6–8 (N=184 patients)						
Model 1: CP+CTC_{BL}	0·560	CP+CTC_{BL}+CTC_{3-5}(< or ≥5 CTC)	0·579	0·019 (–0·018 to 0·055)	5·5 (0·66 to 12·7)	0·02
Model 1: CP+CTC_{BL}	0·562	CP+CTC_{BL}+CTC_{6-8} (< or ≥5 CTC)	0·590	0·029 (–0·019 to 0·065)	9·2 (2·1 to 18·1)	0·002
Overall survival, CTC count available both at weeks 3–5 and 6–8 (N=216 patients)						
Model 1: CP+CTC_{BL}	0·617	CP+CTC_{BL}+CTC_{3-5} (< or ≥5 CTC)	0·634	0·017 (–0·027 to 0·057)	7·2 (0·0 to 30·6)	0·007
Model 1: CP+CTC_{BL}	0·613	CP+CTC_{BL}+CTC_{6-8} (< or ≥5 CTC)	0·633	0·021 (–0·046 to 0·067)	10·1 (2·2 to 20·9)	0·001

CTC=circulating tumour cells. CP=baseline clinicopathological model (appendix pp 3–5). CTC_{BL}=CTC count at baseline. CTC_{3-5}=CTC count at 3–5 weeks. CTC_{6-8}=CTC count at 6–8 weeks.

Table 2: **Assessment of added prognostic information of CTC at baseline and during treatment, by model 1**

(IQR 0–25; range 0–58160). Table 1 shows characteristics of patients and tumours, together with their association with CTC count. CTC count of 5 per 7·5 mL or higher was strongly associated (p<0·0001) with altered performance status, presence of bone or liver metastases, and elevated (ie, above ULNV) carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) concentrations. CTC count was also significantly associated with the number of previous hormone therapy lines and chemotherapy lines received, the use of chemotherapy as further treatment, metastases in the lung or pleura, and "other" metastases. We identified a significantly lower frequency of CTC counts of 5 per 7·5 mL or higher in the HER2-positive subtypes than in other subtypes of breast cancer and in patients receiving anti-HER2 targeted therapy as the initiated treatment (table 1). In the subgroup of patients starting first-line chemotherapy (N=1110), the lower incidence of CTC in the HER2-positive tumour subgroup was not noted (p=0·71), whereas the other the associations reported above were still significant.

Median follow-up for the pooled population was 23 months (IQR 13–42). 1507 (of 1899) patients progressed and 929 (of 1944) died. In Cox regression analyses stratified by study, a CTC count of 5 per 7·5 mL or higher at baseline was a significant negative prognostic factor for progression-free survival (HR 1·92, 95% CI 1·73–2·14, p<0·0001) and overall survival (HR 2·78, 95% CI 2·42–3·19, p<0·0001; figure 2). When using continuous CTC counts, we identified a linear increase in the logarithm of HR for increasing CTC values (appendix p 13).

To assess the added value of CTCs compared with currently used clinical variables, we built full clinicopathological prognostic models for progression-free survival and overall survival, including all the clinicopathological characteristics showing a prognostic effect in multivariate analysis. The clinicopathological model for progression-free survival contained tumour histological subtype and histological grade, number of previous lines of chemotherapy and hormone therapy received for metastatic disease, performance status, and presence of liver or visceral metastasis. Synchronous metastases at diagnosis of breast cancer (de-novo metastatic breast cancer) were included in the clinicopathological model, being associated with longer progression-free survival. The clinicopathological model for overall survival contained tumour histological subtype, number of previous hormone therapies, performance status, and liver metastases. Brain or leptomeningeal metastases also had a significant effect on overall survival, as did metastasis-free interval and age of patient (appendix pp 2–5).

With a resampling procedure, the addition of CTC count status (<5 *vs* ≥5 CTC) at baseline to the clinicopathological models resulted in a significant increase in overall survival prognostication (LR χ^2 64·9 [95% CI 41·3–93·4], p<0·0001, in 1501 patients with full clinicopathological characteristics) and progression-free survival prognostication (LR χ^2 38·4 [95% CI 21·9–60·3], p<0·0001, in 1196 patients with full clinicopathological characteristics). Concordance indices confirmed these results; whereas the clinicopathological models had c-indices of 0·668 for progression-free survival

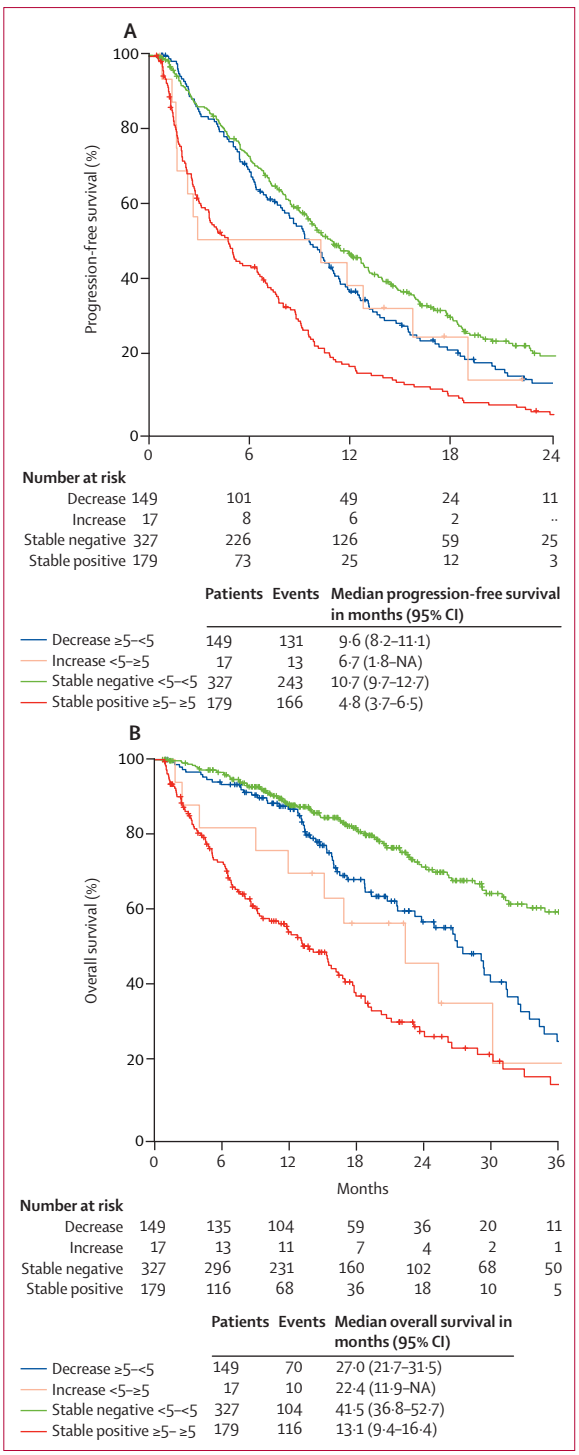

Figure 3: **Kaplan-Meier analysis of progression-free survival and overall survival, by early change in CTC count (landmark analysis at 5 weeks)**
(A) Progression-free survival. (B) Overall survival. CTC=circulating tumour cell.

and 0·714 for overall survival, addition of CTC count to clinicopathological models significantly increased C-indices by 0·016 (95% CI 0·000–0·029) for progression-free survival and by 0·031 (95% CI 0·013–0·047) for overall survival. When modelling CTC count as continuous variable (splines), we identified significant but smaller improvements compared with modelling CTC as a binary variable (cutoff CTC baseline ≥5 per 7·5 mL; table 2).

	Number of patients	Number of events	HR for ≥5 CTC (95% CI)	Interaction test (p value)
Performance status at inclusion				
Full model	1587	756	2·84 (2·44–3·32)	0·84
0	617	242	2·82 (2·14–3·71)	..
1	743	364	2·55 (2·04–3·20)	..
2	171	108	2·24 (1·41–3·55)	..
3 or 4	56	42	1·66 (0·54–5·11)	..
Tumour subtype				
Full model	1880	910	2·73 (2·37–3·14)	0·12
HER2+	474	239	2·42 (1·82–3·22)	..
Hormone receptor +	1166	537	2·83 (2·35–3·41)	..
Triple negative	240	134	3·46 (2·32–5·16)	..
Number of metastatic sites				
Full model	1884	920	2·78 (2·42–3·19)	0·72
≥3 sites	684	372	2·68 (2·14–3·37)	..
<3 sites	1200	548	2·77 (2·31–3·33)	..
Metastatic sites				
Full model	1897	924	2·77 (2·41–3·18)	0·04
Bone only	254	88	2·67 (1·63–4·38)	..
Any CNS metastasis	194	137	1·97 (1·32–2·95)	..
Other	1449	699	3·05 (2·60–3·58)	..
Number of previous treatment lines (hormone therapy or chemotherapy)				
Full model	1711	836	2·79 (2·41–3·23)	0·08
0	790	295	2·88 (2·25–3·69)	..
1	322	183	4·43 (2·95–6·66)	..
2 or more	599	197	2·30 (1·86–2·84)	..
Treatment initiated: including chemotherapy				
Full model	1856	886	2·80 (2·44–3·23)	0·47
Yes	1555	771	2·68 (2·31–3·12)	..
No	301	115	3·07 (2·05–4·60)	..
Treatment initiated: including hormone therapy				
Full model	1856	886	2·80 (2·44–3·23)	0·41
Yes	274	102	3·54 (2·30–5·44)	..
No	1582	784	2·68 (2·31–3·11)	..
Treatment initiated: including any targeted therapy				
Full model	1856	886	2·80 (2·44–3·23)	0·68
Yes	799	309	2·84 (2·20–3·66)	..
No	1057	577	2·84 (2·38–3·39)	..
Treatment initiated: including bevacizumab				
Full model	1856	886	2·80 (2·44–3·23)	0·99
Yes	400	114	3·03 (1·98–4·62)	..
No	1456	772	2·82 (2·42–3·28)	..
Treatment initiated: including anti-HER2 targeted therapy				
Full model	1856	886	2·80 (2·44–3·23)	0·35
Yes	379	184	2·57 (1·83–3·61)	..
No	1477	702	2·85 (2·43–4·34)	..

CTC=circulating tumour cells. HR=hazard ratio.

Table 3: **Subgroup analyses by univariate Cox regression of overall survival on CTC positivity status at baseline**

CTC counts after 3–5 weeks of treatment were available in 672 patients and after 6–8 weeks of treatment were available in 432 patients. Changes in CTC count between baseline and week 3–5 (ie, after one to two cycles of treatment) were strongly associated with progression-free survival and overall survival (p<0·0001 comparing the four groups for progression-free and overall survival, figure 3). When adjusted for baseline CTC count, increased CTC counts (≥5 CTC per 7·5 mL) after 3–5 weeks were also associated with progression-free survival (HR 1·85, 95% CI 1·48–2·32, p<0·0001) as were changes after 6–8 weeks (2·20, 1·66–2·90, p<0·0001). Similarly, after adjustment for baseline CTC count, increased CTC counts after 3–5 weeks were associated with shortened overall survival (HR 2·26, 95% CI 1·68–3·03, p<0·0001) as were changes after 6–8 weeks (HR 2·91, 2·01–4·23, p<0·0001). Of 328 patients with a high baseline CTC count (≥5 CTC per 7·5 mL), an early decrease of CTC to less than 5 CTC per 7·5 mL at week 3–5 was identified in 149 patients (45%; figure 3). Patients exhibiting these early CTC count changes after therapy had a significantly longer overall and progression-free survival than did patients whose CTC count remained above 5 per 7·5 mL (figure 3). We identified much the same associations for changes between CTC count at baseline and at 6–8 weeks of treatment (p<0·0001; appendix pp 14–15).

We next assessed whether CTC changes at 3–5 weeks would further improve prognostication of overall and progression-free survival when compared with the best baseline models—ie, models including baseline clinicopathological characteristics and baseline CTC count. LR tests were significant for progression-free survival (LR 8·2, 95% CI 0·78–20·4, p=0·004, in 436 patients) and overall survival (11·5, 2·6–25·1, p=0·0007, in 568 patients). We obtained much the same results with C-indices and with CTC count at 6–8 weeks (table 2).

A significant prognostic effect of high CTC count (≥5 CTC per 7·5 mL) at baseline and at 3–5 weeks (appendix pp 7–8) on progression-free survival and overall survival was maintained across all subgroups tested, which included breast cancer subtypes and type of treatment started (tables 3, 4).

Baseline CA15-3 was assessed in 1298 patients and raised in 892 (68·7%; median=1·90 ×ULNV; IQR 0·80–6·20). Baseline CEA was assessed in 893 patients and raised in 483 (54·1%; median=1·10×ULNV; IQR 0·40–4·50). None of the participating centres reported CA27-29 concentrations. In univariate analysis, raised CA15-3 was significantly associated with shorter progression-free survival (HR 1·54, 95% CI 1·33–1·78, p<0·0001) and overall survival (1·59, 95% CI 1·31–1·93, p<0·0001) as was raised CEA (progression-free survival HR 1·40,

95% CI 1·20–1·64, p<0·0001; overall survival 1·67, 1·36–2·03, p<0·0001). When added to the clinicopathological models, LR tests showed that the dichotomised serum markers (<ULNV *vs* ≥ULNV) added some prognostic information but increases in C-indices were negligible (appendix pp 9–11). When added to the best performing clinicopathological plus CTC baseline model, only CEA significantly added information based on the LR test (LR 4·1, 95% CI 0·06–12·1, p=0·047, in 754 patients) but the increase in c-index was also small. Results did not change substantially when modelling the serum markers as continuous values (appendix pp 9–11). Importantly, changes in serum tumour markers during treatment, both at 3–5 and 6–8 weeks, did not add any significant prognostic value to the best baseline model, nor to models including CTC changes during treatment (appendix pp 9–11).

Discussion

On the basis of individual patient data from both reported and unreported studies, our results provide firm evidence for the prognostic value of CTC detection at baseline and during treatment. Moreover, our analysis reports the clinical validity of early CTC changes during a new line of treatment in a specific patient, a favourable comparison with serum tumour markers, and show that these findings were not restricted to a specific subgroup (panel).

Non-European data were excluded to ensure that CTC counts and changes were not used by physicians to modify the treatment of patients, as allowed by the US FDA. By its retrospective nature, the limitations to this study are potential selection bias, incomplete data collection, and absence of centralised radiological and pathological review. However, the pre-established protocol, the finite number of CellSearch platforms in Europe, and the high number of patients included might have decreased the effect of these potential biases and allowed us to do analyses with a previously unreached statistical power.

We confirmed the association of CTC count with other known unfavourable prognostic markers (eg, altered performance status), and with bone metastasis, which is usually thought to be associated with more favourable outcome. In patients with no previous treatment for metastatic disease, CTC count was not associated with breast tumour histological subtype; the recorded overall lower incidence of CTCs in HER2-positive breast cancer might be explained by a previous exposure to anti-HER2 therapies which have been shown to substantially decrease the number of CTCs.[11,20] Our main result confirmed that CTC count at baseline is a strong independent prognostic marker that adds value to the existing clinical prognostic variables. Contemporary prognostic models including CTCs are needed to better stratify patients and facilitate risk-directed treatment selection in clinical practice. To show the clinical utility of the prognostic information of CTC count at baseline, a randomised phase 3 trial is now testing CTC count as a method to guide the choice

	Number of patients	Number of events	HR for ≥5 CTC (95% CI)	Interaction test (p value)
Performance status at inclusion				
Full model	1587	1265	1·96 (1·74–2·20	0·58
0	617	464	1·82 (1·50–2·21)	..
1	743	599	1·87 (1·58–2·22)	..
2	171	151	1·44 (0·99–2·11)	..
3 or 4	56	51	1·48 (0·51–4·31)	..
Tumour subtype				
Full model	1835	1475	1·91 (1·72–2·13)	0·16
HER2+	460	367	1·55 (1·24–1·94)	..
Hormone receptor +	1142	911	2·00 (1·74–2·29)	..
Triple negative	233	197	2·15 (1·56–2·96)	..
Number of metastatic sites				
Full model	1852	1491	1·91 (1·72–2·12)	0·87
≥3 sites	668	568	1·77 (1·48–2·12)	..
<3 sites	1184	923	1·91 (1·67–2·19)	
Metastatic sites				
Full model	1852	1491	1·91 (1·72–2·12)	0·24
Bone only	252	176	1·87 (1·33–2·62)	..
Any CNS metastasis	183	167	1·47 (1·03–2·09)	..
Other	1417	1148	2·03 (1·79–2·29)	..
Number of previous treatment lines (hormone therapy or chemotherapy)				
Full model	1711	1366	1·97 (1·76–2·20)	0·12
0	790	581	2·08 (1·75–2·46)	..
1	322	250	2·43 (1·84–3·20)	..
2 or more	599	535	1·63 (1·36–1·96)	..
Treatment initiated: including chemotherapy				
Full model	1811	1461	1·91 (1·71–2·12)	0·87
Yes	1510	1241	1·88 (1·68–2·12)	..
No	301	220	1·93 (1·44–2·58)	..
Treatment initiated: including hormone therapy				
Full model	1811	1461	1·91 (1·71–2·12)	0·26
Yes	274	196	2·36 (1·73–3·22)	..
No	1537	1265	1·83 (1·63–2·05)	..
Treatment initiated: including any targeted therapy				
Full model	1811	1461	1·91 (1·71–2·12)	0·59
Yes	786	604	1·94 (1·63–2·30)	..
No	1025	857	1·88 (1·63–2·17)	..
Treatment initiated: including bevacizumab				
Full model	1811	1461	1·91 (1·71–2·12)	0·53
Yes	397	292	2·18 (1·70–2·78)	..
No	1414	1169	1·85 (1·64–2·09)	..
Treatment initiated: including anti-HER2 targeted therapy				
Full model	1811	1461	1·91 (1·71–2·12)	0·49
Yes	369	293	1·63 (1·26–2·11)	..
No	1442	1168	1·96 (1·74–2·21)	..

CTC=circulating tumour cells. HR=hazard ratio.

Table 4: **Subgroup analyses by univariate Cox regression of progression-free survival on CTC positivity status at baseline**

Panel: Research in context

Systematic review

We searched Medline and conference abstracts (San Antonio Breast Cancer Symposium, American Society of Clinical Oncology congress) to establish the feasibility of this study before contacting the European centres. We used the search terms "circulating tumour cells" and the MeSH terms "neoplastic cells, circulating" and "breast neoplasms". We restricted searches to reports in English published after Jan 1, 2003. The last search was done on March 10, 2012. On the basis of the results of the searches, we believed that data for 1000–1200 individual patients were potentially available, and as a result deemed initiation of the study worthwhile.

Interpretation

Our study shows the correlations between circulating tumour cell (CTC) count and clinical and pathological characteristics, and confirms the independent prognostic effect of CTC count on progression-free survival and overall survival, independent of the patient subgroup. As far as we are aware, our study is the first to show that the CTC count improves the prognostication of metastatic breast cancer when added to full clinicopathological predictive models, whereas serum tumour markers, by contrast, do not.

of the first-line treatment regimen (STIC trial NCT01710605).[21]

In the present cohort of patients who were mostly treated by chemotherapy, we have also confirmed that CTC changes during treatment are significantly associated with progression-free survival and overall survival, and showed that the addition of early CTC changes to a model with clinicopathological factors and CTC at baseline improves prognostic accuracy. This result supports the use of a CTC count change (ie, CTC response) as a potential early indicator of overall survival improvement in clinical trials. However, surrogacy was not assessable because the data collected did not originate from randomised therapeutic trials. To implement changes in CTC count as a method to manage treatment in patients with metastatic breast cancer, two randomised trials have been launched to assess the clinical utility of early CTC changes to drive therapy changes. In the SWOG0500 trial,[22] 120 patients with metastatic breast cancer with no early CTC decrease under first-line chemotherapy were randomised to continue this first-line therapy or to switch early to the second-line chemotherapy; the results showed no significant improvement in overall survival. Notwithstanding the trial's limited statistical power, the absence of an improvement in overall survival draws attention to the fact that existing second-line treatments are unlikely to rescue a spontaneous resistance to first-line cytotoxic regimens. More generally, it has never been proven that early treatment change, driven either by imaging or any other assessment technique, can lead to overall survival benefits. The main aim of the other randomised trial[21] (CirCe01 trial NCT01349842) is to provide evidence for the value of early CTC count-based discontinuation of ineffective, costly, and potentially harmful chemotherapy regimens in third-line or later therapy.

Our study also provides direct evidence to show the superiority of CTC compared with CEA and CA15-3 at each timepoint tested, and shows the limited clinical validity of serum tumour markers, which are currently recommended for monitoring of therapy for metastatic breast cancer by international guidelines.[23] Clinically, these results suggest that serum marker testing at baseline and during the first weeks of treatment can be favourably replaced by CTC count. The promise of circulating tumour DNA for monitoring of metastatic breast cancer has been reported but awaits further validation.[24,25]

In conclusion, CTC quantification using the CellSearch system should now be deemed to have reached the highest level of evidence[26] for clinical validity in patients with metastatic breast cancer. Clinically, CTC-based survival prognostication models, such as those developed in this study, should be thought of as the optimum prognostic models for counselling of patients (eg, when considering two different therapies) and as stratification or adjustment factors in clinical trials. In addition to the interventional trials testing the efficacy of anti-HER2 treatments in metastatic breast cancer considered as HER2-negative but with HER2-positive CTC (DETECT III NCT01619111, CirCe T-DM1 NCT01975142),[21] clinical implementation of molecular and genomic characterisation of CTCs for treatment selection is likely to be the next logical developmental step that can contribute to advancement of precision medicine.[27–29]

Contributors

F-CB, MI, KP, and J-YP were responsible for the initial concept of this study. F-CB and SM designed the study protocol and were in charge of the day-to-day running of the study. F-CB, DJP, TF, FN, RG-C, DM, JAG-S, JS, CC, PG, LM, RZ, AFdL, LDM-A, MI, RL, SJvL, FM-S, M-TS, JV-M, EP, FC, AB, ED-R, JK, S-JD, CR, AR, WJ, EM, VC, SA, CA, LD, E-FS, LZ, JSR-F, KP, and J-YP were all responsible for the reporting of individual patient data. F-CB did the review of the individual data sent by the participating centres. SM and HJ were the study statisticians and did the statistical analyses. F-CB, SM, and J-YP led the interpretation of the data and writing of the report. All authors had input into the data interpretation and preparation of the final report for publication.

Declaration of interests

F-CB, RZ, and J-YP received research grants—unrelated to this study—from Janssen Diagnostics. F-CB, MI, KP, and J-YP have received consultancy fees from Janssen Diagnostics. HJ is employed by the International Drug Development Institute. The other authors declare that they have no competing interests.

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