

## **Circulating Tumor Cells at Each Follow-up Time Point during Therapy of Metastatic Breast Cancer Patients Predict Progression-Free and Overall Survival**

Daniel F. Hayes,<sup>1</sup> Massimo Cristofanilli,<sup>2</sup> G. Thomas Budd,<sup>3</sup> Matthew J. Ellis,<sup>4</sup> Alison Stopeck,<sup>5</sup> M. Craig Miller,<sup>6</sup> Jeri Matera,<sup>6</sup> W. Jeffrey Allard,<sup>6</sup> Gerald V. Doyle,<sup>6</sup> and Leon W.W.M. Terstappen<sup>6</sup>

**Abstract Purpose:** We reported previously that  $\geq 5$  circulating tumor cells (CTC) in 7.5 mL blood at baseline and at first follow-up in 177 patients with metastatic breast cancer (MBC) were associated with poor clinical outcome. In this study, additional follow-up data and CTC levels at subsequent follow-up visits were evaluated.

**Experimental Design:** CTCs were enumerated in 177 MBC patients before the initiation of a new course of therapy (baseline) and 3 to 5, 6 to 8, 9 to 14, and 15 to 20 weeks after the initiation of therapy. Progression-free survival (PFS) and overall survival (OS) times were calculated from the dates of each follow-up blood draw. Kaplan-Meier plots and survival analyses were done using a threshold of  $\geq 5$  CTCs/7.5 mL at each blood draw.

**Results:** Median PFS times for patients with  $< 5$  CTC from each of the five blood draw time points were 7.0, 6.1, 5.6, 7.0, and 6.0 months, respectively. For patients with  $\geq 5$  CTC, median PFS from these same time points was significantly shorter: 2.7, 1.3, 1.4, 3.0, and 3.6 months, respectively. Median OS for patients with  $< 5$  CTC from the five blood draw time points was all  $> 18.5$  months. For patients with  $\geq 5$  CTC, median OS from these same time points was significantly shorter: 10.9, 6.3, 6.3, 6.6, and 6.7 months, respectively. Median PFS and OS times at baseline and up to 9 to 14 weeks after the initiation of therapy were statistically significantly different.

**Conclusions:** Detection of elevated CTCs at any time during therapy is an accurate indication of subsequent rapid disease progression and mortality for MBC patients.

Although systemic treatment of patients with metastatic breast cancer (MBC) results in modest survival prolongation, palliation is the principal goal of treatment (1). Choice of treatment is based on both prognostic and predictive factors. Currently used prognostic factors include clinical features, such as time to

first recurrence, prior therapy, and location and number of metastatic sites (2–4). The predictive factors most commonly used are hormone receptor and HER-2 status for selection of endocrine and trastuzumab therapies, respectively. After initiation of systemic treatment, current methodologies do not often allow for an accurate and early assessment of clinical benefit especially in patients with nonmeasurable disease. Currently, clinicians use history, physical exam, radiographic analysis, and serologic testing. Although each of these tests may be helpful, they are often inaccurate early in a patient's course. Indeed, they may take several months to be definitive. Thus, patients with MBC may be either treated for prolonged periods with an inactive therapy or a potentially active therapy may be discontinued prematurely.

Recently, we reported the results of a prospective, multicenter trial that showed that circulating tumor cell (CTC) levels before starting a new therapy and at first follow-up were a strong predictor of rapid progression and death in patients with MBC about to start any new systemic therapy (5) and in a subset of these patients who were initiating first-line therapy (6). We now report the predictive importance of continued monitoring of CTC levels at subsequent time points beyond first follow-up.

### **Materials and Methods**

**Study design.** A prospective, double-blind, multi-institutional clinical trial was conducted at 20 clinical centers throughout the United States. A total of 177 MBC patients were enrolled and followed with

**Authors' Affiliations:** <sup>1</sup>Department of Internal Medicine and the Comprehensive Cancer Center, University of Michigan Health and Hospital System, Ann Arbor, Michigan; <sup>2</sup>Department of Breast Medical Oncology, M.D. Anderson Cancer Center, Houston, Texas; <sup>3</sup>Department of Medicine, Cleveland Clinic Foundation, Cleveland, Ohio; <sup>4</sup>Department of Medicine, Washington University, St. Louis, Missouri; <sup>5</sup>Department of Medicine, Arizona Cancer Center, Tucson, Arizona; and <sup>6</sup>Immunicon Corp., Huntingdon Valley, Pennsylvania

Received 12/27/05; revised 4/1/06; accepted 5/5/06.

**Grant support:** Immunicon, Inc. (Huntingdon Valley, PA).

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**Note:** Data from this clinical trial have been reported at the 2004 and 2005 Annual Meetings of the American Society of Clinical Oncology and at the 2004 to 2005 San Antonio Breast Cancer Symposium.

The main results of this trial about the outcomes according to circulating tumor cells at baseline and at first follow-up have been previously published by Cristofanilli et al. (see refs. 1 and 2 of this article). The data presented in this article about circulating tumor cells at each subsequent follow-up were presented at the 2004 San Antonio Breast Cancer Symposium but have not been previously published.

**Requests for reprints:** Daniel F. Hayes, University of Michigan, 6312 CCGC, 1500 East Medical Center Drive, Ann Arbor, MI 48109. Phone: 734-615-6725; Fax: 734-615-3947; E-mail: hayesdf@umich.edu.

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doi:10.1158/1078-0432.CCR-05-2821

**Table 1.** CTC counts at the different time points after initiation of therapy

|                       | Baseline | 3-5 weeks | 6-8 weeks | 9-14 weeks | 15-20 weeks |
|-----------------------|----------|-----------|-----------|------------|-------------|
| No. patients          | 177      | 132       | 99        | 129        | 84          |
| ≥5 CTCs, <i>n</i> (%) | 87 (49)  | 41 (31)   | 22 (22)   | 24 (19)    | 14 (17)     |
| Median CTC            | 24       | 32        | 34        | 45         | 15          |
| Mean CTC              | 530      | 359       | 167       | 916        | 156         |
| SD CTC                | 2,634    | 1,539     | 287       | 3,356      | 302         |
| Maximum CTC           | 23,618   | 9,864     | 983       | 16,488     | 970         |

CTC determinations and imaging evaluations. The trial objectives included the use of CTCs to predict response to therapy, progression-free survival (PFS), and overall survival (OS). Principal inclusion criteria were progressive measurable MBC, commencement of a new systemic therapy, and an Eastern Cooperative Oncology Group performance status score of 0 to 2. Prior adjuvant and/or metastatic treatment(s) of any type were permitted. The institutional review board at each center approved the study protocol, and all patients provided written informed consent.

Before starting a new systemic treatment, patients had computed tomography scans of the chest and abdomen, a whole-body bone scan, and a baseline blood draw for enumeration of CTC. Subsequent serial blood specimens were collected at roughly monthly intervals for a period of up to 6 months. Reassessment of disease status was conducted every 9 to 14 weeks depending on treatment type and schedule. Patient responses were determined by the clinical sites using standard International Union Against Cancer criteria (7) without knowledge of CTC results. Patients remained on study for up to 6 months or until they progressed or died, whichever occurred first.

Initial results of this trial have been reported elsewhere and only included CTC determinations before the initiation of therapy (baseline) and at the first follow-up blood draw (5, 6). Progression and survival data for this patient cohort were updated for this report for baseline and first follow-up (3-5 weeks). The number of evaluable patients at each succeeding time point decreases due to patient dropout at preceding time points. Consequently, the power to do landmark analyses at each time point is low and we arbitrarily "pooled" what we felt were important time points along an average patient's clinical course. After the 6- to 8-week time point, we combined the 9- to 11-week and 12- to 14-week time points into 9 to 14 weeks and the 15- to 17-week and 18- to 20-week time points into 15 to 20 weeks. In summary, we compared and evaluated the CTC levels before the initiation of therapy (baseline) and at 3 to 5, 6 to 8, 9 to 15, and 15 to 20 weeks after the initiation of therapy to predict PFS and OS.

**Isolation and enumeration of CTC.** CTCs were isolated and enumerated at each blood draw using the CellSearch System (Veridex LLC, Raritan, NJ). The operational details and preclinical performance data, including accuracy, precision, linearity, and reproducibility, have

**Table 2.** PFS for patients with <5 or ≥5 CTC at different time points

| CTC/<br>7.5 mL                  | Baseline         |               | 3-5 weeks        |               | 6-8 weeks        |               | 9-14 weeks       |                 | 15-20 weeks      |                 |
|---------------------------------|------------------|---------------|------------------|---------------|------------------|---------------|------------------|-----------------|------------------|-----------------|
|                                 | <5               | ≥5            | <5               | ≥5            | <5               | ≥5            | <5               | ≥5              | <5               | ≥5              |
| Patients,<br><i>n</i> (%)       | 90 (51)          | 87 (49)       | 89 (71)          | 37 (29)       | 73 (83)          | 15 (17)       | 91 (89)          | 11 (11)         | 64 (85)          | 11 (15)         |
| Median<br>PFS, mo<br>(95% CI)   | 7.0 (5.8-8.9)    | 2.7 (2.1-4.4) | 6.1 (4.7-8.6)    | 1.3 (0.7-2.1) | 5.6 (4.5-7.6)    | 1.4 (0.6-3.4) | 7.0 (5.1-8.8)    | 3.0 (0.9-4.8)   | 6.0 (3.8-8.7)    | 3.6 (0.7-6.7)   |
| Log-rank <i>P</i>               | 0.0001           |               | <0.0001          |               | 0.0001           |               | 0.0134           |                 | 0.0424           |                 |
| Cox hazard<br>ratio<br>(95% CI) | 1.89 (1.37-2.61) |               | 2.31 (1.53-3.47) |               | 3.03 (1.68-5.48) |               | 2.26 (1.16-4.42) |                 | 2.01 (1.01-4.01) |                 |
| <i>P</i>                        | 0.0001           |               | 0.0001           |               | 0.0009           |               | 0.0301           |                 | 0.0660           |                 |
| PD within<br>3 months<br>(%)*   | 20               | 54            | 19               | 68            | 19               | 53            | NP <sup>†</sup>  | NP <sup>†</sup> | NP <sup>†</sup>  | NP <sup>†</sup> |
| Fisher's<br>exact <i>P</i>      | <0.001           |               | <0.001           |               | 0.009            |               | NP <sup>†</sup>  |                 | NP <sup>†</sup>  |                 |
| PD within<br>6 months<br>(%)*   | 40               | 68            | 43               | 76            | 40               | 73            | 25               | 55              | 17               | 27              |
| Fisher's<br>exact <i>P</i>      | <0.001           |               | 0.001            |               | 0.023            |               | 0.071            |                 | 0.420            |                 |

Abbreviations: PD, progressive disease; NP, not performed.

\*Progressive disease within 3 or 6 months from the time of the baseline blood draw.

<sup>†</sup> Nine to 14 weeks and 15 to 20 weeks are blood draws taken at roughly the 3-month time point and after.

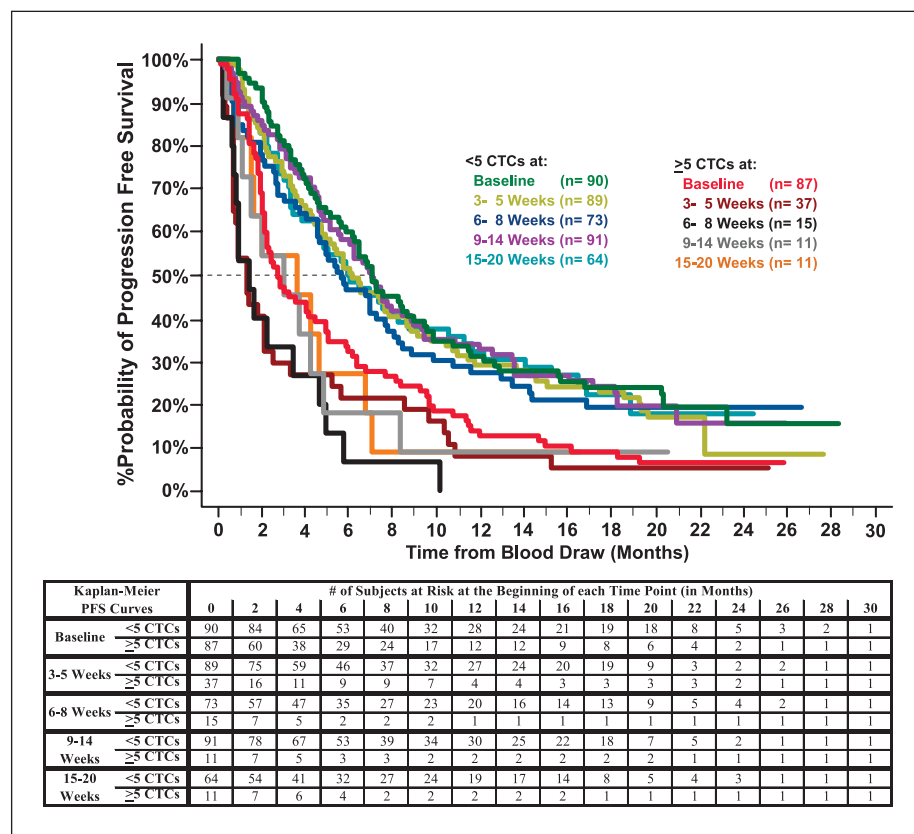


Fig. 1. Kaplan-Meier plots of patients with <5 and ≥5 CTCs at baseline and at 3 to 5 weeks, 6 to 8 weeks, 9 to 14 weeks, and 15 to 20 weeks after the initiation of therapy to predict time to clinical progression or death in 177 patients with MBC. PFS times were calculated from the time of each blood draw.

been described previously (8). Blood samples were drawn into 10 mL EDTA Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ), to which a cellular preservative was subsequently added. Samples were maintained at room temperature and processed within 72 hours after collection. All CTC evaluations were done at one of the two central laboratories (Immunicon, Huntingdon Valley, PA or IMPATH Predictive Oncology, Los Angeles, CA) or at five participating academic centers. The CellSearch System consists of a semiautomated sample preparation system and the CellSearch Epithelial Cell kit to immunomagnetically enrich cells expressing the epithelial cell adhesion molecule (9). Isolated cells are then fluorescently labeled with the nucleic acid dye 4',6-diamidino-2-phenylindole and labeled monoclonal antibodies specific for leukocytes (CD45-allophycocyanin) and epithelial cells (cytokeratin 8,18,19-phycoerythrin). Identification and enumeration of CTCs was done using the CellSpotter Analyzer (Immunicon), a semi-automated fluorescence microscopy system that permits computer-generated reconstruction of cellular images (9). CTCs were defined as nucleated cells lacking CD45 and expressing cytokeratin (8, 9). All assays were done by trained operators who were blinded to patient outcomes.

**Statistical analysis.** PFS was defined as the elapsed time between the date of the blood draw and either the date of clinical progression, death, or the last follow-up (if no progression and/or death were observed during the follow-up period). OS was defined as the elapsed time between the date of the blood draw and either the date of death or the last follow-up (if death was not observed during the follow-up period). Survival curves were compared using log-rank testing for two curves and Wilcoxon testing for multiple curves. A threshold of ≥5 CTC/7.5 mL, the derivation of which has been described previously in detail (5), was used for analysis of PFS and OS at each of the blood draw time points. Patients who progressed according to classic clinical and/or radiographic criteria before any of the follow-up blood draws after the initiation of therapy were excluded from the analysis of PFS for those follow-up blood draws. The median PFS and OS times and the 95% confidence intervals (95% CI) were determined from the

Kaplan-Meier product limit estimate of the survivor functions. Cox proportional hazards regression analysis was used to determine hazard ratios for PFS and OS. The distribution of patients above and below the CTC threshold who progressed within 3 or 6 months from the time of the baseline blood draw or who died within 6 or 12 months from the time of the baseline blood draw was compared using Fisher's exact test. All *P*s reported are two sided, and all analyses were done using Intercooled Stata 9.0 for Windows (StataCorp LP, College Station, TX).

**Role of the funding source.** This study was designed by the sponsor (Immunicon) in collaboration with the clinical investigators and with input from the U.S. Food and Drug Administration's Center for Devices and Radiological Health. An independent clinical research organization collected and monitored all of the clinical and laboratory data. The CTC laboratory data were also collected and verified by the sponsor. Locked and validated databases containing the combined clinical and laboratory data were analyzed separately by both the clinical research organization and the sponsor. The sponsor and clinical investigators jointly decided to publish the results and jointly authored this article.

## Results

**Patient characteristics.** Characteristics of the 177 MBC patients enrolled in the study have been previously reported (5). Nine patients died and five patients withdrew from the study after providing only a baseline blood draw. Of the remaining 163 patients with a follow-up disease assessment, 26 (16%) had a partial response to therapy, 82 (50%) had stable disease, and 55 (34%) had progressive disease at the time of the first follow-up according to classic clinical and/or radiographic criteria. None of the patients showed a complete response to their therapy at any time during the study.

The time between the baseline and follow-up blood draws for the patients reflected standard clinical practice among the

participating physicians. A total of 152 (86%) patients showed evidence of progression during the follow-up period and 102 (58%) patients died. The overall median PFS and OS times for the total patient sample were 5.0 months (95% CI, 4.0-6.4) and 18.4 months (95% CI, 14.6-20.6), respectively. Follow-up times for the 75 patients still alive at the time of the last contact ranged from 2.7 to 28.8 months (mean, 19.5 ± 5.7).

A total of 177, 132, 99, 129, and 84 patients had a blood draw at baseline and 3 to 5, 6 to 8, 9 to 14, and 15 to 20 weeks after initiation of therapy, respectively, and these patients were evaluable for the OS end point. This trial was prospectively double blinded. Thus, clinicians were unaware of the CTC results and laboratory investigators were unaware of the clinical results. Therefore, clinical decisions about progression were based on classic clinical and radiographic criteria. For the PFS end point, any patient who exhibited clinical and/or radiographic evidence of progression before the blood draw time point was not included in the analysis of subsequent blood draws. For PFS, 177, 126, 88, 102, and 75 patients were evaluable at 3 to 5, 6 to 8, 9 to 14, and 15 to 20 weeks after initiation of therapy, respectively.

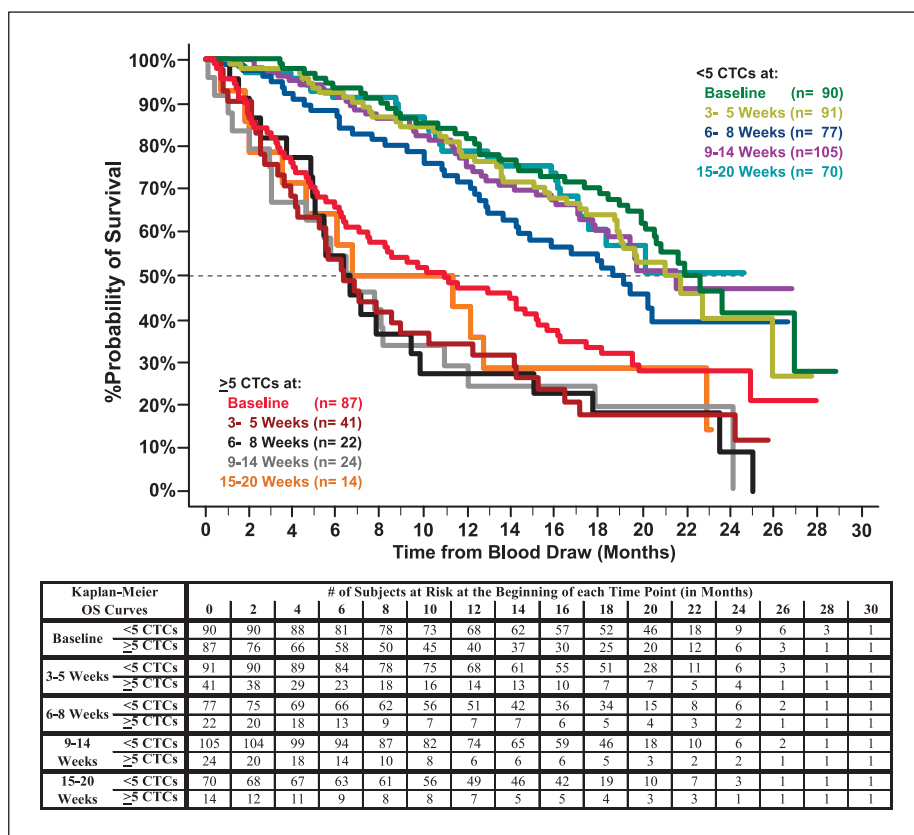
A total of 96 (54%) patients had ≥5 CTCs at one or more of the blood draws. The number of CTCs detected in these patients ranged from 5 to 23,618, and the median number of CTCs varied from 24 to 45. Table 1 summarizes the percentage of patients with ≥5 CTCs as well as the mean, median, SD, and maximum number of CTCs in those patients with ≥5 CTCs at each of the blood draws. As illustrated in Table 1, the percentage of patients with ≥5 CTCs decreased with each succeeding blood draw.

**Analysis of PFS and OS according to CTC levels at each time point.** The median PFS times for those patients with <5 CTCs

ranged from 5.6 to 7.0 months and were significantly longer than the median PFS times for those patients with ≥5 CTCs, which ranged from 1.3 to 3.6 months (Table 2). Patients with elevated CTCs (≥5 CTC/7.5 mL whole blood) at any of the time points had a much higher likelihood of rapid progression than did those with <5 CTCs (Fig. 1). Furthermore, patients with ≥5 CTCs at any of the time points had a much higher likelihood of rapid death than did those with <5 CTCs (Fig. 2). The median OS times for those patients with <5 CTCs ranged from 18.6 to >25.0 months and were substantially longer than the median OS times for those patients with ≥5 CTCs, which ranged from 6.3 to 10.9 months (Table 3).

**Analysis of PFS and OS according to change in CTC level from baseline for 15 to 20 weeks.** To investigate whether a change in CTC predicts rapid progression and mortality, we compared changes in levels between initiation of therapy and an arbitrarily chosen time point of 15 to 20 weeks after initiation of therapy. Figure 3A and B shows the Kaplan-Meier curves for PFS and OS, respectively, using CTC determinations up to 15 to 20 weeks after the initiation of therapy. Four different groups of patients are compared: group 1 (n = 83; Fig. 3A, line 1), patients with <5 CTCs at all blood draw time points; group 2 (n = 38; Fig. 3A, line 2), patients with ≥5 CTCs before the initiation of therapy but who had decreased to <5 CTCs at the time of their final blood draw; group 3 (n = 17; Fig. 3A, line 3), patients with <5 CTCs at baseline and/or 3 to 5 weeks after the initiation of therapy who increased to ≥5 CTCs at the time of their last blood draw; and group 4 (n = 39; Fig. 3A, line 4), patients with ≥5 CTCs at all blood draw time points. PFS and OS times were calculated from the time of the baseline blood draw. Patients with ≥5 CTCs at all time points (group 4) had the shortest

Fig. 2. Kaplan-Meier plots of patients with <5 and ≥5 CTCs at baseline and at 3 to 5 weeks, 6 to 8 weeks, 9 to 14 weeks, and 15 to 20 weeks after the initiation of therapy to predict time to death in 177 patients with MBC. OS times were calculated from the time of each blood draw.



**Table 3.** OS for patients with <5 or ≥5 CTC at different time points

| CTC/<br>7.5 mL                       | Baseline              |                    | 3-5 weeks             |                   | 6-8 weeks             |                  | 9-14 weeks            |                   | 15-20 weeks          |                   |
|--------------------------------------|-----------------------|--------------------|-----------------------|-------------------|-----------------------|------------------|-----------------------|-------------------|----------------------|-------------------|
|                                      | <5                    | ≥5                 | <5                    | ≥5                | <5                    | ≥5               | <5                    | ≥5                | <5                   | ≥5                |
| Patients,<br><i>n</i> (%)            | 90 (51)               | 87 (49)            | 91 (69)               | 41 (31)           | 77 (78)               | 22 (22)          | 105 (81)              | 24 (19)           | 70 (83)              | 14 (17)           |
| Median<br>OS, mo<br>(95% CI)         | 21.9<br>(20.1 to >25) | 10.9<br>(6.4-15.1) | 21.0<br>(18.8 to >25) | 6.3<br>(4.1-10.2) | 18.6<br>(14.2 to >25) | 6.3<br>(4.8-9.8) | 21.5<br>(17.8 to >25) | 6.6<br>(3.0-10.9) | >25<br>(17.1 to >25) | 6.7<br>(2.0-22.9) |
| Log-rank <i>P</i>                    | <0.0001               |                    | <0.0001               |                   | 0.0001                |                  | <0.0001               |                   | 0.0013               |                   |
| Cox hazard<br>ratio<br>(95% CI)      | 2.45 (1.64-3.65)      |                    | 3.37 (2.13-5.34)      |                   | 2.80 (1.62-4.85)      |                  | 3.88 (2.27-6.64)      |                   | 3.09 (1.50-6.34)     |                   |
| <i>P</i>                             | <0.0001               |                    | <0.0001               |                   | 0.0006                |                  | <0.0001               |                   | 0.0048               |                   |
| Death<br>within 6<br>months<br>(%)*  | 7                     | 34                 | 7                     | 39                | 10                    | 23               | 5                     | 29                | 3                    | 21                |
| Fisher's<br>exact <i>P</i>           | <0.001                |                    | <0.001                |                   | 0.156                 |                  | 0.001                 |                   | 0.031                |                   |
| Death<br>within 12<br>months<br>(%)* | 18                    | 53                 | 19                    | 66                | 23                    | 73               | 15                    | 67                | 9                    | 50                |
| Fisher's<br>exact <i>P</i>           | <0.001                |                    | <0.001                |                   | <0.001                |                  | <0.001                |                   | 0.001                |                   |

\*Death within 6 or 12 months from the time of the baseline blood draw.

median PFS (1.8 months; 95% CI, 1.4-2.2), which was significantly different compared with the median PFS of group 3 (5.9 months; 95% CI, 2.0-9.2; *P* = 0.0016), group 2 (6.7 months; 95% CI, 4.0-9.6; *P* < 0.0001), and group 1 (7.2 months; 95% CI, 5.8-9.5; *P* < 0.0001; Fig. 3A). Differences between the curves for the other groups in this figure were not significant.

Figure 3B shows that patients who exceeded the threshold of 5 CTCs at any point after the initiation of therapy are at a significantly higher risk of more rapid death. Patients with ≥5 CTCs at all time points (group 4) had the shortest median OS (4.1 months; 95% CI, 2.8-6.4), which was significantly different compared with the median OS of group 3 (10.6 months; 95% CI, 6.1-16.2; *P* = 0.0022), group 2 (19.8 months; 95% CI, 14.6 to >25; *P* < 0.0001), and group 1 (22.6 months; 95% CI, 20.5 to >25; *P* < 0.0001). Differences between groups 3 and 2 (*P* = 0.0496) and groups 3 and 1 (*P* = 0.0001) were also significant.

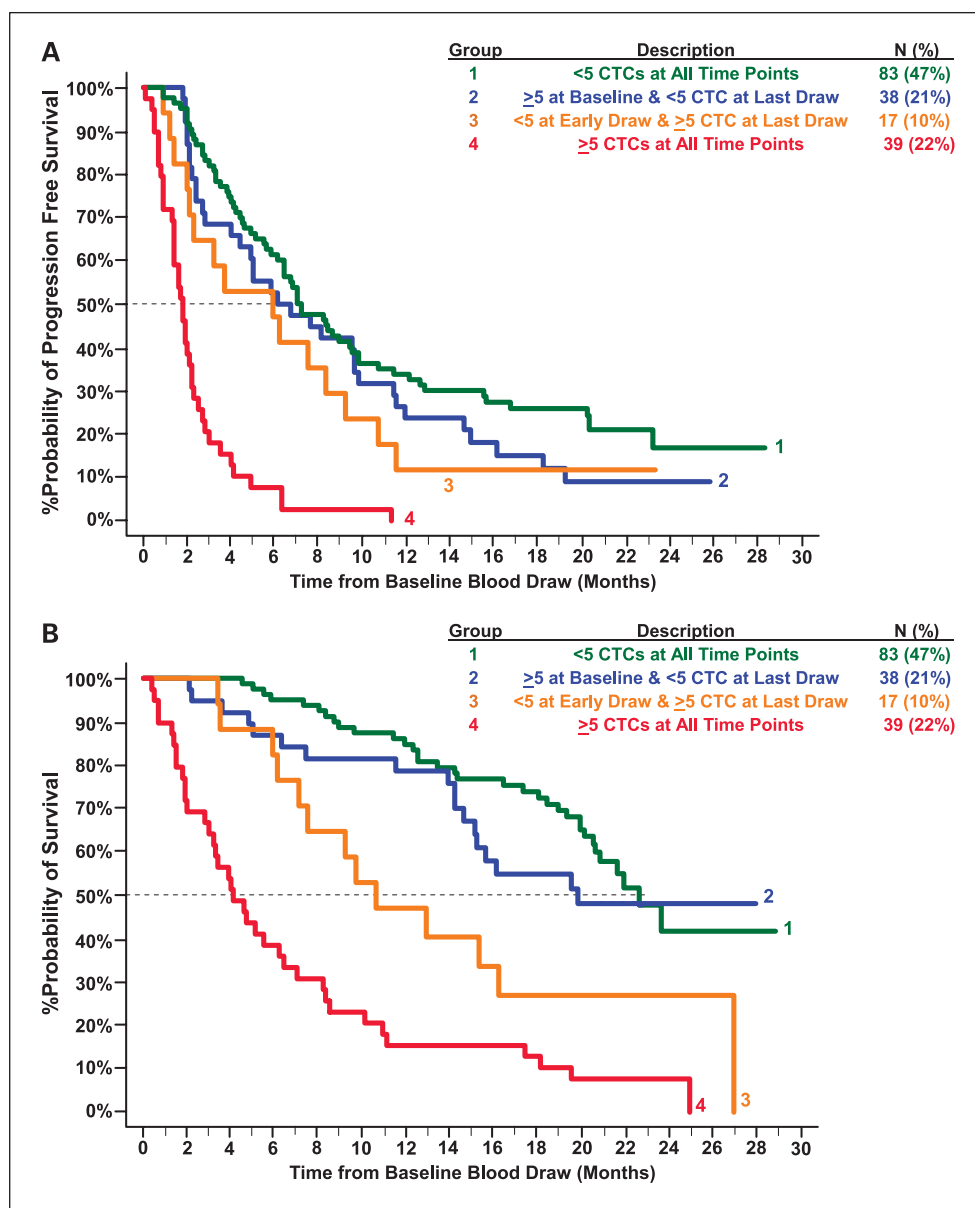
Patients who had ≥5 CTCs at baseline but eventually decreased to <5 CTCs after the initiation of therapy (Fig. 3B, line 2) had approximately the same risk of death (median OS, 19.8 months; 95% CI, 14.6 to >25) as those patients who never exceeded the 5 CTC threshold (median OS, 22.6 months; 95% CI, 20.5 to >25; Fig. 3B, line 1). Interestingly, OS for patients with <5 CTCs at baseline and/or 3 to 5 weeks after the initiation of therapy who eventually exceeded the threshold of 5 CTCs while on therapy (Fig. 3B, line 3) was, as expected, superior to those who always had elevated CTC (Fig. 3B, line 4). However, it was clearly shorter compared with the OS of the patients who either never had elevated CTC levels or for whom elevated CTC levels declined and remained <5/7.5 mL of whole blood

(Fig. 3B, lines 1 and 2). However, the OS for these two groups of patients was clearly worse compared with the OS of the patients who either never had elevated CTC levels or for whom elevated CTC levels declined <5/7.5 mL of whole blood.

**PFS and OS at each point excluding prior elevated CTCs.** Because the clinicians in this study were blinded to CTC data of participating patients, the results shown in Figs. 1-3 used all patients with available CTC results at each time point. In actual practice, however, patients with a positive CTC count of ≥5/7.5 mL whole blood at a preceding time point would probably be considered for a change in therapy. Therefore, we did further exploratory analyses using only those patients (*n* = 87) who had <5 CTCs at 3 to 5 weeks after the initiation of therapy and a subsequent blood draw between 6 and 20 weeks after the initiation of therapy. The median PFS (2.2 months) and OS (15.0 months) times (calculated from the time of the subsequent blood draw) for the patients with ≥5 CTCs at some point 6 and 20 weeks after the initiation of therapy were shorter compared with those patients who never had ≥5 CTCs after the initiation of therapy [median PFS of 5.8 months (*n* = 68); median OS of 19.6 months (*n* = 80)]. Although the numbers of patients with ≥5 CTCs at these subsequent follow-up time points (anywhere from 6 to 20 weeks after the initiation of therapy) were too small (*n* = 6 for PFS and *n* = 7 for OS) to permit a meaningful statistical analysis, the magnitudes of the differences in PFS and OS are consistent with those we have observed when comparing outcomes of patients with elevated versus not elevated CTC in the larger group as a whole (Figs. 1 and 2).



**Fig. 3.** Kaplan-Meier plot of CTC levels before and up to 15 to 20 weeks after the initiation of therapy to predict time to clinical progression or death (A) or the time to death (B) from the date of the baseline blood draw in 177 patients with MBC. Four different groups of patients are compared: group 1 (green curve), 83 (47%) patients with  $<5$  CTCs at all blood draw time points; group 2 (blue curve), 38 (21%) patients with  $\geq 5$  CTCs before the initiation of therapy but who had decreased to  $<5$  CTCs at the time of their final blood draw; group 3 (orange curve), 17 (10%) patients with  $<5$  CTCs at baseline and/or 3 to 5 weeks after the initiation of therapy who increased to  $\geq 5$  CTCs at the time of their last blood draw; and group 4 (red curve), 39 (22%) patients with  $\geq 5$  CTCs at all blood draw time points.



## Discussion

In a previously reported prospective, multi-institutional, double-blinded study, we showed that elevated CTC levels ( $\geq 5$  CTC/7.5 mL whole blood) before and at first follow-up after initiation of a new systemic therapy were strongly associated with short PFS and OS (5). In a separate analysis, we reported that changes in CTC may be a more robust indicator of clinical outcomes than the classically used criteria for determining response (10). Of particular interest was the observation that, although CTCs were elevated in  $\sim 50\%$  of patients before therapy, only 30% remained elevated at first follow-up, suggesting that  $\sim 40\%$  of patients with initially elevated CTCs benefited from the therapy (5). In this prospective longitudinal follow-on study of patients with MBC, CTCs were assessed serially over the course of treatment at additional specified intervals. The results showed that assessment of CTC levels at "any" subsequent follow-up time

points accurately and reproducibly predicted the clinical outcome. Patients who converted from elevated CTCs to nonelevated levels ( $<5$  CTC/7.5 mL) exhibited PFS and OS similar to those whose CTCs were never elevated. This would imply that patients with  $<5$  CTCs seem to be responding to treatment and/or have relatively indolent disease. Moreover, OS of patients who converted from nonelevated CTC levels to elevated CTC levels was decreased relative to those whose CTCs remained low but it was longer than the OS of patients who always exhibited elevated CTC levels.

The results of this study have clinical importance. Several different modalities and agents are active against MBC (1). Although these therapies are not curative, their judicious and timely application can achieve successful palliation and modest improvements in survival. The conundrum the clinician faces is what treatment to use, when to start, and when to change. Therapies are routinely changed due to lack of obvious benefit, onset of toxic secondary affects, or, after providing some initial

benefit, signs of progression. The clinician currently makes these determinations based on information from history and physical examinations, radiological studies, and/or serum marker analyses. Symptoms are notoriously difficult to follow, and the use of imaging is limited because ~50% of patients do not have clinically measurable disease (11–15). Moreover, most imaging modalities are expensive, often inconvenient for the patient, and may require several months or cycles of therapy before providing a reliable indication of clinical status (15). When imaging is not informative, serologic markers, such as routine liver function tests or circulating, soluble tumor markers, including assays for carcinoembryonic and muc-1 antigens (CA 15-3, CA 27.29), can be helpful (16–18). However, the specificity of these assays is limited due to elevations seen in benign, inflammatory, and other nonmalignant conditions (19). Furthermore, early analysis of carcinoembryonic and muc-1 antigens is confounded by the tumor marker spike phenomenon (16, 20, 21), which frequently results in transient elevation of the marker during the first 4 to 8 weeks of therapy and may be difficult to distinguish from true progression. In this regard, we have previously shown that CTC levels are not elevated in subjects with nonmalignant conditions nor are they directly related to tumor burden (5, 8). The results of the current study suggest that patients with elevated CTC later in their treatment course (third cycle or beyond) are very likely to progress in the immediate follow-up period and that a change in therapy may be indicated. The benefit of changing therapy very early in the course of treatment, such as first follow-up, without other obvious clinical and/or radiographic

signs of progression, is unknown. This issue is the objective of a planned prospective randomized clinical trial currently under development. However, standard practice is to change therapy after several weeks or months of therapy if there is evidence of progression. Our data indicate that the observation of elevated CTCs at such a later time point is strongly suggestive that the patient will experience classic clinical progression soon after. We propose that a patient with an elevated CTC level at later time points would benefit from a change in therapy when her CTCs become elevated rather than waiting for more classic signs of progression. This strategy would minimize exposure to the toxicities of further treatment with an agent that is likely to be futile and would avoid delay of initiation of a subsequent treatment regimen that might palliate her symptoms.

In conclusion, the data presented in this report complement our previous publications, which show that elevated CTC levels at baseline and first follow-up (3–5 weeks) are associated with substantially and significantly more rapid progression and mortality in patients with MBC who are about to start any new systemic therapy (5) and in the subset of patients starting first-line systemic therapy (6). The results from the current analysis suggest that elevated CTC levels at any time in the clinical course of a patient with MBC are harbingers of impending progression. Indeed, if these data are validated, CTC levels may ultimately represent a more objective and accurate determination of disease status than classic clinical and/or radiological assessment and as such, when elevated, may indicate the need to change therapy.

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Daniel F. Hayes, Massimo Cristofanilli, G. Thomas Budd, et al.

*Clin Cancer Res* 2006;12:4218-4224.

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