

ORIGINAL ARTICLE

Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer

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ABSTRACT

BACKGROUND

We tested the hypothesis that the level of circulating tumor cells can predict survival in metastatic breast cancer.

METHODS

In a prospective, multicenter study, we tested 177 patients with measurable metastatic breast cancer for levels of circulating tumor cells both before the patients were to start a new line of treatment and at the first follow-up visit. The progression of the disease or the response to treatment was determined with the use of standard imaging studies at the participating centers.

RESULTS

Outcomes were assessed according to levels of circulating tumor cells at baseline, before the patients started a new treatment for metastatic disease. Patients in a training set with levels of circulating tumor cells equal to or higher than 5 per 7.5 ml of whole blood, as compared with the group with fewer than 5 circulating tumor cells per 7.5 ml, had a shorter median progression-free survival (2.7 months vs. 7.0 months, $P < 0.001$) and shorter overall survival (10.1 months vs. > 18 months, $P < 0.001$). At the first follow-up visit after the initiation of therapy, this difference between the groups persisted (progression-free survival, 2.1 months vs. 7.0 months; $P < 0.001$; overall survival, 8.2 months vs. > 18 months; $P < 0.001$), and the reduced proportion of patients (from 49 percent to 30 percent) in the group with an unfavorable prognosis suggested that there was a benefit from therapy. The multivariate Cox proportional-hazards regression showed that, of all the variables in the statistical model, the levels of circulating tumor cells at baseline and at the first follow-up visit were the most significant predictors of progression-free and overall survival.

CONCLUSIONS

The number of circulating tumor cells before treatment is an independent predictor of progression-free survival and overall survival in patients with metastatic breast cancer.

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PREVIOUS STUDIES HAVE SUGGESTED that the presence of circulating tumor cells in patients with metastatic carcinoma is associated with short survival.¹⁻¹⁰ Technical advances have facilitated the detection of rare circulating tumor cells.¹¹⁻¹⁵ The newly developed CellSearch System (Veridex) was designed to detect tumor cells in whole blood. The system is based on the enumeration of epithelial cells, which are separated from the blood by antibody-coated magnetic beads and identified with the use of fluorescently labeled antibodies against cytokeratin and with a fluorescent nuclear stain and fluorescent cytokeratin antibodies.^{16,17} We report the results of a prospective, multicenter, double-blind study to determine the clinical significance of levels of circulating tumor cells in patients with measurable metastatic breast cancer who are starting a new course of systemic therapy.

METHODS

STUDY DESIGN

We conducted a prospective trial at 20 clinical centers in the United States to evaluate the usefulness of measurements of the level of circulating tumor cells in predicting responses to therapy, progression-free survival, and overall survival in patients with metastatic breast cancer. The principal inclusion criteria were progressive, measurable metastatic breast cancer and the commencement of a new systemic therapy. All the patients had Eastern Cooperative Oncology Group (ECOG) scores for performance status of 0 to 2 (with a score of 0 indicating no symptoms, 1 mild symptoms, and 2 moderate symptoms). Prior adjuvant treatment, treatment of metastatic disease, or both were permitted. The protocol included a blinded, centralized review of imaging studies to document an objective response or progressive disease. The institutional review board at each center approved the study protocol, and all patients provided written informed consent.

Before starting a new treatment, patients underwent an evaluation of metastatic sites by means of standard imaging studies and the collection of a blood sample to be used for the enumeration of circulating tumor cells. Another blood sample was collected at the first follow-up visit, approximately three to four weeks after the initiation of the new therapy. Reevaluations of disease status were conducted with the same techniques that were

used at baseline every 9 to 12 weeks, depending on the type of treatment the patient received and the schedule of the treatment. Disease status was assessed according to the criteria of the World Health Organization¹⁸ without knowledge of the levels of circulating tumor cells.

A separate control group comprised 72 premenopausal healthy women and 73 postmenopausal healthy women with no known illness and no history of cancer, 99 women with benign breast diseases, and 101 women with other nonmalignant diseases. The testing laboratories were aware that the specimens were obtained from a control group, but they were blinded to the distinction between no known illness and benign conditions.

ISOLATION AND ENUMERATION OF CIRCULATING TUMOR CELLS

Blood samples were drawn into 10-ml EDTA Vacutainer tubes (Becton Dickinson) to which a cell preservative was added.¹⁹⁻²¹ Samples were maintained at room temperature and processed within 72 hours after collection. All evaluations were performed without knowledge of the clinical status of the patients and the controls at one of three central laboratories (Immunicon, IMPATH Predictive Oncology, or the Cleveland Clinic) or at selected participating centers. The CellSearch System (Veridex) was used for the isolation and enumeration of circulating tumor cells. It consists of a semiautomated system for the preparation of a sample^{22,23} and is used with the CellSearch Epithelial Cell Kit. The procedure enriches the sample for cells expressing the epithelial-cell adhesion molecule with antibody-coated magnetic beads, and it labels the cells with the fluorescent nucleic acid dye 4,2-diamidino-2-phenylindole dihydrochloride. Fluorescently labeled monoclonal antibodies specific for leukocytes (CD45–allophycocyan) and epithelial cells (cytokeratin 8,18,19–phycoerythrin) are used to distinguish epithelial cells from leukocytes. The identification and enumeration of circulating tumor cells were performed with the use of the CellSpotter Analyzer, a semiautomated fluorescence-based microscopy system that permits computer-generated reconstruction of cellular images. Circulating tumor cells were defined as nucleated cells lacking CD45 and expressing cytokeratin.^{16,17} Technical details of the CellSearch and CellSpotter systems, including accuracy, precision, linearity, and reproducibility, have been described elsewhere.²⁴

STATISTICAL ANALYSIS

To achieve 80 percent power (two-sided, with an alpha level of 0.05), we calculated that an enrollment of 175 patients was required, with an interim review to be conducted after the enrollment of 100 patients. Kaplan–Meier estimates of survival were based on the number of circulating tumor cells at baseline and at the first follow-up. For all survival analyses, the time to disease progression or death due to breast cancer was defined as the time between the date when the baseline sample of blood was obtained and the date of clinical progression, death, or the last follow-up visit. Survival curves were compared with the use of log-rank testing. Cox proportional-hazards regression analysis was

used to estimate univariate and multivariate hazard ratios for progression-free survival and overall survival. Results obtained for the first 102 patients enrolled (the training set) were used to select a cutoff level of circulating tumor cells for use in the stratification of patients into two groups, one with a favorable prognosis and the other with an unfavorable prognosis. This cutoff level was then validated with the use of the 75 subsequently enrolled patients (the validation set). To ensure equivalent follow-up times in the training set and the validation set, the follow-up for each patient was truncated at approximately 9 months (38.7 weeks). The distributions of patients above and below the cutoff level in the training set and the validation set were com-

Table 1. Prevalence of Circulating Tumor Cells at Baseline.*

Variable	Number of Subjects†	Number of Circulating Tumor Cells							
		≥2	≥4	≥5	≥6	≥10	≥50	≥100	≥1000
<i>percentage of subjects</i>									
Control subjects									
Normal	145	1	0	0	0	0	0	0	0
Benign breast conditions or other conditions	200	1	0	0	0	0	0	0	0
All subjects with metastatic breast cancer	177	61	53	49	47	38	21	16	3
Therapy									
First-line	83	64	55	52	49	40	18	14	1
Second-line or subsequent	92	59	52	48	46	37	24	17	4
P value		0.54	0.76	0.65	0.65	0.76	0.36	0.68	0.37
Type of therapy									
Hormone therapy, immunotherapy, or both	54	43	35	31	30	20	9	6	2
Chemotherapy alone or combined with other therapy	118	70	63	58	56	47	27	21	3
P value		0.001	0.001	0.002	0.002	0.001	0.01	0.01	1.00
Sites of metastasis									
Visceral	144	62	54	50	48	40	22	17	2
Nonvisceral	32	53	50	47	44	28	19	12	6
P value		0.33	0.70	0.85	0.70	0.23	0.82	0.79	0.22
Estrogen-receptor and progesterone-receptor status									
Positive for either	121	62	54	51	49	39	22	16	2
Negative for both	54	59	52	46	44	37	18	17	4
P value		0.74	0.75	0.62	0.63	0.87	0.69	1.00	0.65
HER2/ <i>neu</i> status									
Positive	45	56	44	40	38	31	16	13	0
Negative	103	66	59	54	52	43	24	17	4
P value		0.27	0.11	0.15	0.11	0.20	0.28	0.63	0.31

* A two-sided Fisher's exact chi-square test was performed to test for statistically significant differences in the proportions of patients with <5 or ≥5 circulating tumor cells per 7.5 ml of whole blood according to variable.

† The analyses according to variable involved fewer than 177 patients, because data for some patients were missing.

pared with the use of Fisher's exact test. The median progression-free survival and median overall survival in the two sets were compared with the use of the nonparametric *k*-sample test for equality of the medians. All *P* values are two-sided. Overall analysis of the prevalence of circulating tumor cells and the analysis of progression-free survival and overall survival were performed according to the intention-to-treat principle.

Estrogen-receptor status, progesterone-receptor status, and HER2/*neu* status were determined at each participating site according to local guidelines. If the HER2/*neu* value was determined by means of immunohistochemistry, values of 0 or 1+ were considered negative and values of 3+ were considered positive. Similarly, HER2/*neu* values of 2+ were considered positive unless the specimen was also analyzed with the use of fluorescence in situ hybridization, in which case institutional criteria for positive and negative values were used for the statistical analysis.

This study was designed by the sponsor (Immunicon) in collaboration with the clinical investigators and with advice from the Center for Devices and Radiological Health of the Food and Drug Administration. An independent clinical research organization (Medical Device Consultants) collected and monitored the clinical and laboratory data. Data on circulating tumor cells were also collected and verified by the sponsor. Locked and validated databases that contained the combined clinical and laboratory data were analyzed separately by the clinical research organization and by the sponsor. The sponsor and the clinical investigators jointly decided to submit the results for publication and jointly prepared the manuscript.

RESULTS

PATIENT CHARACTERISTICS

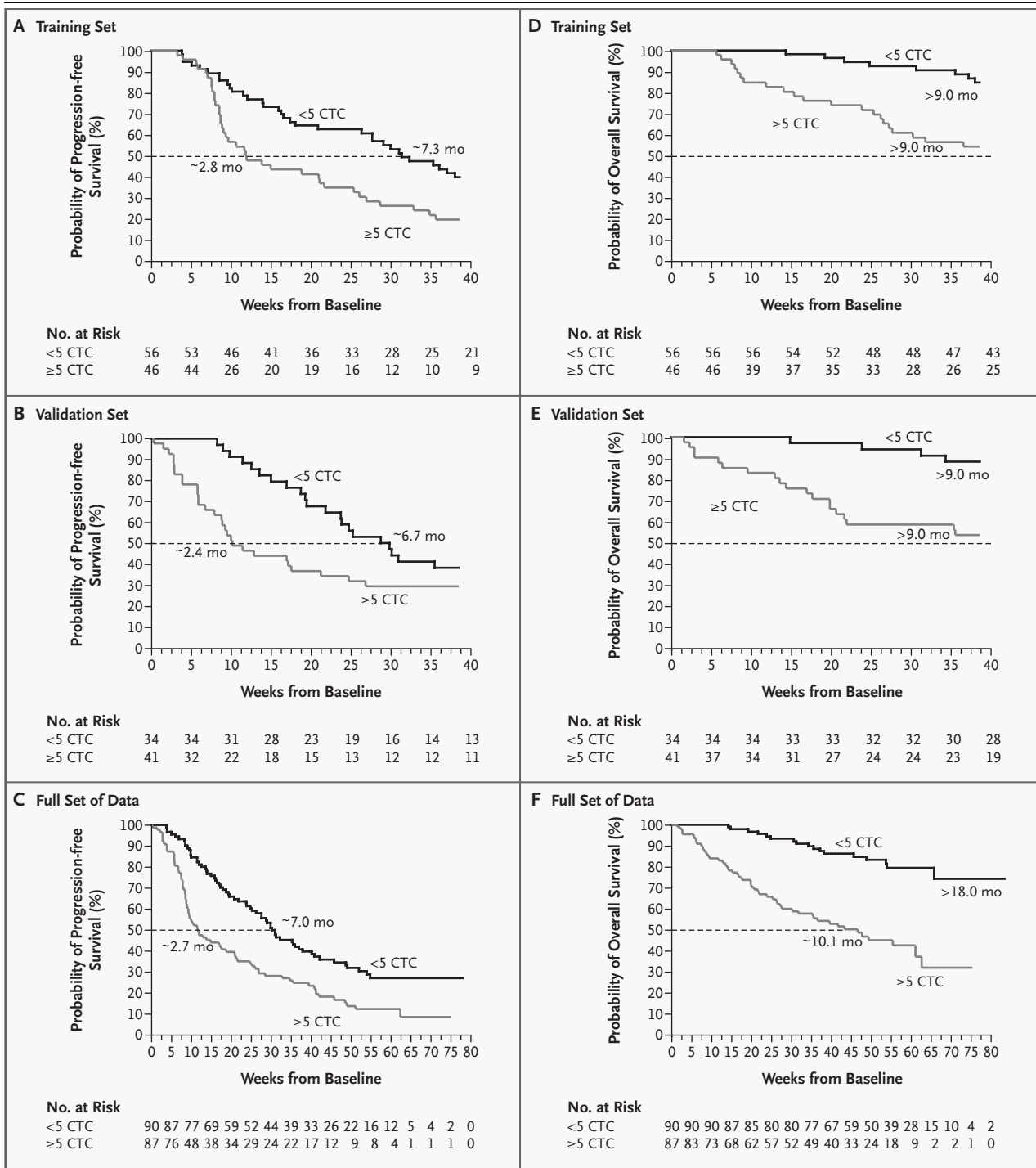
A total of 177 patients were enrolled between November 2001 and January 2003. The average (\pm SD) age of the patients was 58.0 \pm 13.4 years (median, 58); 84 percent of the patients were white. Forty-seven percent were starting their first line of therapy for metastatic disease, 30 percent starting hormonal treatment or immunotherapy and 67 percent starting chemotherapy (alone or in combination with other treatments). Eighteen percent had non-visceral metastatic sites; 68 percent of the tumors were positive for estrogen receptors, progesterone receptors, or both (1 percent, status unknown); and

Figure 1 (facing page). Kaplan–Meier Estimates of Probabilities of Progression-free Survival and Overall Survival in Patients with Metastatic Breast Cancer for Those with <5 Circulating Tumor Cells per 7.5 ml of Whole Blood and Those in the Group with \geq 5 Circulating Tumor Cells per 7.5 ml of Whole Blood before Initiation of a New Line of Therapy.

Progression-free survival and overall survival were calculated from the time of the baseline blood collection. As shown in Panels A, B, D, and E, follow-up times for each patient were truncated at approximately 9 months (38.7 weeks) to ensure an equivalent comparison between patients in the training set and those in the validation set. Panel A shows the probability of progression-free survival in the training set ($P=0.004$ by the log-rank test; hazard ratio for progression in patients with ≥ 5 circulating tumor cells per 7.5 ml of whole blood, 1.97; chi-square=7.89; $P=0.005$). Panel B shows the probability of progression-free survival in the validation set ($P=0.036$ by the log-rank test; hazard ratio for progression in patients with ≥ 5 circulating tumor cells per 7.5 ml of whole blood, 1.81; chi-square=4.32; $P=0.038$). The median progression-free survival and the proportions of patients according to levels of circulating tumor cells were not statistically different in the two sets. The probability of progression-free survival in the full set of data calculated with the use of follow-up times (not truncated) is shown in Panel C ($P<0.001$ by the log-rank test; hazard ratio, 1.95; chi-square=15.33; $P<0.001$). Panel D shows the probability of overall survival in the training set ($P<0.001$ by the log-rank test; hazard ratio for death in patients with ≥ 5 cells per 7.5 ml, 3.98; chi-square=12.64; $P<0.001$). Panel E shows the probability of overall survival in the validation set ($P<0.001$ by the log-rank test; hazard ratio for death in patients with ≥ 5 cells per 7.5 ml, 5.22; chi-square=12.01; $P<0.001$). The median overall survival and the proportions of patients according to levels of circulating tumor cells were not significantly different in the two sets of data. The probability of overall survival among patients in the full set of data calculated with the use of follow-up times (not truncated) is shown in Panel F ($P<0.001$ by the log-rank test; hazard ratio for death in patients with ≥ 5 cells per 7.5 ml, 4.39; chi-square=31.73; $P<0.001$). CTC denotes circulating tumor cells.

26 percent of the tumors were HER2/*neu* 2+ or 3+ (16 percent, status unknown) (Table 1). Sixty-three percent of the patients were still alive at the time of the analysis. Patients in the training set and those in the validation set had similar characteristics.

All but 10 of the 177 patients had a minimal follow-up time of 38.7 weeks for survival after the baseline collection of the blood specimen. No blood specimens were obtained at follow-up visits from 14 (8 percent) of the patients; of these 14 patients, 10 died, and the remaining 4 dropped out of the study before the first follow-up visit. The av-



erage time between the baseline and the first follow-up blood collection for the remaining 163 patients was 4.5 ± 2.4 weeks (range, 1.4 to 16.9; median, 4.0). The results of the imaging studies that were centrally reviewed by two blinded readers

documented a partial response in 26 of 140 patients (19 percent). The average time between the baseline imaging study and the first follow-up imaging study among these 140 patients was 11.9 ± 5.7 weeks (range, 1.9 to 34.1; median, 10.5).

CIRCULATING TUMOR CELLS

Circulating epithelial cells were rare in healthy women (mean, 0.1 ± 0.2 per 7.5 ml of whole blood) and in patients with benign breast disease (mean, 0.1 ± 0.9 per 7.5 ml of whole blood) (Table 1). None of the normal control subjects had 2 or more such cells per 7.5 ml of blood. Two or more circulating tumor cells per 7.5 ml of blood were detected at entry into the study in 61 percent of the patients with metastatic breast cancer (Table 1). Among the various groups of patients, the levels of circulating tumor cells were significantly different only in those who received hormone therapy, immunotherapy, or both, as compared with patients starting chemotherapy.

STRATIFICATION ACCORDING TO LEVELS OF CIRCULATING TUMOR CELLS

To select a level of circulating tumor cells that most clearly distinguished patients with rapid progression of disease from those with slow progression, thresholds of 1 to 10,000 cells for the baseline levels were systematically correlated with progression-free survival for the 102 patients in the training set. The median progression-free survival among patients with levels above or below each threshold differed at the level of 1 circulating tumor cell per 7.5 ml of blood and reached a plateau at approximately 5 cells per 7.5 ml of blood. At the latter level, the Cox proportional-hazards ratio signifying the difference between slow and rapid progression of disease also reached a plateau. Thus, a cutoff of 5 circulating tumor cells per 7.5 ml of blood was chosen to distinguish patients with an unfavorable prognosis from patients with a favorable prognosis. The results were similar when the number of circulating tumor cells measured at the first follow-up visit was correlated with progression-free survival and when the number measured either at baseline or at the first follow-up visit was correlated with overall survival.

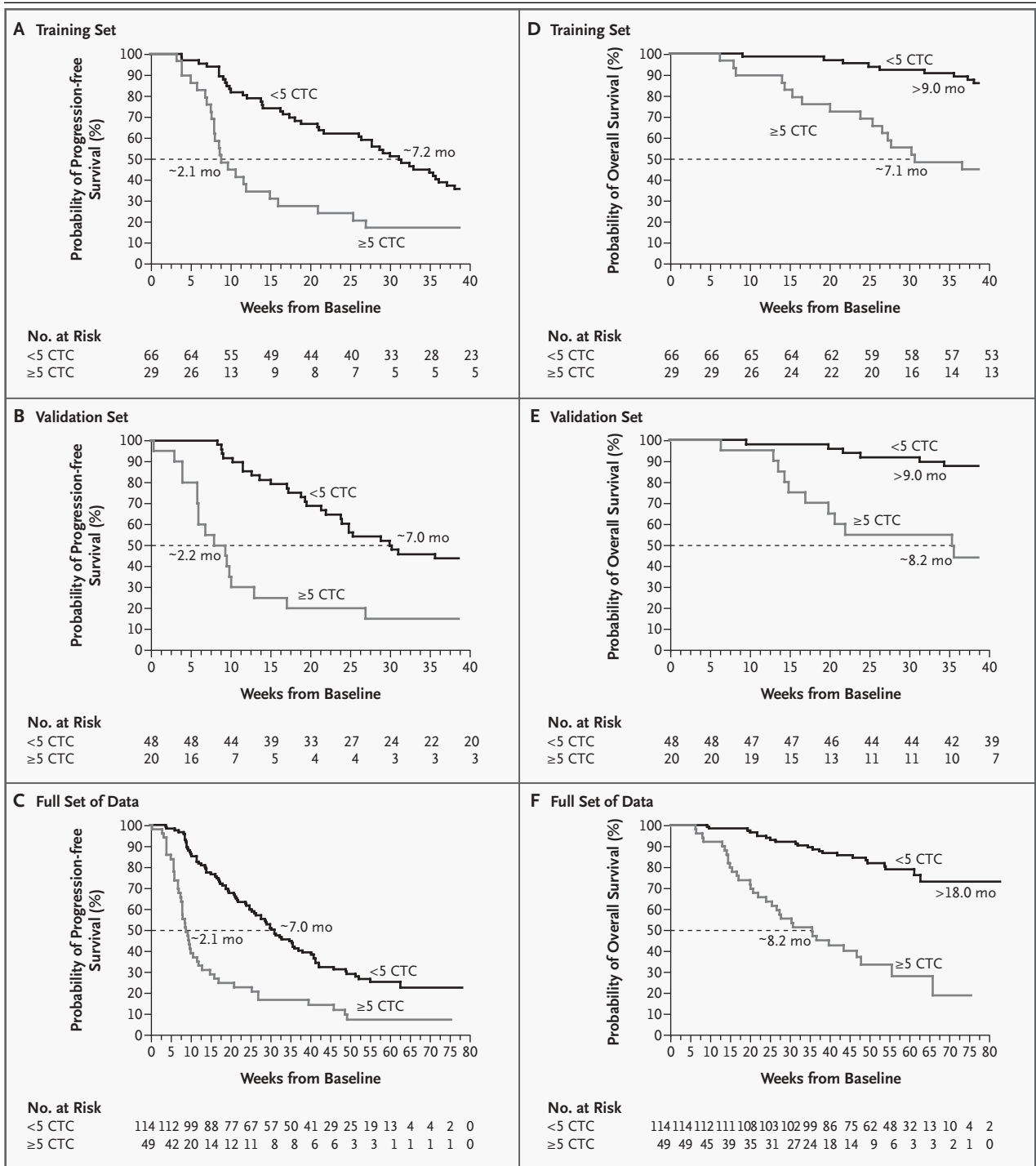
PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL IN THE TRAINING SET AND THE VALIDATION SET

Figure 1 shows Kaplan–Meier curves of progression-free survival and overall survival according to the baseline levels of circulating tumor cells in the training set and the validation set. The Kaplan–Meier estimates for both sets of patients were not significantly different ($P \geq 0.74$). Figure 2 shows Kaplan–Meier survival curves for the levels of cir-

Figure 2 (facing page). Kaplan–Meier Estimates of Probabilities of Progression-free Survival and Overall Survival in Patients with Metastatic Breast Cancer for Those with <5 Circulating Tumor Cells per 7.5 ml of Whole Blood and Those in the Group with ≥ 5 Circulating Tumor Cells in 7.5 ml of Whole Blood at the First Follow-up Visit after Initiation of a New Line of Therapy.

Progression-free survival and overall survival were calculated from the time of the baseline blood collection. As shown in Panels A, B, D, and E, follow-up times for each patient were truncated at approximately 9 months (38.7 weeks) to ensure an equivalent comparison between patients in the training set and those in the validation set. Panel A shows the probability of progression-free survival in the training set ($P < 0.001$ by the log-rank test; hazard ratio for progression in patients with ≥ 5 circulating tumor cells per 7.5 ml of whole blood, 2.50; chi-square=11.20; $P < 0.001$). Panel B shows the probability of progression-free survival in the validation set ($P < 0.001$ by the log-rank test; hazard ratio for progression in patients with ≥ 5 circulating tumor cells per 7.5 ml of whole blood, 3.58; chi-square=14.23; $P < 0.001$). The median progression-free survival and the proportions of patients according to levels of circulating tumor cells (≥ 5 cells per 7.5 ml) were not significantly different in the two sets. The probability of progression-free survival in the full set of data calculated with the use of follow-up times (not truncated) is shown in Panel C ($P < 0.001$ by the log-rank test; hazard ratio for progression in patients with ≥ 5 cells per 7.5 ml, 2.73; chi-square=25.25; $P < 0.001$). Panel D shows the probability of overall survival in the training set ($P < 0.001$ by the log-rank test; hazard ratio for death in patients with ≥ 5 cells per 7.5 ml, 5.50; chi-square=17.35; $P < 0.001$). Panel E shows the probability of overall survival in the validation set ($P < 0.001$ by the log-rank test, hazard ratio for death in patients with ≥ 5 cells per 7.5 ml, 6.12; chi-square=13.24; $P < 0.001$). The median overall survival and the proportions of patients according to levels of circulating tumor cells were not significantly different in the two sets of data. The probability of overall survival among patients in the full set of data calculated with the use of follow-up times (not truncated) is shown in Panel F ($P < 0.001$ by the log-rank test; hazard ratio for death in patients with ≥ 5 cells per 7.5 ml, 5.54; chi-square=38.02; $P < 0.001$). CTC denotes circulating tumor cells.

culating tumor cells at first follow-up. Circulating tumor-cell counts were available at the first follow-up visit for 95 of the 102 patients in the training set and for 68 of the 75 patients in the validation set. Neither progression-free survival nor overall survival was significantly different in the two sets ($P \geq 0.74$). The distribution of patients with levels of circulating tumor cells ≥ 5 per 7.5 ml of blood at baseline and at the first follow-up visit did not differ in the two sets ($P = 0.59$ and $P = 0.23$, respectively).



PREDICTION OF PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL BEFORE INITIATION OF THERAPY

Because the two sets of data were nearly identical, they were combined for the estimation of progression-free survival and overall survival for the en-

tire population. For all 177 patients, the median progression-free survival was approximately 5.0 months (95 percent confidence interval, 4.0 to 6.4) and the median overall survival was more than 18 months (95 percent confidence interval, 14.6 to >18). Of 177 patients, 87 (49 percent) had ≥5 cir-

culating tumor cells per 7.5 ml of blood at baseline. These 87 patients had a significantly shorter median progression-free survival (approximately 2.7 months; 95 percent confidence interval, 2.1 to 4.4) and median overall survival (approximately 10.1 months; 95 percent confidence interval, 6.3 to 14.6) than did patients with <5 circulating tumor cells per 7.5 ml of blood (median progression-free survival, approximately 7.0 months; 95 percent confidence interval, 5.8 to 8.9; overall survival, >18 months) (Table 2 and Fig. 1C and 1F). In the analysis of the patients according to clinical variables, the number of circulating tumor cells at baseline was significantly associated with worse overall survival but was not significantly associated with progression-free survival in patients who were starting hormone therapy, immunotherapy, or both, or with the presence of nonvisceral disease, estrogen-receptor–negative and progesterone-receptor–negative tumors, or HER2–positive cancers (Table 2).

PREDICTION OF PROGRESSION-FREE SURVIVAL AND OVERALL SURVIVAL AFTER INITIATION OF THERAPY

At the first follow-up visit, the number of circulating tumor cells was measured in the 163 patients available for evaluation. The 10 patients who died before the first follow-up visit had high to extremely high counts of circulating tumor cells in the baseline sample (counts of 9, 11, 15, 24, 111, 126, 301, 1143, 4648, and 23,618 per 7.5 ml of blood). Of the 163 remaining patients, 49 (30 percent) with ≥ 5 circulating tumor cells per 7.5 ml of blood at the first follow-up visit had a significantly shorter median progression-free survival (approximately 2.1 months; 95 percent confidence interval, 1.8 to 2.5) and a shorter median overall survival (approximately 8.2 months; 95 percent confidence interval, 5.6 to 11.1) than did the 114 patients (70 percent) with <5 circulating tumor cells per 7.5 ml of blood (progression-free survival, approximately 7.0 months; 95 percent confidence interval, 5.8 to 8.4; overall survival, >18 months) (Fig. 2C and 2F). Analysis of the groups showed that the levels of circulating tumor cells at the first follow-up visit were not significantly associated with progression-free survival only in patients starting hormone therapy, immunotherapy, or both (Table 2).

The median progression-free survival and median overall survival of the 33 patients (data not

shown) with ≥ 5 circulating tumor cells at baseline but <5 per 7.5 ml of blood at the first follow-up visit were approximately 7.6 months and approximately 14.6 months, respectively, and were not statistically different from progression-free survival and overall survival in the 81 patients (data not shown) with <5 circulating tumor cells both at the first follow-up visit and at baseline (progression-free survival, approximately 7.0 months; $P=0.60$; overall survival, >18 months; $P=0.07$). Similarly, the median progression-free survival and overall survival in the group of 5 patients with <5 circulating tumor cells per 7.5 ml of blood at baseline but with ≥ 5 cells at the first follow-up visit were not significantly different from the median progression-free survival and overall survival in the group of 44 patients with ≥ 5 circulating tumor cells both at baseline and at the first follow-up visit (progression-free survival, 2.3 months vs. 2.0 months; $P=0.99$ by the log-rank test; overall survival, 7.1 months vs. 8.2 months; $P=0.89$ by the log-rank test). The median progression-free and median overall survival in the 33 patients with a baseline level of ≥ 5 circulating tumor cells but a level of <5 per 7.5 ml of blood after the initiation of therapy differed significantly from the survival times among the 25 patients who had a decrease in circulating tumor cells from baseline but in whom the levels of circulating tumor cells remained ≥ 5 per 7.5 ml at the first follow-up visit (progression-free survival, 7.6 months vs. 2.1 months; $P=0.002$ by the log-rank test; overall survival, 14.6 months vs. 9.2 months; $P=0.006$ by the log-rank test) (data not shown).

UNIVARIATE AND MULTIVARIATE ANALYSIS OF PREDICTORS OF SURVIVAL

In the univariate analysis, only the line of therapy (first or subsequent), the type of therapy, the time to metastasis, and the levels of circulating tumor cells at baseline and at the first follow-up visit were significantly associated with both progression-free survival and overall survival. Estrogen-receptor and progesterone-receptor status of the tumor and ECOG performance status were significantly associated only with overall survival. Although some of the clinical factors remained relevant in the multivariate analysis (e.g., time to metastasis, HER2/*neu* status, and type of therapy), the levels of circulating tumor cells at baseline and at the first follow-up visit emerged as the strongest predictors of progression-free and overall survival (Table 3).

DISCUSSION

The results of this trial indicate that in metastatic breast cancer the level of circulating tumor cells before a new therapy is initiated and, even more important, the level measured at the first follow-up visit are useful predictors of progression-free survival and overall survival. Circulating tumor-cell levels of ≥ 5 cells per 7.5 ml of blood — a cutoff point

that was prospectively identified in patients in a training set and confirmed in patients in a validation set — gave a reliable estimate of disease progression and survival earlier than estimations made with the use of traditional imaging methods (3 to 4 weeks vs. 8 to 12 weeks after the initiation of therapy, respectively). In a multivariate analysis, the predictive value of the level of circulating tumor cells, either at baseline or at the first follow-up visit, was

Table 2. Progression-free Survival and Overall Survival among Patients with Metastatic Breast Cancer According to the Levels of Circulating Tumor Cells (CTC).

Variable	No. of Patients*	Patients with ≥ 5 CTC no. (%)	Progression-free Survival			Overall Survival		
			Patients with < 5 CTC	Patients with ≥ 5 CTC	P Value	Patients with < 5 CTC	Patients with ≥ 5 CTC	P Value
			mo			mo		
At baseline								
All patients	177	87 (49)	7.0	2.7	<0.001	>18	10.1	<0.001
Therapy								
First-line	83	43 (52)	9.4	4.9	0.003	>18	14.2	0.005
Second-line or subsequent	92	44 (48)	6.4	2.7	0.02	>18	6.4	<0.001
Type of therapy								
Hormone therapy, immunotherapy, or both	54	17 (31)	8.3	8.1	0.44	>18	>18	0.09
Chemotherapy alone or combined with other therapy	118	69 (58)	6.8	2.3	0.002	>18	8.3	<0.001
Sites of metastasis								
Visceral	144	72 (50)	7.0	3.0	<0.001	>18	11.1	<0.001
Nonvisceral	32	15 (47)	9.4	1.9	0.13	>18	7.0	<0.001
Estrogen-receptor and progesterone-receptor status								
Positive for either	121	62 (51)	7.5	2.7	<0.001	>18	11.1	<0.001
Negative for both	54	25 (46)	6.4	2.8	0.15	>18	7.4	0.008
HER2/ <i>neu</i> status								
Positive	45	18 (40)	7.2	5.8	0.36	>18	9.2	0.002
Negative	103	56 (54)	7.0	2.5	<0.001	>18	8.5	<0.001
At first follow-up								
All patients	163	49 (30)	7.0	2.1	<0.001	>18	8.2	<0.001
Therapy								
First-line	80	20 (25)	8.9	1.8	<0.001	>18	11.1	<0.001
Second-line or subsequent	82	29 (35)	5.6	2.3	<0.001	>18	6.4	<0.001
Type of therapy								
Hormone therapy, immunotherapy, or both	53	8 (15)	8.3	2.3	0.15	>18	10.9	0.002
Chemotherapy alone or combined with other therapy	109	41 (38)	7.0	2.0	<0.001	>18	7.1	<0.001
Sites of metastasis								
Visceral	135	38 (28)	7.0	2.2	<0.001	>18	8.2	<0.001
Nonvisceral	27	11 (41)	8.9	1.9	0.03	>18	7.0	<0.001
Estrogen-receptor and progesterone-receptor status								
Positive for either	115	39 (34)	7.5	2.2	<0.001	>18	8.5	<0.001
Negative for both	47	10 (21)	6.8	2.0	<0.001	>18	6.3	<0.001
HER2/ <i>neu</i> status								
Positive	40	7 (18)	8.6	1.2	<0.001	>18	3.5	<0.001
Negative	97	36 (37)	6.4	2.2	<0.001	>18	8.3	<0.001

* The analyses according to variable involved fewer than 177 patients, because data on some patients were missing.

Table 3. Prediction of Progression-free Survival and Overall Survival.*

Variable	Progression-free Survival		Overall Survival	
	Hazard Ratio	P Value	Hazard Ratio	P Value
Analysis with baseline CTC count				
≥5 CTC vs. <5 CTC	1.76	0.001	4.26	<0.001
Second or subsequent line of therapy vs. first	1.73	0.002	2.38	0.001
Chemotherapy vs. hormone therapy, immunotherapy, or both	1.61	0.02	2.54	0.02
ECOG score 2 vs. 1 vs. 0	NS	NS	1.48	0.02
Time to metastasis	NS	NS	0.92	0.03
Analysis with CTC count at first follow-up visit				
≥5 CTC vs. <5 CTC	2.52	<0.001	6.49	<0.001
Positive ER/PR status vs. negative	NS	NS	0.35	<0.001
Second or subsequent line of therapy vs. first	1.58	0.01	2.29	0.006
ECOG score 2 vs. 1 vs. 0	NS	NS	1.53	0.03

* A multivariate Cox regression analysis with a stepwise selection process was used to evaluate the association between the number of circulating tumor cells (CTC) and progression-free survival and overall survival. A stringency level (P value) of 0.05 was used both to include and exclude variables in the analysis. Time to metastasis was included as a continuous variable. The table summarizes results for each variable that demonstrated a statistically significant correlation with progression-free survival and overall survival. The number of circulating tumor cells was the strongest predictor of progression-free survival and overall survival. Because information was not available for all variables, not all subjects were included in the model. ECOG denotes Eastern Cooperative Oncology Group, NS not significant, ER estrogen receptor, and PR progesterone receptor.

independent of the time to metastasis, the site of metastasis (visceral as compared with nonvisceral), and hormone-receptor status.^{25,26}

An unplanned subgroup analysis suggested that the predictive accuracy of the number of circulating tumor cells was not valid in all patient groups for all end points. The number of circulating tumor cells before hormonal treatment was started was not significantly associated with overall survival ($P=0.09$), although the number of circulating tumor cells after the initiation of therapy was significantly associated with overall survival ($P=0.002$).

The CellSearch and CellSpotter assay systems were designed to detect rare epithelial cells in whole blood. One can assume that in patients with metastatic breast cancer most of the cells identi-

fied as circulating tumor cells are malignant, because such cells were detected in only 1 percent of 345 control subjects, none of whom had >3 cells per 7.5 ml of blood. Furthermore, other investigators, using a similar assay, have reported that chromosomal abnormalities in circulating tumor cells obtained from patients with metastatic epithelial cancers matched those in the primary lesion,²⁷ indicating that the circulating cells were derived from the tumor.

Personnel in the testing laboratories were aware that they were analyzing specimens obtained from control subjects and from patients with metastatic disease, but they were completely unaware of the results of clinical and radiographic assessments and the outcomes of the patients who were the main focus of this study. Although we found a difference between the number of detectable circulating epithelial cells in patients with metastatic breast cancer and the number in normal subjects and women with benign breast conditions, the results of our study do not support the use of this assay as a screening tool to detect a new primary cancer or metastatic breast cancer.

The prognostic implications of an elevated level of circulating tumor cells for patients with metastatic disease who are starting a new treatment represent an opportunity to stratify such patients in investigational studies. The very short median progression-free survival in patients with elevated levels of circulating tumor cells at the first follow-up visit suggests that these patients are receiving ineffective therapy. We stress that our results may not be valid for patients who do not have measurable disease or those starting a new regimen of hormone therapy, immunotherapy, or both. This study did not address whether patients with an elevated number of circulating tumor cells might benefit from other therapies. Whether such patients might benefit from other therapies is under investigation.

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In addition to the investigators, the following investigators were members of the Immunicon Clinical Trial Group: C. Atkins, A. Lipton, D. Mintzer, K. Fox, J.D. Sprandio, M. Wax, P. DeGreen, S. Hoffman, D. Greenwald, T. Boyd, R. McCrosky, G. Harrer, P. Cobb, B. Fernbach, and A. Bianco.

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