

Association of Occult Metastases in Sentinel Lymph Nodes and Bone Marrow With Survival Among Women With Early-Stage Invasive Breast Cancer

Armando E. Giuliano, MD

Debra Hawes, MD

Karla V. Ballman, PhD

Pat W. Whitworth, MD

Peter W. Blumencranz, MD

Douglas S. Reintgen, MD

Monica Morrow, MD

A. Marilyn Leitch, MD

Kelly K. Hunt, MD

Linda M. McCall, MS

Andrea Abati, MD

Richard Cote, MD

SENTINEL LYMPH NODE (SLN) DISSECTION has revolutionized the approach to early-stage breast cancer by allowing minimally invasive axillary staging and more intensive examination of the SLN. This has led to the detection of micrometastases¹ and isolated tumor cells of uncertain significance. Some older retrospective studies linked occult metastases to decreased survival,²⁻⁶ but patients were not treated with current standards of adjuvant systemic therapy and their disease generally was staged higher than for contemporary populations. A long-term prospective study of occult metastases in SLNs from 790 contemporary patients with early breast cancer showed that micrometastases and isolated tumor cells did not reduce survival.⁷ By contrast, in the 3884 patients enrolled in the National Surgical Adjuvant Breast and Bowel Project's B-32 study, occult

For editorial comment see p 436.

Context Immunochemical staining of sentinel lymph nodes (SLNs) and bone marrow identifies breast cancer metastases not seen with routine pathological or clinical examination.

Objective To determine the association between survival and metastases detected by immunochemical staining of SLNs and bone marrow specimens from patients with early-stage breast cancer.

Design, Setting, and Patients From May 1999 to May 2003, 126 sites in the American College of Surgeons Oncology Group Z0010 trial enrolled women with clinical T1 to T2N0M0 invasive breast carcinoma in a prospective observational study.

Interventions All 5210 patients underwent breast-conserving surgery and SLN dissection. Bone marrow aspiration at the time of operation was initially optional and subsequently mandatory (March 2001). Sentinel lymph node specimens (hematoxylin-eosin negative) and bone marrow specimens were sent to a central laboratory for immunochemical staining; treating clinicians were blinded to results.

Main Outcome Measures Overall survival (primary end point) and disease-free survival (a secondary end point).

Results Of 5119 SLN specimens (98.3%), 3904 (76.3%) were tumor-negative by hematoxylin-eosin staining. Of 3326 SLN specimens examined by immunohistochemistry, 349 (10.5%) were positive for tumor. Of 3413 bone marrow specimens examined by immunocytochemistry, 104 (3.0%) were positive for tumors. At a median follow-up of 6.3 years (through April 2010), 435 patients had died and 376 had disease recurrence. Immunohistochemical evidence of SLN metastases was not significantly associated with overall survival (5-year rates: 95.7%; 95% confidence interval [CI], 95.0%-96.5% for immunohistochemical negative and 95.1%; 95% CI, 92.7%-97.5% for immunohistochemical positive disease; $P = .64$; unadjusted hazard ratio [HR], 0.90; 95% CI, 0.59-1.39; $P = .64$). Bone marrow metastases were associated with decreased overall survival (unadjusted HR for mortality, 1.94; 95% CI, 1.02-3.67; $P = .04$), but neither immunohistochemical evidence of tumor in SLNs (adjusted HR, 0.88; 95% CI, 0.45-1.71; $P = .70$) nor immunocytochemical evidence of tumor in bone marrow (adjusted HR, 1.83; 95% CI, 0.79-4.26; $P = .15$) was statistically significant on multivariable analysis.

Conclusion Among women receiving breast-conserving therapy and SLN dissection, immunohistochemical evidence of SLN metastasis was not associated with overall survival over a median of 6.3 years, whereas occult bone marrow metastasis, although rare, was associated with decreased survival.

Trial Registration clinicaltrials.gov Identifier: NCT00003854

JAMA. 2011;306(4):385-393

www.jama.com

metastases were associated with a small but statistically significant 1.2% decrease in 5-year survival.⁸

Author Affiliations are listed at the end of this article.
Corresponding Author: Armando E. Giuliano, MD, Cedars-Sinai Medical Center, 310 N San Vicente Blvd, Third Floor, Los Angeles, CA 90048 (armando.giuliano@cshs.org).

Occult metastases in the bone marrow of breast cancer patients have more consistently been associated with decreased survival.⁹⁻¹² Pooled data from 9 clinical studies suggest that patients with bone marrow micrometastases fare worse than those without bone marrow metastases.¹² Many of these studies focused on larger tumors and more advanced disease than currently seen in the United States. Data are lacking for early-stage breast cancer managed by SLN dissection and multimodality therapy.

The American College of Surgeons Oncology Group (ACOSOG) initiated the Z0010 trial in 1999 to determine the prevalence and significance of occult metastases in the SLNs and bone marrow of patients who underwent breast-conserving surgery, SLN dissection, and whole breast irradiation for treatment of clinical T1 or T2 node-negative breast cancer. The Z0010 study also identified node-positive women who became candidates for enrollment in ACOSOG's Z0011 trial.¹³ Herein, we report the major end points of Z0010: prevalence of occult metastases in SLNs and bone marrow, overall survival, and disease-free survival.

METHODS

Study Design

The Z0010 trial, a prospective observational study of patients undergoing breast-conserving therapy and SLN dissection, was approved by the National Cancer Institute and the institutional review boards of participating institutions. Prior to participating, surgeons were required to perform 20 consecutive procedures with SLN identification and accuracy rates of 85% or better based on subsequent complete axillary lymph node dissection or to have completed a postgraduate program with SLN dissection training.¹⁴ Treating clinicians were blinded to the immunohistochemical status of SLNs and bone marrow specimens.

Patients

Women planning to undergo breast-conserving therapy for clinical T1 to

T2N0M0 invasive breast carcinoma were eligible. Participants were required to have a negative pregnancy test result and a functional status (Eastern Cooperative Oncology Group-Zubrod) score of 2 or less. Exclusion criteria were neoadjuvant therapy, prepectoral breast implants, concurrent bilateral malignancies, disease not amenable to lumpectomy, and previous axillary surgery. Written informed consent was obtained prior to enrollment.

Interventions

Bilateral anterior iliac crest bone marrow aspiration biopsy (optional before March 2001) was performed immediately before SLN dissection and lumpectomy. Sentinel lymph node dissection technique was at the discretion of the surgeon. Preoperative lymphoscintigraphy or intraoperative gamma counting was required when tumors were completely medial to the medial edge of the areola. If lumpectomy margins were positive for tumor, reexcision was performed and negative margins were confirmed by the pathologist.

Adjuvant Therapies

Whole-breast irradiation specified in the protocol excluded a third supraclavicular field. The total dose for the breast was 45 to 50 Gy administered in tangential fields with coplanar posterior borders. Adjuvant systemic therapy was determined by treating clinicians based on primary tumor factors and results of hematoxylin-eosin staining.

Immunohistochemical Staining of SLN Specimens

Immunohistochemistry was performed at a central laboratory on hematoxylin-eosin-negative SLNs, with results blinded to clinicians. Sentinel lymph nodes were formalin-fixed and paraffin-embedded, and blocks were cut into 5- μ m sections. Paraffin removal was performed in 10 mmol/L sodium citrate buffer (pH 6) heated at 110°C for 30 minutes in a pressure cooker in a microwave oven. Slides were brought to room temperature, blocked with

horse serum for 20 minutes, and incubated with primary antibody in blocking buffer for 1 hour. Two mouse monoclonal antibodies against cytokeratin were used as the primary immunohistochemical detection system: AE-1 (Signet, Dedham, Massachusetts) against low and intermediate type 1 acidic keratins, and CAM5.2 (Becton Dickinson, San Jose, California) against cytokeratins 8 and 18. Subsequently, slides were washed 3 times with phosphate-buffered saline and incubated with biotinylated secondary antibody (antimouse). After washing them 3 times to remove unbound secondary antibody, slides were stained by 30-minute incubation with avidin-biotin-horseradish peroxidase complexes (Vector Laboratories, Burlingame, California). The chromogen amino-ethyl-carbazole (Sigma-Aldrich, St Louis, Missouri) was used as substrate. Slides were counterstained with hematoxylin. Breast cancer tissue known to be positive for cytokeratin was used as a control.

Immunocytochemical Staining of Bone Marrow Specimens

Bone marrow aspirates were sent to the central laboratory, processed, and stained according to our previously published protocol for bone marrow immunocytochemistry.¹⁵ Briefly, mononuclear cells were separated using the Ficoll density gradient method and centrifuged onto slides. Slides were stained with a cytokeratin cocktail (AE-1 and CAM 5.2), and chromogen Fast Red (Biocare Medical, Concord, California) was used to detect the presence of epithelial cells.

Histopathological and Cytopathological Evaluation of SLN and Bone Marrow Specimens

Pathologists blinded to clinical information assessed cytokeratin-stained SLN (red-brown) and bone marrow (red) cells for morphological characteristics of malignancy (size, nuclear pleomorphism, and increased nuclear-cytoplasmic ratio). All SLN and bone marrow specimens with candidate cells

and more than 10% of randomly selected specimens negative for micrometastasis were re-reviewed by a second pathologist.

More than 55% of SLN cases underwent re-review. In cases without consensus, slides were re-reviewed by both initial observers. In rare cases in which consensus was still not reached, a third pathologist served as arbitrator. Only cases with occult metastases identified by multiple observers were scored as positive for micrometastasis.

All bone marrow slides containing immunocytochemistry-positive or suspicious cells were sent to the National Institutes of Health (NIH) for another review by a cytopathologist. If the central laboratory did not agree with the NIH assessment, an additional review by a third pathologist was performed. Only cases in which multiple reviewers agreed that tumor cells were present were finally scored as positive for micrometastasis. Overall, 95% of cases showed complete or near agreement (suspicious vs positive), and only 5% of cases showed discordance (positive vs negative).

Statistical Analysis

The primary end point was overall survival after patients' initial diagnosis. Patients not known to have died were censored at the date of their last follow-up. A secondary end point was disease-free survival from diagnosis until first recurrence (any site) or death; patients without known recurrence were censored at the date of their last follow-up or death. The study was powered to evaluate the prognostic significance of immunohistochemistry-detected SLN metastases among women with hematoxylin-eosin-negative SLNs, with the assumption that 75% of the target population would have hematoxylin-eosin-negative nodes and that 10% of these women would have immunohistochemistry-positive SLN(s). A target sample size of 5300 women, including those with nodal metastases detected by hematoxylin-eosin, provided 90% power to detect a hazard ratio (HR) of 1.7 (immunohistochemistry-positive

SLNs vs immunohistochemistry-negative SLNs) with a 2-sided significance level of .05.

Comparisons between groups used χ^2 tests for categorical variables and appropriate 2-sample tests (*t* test or Wilcoxon rank sum) for continuous variables. Kaplan-Meier estimates and curves were used to summarize overall survival and disease-free survival. The primary analysis was a log-rank comparison of overall survival between groups. Curves displayed cumulative incidence rather than event-free survival.¹⁶ Univariable and multivariable models were constructed using Cox proportional hazards regression; the prespecified multivariable analyses were adjusted for known prognostic variables (age, tumor type, lymphovascular invasion, estrogen receptor status) and for variables expected to affect survival (adjuvant systemic therapy). Analyses were performed by ACOSOG Statistical Unit with SAS statistical analysis software, version 9.2 (SAS Institute Inc, Cary, North Carolina); all tests were 2-sided and *P* values < .05 were considered significant.

RESULTS

Sentinel Lymph Node Dissection

Between May 10, 1999, and May 30, 2003, 5538 patients at 126 institutions enrolled in the Z0010 trial (FIGURE 1). Of these, 185 were ineligible (multicentric disease, incorrect pathology, absence of pretreatment pregnancy test, and regulatory violations) and 143 did not have the prescribed operation. Of 5210 eligible patients, 5119 (98.3%) had SLNs identified; specified mapping agents were blue dye alone (*n*=751), radioisotope alone (*n*=296), and blue dye plus radioisotope (*n*=4064). There was no statistically significant difference in SLN identification rates among different SLN dissection techniques.

Study Population

Approximately 69% of patients were older than 50 years, 83.3% had clinical stage I and 80.1% had invasive ductal carcinoma (TABLE 1). Median tu-

mor size was 1.4 cm (range, 0-19 cm), and 81.2% of patients had estrogen-receptor-positive tumors. Axillary lymph node dissection was performed in 107 women (2.1%) with hematoxylin-eosin-negative SLNs.

Use of adjuvant therapy among patients with hematoxylin-eosin-negative SLNs was as follows: 2956 of 3247 women (91.0%) received whole breast radiation, 2743 of 3289 (83.4%) received systemic chemotherapy (2061 of 2479 [83.1%] with immunohistochemistry-negative and 269 of 299 [90.0%] with immunohistochemistry-positive SLNs), 2230 of 3289 (67.8%) women received hormonal therapy (1678 of 2479 [67.7%] with immunohistochemistry-negative and 216 of 299 [72.2%] with immunohistochemistry-positive SLNs), and 2498 of 3247 (76.9%) women received whole-breast radiation plus adjuvant systemic therapy (1902 of 2462 [77.3%] with immunohistochemistry-negative and 235 of 299 [78.6%] with immunohistochemistry-positive SLNs).

Results of SLN and Bone Marrow Immunocytochemistry

Of 5119 patients in whom an SLN specimen was identified, 1215 (23.7%) had nodes that were hematoxylin-eosin positive. Of the remaining 3904 patients with nodes that were hematoxylin-eosin negative, 3326 (85.2%) had specimens assessed by immunohistochemistry; of those, 349 specimens (10.5%) contained occult metastases. Five hundred seventy-eight specimens were not assessed by immunohistochemistry: 121 (3.1%) had insufficient tissue and 457 specimens were not sent for processing.

Of 3413 patients (66.7%) who underwent bone marrow biopsy, 104 (3.0%) had occult metastases by immunocytochemistry. Autologous SLN and bone marrow specimens from 2205 patients showed no concordance with respect to occult metastases (κ statistic, -0.01; 95% CI, -0.07 to 0.05; TABLE 2).

Increasing tumor size was associated with SLN metastases identified by hematoxylin-eosin staining or immu-

nohistochemistry. In hematoxylin-eosin–negative SLNs, median tumor size was 1.5 cm (interquartile range [IQR], 1.0-2.0 cm) vs 1.2 cm (IQR, 0.9-1.7 cm) for specimens with vs without immunohistochemical metastases ($P < .001$). There was no significant relationship between tumor size and occult metastases in the bone marrow; median tumor size was 1.4 cm (IQR, 0.83-1.98 cm) vs 1.4 cm (IQR, 1.0-2.0 cm) for specimens with vs without metastases ($P = .87$).

SLN and Bone Marrow Status and Survival

All women were followed up until April 21, 2010, when study data were fro-

zen for analysis. At a median follow-up of 6.3 years, there were 435 deaths and 376 women with disease recurrence. Less than 10% of women had overdue follow-up.

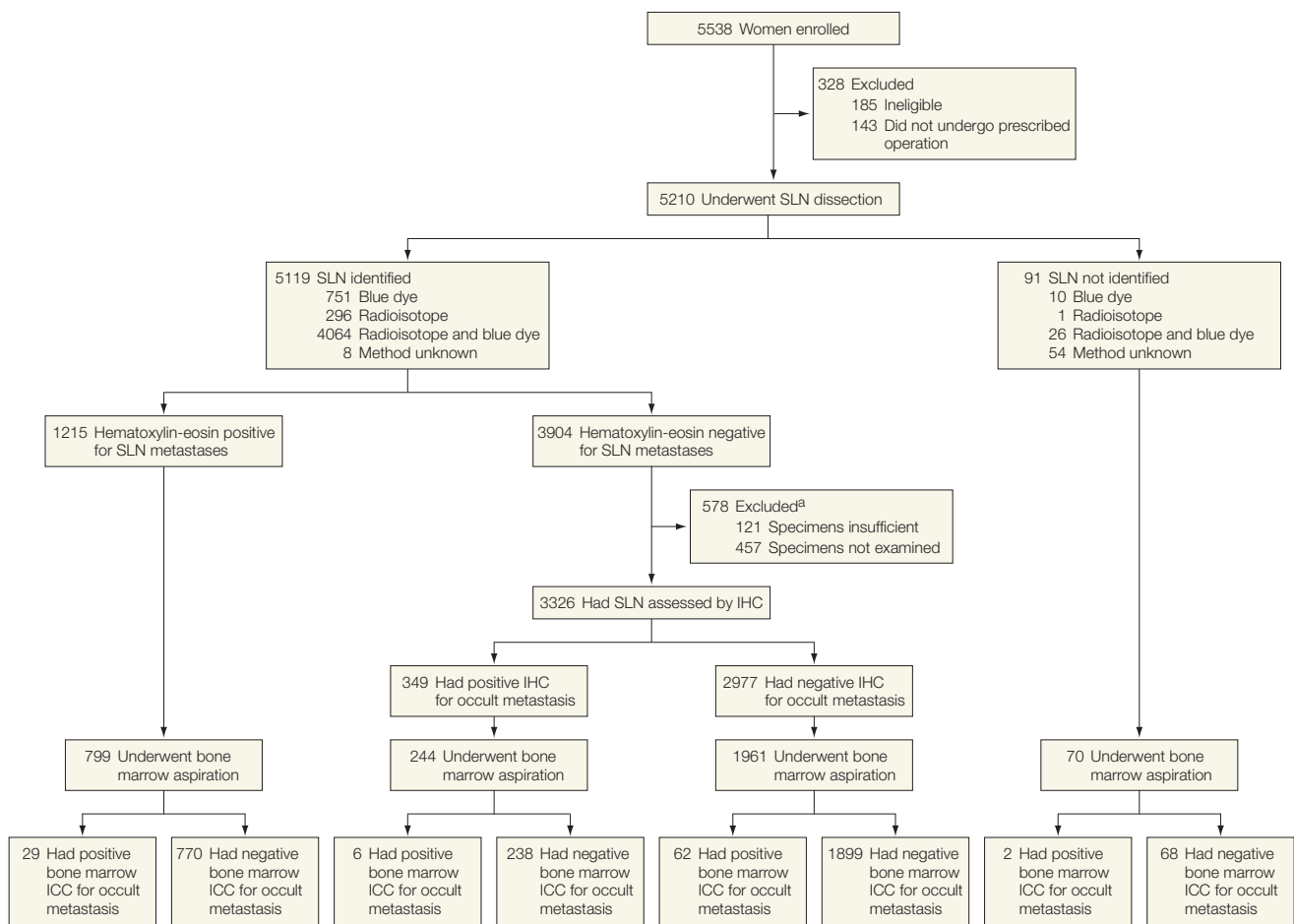
Among patients with hematoxylin-eosin–negative SLNs, immunohistochemical evidence of occult metastases had no significant association with death (FIGURE 2A) or recurrence (Figure 2B). Five-year rates of overall survival for patients with immunohistochemistry-negative SLNs were 95.7% (95% CI, 95.0%-96.5%) and for those with immunohistochemistry-positive SLNs were 95.1% (95% CI, 92.7%-97.5%; $P = .64$). Corresponding 5-year rates of disease-free survival were 92.2%

(95% CI, 91.1%-93.2%) and 90.4% (87.2%-93.8%), respectively ($P = .82$).

Immunohistochemical evidence of SLN metastases was not associated with reduced overall survival on univariable analysis (unadjusted HR, 0.90; 95% CI, 0.59-1.39; $P = .64$) or multivariable analysis (adjusted HR, 0.88; 95% CI, 0.45-1.71; $P = .70$). Age older than 50 years and tumor size larger than 1 cm were independently associated with reduced overall survival.

Occult bone marrow metastases were significantly associated with increased mortality (FIGURE 3A) but not with increased recurrence (Figure 3B). At 5 years, mortality rates were 5.0% (95% CI, 4.2%-5.7%) for patients with

Figure 1. Flowchart of the Z0010 Trial



The trial was designed to evaluate the prevalence and prognostic significance of occult metastases in sentinel lymph nodes and bone marrow.
^aOf the 578 excluded, 334 bone marrow specimens tested negative and 5 tested positive. ICC indicates immunocytochemistry; IHC, immunohistochemistry; and SLN, sentinel lymph node.

immunocytochemistry-negative specimens and 9.9% (95% CI, 3.9%-15.5%) for those with immunocytochemistry-positive specimens ($P = .01$); there were 247 deaths among 3309 women with immunocytochemistry-negative specimens and 15 deaths among 104 women with immunocytochemistry-positive specimens. Overall survival rates were 95.0% (95% CI, 94.3%-95.8%) among women with immunocytochemistry-negative specimens and 90.1% (95% CI, 84.5%-96.1%) for those with immunocytochemistry-positive specimens ($P = .01$). There were 377 disease-free survival events among 3309 patients with immunocytochemistry-negative specimens and 17 among the 104 patients with immunocytochemistry-positive specimens. Five-year disease-free survival rates were 90.8% (95% CI, 89.7%-91.8%) for patients with immunocytochemistry-negative specimens and 86.7% (95% CI, 80.3%-93.7%) for those with immunocytochemistry-positive specimens ($P = .22$). At 5 years, mortality rates for patients with immunocytochemistry-negative specimens were 5.0% (95% CI, 4.2%-5.7%) and 9.9% (95% CI, 3.9%-15.5%) for those with immunocytochemistry-positive bone marrow specimens ($P = .01$); 247 women died among 3309 women with negative specimens and 15 of 104 women with specimens positive for tumors died. Corresponding overall survival rates were 95.0% (95% CI, 94.3%-95.8%) among women with specimens negative for tumors and 90.1% (95% CI, 84.5%-96.1%) for those with specimens positive for tumors ($P = .01$). There were 377 disease-free survival events among 3309 patients with specimens negative for tumors and 17 among the 104 patients with specimens positive for tumors. Five-year disease-free survival rates for these patients with immunocytochemistry-negative specimens were 90.8% (95% CI, 89.7%-91.8%) and 86.7% (95% CI, 80.3%-93.7%) for those with immunocytochemistry-positive specimens ($P = .22$).

Univariable analysis linked bone marrow metastases to reduced overall survival, but multivariable analysis as-

signed significance only to age older than 50 years and tumor size larger than 1.0 cm (TABLE 3). However, because the HR was not significantly reduced by the additional clinicopathological and treat-

ment variables (unadjusted HR, 1.94; 95% CI, 1.02-3.67; $P = .04$ by univariable analysis; adjusted HR, 1.83; 95% CI, 0.79-4.26; $P = .15$ by multivariable analysis), absence of multivariable sig-

Table 1. Age and Tumor Characteristics of Patients Whose Sentinel Lymph Nodes Stained Negative by Hematoxylin-Eosin and Were Subsequently Examined by Immunohistochemistry

Variable	Tumor Status of Sentinel Lymph Node, No. (%)		P Value
	Negative by Hematoxylin-Eosin and Immunohistochemistry (n = 2977)	Positive by Immunohistochemistry (n = 349)	
Age, y			
Median (range)	57 (23-95)	54 (27-87)	
≤50	835 (28.1)	125 (35.8)	.003
>50	2141 (71.9)	224 (64.2)	
Missing, No.	1	0	
Tumor type			
Ductal	2387 (80.3)	262 (75.1)	.002
Lobular	226 (7.6)	45 (12.9)	
Both	77 (2.6)	14 (4.0)	
Other	284 (9.6)	28 (8.0)	
Missing, No.	3	0	
Lymphovascular invasion			
Absent	1921 (90.4)	217 (83.1)	<.001
Present	205 (9.6)	44 (16.9)	
Missing, No.	851	88	
Tumor size, cm			
Median (range)	1.2 (0-19)	1.5 (0.1-5.0)	
≤1.0	1260 (45.1)	101 (30.7)	<.001
1.1-2.0	1202 (43.1)	161 (48.9)	
>2.0	330 (11.8)	67 (20.4)	
Missing, No.	185	20	
Estrogen receptor status			
Positive	2225 (81.1)	268 (83.5)	.30
Negative	518 (18.9)	53 (16.5)	
Missing	234	28	
Progesterone receptor status			
Positive	1828 (67.6)	219 (70.0)	.40
Negative	875 (32.4)	94 (30.0)	
Missing	274	36	

Table 2. Immunochemical Concordance of Autologous Bone Marrow and Sentinel Lymph Node Specimens^a

	No. (%) of Women		
	Immunohistochemical Staining of Sentinel Lymph Node		Total
	Positive	Negative	
Immunocytochemical staining of bone marrow			
Positive	6 (0.3)	62 (2.8)	68 (3.1)
Negative	238 (10.8)	1899 (86.1)	2137 (96.9)
Total	244 (11.1)	1961 (88.9)	

^a χ^2 statistic, -0.01 (95% confidence interval, -0.07 to 0.05).

nificance is consistent with the limited number of immunocytochemistry-positive specimens.

Adjuvant systemic therapy did not have a statistically significant association with the outcomes of patients with SLN occult metastases: 5-year overall survival rate was 96.3% (95% CI, 89.4%-100%) without adjuvant systemic therapy vs 95.7% (95% CI, 93.2%-98.2%) with adjuvant systemic therapy ($P = .74$); the 5-year disease-free survival rate was 91.4% (95% CI, 80.7%-100%) without adjuvant systemic therapy vs 91.0% (95% CI, 87.5%-94.7%) with adjuvant systemic therapy ($P = .87$).

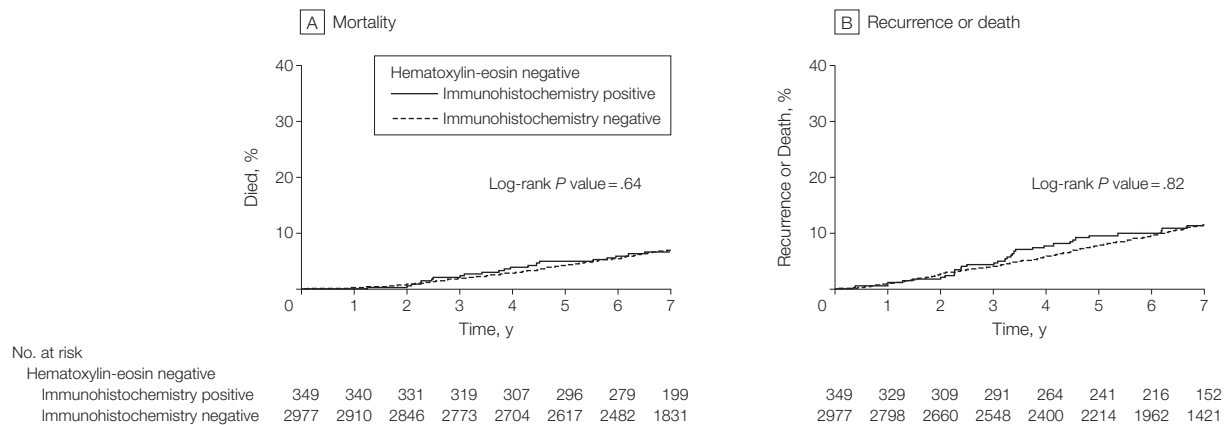
COMMENT

The Z0010 trial is the largest prospective trial to assess immunochemically detected metastases in the SLNs and bone marrow of women with early-stage breast cancer. Occult SLN metastases were detected in 10.5% of patients with hematoxylin-eosin-negative SLNs but were not associated with survival. Occult bone marrow metastases were associated with decreased overall survival only when clinicopathological factors were not considered.

The Z0010 trial was undertaken in part to resolve conflicting data from

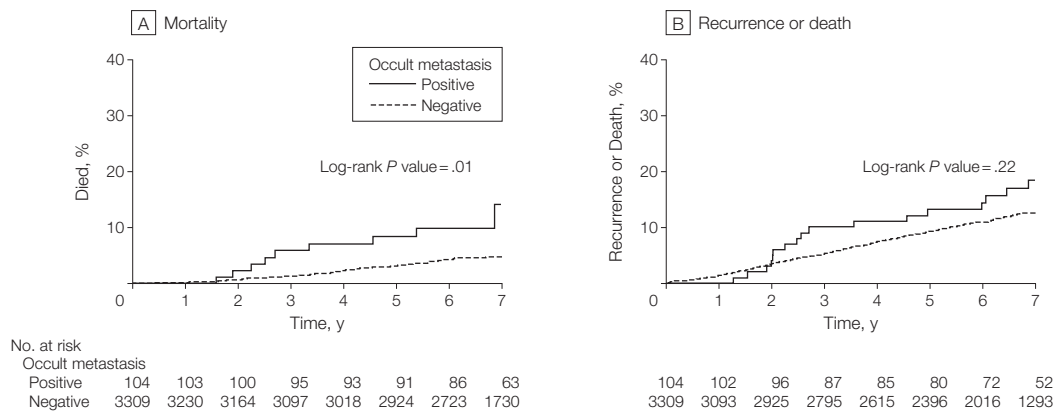
large retrospective studies involving patients with occult metastases (immunohistochemistry-positive or hematoxylin-eosin-negative) in the axillary lymph node dissection specimen. The Ludwig Breast Cancer Study Group identified occult metastases in 20% of patients, about one-third of whom received adjuvant systemic therapy as part of the randomized Ludwig Trial V.⁶ Occult metastases were associated with decreased survival for postmenopausal but not premenopausal women, and the overall decrease was not significant. In a study involving more than 200 000

Figure 2. Sentinel Lymph Node Specimen Results and Cumulative Incidence of Death



A, Patients whose sentinel lymph node specimens were hematoxylin-eosin negative and immunohistochemistry negative vs hematoxylin-eosin negative and immunohistochemistry positive. B, Cumulative incidence of recurrence or death for patients whose sentinel lymph node specimens were hematoxylin-eosin negative and immunohistochemistry negative vs hematoxylin-eosin negative and immunohistochemistry positive.

Figure 3. Bone Marrow Specimen Results and Cumulative Incidence of Death



A, Patients whose bone marrow specimens were negative or positive for occult metastases by immunocytochemistry. B, Cumulative incidence of recurrence or death for patients whose bone marrow specimens were negative or positive for occult metastases by immunocytochemistry. The numbers at risk include 339 women of 578 whose sentinel lymph node specimens were not analyzed by immunohistochemistry.

patients in the Surveillance, Epidemiology and End Results database, survival rate progressively decreased for patients whose nodes were pN0, pN1mic, and pN1,¹⁷ but the authors acknowledged problems with a large retrospective database. Hansen et al⁷ reported findings similar to those of Z0010; however, unlike the Z0010 study, immunohistochemical results often affected decisions regarding adjuvant systemic therapy. In fact, the variable use of adjuvant systemic therapy in these retrospective studies may account for some differences in results.

A retrospective database review by de Boer et al¹⁸ reported outcomes for breast cancer patients treated at 8 cancer centers in the Netherlands. The study included 856 patients with node-negative and 856 with node-positive

(isolated tumor cells or micrometastases) biopsy results who did not receive adjuvant systemic therapy, and 995 patients with node-positive (isolated tumor cells or micrometastases) results who received adjuvant systemic therapy. With a median follow-up of 5.1 years, they noted a significant increase in events among patients with isolated tumor cells and micrometastases who did not receive adjuvant systemic therapy but not among those who received adjuvant systemic therapy. This analysis is difficult to compare with other studies because disease-free survival included contralateral breast cancer and non-breast malignancies, which are not likely to be biologically related to occult metastases from breast cancer. In fact, their study showed no difference

in overall survival with the detection of micrometastases or isolated tumor cells.

In the National Surgical Adjuvant Breast and Bowel Project's B-32 cohort analysis,⁸ 5-year overall survival was 96.4% without occult SLN metastases vs 95.8% with occult metastases. This significant difference was concluded to be insufficient to affect systemic treatment or justify routine immunohistochemistry. This is congruent with conclusions based on Z0010 data. Indeed, data from the 2 trials also are congruent given the differences between these trials. The B-32 protocol required evaluation of 2 closely spaced (0.5-mm) sections intended to detect all metastases larger than 1.0 mm but some metastases smaller than 1.0 mm,⁸ whereas the Z0010 protocol required standard processing similar to that used

Table 3. Univariable and Multivariable Models for Overall Survival of Women Whose Sentinel Lymph Nodes Stained Negative by Hematoxylin-Eosin Stain

Variable	No. of Patients (n = 3902)	No. of Deaths (n = 297)	Univariable Analysis		Multivariable Analysis ^a	
			HR (95% CI)	P Value	HR (95% CI)	P Value
Age, y						
≤50	1131	46	1 [Reference]		1 [Reference]	
>50	2771	251	2.24 (1.64-3.07)	<.001	2.26 (1.30-3.94)	.004
Tumor type						
Ductal	3094	251	1 [Reference]		1 [Reference]	
Lobular	319	18	0.67 (0.41-1.08)	.10	0.84 (0.36-1.97)	.69
Both	97	9	1.16 (0.60-2.26)	.66	1.97 (0.70-5.49)	.20
Other	389	19	0.58 (0.36-0.93)	.02	1.19 (0.51-2.76)	.68
Lymphovascular invasion						
Absent	2380	187	1 [Reference]		1 [Reference]	
Present	301	35	1.52 (1.06-2.19)	0.02	1.03 (0.54-1.96)	.93
Tumor size, cm						
≤1.0	1602	99	1 [Reference]		1 [Reference]	
1.1-2.0	1609	122	1.20 (0.93-1.55)	.15	2.22 (1.34-3.68)	.002
>2.0	455	58	2.14 (1.56-2.94)	<.001	3.22 (1.70-6.12)	<.001
Estrogen receptor status						
Negative	661	77	1 [Reference]		1 [Reference]	
Positive	2943	207	0.55 (0.42-0.72)	<.001	0.64 (0.40-1.04)	.07
Adjuvant systemic therapy						
No	546	58	1 [Reference]		1 [Reference]	
Yes	2743	173	0.56 (0.42-0.75)	<.001	0.60 (0.34-1.02)	.06
Sentinel lymph node immunohistochemistry						
Negative	2977	226	1 [Reference]		1 [Reference]	
Positive	349	23	0.90 (0.59-1.39)	.64	0.88 (0.45-1.71)	.70
Bone marrow immunocytochemistry ^b						
Negative	3309	247	1 [Reference]		1 [Reference]	
Positive	104	15	1.94 (1.02-3.67)	.04	1.83 (0.79-4.26)	.15

Abbreviations: CI, confidence interval; HR, hazard ratio.

^aAdjusted for all variables in the table.

^bThe number at risk and number of deaths are for all women who had bone marrow immunocytochemistry performed. Univariable analysis is for all women with bone marrow immunocytochemistry and multivariable analysis is for women who were hematoxylin-eosin negative.

in routine pathology laboratory practice. This difference resulted in more patients with occult metastasis in the B-32 trial than in the Z0010 trial. The smaller number of patients with immunohistochemistry-detected micrometastases in Z0010 may have been insufficient to detect a small difference in survival. The percentage of participants receiving adjuvant systemic therapy was 78.3% in B-32 vs 86.2% in Z0010. These differences could have attenuated the association between occult metastases and survival in Z0010.

Most patients in the Z0010 trial received adjuvant systemic therapy, reflecting practice patterns in the United States independent of immunohistochemical findings. Thus, although the effect of untreated micrometastases is unknown, it is not relevant to current practice. This conclusion is supported by a population-based study of 24 051 patients in Denmark,¹⁹ which reported that micrometastasis was the sole indication for administration of chemotherapy in only 2.1% of patients. Decisions regarding adjuvant systemic therapy most often reflect consideration of biological or molecular factors associated with the primary tumor.²⁰

Occult metastases of breast cancer in bone marrow reportedly occur in 4% to 48% of patients and consistently have been associated with decreased overall survival.^{12,21,22} These earlier reports included all patients with operable breast cancer and were conducted in an era when patients generally presented with a higher stage of disease. By contrast, the Z0010 trial included only patients with the lowest clinical stage of invasive breast cancer. Because occult bone marrow metastasis is related to stage of disease,⁹ it is not surprising that the incidence of bone marrow metastases is far lower in the Z0010 trial than in prior studies. Technical differences in the assays also may have contributed to differences among studies; immunochemical staining of bone marrow is challenging. In any case, the excellent overall outcome for all patients enrolled supports the low inci-

dence of bone marrow metastases. Balic et al¹⁵ reported a putative stem cell-like phenotype (CD44⁺CD24^{-low}) in immunocytochemistry-positive cells from the bone marrow of 65% of Z0010 patients. This suggests that biological factors in addition to the size of metastasis may determine the tumorigenic potential of metastatic cells.

Recently, there has been considerable interest in the detection of circulating tumor cells in the peripheral blood of patients with cancer, including breast cancer.²³ Studies have used enrichment technologies to isolate and quantify circulating tumor cells, usually in patients with known systemic metastases. Several studies have shown that monitoring circulating tumor cells can identify responders and nonresponders to systemic treatment. Although these technologies have shown considerable promise in patients with metastatic disease, they do not have the sensitivity required to detect circulating tumor cells in patients with early-stage disease, such as those in the Z0010 trial. Newer and more efficient detection methods may address this issue.²⁴

Findings of the Z0010 trial have important implications for clinical practice. Many laboratories routinely perform multiple sections and immunohistochemistry on hematoxylin-eosin–negative SLNs, even though the College of American Pathologists guidelines for SLN processing do not include their use. Data from Z0010 show that occult metastases detected by immunohistochemistry are not associated with survival differences in patients with the earliest stages of breast cancer. Although longer follow-up might reveal small differences in outcome, these are likely to be of no clinical significance, as demonstrated by findings of National Surgical Adjuvant Breast and Bowel Project B-32 trial.

Bone marrow examination with immunocytochemistry may identify high-risk women; however, the incidence in the Z0010 trial was too low to recommend incorporating bone marrow aspiration biopsy into routine practice for patients with the earliest stages of breast cancer. Improved techniques for iso-

lating and detecting occult tumor cells may make their assessment in the bone marrow more efficient and feasible.²⁴

Routine immunohistochemical examination of hematoxylin-eosin–negative SLNs and routine immunocytochemical examination of bone marrow are not clinically warranted for early-stage (clinical T1-T2N0) breast cancer.

Author Affiliations: Division of Surgical Oncology, John Wayne Cancer Institute at Saint John's Health Center, Santa Monica, California (Dr Giuliano); Department of Pathology, University of Southern California Norris Comprehensive Cancer Center, Los Angeles (Dr Hawes); Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, Minnesota (Dr Ballman); Nashville Breast Center, Nashville, Tennessee (Dr Whitworth); Morton Plant Hospital, Clearwater, Florida (Dr Blumencranz); Lakeland Regional Cancer Center, Lakeland, Florida (Dr Reintgen); Memorial Sloan-Kettering Cancer Center, New York, New York (Dr Morrow); University of Texas Southwestern Medical Center, Dallas (Dr Leitch); Department of Surgical Oncology, the University of Texas M. D. Anderson Cancer Center, Houston (Dr Hunt); American College of Surgeons Oncology Group, Durham, North Carolina (Ms McCall); Cytopathology Section, Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland (Dr Abati); and Department of Pathology, University of Miami Miller School of Medicine, Miami, Florida (Dr Cote). Dr Giuliano is now with Cedars-Sinai Medical Center, Los Angeles, California, and Dr Abati is now with DermPath Diagnostics, Port Chester, New York.

Author Contributions: Dr Giuliano had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Giuliano, Reintgen, Morrow, Hunt, Cote.

Acquisition of data: Giuliano, Hawes, Whitworth, Morrow, Leitch, Hunt, Abati, Cote.

Analysis and interpretation of data: Giuliano, Hawes, Ballman, Whitworth, Blumencranz, Morrow, Leitch, Hunt, McCall, Abati, Cote.

Drafting of the manuscript: Giuliano, Hawes, Ballman, Morrow, Hunt, Cote.

Critical revision of the manuscript for important intellectual content: Giuliano, Hawes, Ballman, Whitworth, Blumencranz, Morrow, Leitch, Hunt, McCall, Abati, Cote.

Statistical analysis: Ballman, McCall, Cote.

Obtained funding: Giuliano, Cote.

Administrative, technical, or material support: Giuliano, Hawes, Whitworth, Reintgen, Morrow, Leitch, Hunt, Cote.

Study supervision: Giuliano, Cote.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Giuliano reported that he has received support from ACOSOG for travel to ACOSOG meetings. Dr Hawes reported receiving grant support from the NIH and travel support from ACOSOG and has personal and institutional grants pending from the NIH, the US Department of Defense, California Breast Cancer Research, Whittier Foundation, and V-Force. Dr Ballman reported institutional support from the National Institute of Cancer and other support from AstraZeneca, Lilly, and Novartis. Dr Whitworth reported travel support from ACOSOG. Dr Blumencranz reported institutional support from Morton Plant Hospital, serving on the Florida Society of General Surgeons board of

directors; and institutional payment for lectures from Hologic. Dr Morrow reported grant support from ACOSOG. Dr Leitch reported travel support from ACOSOG and institutional support from ACOSOG for capitation payments for patients enrolled in a study.

Funding/Support: This study was supported by funds from the National Institutes of Health, under the grants entitled "Bone Marrow and Sentinel Node Micrometastases in Breast Cancer" (NCI R01 CA 85840; R. Cote, principal investigator) and American College of Surgeons Oncology Group (NCI U10 CA076001).

Role of the Sponsor: The sponsors had no role in the design and conduct of the study; the collection, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official view of the National Institutes of Health.

Previous Presentation: Presented in part at the annual meeting of the American Society of Clinical Oncology, June 4-8, 2010, Chicago, Illinois.

Additional Contributions: We thank the ACOSOG staff, in particular the leadership of Heidi Nelson, MD (ACOSOG Group Co-Chair, Mayo Clinic, Rochester, Minnesota), David Ota, MD (ACOSOG Group Co-Chair, Duke University, Durham, North Carolina), and Samuel A. Wells Jr, MD (senior clinician, National Cancer Institute, Bethesda, Maryland). All 3 of these physicians contributed to study design and/or manuscript review; none received compensation. We thank Gwen Berry, MHA, of the John Wayne Cancer Institute at Saint John's Health Center, Santa Monica, California, for her editorial assistance, for which she received no compensation beyond her regular salary. We also thank the investigators and their site research teams. Finally, we wish to thank the brave patients with breast cancer who participated in this study and their caregivers.

REFERENCES

- Giuliano AE, Dale PS, Turner RR, Morton DL, Evans SW, Krasne DL. Improved axillary staging of breast cancer with sentinel lymphadenectomy. *Ann Surg*. 1995;222(3):394-399.
- de Mascarel I, Bonichon F, Coindre JM, Trojani M. Prognostic significance of breast cancer axillary lymph node micrometastases assessed by two special techniques: reevaluation with longer follow-up. *Br J Cancer*. 1992;66(3):523-527.
- Fisher ER, Swamidoss S, Lee CH, Rockette H, Redmond C, Fisher B. Detection and significance of occult axillary node metastases in patients with invasive breast cancer. *Cancer*. 1978;42(4):2025-2031.
- Nasser IA, Lee AK, Bosari S, Saganich R, Heatley G, Silverman ML. Occult axillary lymph node metastases in "node-negative" breast carcinoma. *Hum Pathol*. 1993;24(9):950-957.
- Trojani M, de Mascarel I, Bonichon F, Coindre JM, Delsol G. Micrometastases to axillary lymph nodes from carcinoma of breast: detection by immunohistochemistry and prognostic significance. *Br J Cancer*. 1987;55(3):303-306.
- Cote RJ, Peterson HF, Chaiwun B, et al; International Breast Cancer Study Group. Role of immunohistochemical detection of lymph-node metastases in management of breast cancer. *Lancet*. 1999;354(9182):896-900.
- Hansen NM, Grube B, Ye X, et al. Impact of micrometastases in the sentinel node of patients with invasive breast cancer. *J Clin Oncol*. 2009;27(28):4679-4684.
- Weaver DL, Ashikaga T, Krag DN, et al. Effect of occult metastases on survival in node-negative breast cancer. *N Engl J Med*. 2011;364(5):412-421.
- Cote RJ, Rosen PP, Lesser ML, Old LJ, Osborne MP. Prediction of early relapse in patients with operable breast cancer by detection of occult bone marrow micrometastases. *J Clin Oncol*. 1991;9(10):1749-1756.
- Braun S, Pantel K, Müller P, et al. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. *N Engl J Med*. 2000;342(8):525-533.
- Diel IJ, Kaufmann M, Costa SD, et al. Micrometastatic breast cancer cells in bone marrow at primary surgery: prognostic value in comparison with nodal status. *J Natl Cancer Inst*. 1996;88(22):1652-1658.
- Braun S, Vogl FD, Naume B, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. *N Engl J Med*. 2005;353(8):793-802.
- Giuliano AE, Hunt KK, Ballman KV, et al. Axillary dissection vs no axillary dissection in women with invasive breast cancer and sentinel node metastasis: a randomized clinical trial. *JAMA*. 2011;305(6):569-575.
- Posther KE, McCall LM, Blumencranz PW, et al. Sentinel node skills verification and surgeon performance: data from a multicenter clinical trial for early-stage breast cancer. *Ann Surg*. 2005;242(4):593-599.
- Balic M, Lin H, Young L, et al. Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. *Clin Cancer Res*. 2006;12(19):5615-5621.
- Pocock SJ, Clayton TC, Altman DG. Survival plots of time-to-event outcomes in clinical trials: good practice and pitfalls. *Lancet*. 2002;359(9318):1686-1689.
- Chen SL, Hoehne FM, Giuliano AE. The prognostic significance of micrometastases in breast cancer: a SEER population-based analysis. *Ann Surg Oncol*. 2007;14(12):3378-3384.
- de Boer M, van Deurzen CH, van Dijk JA, et al. Micrometastases or isolated tumor cells and the outcome of breast cancer. *N Engl J Med*. 2009;361(7):653-663.
- Tvedskov TF, Jensen MB, Balslev E, Ejlersen B, Kroman N. Stage migration after introduction of sentinel lymph node dissection in breast cancer treatment in Denmark: a nationwide study. *Eur J Cancer*. 2011;47(6):872-878.
- Oakman C, Santarpia L, Di Leo A. Breast cancer assessment tools and optimizing adjuvant therapy. *Nat Rev Clin Oncol*. 2010;7(12):725-732.
- Molino A, Colombatti M, Bonetti F, et al. A comparative analysis of three different techniques for the detection of breast cancer cells in bone marrow. *Cancer*. 1991;67(4):1033-1036.
- Osborne MP, Wong GY, Asina S, Old LJ, Cote RJ, Rosen PP. Sensitivity of immunocytochemical detection of breast cancer cells in human bone marrow. *Cancer Res*. 1991;51(10):2706-2709.
- Cristofanilli M, Braun S. Circulating tumor cells revisited. *JAMA*. 2010;303(11):1092-1093.
- Lin HK, Zheng S, Williams AJ, et al. Portable filter-based microdevice for detection and characterization of circulating tumor cells. *Clin Cancer Res*. 2010;16(20):5011-5018.

Today more than ever before life must be characterized by a sense of Universal responsibility, not only nation to nation and human to human, but also human to other forms of life.

—Dalai Lama (1935-)