Circulating HER-2/erbB-2/c-neu (HER-2) Extracellular Domain as a Prognostic Factor in Patients with Metastatic Breast Cancer: Cancer and Leukemia Group B Study 8662

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ABSTRACT

Purpose: The HER-2/erbB-2/c-neu (HER-2) proto-oncogene is a M, 185,000 transmembrane tyrosine kinase that is amplified and/or overexpressed by 20–40% of breast cancers. HER-2 has been associated with worse prognosis and resistance or sensitivity to specific treatment. We evaluated circulating levels of extracellular domain of HER-2 (ECD/HER-2) in metastatic breast cancer patients and investigated the prognostic and predictive significance of circulating HER-2 levels regarding endocrine therapy or chemotherapy.

Experimental Design: Plasma samples from 242 patients were assayed for circulating ECD/HER-2 levels, using a sandwich enzyme immunoassay. ECD/HER-2 was correlated with clinical data gathered from these patients while they were participating in prospective Cancer and Leukemia Group B (CALGB) trials.

Results: Of 242 patients, 237 were evaluable. Plasma samples from 242 patients were assayed for circulating ECD/HER-2 levels, using a sandwich enzyme immunoassay. ECD/HER-2 was correlated with clinical data gathered from these patients while they were participating in prospective Cancer and Leukemia Group B (CALGB) trials.

Conclusion: Circulating HER-2/erbB-2/c-neu (HER-2) Extracellular Domain as a Prognostic Factor in Patients with Metastatic Breast Cancer: Cancer and Leukemia Group B Study 8662

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mia Group B (CALGB) therapeutic protocols for metastatic breast cancer.

Results: Eighty-nine (37%) of 242 patients had elevated ECD/HER-2 levels (≥10.5 ng/ml). ECD/HER-2 was significantly associated with tumor burden, progesterone receptor levels, and presence of visceral metastases. Patients with elevated pretreatment levels had a significantly shorter OS but not time-to-progression than did those with ECD/HER-2 levels <10.5 ng/ml in univariate analysis. In univariate but not multivariate subset analyses, among patients treated with endocrine therapy (megestrol acetate), elevated initial ECD/HER-2 was associated with worse OS compared with nonelevated patients. However, among patients treated with chemotherapy (mainly anthracycline-containing regimens), OS did not differ significantly. Rates of response to either endocrine therapy or chemotherapy were similar for patients with elevated and nonelevated ECD/HER-2 levels.

Conclusions: ECD/HER-2 levels are elevated in 35–40% of patients with metastatic breast cancer. Elevated ECD/HER-2 levels are associated with a poorer prognosis in these patients. However, no predictive role for ECD/HER-2 was identified, either for endocrine therapy or for anthracycline-based chemotherapy in the metastatic setting.

INTRODUCTION

The HER-2 proto-oncogene is a Mr 185,000 transmembrane tyrosine kinase that participates in signal transduction (1). Although the protein product of HER-2 is expressed in many normal epithelial tissues, this gene is amplified and/or overexpressed in 20–40% of primary breast cancer tissues. Amplification and/or overexpression of HER-2 are associated with a worse clinical outcome in patients with newly diagnosed primary breast cancer (2). Prior studies have suggested that tissue HER-2 amplification or overexpression may be a marker for sensitivity or resistance to both endocrine therapy and chemotherapy (3, 4).

Using MAb s that react with the external domain of the HER-2 proto-oncoprotein product, we and others have developed sandwich assays that detect a soluble truncated form of HER-2 (5–8). This truncated peptide has a Mr of 105,000, and it appears to be the excised ECD of the HER-2 gene product (9). Preliminary studies have suggested that levels of this protein, which we have designated the ECD of Her-2 (ECD/HER-2), are elevated in 20–40% of patients with metastatic breast cancer (5–8).

Because overexpression of HER-2 is associated with worse prognosis and is perhaps a predictive factor for response to therapy and because circulating ECD/HER-2 levels are frequently elevated in patients with metastatic breast cancer, we investigated whether circulating ECD/HER-2 levels might be associated with worse clinical outcome. In 1986, we began prospective collection of plasma samples from patients with metastatic breast cancer who were participating in CALGB therapeutic protocols (CALGB companion protocol 8662). In this report, we have confirmed the observation that ECD/HER-2 levels are frequently elevated in patients with metastatic breast cancer, and that elevated levels of ECD/HER-2 are a determinant of prognosis.

MATERIALS AND METHODS

Patients. Two hundred forty-two patients who were enrolled in CALGB prospective therapeutic trials for metastatic breast disease were entered into companion protocol 8662. Informed consent was obtained for participation in protocol 8662 separately from that obtained for the individual treatment protocols. Table 1 lists the eight treatment protocols from which patients were accrued for companion protocol 8662 and the distribution of patients on each treatment protocol. In the endocrine treatment protocol, patients with ER- or PgR-positive and/or unknown breast cancers were treated with one of three doses of megestrol acetate, whereas patients in the other seven protocols received standard or investigational chemotherapy (Phase I, II, or III agents). Of the 242 patients in companion protocol 8662, 103 patients were treated on endocrine trial and 139 were treated on chemotherapy trials. Patients were entered onto the respective therapeutic trials according to their physicians’ discretion and their eligibility for the therapeutic trial. Selection for type of therapy or trial was not influenced by participation in the companion trial.

Table 1 Patients in CALGB companion protocol 8662 by therapeutic protocol

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of patients</th>
<th>Phase</th>
<th>CALGB protocol no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>242</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine therapy, total</td>
<td>103</td>
<td>III</td>
<td>8741</td>
</tr>
<tr>
<td>Megestrol acetate[α]</td>
<td>8741</td>
<td>III</td>
<td>103</td>
</tr>
<tr>
<td>Chemotherapy, total</td>
<td>139</td>
<td>III</td>
<td>8281</td>
</tr>
<tr>
<td>CAF, VATH, or alternating VATH/CMFViP[β]</td>
<td>8281</td>
<td>III</td>
<td>9</td>
</tr>
<tr>
<td>Cytosine arabinoside, cisplatin[γ]</td>
<td>8343</td>
<td>II</td>
<td>1</td>
</tr>
<tr>
<td>CAMFLVi[δ]</td>
<td>8542</td>
<td>II</td>
<td>16</td>
</tr>
<tr>
<td>Echinomycin[ε]</td>
<td>8641</td>
<td>II</td>
<td>1</td>
</tr>
<tr>
<td>CAF vs. Phase II agent[τ]</td>
<td>8642</td>
<td>III</td>
<td>101</td>
</tr>
<tr>
<td>Trimetrexate[ν]</td>
<td>8742</td>
<td>II</td>
<td>8</td>
</tr>
<tr>
<td>Amonafide[ο]</td>
<td>8841</td>
<td>II</td>
<td>3</td>
</tr>
</tbody>
</table>

[α] Patients were randomly assigned to 160 mg/day, 800 mg/day, or 1600 mg/day (50).
[β] C, cyclophosphamide; A, doxorubicin; F, 5-fluorouracil; V, vinblastine; T, thiopeta; H, halotestin; Vi, vincristine; M, methotrexate; P, prednisone. Ref. 51.
[γ] L, citrovorum factor (leukovorin).
[δ] Ref. 53.
[τ] Patients were randomly assigned to CAF or to treatment with a Phase II agent for four cycles. All of the patients treated with a Phase II agent were crossed over to CAF if there was early progression or after four cycles of the Phase II agent. Agents tested were: trimetrexate, i.v. melphalan, amonafide, carboplatin, and elasmucin. Ref. 54.
[ν] Ref. (55).
[ο] Ref. (56).

The abbreviations used are: CALGB, Cancer and Leukemia Group B; OS, overall survival; ECD, extracellular domain; HER-2, HER-2/neu; erbB-2/c-neu; MAb, monoclonal antibody; PgR, progesterone receptor; ER, estrogen receptor; DFI, disease-free interval; TTP, time to progression; CR, complete response; PR, partial response; RR, relative risk; CI, confidence interval.
patients into EDTA-containing glass tubes at the patients’ treating institutions. Plasma was separated from the cellular component, aliquoted into a freezing tube, and frozen at $-20^\circ$C. Samples were then shipped on dry ice to a central laboratory, where they were thawed, realiquoted into special freezing tubes, refrozen, and stored at $-70^\circ$C until the day of assay. Pretreatment samples were collected within 7 days prior to the first treatment cycle.

**ECR/HER-2 Assay.** ECD/HER-2 levels were determined with a sandwich enzyme immunoassay according to the manufacturer’s instructions (ECD/HER-2 Assay; Oncogene Sciences, Cambridge, MA). Briefly, the assay was constructed with MAb NB3 bound to 96-well plate and enzyme-linked MAb TA1 used as a tracer. On the basis of previous studies, a cutoff of 10.5 ng/ml (mean + 2SD in healthy subjects) was used to distinguish elevated from nonelevated levels (6).

**Variables of Interest.** Clinical data were collected according to the requirements of each therapeutic protocol. ECD/HER-2 was measured at the start of clinical treatment. Other variables analyzed included demographic (age) and pretreatment clinical variables [menopausal status, type of surgery, hormone receptor status, performance status, prior treatment (such as chemo-, radio-, immuno-, and endocrine therapy), number of prior treatments, response to prior treatment, type of primary treatment, site of metastasis, number of metastases, and DFI]. “Prior treatment” included adjuvant systemic treatment and any systemic treatment for metastatic disease. “Response to prior treatment” refers to prior endocrine therapy in the metastatic setting. Of note, only patients with ER- or PgR-positive or unknown receptor status tumors were eligible to participate in the endocrine therapy trial. For those patients, ER status and treatment were, by definition, confounded, and we did not analyze this association.

DFI is the time from original diagnosis of primary breast cancer to first local and/or distant recurrence. OS was measured from the time from date of entry onto the treatment protocol to the date of death. TTP was the interval from the date of entry onto the treatment protocol to the date of disease progression. Response to treatment was CR, PR, or improvement versus no response, according to the response criteria in each therapeutic protocol.

**Statistical Analysis.** We examined several demographic and clinical variables to determine whether they were predictive of TTP or OS in Cox proportional hazards multivariate models. The list of variables we modeled were: age, menopausal status, type of surgery, hormone receptor status, performance status, prior treatment, number of prior treatments, response to prior treatment, type of primary treatment, site of metastasis, number of metastases, and DFI. Of this list of variables, number of prior treatments, primary treatment regimen, performance score, and ER status were the statistically significant predictors of outcome. These variables were then used in subsequent multivariate analysis that included ECD/HER-2. Survival curves were generated using the Kaplan-Meier product limit method. Cox proportional hazard models were used to determine univariate significance of variables on OS and TTP, and also to model the relationship among several variables simultaneously as they affect OS and TTP (10). Logistic regression was used to identify univariate significance of variables on response to treatment and to model the probability of response with several variables simultaneously. Correlation coefficients were calculated to measure associations among variables. Statistical significance refers to two-sided $P$ values of less than 0.05. Significant levels were not adjusted for multiple comparisons. Dichotomous cutoffs were used to generate Kaplan-Meier curves, and to perform univariate and multivariate analysis.

**RESULTS**

**Distribution of ECD/HER-2 Levels**

ECD/HER-2 levels were determined for the 242 patients. Eighty-nine (37%) of these patients had ECD/HER-2 levels $\geq 10.5$ ng/ml (Table 2). Thirty-three (32%) of the 103 patients on endocrine therapy and 56 (40%) of the 139 patients on chemotherapy had ECD/HER-2 levels $\geq 10.5$ ng/ml. ECD/HER-2 levels were elevated in 9 (20%) of 45 patients with nonvisceral metastatic disease and in 58 (45%) of 130 patients with evidence of visceral metastases (Table 2). ECD/HER-2 levels were significantly associated with PgR levels (but not ER levels), number of prior treatments, and visceral metastases (Table 2).

**Association of ECD/HER-2 Levels and Clinical Outcome**

During the time that patients were accrued to companion protocol 8662, the CALGB was conducting a prospectively

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of patients</th>
<th>No. (%) of patients with ECD/HER-2 level $\geq 10.5$ ng/ml</th>
<th>ECD/HER-2 level (mean $\pm$ SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>242</td>
<td>89 (37)</td>
<td>26.2 $\pm$6.2</td>
</tr>
<tr>
<td>Treatment Regimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine therapy</td>
<td>103</td>
<td>33 (32)</td>
<td>12.7 $\pm$1.1</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>139</td>
<td>56 (40)</td>
<td>15.3 $\pm$1.2</td>
</tr>
<tr>
<td>Site of metastatic disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not visceral</td>
<td>45</td>
<td>9 (20)</td>
<td>10.8 $\pm$2.7</td>
</tr>
<tr>
<td>Visceral (lung, liver)</td>
<td>130</td>
<td>58 (45)</td>
<td>16.2 $\pm$1.2</td>
</tr>
<tr>
<td>1 site only</td>
<td>103</td>
<td>45 (44)</td>
<td>15.7 $\pm$1.3</td>
</tr>
<tr>
<td>$&gt;1$ site</td>
<td>27</td>
<td>13 (48)</td>
<td>17.9 $\pm$2.9</td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt;50</td>
<td>67</td>
<td>25 (37)</td>
<td>14.5 $\pm$1.6</td>
</tr>
<tr>
<td>$\geq50$</td>
<td>174</td>
<td>64 (37)</td>
<td>14.1 $\pm$0.9</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>44</td>
<td>14 (32)</td>
<td>14.1 $\pm$2.1</td>
</tr>
<tr>
<td>Post</td>
<td>194</td>
<td>73 (38)</td>
<td>14.2 $\pm$0.9</td>
</tr>
<tr>
<td>ER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>123</td>
<td>50 (41)</td>
<td>13.9 $\pm$1.0</td>
</tr>
<tr>
<td>Negative</td>
<td>71</td>
<td>20 (28)</td>
<td>12.9 $\pm$1.4</td>
</tr>
<tr>
<td>PgR$^a$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>86</td>
<td>37 (43)</td>
<td>14.9 $\pm$1.4</td>
</tr>
<tr>
<td>Negative</td>
<td>64</td>
<td>15 (23)</td>
<td>11.2 $\pm$1.2</td>
</tr>
<tr>
<td>Prior treatment$^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq1$</td>
<td>133</td>
<td>39 (29)</td>
<td>12.4 $\pm$1.0</td>
</tr>
<tr>
<td>$&gt;1$</td>
<td>108</td>
<td>51 (47)</td>
<td>16.5 $\pm$1.3</td>
</tr>
<tr>
<td>DFI $&lt;2$ yr</td>
<td>108</td>
<td>41 (38)</td>
<td>14.8 $\pm$1.3</td>
</tr>
<tr>
<td>$&gt;2$ yr</td>
<td>132</td>
<td>48 (36)</td>
<td>13.8 $\pm$1.0</td>
</tr>
</tbody>
</table>

$^a$ Not all data were available for all patients.

$^b$ Statistically significant association between ECD/HER-2 level in proportions and characteristic category ($P < 0.05$).
randomized study of three separate doses of megestrol acetate (160 mg, 800 mg, and 1600 mg/day) in patients with measurable, or evaluable, ER- or PgR-positive or unknown metastatic breast cancer. All of the other patients on protocol 8662 were treated on chemotherapy protocols, mainly anthracycline-containing regimens. As noted, patients were placed on these protocols according to their physicians’ discretion and their eligibility. Because patients in protocol 8662 were so clearly divided between the two major therapeutic groups, namely, endocrine and chemotherapy, and because results of previous studies have suggested that HER-2 may be related to resistance or sensitivity to specific types of therapies, we also examined each group separately (3, 4). Circulating ECD/HER-2 data are presented for all of the patients, and then separately for those in the megestrol acetate trial and for those in chemotherapy trials.

All Patients

**OS.** Elevated initial ECD/HER-2 levels were univariately associated with shorter OS (P = 0.002; Table 3). Median OS for those patients with an elevated ECD/HER-2 level (≥10.5 ng/ml) was 16.4 months, compared with 22.7 months for patients with a nonelevated ECD/HER-2 level (P = 0.002; Fig. 1A).

We used multivariate analysis to determine whether initial ECD/HER-2 added significant information beyond standard clinical variables that were already statistically significant predictors in the model, namely, number of prior treatments, primary treatment regimen (megestrol acetate or chemotherapy), performance score, and tissue ER content. One hundred ninety-two patients had complete data. After adjusting for other clinical variables, ECD/HER-2 levels were not independently correlated with OS (P = 0.20; Table 4).

**TTP.** We also examined the prognostic significance of ECD/HER-2 and other standard clinical variables with TTP. Median TTP was 6.0 months for patients with elevated (≥10.5 ng/ml) and 7.03 months for those with nonelevated ECD/HER-2 levels (P = 0.96; Table 5; Fig. 2A). Treatment regimen, number of prior treatments, number of metastatic sites, prior radiation therapy, DFI, and tissue PgR content, but not ECD/HER-2 levels, were univariately significantly associated with TTP. Likewise, ECD/HER-2 was not significantly predictive of TTP in the final multivariate models (Table 6).

**Response to Therapy.** Among all of the patients, the response rates (CR + PR) for treatment according to pretreatment ECD/HER-2 status were 34 and 32%, in nonelevated ECD/HER-2 patients and elevated ECD/HER-2 patients, respectively (P = 0.75).

**Patients Enrolled in Endocrine Therapy Protocol**

**OS.** The median OS for patients on megestrol acetate with elevated pretreatment ECD/HER-2 was 20.2 months compared to 16.8 months for patients with nonelevated ECD/HER-2 (P = 0.007; Table 3).

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**Table 3**  RR of death associated with ECD/HER-2 by univariate model

<table>
<thead>
<tr>
<th>Patients group</th>
<th>RR of death</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n = 242)</td>
<td>1.54</td>
<td>1.18–2.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Endocrine therapy (n = 103)</td>
<td>1.82</td>
<td>1.18–2.81</td>
<td>0.007</td>
</tr>
<tr>
<td>Chemotherapy (n = 139)</td>
<td>1.26</td>
<td>0.89–1.78</td>
<td>0.19</td>
</tr>
</tbody>
</table>

**Fig. 1** OS of patients with metastatic breast cancer by pretreatment ECD/HER-2 level. A, all patients (n = 242; ECD/HER-2, <10.5 ng/ml: n = 153; ECD/HER-2, ≥10.5 ng/ml: n = 89). B, patients who participated in a randomized trial of three doses of megestrol acetate (n = 103; ECD/HER-2, <10.5 ng/ml: n = 70; ECD/HER-2, ≥10.5 ng/ml: n = 33). C, patients who participated in chemotherapy trials (n = 139; ECD/HER-2, <10.5 ng/ml: n = 83; ECD/HER-2, ≥10.5 ng/ml: n = 56). - - -, ECD/HER-2 <10.5 ng/ml; ---, ECD/HER-2 ≥10.5 ng/ml. OS was determined from time of entry onto therapeutic protocol.
pared with 27.8 months for patients with nonelevated ECD/HER-2 (P = 0.007; Table 3, Fig. 1B). Seventy-eight patients had complete data for multivariate analysis. After accounting for the number of prior treatments, performance score, and tissue ER content, initial ECD/HER-2 was multivariately associated with OS for patients treated with endocrine therapy, although this interaction did not reach conventional levels of statistical significance (RR, 1.63; 95% CI, 0.98–2.72; P = 0.063; Table 4).

TTP. Among patients treated with megestrol acetate, median TTP was 5.95 months for patients with elevated ECD/HER-2 levels (≥10.5 ng/ml) and 7.36 months for those with nonelevated ECD/HER-2 levels (P = 0.90; Table 5, Fig. 2B). Initial ECD/HER-2 was not significantly predictive of TTP in univariate and multivariate analysis (Tables 5 and 6).

Response to Therapy. There was no statistically significant difference in response rates to megestrol acetate between patients with elevated and those with nonelevated ECD/HER-2 levels (response rate = 28% versus 37% for patients with nonelevated ECD/HER-2 versus elevated ECD/HER-2, respectively; P = 0.41). Prior studies have suggested that inclusion of stable disease with CR and PR in consideration of “clinical benefit” is appropriate (11). However, when analyzed in this fashion, clinical benefit was no different between patients with elevated and nonelevated ECD/HER-2 levels (80% versus 78%, respectively; P = 0.79).

Patients Enrolled in Chemotherapy Protocols

OS. For patients who participated in chemotherapy trials, the median OS was 15.6 months in the elevated ECD/HER-2 group and 19.0 months in the nonelevated group (P = 0.19; Fig. 1C).

Initial ECD/HER-2 was not associated with OS for patients treated with chemotherapy in univariate and multivariate analysis (Tables 3 and 4).

TTP. For patients who participated in chemotherapy trials, the median TTP was 6.01 months in the elevated ECD/HER-2 group and 6.64 months in the nonelevated group (P = 0.88; Fig. 2C). In univariate and multivariate analysis, pretreat-

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**Table 4** RR of death associated with ECD/HER-2 by multivariate Cox proportional hazards model<sup>a</sup>

<table>
<thead>
<tr>
<th>Patients group</th>
<th>RR of death</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n = 192)</td>
<td>1.24</td>
<td>0.89–1.74</td>
<td>0.20</td>
</tr>
<tr>
<td>Endocrine therapy (n = 78)</td>
<td>1.63</td>
<td>0.98–2.72</td>
<td>0.06</td>
</tr>
<tr>
<td>Chemotherapy (n = 114)</td>
<td>1.05</td>
<td>0.68–1.64</td>
<td>0.82</td>
</tr>
</tbody>
</table>

<sup>a</sup>The multivariate models include the additional variables of primary treatment (only for all of the patients), number of prior treatments, performance status, and ER status.

**Table 5** RR of progression associated with ECD/HER-2 by univariate model

<table>
<thead>
<tr>
<th>Patients group</th>
<th>RR of progression</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n = 242)</td>
<td>1.01</td>
<td>0.77–1.31</td>
<td>0.96</td>
</tr>
<tr>
<td>Endocrine therapy (n = 103)</td>
<td>1.03</td>
<td>0.67–1.57</td>
<td>0.90</td>
</tr>
<tr>
<td>Chemotherapy (n = 139)</td>
<td>0.97</td>
<td>0.69–1.37</td>
<td>0.88</td>
</tr>
</tbody>
</table>

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*Fig. 2 TTP of patients with metastatic breast cancer by pretreatment ECD/HER-2 level. A, all patients (n = 242; ECD/HER-2, <10.5 ng/ml: n = 153; ECD/HER-2, ≥10.5 ng/ml: n = 89). B, patients who participated in a randomized trial of three doses of megestrol acetate (n = 103; ECD/HER-2, <10.5 ng/ml: n = 70; ECD/HER-2, ≥10.5 ng/ml: n = 33). C, patients who participated in chemotherapy trials (n = 139; ECD/HER-2, <10.5 ng/ml: n = 83; ECD/HER-2, ≥10.5 ng/ml: n = 56). - - -, ECD/HER-2 <10.5 ng/ml; —, ECD/HER-2 ≥10.5 ng/ml. TTP was determined from time of entry onto therapeutic protocol.*
Circulating ECD/HER-2 as a Prognostic Factor in Breast Cancer

Response to Therapy. There was no statistically significant difference in response rates to chemotherapy between patients with elevated and nonelevated pretreatment ECD/HER-2 levels (29 and 38%, respectively, \( P = 0.26 \)). Likewise, clinical benefit was nearly identical for patients with elevated and nonelevated ECD/HER-2 levels (65 versus 67%, respectively; \( P = 0.68 \)).

Because the chemotherapy subgroup represented a relatively heterogeneous population, we analyzed an additional subset of patients who received an anthracycline-containing regimen as first-line chemotherapy for metastatic disease. Of the identified 67 patients, 42 patients were enrolled in CALGB clinical protocol 8642, 16 patients in clinical protocol 8542, and 9 patients in clinical protocol 8281. Ten (40%) of 25 patients with elevated ECD/HER-2 responded, compared with 19 (51%) of 37 patients with nonelevated levels. These results were not statistically significant (\( P = 0.84 \)). Likewise, OS and TTP were not different (data not shown).

DISCUSSION

The results of this study confirm those previously published that MAb-based sandwich assays detect elevated levels of ECD of the HER-2 proto-oncogene in approximately 20–40% of patients with metastatic breast cancer (5–8, 12–20). Levels of this ECD/HER-2 were most likely to be elevated in patients who had more advanced disease, especially those with visceral metastases. This observation probably explains the association of ECD/HER-2 with the number of prior treatments, because patients who have had failed prior treatment are more likely to have greater burden of disease. Of interest, initial circulating ECD/HER-2 levels in patients with metastatic disease were independent of ER, patient age, menopausal status, initial primary surgery (mastectomy or breast-conserving therapy), performance status, or DFI from primary treatment to first metastasis. Therefore, like most other circulating tumor markers, such as CA15–3 and CEA, circulating ECD/HER-2 levels appear to be, at least in part, a function of tumor burden (21, 22).

In most other studies, HER-2 has been correlated with negative ER content, but we observed a positive correlation between ECD/HER-2 and ER and PgR. It must be noted that most prior studies reviewed the incidence of HER-2 positivity in the adjuvant setting, whereas our study was performed in the metastatic setting. Therefore, we may have specifically selected for those ER-positive patients with poorer prognosis. The poor prognosis of such patients may be mediated, in part, by HER-2. One might expect a higher percentage of metastatic ER-positive patients to be HER-2 positive, when compared with ER-positive patients in the adjuvant setting. Furthermore, we also specifically studied a group of patients, representing nearly 50% of our study population, who had progressed on first-line endocrine therapy and were participating in a study of second-line megestrol acetate. Again, these patients, who were mostly ER positive, may have had more aggressive disease than a group of unselected patients with newly diagnosed early breast cancer.

Many other circulating tumor markers have been proposed for breast cancer. Of these, CA15–3 and CEA are the most intensively studied and the most commonly used in the clinic (23). However, the principal utility of these markers has been to monitor clinical course in patients undergoing endocrine- and/or chemotherapy (21, 24–26). In general, the prognostic associations of initial CA15–3 and CEA in patients with metastatic disease have been related to tumor burden (21, 24). These observations suggest that CA15–3 levels simply reflect tumor burden and not biology or relative resistance. Although increased staining with MAbs that react with antigens related to the CA15–3 antigen have been associated with improved overall prognosis in patients with newly diagnosed breast cancer (27–30), there are no reports linking either this family of high molecular weight mucin-like antigens or CEA with relative resistance to either endocrine- or chemotherapy.

In contrast to circulating CA15–3 or CEA, which seem to reflect mostly tumor burden, circulating ECD/HER-2 levels may reflect biological or relative resistance as well. Prior investigations have suggested a relationship with HER-2 and resistance or sensitivity to specific systemic therapies (3, 4). Of relevance to this study, we were unable to detect any interaction between circulating ECD/HER-2 and response to either endocrine therapy or chemotherapy.

Elevated ECD/HER-2 levels were significantly associated with worse OS in univariate analysis in patients receiving endocrine therapy, but not among patients treated with chemotherapy. However, because this study did not have prospectively assigned untreated control groups for either endocrine treatment or chemotherapy, we cannot assess the predictive utility of ECD/HER-2 using TTP or OS as end points (31). At most, we can conclude from these findings that HER-2 is a poor “prognostic” factor. In contrast, one can assume that the response rate among patients who did not receive treatment is zero. With this assumption, this investigation failed to detect any association between pretreatment ECD/HER-2 levels and benefit from either endocrine- or chemotherapy.

Several studies have suggested that tissue overexpression or amplification of HER-2 may be associated with resistance to endocrine therapy in adjuvant and metastatic breast cancer setting (32–38). Prior investigations by us and others have reported similar observations for circulating ECD/HER-2 (8, 19). In contrast, results from one study in the adjuvant setting and three studies in metastatic breast cancer have not supported these findings (20, 39–41). Of those studies, only one had prospectively assigned randomized untreated control arms (32). The retrospective nature of these studies, including our current in-

### Table 6

<table>
<thead>
<tr>
<th>Patients group</th>
<th>RR of progression</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (( n = 192 ))</td>
<td>0.76</td>
<td>0.55–1.06</td>
<td>0.11</td>
</tr>
<tr>
<td>Endocrine therapy (( n = 78 ))</td>
<td>0.86</td>
<td>0.52–1.43</td>
<td>0.57</td>
</tr>
<tr>
<td>Chemotherapy (( n = 114 ))</td>
<td>0.71</td>
<td>0.45–1.11</td>
<td>0.14</td>
</tr>
</tbody>
</table>

* The multivariate models include the additional variables of primary treatment (only for all of the patients), number of prior treatments, performance status, and ER status.
vestigation, almost certainly contributes to these conflicting results.

We did not observe an interaction between pretreatment ECD/HER-2 levels and sensitivity to chemotherapy, even when we analyzed only those who received anthracycline-containing regimens as first-line chemotherapy. This finding appears to be contrary to the hypothesis that HER-2-overexpressed patients have relative sensitivity to anthracycline-containing regimens. The association of overexpression of HER-2 in primary breast cancer tissue and relative sensitivity to anthracyclines has been suggested in six previously reported studies (4, 42–48). All of these suggest higher benefit from anthracycline treatment in HER-2-positive patients than in HER-2-negative patients. Nonetheless, the prognosis in HER-2-positive patients was usually worse than in HER-2-negative patients even after anthracycline-containing regimens.

However, three retrospective studies have investigated the correlation between anthracycline sensitivity and HER-2 status in metastatic breast cancer patients (13, 14, 49). In these studies, the response rates in patients with HER-2-overexpressing and -nonoverexpressing tumors were similar or were better in HER-2-positive patients when anthracycline was administered. In fact, our results could be interpreted to be consistent with these findings, because they suggest that HER-2-positive patients did equally well with anthracycline-containing regimens as HER-2-negative patients. To truly test this hypothesis, a control group of patients treated with a uniform, non-anthracycline-containing regimen as first-line therapy is required (4, 13, 31). Such a control group was not available in this study. However, it is possible that metastatic breast cancer behaves differently from primary breast cancer as regards anthracycline-containing regimens. The association between HER-2 and benefit from anthracyclines in metastatic breast cancer requires further evaluation.

In summary, these results suggest that the external domain of the HER-2 proto-oncogene product levels are elevated in 40% of patients in metastatic breast cancer. Furthermore, elevated circulating ECD/HER-2 levels were associated with worse survival in patients with metastatic breast cancer. However, in this study, elevated ECD/HER-2 levels were not associated with resistance to endocrine therapy or with sensitivity to anthracycline-based chemotherapy. Therefore, the precise utility of detection of circulating ECD/HER-2 remains undefined.

REFERENCES


Circulating ECD/HER-2 as a Prognostic Factor in Breast Cancer


Circulating HER-2/erbB-2/c-neu (HER-2) Extracellular Domain as a Prognostic Factor in Patients with Metastatic Breast Cancer: Cancer and Leukemia Group B Study 8662

Daniel F. Hayes, Hideko Yamauchi, Gloria Broadwater, et al.


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