**The Biology Behind**

**The Prognostic and Predictive Values of ECD-HER-2**


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**Abstract**

The search for a simple, sensitive test to reliably determine prognosis and predict response to therapy in patients with cancer is an important area of translational research. In this issue of Clinical Cancer Research, Hayes et al. report the results of an ancillary Cancer and Leukemia Group B protocol designed to determine whether the circulating extracellular domain of HER-2 (ECD-HER-2) is indicative of prognosis or predictive of response to therapy in women with metastatic breast cancer. Results were drawn from a sample of 242 patients of whom 89 had elevated values of the protein. These women had been enrolled in a variety of Cancer and Leukemia Group B protocols evaluating either the efficacy of dose in the use of megestrol acetate as second-line hormonal treatment or in patients enrolled into several chemotherapeutic protocols, many containing doxorubicin. They report that patients with pretreatment elevation of ECD-HER-2 had a worse prognosis than those who did not, but that there was no convincing correlation of elevated ECD-HER-2 with response to either endocrine or chemotherapy. Although the small number of patients and the retrospective study design allows one to draw only tentative conclusions, this report raises several important issues for the conduct of translational research and points to several new hypotheses for future testing.

**Introduction**

The existence of receptors for growth factors affecting epithelium was first introduced when Cohen identified a substance found in the submaxillary gland that promoted early eyelid separation by increasing epidermal growth and keratinization. The cognate receptor for this EGF (EGFR) was shown to be the cellular homologue of the v-erbB oncogene. The EGFR family now includes four members; EGF (HER-1); ErB-B-2 (HER-2/neu, homologue of the human EGFR, or c-neu, homologue of the rat proto-oncogene neu); ErB-B-3 (HER-3); and ErB-B-4 (HER-4). The Her-2/neu gene (ERB-B2) was identified in ethyl nitrosourea-induced rat neuroglioblastomas as a member of the EGFR family by Schechter et al. (3). This oncogene had homology with the erbB gene that encodes EGFR in the region of erbB encoding the tyrosine kinase domain. Activation of an EGFR family member promotes receptor heterodimerization and auto- or trans-tyroosine phosphorylation that activates signal transduction cascades in response to extracellular ligands, including EGF and heregulin; HER-2/neu is the preferred partner of the other family members, despite the inability to identify a bona fide HER-2/neu ligand. Heterodimerization expands the potential array of signaling consequences that occur after receptor activation by a single ligand. Members of the EGFR family are normally expressed in tissues throughout the body. Amplification of both EGFR and HER-2/neu and receptor activation occurs in a wide variety of human tumors. HER-2/neu is constitutively activated by mutation or overexpression and causes transformation in several models. It is now known to participate in several aspects of the malignant phenotype including cell growth, angiogenesis, survival, and metastasis. The realization that HER-2/neu can cooperate with other EGFR family members in malignant growth and transformation culminated in the development of trastuzumab, an anti-HER-2/neu receptor antibody that has therapeutic effects when used alone or in combination with chemotherapy in the treatment of breast cancer.

HER-2/neu exists as a transmembrane protein with a molecular mass of 185 kDa. The ECD of EGFR family members is marked by two consensus, cysteine-rich motifs. Ligands such as EGF are synthesized as integral membrane protein precursors with ECDs that contain EGF-like sequences. The ECDs have been shown to bind to and activate members of the EGFR family on adjacent cells. The ECDs have been shown to bind to and activate members of the EGFR family on adjacent cells (6). Using the human breast carcinoma cell line SK-BR-3, Zabrecky et al. (7) detected HER-2/neu-like activity in conditioned medium from cultures of SK-BR-3 cells. Two monoclonal antibodies specific for the extracellular domain immunoprecipitated a protein with a molecular mass of ~105 kDa that was generated by posttranslational processing. This polypeptide competed with generated HER-2/neu for binding to several monoclonal antibodies. Authors including Hayes and colleagues (8, 9) found that this polypeptide could be detected in the blood and other body fluids of patients with breast cancer. These data provide the background for the current study.

**Evidence That HER-2/neu Is a Prognostic Indicator**

It stands to reason that if HER-2/neu overexpression is a prognostic indicator and if the amount of circulating ECD-HER-2 correlates with the content of HER-2/neu in the primary tumor, then the measurement of ECD-HER-2 should also be of prognostic importance. The prognostic value of HER-2/neu overexpression at
the time of diagnosis was suggested by Slamon et al. (10, 11), based on an analysis of HER-2/neu gene amplification in primary human breast cancer specimens. The biological basis for this work is well founded because cells transformed by HER-2/neu display a more aggressive phenotype, i.e., greater proliferative and metastatic capacity, lesser cellular differentiation, greater topoisomerase II α activity, and a higher incidence of mutant p53. Its rapidity and relative resistance to treatment distinguishes the growth of tumors expressing HER-2/neu in experimental animals (12). Despite the initial reports on the prognostic significance of HER-2/neu, additional studies did not immediately confirm these results (13). These early discrepancies were likely attributable to differences in measuring the expression of the protein, the use of different techniques, and because of differences in defining a positive test. It is now generally accepted that immunohistochemistry performed with a validated antibody that stains ≥2+ correlates with gene amplification measured by fluorescence in situ hybridization positivity in 97–99% of cases (14). A recent review by Yamauchi et al. (15) analyzed the world’s literature on the value of HER-2/neu as a prognostic indicator when measured at the time of initial diagnosis. This overview concluded that HER-2/neu is a weak to moderate negative prognostic indicator. Thus, the data for HER-2/neu as a prognostic indicator at the time of diagnosis exists but is tempered by the variations in technical expertise in processing tissue and conducting the appropriate assays.

The prognostic significance of overexpressed HER-2/neu in metastatic sites is less well understood because most studies focused on its impact at the time of diagnosis. In a recent analysis of disease progression using both immunohistochemistry and fluorescence in situ hybridization, Edgerton et al. (16) reported a 20% discordance between primary tumors and distant visceral metastases. This is in contrast to recent work by Simon et al. (17), who found a 5% discordance between the primary tumor and disease within axillary lymph nodes. Therefore, on the basis of existing data, one would anticipate that elevated ECD-HER-2 found in patients with metastatic breast cancer might also be of prognostic value.

Evidence That HER-2/neu Is a Predictive Indicator of Response to Therapy

A useful predictive indicator should identify those patients most likely to respond to a particular form of treatment. The overview conducted by Yamauchi et al. (15) found that HER-2/neu was a weakly to moderately negative predictive factor for response to hormonal therapy, a moderately negative predictive factor for response to alkylating agents, and moderately positive predictive factor for response to anthracyclines. There were insufficient data to draw conclusions regarding responsiveness to taxanes or radiotherapy.

There is ample reason to anticipate that elevated ECD-HER-2 might indicate alterations in response to hormonal therapy. Several clinical studies suggested that overexpression of HER-2/neu correlated with resistance to endocrine therapy (18–20). These retrospective analyses are consistent with laboratory findings that transfection of hormone-dependent breast cancer cells with HER-2/neu produced estrogen independence and tamoxifen resistance (12, 21). Slamon and colleagues found that overexpression of HER-2/neu created hormone independence, and that the expression of HER-2/neu led to the activation of a cellular program that resulted in down-regulation of the estrogen receptor (21). These data would appear to form a solid basis for the clinical observations that patients with tumors that overexpressed HER-2/neu fared worse than expected when treated with drugs designed to interrupt hormone-mediated signaling. Furthermore, an early report suggested that patients whose tumors overexpressed HER-2/neu treated with tamoxifen did worse than those who did not receive treatment (18–20). In fact, Benz et al. (12) found that overexpression of HER-2/neu created a growth advantage for cells treated with tamoxifen.

The current report from the CALGB suggests that patients with elevated ECD-HER-2 receiving hormonal therapy with megestrol acetate did no worse than those with non-elevated values, i.e., the shorter median time to progression and lower response rates seen in patients with elevated ECD-Her-2 did not reach statistical significance. However, these data must be interpreted with caution because of the small sample size, the retrospective study design, and the unique characteristics of its participants. For example, only 5–10% of newly diagnosed patients whose tumors express HER-2/neu also express estrogen receptors. Yet as pointed out by the authors, the majority of patients in this series with elevated circulating levels of ECD-HER-2 also expressed hormone receptors. This is likely because of the inclusion of patients who had progressed on front-line hormonal therapy with tamoxifen. The current report is consistent with an earlier retrospective evaluation of the predictive value of Her-2/neu (and p53) in patients who participated in CALGB protocol 8541, a study designed to determine the effect of dose and dose intensity on the response to CAF in the adjuvant setting in patients with estrogen receptor-positive, node-positive breast cancer (22). Of 650 patients analyzed for HER-2/neu status, ~50% received tamoxifen. Sixty-four of the 322 patients receiving tamoxifen and 91 of the 328 patients not receiving tamoxifen overexpressed HER-2/neu. Analyzing these 155 patients, the investigators found no influence of HER-2/neu or p53 status on risk of disease recurrence and survival. In contrast, Yamauchi et al. (23) found previously that elevated ECD-HER-2 identified a group of patients less likely to benefit from hormonal therapy with droloxifene. Therefore, the utility of finding elevated ECD-HER-2 in predicting front-line response to hormonal therapy remains an unresolved and important question.

The fact that some but not all clinical studies demonstrated relative resistance to hormonal therapy is not surprising, given their small number of patients and retrospective nature. This creates difficulties in accurately knowing the precision of the measurements of both Her-2/neu and estrogen receptor. Finally, it is optimistic to assume that observations made in relatively simple laboratory models can be directly applied to the study of human malignancies in the far more complex setting of a patient with breast cancer.

Response to Chemotherapy

Retrospective analyses of clinical trials have promulgated the concept that HER-2/neu overexpression predicts altered sensitivity to chemotherapy including resistance to CMF (24, 25) and sensitivity to CAF (26). In contrast to the influence of HER-2/neu on hormonal sensitivity, little data exist to support a biological basis for increased sensitivity to chemotherapy in cells that overexpress
Her-2/neu overexpression and effects of chemosensitivity. In addition, Orr et al. (27) transplanted human mammary epithelial cells with Her-2/neu and studied the sensitivity of a panel of chemotherapeutic drugs in isogenic pairs either non-enriched for HER-2/neu or enriched for HER-2/neu by fluorescent labeling of the surface HER-2/neu and sorting for an enriched population of cells (>85%). These studies showed no differences in sensitivity to cisplatin, doxorubicin, 5-fluorouracil, paclitaxel, methotrexate, or flavopiridol (27). Thus, it would appear that the signaling cascade initiated by increased activation of this oncogene has little direct effect on sensitivity to most chemotherapeutic drugs in human mammary epithelium.

If HER-2/neu is insufficient to produce changes in drug sensitivity, it does not rule out the possibility that it might cooperate with other members of the EGFR family via heterodimerization induced by EGF-like ligands or with other cellular proteins present in breast cancer to produce alterations in drug sensitivity. For example, overexpression of both HER-2/neu and mutated H-ras produced resistance to doxorubicin in MCF-10A cells, and this effect was associated with the expression of P-glycoprotein. In addition, overexpression of heresulin B-2 in MCF-7 cells increased sensitivity to topoisomerase II inhibitors, doxorubicin and etoposide (28). Our own laboratory found that activation of EGFR had profound effects on the expression and posttranslation modification of P-glycoprotein, the MDR1 gene product. For example, activation of the receptor led to phosphorylation of P-glycoprotein (29), activation of downstream signaling through the phospholipase C limb of the calcium messenger system that culminated in a mitogen-activated protein kinase-mediated increase in transcription of the MDR1 gene (30). These results are consistent with the cooperative effects on signal transduction pathways one might expect from overexpression of Her-2/neu and mutations in H-Ras. Yet Pegram et al. (26) did not detect a multidrug resistance phenotype in cell lines expressing HER-2/neu. Therefore, unlike the effects of HER-2/neu on hormone dependence and responsiveness to hormonal therapies, fewer data exist for a direct interaction between HER-2/neu and chemotherapy.

The current study found no correlation between elevated ECD-HER-2 and response to chemotherapeutic regimens, many of which included doxorubicin, i.e., there was no statistically significant difference in the shorter time to progression, lower response rates, nor clinical benefit response rates between these groups. CALGB 8541 (described above) found that patients whose primary tumors overexpressed Her-2/neu that had been treated with “high-dose CAF” (600 mg/m² cyclophosphamide, 60 mg/m² doxorubicin, and 600 mg/m² 5-fluorouracil) fared better than those treated with lower doses or dose intensities. In contrast, this dose-response relationship was not observed in patients who harbored Her-2/neu-negative tumors (31). In earlier studies, patients overexpressing Her-2/neu were found to be relatively resistant to CMF (24, 25). Since then, other studies have retrospectively analyzed the response to anthracyclines as a function of HER-2/neu status in patients with metastatic disease (32, 33). These studies also suggested a positive correlation between HER-2/neu overexpression and favorable response to anthracycline-containing regimens. Therefore, as in the case with hormonal therapy, the ability of HER-2/neu to predict response to chemotherapy remains an unanswered question.

The analysis by Yamauchi et al. (23) clearly demonstrated the predictive value of HER-2/neu overexpression and response to trastuzumab (23). In this regard, it has been shown that the combination of the antibody plus taxanes and platinum compounds produces synergistic cell kill (34). Although the mechanism underlying the synergy produced by disabling the receptor is unclear, the response to cisplatin may be enhanced by an effect on DNA repair (35).

Future Directions

The presence of ECD-HER-2 in the circulation raises several interesting questions:

(a) Does this cleaved protein bind to and block the effect of trastuzumab? It has been shown that only 15–20% of patients whose tumors overexpress HER-2/neu respond to trastuzumab given alone in the treatment of metastatic breast cancer (36). At least 30% of patients have increased circulating ECD-HER-2 (8, 9). Using SK-B-R breast cancer cells that overexpress HER-2/neu, Zabrecky et al. (7) demonstrated that the cleaved extracellular domain was capable of binding to and blocking the function of a variety of anti-HER-2/neu antibodies. Therefore, it will be of considerable importance to determine whether elevated ECD-HER-2 identifies a group of patients who have less benefit from trastuzumab, and if so, can approaches be developed to circumvent this effect.

(b) Can release of ECD-HER-2 into the circulation “immediately” after chemotherapy be used as a marker of early response to therapy? Currently, the choice of treatment for metastatic disease is empiric and unsatisfactory; the complete response rate is <20%, the median duration of response is 9 months, and the median survival is 12–24 months. Yet, virtually all patients treated with chemotherapy suffer significant side effects. A standard approach is to administer several cycles of chemotherapy (unless the disease has obviously progressed), before re-evaluating with blood tests and tumor imaging. As a result, patients with a limited life expectancy can use up months before a given treatment is deemed ineffective. By this time, progression of disease compounded by deterioration of performance status may preclude the individual from participation in promising clinical trials or from tolerating standard treatments. Therefore, it would be useful to develop a means to predict response to treatment in individual patients. If ECD-HER-2 is released into the circulation after tumor destruction, an early rise in this marker might indicate a patient with a greater likelihood of responding than one in which treatment produced no further increase in the marker. If the kinetics of release were right, this test might be analogous to the release of intracellular enzymes from damaged myocardium as an indicator of ischemic damage. Conversely, the failure to see an increase might strongly predict for those patients who would derive little benefit from additional cycles of the same chemotherapeutic regimen.

In summary, the CALGB report of the ability to assess ECD-HER-2 in the cooperative group setting and to evaluate this marker for its prognostic and predictive values in patients...
with metastatic breast cancer is an example of the application of a basic laboratory discovery to the care of patients. Information gleaned from this report should provide the basis for future prospective studies that will clarify the role of this marker in the prognosis and treatment of breast cancer.

References

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