

Human Epidermal Growth Factor Receptor 2 as a Prognostic Indicator in Osteogenic Sarcoma

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Prognostic biologic factors that can be assessed at the time of diagnosis for patients with osteogenic sarcoma have not been identified. The current study was designed to evaluate the prognostic significance of the human epidermal growth factor receptor 2 as it relates to histologic response to preoperative chemotherapy and event-free survival. A retrospective immunohistochemical study was performed on material from patients who were newly diagnosed with osteogenic sarcoma who were treated according to the T12 protocol from the authors' institution between 1986 to 1993. Staining for HER2/erbB-2 was accomplished using standard monoclonal antibodies and methods. At the time of initial biopsy, 42.6% of the samples showed HER2/erbB-2 overexpression. Higher levels of expression were observed in samples from patients with clinically detectable metastases at initial presentation and at relapse. Expression of HER2/erbB-2 correlated with inferior event-free survival in patients with non-metastatic disease (47% versus 79% at 5 years). In addition, HER2/erbB-2 expression was associated with significantly less tumor necrosis af-

ter preoperative chemotherapy as determined by the Huvos grading system. These data suggest that HER2/erbB-2 should be evaluated prospectively as a prognostic indicator and clinical trials using antibodies that target this receptor should be considered for the treatment of patients with osteogenic sarcoma.

List of Abbreviations Used

HER2	human epidermal growth factor receptor 2
EGFr	epidermal growth factor receptor
EGF	epidermal growth factor
TGF α	transforming growth factor alpha

Glossary

c-fos, c-myc, c-met, c-sis = Examples of oncogenes implicated in osteogenic sarcoma.
Rb = Tumor suppressor gene responsible for retinoblastoma.
p53 = Tumor suppressor gene coding for a transcription factor implicated in several cancers including osteosarcoma.
3B5 antibody = A clone of the anti-HER2/erbB-2 monoclonal antibody specific for the carboxyl domain of the HER2/erbB-s gene product.

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NIH-313 cells = A mouse fibroblast cell line.

HER2 gene = Gene encoding for human epidermal growth factor receptor 2, also referred to as erbB-2 and neu.

HER2/erbB-2 = The protein coded by the HER2 gene, also referred to HER2, erbB-2, and HER2/neu.

rhuMAB HER2 = Recombinant humanized monoclonal antibody to HER2.

Osteogenic sarcoma is the most common primary malignancy of bone. The current treatment protocols use systemic adjuvant chemotherapy and surgical ablation of all sites of measurable disease. Patients without clinically detectable metastases at presentation have achieved 5-year disease-free survival rates approaching 70%.⁸ The remainder of patients will have recurrent disease develop, with the lungs being the most common site for recurrence. Patients with recurrent disease rarely respond to salvage chemotherapy.^{8,9,19}

Prognostic indicators, other than clinical stage, identifiable at diagnosis to determine which patients ultimately will not respond to current treatment protocols are scarce and currently are of insufficient predictive value to stratify therapy. Clinical stage is the most important predictor of outcome at diagnosis. Approximately 15% to 20% of patients initially present with metastatic disease (Stage III).⁵ Historically, these patients have a very poor prognosis with only approximately 10% achieving clinically significant survival despite aggressive metastasectomies and intensive chemotherapy.¹⁰ However, recent investigations have shown a more favorable prognosis when lung metastases are resected surgically in conjunction with aggressive chemotherapy.¹ Other determinants investigated for their relation to outcome such as tumor size, tumor location, and serum chemistries (lactate dehydrogenase or alkaline phosphatase) have not been consistently predictive of relapse in different studies.^{8,9,19,20}

The most powerful predictor of outcome is

the histologic response of the tumor to preoperative chemotherapy.¹⁵ Tumor necrosis determined by histologic examination is graded according to the system of Huvos.¹⁵ Briefly, Grade 1 indicates no or minimal evidence of necrosis (0% to 50%); Grade 2 indicates areas of necrotic material with other areas of viable tumor (51% to 90%); Grade 3 indicates only scattered foci of viable tumor are present (91% to 99%); and Grade 4 indicates no viable tumor seen after extensive sampling (100%). Grades 3 and 4 are associated with superior event-free survival. Unfortunately, attempts at changing or intensifying chemotherapeutic regimens postoperatively based on poor histologic response in the resected primary tumor specimen have not improved outcome. The use of more intensive preoperative therapy in subsequent investigations has improved the histologic response but has not improved event-free survival.^{9,11}

Clearly a need exists for the capability to stratify patients into high- and low-risk categories. As such, the molecular basis for tumor development and the identification of biologic markers for prognosis in osteogenic sarcoma has been under intense investigation.^{2,6,7,22} Oncogenes and tumor suppressor genes have been a major focus of this research. Oncogenes are altered versions of normal genes that code for the regulatory proteins of cell growth such as growth factors and their cell surface receptors. Normal genes that have the ability to become oncogenes are called proto-oncogenes. Tumor suppressor genes differ from oncogenes in that their absence is associated with malignant transformation. Numerous oncogenes and tumor suppressor genes in osteogenic sarcoma have been identified including c-fos, c-myc, c-met, c-sis, Rb, and p53.

The proto-oncogene erbB-2 located on human chromosome 17 at q21 encodes HER2. The epidermal growth factor receptor, a member of the tyrosine kinase growth factor receptor family, is a transmembrane glycoprotein commonly expressed in many normal human tissues. As a transmembrane protein, the ex-

tracellular domain of the EGFr has a ligand-specific binding site for growth factors, namely EGF and TGF α . The intracellular domain is a tyrosine kinase, which once activated by extracellular ligand, leads to the induction of a cascade of mechanisms responsible for cell growth. Activation of the proto-oncogene erb-B results in the overexpression of EGFr in many types of human tumors. HER2 is structurally homologous to EGFr, although the authentic ligand for HER2 has yet to be identified.

Overexpression of HER2/erbB-2 has been shown to be associated with tumorigenesis and enhanced tumorigenicity *in vitro* and *in vivo*. It has been established that overexpression of erbB-2 correlates with a poorer prognosis in patients with breast cancer.¹⁷ One prior study has shown a correlation between erbB-2 expression with early pulmonary metastases and inferior survival in patients with osteogenic sarcoma.¹² The current study was designed to evaluate HER2/erbB-2 as a possible prognostic indicator at the time of diagnosis of osteogenic sarcoma in humans. Archival paraffin-embedded tissue was identified from a cohort of patients who were treated uniformly at one institution. The material was immunohistochemically stained for HER2/erbB-2. Protein expression was related to histologic necrosis in tumors after preoperative chemotherapy and event-free survival.

MATERIALS AND METHODS

Patients

All patients with newly diagnosed, previously untreated, high-grade osteogenic sarcoma who presented to the author's institution between 1986 and 1993 were offered the opportunity to participate in the T12 protocol.¹¹ The study was reviewed by the Institutional Review Board. Informed consent was obtained from all patients. Patients were assigned randomly to one of two possible treatment regimens. Regimen 1, the control regimen, was modeled after the T10 protocol.⁸ Regimen 2, the experimental regimen, was designed to administer more intensive preoperative chemotherapy (T12 protocol). Briefly, the T10 protocol calls for high-dose

methotrexate and the combination of bleomycin, cyclophosphamide, and dactinomycin preoperatively.

Postoperatively, the patients were stratified according to histologic response. Patients with a good histologic response (Grades 3 or 4) received the same agents with the addition of doxorubicin. Patients with a poor histologic response (Grades 1 or 2) received the same agents with the addition of doxorubicin and cisplatin. Patients with resectable pulmonary metastases underwent thoracotomy after ablation of the tumor at the primary site. The results of the T12 trial, which have been published previously, failed to show significant difference in event-free survival between the two groups (78% and 73%).¹¹ As such, the entire group of patients could be investigated as one cohort. Histologic necrosis according to the grading system as described above¹⁵ was determined by one pathologist.

Immunohistochemistry

All identifiable archival material from patients participating in the T12 protocol study was retrieved from the department of pathology. Bone specimens were decalcified overnight, sectioned, deparaffinized, and rehydrated. The 3B5 antibody (Becton Dickinson, Franklin Lakes, NJ) was used to stain for HER2/erbB-2 according to standard methods.¹⁴ Positive and negative controls were included for each run. Expression of HER2/erbB-2 was confirmed with the Herceptest kit (Dako Corporation, Carpinteria, CA) according to the manufacturer's instructions. Each case was scored by a pathologist blinded to the patient's identity. The cases were scored as 0 (no staining), 1+ (1% to 25% positively staining cells), 2+ (26% to 50% positively staining cells), 3+ (51% to 75% positively staining cells), and 4+ (76% to 100% positively staining cells). For HER2/erbB-2, staining is localized to the cell membrane (Fig 1). Staining of 2+ or greater with the 3B5 antibody was considered a positive result and correlated with at least 2+ staining using the controls and criteria provided in the Herceptest kit. This staining intensity is the minimum required for a patient with breast cancer to be enrolled in the anti-HER2 monoclonal antibody clinical trials.

Statistical Methods

Event-free survival, the primary outcome variable in the current study, is defined as the interval from diagnosis to relapse, progression, death, or last fol-

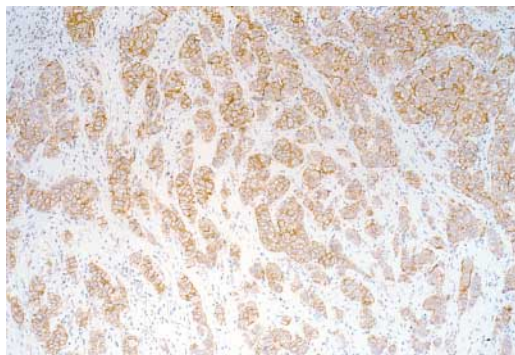


Fig 1. Immunohistochemical staining of osteogenic sarcoma cells showing high levels of HER2/erbB-2 expression. The brown color in the cell membrane represents positive staining.

lowup. The permutation test based on the log-rank statistic was used to compare event-free survival rates between patients with positive versus negative staining for HER2/erbB-2. The permutation procedure was used because of the small number of failures in these data, a situation in which the log-rank test is unreliable. The Fisher's exact test was used to evaluate the difference in histologic necrosis after preoperative chemotherapy between positive and negative HER2/erbB-2 expression.

RESULTS

Seventy-three patients entered the T12 protocol. Archival pathologic material was identified in 53 patients (73%). Initial biopsy material was identified in 47 patients (64%). The clinical profiles of the patients with pathologic material for which HER2/erbB-2 expression could be assessed were not markedly different from those patients with nonassessable archival material in terms of age, gender, and presence of metastatic disease (Table 1). Patients with Grade 4 tumor necrosis had no assessable material at the definitive surgery and therefore are overrepresented in the group of patients with nonassessable tissue. When analysis was limited to patients for whom biopsy material was available, there was no difference between patients with assessable tissue and patients with nonassessable tissue.

TABLE 1. Clinical Characteristics of Patients in the T12 Protocol

Clinical Characteristics	Number
Number of patients	73
Assessable	53
Nonassessable	20
Median age at diagnosis (years)	16.9
Male:female	42:31
Histologic response	
Good	30
Poor	43
Lung metastases at diagnosis	
Absent	63
Present	10

Therefore, the authors considered this group to be representative of the entire cohort of patients enrolled in the T12 protocol.

The relationship of metastatic disease, recurrence, histologic response to preoperative chemotherapy and HER2/erbB-2 expression are shown in Table 2. Twenty-four of the 53 patients (45%) had overexpression of HER2/erbB2. Of the six patients who presented with lung metastases, 50% expressed HER2/erbB-2. Resection samples from 10 of 13 (77%) patients with pulmonary metastases had HER2/erbB-2 overexpression.

Thirty-three of the biopsy samples that were stained with the 3B5 antibody also were stained with the Herceptest kit. Twenty-nine samples had equivalent grading between the two assays. The remaining four samples stained greater with 3B5 compared with Herceptest kit staining.

TABLE 2. Expression of HER2/erbB-2 in Patients With Assessable Tissue

HER2/erbB-2 Expression	Positive	Negative
Overall (n = 53)	45%	55%
Lung metastases at diagnosis (n = 6)	50%	50%
Recurrence (n = 13)	77%	23%
Response to chemotherapy		
good (n = 18)	22%	78%
poor (n = 35)	57%	43%

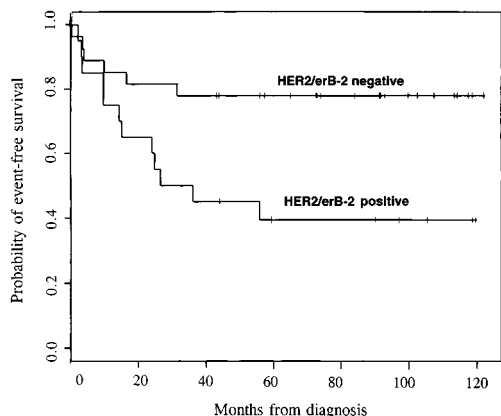


Fig 2. Kaplan-Meier curve for patients without metastatic disease at the time of diagnosis (n = 47) with the absence or presence of HER2/erbB-2 overexpression.

Overexpression of HER2/erbB-2 correlated significantly with tumor necrosis after preoperative chemotherapy. Patients with positive HER2/erbB-2 staining had a poorer response to preoperative chemotherapy compared with those with negative staining (83.3% versus 51.7%, $p = .02$). Event-free survival was significantly inferior in patients with HER2/erbB-2 overexpression (78% versus 40% at 5 years, $p = .01$). When patients with metastatic disease at presentation are excluded, the difference in event-free survival remained significant (79% versus 47% at 5 years, $p = .05$) (Fig 2).

DISCUSSION

The purpose of the current study was to assess HER2/erbB-2 as a possible prognostic factor for patients with osteogenic sarcoma. Currently there are no predictive biologic factors available that can be evaluated at the time of diagnosis. In this cohort of patients, the expression of HER2/erbB-2 correlated with event-free survival and histologic response to preoperative chemotherapy.

It is well known that overexpression of HER2/erbB-2 correlates strongly with inferior clinical outcome in patients with breast cancer.¹⁶ Patients with overexpression of HER2/

erbB-2 have a significantly poorer survival rate compared with patients with little expression of HER2/erbB-2. Patients with lymph node involvement and evidence of erbB-2 gene amplification experienced earlier relapse times and shortened survival compared with patients without evidence of gene amplification. Overexpression of HER2/erbB-2 also is observed in tumors of the ovary, stomach, kidney, colon, bladder, and salivary gland.^{18,21} In addition, several lines of evidence support the direct role of HER2/erbB-2 expression in tumorigenesis. When the oncogene is transfected into mouse fibroblast cells (NIH-3T3), malignant transformation results and these cells are tumorigenic in the mouse.⁴ Furthermore, specific antibodies to the extracellular domain of the HER2 gene inhibit the growth in vitro of tumors that overexpress the gene. These data suggest a direct role for HER2/erbB-2 for malignant transformation and enhanced tumorigenicity.

Onda et al¹² were the first to show the expression of HER2/erbB-2 in primary human osteogenic sarcomas. They found an association with high levels of HER2/erbB-2 and pulmonary metastases and poor outcome. The current study has confirmed and extended these findings. In the previous study, 42% of patients with osteogenic sarcoma had overexpression of HER2/erbB-2. This compares with the authors' observation of 45% of patients having an overexpression of HER2/erbB-2. A slightly higher frequency of HER2/erbB-2 expression was observed in patients with pulmonary metastases at the time of presentation. The authors observed a significantly higher percentage of patients with a poor histologic response after preoperative chemotherapy in patients with the gene overexpression. The current study expands on the previously reported results¹² by showing an inferior outcome in a larger cohort of patients who were treated uniformly. Because inferior outcome is seen in patients with localized disease, perhaps HER2/erbB-2 should be evaluated prospectively as a potential prognostic factor.

Certain correlations exist between expres-

sion of HER2/erbB-2 expression and the clinical characteristics of osteosarcoma. First, inferior event-free survival is related to HER2/erbB-2 expression. At 5 years, 78% of patients without evidence of HER2/erbB-2 expression were alive and free of disease compared with 40% of patients with expression of the gene. Second, an association between histologic response to preoperative chemotherapy and HER2/erbB-2 expression exists. Only 17% of patients with HER2/erbB-2 overexpression had a good response to chemotherapy compared with 48% without the gene overexpression. These data suggest that HER2/erbB-2 plays a role in tumor biology and aggressive tumor growth. The expression of HER2/erbB-2 may be a useful marker in determining patient prognosis before the initiation of treatment.

In addition to identifying prognostic factors, new therapeutic strategies are essential for treating patients with osteogenic sarcoma because current treatment agents are limited and a significant number of patients ultimately will not respond to these agents. It would be highly desirable to identify new factors that could be targeted against osteogenic sarcoma activity and incorporated into the front-line therapy. Because HER2/erbB-2 overexpression significantly correlates with inferior probability of event-free survival, the HER2 antigen might be an attractive target for intervention in patients with osteogenic sarcoma particularly in those patients with metastatic disease who infrequently respond to existing cytotoxic chemotherapy. The results of rhuMAB HER2 (a recombinant humanized monoclonal antibody to HER2) in the treatment of breast cancer, with and without combination chemotherapy, have been encouraging.³ Objective responses including lack of disease progression, partial remission, and one complete remission have been observed in patients with metastatic breast cancer. Higher response rates without increased toxicity were observed in combination with chemotherapy compared with chemotherapy alone.¹³ These results coupled with the authors' findings of inferior event-free sur-

vival associated with HER2/erbB-2 expression have led to the development of multiinstitutional Phase II trials of rhuMAB HER2 in osteogenic sarcoma. A trial of weekly HER2 antibody administration in patients with refractory osteogenic sarcoma currently is planned as a Phase II trial. In addition, rhuMAB HER2 in combination with chemotherapy for patients with newly diagnosed metastatic disease is under consideration.

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