Phase II Trial of Trastuzumab in Combination With Cytotoxic Chemotherapy for Treatment of Metastatic Osteosarcoma With Human Epidermal Growth Factor Receptor 2 Overexpression: A Report From the Children's Oncology Group

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A B S T R A C T

Purpose

Despite efforts to intensify chemotherapy, survival for patients with metastatic osteosarcoma remains poor. Overexpression of human epidermal growth factor receptor 2 (HER2) in osteosarcoma has been shown to predict poor therapeutic response and decreased survival. This study tests the safety and feasibility of delivering biologically targeted therapy by combining trastuzumab with standard chemotherapy in patients with metastatic osteosarcoma and HER2 overexpression.

Patients and Methods

Among 96 evaluable patients with newly diagnosed metastatic osteosarcoma, 41 had tumors that were HER2-positive by immunohistochemistry. All patients received chemotherapy with cisplatin, doxorubicin, methotrexate, ifosfamide, and etoposide. Dexrazoxane was administered with doxorubicin to minimize the risk of cardiotoxicity from treatment with trastuzumab and anthracycline. Only patients with HER2 overexpression received concurrent therapy with trastuzumab given for 34 consecutive weeks.

Results

The 30-month event-free and overall survival rates for patients with HER2 overexpression treated with chemotherapy and trastuzumab were 32% and 59%, respectively. For patients without HER2 overexpression, treated with chemotherapy alone, the 30-month event-free and overall survival rates were 32% and 50%, respectively. There was no clinically significant short-term cardiotoxicity in patients treated with trastuzumab and doxorubicin.

Conclusion

Despite intensive chemotherapy plus trastuzumab for patients with HER2-positive disease, the outcome for all patients was poor, with no significant difference between the HER2-positive and HER2-negative groups. Although our findings suggest that trastuzumab can be safely delivered in combination with anthracycline-based chemotherapy and dexrazoxane, its therapeutic benefit remains uncertain. Definitive assessment of trastuzumab's potential role in treating osteosarcoma would require a randomized study of patients with HER2-positive disease.

J Clin Oncol 30:2545-2551. © 2012 by American Society of Clinical Oncology

INTRODUCTION

Among the 600 new patients with osteosarcoma diagnosed in North America each year, 20% will have clinical evidence of metastatic disease.¹ Although the routine use of intensive chemotherapy has significantly improved survival for patients with localized disease, patients with metastatic osteosarcoma continue to do poorly. Prospects for long-term survival are even worse for patients with metastases to bone

or bilateral pulmonary metastases. These adverse clinical features confer a poor prognosis, with a 2-year event-free survival (EFS) rate of 15% to 20%.²⁻⁵ Substantial improvements in the survival of children with osteosarcoma will likely require the creative incorporation of noncytotoxic agents that address biologic features unique to this disease.

With the retrospective establishment of a correlation between overexpression of the membrane-bound receptor human epidermal growth factor

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Submitted June 10, 2011; accepted April 24, 2012; published online ahead of print at www.jco.org on June 4, 2012.

Supported by the Chair's Grant No. U10 CA98543 and Human Specimen Banking Grant No. U24 CA114766 of the Children's Oncology Group from the National Cancer Institute, National Institutes of Health, Bethesda, MD. Additional support for research was provided by a grant from the WWW (QuadV) Foundation to the Children's Oncology Group.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

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0732-183X/12/3020-2545/\$20.00

DOI: 10.1200/JCO.2011.37.4546

receptor 2 (HER2) and poor outcome among patients with osteosarcoma, HER2 emerged as a promising candidate for targeted biologic therapy.⁶⁻⁹ The development of trastuzumab, a humanized monoclonal antibody that binds specifically to HER2, presented an opportunity to exploit this target. Breast cancer studies have demonstrated enhanced therapeutic responses in women with high levels of HER2 expression when trastuzumab is combined with cytotoxic chemotherapy.¹⁰⁻¹⁶

Despite the promise of such targeted antineoplastic therapy, cardiotoxicity is a potentially serious adverse effect of trastuzumab. The risk of myocardial damage is increased when this antibody is administered in combination with chemotherapeutic regimens that include anthracyclines, a core element of osteosarcoma therapy.¹⁷⁻¹⁹

Given the adverse prognostic impact of high-level HER2 expression on outcome in osteosarcoma, we conducted a clinical trial designed to test the feasibility and safety of treating children with newly diagnosed metastatic osteosarcoma with trastuzumab in combination with cytotoxic chemotherapy. Dexrazoxane was included in an attempt to minimize the potential cardiotoxicity of this regimen.²⁰⁻²²

PATIENTS AND METHODS

Eligibility

Patients younger than 32 years of age with measurable, newly diagnosed, high-grade metastatic osteosarcoma with bone, bone and lung, bilateral lung (any number), or unilateral lung metastases with at least four lung nodules were eligible for this clinical trial. Participants were required to have normal left ventricular function (left ventricular fractional shortening [LVFS] of $\geq 28\%$ or left ventricular ejection fraction $\geq 50\%$), normal renal function (creatinine $\leq 1.5 \times$ normal or glomerular filtration rate ≥ 70 mL/min/1.73 m²), adequate bone marrow function (absolute neutrophil count $> 1,000/\mu\text{L}$ and platelets $> 100,000/\mu\text{L}$), and an Eastern Cooperative Oncology Group performance status ≤ 2 (Karnofsky/Lansky score ≥ 50). Patients with a history of pericarditis, myocarditis, and symptomatic dysrhythmias or conduction disturbances were excluded.

Before enrollment onto the therapeutic trial, all patients were required to register on a companion tumor biology study that included testing for HER2 expression. Initially, the therapeutic study was limited to patients whose tu-

mors demonstrated high levels of HER2 expression. After enrollment of the first 14 patients, eligibility was modified to include all newly diagnosed patients with the poor-risk staging characteristics defined earlier. This change was introduced to allow early initiation of therapy while awaiting results of HER2 testing. Although the chemotherapy backbone was the same for all patients, only patients with overexpression of HER2 were treated with trastuzumab.

Determination of HER2 Status

HER2 status was established using immunohistochemical staining performed and graded by an independent, centralized testing facility (LabCorp, Burlington, NC). Expression of HER2 in biopsy specimens was measured using the Dako Herceptest kit (Dako, Copenhagen, Denmark) with the CB11 antibody. Staining was graded as 0 (presence of staining in $< 10\%$ of tumor cells), 1+ (staining in 10% to 50% of tumor cells), or 2+ (staining in $> 50\%$ of tumor cells).⁷ In contrast to the grading protocol used for breast cancer where only membranous staining is counted, cytoplasmic, membrane, and nuclear staining were all considered positive in this grading system, reflecting the different staining pattern found to be prognostic in osteosarcoma. Only patients with 2+ levels of staining were considered for inclusion of trastuzumab in their treatment regimen. Patients graded as 0 or 1+ were treated with the same chemotherapy backbone but without trastuzumab. Although HER2 gene amplification typically mediates high expression levels in breast cancer, it is rarely found in osteosarcoma with HER2 overexpression, precluding the use of copy number assessment to define patient inclusion. A complete discussion of the HER2 grading system used in this study is provided in the Data Supplement.

Chemotherapy Protocol

Chemotherapy for all patients consisted of a 10-week induction phase followed by 21 weeks of postinduction therapy (Fig 1). Patients with HER2-positive disease also received a total of 34 doses of trastuzumab (4 mg/kg loading dose followed by 2 mg/kg given weekly). The first 14 patients all received trastuzumab starting on week 1 of treatment. For all subsequent patients with HER2-positive disease, trastuzumab was introduced no later than week 6 concurrent with the second course of doxorubicin and cisplatin (Fig 1).

Local Control and Management of Metastatic Disease

Resection of the primary site and all metastatic foci of disease was strongly recommended. For patients with metastatic bony disease that was considered unresectable as a result of potential functional impairments or unacceptable morbidity, external-beam radiotherapy was recommended. Where tissue tolerances permitted, focal radiation doses of 66 Gy were suggested.

1	4	5	6	9	10	11	13	16	17	20	21
Dox	MTX	MTX	Dox	MTX	MTX	Surgery	Ifos	MTX	Dox	MTX	Ifos
Cisplat			Cisplat				Etop		Cisplat		Etop
For patients with HER2-positive disease only, trastuzumab is given on a weekly schedule and may extend beyond the completion of chemotherapy until 34 total doses of trastuzumab are completed.											
24	25	28	29	32	33	34					
MTX	Dox	MTX	Dox	MTX	MTX	Ifos					
	Cisplat		Ifos*			Etop					
With or without trastuzumab, as described above											
<u>DOSES:</u>											
Dox = doxorubicin 37.5 mg/m ² per day IV bolus × 2 days with dexrazoxane 375 mg/m ² /dose											
Cisplat = cisplatin 60 mg/m ² per day × 2 days											
MTX = methotrexate 12 g/m ² per day + leucovorin 15 mg every 6 hours × 10 doses starting 24 hours after methotrexate											
Ifos = ifosfamide 2.8 g/m ² per day × 5 days with mesna											
Ifos* = ifosfamide 1.8 g/m ² per day × 5 days with mesna											
Etop = etoposide 100 mg/m ² per day × 5 days											
G-CSF, given with all courses except methotrexate											
Trastuzumab = trastuzumab 4 mg/kg week 1, then 2 mg/kg weekly for a total of 34 doses											

Fig 1. Treatment schema. G-CSF, granulocyte colony-stimulating factor; HER2, human epidermal growth factor receptor 2; IV, intravenous.

Assessment of Response

Clinical response to therapy was assessed after 10 weeks of induction chemotherapy and at the completion of planned protocol therapy. Assessment included whole-body technetium-99m bone scan, computed tomography scan of the chest, and computed tomography or magnetic resonance imaging of metastatic disease.

Histologic response to therapy was evaluated in whatever site of disease was resected at the end of induction. The percent necrosis in the resected tissue as assessed by the institutional pathologist was considered the histologic response for the patient.

Assessment of Toxicity

Toxicity was graded according to Common Toxicity Criteria (version 2). Potential additive cardiac toxicity from the delivery of trastuzumab in combination with anthracycline was monitored using a combination of real-time functional assessment with echocardiograms²³⁻²⁶ and collection of blood samples for batched biochemical studies which included cardiac troponin T (cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP).²⁷⁻³² Results of the biochemical studies were not used to modify therapy.

Echocardiograms were obtained at baseline before starting therapy, at the end of induction (after delivery of doxorubicin 150 mg/m²), before the fifth dose of doxorubicin (after delivery of doxorubicin 300 mg/m²), and at the end of treatment, after administration of a total of doxorubicin 375 mg/m² with or without trastuzumab.

Immediate reporting of cardiac toxicity was mandatory. Any grade 2 cardiac toxicity required withholding of both doxorubicin and trastuzumab. Changes in left ventricular function requiring interruption of anthracycline and trastuzumab delivery included the development of an asymptomatic resting left ventricular ejection fraction below the institutional lower limit of normal, a decline in the resting left ventricular ejection fraction to $\leq 80\%$ of baseline, or a decrease in LVFS to $\leq 24\%$. Further treatment with both anthracycline and trastuzumab could be resumed only if a repeat echocardiogram in 2 to 4 weeks demonstrated normalization of left ventricular function. Clinical evidence of irreversible congestive heart failure required discontinuation of both trastuzumab and doxorubicin.

Outcome Measures

EFS was taken to be the time from enrollment until disease progression, diagnosis of a second malignant neoplasm (SMN), death, or last patient contact, whichever occurred first. Patients who experienced disease progression, SMN, or death were considered to have experienced an EFS event; otherwise, the patient was considered as censored at last contact.

The survivor functions associated with EFS and survival were estimated using the Kaplan-Meier method.³³ The relative risks for an EFS event and death were compared across groups using the log-rank test.³³ Data current to June 30, 2009, were used in the analysis.

RESULTS

Patient Characteristics

A total of 101 patients (96 evaluable patients) were enrolled onto this study from July 2001 until November 2005. Forty-one patients were HER2 positive (2+ HER2 expression), and 55 patients were HER2 negative (either 0 or 1+ HER2 expression levels).

Ineligible/Inevaluable Patients

Three patients were excluded from analysis because they were ineligible (two because of delay in initiating protocol therapy and one because of misdiagnosis). Two patients were inevaluable as a result of inappropriate treatment with trastuzumab despite HER2 expression levels below the protocol-specified threshold.

Frequency of HER2-Positive Patients

Initially, only HER2-positive patients were eligible for enrollment. The first 14 patients enrolled (all HER2 positive) represented

Table 1. Patient Demographics and Clinical Characteristics

Characteristic	HER2-Positive Patients (n = 41)		HER2-Negative Patients (n = 55)		Total Patients (N = 96)	
	No.	%	No.	%	No.	%
Primary site						
Leg/foot	34		48		82	
Arm/hand	5		4		9	
Pelvis	2		1		3	
Other	0		2		2	
Metastatic site						
Unilateral lung	1	2.4	0	0.0	1	1.0
Bilateral lung	26	63.4	33	60.0	59	61.5
Bone only	5	12.2	9	16.4	14	14.6
Bone and lung	9	22.0	13	23.6	22	22.9
Mean age, years	13.8		15.1		14.5	
Sex						
Male	20		33		53	
Female	21		22		43	

Abbreviation: HER2, human epidermal growth factor receptor 2.

33% of the patients submitted for HER2 expression analysis before amendment of study eligibility to permit enrollment of all patients regardless of HER2 expression status. Subsequent to the eligibility amendment, 35% of the enrolled patients were HER2 positive (2+ HER2 expression).

Sites of Metastasis and Distribution by HER2 Status

Patients with bilateral lung metastases made up the largest subset, constituting 62% of those eligible for analysis. Another 15% of patients had metastases only to bony sites, whereas 23% of eligible patients had both lung and bone metastases (Table 1). Only one HER2-positive patient was enrolled on the basis of $\geq$ four unilateral lung nodules.

Histologic Response

Histologic response after induction chemotherapy was available for 27 of 41 HER2-positive patients and 35 of 55 HER2-negative patients. Using a threshold of more than 90% tumor necrosis to segregate good responders from poor responders, 15 (56%) of 27 HER2-positive patients had a good histologic response compared with 14 (40%) of 35 HER2-negative patients demonstrating a good histologic response.

Survival

Median follow-up time for patients alive at last contact was 41.6 months. Among patients whose tumors overexpressed HER2, all of whom were treated with trastuzumab, 30-month EFS was 32% (95% CI, 18% to 46%), with 30-month overall survival of 59% (95% CI, 43% to 73%). For patients with tumors that did not overexpress HER2, none of whom received trastuzumab, 30-month EFS was 32% (95% CI, 20% to 45%), with a 30-month overall survival of 50% (95% CI, 36% to 63%). The minimal differences in EFS and overall survival between these cohorts were not statistically significant ($P = .54$ for EFS; $P = .58$ for overall survival).

Fig 2. Survival by human epidermal growth factor receptor 2 (HER2) status and sites of metastasis. (A) Event-free survival (EFS) for all patients. (B) Overall survival for all patients. (C) EFS for lung only metastases. (D) EFS for combined lung and bone metastases.

Subset analysis of EFS based on site of metastasis revealed no significant differences in outcome between HER2-positive and HER2-negative patients presenting only with bone metastases (30-month EFS, 40% v 34%, respectively). There was also no significant difference in outcome between patients with disease exclusively metastatic to lung (30-month EFS was 33% for HER2-positive patients v 43% for HER2-negative patients; $P = .79$). The relatively small cohort of patients with metastases to both bone and lung fared somewhat more poorly than patients without bony metastases, with no significant difference in outcome based on HER2 status (Fig 2).

Toxicity Results

Cardiac toxicity. No patient developed clinical evidence of congestive heart failure. Both diastolic and systolic blood pressure decreased over time in all patients, although neither the magnitude of the decline nor the differences between treatment arms ($\pm$ trastuzumab) was statistically significant. There was also a trend toward decreasing LVFS, although this was not statistically significant (Fig 3).

LVFS data were available for only 47 of 96 eligible patients. Serial measurements of LVFS were submitted for only 12 HER2-positive and 12 HER2-negative patients, raising concern that our findings may not accurately reflect therapy-related changes in myocardial function across the entire patient cohort. To address this concern, we compared patients with and without LVFS data across multiple parameters (age,

sex, race, primary tumor site, and proportion of patients treated with trastuzumab) and found no statistically significant differences between groups that would have biased our conclusions. Nonetheless, comments regarding the absence of subclinical changes in cardiac function must be tempered by the incompleteness of our LVFS data. The Data Supplement contains a table of LVFS data and comparative analysis of patient subgroups for which this data were available.

Blood assays included serum cTnT, a measure of acute injury to cardiac myocytes, and serum NT-proBNP, a measure of cardiac neurohormonal activation associated with cardiomyopathy from either pressure or volume overload. Serum cTnT assays were remarkable for the striking absence of any elevations over the course of protocol therapy for either treatment arm (HER2 positive and HER2 negative). All measured cTnT levels were less than 0.01 ng/mL regardless of HER2 status and treatment assignment to trastuzumab. Although there was a slight increase in serum NT-proBNP levels with increasing weeks of treatment, the difference in the rate of change in serum NT-proBNP between patients treated with or without trastuzumab was not significant ($P = .39$; Fig 4).

Noncardiac toxicity. As expected, this regimen was myelosuppressive, with nearly all patients developing grade 3 or 4 neutropenia and the vast majority of patients requiring blood product support. Although neutropenia and fever with neutropenia occurred with nearly equal frequency among HER2-positive and HER2-negative

Fig 3. Changes in myocardial function during treatment: Solid lines represent best prediction of changes in myocardial function over time during protocol therapy. The standardized left ventricular fractional shortening (z score) decreases slightly as time from enrollment increases in both treatment arms. Neither downward trend nor the difference in slope between the two treatment arms is statistically significant.

patients, there was an excess of infectious complications in the HER2-positive group treated with trastuzumab (45% v 33%, respectively, in course 3). Renal salt wasting was also seen in a higher proportion of HER2-positive patients versus HER2-negative patients over the final 14 weeks of protocol therapy (hypokalemia, 36% v 13%; hypocalcemia, 13% v 4%; hypophosphatemia, 23% v 8%, respectively). In addition, in HER2-positive patients, compared with HER2-negative patients, there were more reports of nausea (16% v 4%, respectively) and emesis (10% v 4%, respectively) during the same interval (Table 2). Only the differences in hypokalemia reached statistical significance ($P = .01$).

Death or First Event As a Result of Causes Other Than Disease Progression

SMNs. Three patients developed secondary myeloid leukemia. Two of these patients were in the HER2-positive cohort treated with trastuzumab.

Fig 4. Serum measurements of N-terminal pro-brain natriuretic peptide (NT-proBNP), a marker of cardiomyopathy. Changes in NT-proBNP levels in human epidermal growth factor receptor 2 (HER2)–negative patients treated without trastuzumab and HER2-positive patients treated with trastuzumab.

Table 2. Noncardiac Toxicities

Toxicity	% of Patients					
	Weeks 1-12		Weeks 13-23		Weeks 24-38	
	HER2 Positive	HER2 Negative	HER2 Positive	HER2 Negative	HER2 Positive	HER2 Negative
Nausea	30.0	16.4	9.7	17.1	16.1	4.2
Emesis	27.5	16.4	16.7	8.6	9.7	4.2
Stomatitis	17.1	7.3	15.6	5.7	12.9	12.5
Neutropenia	90.2	78.2	90.6	94.3	100.0	95.7
F/N	30.0	33.3	37.5	47.1	58.1	54.2
F/N with infection	15.0	21.8	25.8	31.4	32.3	16.7
Infection without neutropenia	17.5	7.3	12.9	8.6	12.9	16.7
Platelet transfusion	43.2	44.0	56.7	70.6	71.0	87.5
Packed RBC transfusion	68.4	60.4	71.0	85.3	74.2	91.7
Hypokalemia	17.1	12.7	50.0	11.4	35.5	12.5
Hypocalcemia	7.5	7.3	3.2	2.9	12.9	4.2
Hypophosphatemia	12.5	1.9	15.6	17.6	22.6	8.3
Hypomagnesemia	2.5	1.9	0.0	0.0	3.2	4.2
AST	19.5	12.7	9.4	5.7	9.7	12.5
ALT	68.3	51.9	61.3	47.1	54.8	54.2

NOTE. All HER2-positive patients received trastuzumab. No trastuzumab was given to HER2-negative patients. All analyzed toxicities are grade 3 or 4. Abbreviations: F/N, fever with neutropenia; HER2, human epidermal growth factor receptor 2.

Toxic deaths. There were two toxic deaths on treatment. One HER2-negative patient developed sepsis with acute respiratory distress syndrome 7 months after beginning chemotherapy. A second patient who was HER2 positive died of renal failure after presenting with severe dehydration in the setting of fever with neutropenia, 12 days after starting chemotherapy. Although this patient was HER2 positive, no trastuzumab had been given because the patient's HER2 status had not yet been determined.

DISCUSSION

Since 1986, when the combination of cisplatin, doxorubicin, and methotrexate was established as the standard backbone of contemporary osteosarcoma therapy, improving the survival of patients with osteosarcoma has proven to be an enormously difficult challenge.³⁴ Recognizing that prior generations of clinical trials have pushed the tolerable limits of dose-intensified cytotoxic therapies,³⁵ we sought to exploit a unique feature of osteosarcoma biology through the inclusion of a targeted biologic agent. Our study established the feasibility of combining intensive anthracycline-based chemotherapy with trastuzumab, a humanized monoclonal antibody targeting HER2. No measurable acute myocardial injury occurred with the concurrent delivery of trastuzumab and anthracycline coupled with the cardioprotective agent, dexrazoxane. The outcome for HER2-positive patients treated with trastuzumab did not seem different than the outcome for HER2-negative patients treated without this agent. It is possible that the inclusion of this targeted biologic therapy may have mitigated the adverse prognostic impact of HER2 overexpression. Unless further randomized study of HER2 in osteosarcoma is undertaken, however,

any therapeutic advantage to its inclusion must be regarded as speculative.

In 1996, Japanese investigators reported an inverse correlation between HER2 expression levels and outcome based on a retrospective analysis of 26 patients.⁶ HER2 was overexpressed in 42% of patients. Overexpression of HER2 was associated with metastasis at presentation, a greater risk of lung metastases developing within 6 months of diagnosis, poor survival at 3 years (14% v 84% for patients without HER2 overexpression), and inferior histologic response to chemotherapy. Although gene amplification is almost universally demonstrated in patients with breast cancer with HER2 overexpression, Onda et al⁶ found no gene amplification in osteosarcoma specimens with immunohistochemical evidence of increased HER2 expression. A subsequent retrospective analysis of 47 patients with osteosarcoma by Gorlick et al⁷ demonstrated a similar frequency of HER2 overexpression by immunohistochemistry (43%). As in the prior Japanese study, HER2 overexpression was associated with poor histologic response to therapy and inversely correlated with EFS.

After the initiation of patient accrual on our study, a series of additional reports were published that analyzed the prognostic significance of HER2. Although several of these studies offered further support for the adverse prognostic significance of HER2 overexpression,^{8,36-38} other studies challenged the prognostic value of HER2 expression in osteosarcoma.³⁹⁻⁴²

Although we acknowledge the controversy regarding the reliability, consistency, and predictive value of HER2 expression levels in osteosarcoma, our study was not designed to resolve this important question of prognostic validity. Patients in our study were stratified according to HER2 expression and treated with different therapy ($\pm$ trastuzumab) based on characterization of HER2 expression levels by immunohistochemistry. We expect that questions about the prognostic impact of HER2 will be resolved after completion of a forthcoming review of the HER2 status, histologic response, and EFS of 239 patients with localized disease treated in a companion study (Children's Oncology Group P9754 trial) with no differences in protocol therapy based on HER2 expression status. This analysis is currently under way.

Approximately 35% of all patients who were eligible based on stage proved to be HER2 positive, which is only slightly lower than the anticipated 40% frequency of HER2 overexpression. The distribution of metastatic sites was similar between HER2-negative and HER2-positive patients. There did not seem to be any differences in EFS or overall survival based on sites of metastasis or HER2 expression status. If HER2 overexpression is associated with inferior prognosis in osteosarcoma, the similar outcome for HER2-negative and HER2-positive patients may suggest benefit from the addition of trastuzumab.

Ultimately, the potential therapeutic value of trastuzumab in osteosarcoma therapy can only be established in the context of a randomized trial where HER2-positive patients are assigned to treat-

ment with or without this antibody. Before such a study is undertaken, however, we must be convinced of the prognostic importance of HER2 expression in osteosarcoma and the adequacy of our means of consistently establishing HER2 expression levels in this disease.

Our study suggests that trastuzumab can be safely integrated with anthracycline-based chemotherapy given in combination with dexrazoxane for cardioprotection. Protection afforded by dexrazoxane cannot be fully assessed because of our nonrandomized design. However, the absence of clinically significant cardiac toxicity suggests that our strategy of cardioprotection may permit the safe inclusion of other potentially cardiotoxic agents in the treatment of osteosarcoma.

Any further exploration of the potential role of trastuzumab in osteosarcoma therapy will likely depend on the outcome of studies assessing the impact of HER2 status on response and survival in the recently completed Children's Oncology Group nonmetastatic osteosarcoma study. If the adverse prognostic impact of HER2 expression is validated in that large study of nonmetastatic patients, further endeavors to incorporate trastuzumab into osteosarcoma therapy would seem justified.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory**

Role: George Douglas Letson, Stryker Corporation (C); Neyssa Marina, Millennium Pharmaceuticals (U) **Stock Ownership:** None **Honoraria:** None **Research Funding:** Gene P. Siegal, National Institutes of Health, Haley's Hope Memorial Support Fund for Osteosarcoma, Thomas Logan RAID Fund for Ewing's Sarcoma Research **Expert Testimony:** None **Other Remuneration:** None

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REFERENCES

1. Lewis DR, Ries G: Cancers of the bone and joint. SEER Survival Monograph. http://seer.cancer.gov/publications/survival/surv_bone_joint.pdf
2. Meyers PA, Heller G, Healey J, et al: Chemotherapy for nonmetastatic osteosarcoma: The Me-

morial Sloan Kettering experience. *J Clin Oncol* 10:5-15, 1992

3. Harris MB, Gieser P, Goorin AM, et al: Treatment of metastatic osteosarcoma at diagnosis: A Pediatric Oncology Group study. *J Clin Oncol* 16: 3641-3648, 1998

4. Bielack SS, Kempf-Bielack B, Delling G, et al: Prognostic factors in high grade osteosarcoma of the

extremities or trunk: An analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol* 20:776-790, 2002

5. Kager L, Zoubek A, Potschger U, et al: Primary metastatic osteosarcoma: Presentation and outcome of patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol* 21:2011-2018, 2003

6. Onda M, Matsuda S, Higaki S, et al: ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. *Cancer* 77:71-78, 1996
7. Gorlick R, Huvois AG, Heller G, et al: Expression of HER2/erbB-2 correlates with survival in osteosarcoma. *J Clin Oncol* 17:2781-2788, 1999
8. Scotlandi K, Manara MC, Hattlinger CM, et al: Prognostic and therapeutic relevance of HER2 expression in osteosarcoma and Ewing's sarcoma. *Eur J Cancer* 41:1349-1361, 2005
9. Hynes NE: Amplification and overexpression of the erbB-2 gene in human tumors: Its involvement in tumor development, significance as a prognostic factor, and potential as a target for cancer therapy. *Semin Cancer Biol* 4:19-26, 1993
10. Slamon DJ, Clark GM, Wong SG, et al: Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/new oncogene. *Science* 235:177-182, 1987
11. Hancock MC, Langton BC, Chan T, et al: A monoclonal antibody against the c-erbB-2 protein enhances the cytotoxicity of cis-diamminedichloroplatinum against human breast and ovarian tumor cell lines. *Cancer Res* 51:4575-4580, 1991
12. Pietras RJ, Fendly BM, Chazin VR, et al: Antibody to HER2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene* 9:1829-1838, 1994
13. Pegram MD, Lipton A, Hayes DF, et al: Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J Clin Oncol* 16:2659-2671, 1998
14. Slamon DJ, Leyland-Jones B, Shak S, et al: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 344:783-792, 2001
15. Romond EH, Perez EA, Bryant J, et al: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 353:1673-1684, 2005
16. Mariani G, Fasolo A, De Benedictis E, et al: Trastuzumab as adjuvant systemic therapy for HER2-positive breast cancer. *Nat Clin Pract Oncol* 6:93-104, 2009
17. Tan-Chiu E, Yothers G, Romond E, et al: Assessment of cardiac dysfunction in a randomized trial comparing doxorubicin and cyclophosphamide followed by paclitaxel, with or without trastuzumab as adjuvant therapy in node positive, human epidermal growth factor receptor 2-expressing breast cancer: NSABP B-31. *J Clin Oncol* 23:7811-7819, 2005
18. Suter TM, Procter M, van Veldhuisen DJ, et al: Trastuzumab-associated cardiac adverse events in the Herceptin Adjuvant Trial. *J Clin Oncol* 25:3859-3865, 2007
19. Perez EA, Suman VJ, Davidson NE, et al: Cardiac safety analysis of doxorubicin and cyclophosphamide followed by paclitaxel with or without trastuzumab in the North Central Cancer Treatment Group N9831 Adjuvant Breast Cancer Trial. *J Clin Oncol* 26:1231-1238, 2008
20. Speyer JL, Green MD, Zeleniuch-Jacquotte A, et al: ICRF-187 permits longer treatment with doxorubicin in women with breast cancer. *J Clin Oncol* 10:117-127, 1992
21. Wexler LH, Andrich MP, Venzon D, et al: Randomized trial of the cardioprotective agent ICRF-187 in pediatric sarcoma patients treated with doxorubicin. *J Clin Oncol* 14:362-372, 1996
22. Swain SM, Whaley FS, Berber MC, et al: Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. *J Clin Oncol* 15:1318-1332, 1997
23. Colan SD, Parness IA, Spevak PJ, et al: Developmental modulation of myocardial mechanics: Age and growth-related alterations in afterload and contractility. *J Am Coll Cardiol* 19:619-629, 1992
24. Lipshultz SE, Easley KA, Orav EJ, et al: Cardiac structure and function in children infected with human immunodeficiency virus: The prospective P2C2 HIV multicenter study. *Circulation* 97:1246-1256, 1998
25. Sluysmans T, Colan SD: Theoretical and empirical derivation of cardiovascular allometric relationships in children. *J Appl Physiol* 99:445-457, 2005
26. Mair J, Artner-Sworzak E, Lechleitner P, et al: Cardiac troponin T in diagnosis of acute myocardial infarction. *Clin Chem* 37:845-852, 1991
27. Trachtenberg BH, Landy DC, Franco VI, et al: Anthracycline-associated cardiotoxicity in survivors of childhood cancer. *Pediatr Cardiol* 32:342-353, 2011
28. Lipshultz SE, Rifai N, Dalton VM, et al: The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. *N Engl J Med* 351:145-153, 2004
29. Lipshultz SE, Rifai N, Sallan SE, et al: Predictive value of cardiac troponin T in pediatric patients at risk for myocardial injury. *Circulation* 96:2641-2648, 1997
30. Maeda K, Tsuramoto T, Wada A, et al: Plasma brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction. *Am Heart J* 135:825-832, 1998
31. Favilli S, Frenos S, Lasagni D, et al: The use of B-type natriuretic peptide in pediatric patients: A review of the literature. *J Cardiovasc Med* 10:298-302, 2009
32. Kalbfleisch JD, Prentice RL: *The Statistical Analysis of Failure Time Data*. New York, NY, John Wiley & Sons, 2002
33. Link MP, Goorin AM, Miser AW, et al: The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. *N Engl J Med* 314:1600-1606, 1986
34. Goorin AM, Harris MB, Bernstein M, et al: Phase II/III trial of etoposide and high-dose ifosfamide in newly diagnosed metastatic osteosarcoma: A Pediatric Oncology Group trial. *J Clin Oncol* 20:426-433, 2002
35. Zhou H, Randall RL, Brothman AR, et al: HER-2/neu expression in osteosarcoma increases risk of lung metastasis and can be associated with gene amplification. *J Pediatr Hematol Oncol* 25:27-32, 2003
36. Fellenberg J, Krauthoff A, Pollandt A, et al: Evaluation of the predictive value of Her-2/neu gene expression on osteosarcoma therapy in laser microdissected paraffin-embedded tissue. *Lab Invest* 84:113-121, 2004
37. Hughes DP, Thomas DG, Giordano TJ, et al: Cell surface expression of epidermal growth factor receptor and Her-2 with nuclear expression of Her-4 in primary osteosarcoma. *Cancer Res* 64:2047-2053, 2004
38. Akatsuka T, Wada T, Kokai Y, et al: ErbB2 expression is correlated with increased survival of patients with osteosarcoma. *Cancer* 94:1397-1404, 2002
39. Maitra A, Wanzer D, Weinberg AG, et al: Amplification of the HER2/neu oncogene is uncommon in pediatric osteosarcomas. *Cancer* 92:677-683, 2001
40. Thomas DG, Giordano TJ, Sanders D, et al: Absence of HER2/neu gene expression in osteosarcoma and skeletal Ewing's sarcoma. *Clin Cancer Res* 8:788-793, 2002
41. Anninga JK, van de Vijver MJ, Cleton-Jansen AM, et al: Overexpression of the HER-2 oncogene does not play a role in high grade osteosarcomas. *Eur J Cancer* 40:963-970, 2004
42. Kilpatrick SE, Geisinger KR, King TS, et al: Clinicopathologic analysis of HER-2/neu immunorepression among various histologic subtypes and grades of osteosarcoma. *Mod Pathol* 14:1277-1283, 2001