HER-2 Involvement in Osteosarcoma

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Abstract The major goals of translational research in osteosarcoma entail the identification of prognostic factors and therapeutic targets. Given the relevance of epidermal growth factor receptor pathway to breast cancer and the finding that HER-2 was expressed in a proportion of osteosarcoma, it was reasonable to investigate this pathway further. Investigations of HER-2 in osteosarcoma have led to the publication of numerous conflicting reports with regard to the level and prognostic value of HER-2 expression, which are reviewed and discussed. Numerous lessons provided by this research experience are described. This pathway has also been explored as a therapeutic target with at least one study of trastuzumab for the treatment of osteosarcoma completed. Other studies utilizing alternative approaches to target the HER-2 receptor for the treatment of osteosarcoma have been considered.

Keywords Osteosarcoma • Human epidermal growth factor receptor (HER-2) • Trastuzumab • Immunohistochemistry • Targeted therapy

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Introduction

The major goals of translational research in osteosarcoma entail the identification of prognostic factors and therapeutic targets. The human epidermal growth factor receptor (HER-2) pathway has been demonstrated to have biological relevance in breast cancer, and its inhibition has been shown to have clinically significant results. A portion of osteosarcomas express HER-2. The level of HER-2 expression and its prognostic relevance in osteosarcoma remains controversial. This chapter reviews and discusses the germane literature and clinical implications.

HER-2 Biology

HER-2 was first described by multiple groups in the 1980s, which has led to its multiple names in the literature. The term *neu* was derived from studies in rats which developed neuroglioblastomas following in utero exposure to ethylnitrosourea. The *neu* gene transformed NIH3T3 cells. The protein product was shown to have homology with the epidermal growth factor receptor (EGFR) encoded by the *erb-B* gene [1]. Using the avian erythroblastosis virus transforming gene, v-*erbB*, which has similarity to human EGFR, as a probe, the human homologue of the rat *neu* gene was isolated. Due to it similarity to EGFR, it was named HER-2 [2]. Using the same probe, another group isolated two genes: *c-erB-1*, encoding EGFR, and *c-erB-2* [3]. In a mammary carcinoma cell line, another group also using the v-*erB* probe described MAC117 [4]. Subsequently, *neu*, *c-erB-2*, MAC117, and HER-2 were all shown to be the same gene by having sequence homology and the same chromosome locus.

Like its homologue, EGFR, HER-2 is a transmembrane tyrosine kinase receptor [5]. While EGFR is localized to chromosome 7, HER-2 is found on chromosome 17q21. During fetal development, HER-2 is widely expressed in tissues including placenta, liver, kidney, lung, and brain. Lower levels of expression are also seen in adult tissues: kidney, liver, skin, lung, jejunum, uterus, stomach, and colon. The HER-2 null mouse is embryonic lethal due to complete absence of cardiac trabeculae [6]. Conditional knockout of HER-2 in mouse ventricular cardiomyocytes leads to the development of severe dilated cardiomyopathy [7]. HER-2 is expressed throughout mammary duct development from the nulliparous mouse to lactation [8]. The dominant negative truncated HER-2 receptor expressed under the control of a mouse mammary cell specific promoter leads to a failure to form lactationally active lobuloaveoli [9]. In addition, HER-2 expression has been implicated in the development of the sympathetic nervous system, peripheral nerves, as well as spinal cord oligodendrocytes [10–12].

There are four members of the family of epidermal growth factor receptor tyrosine kinases: ErbB-1 (EGFR), ErbB-2 (HER-2), ErbB-3 (HER-3), and ErbB-4 (HER-4). All of these receptors need to dimerize to initiate the signaling cascade and frequently form heterodimers. HER-2 is unique in that it is the only member of this family for which there is no known ligand. However, it has been shown to be the

preferred partner for the other members to form heterodimers. Heterodimers with HER-2 as a partner have enhancement and prolongation of intracellular signaling [13]. HER2 heterodimerization and activation has been implicated in multiple downstream signaling cascades including mitogen-activated protein kinase (MAPK), PI3K/Akt, mTOR, Src kinase, and Signal Transducer and Activator of Transcription (STAT). The complexity of these downstream effects is mediated by the differing ligand affinity between the different members of the HER family and the specific heterodimer at the time of activation [14, 15].

HER-2 overexpression has been shown to be tumorigenic. Transfection of NIH3T3 cells with HER-2 transforms the cells and leads to tumor formation in mice. The tumorigenicity is associated with level of expression of HER-2 within the transformed cells [16, 17]. Transgenic mice expressing HER-2 under the control of a mouse mammary cell specific promoter form mammary tumors consistent with adenocarcinomas at 4 months of age. Ultimately most of the mice develop lung metastases as well [18].

HER-2 in Breast Cancer

HER2 has been shown to be overexpressed in many human adenocarcinomas including breast, ovaries, lung, stomach, and salivary gland [19]. It has been the most thoroughly evaluated in breast cancer. Shortly after HER-2 was described as a possible oncogene, it was demonstrated to be amplified in greater than 30 % of breast cancers. This initial evaluation also noted a trend for the increased number of copies being associated with increased number of involved lymph nodes at diagnosis. In addition, when the authors evaluated a cohort of node positive patients, HER2 amplification was significantly associated with the number of involved nodes as well as shorter time to relapse and shorter overall survival [20]. Amplification of HER-2 was also demonstrated to correlate with overexpression by Northern, Western, and immunohistochemistry. Western blot analysis was most discordant because of excessive stromal elements in the tumor tissue. In 10 % of the cases, while there was no evidence of amplification, there was clear overexpression at the level of RNA and protein [21]. This suggests that gene amplification may not be the only mechanism leading to HER-2 overexpression in breast cancer.

Trastuzumab is a humanized mouse monoclonal antibody directed at the extracellular domain of HER-2. In xenograft models of human breast cancer cell lines overexpressing HER-2, trastuzumab was shown to have a dose-dependent antitumor activity that was additive with paclitaxel or doxorubicin [22]. In a phase II study of single-agent trastuzumab in women with relapsed, metastatic breast cancer overexpressing HER-2, 5 of 43 evaluable patients (11.6 %) exhibited response to treatment; 37 % had some response or stable disease. One patient exhibited compete response. HER-2 status was defined by immunohistochemistry demonstrating membrane staining in greater than 25 % of cells [23]. When trastuzumab was added to cisplatin in women with relapsed, metastatic breast cancer overexpressing HER-2 the overall response

rate was 23 %. HER-2 status was determined by immunohistochemistry grading 0–3: 2+ and 3+ were considered eligible for study participation [24]. In a phase III study of women with metastatic breast cancer overexpressing HER-2, many of whom had received prior chemotherapy, women were randomized to chemotherapy with or without trastuzumab. The addition of trastuzumab prolonged median time to progression from 4.6 months to 7.4 months ($p<0.001$). The eligibility criterion for HER-2 overexpression was immunohistochemical membrane staining of 2+ or 3+ on 10 % of the tumor cells [25]. Trastuzumab exhibited survival advantage when combined with adjuvant chemotherapy in women with operable HER-2 positive breast cancer. In a report of two randomized trials, the patients in the trastuzumab group had an event free survival of 87.1 % compared to 75.4 % in the control arm. The overall survival rates for the trastuzumab and control group at 3 years were 94.3 % and 91.7 %, respectively. For participation on this study, the tumors required to have immunohistochemical staining of 3+ on greater than 10 % of the tumor cells or demonstration of amplification of HER-2 by fluorescence in situ hybridization [26]. The different criteria for HER-2 positivity led to significant confusion about the relevance of HER-2 in breast cancer. Contradictory reports with varying percentages of HER-2 positive tumors using different antibodies added to the controversy [27].

During the initial pivotal trials of trastuzumab in breast cancer, cardiac dysfunction became readily apparent as a major toxicity of treatment. This led to the establishment of an independent Cardiac Review and Evaluation Committee (CERC). The results of the review of the CERC revealed that 3–7 % of patients treated with trastuzumab alone experienced cardiac dysfunction. This compares to 1 % of patients treated with paclitaxel alone and 8 % of patients treated with anthracyclines. When trastuzumab is combined with paclitaxel or anthracyclines, the rates of cardiac toxicity increased to 13 % and 27 %, respectively. If trastuzumab was combined with other chemotherapy, the rates of cardiac dysfunction remained between 3 and 6 %. The majority of patients with cardiac dysfunction required post-treatment medical therapy. Functional impairment was most pronounced in the patients receiving trastuzumab in combination with anthracyclines, occurring in 16 % versus no greater than 4 % in all the other regimens. However, given the improvement in time to treatment failure associated with trastuzumab, the authors conclude that the risk of cardiac dysfunction is justified. The addition of trastuzumab to chemotherapy other than anthracyclines led to similar outcomes observed with the anthracyclines based regimens [28].

HER-2 in Osteosarcoma Cell Lines

Unlike in breast cancer cells, in osteosarcoma cell lines HER-2 displays primarily cytoplasmic or mixed membranous and cytoplasmic staining. Hughes, et al examined the expression pattern of all the members of the family of epidermal growth factor receptors in primary as well as established osteosarcoma cell lines. They evaluated the expression patterns by immunohistochemistry, western blot, polymerase chain reaction (PCR), and flow cytometry. They demonstrated that HER-3

was not expressed in osteosarcoma. EGFR expression was detectable in a primarily membranous pattern by immunohistochemistry in most of the cell lines studied. The expression of EGFR was confirmed by PCR as well as western blot. However, flow cytometry revealed minimal surface EGFR expression, which the authors suggest may be secondary to internalization in endocytosed vesicles. Supporting this assertion, the authors found that EGFR immunohistochemistry staining in archival specimens displayed a diffuse pattern consistent with localization of the activated receptor within the cytoplasm. HER-4 demonstrated diffuse and nuclear patterns of staining by immunohistochemistry in the primary tumor samples, and primarily nuclear localization in the archival tissue. The protein levels by western blot were consistent with the levels of expression detected by immunohistochemistry. HER-2 demonstrated primarily a diffuse pattern of staining consistent with cytoplasmic localization by immunohistochemistry in both the primary cell lines as well as the archival samples of osteosarcoma. The expression of HER-2 by immunohistochemistry was less intense than that seen by EGFR. The expression levels by immunohistochemistry were consistent with the levels of messenger RNA detected by PCR and protein by western blots. Unexpectedly, despite the lack of detection of HER-2 on the membrane by immunohistochemistry, flow cytometry revealed higher quantities of HER-2 than EGFR on the surface of the primary osteosarcoma cell lines [29].

The detection by flow cytometry of HER-2 on the cell surface of osteosarcoma cell lines has been corroborated by two other studies. Hassan et al. demonstrated in primary as well as established osteosarcoma cell lines that HER-2 is detectable in greater quantities than EGFR [30]. Scotlandi et al. found that 62 % of the primary and established osteosarcoma cell lines tested demonstrated HER-2 expression by flow cytometry, albeit at lower levels than the breast and ovarian cancer cell lines used as positive controls. None of the osteosarcoma cell lines demonstrated amplification of the *HER2* gene by fluorescence in situ hybridization. When treated with trastuzumab, the primary cell lines demonstrated only modest growth inhibition. In contrast, the established osteosarcoma cell line, SaoS-2, showed similar growth inhibition to the positive control breast cancer cell line. However, since the insulin-like growth factor receptor (IGF-1R) is known to play a role in resistance to treatment with trastuzumab and because IGF-1R has been implicated in the pathogenesis of osteosarcoma, the combination of trastuzumab with an antibody targeting IGF-1R was found to have significant growth inhibitory effects, greater than with either antibody alone [31]. Unlike the data in cell lines, the studies in patient samples have described conflicting results regarding whether HER-2 is expressed in osteosarcoma and its role in defining prognosis.

HER-2 Is a Negative Prognostic Indicator in Osteosarcoma

Six studies have demonstrated that HER-2 expression in osteosarcoma portends a poor outcome. Onda et al. in 1996 first described HER-2 expression in osteosarcoma. Using frozen and paraffin embedded tissue from 26 patients, they evaluated HER-2 expression by immunoblotting, immunohistochemical staining, and Southern

blotting, which they correlated with known clinical outcomes. They found that 42 % of tissues demonstrated various levels of expression by immunoblotting, which was scored from 0 to 3+ (no staining, weak, moderate, and high, respectively). This was corroborated by immunohistochemistry, revealing a primarily membranous pattern of staining. Southern blot analysis did not reveal any amplification of the *HER-2* gene. Patients whose tumors expressed HER-2 (1 to 3+) had significantly worse response to preoperative chemotherapy and survival as measured by Kaplan-Meier curves. In this series, patients who had no HER-2 expression demonstrated a 1-year survival rate of 100 % and 3-year survival rate of 84 %. In contrast those with weak to high expression of HER-2 had significantly worse outcomes with 1- and 3-year survival rates of 61 % and 14 %, respectively [32].

In another, single-institution, retrospective analysis, Gorlick et al. reviewed 53 patients treated on the T12 protocol. This randomized trial found no survival benefit to dose intensification of the preoperative chemotherapy, allowing all the samples to be treated as a single cohort [33]. HER-2 expression levels were evaluated by immunohistochemistry and scored according to according to the percentage of cells staining positive: 0 (no staining), 1+ (1–25 %), 2+ (26–50 %), 3+ (51–75 %), and 4+ (76–100 %). HER-2 staining localized primarily to the cell membrane. Overexpression was defined as greater than 2+ staining. HER-2 was overexpressed in 45.3 % of the patients' tumors, which was similar to the 42.6 % detected from the initial biopsy specimens. Overexpression of HER-2 was found to be correlated with decreased response to preoperative chemotherapy and a worse event-free survival. At 5-years, patients whose tumors overexpressed HER-2 had a 40 % event-free survival compared to 78 % for patients with low or undetectable levels of HER-2 expression. The difference in event-free survival remained significant even when the 13 % of patients who presented with metastatic disease were excluded from the analysis (47 % versus 79 %) [34].

Zhou et al. reviewed HER-2 expression from 25 patients treated at their institution from 1981 to 1996. They included in their analysis 25 primary tumor samples and 12 specimens from metastatic lung lesions. They evaluated the samples using immunohistochemistry for levels of HER-2 expression and FISH for amplification. Immunohistochemistry was defined as positive if greater than 25 % of tumor cells demonstrated immunoreactivity. Amplification was defined as positive if greater than 10 % of the cells demonstrated more than two signals or if more than three cells showed a large number of signals by FISH probe for the *HER-2* gene. They found focal to diffuse cytoplasmic staining in the majority of the tumor cells staining positive for HER-2. HER-2 expression was detectable in 44 % of the primary tumor samples and 58 % of the pulmonary metastases. HER-2 expression was not found to be correlated with response to chemotherapy. However, patients whose tumors stained positive for HER-2 were found to have a significantly worse metastasis-free survival. In this cohort, 19 patients presented with localized disease at diagnosis. Of those 19 patients, 7 had tumors staining positive for HER-2, and 5 went on to develop recurrences. To evaluate for amplification of the *HER*-2 gene FISH was performed on 12 samples. Increased signal consistent with amplification was observed in 6 of 7 immunostain-positive samples and 2 of 5 immunostain-negative samples. In

the two immunostain-negative samples which were found to have amplification of *HER-2*, the immunohistochemistry revealed focal HER-2 staining which did not meet the criteria for positive [35]. As discussed above, the cytoplasmic staining of HER-2 has uncertain biologic significance because of the protein's known function as a transmembrane receptor. In colon cancer, cytoplasmic staining for HER-2 has been demonstrated to correlate with a worse overall survival. Western blots were performed to corroborate protein expression on tumors that had both cytoplasmic and membranous staining and those that demonstrated only cytoplasmic staining. In the tumors that demonstrated both cytoplasmic and membranous pattern of staining, they found two bands: one at 185-kDa (corresponding to the expected size of the HER-2 protein) and one at 155-kDa. In the tumors that stained positive for HER-2 solely in the cytoplasm, only the 155-kDa band was detected by western blot [36]. The biological significance of this truncated version of HER-2 has not been examined. Given the discrepancy in size, it also raises the concern that cytoplasmic staining for HER-2 may indicate false positive staining and possible cross-reactivity with another protein expressed by these tumors.

In 2004, Fellenberg et al. attempted to address some of these issues with immunohistochemistry by assessing HER-2 expression at the level of mRNA by real-time reverse-transcription PCR (RT-PCR). To enrich the samples, they used laser microdissection to isolate osteosarcoma cells for analysis. They evaluated 17 pretreatment biopsies from a single institution using histologic response as their primary clinical endpoint. They found that HER-2 mRNA could be detected in all the samples tested. HER-2 expression was significantly elevated in patients who demonstrated a poor histologic response to preoperative chemotherapy. For internal validation, they corroborated their findings by testing two different areas of the tumors to ensure reproducibility. When they analyzed the samples for protein expression by immunohistochemistry, they found strong cytoplasmic staining in all the samples. There was no correlation between mRNA levels and protein expression of HER-2 [37]. This study serves as a proof of concept, that they were able to enrich tumor cells and perform RT-PCR on paraffin-embedded tissue. Using histologic response as the primary clinical endpoint, did not provide data on the significance of HER-2 overexpression on survival. Secondarily, the lack of correlation between mRNA and protein levels may have implications for the clinical significance of cytoplasmic staining for HER-2 by immunohistochemistry.

In the same year, Ferrari et al. published a report on a cohort of 19 patients who presented with localized disease who subsequently experienced a pulmonary relapse. They evaluated differences in the expression pattern between the primary tumor and the subsequent pulmonary metastasis. They examined HER-2 expression by immunohistochemistry according to the percentage of cells staining positive on the membrane, 0 to 4+. The tumor was considered to be positive if it exhibited 2+ or greater staining. They found HER-2 to be expressed in 32 % of the primary tumors and 53 % of the patients had at least one nodule expressing HER-2. The accordance rate, defined as the presence of the same expression pattern in the primary and metastatic samples, was 42 %. Patients with HER-2 positive primary tumors had a shorter recurrence-free interval of 17.2 months versus 31.8 months for patients with HER-2

negative primary tumors. Likewise, patients with HER-2 positive primary tumors were more likely to recur with multiple pulmonary metastases [38].

A large, single-institution, retrospective analysis of HER-2 expression in osteosarcoma in 84 patients treated on two similar protocols was published by Scotlandi et al. in 2005. They examined pretreatment biopsy specimens, using two different antibodies, and for half of the specimens three different antibodies. They defined expression as having greater than 25 % of the cells stain positive. They detected HER-2 expression in 32 % of the samples with a pattern of focal to diffuse cytoplasmic staining. Between the two antibodies tested they found a concordance rate of 78 %. For the samples tested with the third antibody similar results were obtained with 28 % of the samples positive for HER-2 expression. Patients whose tumors expressed HER-2 were found to have a higher rate of relapse and a worse event-free survival. Patients with HER-2 negative tumors exhibited an event-free survival of greater than 60 % compared to approximately 40 % for those expressing HER-2 [31]. This analysis demonstrated cytoplasmic staining for HER-2 in osteosarcoma using multiple antibodies. The high-rate of concordance in the pattern of staining between these three antibodies suggests that the cytoplasmic staining is less likely to be due to cross-reactivity with another protein. However, the clinical significance of HER-2 in osteosarcoma remains controversial as several studies have found contradictory results in HER-2 expression.

HER-2 Is Not Prognostic in Osteosarcoma

At the same time as the retrospective analyses discussed previously demonstrated the correlation with poor prognosis in patients whose tumors expressed HER-2, eight studies also reported that HER-2 expression is not prognostic in osteosarcoma. Maitra et al., in 2001, using immunohistochemistry and FISH examined 21 diagnostic biopsy specimens from a single institution. For immunohistochemistry analysis, they defined as positive only cell membrane staining, excluding cytoplasmic and nuclear staining, and graded according to a four-tier grading scheme: negative, low, medium, and high. They did not find HER-2 overexpression by immunohistochemistry in any of the samples. Likewise, they did not detect any amplification of the *HER-2* gene by FISH [39].

Kilpatrick et al., in the same year, reported on a retrospective analysis from two centers between 1985 and 2000. They examined HER-2 expression by immunohistochemistry comparing two different antibodies as well as decalcified versus nondecalcified specimens. They delineated between membranous and cytoplasmic staining, but did not exclude cytoplasmic staining. Cytoplasmic staining was scored from 0 to 3+. Positive was defined as 2+ or 3+: weak to moderate staining in more than 10 % of cells; or moderate to strong staining in more than 10 % of cells. None of the osteosarcoma specimens demonstrated staining for HER-2 on the cell membrane. Focal cytoplasmic staining in more than 10 % of the cells was found in

83 and 98 % of the samples, using the different antibodies. There was poor agreement between the antibodies in the extent of cytoplasmic staining, even when they were collapsed to positive and negative. Neither antibody demonstrated correlation with response to preoperative chemotherapy, metastasis, or survival [40].

Thomas et al. performed a retrospective analysis of osteosarcomas in a single-institution from 33 patients that included 25 primary biopsies, 29 resections after chemotherapy, 9 pulmonary metastatic lesions, and 6 other lesions. The samples were analyzed by immunohistochemistry and RT-PCR. They graded the immunohistochemical staining according to a five-tier system: negative, cytoplasmic, low-positive membranous, medium-positive membranous, and high-positive membranous. None of the samples demonstrated staining for HER-2 on the cell membrane, but 47 % of the specimens did demonstrate diffuse cytoplasmic staining. None of the samples had HER-2 mRNA amplifiable by RT-PCR. mRNAs from housekeeping genes were amplifiable suggesting that this was not due to issues with technique or failure of RNA extraction. Likewise they were able to RT-PCR HER-2 from a breast cancer specimen, suggesting that this negative result is not secondary to failure of the primers [41]. Since they were unable to detect the corresponding mRNA in the samples, the authors concluded that the cytoplasmic staining of HER-2 should be discounted as a positive finding.

Another single-institution, retrospective analysis was performed by Anninga et al. They included in their analysis 15 pretreatment biopsy specimens as well as 12 specimens including post-chemotherapy resections or pulmonary, distant bone, or local relapse specimens. They evaluated the samples by quantitative real-time RT-PCR (qPCR) and by immunohistochemistry. Tumor samples were scored 0 to 3+ according to the level of membrane staining. Cytoplasmic staining was not considered positive. Of the 27 evaluable specimens, only one sample (from a pretreatment biopsy) displayed membranous staining, which was scored as moderate. Focal cytoplasmic staining was detected in two other samples. None of the samples had overexpression of HER-2 mRNA when compared to a HER-2 overexpressing cell line. The levels of HER-2 expression detected by qPCR were described as within the range of normal breast tissue. In the one sample with HER-2 membranous staining, FISH did not reveal *HER-2* amplification [42]. The authors likewise concluded that HER-2 does not play a significant role in osteosarcoma.

A collaborative project, involving four institutions, evaluated HER-2 expression in 22 samples from 20 patients. They were all reviewed at one institution by immunohistochemistry and fluorescence in situ hybridization. Immunohistochemistry was graded from 0 to 3+ according to level (>10 % of cells) and intensity (mild, moderate, strong) of membranous staining. Scores of 0 and 1+ were considered to be negative. Four of the samples (18 %) showed focal positivity for HER-2 (1+ grading). None of the samples revealed amplification of HER-2 by fluorescence in situ hybridization. When the authors interpreted 1+ staining as positive, univariate analysis did not reveal a statistically significant difference in survival in the two groups [43]. The authors note that major concerns of this study include the small sample size and the limited follow-up, as median survival had not been reached at the time of publication.

Somers et al reviewed 34 samples from 18 patients in a single-institution. They examined tumor samples on tissue microarrays for HER-2 expression by immunohistochemistry and amplification by chromogenic in situ hybridization (CISH). They graded the immunostaining from 0 to 3+ according to intensity of membrane staining. Cytoplasmic staining was graded as 0. They found that four osteosarcoma specimens from two patients displayed HER-2 immunostaining. Two revealed cytoplasmic staining (0), and two cytoplasmic and membranous staining (1+). None of the samples were evaluated as having overexpression of HER-2 by immunohistochemistry. None of the samples demonstrated *HER2* gene amplification by CISH. In 39 % of the tumors, aneuploidy (having multiple signals to the CISH probe) was detected in less than 10 % of the cells. They also noted that four samples exhibited three nuclear signals in greater than 50 % of the cells, which they state is suggestive for trisomy 17. None of the tumors with increased signal by CISH probe displayed expression for HER-2 [44]. Since there was no concordance between the increased chromogenic signal and immunohistochemistry, the authors concluded that the increased signal should not be interpreted as amplification of the gene. These findings if interpreted by the criteria used by Zhou et al. would have been described as positive for amplification.

HER-2 gene amplification was evaluated by Willmore-Payne et al using FISH as well as multiplex and monoplex PCR. They also performed immunohistochemistry on the samples, grading from 0 to 3+. Cytoplasmic staining was graded as 0. In the initial 21 cases evaluated by multiplex PCR and FISH, there was no evidence of *HER-2* gene amplification. Of these cases, 11 demonstrated cytoplasmic staining for HER-2 by immunohistochemistry, which were all graded as 0. No samples demonstrated membranous staining. Given the negative findings, they obtained an additional 35 paraffin blocks from 26 patients from another institution to perform monoplex PCR and FISH. Again, they were not able to detect any *HER-2* gene amplification. In these 26 patients, they detected two samples with cytoplasmic staining for HER-2 by immunohistochemistry, and one sample with 1+ membranous staining [45]. The authors concluded that HER-2 is not amplified or expressed in osteosarcoma.

Bakhshi et al. evaluated HER-2 expression by immunohistochemistry in 63 patients. They delineated the pattern of staining as cytoplasmic versus membranous. They graded the samples according to the percentage of cells stained: 0, 0–10 %; 1+, 11–30 %; 2+, 31–50 %; 3+, 51–100 %. They observed HER-2 staining (1+ and greater) in 47.6 % of samples. All of the samples demonstrated cytoplasmic staining, and four samples demonstrated both cytoplasmic and membranous staining. Positive staining for HER-2 was not correlated with metastatic disease at presentation [46]. The authors did not evaluate HER-2 for any clinical outcomes. Also confounding the results is that almost half of the patients presented with metastatic disease. The high proportion of patients presenting with advance disease which is the most powerful predictor of outcome in osteosarcoma may obscure the relevance of a biological marker.

HER-2 Is a Positive Prognostic Indicator in Osteosarcoma

Adding to the controversy over the relevance of HER-2 in osteosarcoma, Akatsuka et al. published a report of 81 patients with localized disease from two centers. They evaluated initial biopsy specimens for HER-2 expression by immunohistochemistry. The samples were graded from 0 to 3+ based on the percentage of cells staining positive: 0, negative; 1+, 1–30 %; 2+, 31–75 %; 3+, 76–100 %. The section with the highest degree of staining was used as representative, and overexpression was defined as tumors with 2+ or 3+ staining. They found that 63 % of the tumors had overexpression of HER-2. HER-2 expression did not correlate with response to chemotherapy. Overexpression of HER-2 was significantly correlated with event-free survival. At 5 years, the event-free survival of patients with overexpression of HER-2 was 72 % compared to 46 % for patients without HER-2 overexpression [47]. In a separate report, these authors also demonstrate that the rate of HER-2 expression is lower in metachronous pulmonary metastases as compared to initial biopsy specimens. They suggest that HER-2 does not play a role in the development of lung metastasis [48].

Summary of HER-2 Studies

All of these studies (summed in Table 1) provide limited clarity of the role of HER-2 in osteosarcoma. The confounding features include issues with immunohistochemistry staining and gene amplification. In the studies examining the levels of expressions of HER-2 by immunohistochemistry, there are differences in the antibodies being used, differences in the interpretations of positive staining, and differences in the grading systems used to define overexpression; recapitulating the experience in breast cancer during the incipient years following the identification of HER-2. In the studies revealing that HER-2 is not prognostic in osteosarcoma, the point of contention lies in whether HER-2 is truly overexpressed in osteosarcoma. Two studies which identified defined positive HER-2 staining in osteosarcoma demonstrated that it was not associated with a worse prognosis. The study by Bakhshi et al. was complicated by the increased numbers of patients presenting with advance disease. In contrast, Akatsuka et al. demonstrated positive HER-2 staining, but showed that it improved survival.

In regards to the second confounding feature, in breast cancer, the basis of overexpression of HER-2 is gene amplification in the majority of tumors. In osteosarcoma, there is evidence that gene amplification of *HER-2* is not involved in the pathogenesis. Again, there is disagreement in the literature in terms of the definition of positive criteria for gene amplification.

A meta-analysis published in 2010 evaluated the association of HER-2 overexpression with prognosis in osteosarcoma. Of the 28 evaluable reports, 23 were excluded.

Table 1 Studies evaluating HER-2 as a prognostic indicator in osteosarcoma

Study	Assay	*n*	Positive (%)	Outcome
Onda	Immunoblotting IHC Southern	26	Membranous: 42 0	Survival: 1-yr, 3-yr Neg: 100 %, 84 % Pos: 61 %, 14 %
Gorlick	IHC	53	Membranous: 42.6	Event-free survival: 5-yr Neg: 78 % Pos: 40 %
Zhou	IHC FISH	25 primary 12 metastases 7 IHC pos 5 IHC neg	Cytoplasmic: 44 Cytoplasmic: 58 85.7 40	Metastasis-free survival Worse
Fellenberg	RT-PCR IHC	10 good response 7 poor response NR	0 85 Cytoplasmic: 100	Histologic response: mRNA levels 94 % predictive of histologic response
Ferrari	IHC	17	Primary: 32 Metastases: 53	Recurrence-free interval: Neg: 31.8 months Pos: 17.2 months
Scotlandi	IHC	84	28–32	Event-free survival: Worse
Maitra	IHC FISH	21	0 0	NR
Kilpatrick	IHC	41	Membranous: 0 Cytoplasmic: 83–98	Response to chemotherapy, metastasis, survival: No association
Thomas	IHC RT-PCR	66	Membranous: 0 Cytoplasmic: 47 0	NR
Anninga	RT-PCR IHC FISH	27 27 1	0 Membranous: 3.7 Cytoplasmic: 7.4 0	NR
Tsai	IHC FISH	22 22	Focal: 18 0	No association (limited follow-up)
Somers	IHC microarray CISH microarray	34 34	Membranous and cytoplasmic: 5.8 Cytoplasmic: 5.8 0	NR
Willmore-Payne	FISH PCR IHC	47 46	0 0 Membranous: 0 Cytoplasmic: 4.3	NR
Bakhshi	IHC	63	Cytoplasmic: 41.2 Membranous and cytoplasmic: 6.3	Expression in patients with metastatic disease and grade: No difference
Akatsuka	IHC	81	63 %	Event-free survival: 5-yr Neg: 46 Pos: 72

IHC immunohistochemistry, *NR* not reported, *yr* year, *RT-PCR* reverse-transcription polymerase chain reaction, *FISH* fluorescent in situ hybridization, *CISH* chromogenic in situ hybridization

In the remaining five reports, the authors had difficulty with standardization of the cohorts as the reports as described above used different modalities to evaluate HER-2 overexpression, different antibodies, and different criteria for the evaluation of immunohistochemistry staining. The authors conclude that HER-2 positivity revealed a trend for a 1.26-fold higher risk of death, which was not statistically significant [49]. Another major confounder of the meta-analysis was the lack of standardization of the populations and the treatments across the studies.

More recently, results from the Children's Oncology Group have been presented. They evaluated 191 samples from 149 patients for whom there were confirmed histologic diagnosis of osteosarcoma, adequate staining, and survival information. Most of the patients were enrolled on clinical trial and had standardized treatment. HER-2 overexpression was evaluated by immunohistochemistry and graded according to the percentage of cells staining positive: negative (no staining), 1+ (0–25 %), 2+ (26–50 %), 3+ (51–75 %), and 4 (>75 %). Positive for HER-2 overexpression was defined by a grade of 3+ or 4+. According to these criteria, the investigators found that HER-2 was overexpressed in 13.4 % of the samples evaluated. HER-2 overexpression did not correlate with survival [50].

Trastuzumab in Osteosarcoma

Given the promising clinical benefit of trastuzumab in breast cancer and the early retrospective analyses in osteosarcoma, the Children's Oncology Group initiated a phase II trial of trastuzumab in patients with metastatic osteosarcoma. Eligible patients were required to have newly diagnosed metastatic disease, defined as bone, bone and lung, bilateral lung, or greater than four unilateral lung metastases. Immunohistochemistry staining was used to evaluate the HER-2 status of the patients' biopsy specimen performed and graded by an independent, centralized facility. The specimens were graded according the percentage of cells staining positive for HER-2 (membranous, cytoplasmic, and nuclear staining all considered positive): 0, <10 %; 1+, 10–50 %; 2+, >50 %. Patients with 2+ staining received trastuzumab in addition to the five-drug regimen of methotrexate, doxorubicin, cisplatin, ifosfamide, and etoposide. Patients treated with trastuzumab initiated therapy prior to week 6 and continued weekly until they had completed the course of 34 doses. The primary outcome of event-free survival was compared to patients without HER-2 expression receiving five-drug chemotherapy without trastuzumab. Between July 2001 and November 2005, 96 evaluable were enrolled on the study: 41 HER-2 positive and 55 HER-2 negative. Of the patient samples submitted for review, 33–35 % demonstrated HER-2 positive expression. The results of the trial were disappointing. There was no difference in the event-free and overall survival in the two treatment arms. The 30-month event-free survival was 32 % in both the trastuzumab arm and the non-HER-2 expressing arm. The 30-month overall survival was 59 % in the trastuzumab cohort and 50 % in the non-HER-2 expressing cohort. Despite the high-doses of anthracyclines, there was no increase in cardiac toxicity in the trastuzumab treated arm [51]. The addition of trastuzumab to

cytotoxic chemotherapy was well tolerated in this group of patients. The lack of clinical benefit noted in the trial may have been due to trastuzumab overcoming the negative prognostic effects associated with the overexpression of HER-2.

Lessons Learned

There is limited value of institutional retrospective analyses in defining possible targeted therapy. The sample sizes available are too small to detect differences in subpopulations. Secondarily, inconsistencies in techniques make reproducibility and validation very challenging. Target validation requires proving *in vivo*, using available rodent and canine models, the tumor localization and efficacy of the therapeutic agent. In conjunction, understanding the biologic basis for the targets and the mechanisms of cellular dependencies will lend confidence to the applicability of the therapeutic agent for targeted therapy. These requirements need to be standardized in the foundation of a coherent drug development plan in osteosarcoma.

Conclusion

HER-2 expression, like P-glycoprotein, is too controversial and cannot be used as a prognostic factor or in the treatment of osteosarcoma.

References

1. Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI, Weinberg RA (1984) The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature 312(5994):513–516
2. Coussens L, Yang-Feng TL, Liao YC, Chen E, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J, Francke U et al (1985) Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. Science 230(4730):1132–1139
3. Semba K, Kamata N, Toyoshima K, Yamamoto T (1985) A v-erbB-related protooncogene, c-erbB-2, is distinct from the c-erbB-1/epidermal growth factor-receptor gene and is amplified in a human salivary gland adenocarcinoma. Proc Natl Acad Sci U S A 82(19):6497–6501
4. King CR, Kraus MH, Aaronson SA (1985) Amplification of a novel v-erbB-related gene in a human mammary carcinoma. Science 229(4717):974–976
5. Bargmann CI, Hung MC, Weinberg RA (1986) The neu oncogene encodes an epidermal growth factor receptor-related protein. Nature 319(6050):226–230. doi:10.1038/319226a0
6. Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C (1995) Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature 378(6555):394–398. doi:10.1038/378394a0
7. Ozcelik C, Erdmann B, Pilz B, Wettschureck N, Britsch S, Hubner N, Chien KR, Birchmeier C, Garratt AN (2002) Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes

leads to dilated cardiomyopathy. Proc Natl Acad Sci U S A 99(13):8880–8885. doi:10.1073/pnas.122249299
8. Schroeder JA, Lee DC (1998) Dynamic expression and activation of ERBB receptors in the developing mouse mammary gland. Cell Growth Differ 9(6):451–464
9. Jones FE, Stern DF (1999) Expression of dominant-negative ErbB2 in the mammary gland of transgenic mice reveals a role in lobuloalveolar development and lactation. Oncogene 18(23):3481–3490. doi:10.1038/sj.onc.1202698
10. Britsch S, Li L, Kirchhoff S, Theuring F, Brinkmann V, Birchmeier C, Riethmacher D (1998) The ErbB2 and ErbB3 receptors and their ligand, neuregulin-1, are essential for development of the sympathetic nervous system. Genes Dev 12(12):1825–1836
11. Morris JK, Lin W, Hauser C, Marchuk Y, Getman D, Lee KF (1999) Rescue of the cardiac defect in ErbB2 mutant mice reveals essential roles of ErbB2 in peripheral nervous system development. Neuron 23(2):273–283
12. Park SK, Miller R, Krane I, Vartanian T (2001) The erbB2 gene is required for the development of terminally differentiated spinal cord oligodendrocytes. J Cell Biol 154(6):1245–1258. doi:10.1083/jcb.200104025
13. Graus-Porta D, Beerli RR, Daly JM, Hynes NE (1997) ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. EMBO J 16(7):1647–1655. doi:10.1093/emboj/16.7.1647
14. Hynes NE, MacDonald G (2009) ErbB receptors and signaling pathways in cancer. Curr Opin Cell Biol 21(2):177–184. doi:10.1016/j.ceb.2008.12.010
15. Olayioye MA, Neve RM, Lane HA, Hynes NE (2000) The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 19(13):3159–3167. doi:10.1093/emboj/19.13.3159
16. Di Fiore PP, Pierce JH, Kraus MH, Segatto O, King CR, Aaronson SA (1987) erbB-2 is a potent oncogene when overexpressed in NIH/3 T3 cells. Science 237(4811):178–182
17. Hudziak RM, Schlessinger J, Ullrich A (1987) Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3 T3 cells. Proc Natl Acad Sci U S A 84(20):7159–7163
18. Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ (1992) Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. Proc Natl Acad Sci U S A 89(22):10578–10582
19. Hynes NE (1993) 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(1):19–26
20. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235(4785):177–182
21. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A et al (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244(4905):707–712
22. Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J (1998) Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. Cancer Res 58(13):2825–2831
23. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L (1996) Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol 14(3):737–744
24. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, Baly D, Baughman SA, Twaddell T, Glaspy JA, Slamon DJ (1998) 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(8):2659–2671

25. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344(11):783–792. doi:10.1056/NEJM200103153441101
26. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Fehrenbacher L, Kutteh LA, Vogel VG, Visscher DW, Yothers G, Jenkins RB, Brown AM, Dakhil SR, Mamounas EP, Lingle WL, Klein PM, Ingle JN, Wolmark N (2005) Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 353(16):1673–1684. doi:10.1056/NEJMoa052122
27. Press MF, Hung G, Godolphin W, Slamon DJ (1994) Sensitivity of HER-2/neu antibodies in archival tissue samples: potential source of error in immunohistochemical studies of oncogene expression. Cancer Res 54(10):2771–2777
28. Seidman A, Hudis C, Pierri MK, Shak S, Paton V, Ashby M, Murphy M, Stewart SJ, Keefe D (2002) Cardiac dysfunction in the trastuzumab clinical trials experience. J Clin Oncol 20(5):1215–1221
29. Hughes DP, Thomas DG, Giordano TJ, Baker LH, McDonagh KT (2004) Cell surface expression of epidermal growth factor receptor and Her-2 with nuclear expression of Her-4 in primary osteosarcoma. Cancer Res 64(6):2047–2053
30. Hassan SE, Bekarev M, Kim MY, Lin J, Piperdi S, Gorlick R, Geller DS (2012) Cell surface receptor expression patterns in osteosarcoma. Cancer 118(3):740–749. doi:10.1002/cncr.26339
31. Scotlandi K, Manara MC, Hattinger CM, Benini S, Perdichizzi S, Pasello M, Bacci G, Zanella L, Bertoni F, Picci P, Serra M (2005) Prognostic and therapeutic relevance of HER2 expression in osteosarcoma and Ewing's sarcoma. Eur J Cancer 41(9):1349–1361. doi:10.1016/j.ejca.2005.03.015
32. Onda M, Matsuda S, Higaki S, Iijima T, Fukushima J, Yokokura A, Kojima T, Horiuchi H, Kurokawa T, Yamamoto T (1996) ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. Cancer 77(1):71–78. doi:10.1002/(SICI)1097-0142(19960101)77:1<71::AID-CNCR13>3.0.CO;2-5
33. Meyers PA, Gorlick R, Heller G, Casper E, Lane J, Huvos AG, Healey JH (1998) Intensification of preoperative chemotherapy for osteogenic sarcoma: results of the Memorial Sloan-Kettering (T12) protocol. J Clin Oncol 16(7):2452–2458
34. Gorlick R, Huvos AG, Heller G, Aledo A, Beardsley GP, Healey JH, Meyers PA (1999) Expression of HER2/erbB-2 correlates with survival in osteosarcoma. J Clin Oncol 17(9):2781–2788
35. Zhou H, Randall RL, Brothman AR, Maxwell T, Coffin CM, Goldsby RE (2003) Her-2/neu expression in osteosarcoma increases risk of lung metastasis and can be associated with gene amplification. J Pediatr Hematol Oncol 25(1):27–32
36. Osako T, Miyahara M, Uchino S, Inomata M, Kitano S, Kobayashi M (1998) Immunohistochemical study of c-erbB-2 protein in colorectal cancer and the correlation with patient survival. Oncology 55(6):548–555
37. Fellenberg J, Krauthoff A, Pollandt K, Delling G, Parsch D (2004) Evaluation of the predictive value of Her-2/neu gene expression on osteosarcoma therapy in laser-microdissected paraffin-embedded tissue. Lab Invest 84(1):113–121. doi:10.1038/sj.labinvest.3700006
38. Ferrari S, Bertoni F, Zanella L, Setola E, Bacchini P, Alberghini M, Versari M, Bacci G (2004) Evaluation of P-glycoprotein, HER-2/ErbB-2, p53, and Bcl-2 in primary tumor and metachronous lung metastases in patients with high-grade osteosarcoma. Cancer 100(9):1936–1942. doi:10.1002/cncr.20151
39. Maitra A, Wanzer D, Weinberg AG, Ashfaq R (2001) Amplification of the HER-2/neu oncogene is uncommon in pediatric osteosarcomas. Cancer 92(3):677–683
40. Kilpatrick SE, Geisinger KR, King TS, Sciarrotta J, Ward WG, Gold SH, Bos GD (2001) Clinicopathologic analysis of HER-2/neu immunoexpression among various histologic subtypes and grades of osteosarcoma. Mod Pathol 14(12):1277–1283. doi:10.1038/modpathol.3880474
41. Thomas DG, Giordano TJ, Sanders D, Biermann JS, Baker L (2002) Absence of HER2/neu gene expression in osteosarcoma and skeletal Ewing's sarcoma. Clin Cancer Res 8(3):788–793

42. Anninga JK, van de Vijver MJ, Cleton-Jansen AM, Kristel PM, Taminiau AH, Nooij M, Egeler RM, Hogendoorn PC (2004) Overexpression of the HER-2 oncogene does not play a role in high-grade osteosarcomas. Eur J Cancer 40(7):963–970. doi:10.1016/j.ejca.2003.10.025
43. Tsai JY, Aviv H, Benevenia J, Chang VT, Patterson F, Aisner S, Hameed M (2004) HER-2/neu and p53 in osteosarcoma: an immunohistochemical and fluorescence in situ hybridization analysis. Cancer Invest 22(1):16–24
44. Somers GR, Ho M, Zielenska M, Squire JA, Thorner PS (2005) HER2 amplification and overexpression is not present in pediatric osteosarcoma: a tissue microarray study. Pediatr Dev Pathol 8(5):525–532. doi:10.1007/s10024-005-0044-5
45. Willmore-Payne C, Holden JA, Zhou H, Gupta D, Hirschowitz S, Wittwer CT, Layfield LJ (2006) Evaluation of Her-2/neu gene status in osteosarcoma by fluorescence in situ hybridization and multiplex and monoplex polymerase chain reactions. Arch Pathol Lab Med 130(5):691–698. doi:10.1043/1543-2165(2006)130[691:EONGSI]2.0.CO;2
46. Bakhshi S, Gupta A, Sharma MC, Khan SA, Rastogi S (2009) Her-2/neu, p-53, and their coexpression in osteosarcoma. J Pediatr Hematol Oncol 31(4):245–251. doi:10.1097/MPH.0b013e318197947e
47. Akatsuka T, Wada T, Kokai Y, Kawaguchi S, Isu K, Yamashiro K, Yamashita T, Sawada N, Yamawaki S, Ishii S (2002) ErbB2 expression is correlated with increased survival of patients with osteosarcoma. Cancer 94(5):1397–1404
48. Akatsuka T, Wada T, Kokai Y, Sawada N, Yamawaki S, Ishii S (2001) Loss of ErbB2 expression in pulmonary metastatic lesions in osteosarcoma. Oncology 60(4):361–366, doi:58533
49. Li YG, Geng X (2010) A meta-analysis on the association of HER-2 overexpression with prognosis in human osteosarcoma. Eur J Cancer Care 19(3):313–316. doi:10.1111/j.1365-2354.2008.00970.x
50. Gorlick R, Barkauskas DA, Krailo M, Piperdi S, Sowers R, Gorlick S, Gill J, Geller D, Randall RL, Janeway KA, Schwartz CL, Grier H, Meyers P, Bernstein M, Marina N (2012) HER-2 expression is not prognostic in osteosarcoma: A report of a Children's Oncology Group Prospective Biology Study. Connective Tissue Oncology Society, 2012 meeing abstract
51. Ebb D, Meyers P, Grier H, Bernstein M, Gorlick R, Lipshultz SE, Krailo M, Devidas M, Barkauskas DA, Siegal GP, Ferguson WS, Letson GD, Marcus K, Goorin A, Beardsley P, Marina N (2012) 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. J Clin Oncol 30(20):2545–2551. doi:10.1200/JCO.2011.37.4546