

The Association of P-Glycoprotein with Response to Chemotherapy and Clinical Outcome in Patients with Osteosarcoma

A Meta-Analysis

Emilios E. Pakos, M.D.¹
John P. A. Ioannidis, M.D.¹⁻³

¹ Clinical and Molecular Epidemiology Unit, Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece.

² Biomedical Research Institute, Foundation for Research and Technology-Hellas, Ioannina, Greece.

³ Division of Clinical Care Research, Department of Medicine, Tufts-New England Medical Center, Boston, Massachusetts.

The authors thank Dr. Yong Bum Park for providing additional data and clarifications from his study.

Address for reprints: John P. A. Ioannidis, M.D., Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina 45110, Greece. Fax: (011) 30 2651097867; E-mail: jioannid@cc.uoi.gr

Received October 15, 2002; revision received April 25, 2003; accepted April 29, 2003.

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DOI 10.1002/cncr.11546

BACKGROUND. There is controversy regarding whether P-glycoprotein (Pgp) may be a prognostic factor for the response to chemotherapy and clinical disease progression in patients with osteosarcoma.

METHODS. The authors conducted a meta-analysis of 14 studies ($n = 631$ patients) that evaluated the correlation between Pgp and histologic response to chemotherapy and clinical disease progression (death, metastasis, or recurrence). Data were synthesized in receiver operating characteristic curves and with fixed-effects and random-effects likelihood ratios and risk ratios.

RESULTS. Pgp had no discriminating ability for identifying poor responders versus good responders to chemotherapy: The positive likelihood ratio was 1.15 (95% confidence interval [95% CI], 0.93–1.43), and the negative likelihood ratio was 0.88 (95% CI, 0.65–1.18; random-effects calculations). There was some between-study heterogeneity, but no study showed strong discriminating ability. Conversely, Pgp positivity increased the risk of disease progression 1.92-fold (95% CI, 1.18–3.13; random-effects calculations) with some between-study heterogeneity that disappeared when only studies that employed immunohistochemistry were considered (risk ratio, 2.23; 95% CI, 1.37–3.64). The results were robust in various sensitivity analyses, although smaller studies tended to show stronger associations with the risk of disease progression compared with larger studies ($P = 0.03$).

CONCLUSIONS. The available evidence showed conclusively that Pgp was not associated with the histologic response of patients with osteosarcoma to combination chemotherapy regimens. Conversely, Pgp positivity, as determined by immunohistochemistry, was a strong correlate of more rapid disease progression, although there was heterogeneity across the performed studies that, to some extent, may have reflected bias, differential measurements of Pgp, or confounding with other risk factors. *Cancer* 2003;98:581–9. © 2003 American Cancer Society.

KEYWORDS: P-glycoprotein, osteosarcoma, chemotherapy, survival, metastasis, meta-analysis.

The prognosis of patients with osteosarcoma remains guarded, despite advances in surgical treatment and the availability of new chemotherapeutic agents in recent years. Several prognostic risk factors have been proposed, including tumor size, tumor grade, tumor site, age at diagnosis, and response to chemotherapy.¹ In particular, histologic response to chemotherapy seems to carry considerable predictive information.^{2,3} Chemotherapy often may not achieve the expected results due to drug resistance of tumor cells. The classic example is multidrug resistance (MDR) mediated by the *MDR1* gene.⁴

The protein product of *MDR1*, P-glycoprotein (Pgp), is a plasma membrane protein that acts as an adenosine triphosphate dependent efflux pump responsible for removing cytotoxic molecules, including chemotherapeutic agents, from cells.⁴ Therefore, high levels of *MDR1* gene expression may be a prime mechanism whereby drug resistance may develop in a tumor. Several studies have tried to investigate the role of Pgp in osteosarcoma, but the overall results remain controversial.⁵⁻¹⁸

Single studies are limited unavoidably in terms of sample size, even when multicenter efforts are undertaken. However, the total accumulated data provide a considerable body of evidence. Given the amount of accumulated data, a quantitative synthesis using rigorous methods was deemed important. Therefore, we conducted a comprehensive meta-analysis of all available studies relating Pgp with the response to chemotherapy and outcome of patients with osteosarcoma. The objective of this analysis was to arrive at summary estimates of association, to estimate the between-study heterogeneity, and to find possible explanations for the presence of potential heterogeneity between studies.

MATERIALS AND METHODS

Identification and Eligibility of Relevant Studies

We considered all studies that examined the association of Pgp with osteosarcoma outcomes in at least 10 patients. Sources included MEDLINE and EMBASE (last search update, August, 2002). The search strategy was based on combinations of the terms *osteosarcoma*, *P-glycoprotein*, *MDR1*, and *multidrug resistance*. References of retrieved articles also were screened, and investigators were contacted and asked to supplement additional data and clarifications when key information relevant to the meta-analysis was missing.

All studies that examined the role of Pgp in the response to chemotherapy and/or to clinical outcome (death and metastatic disease with or without including local recurrence) were eligible for our meta-analysis. We accepted all studies that measured Pgp regardless of the method of detection (immunohistochemistry [IHC] or reverse transcriptase-polymerase chain reaction [RT-PCR]). Whenever reports pertained to overlapping patients, we retained only the largest study to avoid duplicating information.

Definitions and Standardizations

We identified studies regardless of when Pgp measurements had been performed but preferred studies in which the main quantitative synthesis data were ob-

tained from Pgp evaluations of specimens that were obtained before chemotherapy. Pgp may be detected at higher levels postchemotherapy.^{7,17}

We used prespecified rules to standardize, as much as possible, the definition of Pgp positivity, especially for studies that provided no specific definition or that used different cutoff levels to define a positive Pgp reading. For studies that used IHC, we defined Pgp positivity as positive cell staining in $\geq 10\%$ of tumor cells, a definition that was employed by the majority of studies. When only different definitions were used in the original studies, we used the cutoff level that was closest to the 10% cutoff level. For studies that used RT-PCR only, we defined Pgp positivity as *MDR1* levels > 1.0 . For studies that used both IHC and RT-PCR, we used the IHC data. Analyses using the RT-PCR data instead of the IHC data from the same studies provided similar results (data not shown).

We defined response to chemotherapy according to the percentage of histologic necrosis of tumor cells in specimens after patients received preoperative or postoperative chemotherapy. A cutoff level of 90% necrosis was used for separating responders from nonresponders. For studies that used the Huvos grading system to evaluate histologic necrosis,² responders included patients with Grade 3 or 4 responses.

Clinical outcomes were standardized to capture 24 months of follow-up in all studies. All studies had at least 24 months of follow-up, and censoring before 24 months in the pertinent studies was very uncommon. Disease progression was defined as recurrence, development of systemic disease (metastasis), or death. We accepted the intention of each primary study to count or not to count local recurrences. Composite disease progression outcomes should be viewed with some caution, because local recurrence also may be dependent on the type of surgery and does not have the same impact as a distant metastasis. For patients who developed a recurrence with metastatic disease and eventually died, the time of event was the time of recurrence/metastasis.

Data Extraction

Two investigators independently extracted the data and reached consensus on all items. We extracted data on characteristics of studies and patients, measurements, and results. In more detail, for each report, we recorded author names, journal and year of publication, country of origin, years of patient enrollment, number of patients analyzed, tumor stage and grade, demographics, chemotherapy and surgery used, timing of Pgp assessment (prechemotherapy or postchemotherapy), type of Pgp measurements, definition(s)

of Pgp positivity, and blinding of Pgp measurements to the study outcomes. The main outcome results consisted of 2×2 tables showing the histologic response or lack of response to chemotherapy and the presence or absence of disease progression during 24 months of follow-up according to the presence or absence of Pgp in prechemotherapy assessments. We also recorded the number of patients censored without disease progression prior to 24 months.

Statistical Analysis

Data on the diagnostic performance of Pgp for determining histologic response to chemotherapy were evaluated by constructing a summary receiver operating characteristic (SROC) curve and by estimating the combined positive and negative likelihood ratios (LR + and LR -, respectively).

For a diagnostic or predictive test, the sensitivity (true-positive rate) and specificity (one minus false-positive rate) are correlated; therefore, it is not correct to estimate these two quantities independently. To bypass this problem, the SROC method may be used. The SROC curve is estimated by the regression $D = a + bS$, where D is the difference of the logits of the true-positive and false-positive rate, and S is the sum of these logits.¹⁹ Both weighted and unweighted regressions were estimated. The SROC curve shows the trade-off between sensitivity and specificity across the included studies.

Likelihood ratios also are metrics that combine both sensitivity and specificity in their calculation. LR + was defined as the ratio of sensitivity over 1 minus specificity, and LR - was defined as the ratio of 1 minus sensitivity over specificity. When there is absolutely no discriminating ability for a diagnostic or predictive test, both likelihood ratios equal 1. The higher the LR + and the lower the LR -, the better the discriminating ability. Although there is no absolute cutoff level, a good diagnostic test may have LR + > 5 and LR - < 0.2. Study specific LR values were combined with fixed-effects and random-effects models, and between-study heterogeneity was assessed with the Q statistic.²⁰

Data concerning the predictive ability of Pgp for 24-month clinical outcomes were combined across studies in a similar fashion using fixed-effects and random-effects estimates for the synthesis of risk ratios for disease progression.²⁰ The risk ratio shows the rate of disease progression in the group with Pgp divided by the rate of disease progression in the group without Pgp. Between-study heterogeneity in the risk ratios was assessed with the Q statistic.²⁰

Fixed-effects models assume that differences be-

tween the results of the combined studies are due entirely to chance. Random-effects models allow for the possibility that results may differ genuinely between studies. In the presence of between-study heterogeneity, random-effects models provide wider confidence intervals (CIs).²¹ We generally present random-effects estimates, unless stated otherwise.

Sensitivity analyses examined the effect of removing outlier studies (with association estimates differing over six-fold from the summary estimate), studies with postchemotherapy Pgp measurements, studies that did not use IHC, studies that did not use the 10% IHC cutoff level, and studies that did not state whether Pgp measurements were blinded to outcomes. We also used appropriate bias diagnostics to examine whether there was evidence that the results differed in small studies compared with large studies²² or whether results were changing gradually over time with the publication of more recent studies.²³

Analyses were conducted in using SPSS (version 10.0; SPSS, Inc., Chicago, IL), Meta-Analyst (Joseph Lau, Boston, MA) and Meta-Test (Joseph Lau) software packages. P values are two-tailed.

RESULTS

Eligible Studies

Twenty-seven reports were retrieved. Of those, 13 studies were excluded because they overlapped with another larger study ($n = 2$ studies^{24,25}), they included no data on response to chemotherapy or clinical disease progression ($n = 6$ studies²⁶⁻³¹), they included no Pgp measurements ($n = 2$ studies^{32,33}), or they included < 10 osteosarcomas or no osteosarcomas ($n = 3$ studies³⁴⁻³⁶). Fourteen independent studies were entered in the meta-analysis (Table 1). Four studies had been conducted in America, four studies had been conducted in Europe, and six studies had been conducted in Asian populations. Overall, 631 patients with osteosarcomas and Pgp determinations were included, among whom 597 patients (14 studies) had data available on histologic response to chemotherapy, and 479 patients (8 studies) had data available on clinical disease progression. Only 13 patients had documented metastatic disease at the time of Pgp evaluation (for those 13 patients, disease progression during follow-up would refer to death or metastasis at a different site). Eleven studies stated that the included osteosarcomas were of high grade, whereas no grading information was available in 3 studies.^{9,14,18} Patient populations generally were young, with the mean or median age ranging between 14 years and 27 years across studies (Table 1). All analyzed patients with osteosarcomas were treated with combination chemotherapy regimens (three or four drugs), including

TABLE 1
Characteristics of Eligible Studies

Reference	No. analyzed	No. with metastatic disease	Age (yrs)	Pgp method	Pgp cutoff (%)	Blinding	Chemotherapy response (criteria)	Two-yr disease progression ^a
Wunder et al. ⁷	123	0	20 (mean)	RT-PCR	1.0	NR	29/119 (N90)	37/123
Gorlick et al. ⁶	20	3	14 (mean)	IHC	> 0	Yes	7/20 (Huvos)	NR
Serra et al. ⁸	38	0	19 (mean)	IHC	≥ 15	NR	11/36 (N90)	11/38
Burak et al. ⁹	18	0?	27 (mean)	IHC	≥ 10	Yes	4/18 (N90)	NR
Hornicek et al. ¹⁰	33	0	20 (mean)	IHC	> 0	NR	7/20 (N90)	13/33
Kusuzaki et al. ¹⁸	10	2	20 (mean)	IHC	≥ 10	Yes	3/10 (N90)	NR
Baldini et al. ¹³	92	0	~14 (median)	IHC	≥ 10	Yes	67/92 (N90)	17/92
Park et al. ¹¹	52	4	21 (mean)	IHC	≥ 10	Yes	26/52 (N90)	20/52
Kumta et al. ¹⁴	45	0	18 (mean)	IHC	> 25	NR	20/45 (Huvos)	NR
Chan et al. ¹²	61	4	5-21	IHC	> 0	Yes	15/46 (N90)	21/61
Yamamoto et al. ¹⁵	28	0	~15 (median)	IHC	> Weak	Yes	11/28 (N90)	5/28
Oda et al. ⁵	25	0?	NR	IHC	> 10	Yes	11/25 (N90)	NR
Radig et al. ¹⁶	52	0?	NR	IHC	> 50	NR	27/52 (N90)	8/52
Posl et al. ¹⁷	34	0	19 (mean)	IHC	≥ 10	NR	26/34 (N90)	NR

Pgp: P-glycoprotein; IHC: immunohistochemistry; RT-PCR: reverse-transcriptase polymerase chain reaction; NR: not reported; N90: histologic response based on > 90% tumor cell necrosis.

^a Censoring before 2-years of follow-up occurred in 5 patients in Wunder et al.,⁷ 1 patient in Chan et al.,¹² 9 patients in Yamamoto et al.,¹⁵ and 9 patients in Radig et al.¹⁶

doxorubicin, cisplatin, methotrexate, ifosfamide, etoposide, taxol, carboplatin, pirarubicin, bleomycin, or cyclophosphamide in various combinations. All patients received doxorubicin. Surgery was comprised of resection, limb salvage, or amputation procedures.

Only IHC data were available for Pgp determinations in all eligible studies, with two exceptions: in one study, RT-PCR⁶ data also were available on the same samples, but IHC data have been entered in the main meta-analysis (inclusion of the RT-PCR data instead does not change any conclusions); in another study,⁷ only RT-PCR data were available, and we examined the meta-analysis results including or excluding this study. The latter study also included some patients for whom only postchemotherapy Pgp measurements were available; otherwise, prechemotherapy Pgp determinations were available in for all other patients in the meta-analysis. Six studies used the 10% cutoff level for Pgp positivity, whereas different thresholds (0–50%) were used in the remaining reports (Table 1). Eight studies clearly stated that Pgp determinations were blinded to outcomes. The percentage of histologic responses to chemotherapy ranged between 22% and 76%, whereas rates of 2-year disease progression ranged between 18% and 39% across studies.

Data Synthesis: Response to Chemotherapy

Figure 1 shows that Pgp positivity did not predict whether histologic response rates > 90% would occur. The SROC curve passed very close to the diagonal suggestive of total lack of discriminating performance. According to the SROC, a sensitivity of 50% corre-

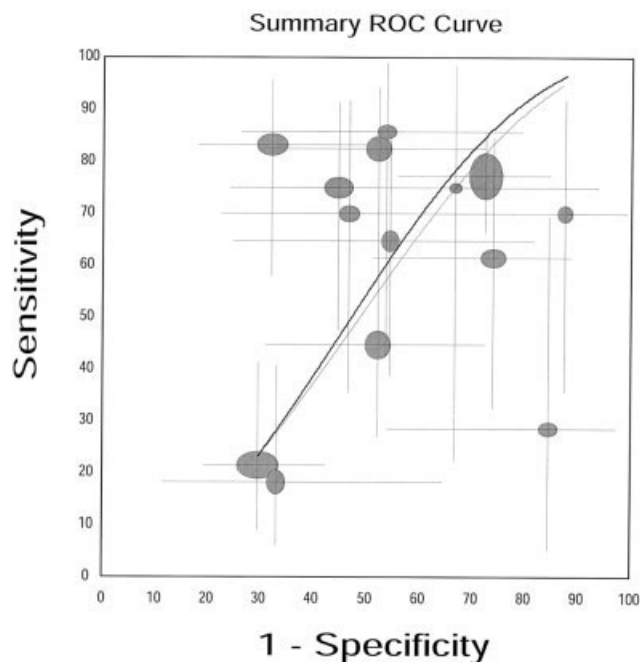


FIGURE 1. Summary receiver operating characteristic (SROC) curve for the discriminating ability of P-glycoprotein (Pgp) in separating good responders and poor responders to chemotherapy based on histologic criteria. Each study is illustrated by an ellipse demonstrating the sensitivity and specificity of Pgp positivity for predicating a poor histologic response to chemotherapy along with lines extending to the respective 95% confidence intervals. The ellipse axes are proportional to the weight of the study in terms of specificity and sensitivity. Also shown are weighted (thick line) and unweighted (thin line) SROC curves summarizing these data.

TABLE 2
Likelihood Ratios for the Association between the Presence of P-Glycoprotein and No Histologic Response to Chemotherapy^a

Studies	No. of studies/ patients	Likelihood ratio (95% CI)	
		Positive	Negative
All	14/597	1.15 (0.93–1.43) ^b	0.88 (0.65–1.18) ^b
Excluding outlier studies	14/597	1.15 (0.93–1.43) ^b	0.88 (0.65–1.18) ^b
Excluding studies with postchemotherapy Pgp	13/478	1.15 (0.89–1.49) ^b	0.87 (0.63–1.22) ^b
Excluding studies with non-IHC measurements	13/478	1.15 (0.89–1.49) ^b	0.87 (0.63–1.22) ^b
Excluding studies without 10% IHC cut-off	6/231	1.13 (0.70–1.83) ^b	0.89 (0.58–1.38) ^c
Excluding studies with unstated blinding	8/291	1.17 (0.81–1.69) ^b	0.80 (0.45–1.41) ^b

95% CI: 95% confidence interval; Pgp: P-glycoprotein; IHC: immunohistochemistry.

^a All figures were based on random effects calculations.

^b $P < 0.05$ for between-study heterogeneity.

^c $0.05 < P < 0.10$ for between-study heterogeneity.

sponded to a specificity of 53%, and a specificity of 50% corresponded to a sensitivity of 55% in the weighted analysis. Unweighted estimates were similar (Fig. 1). No study showed any particularly strong discriminating performance overall.

In the main analysis and in various sensitivity analyses, LR + remained in the range of 1.13–1.17, and LR – remained in the range of 0.80–0.89, values characteristic of very poor discriminating performance (Table 2). The 95% CIs typically exclude by far two-fold discriminating effects for either metric. It is noteworthy that there was significant between-study heterogeneity for both metrics, both in the overall analysis and after excluding various studies in sensitivity analyses. Evaluation of bias diagnostics showed that there was no evidence that the results of large studies differed from the results of smaller studies, and the magnitude of the LR + and LR – did not change markedly as more studies were published over time.

Data Synthesis: Disease Progression at 24 Months

Pgp positivity was associated with a worse prognosis regarding the risk of disease progression within 2 years (Fig. 2). The risk of disease progression approximately doubled; however, there was significant between-study heterogeneity in these results. Various sensitivity analyses showed a persistent, increased risk of disease progression (Table 3), and between-study heterogeneity was no longer significant when the analyses were limited to IHC studies. Twenty-four of 479 patients (5%) were censored without disease progression before they reached 2 years of follow-up. Their exclusion from the calculations also strengthened the observed association.

There was no evidence that the observed association had changed significantly over time, although

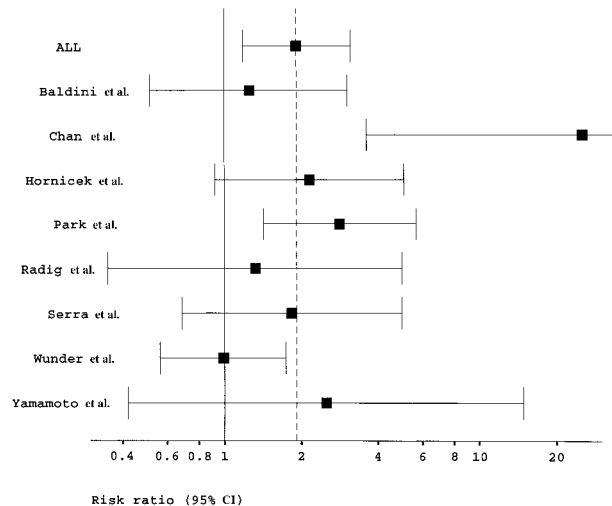


FIGURE 2. Meta-analysis of the association between P-glycoprotein positivity and the risk of disease progression at 2 years. Each study is shown by the name of the lead author, the number of patients, and the risk ratio with 95% confidence intervals. Also shown are summary risk ratio (ALL) and 95% confidence intervals according to random-effects calculations.

formal statistical significance would be claimed only after results published up until the end of year 2000 were analyzed. There was a suggestion that larger, more precise studies provided more conservative estimates compared with smaller, less precise studies ($P = 0.03$). The largest study in the field failed to show an association between Pgp and the risk of disease progression.⁷

DISCUSSION

This meta-analysis showed convincingly that Pgp positivity does not predict whether patients with osteo-

TABLE 3
Risk Ratio for Association between P-Glycoprotein and Disease Progression within 24 Months

Studies	No. of studies/ patients	Risk ratio (95% CI)	
		Random effects	Fixed effects
All	8/479	1.92 (1.18–3.13) ^a	2.03 (1.50–2.76)
Excluding outlier studies	7/418	1.61 (1.15–2.27)	1.55 (1.13–2.12)
Excluding studies with postchemotherapy Pgp measurements	7/356	2.23 (1.37–3.64)	2.71 (1.87–3.93)
Excluding studies with non-IHC measurements	7/356	2.23 (1.37–3.64)	2.71 (1.87–3.93)
Excluding studies without 10% IHC cutoff	2/144	1.98 (0.89–4.39)	1.97 (1.15–3.37)
Excluding studies with unstated blinding	4/233	3.00 (1.16–7.75) ^a	3.45 (2.11–5.65)
Excluding subjects censored before 2 years	8/455	2.91 (1.26–6.71) ^a	2.67 (1.75–4.06)

95% CI: 95% confidence interval; Pgp: P-glycoprotein; IHC: immunohistochemistry.

^a $P < 0.05$ for between-study heterogeneity.

sarcoma who undergo combination chemotherapy will have a histologic response to treatment with > 90% necrosis. Conversely, we found that Pgp positivity in these patients seemingly doubled the risk of disease progression over a follow-up of 2 years. We should caution, however, that there was considerable heterogeneity in the results of the various studies that were included in this meta-analysis.

Heterogeneity for disease progression was driven in particular by two studies with opposing results. Chan et al.¹² described a very impressive correlation between Pgp and clinical disease progression. Conversely, Wunder et al.⁷ found no such association in the largest study conducted to date, but their CIs suggest that a modest association with disease progression could not be ruled out. The remaining studies suggest overall a modest but significant association of Pgp with clinical outcome. Wunder et al. used RT-PCR instead of IHC determinations. Although RT-PCR may be a more sensitive method,³⁷ it is unclear whether levels of mRNA correspond to IHC positivity. One study⁶ found extensive discrepancies in Pgp positivity, depending on whether IHC or RT-PCR was used, and two other studies found only modest concordance between the two methods.^{15,37} Moreover, Wunder et al. included both prechemotherapy and postchemotherapy measurements, and there is evidence that chemotherapy may increase Pgp expression significantly.¹⁷ Nevertheless, a strict analysis excluding that study yielded practically identical results showing a lack of correlation of Pgp with histologic response and very similar results showing a correlation between Pgp and the risk of disease progression. Chan et al. used a different categorization for Pgp positivity than most other studies in the field. Their results may have been more comparable with other studies if the 10% cutoff level had been used instead. Nevertheless, we should acknowledge that the clinically optimal method for

detection of Pgp and the optimal cutoff level thereof are unknown.

P-glycoprotein has been proposed as a significant predictor of response to chemotherapy and/or clinical outcome^{38–43} for several malignancies, including soft tissue sarcomas in children,³⁸ ovarian and small cell lung carcinomas,³⁹ neuroblastoma,^{40,41} and hematologic malignancies.^{42,43} However, several studies also failed to find an association with prognosis of specific malignancies, such as rhabdomyosarcoma⁴⁴ and Ewing sarcoma,⁴⁵ as well as types of lymphomas,⁴⁶ gastric carcinoma,⁴⁷ and mesothelioma.⁴⁸ Thus, the literature on a potential prognostic role of Pgp in sarcomas in particular has been ambivalent.

The dissociation of chemotherapy response and clinical disease progression in this meta-analysis is a challenging finding. Unless the positive association with clinical disease progression was due to bias, the findings suggest that the mechanism of action of Pgp may be independent of regulation of the response of chemotherapy. The lack of correlation of Pgp with percent necrosis should be interpreted with the understanding that percent necrosis is only a crude predictor of clinical outcome. Pgp may be related to other osteosarcoma features, such as aggressiveness, advanced or long-standing disease, or metastatic potential, as suggested for other malignancies.⁴⁹ Subclinical loci of metastatic cells may be eliminated differentially by chemotherapy, depending on Pgp status, whereas the differential effect on the main tumor mass may be less prominent. Furthermore, the chemotherapeutic regimens used in these studies always included doxorubicin (the typical Pgp substrate), but other agents that are not Pgp substrates also were employed, such as high-dose methotrexate, cisplatin, ifosfamide, bleomycin, and cyclophosphamide. Alternatively, the available methods for characterizing response to chemotherapy may not be sensitive enough to differenti-

ate subtle variants of the cellular response that may be important for predicating the eventual course of the disease.

Some limitations of this meta-analysis should be acknowledged. First, publication bias is a threat, especially in view of the fact that the largest study in the field failed to show an association of Pgp with disease progression, and smaller studies claimed overall more prominent associations. However, even the largest study did not exclude a modest association by its 95% CIs. Second, we tried to retrieve additional information that was not available from the published reports and to standardize definitions of measurements and outcomes. Nevertheless, some differences in the definitions for measurements and outcomes may be unavoidable in meta-analyses of prognostic factors; thus, conclusions need to be interpreted cautiously.^{50,51} The methods used to determine histologic necrosis are varied, and sometimes the assessments may be crude. Nevertheless, there is no indication that specific individual studies performed assessments of systematically worse quality compared with other studies. Some misclassification errors are unavoidable. However, it is unlikely that misinterpretations would be more common in Pgp positive patients versus Pgp negative patients.

Third, even with 14 studies included and approximately 600 patients analyzed, small associations between histologic response and Pgp (e.g., LR + = 1.2) may have been missed. However, it is unlikely that such associations are relevant clinically. Furthermore, because osteosarcomas are not very common on a population basis, the sample size of the meta-analysis is one of the largest to date among studies targeting patients with this malignancy. Fourth, the estimates that we obtained were unadjusted for other parameters that may be related to outcomes in patients with osteosarcoma. In particular, it is known that tumor size is a strong predictor⁷ with an almost 3-fold risk for disease progression in tumors > 9 cm compared with smaller tumors. Histologic tumor type and chemotherapeutic regimens also may affect prognosis.⁵² However, there is no evidence to suggest that Pgp is associated with larger tumor size or specific histologic types, whereas treatment in the analyzed studies was not determined based on Pgp results.

The prognostic value of Pgp in patients with osteosarcoma also should be examined in the context of other proposed molecular markers, including p53,⁵³ ErbB2,⁵⁴ heat shock proteins,³³ and DNA sequence copy number increases,⁵⁵ among others. Although one study showed a positive correlation between p53 mutation and Pgp,¹¹ two other studies showed no such correlation.^{24,25} No correlation has been demon-

strated between ErbB2 and Pgp, although modest associations cannot be excluded.²⁴ The clinical behavior of osteosarcoma may be determined by large numbers of different genes acting in consort. The use of newer technologies, such as microarray analysis,⁵⁶ may unravel the relative contribution of these genes and may help understand better the role of Pgp in the regulation of the clinical prognosis of patients with osteosarcoma.

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