A Meta-Analysis on the Interaction between HER-2 Expression and Response to Endocrine Treatment in Advanced Breast Cancer

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Abstract

Purpose: Experimental data suggest a complex cross-talk between HER-2 and estrogen receptor, and it has been hypothesized that HER-2-positive tumors may be less responsive to certain endocrine treatments. Clinical data, however, have been conflicting. We have conducted a meta-analysis on the interaction between the response to endocrine treatment and the overexpression of HER-2 in metastatic breast cancer.

Experimental Design: Studies have been identified by searching the Medline, Embase, and American Society of Clinical Oncology abstract databases. Selection criteria were (*a*) metastatic breast cancer, (*b*) endocrine therapy (any line of treatment), and (*c*) evaluation of HER-2 expression (any method). For each study, the relative risk for treatment failure for HER-2-positive over HER-2-negative patients with 95% confidence interval was calculated as an estimate of the predictive effect of HER-2. Pooled estimates of the relative risk were computed by the Mantel-Haenszel method.

Results: Twelve studies (n = 2,379 patients) were included in the meta-analysis. The overall relative risk was 1.42 (95% confidence interval, 1.32-1.52; $P < 0.00001$; test for heterogeneity = 0.380). For studies involving tamoxifen, the pooled relative risk was 1.33 (95% confidence interval, 1.20-1.48; $P < 0.00001$; test for heterogeneity = 0.97); for studies involving other hormonal drugs, a pooled relative risk of 1.49 (95% confidence interval, 1.36-1.64; $P < 0.00001$; test for heterogeneity = 0.08) was estimated. A second meta-analysis limited to tumors that were either estrogen receptor positive, estrogen receptor unknown, or estrogen receptor negative/progesterone receptor positive yielded comparable results.

Conclusions: HER-2-positive metastatic breast cancer is less responsive to any type of endocrine treatment. This effect holds in the subgroup of patients with positive or unknown steroid receptors.

The HER-2 (also known as c-*erb*B-2 or *neu*) oncoprotein is one of the four transmembrane receptors of the *erb*B family. Different from the other receptors of the family, HER-2 has unique receptor features: it binds no known specific ligand, but it seems to exert its biological activity by serving as a preferential partner of the other *erb*B receptors. Indeed, it forms heterodimers with the other members of the *erb*B family after their binding to specific ligands, thus enhancing and prolonging cell signaling (1–4). Interestingly, overexpression of HER-2 also induces its spontaneous homodimerization thereby leading to activation of the tyrosine kinase moiety of the intracytoplasmic domain without the need for ligand (5). Proliferation of breast cells is dependent on the regulatory action of both steroid hormones and growth factors, and a body of experimental evidence suggests the presence of complex cross-talk and interactions between the HER-2/tyrosine kinase pathway and the estrogen receptor (ER) pathway (6–8). In particular, it seems that HER-2-overexpressing tumor cells might grow in estrogen-depleted condition and be resistant to selective ER modulators, such as tamoxifen, compared with HER-2-negative cells (8–11).

Overexpression of HER-2, generally due to gene amplification, is found in 20% to 30% of human breast cancers and it has been postulated that this overexpression may modulate the clinical sensitivity of tumors to endocrine treatments. However, clinical studies have yielded conflicting results in both adjuvant and metastatic settings.

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In particular, in the metastatic setting, several groups have identified a correlation between HER-2 overexpression and a lower response rate to endocrine treatment (12–21), whereas others have not (22–26). Instances of conflicting results may be related to the lack of a standardized methodology for HER-2 analysis, to flawed experimental designs, but also to the low statistical power of the single studies.

We have conducted a meta-analysis of the pertinent published studies in an attempt to gain insights into the association between HER-2 and response to different types of endocrine therapy in metastatic breast cancer.

Methods

Before starting the review process, the authors agreed on a protocol that contained all aspects of the meta-analysis.

Study identification. Studies were identified by a computerized search of the Medline (1966-2002), Cancerlit (1966-2002), and Embase (1990-2002) databases using the following text words: "breast cancer and (c-erbB-2 or c-erbB2 or Neu or HER2)." A computerized search of the Proceedings of the Annual Meetings of the American Society of Clinical Oncology held between 1998 and 2002 was run to identify relevant studies published in abstract form. Lastly, all review articles and all cross-referenced studies from retrieved articles were screened for pertinent articles.

Selection criteria. To be included in the meta-analysis, retrieved studies had to fulfill the following simple inclusion criteria: (*a*) advanced breast cancer, (*b*) endocrine therapy (any line of treatment), and (*c*) evaluation of HER-2 expression (any method). Studies meeting these criteria were excluded from the analysis if any of the following cases occurred: (*a*) the response rate stratified by HER-2 status was neither reported in nor derivable from the original article and the principal investigator refused or was unable to provide this information on request and (*b*) the article was an earlier report of data updated in a subsequent article to be included in the meta-analysis.

Data extraction. Data were independently extracted from each report by G.A. and E.M., who were blinded to each other, using a data recording form developed for this purpose. After extraction, data were reviewed and compared by M.D.L. Instances of disagreement between the two data extractors were resolved by consultation. When needed, additional information about a specific study was gathered by directly querying the principal investigator.

Information about the type endocrine therapy administered was collected for almost all the studies. Two main groups were created: the tamoxifen group, which included trials that used tamoxifen, and the no tamoxifen group, which included trials using all other kinds of hormonal treatment. When the drug used was not specified, the study was included in the no tamoxifen group.

End point for analysis. Treatment failure was chosen as a clinically meaningful end point of the meta-analysis. It was defined as progression of the disease within 6 months of treatment onset. In fact, there is a general acceptance that endocrine therapy-induced stabilization of advanced breast cancer lasting $\geq$6 months should be deemed a therapeutic achievement (clinical benefit) because it results in a survival comparable with that of partial responding patients (27).

Statistical analysis. The risk of treatment failure was taken as a measure of the resistance of the disease to the hormonal therapy. The correlation between HER-2 expression and treatment failure rate within each single study was expressed as relative risk (RR) for treatment failure of HER-2-positive over HER-2-negative patients. Thus, a RR equal to 1 indicates a lack of association between HER-2 status and treatment failure rate; a RR higher than 1 corresponds to a direct correlation between treatment failure rate and HER-2-positive status (i.e., a tendency of HER-2-positive patients to have a higher rate of treatment failure), an inverse correlation being indicated by a RR lower than 1 (i.e., a tendency of HER-2-positive patients to have a lower rate of treatment failure).

A pooled estimate (meta-analysis) of the RRs of the individual studies was computed by a fixed effect model according to Mantel and Haenszel (28). Homogeneity assumption was checked by a χ^2 test with a *df* equal to the number of analyzed studies minus 1.

A sensitivity analysis was carried out by recalculating the pooled RR estimate for different subgroups of studies based on relevant clinical features. This analysis serves to determine whether the pooled estimates are stable or whether they depend on some features of the studies included in the meta-analysis. Consequently, it shows whether the overall result would be affected by a change in the meta-analysis selection criteria.

An estimate of the potential publication bias was carried out by plotting the single study RR on a log-scale against respective SE (funnel plot). A simulation was also carried out by recalculating the overall RR after the inclusion of increasing numbers of simulated negative trials. This serves to evaluate how many potentially unpublished negative trials would be necessary to make the overall results nonstatistically significant.

Quality score. A quality score was assigned to each study based on the sample size. Three clusters of studies were identified: those with <90 patients (score 0), those with 90 to 200 (score 1), and those with >200 patients (score 2). It was necessary to assign this quality score because the assumption underlying the use of the Mantel-Haenszel pooled estimator may not hold for studies with a very low number of patients. Because of its subjective nature, however, the quality score was not used to weigh further the contribution of each study to the meta-analysis. The quality score was used as a stratification factor in the sensitivity analysis to evaluate whether the pooled results of the meta-analysis are independent of the size of the studies included.

Results

Results of literature search. A total of 15 studies were identified that met the meta-analysis inclusion criteria (12–26). The studies by Nicholson et al. (12) and Leitzel et al. (15) were excluded because subsequent articles containing updated results were included in the meta-analysis, whereas the study by Bezwoda (25) was excluded because it contained insufficient information and it was not possible to get further details. All the remaining 12 studies were used in the pooled analysis (2,379 patients). Table 1 lists the studies identified and their main characteristics.

HER-2 overexpression assessment. HER-2 expression was evaluated in a variable proportion of the patient population of each study (Table 2). However, in 7 of 12 studies, HER-2 status was evaluated in at least 90% of the whole study population; only 2 small study showed a very low proportion of patients assessed for HER-2 status (16, 26). These figures indicate that the series under study are fairly representative of the patient populations of the individual studies.

HER-2 expression was evaluated with different methods: immunohistochemical detection of the p185 protein (13, 18, 19, 22, 24), evaluation of gene amplification by Southern blot (14, 19) and ELISA (16, 20, 21, 23, 26), or chemoluminescent immunoassay (17) determination of circulating levels of HER-2 in the patient serum. Differences in the cutoff values chosen to distinguish between negative and positive HER-2 status complicate the picture even further, so that each study used its own particular methodology. Despite the total lack of methodologic standardization, the prevalence of HER-2-positive tumors is reasonably constant among studies, ranging from 19% to 35% (Table 2). This is in line with the reported proportion of HER-2-positive tumors in different settings (29).

Table 1. Identified studies and their inclusion in the meta-analysis

Year	Author	Evaluable patients	Drug	Line of treatment	Inclusion	Quality
1990	Nicholson	61	Tamoxifen	First-line	No*	0
1992	Wright	65	Tamoxifen or ovarian ablation	First-line	Yes	0
1995	Leitzel	300	Megestrol or fadrazole	Second-line	No†	2
1995	Archer	92	Tamoxifen (64); goserelin (6); tamoxifen + goserelin (19); megestrol (3)	First-line	Yes	1
1995	Berns	126	Tamoxifen or others	First-line	Yes	1
1996	Willsher	52	Tamoxifen (59); tamoxifen + goserelin (8)	First-line	Yes	0
1997	Yamauchi	94	Droloxifene	First-line	Yes	1
1998	Elledge	204	Tamoxifen	First-line	Yes	2
1998	Fehm	23	Not specified	First-line	Yes	0
1999	Houston	241	Tamoxifen	First-line	Yes	2
2000	Bezwoda	35	Tamoxifen	First-line	No	0
2000	Jukkola	106	Undefined Hormonal	First-line	Yes	1
2001	Hayes	103	Megestrol (160, 800, or 1,600 mg/d)	First-line and second-line	Yes	1
2002	Lipton	711	Megestrol or fadrazole or letrozoleSecond-line	Second-line	Yes	2
2003	Lipton	562	Tamoxifen or letrozole	First-line	Yes	2

*The study by Wright is an update of this study.
†The study by Lipton (2002) is an update of this study.

Steroid receptor information. Steroid receptor information was available for most studies in the meta-analysis (Table 3) for a total of 2,288 of 2,385 (95.9%) patients (2,379 of whom evaluable for the meta-analysis). Among these, 1,368 (59.8%) were ER positive, 357 (15.6%) were either ER positive or progesterone receptor (PgR) positive, and 368 (16.1%) had unknown ER status. The study by Berns et al. (14) did not report steroid receptor information for the 126 patients with HER-2 status evaluation; however, it was assumed that the proportion of ER-positive patients was approximately superimposable to that of the whole study population (ER positive: 193 of 259 = 59%). Two small studies (17, 19) lacked clear information about steroid receptor status. However, given the selection criteria of these studies, it is reasonable to assume that ER-positive patients represent a vast proportion of subjects in this series.

Correlation between HER-2 and treatment failure. Treatment failure rates according to HER-2 expression were reported in all

Table 2. HER-2 expression assessment in the various studies

Author	No. (%) evaluable patients	HER-2 determination	Cutoff	No. (%) HER-2+
Archer	92/92 (100)	Immunohistochemistry	≥1 stained tumor cell membranes	24/92 (26)
Berns	126/259 (49)	Southern blot	>2 gene copies	24/126 (19)
Elledge	204/349 (58)	Immunohistochemistry	Score ≥2 (≥1% stained cells)	44/204 (22)
Fehm	23/23 (100)	Chemoluminescent immunoassay serum	>120 fmol/mL	7/23 (30)
Houston	241/241 (100)	Immunohistochemistry	≥1 stained tumor cell membranes	76/241 (32)
Hayes	103/368 (28)	ELISA serum	≥10.5 ng/mL	33/103 (32)
Jukkola	106/106 (100)	PCR, Southern blot, immunohistochemistry	NA	23/106 (22)
Lipton first-line	562/907 (62)	ELISA serum	>15 ng/mL	164/562 (29)
Lipton second-line	711/719 (99)	ELISA serum	>15 ng/mL	217/711 (30)
Willsher	52/73 (71)	ELISA serum	>20 ng/mL	13/52 (25)
Wright	65/72 (90)	Immunohistochemistry	≥50%	14/65 (22)
Yamauchi	94/363 (26)	ELISA serum	>5,000 units/mL	32/94 (34)

Table 3. Steroid receptor status information

Author	ER status	ER+ (%)	ER unknown (%)	ER−/PgR+ (%)
Archer	ER+, ER−	55/92 (60)	—	NA
Berns	ER+, ER−	193/259 (59)	—	NA
Elledge	ER+	204/204 (100)	—	NA
Fehm	NA	NA	NA	NA
Hayes	ER+, ER unknown, ER−/PgR+	NA	NA	NA
Houston	ER+, ER−	189/241 (78)	—	NA
Jukkola	NA	NA	NA	NA
Lipton first-line	ER+, ER unknown, ER−/PgR+	ER+/PgR+: 219/562 (39); ER+/PgR− or ER−/PgR+: 155/562 (28)188/562 (33)	NA	
Lipton second-line	ER+, ER unknown, ER−/PgR+	ER+/PgR+: 391/719 (54); ER+/PgR− or ER−/PgR+: 200/719 (28) 128/719 (18)	NA	
Willsher	ER+, ER unknown, ER−	32/52 (62)	15/52 (28)	NA
Wright	ER+, ER−	30/65 (46)	—	NA
Yamauchi	ER+, ER unknown, ER−/PgR+	55/94 (59)	37/94 (39)	2/94 (2)

but one study (24). However, this information could be derived from the progression-free survival curves presented in the article.

The RR of treatment failure was calculated for each study in the meta-analysis. The data of the 2,379 patients were first analyzed globally. Single-study RRs ranged from 1.06 to 3.81. Overall, the estimated pooled RR for all the studies was 1.42 [95% confidence interval (95% CI), 1.32-1.52], showing a highly significant correlation between HER-2 positivity and treatment failure ($P < 0.00001$; Fig. 1). The result of the test for heterogeneity ($P = 0.380$) shows that the differences among single-study figures may be explained by chance and confirms the appropriateness of pooling the data. Interestingly, the correlation between HER-2 positivity and treatment failure is maintained regardless of the type of endocrine therapy administered. The RR was 1.33 (95% CI, 1.20-1.48; $P < 0.00001$; test for heterogeneity = 0.97) for studies involving tamoxifen (tamoxifen group) and 1.49 (95% CI, 1.36-1.64; $P < 0.00001$) for studies regarding other endocrine agents (no tamoxifen group), respectively. However,

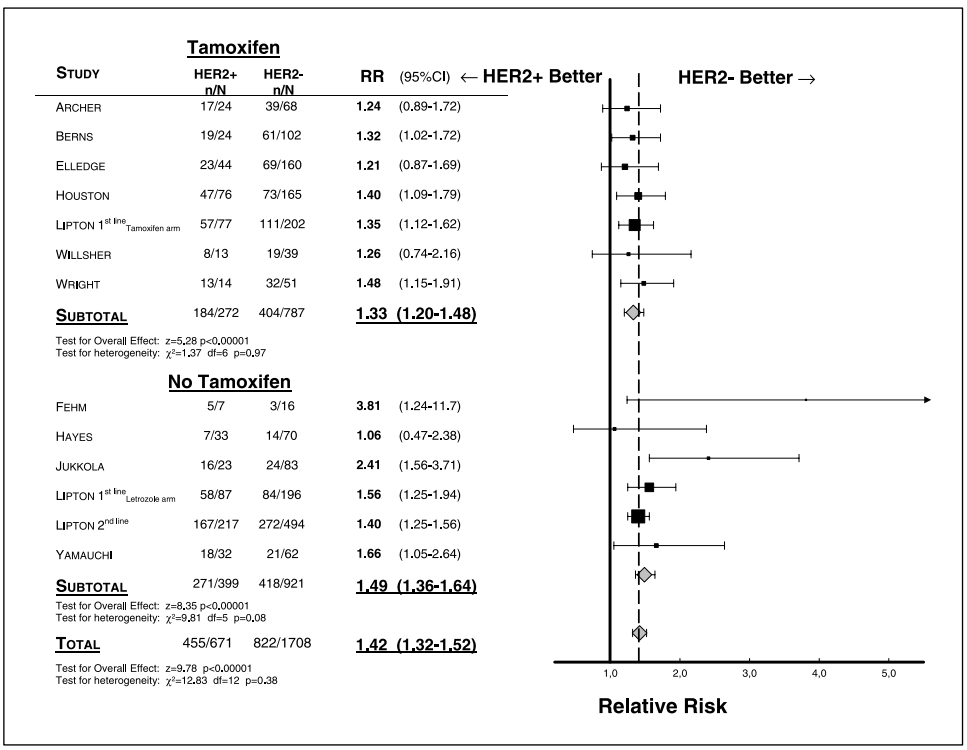

Fig. 1. RR of treatment failure for HER-2-positive versus HER-2-negative for each study in the meta-analysis and respective pooled estimates.

in this latter group, the effect of HER-2 as regard to tumor responsiveness showed a trend to heterogeneity although not significant (test for heterogeneity = 0.08; Fig. 1).

HER-2 overexpression is usually correlated with ER-negative status; thus, to avoid the potential confounding effect of the small number of ER-negative patients included in the global analysis, a second meta-analysis was done using data of patients who were either ER positive, ER unknown, or ER negative/PgR positive (n = 1,925). This meta-analysis was possible only for 8 of the 12 studies. The results are reported in Fig. 2. The overall estimate shows an even higher correlation between HER-2 overexpression and treatment failure (overall RR, 1.45; 95% CI, 1.34-1.57; $P < 0.00001$). The test for heterogeneity ($P = 0.270$) shows the appropriateness of combining data. In the tamoxifen group, the RR was 1.48 (95% CI, 1.29-1.70; $P < 0.00001$; test for heterogeneity = 0.09), and in the no tamoxifen group, the RR was 1.43 (95% CI, 1.30-1.58; $P < 0.00001$; test for heterogeneity = 0.64). Again, despite the type of hormone therapy, patients with HER-2-positive tumors had a worse outcome compared with women whose tumor was for HER-2 negative.

A sensitivity analysis was done to check if modification of the inclusion criteria of the meta-analysis affected the final result. This was done by limiting the meta-analysis to some clinically relevant subgroups of studies. As shown in Table 4, the overall estimated RR is fairly stable irrespective of the study quality score, line of treatment, and HER-2 evaluation technique.

Estimation of the publication bias. Meta-analyses of published studies could emphasize positive results because of the so-called publication bias, which is a tendency of authors and editors not to publish trials with negative results (i.e., results that do not confirm the hypothesis under evaluation). A "funnel plot" can be generated to estimate whether a meta-analysis may have been affected by such a bias. In situations in which the publication bias is absent or negligible, the distribution of the studies on the plot resembles a funnel, with the apex converging on the average meta-analytic RR estimate. As shown in Fig. 3, the distribution of the studies in our meta-analysis is clearly funnel-shaped, which indicates that a potential publication bias should be limited.

However, given the subjective nature of the funnel plot evaluation, we also used a simulation process to try to estimate how additional unpublished negative trials would have affected the meta-analytic results. We thus generated hypothetical negative studies, each of which was a replicate of the most negative real study in the meta-analysis [i.e., the Hayes et al. study (26)]. The results of the simulation are reported in Table 5. Even when as many as 10 negative simulated studies were added to the analysis, the correlation between HER-2 overexpression and treatment failure remained statistically significant (RR, 1.36; 95% CI, 1.26-1.45) and no heterogeneity among studies emerged throughout the simulation. This shows that, even if some negative trials may have not been published, these should not have greatly affected the results of the meta-analysis.

Discussion

It has yet to be established whether HER-2 overexpression is predictive of breast cancer resistance to endocrine treatment, because conflicting results are reported in both adjuvant and metastatic settings.

To address the latter issue, we conducted a meta-analysis of the published studies to obtain an overall pooled estimate of the association between HER-2 overexpression and treatment failure rate. We found a significant direct correlation between

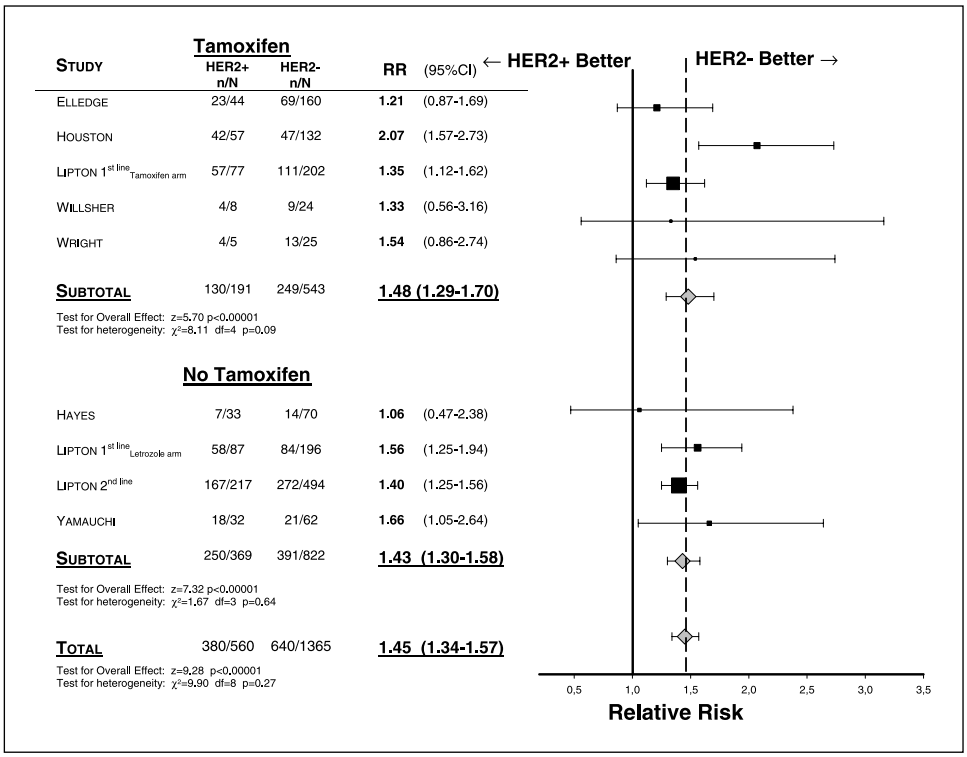

Fig. 2. RR of treatment failure for HER-2-positive versus HER-2-negative for ER-positive/ER-unknown patients and respective pooled estimates.

Table 4. Sensitivity analysis

Study selection	No. studies	No. patients	RR (95% CI)	Test for overall effect (*P*)	Test for heterogeneity (*P*)
Quality score = 2	4	1,718	1.39 (1.28-1.50)	<0.00001	0.86
Quality score = 1 or 2	9	2,239	1.40 (1.31-1.51)	<0.00001	0.37
First-line only	10	1,613	1.43 (1.31-1.57)	<0.00001	0.24
Immunohistochemistry only	4	602	1.33 (1.14-1.55)	0.0003	0.72
ELISA serum	5	1,532	1.40 (1.29-1.53)	<0.00001	0.89
Other than immunohistochemistry or ELISA	3	255	2.00 (1.08-3.72)*	0.03*	0.008
All	12	2,379	1.42 (1.32-1.52)	<0.00001	0.38

*Random effect model.

HER-2 overexpression and the risk of disease progression while on endocrine treatment. Furthermore, we showed a lack of statistical heterogeneity among studies, which suggests that contradictory results were related to chance.

Although many trials addressing the relationship between endocrine resistance and HER-2 expression/amplification have been conducted in the adjuvant setting (30–35), in the present study, we decided to include only studies involving the metastatic setting. In the adjuvant trials, individual response is impossible to categorize; thus, the use of aggregated data to estimate treatment failure and treatment success rather than raw data from the single individual would have been not appropriate (36).

Because of the difference in biological activity between tamoxifen and other types of endocrine therapy, such as aromatase inhibitors and steroidal pure antiestrogens (such as fulvestrant), it has been suggested that the use of the latest, unlike tamoxifen, may overcome resistance of HER-2-positive tumors. A recent randomized trial of neoadjuvant therapy (37) showed that, in the subset of ER-positive, epidermal growth factor receptor–positive, and/or HER-2-positive, letrozole was significantly more effective than tamoxifen. However, in more conventional treatment settings (adjuvant and/or metastatic), this finding has not been confirmed yet. In a large randomized trial of first-line hormone therapy for metastatic breast cancer that is included in the present meta-analysis (21), patients with normal serum HER-2 receiving letrozole showed a significantly greater objective response rate and clinical benefit and longer time to progression and time to treatment failure than patients receiving tamoxifen. However, in patients with elevated serum HER-2, there was no significant difference between letrozole and tamoxifen in objective response rate and clinical benefit, although a strong trend favoring longer duration of response with letrozole was observed. Whether letrozole or any other form of endocrine treatment could be better than tamoxifen in the HER-2-positive group of patients cannot be assessed by this meta-analysis. The heterogeneity of treatments among the included studies makes it difficult to extrapolate any firm conclusion about this issue. Nevertheless, the vast majority of the patients in the no tamoxifen group received letrozole and the vast majority of patients in the tamoxifen group received tamoxifen or another selective ER modulator. Our results suggest that, overall, endocrine therapy is less effective in patients with a HER-2-positive tumor and that the increased risk of treatment failure for HER-2-positive breast cancer may hold irrespective of the drug administered at least in the metastatic setting.

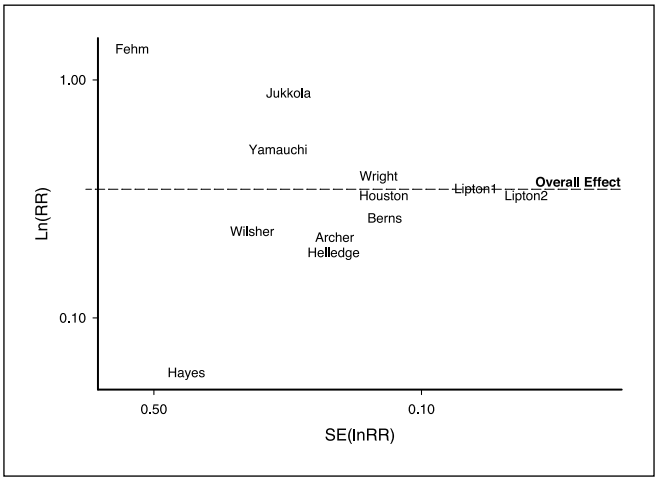

Fig. 3. Funnel plot for the visual estimation of the potential publication bias.

Some methodologic issues of our work are worth of discussion. The present study is a meta-analysis of nonrandomized observational trials. In general, randomization provides a more unbiased estimate because it controls for potential unknown confounders, and it should be the preferred approach whenever possible. In some research settings, however, randomized trials are rarely, if ever, feasible (i.e., translational studies in oncology). However, also for observational studies, pooled estimates are by far superior to the usual assessment of consistency constituted by tallies of the percentage of positive, negative, and null studies. Indeed, the meta-analytic method applied to this setting has produced very informative data (38, 39). The overt clinical heterogeneity of the patient populations included in this study could be considered a potential problem in interpreting the results of the present analyses. There is, in fact, a substantial diversity in the studies in terms of HER-2 status evaluation technique, drug used, and line of treatment. However, despite this clinical

Table 5. Estimation of the publication bias (explanation in the text)

Studies	RR (95% CI)	Test for overall effect (*P*)	Test for heterogeneity (*P*)
All	1.42 (1.32-1.52)	<0.00001	0.38
All + 1 simulated negative	1.41 (1.31-1.51)	<0.00001	0.40
All + 2 simulated negative	1.40 (1.31-1.50)	<0.00001	0.45
All + 3 simulated negative	1.39 (1.30-1.49)	<0.00001	0.49
All + 4 simulated negative	1.39 (1.29-1.49)	<0.00001	0.53
All + 5 simulated negative	1.38 (1.29-1.48)	<0.00001	0.57
All + 7 simulated negative	1.37 (1.28-1.47)	<0.00001	0.63
All + 10 simulated negative	1.36 (1.26-1.45)	<0.00001	0.72

heterogeneity, there is no statistical heterogeneity among the study results. This shows that it is entirely appropriate to use an overall estimate of the effect of HER-2 expression and suggests that the clinical variability could rather be considered a strength of our results. Indeed, a great variability among studies is equivalent to adding "noise" to the analysis, which very unlikely would convert a null result into a positive finding; rather, it is more likely that a positive correlation between HER-2 overexpression and treatment resistance would be masked or weakened by this "noise." Furthermore, as suggested by the sensitivity analysis, the correlation is fairly stable among subgroups of studies, thereby suggesting that it may be a general feature of breast tumor responsiveness to endocrine treatment across different clinical situations. Another potential problem of meta-analysis of published studies is the tendency to inflate "positive" results because of a general inclination for "negative" studies (i.e., studies that do not confirm the research hypothesis) to be underrepresented in the literature. However, the "funnel" analysis combined with a simulation procedure indicated that this bias, although theoretically possible, should be negligible in the setting of our meta-analysis.

According to the American Society of Clinical Oncology Expert Panel on Tumor Markers, which proposed a tumor marker utility grading system (40), most of the studies in this meta-analysis can be regarded as level III studies (14, 16, 18–22, 24, 26); a few are level IV (13, 17, 23). The latter, which may correspond to those assigned a quality score of 0 in our work, did not affect the overall meta-analytic result as shown by the sensitivity analysis. Consequently, it can be argued that the correlation observed between HER-2 overexpression and a lower response to endocrine therapy approaches level I of evidence. However, the reported correlation should not to be regarded as a certain causal association. Because the analyzed trials did not include a control arm (i.e., an arm in which the therapeutic intervention was not administered), we cannot assess whether our results derive from resistance to endocrine therapy or simply from more aggressive biological behavior of HER-2-positive tumors. However, the *consistency* of our results across different settings (i.e., adjuvant, neoadjuvant, and metastatic) and the *biological plausibility* of our hypothesis support the role of HER-2 in the development of endocrine resistance in human breast cancer.

Because of the lack of a control arm without therapeutic intervention, the benefit ratio (i.e., the ratio of the benefit from treatment between groups of patients characterized by different status of the hypothetical predictive factor) could not be assessed (41). Furthermore, although a clear-cut association between HER-2 positivity and treatment emerges from our analysis, the variability of the studies may have reduced the strength of the effect by adding noise to the pooled analysis. Therefore, although we show a >40% increase of the risk of treatment failure for HER-2-positive advanced breast cancer, it may not allow an accurate evaluation of the predictive strength of the marker. Predictive strength should more appropriately be evaluated in the scenario of randomized trials of adjuvant therapy.

Increasing evidences from preclinical models show that the cross-talk between tyrosine kinase receptors and ER may be crucial for the development of the endocrine resistance. Other downstream molecules, such as the coregulator AIB1 or the protein actMap-kinase (42–44), are implicated in this cross-talk and might play an important role in determining response to endocrine therapy. A recent study (45) in patients receiving tamoxifen adjuvant therapy suggests that HER-2 is associated with resistance only in tumors that also express high levels of AIB1, an ER coactivator that is turned on by HER-2 signaling. Thus, measuring only HER-2 may provide only part of the information and may cause some variability in small clinical trials.

From a clinical standpoint, our meta-analysis shows that, for metastatic breast cancer, the results of all published studies are consistent with an association between HER-2 overexpression and higher rates of failure of endocrine treatment. These results may have direct clinical implications. Indeed, endocrine therapy alone should not be considered a first-choice treatment for patients with ER-positive/HER-2-positive metastatic breast tumors; rather, the treatment strategy for such patients should prefer chemotherapy alone or in combination with trastuzumab. Alternatively, the combination of endocrine therapy with trastuzumab may potentially overcome the partial resistance of such tumors to hormonal manipulations. This latter strategy has been proven effective in preclinical models (8) and randomized trials investigating it in the clinical setting are expected to deliver results in 2005. Finally, the exploitation of endocrine agents, such as the "pure" antiestrogen fulvestrant, the antiproliferative action of which *in vitro* is not affected by HER-2 status (46), may be useful. However, clinical data demonstrating its efficacy in this specific context are still lacking. Better designed clinical trials able to answer to these new challenging biological questions are required to further clarify mechanism of endocrine resistance and to investigate alternatives treatment strategies designed to prevent resistance.

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