HER2 Status and Efficacy of Adjuvant Anthracyclines in Early Breast Cancer: A Pooled Analysis of Randomized Trials

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Background Adjuvant chemotherapy with anthracyclines improves disease-free and overall survival compared with non–anthracycline-based adjuvant chemotherapy regimens in the treatment of early breast cancer. The role of *HER2* status as a marker of anthracycline responsiveness has been explored by subset analyses within randomized clinical trials, with inconsistent results. We performed a pooled analysis of the interaction between *HER2* status and the efficacy of adjuvant anthracyclines based on the published subset data.

Methods We searched literature databases to identify randomized trials that compared anthracycline-based with non–anthracycline-based adjuvant chemotherapy regimens in the treatment of early breast cancer and reported efficacy data according to *HER2* status. Log hazard ratios (HRs) for disease-free and overall survival were pooled across the studies according to *HER2* status by inverse variance weighting. A pooled test for treatment by *HER2* status interaction was performed by weighted linear meta-regression. All statistical tests were two-sided.

Results Eight studies (with 6564 randomly assigned patients, of whom 5354 had *HER2* status information available) were eligible for this analysis. In *HER2*-positive disease (n = 1536 patients), anthracyclines were superior to non–anthracycline-based regimens in terms of disease-free (pooled HR of relapse = 0.71; 95% confidence interval [CI] = 0.61 to 0.83; $P < .001$) and overall (pooled HR of death from any cause = 0.73; 95% CI = 0.62 to 0.85; $P < .001$) survival. In *HER2*-negative disease (n = 3818 patients), anthracyclines did not improve disease-free (HR = 1.00; 95% CI = 0.90 to 1.11; $P = .75$) or overall (HR = 1.03; 95% CI = 0.92 to 1.16; $P = .60$) survival. The test for treatment by *HER2* status interaction yielded statistically significant results: for disease-free survival, the chi-square statistic for interaction was 13.7 ($P < .001$), and for overall survival, it was 12.6 ($P < .001$).

Conclusions The added benefits of adjuvant chemotherapy with anthracyclines appear to be confined to women who have *HER2* overexpressed or amplified breast tumors.

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The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) overview of randomized trials of polychemotherapy in early-stage breast cancer demonstrated that anthracycline-based regimens are superior to non–anthracycline-based regimens with respect to disease-free and overall survival (1). However, the magnitude of the absolute benefit from adjuvant anthracyclines, although statistically significant, was modest and not consistent across individual trials (2–7). An additional concern about the widespread use of anthracyclines as adjuvant treatment for early-stage breast cancer is related to the small but clinically significant risks of cardiotoxicity and secondary leukemia that are associated with these agents. Exploratory studies (8–15) have suggested that the benefit of adjuvant treatment with anthracyclines is confined mainly to women whose breast tumors have overexpressed and/or amplified *HER2*. The association between *HER2* status and responsiveness to adjuvant anthracyclines may be related to the tumor level of topoisomerase II alpha, the molecular target of anthracyclines. In fact, the gene encoding topoisomerase II alpha is located adjacent to the *HER2* oncogene on chromosome 17, and both genes are frequently coamplified in breast tumors (16). In this regard, some retrospective studies (8,9) that have investigated

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the predictive value of aberrations in the topoisomerase II alpha gene in patients treated with anthracyclines have suggested that the additional benefit of adjuvant anthracyclines might be limited to women whose breast tumors have amplification of both the *HER2* and topoisomerase II alpha genes.

Data from several randomized clinical trials that compared anthracycline-based adjuvant chemotherapy with non–anthracycline-based regimens have been subjected to subgroup analysis to examine whether the *HER2* status of a breast tumor is a predictive marker for response to adjuvant treatment with anthracyclines (8–15). Only two of these studies (10,15) reported a positive association between *HER2* positivity and a better response to anthracycline-based regimens than to non–anthracycline-based regimens. Possible reasons for this inconsistency include differences among the studies in the methods used to assess *HER2* status, incomplete specimen collection, and the administration, in some trials, of outdated or suboptimal adjuvant regimens. Furthermore, subset analyses within individual studies that are intended to investigate the effects of an intervention for specific subgroups of patients are inevitably plagued by chance effects and lack of power and should be regarded as exploratory (17).

To better assess the differential efficacy of adjuvant anthracyclines according to *HER2* status, we performed a meta-analysis of data from published subset analyses that were conducted in the context of randomized clinical trials that compared anthracycline-based adjuvant regimens with non–anthracycline-based adjuvant regimens in women with early-stage breast cancer. The aim of this meta-analysis was to assess the presence and degree of interaction between *HER2* status and use of adjuvant anthracyclines in the treatment of early breast cancer.

CONTEXT AND CAVEATS

Prior knowledge

In the treatment of early breast cancer, anthracycline-based adjuvant chemotherapy improves disease-free and overall survival compared with non–anthracycline-based adjuvant chemotherapy. However, it is unclear whether the *HER2* status of breast tumors is a marker of anthracycline responsiveness.

Study design

A pooled analysis of data from eight randomized controlled trials that compared anthracycline-based with non–anthracycline-based adjuvant chemotherapy regimens in the treatment of early breast cancer.

Contribution

The added benefits of adjuvant chemotherapy with anthracyclines appear to be limited to women whose breast tumors have overexpressed or amplified *HER2*.

Implications

Patients with *HER2*-negative breast tumors derive no added benefits from adjuvant chemotherapy with anthracyclines.

Limitations

Only eight randomized clinical trials published data on the effects of adjuvant anthracyclines according to *HER2* status. Summary results rather than individual patient data were analyzed, and there was no centralized reassessment of *HER2* status. The methods used to determine *HER2* status differed among the trials.

Methods

Identification and Selection of Trials

For this meta-analysis, we sought data from all English-language published (as full papers or as conference abstracts) and unpublished randomized trials that compared anthracycline-based adjuvant chemotherapy with non–anthracycline-based adjuvant chemotherapy in the treatment of early breast cancer and reported efficacy data according to *HER2* status. All trials had to fulfill the following criteria to be included in this meta-analysis: 1) the patients had to have been randomly assigned; 2) the trial had to be designed to compare anthracycline-based with non–anthracycline-based chemotherapeutic regimens in the adjuvant treatment of early breast cancer; and 3) hazard ratios for disease-free and overall survival according to *HER2* status either had to be reported or could be computed from the data presented. Studies in which hazard ratios for disease-free and/or overall survival according to *HER2* status were not reported or could not be computed from the data presented were excluded.

We searched for relevant trials 1) by performing computer-aided literature searches of the MEDLINE and CANCERLIT databases; 2) by examining the reference lists of published trials, review articles, and editorials on *HER2* status and efficacy of adjuvant anthracyclines; 3) by searching meetings abstracts; and 4) by consulting the US National Cancer Institute Physicians Data Query Clinical Protocol. For database searches, the following strategy was used: "Breast Neoplasms" [MeSH] AND "Chemotherapy, Adjuvant" [MeSH] AND ("Anthracyclines" [MeSH] OR "Anthracyclines/therapeutic use" [MeSH]) AND ("Receptor, erbB-2" [MeSH] OR "Genes, erbB-2" [MeSH]). The databases were searched for papers published through November 30, 2006.

Data Abstraction

The following data were abstracted from each study: the number of randomly assigned patients, the number of patients whose tumors were assessed for *HER2*, the description of treatment regimens, the year of publication or disclosure (for data presented as conference abstracts only), the outcome measures, and the treatment effect estimates. Hazard ratios (HRs) for breast cancer recurrence and all-cause mortality were extracted (along with 95% confidence intervals [CIs]) by using the non–anthracycline-based adjuvant chemotherapy arm as the reference group. All data were checked for internal consistency; principal investigators of the studies were contacted to retrieve missing information or to clarify inconsistencies.

Statistical Methods

The primary endpoints of this analysis were disease-free and overall survival. This meta-analysis was performed using a fixed-effects model. All trials reported *HER2*-specific hazard ratios with 95% confidence intervals. In the Belgian study (8), which compared two dose levels of epirubicin plus cyclophosphamide with cyclophosphamide, methotrexate, and 5-fluorouracil (CMF), the hazard ratios for the two epirubicin-containing arms vs CMF were pooled by inverse variance weighting. Log hazard ratios for disease-free and overall survival were pooled across the studies, both overall and according

Table 1. Characteristics of the eight studies included in the pooled analysis of anthracycline-based adjuvant chemotherapy vs non–anthracycline-based adjuvant chemotherapy for early breast cancer*

Study name or location (reference)	No. of randomly assigned patients	Study arms	Patient inclusion criteria
NSABP B11 (10)	682	PF vs PAF	Node positive, ER negative, and/or PgR negative
NSABP B15 (11)	2295	CMF vs AC	Node positive and age < 49 y if PgR positive or node positive and age ≤ 59 y if PgR negative
GUN 3 (12)	220	CMF vs CMF/EV	Node positive, stage II breast cancer, and premenopausal or node positive, postmenopausal, and ER negative or Stage III, either pre- or postmenopausal
Belgium (8)	777	CMF vs HEC/EC	Node positive, pre- or postmenopausal
Milan, Italy (13)	552	CMF vs CMF→A	Node positive (1–3 nodes)
DBCG 89D (9)	980	CMF vs FEC	Node negative, premenopausal, and grade II–III or node positive, premenopausal, and hormone receptor negative/unknown or node positive or tumor size > 5 cm, postmenopausal, and hormone receptor negative
GOIRC (14)	348	CMF vs E	Node negative and hormone receptor negative or node positive and pre- or postmenopausal
NCIC MA5 (15)	710	CMF vs CEF	Node positive and premenopausal

* NSABP = National Surgical Adjuvant Breast and Bowel Project; PF = L-phenylalanine mustard and 5-fluorouracil; PAF = L-phenylalanine mustard, 5-fluorouracil, and doxorubicin; ER = estrogen receptor; PgR = progesterone receptor; CMF = cyclophosphamide, methotrexate, and 5-fluorouracil; AC = doxorubicin, cyclophosphamide; GUN = Gruppo Universitario Napoletano; EV = epirubicin, vinblastine; HEC = high-dose epirubicin, cyclophosphamide; EC = epirubicin, cyclophosphamide; CMF→A = CMF followed by doxorubicin; DBCG = Danish Breast Cancer Cooperative Group; FEC = 5-fluorouracil, epirubicin, and cyclophosphamide; GOIRC = Gruppo Oncologico Italiano di Ricerca Clinica; E = epirubicin; NCIC = National Cancer Institute of Canada; CEF = cyclophosphamide, epirubicin and 5-fluorouracil.

to *HER2* status by inverse variance weighting. Formal tests for treatment by *HER2* status interaction were performed for each study, and the results of such tests were compared with those of interaction tests reported by the individual studies. A pooled test for treatment by *HER2* status interaction was performed using a weighted linear meta-regression of the log hazard ratio with *HER2* status as a binary covariate (positive/negative). Publication bias was assessed by visual inspection of funnel plots for study size against treatment effect (18) and with Egger's regression asymmetry test (19). Sensitivity analyses were performed to examine whether the strength of interaction between *HER2* status and the efficacy of adjuvant anthracycline treatment was associated with the method of *HER2* assessment, the type of anthracycline-based regimen used, or the proportion of patients who were assessed for *HER2* status. All analyses were conducted with the use of Stata statistical software (version 9, Stata Corp., College Station, TX). All *P* values were two-sided and the cutoff for statistical significance was .05.

Results

We identified nine randomized trials (8–15,20) that compared anthracycline-based with non–anthracycline-based regimens and that analyzed outcomes according to *HER2* status; seven trials (8–11,13–15) were published as full articles in peer-reviewed journals, and two (12,20) were published as conference abstracts. One of the trials published as a conference abstract (20) was excluded because it reported *P* values but no other data that would allow us to estimate the hazard ratio according to *HER2* status. Table 1 presents the characteristics of the eight trials included in the meta-analysis.

HER2 Status Assessment

The proportion of randomly assigned patients who had the *HER2* status of their breast tumors assessed ranged from 46% to 94% (Table 2). Overall, 5354 (82%) of the 6564 randomly assigned patients were assessed for *HER2* status. Among the patients whose *HER2* status was assessed, 1536 (29%) had tumors that overexpressed the HER2 protein or had amplification of the *HER2* gene. *HER2* status was evaluated by immunohistochemical assay alone in five studies (10–14); by fluorescence in situ hybridization alone or as a complement to immunohistochemical assay in two studies (8,9); and by immunohistochemical assay, fluorescence in situ hybridization, and the polymerase chain reaction in one study (15). Despite this heterogeneity, the prevalence of *HER2*-positive tumors was reasonably constant among the eight studies, ranging from 19% to 37%. Details about *HER2* testing and the scoring criteria from the individual studies are listed in Table 2.

Interaction Between *HER2* Status and Treatment Effect

Data on disease-free survival were available for six studies (8–11,13,15) No disease-free survival data were available in the Gruppo Universitario Napoletano 3 study (12). Methodologic issues concerning an inconsistency between overall survival and disease-free survival data prevented inclusion of disease-free survival data from the Gruppo Oncologico Italiano di Ricerca Clinica study (14) in the present analysis. Data on overall survival were reported in all studies except the Belgian study (8). Overall, compared with non–anthracycline-based adjuvant chemotherapy regimens, anthracycline-based adjuvant chemotherapy was associated with a statistically significant reduction in the risk of relapse (pooled HR = 0.90; 95% CI = 0.82 to 0.98; $P = .01$) and a borderline statistically significant reduction in the risk of death from any cause (pooled HR = 0.91; 95% CI = 0.79 to 1.04; $P = .056$) (Figs. 1 and 2). There was no statistically significant heterogeneity among the trials for either outcome variable.

Among patients with *HER2*-positive breast tumors, anthracycline-based adjuvant chemotherapy was associated with a marked reduction in the risks of relapse (pooled HR = 0.71, 95% CI = 0.61 to 0.83; $P < .001$) and death (pooled HR = 0.73, 95% CI = 0.62 to 0.85; $P < .001$). Conversely, among patients with *HER2*-negative breast tumors, there was no difference between those who received

Table 2. Assessment of *HER2* status in the eight studies included in the meta-analysis*

Study name or location, reference	*HER2* status		*HER2* assay method		
	No. of patients assessed/No. of patients randomly assigned (%)	No. of patients *HER2* positive/No. of patients assessed (%)	Central testing	Type of assay (antibody)	Scoring criteria for *HER2* positivity
NSABP B11[10]	638/682 (94%)	239/638 (37%)	Yes	IHC (cocktail)	Fishnet appearance†
NSABP B15[11]	2.034/2.295 (89%)	599/2.034 (29%)	Yes	IHC (cocktail)	Fishnet appearance†
GUN 3[12]	123/220 (56%)	30/123 (24%)	Not reported	IHC (MAB1)	Not reported
Belgium[8]	354/777 (46%)	73/354 (21%)	Yes	FISH	Staining intensity ratio > 2
Milan, Italy[13]	506/552 (92%)	95/506 (19%)	Yes	IHC (CB11)	Strong membrane labeling
DBCG 89D[9]	805/980 (82%)	246/805 (31%)	Yes	HercepTest and FISH if 2+‡	+3 staining intensity ratio > 2§
GOIRC[14]	266/348 (76%)	91/266 (34%)	Yes	IHC (CB11)	Strong membrane labeling in >50% stained cells
NCIC MA5[15]	28/710 (88%)	163/628 (26%)	Yes	FISH	Staining intensity ratio > 2

* NSABP = National Surgical Adjuvant Breast and Bowel Project; IHC = immunohistochemistry; GUN = Gruppo Universitario Napoletano; MAB 1 = monoclonal antibody 1; FISH = fluorescence in situ hybridization; CB11 = monoclonal antibody CB11; DBCG = Danish Breast Cancer Cooperative Group; GOIRC = Gruppo Oncologico Italiano di Ricerca Clinica; NCIC = National Cancer Institute of Canada.

† Positive if any tumor cell showed definite membrane staining.

‡ All *HER2* (2+) positive tumors underwent complementary FISH to investigate *HER2* gene amplification.

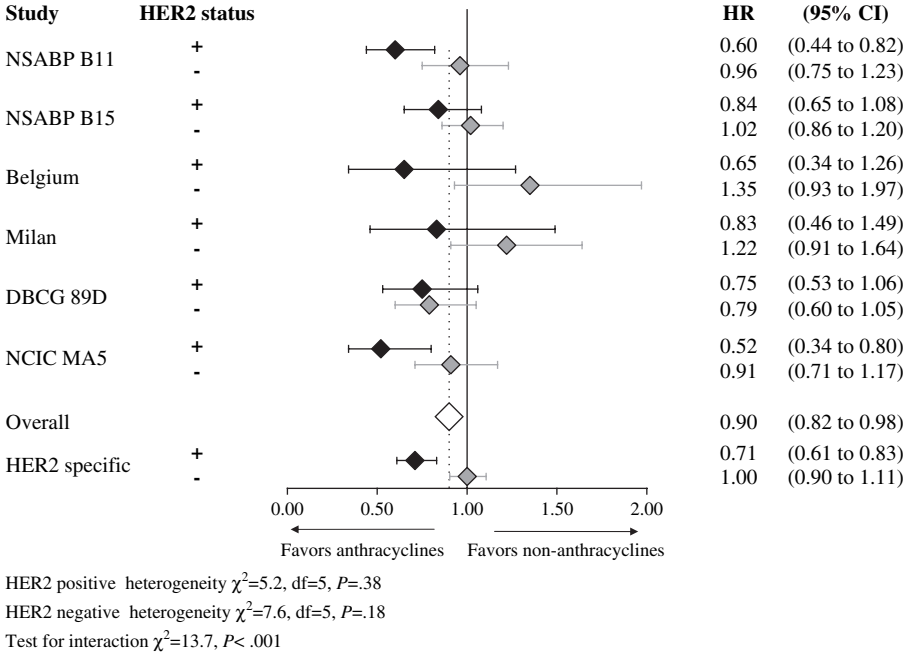
§ All *HER2* (3+) positive tumors and *HER2* (2+) positive tumors with *HER2* amplification investigated by FISH (fluorescence in situ hybridization; ≥ ratio 2) were considered to be *HER2* positive.

anthracycline-based adjuvant chemotherapy and those who received non–anthracycline-based adjuvant chemotherapy with respect to disease-free (pooled HR of relapse = 1.00, 95% CI = 0.90 to 1.11; $P = .75$) or overall (pooled HR of death from any cause = 1.03, 95% CI = 0.92 to 1.16; $P = .60$) survival (Figs. 1 and 2). The test for treatment by *HER2* status interaction yielded statistically significant results: for disease-free survival, the chi-square statistic for interaction was 13.7 ($P < .001$), and for overall survival it was 12.6 ($P < .001$), allowing us to reject the null hypothesis that the effect of adjuvant anthracyclines is independent of *HER2* status (Table 3).

Neither a visual assessment of funnel plots nor formal statistical tests (Egger's test for bias $P > .3$; Begg's rank correlation test $P > .5$) indicated the existence of publication bias, suggesting that studies in which anthracyclines showed a differential effect on disease-free and/or overall survival according to *HER2* status were not more or less likely to be published than those that did not show such an effect.

Sensitivity Analyses

To examine whether the overall interaction observed between *HER2* status and the use of anthracyclines was due to an effect that was present only in some groups of trials, we conducted sensitivity analyses in which patients were grouped according to the assessment method used to determine the *HER2* status of their tumors (immunohistochemistry vs fluorescence in situ hybridization), the



HER2 positive heterogeneity χ^2=5.2, df=5, P=.38

HER2 negative heterogeneity χ^2=7.6, df=5, P=.18

Test for interaction χ^2=13.7, P< .001

Fig. 1. Disease-free survival. Hazard ratios (HR, **diamonds**) and 95% confidence intervals (CIs, **horizontal bars**) of anthracycline-based vs non–anthracycline-based regimens in *HER2*-positive (**black diamonds**) and *HER2*-negative (**gray diamonds**) patients. Hazard ratios and their 95% confidence intervals (CIs) were abstracted from each study and combined to obtain a pooled estimate (**white diamond, dotted line**) of the effect of anthracycline-based regimens. Chi-square (χ^2) test statistics were used to test for the presence of heterogeneity across studies both in *HER2*-positive and in *HER2*-negative patients. A pooled test for treatment by *HER2* status interaction was performed using an inverse variance weighted linear meta-regression; the χ^2 statistic assessing the interaction was 13.7, $P < .001$. NSABP = National Surgical Adjuvant Breast and Bowel Project; DBCG = Danish Breast Cancer Cooperative Group; NCIC = National Cancer Institute of Canada.

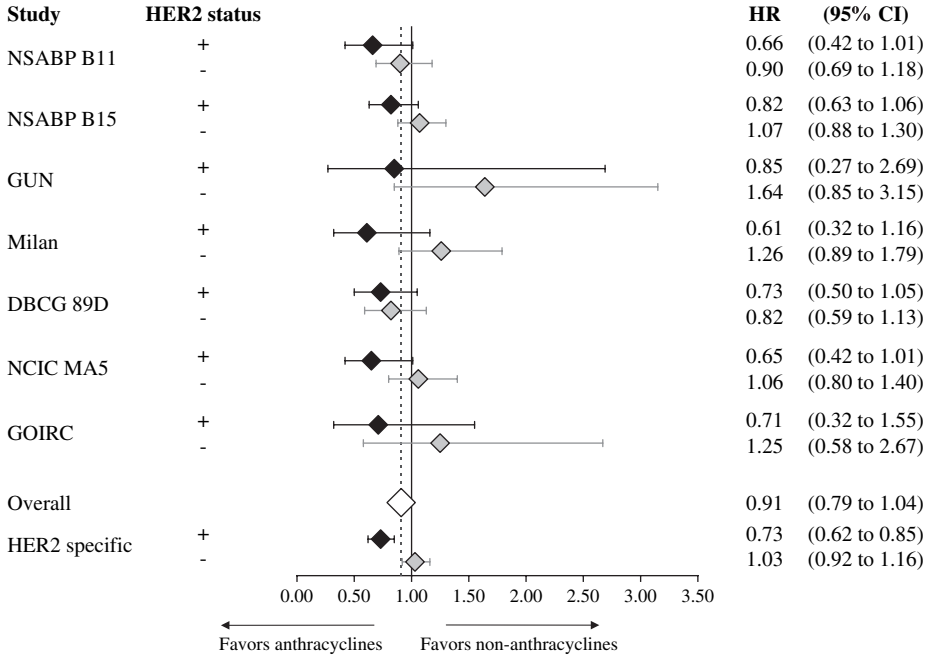


Fig. 2. Overall survival. Hazard ratios (HR, **diamonds**) and 95% confidence intervals (CIs, **horizontal bars**) of anthracycline-based vs non–anthracycline-based regimens in *HER2*-positive (**black diamonds**) and *HER2*-negative (**gray diamonds**) patients. Hazard ratios and their 95% confidence intervals were abstracted from each study and combined to obtain a pooled estimate (**white diamond, dotted line**) of the effect of anthracycline-based regimens. Chi-square (χ^2) test statistics were used to test for the presence of heterogeneity across studies both in *HER2*-positive and in *HER2*-negative patients. A pooled test for treatment by *HER2* status interaction was performed using an inverse variance weighted linear meta-regression; the χ^2 statistic assessing the interaction was 12.6, $P < .001$. NSABP = National Surgical Adjuvant Breast and Bowel Project; GUN = Gruppo Universitario Napoletano; DBCG = Danish Breast Cancer Cooperative Group; NCIC = National Cancer Institute of Canada.

type of anthracycline-based regimen they received (doxorubicin vs epirubicin), and the proportion that was assessed for *HER2* status (>60% vs ≤60%). The latter cutpoint was chosen because all but two studies had large proportions (ie, >75%) of patients assessed for *HER2* status. The magnitudes of the differential effects of anthracyclines in *HER2*-positive patients and in *HER2*-negative patients were remarkably similar across groups of trials (Fig. 3), suggesting that the interaction between anthracycline use and *HER2* status is independent of the type of *HER2* assay, the proportion of patients assessed for *HER2* status, and the type of anthracycline.

Discussion

We conducted this meta-analysis to examine whether anthracycline-based adjuvant chemotherapy would be more efficacious than non–anthracycline-based adjuvant chemotherapy in *HER2*-positive women but not in *HER2*-negative women. The pooled estimates of the benefits associated with anthracyclines in all patients included in this meta-analysis are consistent with those reported by the EBCTCG systematic overview (1) both in terms of disease-free survival (HR of relapse = 0.90 in our analysis vs 0.89 in the EBCTCG overview) and overall survival (HR of death from any cause = 0.91 vs 0.84, respectively). Our results confirm that the added benefit of adjuvant chemotherapy with anthracyclines is confined to women who have breast tumors in which *HER2* is overexpressed or amplified. Most of the studies included in this analysis showed this association (8,9,11–14), and two studies (10,15) reported a statistically significant interaction between *HER2* status and adjuvant anthracyclines for disease-free survival. The statistically significant interaction for both disease-free and overall survival revealed by our meta-analysis provides convincing statistical evidence of a quantitative interaction between *HER2* status and responsiveness to adjuvant anthracyclines.

Our study has several limitations that should be considered when interpreting the data. First, as with all meta-analyses, our findings and interpretations are limited by the quality and quantity of available evidence (ie, trials) on the effects of adjuvant anthracyclines according to *HER2* status. Second, we used published summary results rather than individual patient data, and it was not possible to check the original data and analyses, to undertake additional evaluations, or to perform a centralized reassessment of participants' *HER2* status. Third, a number of unpublished studies might exist, which could lead to possible publication bias if studies that did not show an interaction between *HER2* status and treatment with anthracyclines were less likely to be published. However,

Table 3. Test for treatment by *HER2* status interaction*

Study name or location, reference	Disease-free survival		Overall survival	
	χ^2	*P*	χ^2	*P*
NSABP B11[10]	5.4	.02	2.0	.15
NSABP B15[11]	1.7	.19	2.6	.11
GUN 3[12]	–	–	0.9	.33
Belgium[8]	3.7	.06	–	–
Milan, Italy[13]	1.3	.25	3.8	.05
DBCG 89D[9]	0.05	.82	0.2	.63
GOIRC[14]	–	–	1.00	.32
NCIC MA5[15]	5.0	.02	3.6	.07
Overall	13.2	<.001	12.2	<.001

* NSABP = National Surgical Adjuvant Breast and Bowel Project; GUN = Gruppo Universitario Napoletano; DBCG = Danish Breast Cancer Cooperative Group; GOIRC = Gruppo Oncologico Italiano di Ricerca Clinica; NCIC = National Cancer Institute of Canada; – = not available.

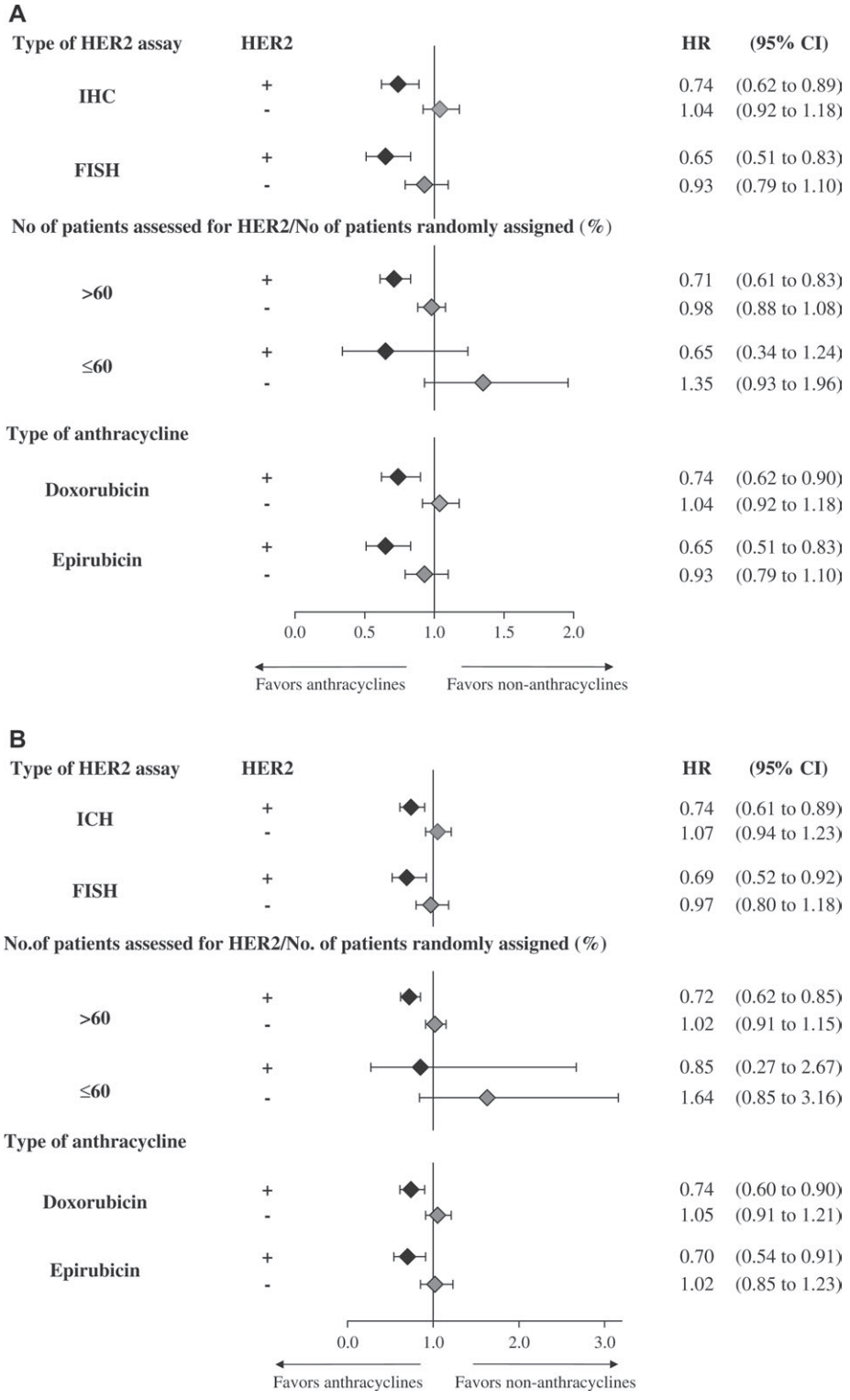


Fig. 3. Sensitivity analyses. **A**) Disease-free survival. **B**) Overall survival. Pooled hazard ratios (HRs, **diamonds**) and 95% confidence intervals (CIs, **horizontal bars**) of anthracycline-based vs non–anthracycline-based regimens for *HER2*-positive (**black diamonds**) and *HER2*-negative (**shaded diamonds**) patients in each of the different subgroups examined. IHC = immunohistochemistry; FISH = fluorescent in situ hybridization.

we found no indication of publication bias by using statistical methods that are designed to detect it. A fourth limitation of our meta-analysis is the variability in methods used to assess *HER2* status. However, a sensitivity analysis for methods of *HER2* assessment (immunohistochemistry vs fluorescence in situ hybridization) showed no substantial differences in the degree of interaction between *HER2* status and the efficacy of adjuvant anthracyclines according to type of *HER2* assay.

Further support for our results comes from studies that have compared different dose intensities of adjuvant anthracyclines (8,21,22). These studies have shown that the benefit of more intensive anthracycline-based regimens is limited to *HER2*-positive patients.

Interpretation of our results must also take into account that *HER2* is not the true anthracycline molecular target and that changes in expression of the topoisomerase II alpha gene or protein may be a more accurate and biologically related predictive

factor of response to anthracyclines. Indeed, several studies have suggested that the added benefit of adjuvant anthracyclines may be restricted to the small proportion of patients whose tumors have amplification of or deletions in the topoisomerase II alpha gene itself or in topoisomerase II alpha protein expression (8,9,23). Furthermore, topoisomerase II alpha protein levels are regulated by gene amplification and by the tumor proliferation rate (24), which suggests that anthracycline superiority may also be seen in the subset of *HER2*-negative tumors that overexpress topoisomerase II alpha protein. Clarification of whether topoisomerase II alpha status rather than *HER2* status is actually predictive of increased sensitivity to anthracyclines will require a meta-analysis of individual patient data from randomized clinical trials that have compared adjuvant anthracyclines with non–anthracycline-based regimens and that include patients for whom both the *HER2* status and the topoisomerase II alpha status are known.

In conclusion, our results suggest that *HER2* status is a predictor of responsiveness to adjuvant anthracycline therapy for early breast cancer. The absence, in our study, of any effect of anthracyclines observed in patients with *HER2*-negative disease suggests that this group of patients could be spared unnecessary toxic effects related to the use of this class of agents and raises questions as to the appropriateness of control arms in randomized clinical trials in which anthracycline-based regimens are used in unselected patient populations.

References

1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* 2005;365:1687–1717.
2. Fisher B, Brown AM, Dimitrov NV, et al. Two months of doxorubicin-cyclophosphamide with and without interval reinduction therapy compared with 6 months of cyclophosphamide, methotrexate and fluorouracil in positive-node breast cancer patients with tamoxifen non responsive tumours: results from the National Surgical Adjuvant Breast and Bowel Project B-15. *J Clin Oncol.* 1990;8:1483–1496.
3. Fisher B, Anderson S, Tan-Chiu E, et al. Tamoxifen and chemotherapy for axillary node-negative, estrogen receptor-negative breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-23. *J Clin Oncol.* 2001;19:931–942.
4. Piccart MJ, Di Leo A, Beauduin M, et al. Phase III trial comparing two dose levels of epirubicin combined with cyclophosphamide with cyclophosphamide, methotrexate, and fluorouracil in node-positive breast cancer. *J Clin Oncol.* 2001;19:3103–3110.
5. Bang SM, Heo DS, Lee KH, et al. Adjuvant doxorubicin and cyclophosphamide versus cyclophosphamide, methotrexate, and 5-fluorouracil chemotherapy in premenopausal women with axillary lymph node positive breast carcinoma. *Cancer.* 2000;89:2521–2526.
6. Colozza M, Bisagni G, Mosconi AM, et al. Epirubicin versus CMF as adjuvant therapy for stage I and II breast cancer: a prospective randomised study. *Eur J Cancer.* 2002;38:2279–2288.
7. Moliterni A, Bonadonna G, Valagussa P, Ferrari L, Zambetti M. Cyclophosphamide, methotrexate and fluorouracil with and without doxorubicin in the adjuvant treatment of resectable breast cancer with one to three positive axillary nodes. *J Clin Oncol.* 1991;9:1124–1130.
8. Di Leo A, Gancberg D, Larsimont D, et al. HER-2 amplification and topoisomerase II alpha gene aberrations as predictive markers in node-positive breast cancer patients randomly treated either with an anthracycline-based therapy or with cyclophosphamide, methotrexate, and 5-fluorouracil. *Clin Cancer Res.* 2002;8:1107–1116.
9. Knoop AS, Knudsen H, Balslev E, et al. Retrospective analysis of topoisomerase IIa amplifications and deletions as predictive markers in primary breast cancer patients randomly assigned to cyclophosphamide, methotrexate, and fluorouracil or cyclophosphamide, epirubicin, and fluorouracil. *J Clin Oncol.* 2005;23:7483–7490.
10. Paik S, Bryant J, Park C, et al. ErbB-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. *J Natl Cancer Inst.* 1998;90:1361–1370.
11. Paik S, Bryant J, Tan-Chiu E, et al. HER2 and choice of adjuvant chemotherapy for invasive breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-15. *J Natl Cancer Inst.* 2000;92:1991–1998.
12. De Laurentiis M, Caputo F, Massarelli E, Forestieri V, Vernaglia A, Carlomagno C. HER2 expression and anthracycline effect: results from the Naple GUN 3 randomized trial. *Proc Am Soc Clin Oncol.* 2001;20: A133.
13. Moliterni A, Menard S, Valagussa P, et al. HER2 overexpression and doxorubicin in adjuvant chemotherapy for resectable breast cancer. *J Clin Oncol.* 2003;21:458–462.
14. Colozza M, Sidoni A, Mosconi AM, et al. HER2 overexpression as a predictive marker in a randomized trial comparing adjuvant cyclophosphamide/methotrexate/5-fluorouracil with epirubicin in patients with stage I/II breast cancer: long-term results. *Clin Breast Cancer.* 2005;6:253–259.
15. Pritchard KI, Shepherd LE, O'Malley FP, et al. HER2 and responsiveness of breast cancer to adjuvant chemotherapy. *N Engl J Med.* 2006;354: 2103–2111.
16. Jarvinen TAH, Tanner M, Rantanen V, et al. Amplification and deletion of topoisomerase IIa associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. *Am J Pathol.* 2000;156:839–847.
17. Oxman AD, Guyatt GH. A consumers' guide to subgroup analyses. *Ann Intern Med.* 1992;116:78–84.
18. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics.* 1994;50:1088–1101.
19. Egger M, Smith GD, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315:629–634.
20. Vera R, Albanell J, Lirola JL, Bermejo B, Sole LA, Baselga J. HER2 overexpression as a predictor of survival in a trial comparing adjuvant FAC and CMF in breast cancer. *Proc Am Soc Clin Oncol.* 1999;18:A265.
21. Del Mastro L, Bruzzi P, Nicolo G, et al. HER2 expression and efficacy of dose-dense anthracycline-containing adjuvant chemotherapy in breast cancer patients. *Br J Cancer.* 2005;93:7–14.
22. Muss HB, Thor AD, Berry DA, et al. C-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. *N Engl J Med.* 1994;330:1260–1266.
23. Di Leo A, Claudino WM, Pestrin M, Licitra S, Biganzoli L. Using specific cytotoxics with a targeted mind. *Breast.* 2007;16:S120–S126.
24. Jarvinen TA, Kononen J, Pelto-Huikko M, Isola J. Expression of topoisomerase IIa is associated with rapid cell proliferation, aneuploidy, and c-erb B-2 overexpression in breast cancer. *Am J Pathol.* 1996; 148:2073–2082.

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