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ORIGINAL REPORT

Topoisomerase IIα Amplification Does Not Predict Benefit From Dose-Intense Cyclophosphamide, Doxorubicin, and Fluorouracil Therapy in *HER2*-Amplified Early Breast Cancer: Results of CALGB 8541/150013

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ABSTRACT

Purpose

We have demonstrated that patients with *HER2*-amplified tumors derive more benefit from higher doses of doxorubicin-containing chemotherapy (cyclophosphamide, doxorubicin, and fluorouracil [CAF]). Because topoisomerase IIα (Topo-IIα) is a target for doxorubicin and is coamplified in 20% to 50% of *HER2*-amplified tumors, we postulated that Topo-IIα copy number might account for the benefit from CAF dose escalation in *HER2*-positive tumors. To address this hypothesis, we examined Topo-IIα and *HER2* copy number, CAF dose, and clinical outcomes in Cancer and Leukemia Group B (CALGB) 8541.

Patients and Methods

Topo-IIα and *HER2* copy number were measured by fluorescent in situ hybridization (FISH) using a triple-probe system, which includes Topo-IIα, *HER2*, and chromosome 17 (CEP17). Topo-IIα and/or *HER2* were classified as amplified (≥ two copies/CEP17, deleted (≤ 0.67 copies/CEP17) and normal copy number (> .67 to < 2.0 copies/CEP17).

Results

Topo-IIα/*HER2*/CEP17 measurement was successful in 624 of 687 cases. *HER2* was amplified in 117 cases (19%). Topo-IIα was amplified in 41 cases (7%) and deleted in 69 cases (11%). Topo-IIα amplification was highly correlated with *HER2* amplification (39 of 41; $P < .0001$), *HER2* by immunohistochemistry, and by dual-probe FISH. Topo-IIα was deleted in both the *HER2*-amplified (30 of 69; 43%), normal (22 of 69; 32%) and *HER2*-deleted tumors (17 of 69; 25%). Although Topo-IIα–amplified tumors were nearly always *HER2* amplified, these tumors did not receive benefit from increasing the dose of CAF ($P = .15$).

Conclusion

The correlative companion study CALGB 8541-150013 does not support the hypothesis that Topo-IIα amplification is the mechanism behind benefit from increased dose of anthracyclines in *HER2*-positive breast cancer.

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INTRODUCTION

The use of chemotherapy to treat early-stage breast cancer is proven to extend survival[1]; however, the best chemotherapeutic regimen has not been determined. Although doxorubicin-containing regimens are standard for higher-risk patients, they tend to have higher toxicity, in particular, a 1% to 3% risk of congestive heart failure in patients who receive doses of more than 300 mg/m^2.[2] In addition, newer nonanthracycline treatment options are available; however, methods to select which patients are most likely to benefit from anthracyclines are not.[3,4] Better understanding of the predictive value of certain biologic markers should allow us to identify chemotherapy regimens that are most likely to be effective with the least amount of toxicity.

The *HER2* or *ErbB2* oncogene is overexpressed in approximately 25% to 30% of human breast cancer specimens and is associated with a worse outcome in many studies.[5,6] Accumulating evidence has shown that *HER2* expression is associated with improved outcome after doxorubicin-based therapy.[7,8] The Cancer and Leukemia Group B (CALGB) has retrospectively evaluated *HER2* expression using various methods and has shown that patients with

tumors that overexpress *HER2* who received high-dose cyclophosphamide, doxorubicin, and fluorouracil (CAF) had a better outcome compared with patients with *HER2*-positive tumors who received moderate or low-dose CAF.[7,9] In National Surgical Adjuvant Breast and Bowel Project B11, patients with estrogen receptor (ER) or progesterone receptor (PR) –negative disease were randomly assigned between melphalan and fluorouracil versus melphalan, doxorubicin, and fluorouracil.[10] Only patients whose tumors were *HER2* positive by immunohistochemistry were found to benefit from the addition of doxorubicin (hazard ratio = 0.60; 95% CI, 0.47 to 0.92). Pritchard et al[11] evaluated the role of *HER2*, measured by fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC) in premenopausal women with node-positive breast cancer who had received either adjuvant cyclophosphamide, epirubicin, and fluorouracil (CEF) or cyclophosphamide, methotrexate, and fluorouracil (CMF). In patients whose tumors showed amplification of *HER2*, CEF was superior to CMF on the basis of relapse-free survival, whereas tumors that lacked amplification of *HER2* saw no such incremental benefit.

Topoisomerase IIα (Topo-IIα) is a DNA-modifying enzyme that binds to the double helix to release torsional stress and create double-strand breaks that allow replication to occur. Drugs that interfere with Topo-IIα include the anthracyclines (doxorubicin, daunorubicin), etoposide, teniposide, and amsacrine. These agents act by binding covalently with Topo-IIα after double-strand breaks have occurred, inducing lethal cellular damage by inhibition of religation. Increase in Topo-IIα expression is associated with sensitivity to these agents, both in cell lines and tumors, as a result of increased substrate on which the drug may act.[12,13] Topo-IIα occurs in the same amplicon on chromosome 17 (CEP17) as *HER2*, and Jarvinen et al[14] have shown in breast cancer cell lines and primary tumors that amplification or deletion of the Topo-IIα gene is frequent in *HER2* tumors. Of 57 *HER2*-amplified primary breast carcinomas, 25 (44%) showed *ErbB2*-topoIIα coamplification, and 24 (42%) showed a physical deletion of the Topo-II α gene. These studies showed a consistent relationship between deletion of the gene and decreased protein expression.[15]

Our previous work has shown that activation of the *HER* family of receptors is associated with upregulation of Topo-IIα and increase in sensitivity to doxorubicin.[16] This suggests an alternative mechanism of sensitivity to anthracyclines, which involves receptor activation rather than coamplification. In an attempt to understand the mechanism of this observation, we evaluated Topo-IIα activity on activation of the *HER2* receptor using a chimeric receptor model.[17] We showed that the increase in sensitivity to doxorubicin can be explained by increased enzymatic activity of Topo-IIα as measured by decatenating and cleavage assays.[18] In addition, this effect could be reversed by the anti-*HER2* antibody, trastuzumab, in breast cancer cells that overexpress *HER2* (BT474). Chimeric cells were also more sensitive to etoposide; therefore, it is likely that doxorubicin sensitivity is mediated by Topo-IIα.

Between January 1985 and May 1991, CALGB 8541 randomly assigned patients with node-positive breast cancer to three treatment arms evaluating different dose and scheduling of CAF: arm 1 (low-dose), 300 mg/m^2 of cyclophosphamide, 30 mg/m^2 of doxorubicin, and 300 mg/m^2 of fluorouracil every 28 days for four cycles; arm 2 (moderate dose), 400 mg/m^2 of cyclophosphamide, 40 mg/m^2 of doxorubicin, and 400 mg/m^2 of fluorouracil every 28 days for six cycles, and arm 3, 600 mg/m^2 of cyclophosphamide, 60 mg/m^2 of doxorubicin, and 600 mg/m^2 of fluorouracil every 28 days for four cycles.[19] At a median follow-up of 9 years, disease-free survival (DFS) and overall survival (OS) for patients on the moderate- and high-dose arms are superior to the corresponding survival measures for patients on the low-dose arm (two-sided $P < .0001$ and two-sided $P = .004$, respectively), with no difference in DFS or OS between the moderate- and the high-dose arms. This study attempted to address whether Topo-IIα amplification is responsible for the benefit seen with increasing the dose of anthracycline in CALGB 8541.

PATIENTS AND METHODS

Patients

A subgroup of patients registered to CALGB 8541 who received adjuvant doxorubicin and whose tumors had been previously evaluated for *HER2* by PathVysion FISH (Abbott Molecular; Abbott Park, IL) were included in this retrospective study, provided that sufficient invasive cancer remained in the block to provide representative sections of the primary tumor for assay. Six hundred eighty-seven patient samples matching these criteria were available from the CALGB Pathology Coordinating Office. Topo-IIα/*HER2*/CEP17 measurement was successful in 624 (91%) of 687 available cases. There was no significant difference in patient characteristics between the 624 patients assessable for Topo-IIα compared with the entire 1,572 patients registered to CALGB 8541. Age, tumor size, and number of positive nodes were compared using the Wilcoxon Rank-Sum test. The χ^2 test was used to test for differences in menopausal status, receptor status, and the three dose levels of CAF (Table 1). There was no significant difference in patient characteristics between patients assessable for Topo-IIα across the three CAF dose arms. Age, tumor size, and number of positive nodes were compared using the Wilcoxon rank-sum test. The χ^2 test was used to test for differences in menopausal status, receptor status, and the three dose levels of CAF (Table 2).

As only existing pathologic specimens were studied that were not identifiable to the investigators on this study, additional informed consent was not required. Samples provided to laboratory investigators were

Table 1. Patient Characteristics

Characteristic	Patients With Topoisomerase IIα Assessment (n = 624)	All Treated Patients (n = 1572)	*P* (assessed to all treated)
Age, years			
Median	50	49	.13
Range	22-77	22-81	
Tumor size, cm			
Median	2.6	2.6	.26
Range	0.3-12.0	0.1-12.0	
No. of positive nodes			
Median	3.0	3.0	.17
Range	1-54	1-54	
Premenopausal, %	39.7	43	.12
Receptor positive, %	72.3	72	.86
Dose of CAF, %			
Low	32	33	
Moderate	33	33	.82
High	35	34	

NOTE. Age, tumor size and No. of positive nodes were compared using the Wilcoxon rank–sum test. The χ^2 test was used to test for differences in percentage premenopausal, receptor positive, and in the three dose levels of CAF.
Abbreviation: CAF, cyclophosphamide, doxorubicin, and fluorouracil.

Table 2. Dose of CAF Effect in the Analyzed Subset (n = 624)

Characteristic	Dose of CAF			P
	Low	Moderate	High	
Age, years				
Median	51	50	49	.62
Range	22-76	26-75	29-77	
Tumor size, cm				
Median	3.0	3.0	2.5	.24
Range	0.4-11.5	0.5-12.0	0.3-9.5	
No. of positive nodes				
Median	3.0	3.0	3.0	.83
Range	1-29	1-54	1-38	
Premenopausal, %	39	43	38	.58
Receptor positive, %	74	70	73	.64

NOTE. Age, tumor size, and No. of positive nodes were compared using the Kruskal–Wallis test. The χ^2 test was used to test for differences in percentage premenopausal and receptor positive.
Abbreviation: CAF, cyclophosphamide, doxorubicin, and fluorouracil.

stripped of identifiers, and relinking of clinical outcome with laboratory findings was performed at the CALGB Statistical Center. This study was reviewed and approved by the institutional review board at the institutions where the laboratory work was performed.

HER2 by CB11 immunohistochemistry has been previously performed and reported. PathVysion FISH for *HER2*[10] and S-phase by flow cytometry have been previously reported.[20-22]

FISH for HER2 *and Topo-IIα*

FISH for *HER2* and Topo-IIα was performed using the Vysis LSI FISH Probe, Topo-IIα(17.21-22)/HER2(17q11.2-q12/)CEP17(17p11.1-q11.1) Tricolor Probe in the laboratory of L.D. The system is similar to that used for the VYSIS PathVysion kit for *HER2*, which is approved by the United states Food and Drug Administration. We used the VP2000 automated tissue processor and HYBRITE denaturation hybridization system from Abbott Molecular for all assays.[23] Acid pretreatment and protease digestion to breakdown formaldehyde cross-links was performed (Vysis Paraffin pretreatment kit), followed by sodium chloride-sodium citrate (SSC) and formamide denaturation (72°C, 5 minutes). After dehydration, the *HER2*/CEP17/Topo-IIα probe cocktail was added, and coverslips were applied and sealed with rubber cement. Slides were incubated in a humidity chamber overnight for 18 hours at 37°C. On the following day, slides were washed in a stringency buffer (SSC, NP40), dried on a slide warming tray and incubated with 4'-6-diamidino-2-phenylindole (DAPI) for nuclear identification. Slides were stored in the dark, at −20°C. Nonamplified and amplified control slides (fixed cell lines embedded in paraffin, MDA-MB-231 and Hs578T, respectively) were analyzed with each assay, provided in the Vysis kit. Twenty nuclei were scored individually for Topo-IIα, *HER2*, and CEP17 probes. By convention, Topo-IIα and/or *HER2* were considered amplified when the ratio of Topo-IIα/CEP17 signals or *HER2*/CEP17 signals was ≥ 2.00. A case was considered deleted when the ratio of signals was ≤ 0.67. Cases with signal ratios between 0.67 and 2.00 were considered normal copy number in this study.[15]

Statistical Methods

All FISH data were submitted to the CALGB Statistical Center as a continuous variable for analysis. Statistical analyses were performed by CALGB statisticians using SAS 9.1 (SAS Institute, Cary, NC). We used a multivariate Cox proportional hazards regression model to test the interaction of treatment arm and Topo-IIα while adjusting for important covariates. The relationship between Topo-IIα amplification with *HER2* amplification by FISH was determined by contingency tables and test for association with the χ^2 test. The χ^2 test was also used to test for associations between Topo-IIα amplification and deletion with ER/PR status. The Kruskal-Wallis test was used to evaluate for differences in the mean rank values for *HER2* IHC, FISH ratio, S phase, and ER-positive status in the three Topo-IIα categories (amplified, deleted, and normal). Kaplan-Meier estimates were used to graphically display outcomes for DFS and OS by dose (low, moderate, high) and by marker/method for amplified versus nonamplified *HER2* or Topo-IIα amplified/deleted/diploid.

RESULTS

We performed FISH for *HER2*, Topo-IIα, and CEP17 using the triple-probe system on 687 of 687 available cases. Topo-IIα/*HER2*/CEP17 measurement was successful in 624 (91%) of 687 available cases. *HER2* was amplified in 117 cases (19%) and deleted in 18 cases (3%). Topo-IIα was amplified in 41 cases (7%) and deleted in 69 cases (11%). A comparison of *HER2* FISH status in CALGB 8869 using PathVysion compared with *HER2* FISH using tricolor system (Vysis) in the CALGB 9344 study showed the sensitivity and specificity of 95% and 97% for Topo-IIα by triple probe and for Topo-IIα by dual probe (L.D.), respectivelyl. The Pearson correlation coefficient of FISH dual probe to tricolor probe is 0.88 ($P < .001$).

Topo-IIα was deleted in both the *HER2*-amplified (30 of 69; 43%), normal (22 of 69; 32%), and *HER2*-deleted tumors (17 of 69; 25%). A scatterplot of *HER2* versus Topo-IIα copy number reveals a biphasic relationship between these two amplicons, with *HER2*-amplified tumors either showing coamplification of Topo-IIα or reduction in copy number of Topo-IIα (median, 0.83) compared with *HER2* nonamplified tumor samples (Fig 1). Of note, Topo-IIα was never amplified in more than five copies per cell, despite high levels of *HER2* amplification of up to 14 copies: CEP17.

Topo-IIα amplification was highly correlated with *HER2* amplification (39 of 41; $P < .0001$), *HER2* by IHC (CB11; $P < .0001$), and by dual-probe *FISH* ($P < .0001$; Table 3). The median S phase in the

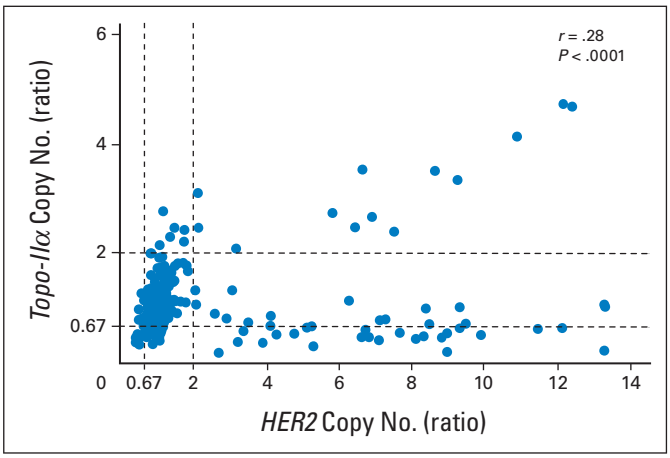

Fig 1. Topoisomerase II α copy number versus HER2 copy number. Topoisomerase IIα and HER2 copy number was determined using Vysis Triple Probe kit (HER2, TOP2A, CEP17). The topoisomerase IIα ratio was measured by counting the number of TOP2A signals/nucleus in a minimum of 20 nuclei over the number of CEP17 signals/nucleus in a minimum of 20 nuclei. The HER2 ratio was measured by counting the number of HER2 signals/nucleus in a minimum of 20 nuclei over the number of CEP17 signals/nucleus in a minimum of 20 nuclei. Topoisomerase IIα ratio (Y-axis) is plotted against HER2 ratio (X-axis) and the correlation coefficient calculated using SAS 9.1.

Table 3. Association of Topo–IIα Amplification With Disease and Treatment Variables

Variable	Topo–IIα Amplified	Topo–IIα Deleted	Topo–IIα Normal	*P*
HER2 IHC 3+, No. of cases	90	50	1.0	< .0001
FISH, ratio	4.5	1.11	1.05	< .0001
S phase, % nuclei	17.5	14	12	.12
ER, % nuclei	55	61	67	.20

NOTE. The values displayed for *HER2* IHC, FISH ratio, and S phase are medians and for ER the percentage positive. Differences in the mean ranks or proportions are compared using the Kruskal–Wallis test.
Abbreviations: Topo–IIα, topoisomerase IIα; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; ER, estrogen receptor.

Topo-IIα–nonamplified group was 12, whereas it was 17.5 in the Topo-IIα–amplified group ($P = .066$). Topo-IIα amplification did not show a statistically significant association with ER and/or PR negativity ($P = .15$). Topo-IIα deletion was also correlated with *HER2* amplification ($P < .0001$) and overexpression ($P < .0001$), but not with ER/PR or S phase.

To determine whether *HER2* amplification remained a predictor of benefit from anthracycline in this subset of patients evaluated by the triple probe, we performed an interaction analysis of *HER2* ratio with CAF dose. *HER2*-amplified tumors treated with moderate- and higher-dose CAF regimens had an improved DFS and OS compared with those treated with the low-dose CAF regimen in an unadjusted analysis ($P = .0032$). Although the trend was similar, the interaction between *HER2* and CAF in the Cox proportional hazards model adjusted for CAF dose, number of positive nodes, tumor size, and menopausal status was of borderline significance ($P = .079$; Fig 2).

The benefit of CAF dose in Topo-IIα–amplified tumors was then evaluated. Despite the fact that *HER2* amplification suggested benefit from CAF dose escalation, Topo-IIα amplification did not account for this benefit, as no interaction of CAF dose was seen in Topo-IIα–amplified cases. An unplanned analysis of Topo-IIα copy number ≤ 0.67 seemed to show more benefit from an increased dose of CAF in the Kaplan-Meier plot. However, the interaction of Topo-IIα and dose of CAF was not statistically significant in the Cox proportional hazards multivariate model. Similarly, Topo-IIα normal cases showed no benefit from increasing CAF dose (Fig 3).

Multivariate Cox proportional hazards models with either DFS or OS as the dependent variable and other independent variables (CAF treatment arm, number of positive nodes [square root transformed], tumor size [≤ 2 *v* > 2 cm], menopausal status [pre *v* peri/post], Topo-IIα [deleted *v* not], and the interaction of Topo-IIα and CAF treatment assignment) resulted in nonstatistically significant interaction (*P* values of .24 and .21, respectively). No interaction with dose was observed in the *HER2* normal/deleted group or Topo-IIα normal/deleted groups.

In view of the biologic connection between *HER2* and Topo-IIα and the putative association between Topo-IIα and anthracyclines, we evaluated whether Topo-IIα contributed to the benefit from escalating doses of CAF in the *HER2*-amplified cases. In a multivariate Cox proportional hazards model adjusting for CAF dose, number of nodes, tumor size, and menopausal status, *HER2* amplification predicted benefit from CAF dose escalation, but in the current analysis, Topo-IIα amplification with or without *HER2* amplification did not account for this benefit (Table 4).

DISCUSSION

The mechanism by which patients with *HER2*-amplified tumors benefit more from anthracyclines is unknown. We attempted to address one hypothesis, that Topo-IIα amplification was responsible for this effect, with negative findings. It is particularly notable that *HER2*-amplified tumors continued to show benefit from increasing doses of CAF in this subset tested with the *HER2*, CEP17, Topo-IIα triple probe, yet Topo-IIα amplification did not stratify the patients in this way. This suggests that an alternative mechanism to coamplification should be considered and perhaps lies in the fact that Topo-IIα gene expression is not well represented by copy number as measured by FISH.

A number of retrospective studies have evaluated the role of Topo-IIα amplification as a predictive marker of response to anthracyclines in breast cancer.[26,26a,26b] Di Leo et al[27] evaluated *HER2* and Topo-IIα gene aberrations by FISH in a series of 430 primary breast cancer samples of patients with node-positive breast cancer treated with one of two epirubicin-containing regimens versus CMF. In this study, Topo-IIα evaluation suggested that the superiority of anthracyclines over CMF in *HER2*-amplified patients could be confined to the subgroup of Topo-IIα–amplified tumors. In contrast, Knoop et al[28] retrospectively identified and analyzed tumor tissue for *HER2* positivity and for Topo-IIα amplification and deletion from 805 of

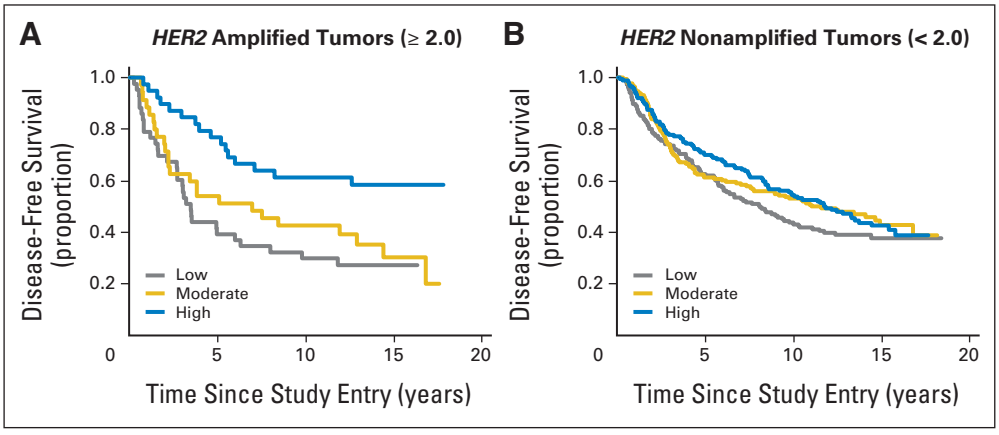

Fig 2. Disease-free survival by cyclophosphamide, doxorubicin, and fluorouracil (CAF) dose in HER2 amplified and nonamplified tumors. Kaplan-Meier estimates were calculated using SAS 9.1 for disease-free survival by CAF dose (low, moderate, high) for HER2 amplified versus HER2 nonamplified tumors. $P = .079$, interaction term for the three arms.

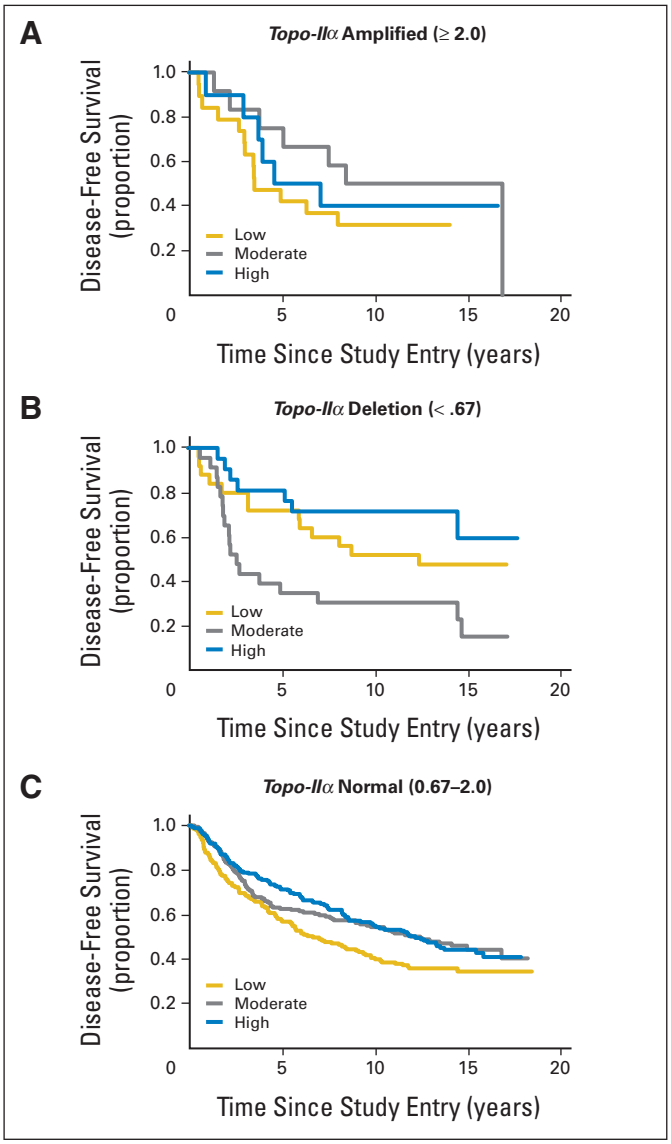

Fig 3. Disease-free survival by cyclophosphamide, doxorubicin, and fluorouracil (CAF) dose in tumors with topoisomerase (topo) II α amplification, deletion and normal copy number. Kaplan-Meier estimates were calculated using SAS 9.1 for disease-free survival by CAF dose (low, moderate, high) for tumors with topo IIα amplification, topo IIα deletion and topo IIα diploid (normal) copy number. $P = .15$, interaction term for the three arms.

980 patients randomly assigned to CMF versus CEF in the Danish Breast Cancer Cooperative Group trial 89D. Topo-IIα changes were identified in 23% of the 773 evaluated tumors: 12% had Topo-IIα amplification and 11% had Topo-IIα deletions. They reported improved recurrence-free survival both in patients with Topo-IIα amplification and in patients with Topo-IIα–deleted tumors who were treated with CEF compared with CMF. Patients with normal Topo-IIα genotype had a similar outcome in both treatment arms. This finding was not expected, as the hypothesis was that the deletions predicted resistance to anthracyclines.

Table 4. Variable of Interaction With CAF Dose*

Variable of Interaction	*P*
HER2 positive *v* *HER2* negative	.079
HER2 positive or Topo–IIα amplified (≥ 2.0) *v* all others	.34
HER2 positive and Topo–IIα amplified (≥ 2.0) *v* all others	.77

Abbreviations: CAF, cyclophosphamide, doxorubicin, and fluorouracil; Topo–IIα, topoisomerase IIα.
*All terms were modeled as dichotomous.

In the Breast Cancer International Research Group 06 trial, correlation between response to anthracyclines and Topo-IIα aberrations was prospectively planned. Patients with Topo-IIα amplification had better DFS after adjuvant therapy in both the trastuzumab-containing arm without anthracycline and in the nontrastuzumab-containing arm that included an anthracycline.[30,31] The investigators did not find a significant level of Topo-IIα deletion, and interpreted these data to mean that Topo-IIα amplification was a good surrogate of benefit from anthracycline.

O'Malley et al[32] evaluated the predictive value of Topo-IIα in premenopausal women with node-positive breast cancer randomly assigned in the MA.5 trial to CEF versus CMF. Cox model analysis suggested that Topo-IIα protein overexpression was highly predictive of a better DFS with CEF than with CMF. In this study, both Topo-IIα amplification and deletion predicted benefit from CEF versus CMF for both DFS and OS. Analysis from the United Kingdom National Epirubicin Adjuvant Trial (NEAT), which compared CMF with epirubicin, followed by CMF, included *HER2*, Topo-IIα, Ki67, and chromosome 17 polysomy.[33] The results suggest that the most powerful predictor of benefit from adjuvant anthracycline is chromosome 17 polysomy, perhaps as a marker of chromosome instability. No effect was observed for differing Topo-IIα status.

In our current study, Topo-IIα was coamplified in 33% of *HER2*-amplified tumors and was rarely seen in non–*HER2*-amplified tumors. Topo-IIα deletion was numerically more common than amplification (43% *v* 33%) although not statistically different. Of note, *HER2*-amplified tumors seemed to show two patterns of Topo-IIα copy number, either amplified or reduced in number compared with *HER2*-nonamplified tumors. This supports the contention that *HER2* amplification is associated with Topo-IIα copy number alterations, an indirect indicator of genomic instability. The association with sensitivity to anthracyclines may be explained by the fact that tumors with greatest degree of genomic instability showed the highest levels of Topo-IIα mRNA as shown by Carter et al[24] using multiple gene expression data sets.

It is also apparent, from recent high-density genomic mapping, that FISH analysis may not accurately predict amplification at a particular locus. Indeed, Lezon-Geyda et al[34] have shown that the Topo-II locus is rarely amplified using Representational Oligonucleotide Microarray Analysis, even when the FISH probe suggests this is the case. Hence it seems more likely that Topo-II copy number alterations may be a surrogate for a more global event (ie, genomic instability).

On the basis of this study and others cited, we do not support the hypothesis that Topo-IIα amplification alone explains the benefit of anthracyclines in *HER2* tumors. CALGB 8541 did not include a nonanthracycline arm; however, in this retrospective subset analysis, Topo-IIα does not explain the reported benefit from higher doses of doxorubicin-containing chemotherapy in CALGB 8541. Further studies are required to define the relationship between

Topo-IIα copy number, RNA, and protein in data sets with treatment response information.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group: Polychemotherapy for early breast cancer: An overview of the randomized trials. Lancet 352: 930-942, 1998

2. Buzdar AU, Kau SW, Smith TL, et al: Ten-year results of FAC adjuvant chemotherapy trial in breast cancer. Am J Clin Oncol 12:123-128, 1989

3. Jones S, Savin M, Holmes F, et al: Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide as adjuvant therapy for operable breast cancer. J Clin Oncol 24:5381-5387, 2006

4. Slamon D, Eiermann W, Robert N, et al: BCIRG006: 2nd interim analysis phase III randomized trial comparing doxorubicin plus cyclophosphamide followed by docetaxel with doxorubicin plus cyclophosphamide followed by docetaxel and trastuzumab with docetaxel, carboplatin and trastuzumab in HER2 positive early breast cancer patients. 29th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 14-17, 2006 (abstr 52)

5. Borg A, Baldetorp B, Ferno M, et al: *ErbB2* amplification in breast cancer with a high rate of proliferation. Oncogene 6:137-143, 1991

6. van de Vijver MJ, Peters JL, Mooi WJ, et al: Neu-protein overexpression in breast cancer: Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. N Engl J Med 319:1239-1245, 1988

7. Thor AD, Berry DA, Budman DR, et al: ErbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. J Natl Can Inst 90: 1346-1360, 1998

8. Paik S, Bryant J, Wolmark N: ErbB-2 Overexpression and response to chemotherapy: NSABP study. Breast Cancer Res Treat 50:3, 1998 (abstr 19)

9. Dressler LG, Berry DA, Broadwater G, et al: Comparison of HER2 status by fluorescence in situ hybridization and immunohistochemistry to predict benefit from dose escalation of adjuvant doxorubicin-based therapy in node-positive breast cancer patients. J Clin Oncol 23:4287-4297, 2005

10. Paik S, Bryant J, Park C, et al: ErbB-2 and response to doxorubicin inpatients with axillary lymph node-positive, hormone receptor-negative breast cancer. J Natl Cancer Inst 90:1361-1370, 1998

11. Pritchard K, Shepherd L, O'Malley F, et al: Her 2 and responsiveness of breast cancer to adjuvant chemotherapy. N Engl J Med 354:2103-2111, 2006

12. Smith K, Houlbrook S, Greenall M, et al: Topoisomerase II alpha co-amplification with erbB-2 in human primary breast cancer and breast cancer cell lines: Relationship to m-AMSA and mitoxantrone sensitivity. Oncogene 8:933-938, 1993

13. Kim R, Hirabayashi N, Nishiyama M, et al: Expression of MDR1, GST and Topoisomerase II as an indicator of clinical response to Adriamycin. Anticancer Res 11:429-431, 1991

14. Järvinen TA, Tanner M, Rantanen V, et al: Amplification and deletion of topoisomerase II alpha associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. Am J Pathol 156:839-847, 2000

15. Järvinen TA, Tanner M, Barlund M, et al: Characterization of topoisomerase II alpha amplification and deletion in breast cancer. Genes Chromosomes Cancer 26:142-150, 1999

16. Harris LN, Yang L, Liotcheva V, et al: Induction of topoisomerase II activity after ErbB2 activation is associated with a differential response to breast cancer chemotherapy. Clin Cancer Res 7:1497-1504, 2001

17. Rowe TC, Chen GL, Hsiang Y, et al: DNA damage by antitumor acridines mediated by mammalian topoisomerase II. Cancer Res 46:2021-2026, 1986

18. DiGiovanna M, Stern D: Activation state-specific monoclonal antibody detects tyrosine phosphorylated p185 neu/erbB-2 in a subset of human breast tumors overexpressing this receptor. Cancer Res 55:1946-1955, 1995

19. Budman DR, Berry DA, Cirrincione CT, et al: Dose and dose intensity as determinants of outcome in the adjuvant treatment of breast cancer. J Natl Cancer Inst 90:1205-1211, 1998

20. DiGiovanna M, Carter D, Flynn S, et al: Functional assay for HER2/neu demonstrates active signaling in a minority of HER2/neu overexpressing invasive human breast tumors. Br J Cancer 74:802-806, 1996

21. Thor A, Liu S, Edgerton S, et al: Activation (tyrosine phosphorylation)of Erb B-2(HER2/neu): A study of incidence and correlation with outcome in breast cancer. J Clin Oncol 18:3230-3239, 2000

22. Harris L, Tang C, Yang C, et al: Induction of chemotherapy sensitivity in MCF-7 breast cancer cells by heregulin. Clin Cancer Res 4:1005-1012, 1998

23. Jacobson K, Thompson A, Browne G, et al: Automation of fluorescence in situ hybridization pretreatment: A comparative study of different sample types. Mol Diagn 5:209-220, 2000

24. Carter S, Eklund A, Kohane I, et al: A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcomes in multiple human cancers. Nat Genet 38:1043-1048, 2006

25. Jacobson K, Morrison L, Henderson B, et al: Gene copy mapping of the ERBB2/TOP2A region in breast cancer. Genes Chromosomes Cancer 40:19-31, 2004

26. Schwartz R, Schnitt S, Rousseau A, et al: Topoisomerase IIa expression and response to anthracycline-based neoadjuvant chemotherapy: Results from a randomized clinical trial. Mod Pathol 16:195, 2003

26a. Knoop A, 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: Danish Breast Cancer Group. J Clin Oncol 23:7483-7490, 2005

26b. Tanner M, Isola J, Wiklund T, et al: Topoisomerase IIa gene amplification predicts favorable treatment response to tailored and dose-escalated anthracycline-based adjuvant chemotherapy in HER-2/neu amplified breast cancer: Results from the randomized trial SBG 9401. J Clin Oncol 24:2428-2436, 2006

27. Di Leo A, Gancberg D, Larsimont D, et al: HER-2 amplification and topoisomerase II 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 8:1107-1116, 2002

28. Reference deleted

29. Reference deleted

30. Slamon D, Eiermann W, Robert N, et al: Phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (AC –> T) with doxorubicin and cyclophosphamide followed by

docetaxel and trastuzumab (AC –> TH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2 positive early breast cancer patients: BCIRG 006 study. Breast Cancer Res Treat 94:S5, 2005 (suppl 1)

31. Press M, Bernstein L, Sauter G, et al: Topoisomerase II-alpha gene amplification as a predictor of responsiveness to anthracycline containing chemotherapy in the Cancer International Research Group 006 clinical trial of trastuzumab (Herceptin) in the adjuvant setting. San Antonio Breast Cancer Symposium, San Antonio, TX, December 8-11, 2005 (abstr 1045)

32. O'Malley FP, Chia S, Tu D, et al: Topoisomerase II alpha protein overexpression has predictive utility in a randomized trial comparing CMF to CEF in premenopausal women with node positive breast cancer (NCIC CTG MA. 5). Breast Cancer Res Treat 100:S18, 2006 (suppl 1; abstr 38)

33. Bartlett JMS, Munro A, Dunn JA, et al: Chromosome 17 polysomy as a predictor of anthracycline response: Emerging evidence from the UK NEAT adjuvant breast cancer trial. San Antonio Breast Cancer Conference, San Antonio, TX, December 10-14, 2008 (abstr 45)

34. Lezon-Geyda K, Hicks JB, Tuck DP, et al: Genomic rearrangements in breast cancer: Clinical implications. Biol Ther Breast Cancer 8:7-11, 2007

