VOLUME 29 · NUMBER 7 · MARCH 1 2011

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Alteration of Topoisomerase II–Alpha Gene in Human Breast Cancer: Association With Responsiveness to Anthracycline-Based Chemotherapy

Michael F. Press, Guido Sauter, Marc Buyse, Leslie Bernstein, Roberta Guzman, Angela Santiago, Ivonne E. Villalobos, Wolfgang Eiermann, Tadeusz Pienkowski, Miguel Martin, Nicholas Robert, John Crown, Valerie Bee, Henry Taupin, Kerry J. Flom, Isabelle Tabah-Fisch, Giovanni Pauletti, Mary-Ann Lindsay, Alessandro Riva, and Dennis J. Slamon

From the Norris Comprehensive Cancer Center, University of Southern California; Jonsson Comprehensive Cancer Center, University of California Los Angeles, Los Angeles; City of Hope National Medical Center, Duarte; Cepheid, Sunnyvale, CA; University Medical Center Hamburg-Eppendorf, University of Hamburg, Hamburg; Frauenklinik vom Roten Kreuz, Munich, Germany; International Drug Development Institute, Louvain-la-Neuve, Belgium; Maria Sklodowska-Curie Centre, Warsaw, Poland; Hospital Universitario San Carlos, Madrid, Spain; Inova Fairfax Hospital Institute of Research, Falls Church, VA; Irish Cooperative Oncology Research Group, St Vincent's University Hospital, Dublin, Ireland; Cancer International Research Group; and sanofi-aventis, Paris, France.

Submitted April 29, 2010; accepted September 23, 2010; published online ahead of print at www.jco.org on December 28, 2010.

Supported by sanofi-aventis (BCIRG-005 and -006 clinical trials) and Genentech (H0648 clinical trial); by Grants No. CA48780 from the National Cancer Institute, DAMD17-03-1-0626 from the US Army Medical Research and Development Command, and 12IB-0155 from the California Breast Cancer Research Program Expedition Inspiration and by the Breast Cancer Research Foundation (M.F.P.); by Grant No. CA77398 from the National Cancer Institute (L.B., J.S.-H.); and by grant support from the Department of Defense Breast Cancer Research Innovator Award and Revlon/University of California Los Angeles Women's Program (D.J.S.).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Michael F. Press, MD, PhD, University of Southern California/Norris Comprehensive Cancer Center, 1441 Eastlake Ave, NOR5409, Los Angeles, CA 90033; e-mail: press@usc.edu.

© 2010 by American Society of Clinical Oncology

0732-183X/11/2907-859/$20.00

DOI: 10.1200/JCO.2009.27.5644

ABSTRACT

Purpose
Approximately 35% of *HER2*-amplified breast cancers have coamplification of the topoisomerase II-alpha (*TOP2A*) gene encoding an enzyme that is a major target of anthracyclines. This study was designed to evaluate whether *TOP2A* gene alterations may predict incremental responsiveness to anthracyclines in some breast cancers.

Methods
A total of 4,943 breast cancers were analyzed for alterations in *TOP2A* and *HER2*. Primary tumor tissues from patients with metastatic breast cancer treated in a trial of chemotherapy plus/minus trastuzumab were studied for amplification/deletion of *TOP2A* and *HER2* as a test set followed by evaluation of malignancies from two separate, large trials for changes in these same genes as a validation set. Association between these alterations and clinical outcomes was determined.

Results
Test set cases containing *HER2* amplification treated with doxorubicin and cyclophosphamide (AC) plus trastuzumab, demonstrated longer progression-free survival compared to those treated with AC alone ($P = .0002$). However, patients treated with AC alone whose tumors contain *HER2/TOP2A* coamplification experienced a similar improvement in survival ($P = .004$). Conversely, for patients treated with paclitaxel, *HER2/TOP2A* coamplification was not associated with improved outcomes. These observations were confirmed in a larger validation set, where *HER2/TOP2A* coamplification was again associated with longer survival when only anthracycline-containing chemotherapy was used for treatment compared with outcome in *HER2*-positive cancers lacking *TOP2A* coamplification.

Conclusion
In a study involving nearly 5,000 breast malignancies, both test set and validation set demonstrate that *TOP2A* coamplification, not *HER2* amplification, is the clinically useful predictive marker of an incremental response to anthracycline-based chemotherapy. Absence of *HER2/TOP2A* coamplification may indicate a more restricted efficacy advantage for breast cancers than previously thought.

J Clin Oncol 29:859-867. © 2010 by American Society of Clinical Oncology

INTRODUCTION

Anthracycline-based chemotherapy is the mainstay of current adjuvant treatments for early-stage breast cancer. This is supported by a meta-analysis of several randomized studies showing slightly higher (approximately 4%) disease-free survival (DFS) and overall survival (OS) rates achieved with anthracycline-based versus nonanthracycline chemotherapies.[1] However, anthracyclines have significant long-term toxicities including cardiac dysfunction and/or induction of myelodysplasia and acute leukemias.[2-4]

Several studies have reported an association between *HER2* amplification/overexpression and increased responsiveness to anthracycline-based chemotherapy[5-9]; however, underlying biologic mechanism(s) are unclear. Indeed, in vitro and in vivo studies indicate that *HER2* overexpression alone does not alter anthracycline sensitivity.[10] *HER2* is located on the long arm of chromosome 17 (17q11.2-12) in close proximity to topoisomerase II-α (*TOP2A*) at 17q21-22. Although *HER2* is considered the target of the amplification event, *HER2* amplicon size is variable and contains other genes[11-14] occasionally including

TOP2A.[12,15-17] Because *TOP2A* is a target of anthracyclines, it is possible that this gene, not *HER2*, is the link between *HER2*-positive disease and anthracycline responsiveness.[18] The objectives of this study were three-fold: determine the nature and frequency of *TOP2A* copy-number alterations in clinically annotated breast cancers using molecularly validated cutoffs; determine how often these alterations are found in both *HER2*-positive and -negative breast cancers; and evaluate any association between such alterations and response to anthracycline-based chemotherapy. We addressed these questions using a retrospective evaluation of 4,943 breast cancers. The first group was a hypothesis generating test set of 339 cancers from women enrolled in a trial of *HER2*-positive metastatic disease in which patients were treated with anthracycline-based or nonanthracycline chemotherapy plus/minus trastuzumab. Clinical response data was then correlated with the presence or absence of *HER2* and *TOP2A* alterations. To validate any observed associations from the test set, we next evaluated 4,604 samples from two larger studies, Breast Cancer International Research Group (BCIRG) -006 (2,990 patients) and BCIRG-005 (1,614 patients). This validation set was used to define the frequency of *TOP2A* copy-number changes in *HER2*-amplifed and *HER2*-normal patients and determine whether *TOP2A* or *HER2* alterations were correlated with anthracycline response.

METHODS

Patients

Test set patients (Figs 1, 2) consisted of patients enrolled in the original randomized phase III trastuzumab registration study (H0648g) designed to evaluate chemotherapy plus/minus trastuzumab in patients with *HER2*-positive metastatic breast cancer.[19] Validation set patients consisted of participants in the BCIRG-005 and BCIRG-006 adjuvant breast cancer trials which accrued 3,298 and 3,222 patients, respectively, between August 2000 and March 2004. BCIRG-005 evaluated combination versus sequential chemotherapy in *HER2*-normal, node-positive, early-stage breast cancers[20] and BCIRG-006 studied node-positive and high-risk, node-negative, *HER2*-amplified breast cancer[21,22] comparing two different adjuvant trastuzumab/chemotherapy regimens (one with and one without anthracyclines) to anthracycline-based chemotherapy alone. Details of patient tissue samples and clinical study designs are described separately (Appendix, online only).

Tissue Analyses: Validation of Probes and Cutoffs Used to Determine HER2/TOP2 Amplification/Deletion

TOP2A and *HER2* amplification/deletion status was determined by fluorescent in situ hybridization (FISH) using commercial probes (Abbott-Vysis, Inc; Downers Grove, IL). [22,23] Analysis of both genes was performed simultaneously using SpectrumGreen-labeled *HER2* and SpectrumOrange-labeled *TOP2A*, respectively. Chromosome-17 centromere numbers were determined using a SpectrumAqua-labeled chromosome enumeration probe (CEP17). *TOP2A* and *HER2* copy numbers were determined in a minimum of 20 interphase, nonoverlapping, tumor cell nuclei and compared with chromosome 17 centromeres in those same nuclei. To insure that probes and cutoffs used to generate the amplification/deletion status of *TOP2A* and *HER2* in the test and validation sets were correct, the status of these two genes was first determined in a molecularly characterized panel of known material using amplicon mapping techniques (Appendix).

HER2 amplification was defined as a *HER2* gene-to-CEP17 ratio ≥ 2.0, which is the US Food and Drug Administration–approved ratio, rather than the American Society of Clinical Oncology-College of American Pathologists guideline ratio for reasons published elsewhere.[24] The identical ratio was used to define *TOP2A* gene amplification[25,26] since both genes are part of the same

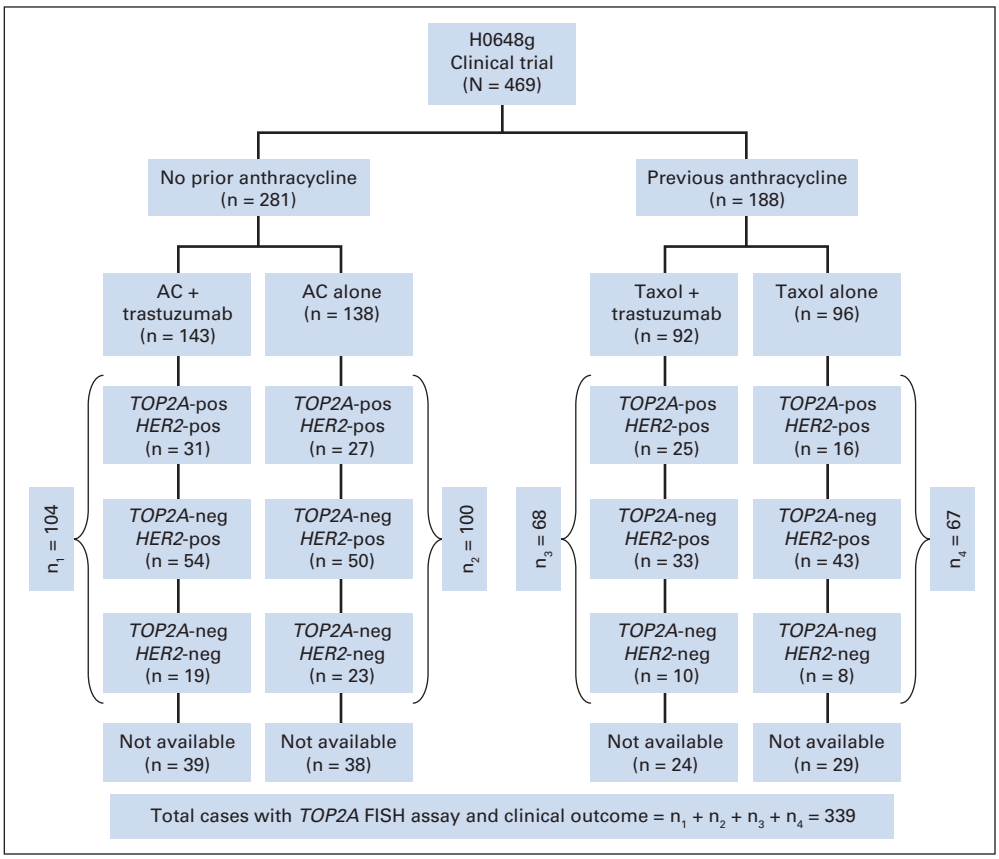

Fig 1. Specimen accountability in the H0648 test set clinical trial. This schematic diagram summarizes the number of women entered in each treatment arm of the H0648g pivotal clinical trial and the breast cancer specimens analyzed by fluorescent in situ hybridization (FISH) in each treatment arm. A, anthracycline (doxorubicin or epirubicin); C, cyclophosphamide; *HER2*-pos, *HER2* gene amplification; *HER2*-neg, lacking *HER2* gene amplification; *TOP2A*-pos, *TOP2A* gene amplification; *TOP2A*-neg, lacking *TOP2A* gene amplification including both *TOP2A* normals and *TOP2A* gene deletions.

 © 2010 by American Society of Clinical Oncology

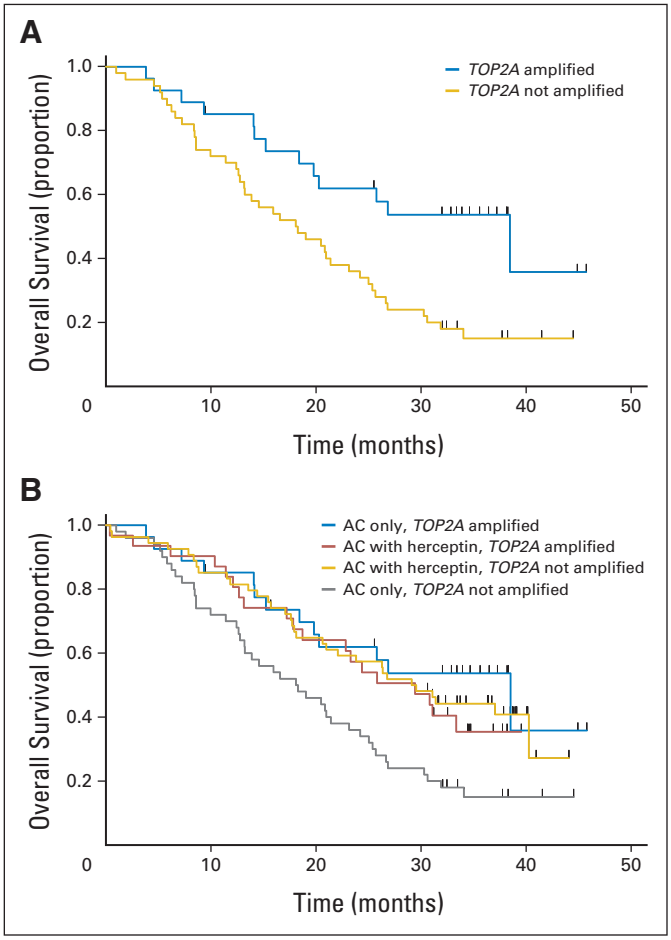

Fig 2. Overall survival of women with *HER2*-gene amplified metastatic breast cancer treated in the test set H0648 clinical trial with anthracycline-containing chemotherapy. (A) Women treated with doxorubicin and cyclophosphamide (AC) alone (n = 77) comparing those with *TOP2A*-amplified tumors (*TOP2A* fluorescent in situ hybridization [FISH] ratio ≥ 2.00; n = 27) with those whose tumors are not *TOP2A* amplified (*TOP2A* FISH ratio < 2.00; n = 50; log-rank test $P = .004$). (B) Overall survival of women with *HER2*-amplified metastatic breast cancer treated with AC alone chemotherapy compared with AC plus trastuzumab by *TOP2A* status. Three of the subsets have similar overall survival (women whose cancers were *HER2*/*TOP2A* coamplified and were treated with AC alone; women whose cancers were *HER2*/*TOP2A* coamplified and were treated with AC plus trastuzumab; women whose tumors were *HER2* amplified but not *TOP2A* amplified and were treated with AC plus trastuzumab), which was significantly longer than the subset of women whose cancers were *HER2* amplified but not *TOP2A* amplified and were treated with AC alone.

amplification event. All specimens from the three trials were retested by FISH for this study, blinded to the original results and categorized as either amplified/deleted or nonamplified for *TOP2A* and *HER2*. Concordance between original and current FISH results for *HER2* status in H0648g test set patients was 97%.[23] *HER2* status was also repeated for the validation set to determine if comparable FISH results were seen in a tissue microarray format resulting in a 99.8% and 99.6% concordance between the original and current analyses for BCIRG-005 and BCIRG-006, respectively.[22]

Statistical Methods

Clinical outcomes (defined as overall response rates, progression-free survival [PFS], and OS in H0648 and DFS and OS in BCIRG-005 and BCIRG-006) were compared in *TOP2A*-amplified and nonamplified subgroups using χ^2, Mantel-Haenszel, and log-rank tests (Appendix).[27,28] In addition, in trial BCIRG-006, used as the validation set for the effect of *TOP2A* amplification in the adjuvant setting, a Cox regression model was fitted with an indicator for *TOP2A* amplification, an indicator for randomized treatment, and an interaction term between these two indicators.

RESULTS

Association Between Clinical Outcomes and TOP2A *Alterations in Test Set Patients*

Of 469 participants in the *HER2*-positive metastatic study H0648g,[19] 339 specimens (72%) were available for this analysis. Clinical characteristics did not differ between the original study population and current test set patients (Table 1). FISH analyses confirmed that 279 (82%) of 339 were *HER2* amplified, while 60 (18%) of 339 were not (Table 1). Using molecularly validated cutoff ratios for *TOP2A* (Appendix), a total of 99 (29.2%) of 339 test set patients showed *TOP2A* coamplification, while 47 (13.9%) of 339 had *TOP2A* deletion (Table 2). The remaining 193 (56.9%) of 339 did not contain *TOP2A* alterations (Table 2). Of note, *TOP2A* amplification was not detected in any *HER2*-normal cancers (Table 2) while two (3%) of 60 had evidence of *TOP2A* deletion.

Clinical outcomes from 204 patients treated with the doxorubicin and cyclophosphamide (AC) regimen and 135 treated with the paclitaxel regimen (each alone or in combination with trastuzumab) were reviewed. A total of 281 (60%) of 469 patients in the original trial were randomly assigned to AC therapy of which 143 received trastuzumab while 138 did not (Fig 1). *TOP2A* results were available for 204 (73%) of these malignancies. Among 162 patients treated with anthracycline-based therapy, either alone or in combination with trastuzumab, those with *HER2*/*TOP2A* coamplification showed trends toward longer median PFS (7.6 *v* 6.7 months; $P = .064$) and OS (30.8 *v* 21.7 months; $P = .069$) compared to those without coamplification (Appendix Tables A2, A3, online only). Trastuzumab treatment was associated with significantly improved PFS for both *HER2*/*TOP2A*-coamplified cancers (8.6 *v* 7.1 months; $P = .034$) as well as those lacking *TOP2A* coamplification (7.3 *v* 5.6 months; $P = .0026$, Appendix Table A2). However, for patients treated with AC alone, there was a distinct difference in outcomes between *HER2*/*TOP2A*-coamplified cancers and those with only *HER2* amplification. In this group, *HER2*/*TOP2A* coamplified patients demonstrated a trend toward longer PFS (7.1 *v* 5.6 months; $P = .11$) and a statistically significant increase in OS (38.5 *v* 18.2 months; $P = .004$; Figs 2A, 2B; Appendix Tables A2, A3) despite the fact that these patients had not received trastuzumab. Women whose breast cancers had *TOP2A* deletions experienced clinical outcomes that were not significantly different from women who had *TOP2A*-normal cancers.

To determine whether the association was specifically related to anthracycline-based therapy as opposed to other chemotherapy, the same analysis was performed for the 135 patients treated with a non-anthracycline regimen (ie, paclitaxel plus/minus trastuzumab). Unlike patients receiving anthracycline-based chemotherapy, patients treated with paclitaxel alone showed no differences in PFS or OS related to presence or absence of *TOP2A* coamplification (PFS, 4.3 *v* 2.8 months; $P = .20$; OS, 18.4 *v* 20.6 months; $P = .96$, Appendix Tables A4, A5, online only). Overall data from the test set indicated that *HER2*-positive patients receiving chemotherapy without trastuzumab

© 2010 by American Society of Clinical Oncology

Table 1. Comparison of Characteristics for Women in H0648g Test Set Clinical Trial Whose Breast Cancers Were Analyzed for *TOP2A* Gene Amplification

Characteristic	All Women in H0648g Clinical Trial[19] (n = 469)		Women					
			With Analysis of *TOP2A* Gene Amplification (n = 339)				Not Analyzed for *TOP2A* Gene Amplification (n = 130)	
			Yes		No			
	No.	%	No.	%	No.	%	No.	%
HER2 gene amplification by FISH			279 of 339	82	60 of 339	18	Not applicable	
Yes	344 of 451	76						
No	107 of 451	24						
Mean age, years	52		52		53		53	
SD	10.7		10.4		11.2		11.1	
Range	25-77		25-77		27-76		26-73	
Karnofsky score								
90-100	309 of 457	67.6	187 of 271	69.0	39 of 57	68.4	83 of 129	64.3
< 90	148 of 457	32.4	84 of 271	31.0	18 of 57	31.6	46 of 129	35.7
Median No. of involved nodes at diagnosis	2		2		3		2	
Range	0-42		0-30		0-32		0-42	
Missing	67		39		11		17	
Prior therapy								
Chemotherapy*	314 of 464	67.7	193 of 276	69.9	33 of 58	56.9	88 of 130	67.7
Hormone†	265 of 461	57.5	153 of 275	55.6	35 of 57	61.4	77 of 129	59.7
Radiation†	279 of 463	60.3	170 of 276	61.6	32 of 58	55.2	77 of 129	59.7
Metastatic sites at enrollment								
≤ 1	154 of 465	33.1	98 of 277	35.4	19 of 58	32.8	37 of 130	28.5
2	154 of 465	33.1	93 of 277	33.6	20 of 58	34.5	41 of 130	31.5
≥ 3	157 of 465	33.8	86 of 277	31.0	19 of 58	32.8	52 of 130	40.0
Median disease-free interval, months	22.2		21.1		26.6		22.0	
Range	0-225		0-225		0-152		0-223	
Missing	5		2		2		1	
HER2 status (by IHC)								
2+	120 of 469	25.6	30 of 279	10.8	49 of 60	81.7	41 of 130	31.5
3+	349 of 469	74.4	249 of 279	89.2	11 of 60	18.3	89 of 130	68.5

Abbreviations: FISH, fluorescent in situ hybridization; SD, standard deviation; IHC, immunohistochemistry.
*Adjuvant chemotherapy only.
†As adjuvant, for metastasis or both.

have an incremental improvement in clinical outcome associated with anthracyclines only if their cancers contain *TOP2A* coamplification.

Association Between Clinical Outcomes and TOP2A *Alteration in Validation Set Patients*

TOP2A status was next determined for 2,990 (93.1%) of 3,222 of the *HER2*-amplified tumors from BCIRG-006 (Appendix Figs A1, A2, online only). A total of 1,057 (35.4%) of 2,990 showed *HER2*/*TOP2A* coamplification while 1,788 (59.8%) of 2,990 were *TOP2A*-normal and 145 (4.8%) of 2,990 *TOP2A* deleted. Clinical characteristics of patients with *TOP2A* alterations did not differ in age, Karnofsky performance status, axillary node status, stage, or treatment arm (data not shown). Like test set patients, validation set patients were analyzed for clinical outcomes according to their *TOP2A* status. Overall, these data demonstrate that regardless of treatment arm, *HER2*/*TOP2A* coamplification is associated with a significantly longer DFS and OS ($P < .001$ and $P < .001$, respectively) when compared to women whose cancers do not contain *TOP2A* amplification. In *TOP2A*-normal patients who constitute 60% to 65% of *HER2*-positive cancers, trastuzumab significantly improves clinical outcomes whether used as doxorubicin, cyclophosphamide, docetaxel, and trastuzumab (ACTH) and docetaxel, carboplatin, and trastuzumab (TCH; DFS, $P < .001$; OS, $P = .024$; Fig 3; Table 3) with no difference in DFS or OS between the two regimens ($P = .32$ and $P = .67$, respectively). In these patients, trastuzumab resulted in significantly improved outcome for all comparisons (ie, AC→T *v* AC→TH, AC→T *v* TCH, or AC→T *v* AC→TH+TCH; Table 3). However, consistent with test set data, validation set patients demonstrated that for the 35% of *HER2*-positive breast cancers with *TOP2A* coamplification receiving anthracycline-based chemotherapy alone (ie, AC→T), there were significant improvements in both DFS and OS ($P < .001$ and $P = .019$, respectively); similar to that seen with trastuzumab-containing regimens (Fig 3). The test for interaction for an association between *TOP2A* coamplification and incremental anthracycline benefit was significant ($P = .045$, Table 3).

Frequency of TOP2A *Alterations in* HER2*-Normal Breast Cancers and Association With Anthracycline Benefit*

To determine the frequency of genomic alterations of *TOP2A* in breast cancers without *HER2* amplification, we analyzed samples from the BCIRG-005 adjuvant study, a trial that accrued

 © 2010 by American Society of Clinical Oncology

Table 2. *HER2* and *TOP2A* Gene Amplification in the Test Set Clinical Trial by Fluorescent In Situ Hybridization Assay and Received Chemotherapy

	HER2 Gene					
	Not Amplified		Amplified		Totals	
Patients	No.	%	No.	%	No.	%
All patients with *TOP2A* results						
TOP2A deleted	2	3	45	16	47	14
TOP2A normal	58	97	135	48	193	57
TOP2A amplified	0	0	99	36	99	29
Total	60	100	279	100	339	100
Patients with *TOP2A* results receiving AC regimens						
TOP2A deleted	1	2	27	17	28	14
TOP2A normal	41	98	77	47	118	58
TOP2A amplified	0	0	58	36	58	28
Total	42	100	162	100	204	100
Patients with *TOP2A* results receiving paclitaxel regimens						
TOP2A deleted	1	6	18	15	19	14
TOP2A normal	17	94	58	50	75	56
TOP2A amplified	0	0	41	35	41	30
Total	18	100	117	100	135	100

NOTE. The H0648g pivotal clinical trial of trastuzumab in metastatic breast cancer was sponsored by Genentech.
Abbreviation: AC, doxorubicin and cyclophosphamide.

only *HER2*-negative patients.[20] All patients in this trial received anthracycline-based therapy as part of a combination or sequential regimen (ie, TAC [docetaxel plus doxorubicin plus cyclophosphamide] or ACT). At the time of this analysis, 611 disease-related events had occurred demonstrating no difference between the two treatment arms. Analysis for *TOP2A* in this portion of the validation set allowed assessment of the frequency and nature of *TOP2A* alterations in the absence of *HER2* amplification and whether such changes were associated with different DFS/OS event rates. In 1,614 of these *HER2*-normal cases, no *TOP2A* amplification was observed while 42 (2.6%) were *TOP2A* deleted. These deletions were not differentially associated with either DFS or OS.

DISCUSSION

Anthracyclines are among the most frequently prescribed cytotoxics in the treatment of breast cancer; however, not all patients benefit equally and these agents have significant potential long-term toxicities.[2-4,29-31] Attempts to identify patients most likely to benefit from their use, have resulted in a remarkably consistent observation. Data from numerous large clinical studies, performed by multiple groups, conducted over three decades demonstrate that only those breast cancers containing *HER2* amplification/overexpression appear to incrementally benefit from anthracycline- versus nonanthracycline-based regimens.[5-9,32-34] Conversely, the remaining 75% to 80% of breast cancers that are *HER2* normal do not.[5-9,32-34] A recently published meta-analysis of composite data from more than 5,000 breast cancers from these and other studies clearly confirms this association.[35] One of the more recent of these studies is National Cancer Institute of Canada Mammary-5 trial comparing cyclophosphamide, high-dose epirubicin, and fluorouracil (CEF) to cyclophosphamide, methotrexate, and fluorouracil. It again confirmed that only cancers with *HER2* amplification had superior relapse-free survival (RFS; $P = .003$) and OS ($P = .06$) when treated with CEF while those lacking *HER2* amplification received no incremental benefit in either RFS or OS from this regimen.[8] An important subsequent analysis showed that incremental CEF outcome benefits were found in those patients whose cancers also overexpressed the TOP2A protein[36] or had *TOP2A* gene amplification/deletion.[37] The Danish Breast Cancer Cooperative Group also compared cyclophosphamide, methotrexate, and fluorouracil to CEF and recently reported that incremental anthracycline benefits were restricted to *TOP2A* or *TOP2A/TIMP*–altered subgroups of breast cancer.[38,39] While the exact biologic mechanisms underlying an association between *HER2* amplification and increased anthracycline response remain unclear, these data as well as other studies[40] implicate *TOP2A* alterations as a potential molecular basis for superior anthracycline sensitivity. This hypothesis gains added credence from studies demonstrating that *HER2* overexpression alone does not enhance sensitivity to anthracyclines.[10] Together, these observations provide insight into a possible mechanism(s) regarding why *HER2*-amplified breast cancers are uniquely associated with increased anthracycline sensitivity and implicate a potential biomarker for increased response to anthracyclines.[41]

Anthracyclines inhibit TOP2A protein activity, a key enzyme in DNA replication and RNA transcription.[42] Moreover, in vitro studies indicate that sensitivity to TOP2A inhibitors is dependent on *TOP2A* expression levels in cancer cells.[42-44] The *TOP2A* gene is located at chromosome 17q21-22 in close proximity to *HER2* resulting in a proportion of *HER2*-amplified breast cancers also containing coamplification of *TOP2A*.[12,45] This in turn is associated with overexpression of TOP2A protein and potential increased sensitivity to TOP2A inhibitors.[12,42,45,46] Initial smaller studies of *TOP2A* alterations in breast cancer suggested they were frequently found in *HER2*-amplified tumors.[47,48] Subsequent larger studies showed that between 33% to 60% of *HER2*-positive cancers contain concurrent *TOP2A* amplification[25,26,45] while 20% to 42% are *TOP2A* deleted.[26,45,48-50] Some published reports of *HER2*-normal breast cancers[47,48] find

© 2010 by American Society of Clinical Oncology

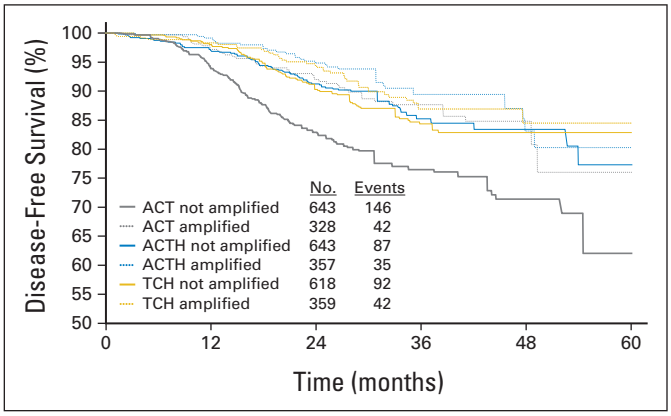

Fig 3. Clinical outcome of women stratified by *TOP2A* status and by treatment arm in the Breast Cancer International Research Group (BCIRG) 006 clinical trial: comparison of disease-free survival (DFS) of women whose breast cancers lacked *TOP2A* gene coamplification and were treated on the doxorubicin, cyclophosphamide, and docetaxel (ACT) control treatment arm versus DFS of women whose breast cancers had *TOP2A* gene coamplification and were treated on the ACT control treatment arm and versus DFS of women whose breast cancers lacked *TOP2A* gene coamplification but were treated on either the doxorubicin, cyclophosphamide, docetaxel, and trastuzumab (ACTH) or the docetaxel, carboplatin, and trastuzumab (TCH) experimental treatment arms and with DFS of women whose breast cancers had *TOP2A* gene coamplification and were treated on either the ACTH or TCH experimental treatment arms. The number of patients and events in each treatment arm is listed for each arm. Comparisons of clinical outcome by *TOP2A* status (coamplified *v* not coamplified) and treatment arm are illustrated elsewhere.[21] Table 3 provides the corresponding tests for treatment effects and interaction terms. The BCIRG-006 study compared two different experimental trastuzumab plus chemotherapy regimens with chemotherapy alone. The control arm (AC→T) consisted of four cycles of doxorubicin 60 mg/m^2 plus cyclophosphamide 600 mg/m^2 every 3 weeks followed by four cycles of docetaxel 100 mg/m^2 every 3 weeks. Patients in the first experimental arm (ACTH) received the same chemotherapy with the addition of trastuzumab beginning with the first docetaxel dose followed by 2 mg/kg/week for 1 year. A second, nonanthracycline experimental arm (TCH) consisted of docetaxel 75 mg/m^2 plus carboplatin at an area under the curve of 6 every 2 weeks for six cycles concurrently with trastuzumab. Trastuzumab was administered at 4 mg/kg for the first dose followed by 2 mg/kg/week until completion of chemotherapy, then at 6 mg/kg every 3 weeks to complete 1 year of treatment.

TOP2A infrequently amplified or deleted while others, based on relatively few patients, report a 10% to 20% *TOP2A* amplification/deletion rate[25,26] causing confusion regarding this matter. This wide variability presents a challenge for assessing any true association of this alteration with clinical outcomes and is likely related to different FISH ratio cut points used to define *TOP2A* amplification (≥ 1.5 or ≥ 2.0) and/or *TOP2A* deletions (≤ 0.67, ≤ 0.7, ≤ 0.8, or < 1.0).[25,26,45,48,51,52]

In this study of almost 5,000 new breast cancers from three separate trials, the following questions were addressed: what is the prevalence of *TOP2A* alterations? What is its concordance with *HER2* amplification? And what is the association (if any) with incremental anthracycline sensitivity? Utilizing methods and probes validated from physical mapping of the 17q12-q21 amplicon, we find that 35% of *HER2*-amplified cancers contain *TOP2A* coamplification, 5% have deletions, and 60% are *TOP2A* normal. In addition, analysis of 1,614 *HER2*-normal cases revealed no *TOP2A* amplification but a 3% deletion rate. These data are consistent with other reports that *TOP2A* amplification, when present, is seen with *HER2* coamplification[41,45,48] and contrast with smaller studies reporting *TOP2A* amplification in *HER2*-normal breast cancers.[26,53] Because of differences in methods and cutoffs to assess *HER2* and *TOP2A* status,[54] we again believe this published variability in *TOP2A* alterations is largely due to technical rather than biologic factors.

There are also conflicting data regarding an association between *TOP2A* alterations and anthracycline sensitivity. In this study, we find such an association. Conversely, an analysis of almost 2,000 patients reported previously that *HER2* and *TOP2A* have "only a clinically modest and statistically borderline predictive value."[55] However, these investigators noted difficulty in reproducing FISH results between and within laboratories involved in this study, demonstrating a concordance rate of only 69.2%. In this analysis of approximately 5,000 patients, there was no similiar difficulty, with a more than 97% concordance between prior and current analyses. A small study of 41 patients with *TOP2A* gene amplification also recently reported no association between TOP2A status and anthracycline dose-response[56]; however, all patients received the drug and no information was provided regarding distribution of patients across three anthracycline dose strata. Assuming an even distribution, only 14 patients would exist in each treatment arm resulting in a lack of sufficient statistical power to demonstrate any association. In a separate study of 2,123 patients, all of whom received identical anthracycline doses either in combination or sequence with other drugs, no association was found between *TOP2A* alterations and anthracycline sensitivity; however, there was an association with *HER2* amplification.[57] As noted by

Table 3. Treatment Effect and Interaction Between Treatment Effect and *TOP2A* Amplification Based on the BCIRG-006 Validation Set Trial

	TOP2A						
	Nonamplified (n = 1,904)			Amplified (n = 1,044)			
Regimen	Hazard Ratio	95% CI	*P*	Hazard Ratio	95% CI	*P*	Interaction Test *P*
ACT *v* ACTH	0.53	0.40 to 0.69	< .001	0.80	0.51 to 1.25	.34	.117
ACT *v* TCH	0.57	0.44 to 0.74	< .001	0.92	0.60 to 1.42	.65	.063
ACT *v* ACTH + TCH	0.55	0.44 to 0.68	< .001	0.85	0.59 to 1.25	.41	.045

NOTE. The BCIRG-006 study compared two different experimental trastuzumab plus chemotherapy regimens with chemotherapy alone. The control chemotherapy alone arm (ACT) consisted of doxorubicin and cyclophosphamide followed by docetaxel. Patients in the first experimental arm of anthracycline-containing chemotherapy (ACTH) received the same chemotherapy with the addition of trastuzumab beginning with the first docetaxel. A second, nonanthracycline experimental arm (TCH) consisted of docetaxel plus carboplatin concurrently with trastuzumab.

Abbreviations: BCIRG, Breast Cancer International Research Group; ACT, doxorubicin, cyclophosphamide, docetaxel; ACTH, doxorubicin, cyclophosphamide, docetaxel, and trastuzumab; TCH, docetaxel, carboplatin, and trastuzumab.

 © 2010 by American Society of Clinical Oncology

the authors[57] and in the accompanying editorial,[58] "the predictive information we need most in this area cannot be augmented from any analyses of the trial" given that all patients received an anthracycline. Finally, a recent study of pooled data from two trials (National Epirubicin Adjuvant Trial/BR9601)[59,60] found no association between *TOP2A* status and response to anthracycline-based chemotherapy. However, this study inexplicably used different gene-to-centromere FISH ratios (ie, *HER2/CEP17* $\geq$ 2.0 and *TOP2A/CEP17* $\geq$ 1.5) for two genes in the same amplicon.[60] No scientific rationale is offered for use of different ratios to assess loci within the same 17q12-q21 amplicon, especially since both are compared to the identical control probe (CEP17). Given that $\geq$ 2.0 is the established, US Food and Drug Administration–approved FISH ratio defining *HER2* amplification, as well the ratio used for this study, we would estimate that approximately 55% of the *TOP2A*-amplified patients from the National Epirubicin Adjuvant Trial/BR9601 report are actually *TOP2A* normal with *TOP2A*/CEP17 ratios between 1.5 to 2.0. It is not surprising that inclusion of so many potential false-positive patients results in failure to demonstrate any association between *TOP2A* and anthracycline response.

Conversely, our test set of 339 patients indicates that patients with *HER2*-positive breast cancers containing *TOP2A* coamplification have longer PFS and improved OS when compared to *TOP2*-normal patients if they receive anthracyclines. Outcome data within the test set treatment arms show that *HER2*-positive patients receiving only AC have similar PFS/OS improvements to those receiving AC plus trastuzumab if their malignancies contain *TOP2A* coamplification (eg, Fig 2). *HER2*-positive cancers lacking *TOP2A* coamplification show no such incremental benefit from anthracycline compared to nonanthracycline treatment. The much larger validation set consisted of 4,604 patients from the BCIRG-005/-006 adjuvant trials and confirms a significant association between *TOP2A* coamplification and improved DFS/RFS as well as OS in women treated with anthracyclines. No association between *TOP2A* coamplification and outcome was observed in nonanthracycline-based treatment arms (taxol in the H0648 study and TCH in BCIRG-006) underscoring the biologic and therapeutic significance of an association between *TOP2A* amplification and incremental anthracycline sensitivity and indicating that *TOP2A* amplification is a predictive biomarker for anthracycline-based chemotherapies. It has also been suggested that *TOP2A* deletions are associated with increased anthracycline response, however, there are only two such studies, both showing nonsignificant trends between *TOP2A* deletions and anthracycline response.[38,53] We are unaware of any other study reporting such an association and our current data, do not support this hypothesis. Moreover, compelling preclinical studies indicate that *TOP2A* deletions are associated with anthracycline resistance rather than improved responsiveness.[42,45]

Results from this study demonstrate that women whose cancers have *HER2*/*TOP2A* coamplification (approximately 8% of breast cancers) experience equivalent DFS, RFS, or OS outcomes whether treated with a trastuzumab-containing regimen or an anthracycline-based regimen without trastuzumab. Breast cancers containing both alterations (*HER2*/*TOP2A* coamplification) benefit equally when treated with either trastuzumab or anthracyclines; however, they appear to receive no additional benefit from combining trastuzumab with anthracyclines. The use of anthracyclines, particularly in combination with trastuzumab, is associated with significant additional long-term toxicities.[29,31,61] The findings from multiple studies[6,8,33,51,52,62-68] as well as a published meta-analysis[35] indicate that the incremental benefit from anthracyclines reported in breast cancer is restricted to *HER2*-positive patients. This study of 4,943 patients confirms these findings and demonstrates that this differential anthracycline benefit is associated with *TOP2A* coamplification. Taken together, these data indicate that anthracycline-based adjuvant therapies, with their attendant short and long-term risks, should only be considered for the approximately 8% of human breast cancers that have *HER2/TOP2A* coamplification and, then, only in patients who do not receive a *HER2*-targeted therapy like trastuzumab.

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.

Employment or Leadership Position: Marc Buyse, International Drug Development Institute (C); Isabelle Tabah-Fisch, sanofi-aventis (C) **Consultant or Advisory Role:** Michael F. Press, Genentech (C), GlaxoSmithKline (C); Wolfgang Eiermann, sanofi-aventis (C), Roche (C), Novartis (C), AstraZeneca (C), Merck (C), Genentech (C); Miguel Martin, Roche (C); Nicholas Robert, Roche (C), sanofi-aventis (C) **Stock Ownership:** Marc Buyse, International Drug Development Institute; Isabelle Tabah-Fisch, sanofi-aventis **Honoraria:** Michael F. Press, Genentech, GlaxoSmithKline; Wolfgang Eiermann, sanofi-aventis, Roche, Merck, Novartis, AstraZeneca; Miguel Martin, Roche; Nicholas Robert, Roche, sanofi-aventis; John Crown, sanofi-aventis **Research Funding:** Michael F. Press, Genentech, GlaxoSmithKline, Ventana Medical Systems; Nicholas Robert, Roche, sanofi-aventis; John Crown, sanofi-aventis; Mary-Ann Lindsay, sanofi-aventis, Genentech **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Michael F. Press, Dennis J. Slamon
Financial support: Michael F. Press
Administrative support: Michael F. Press, Ivonne E. Villalobos, Isabelle Tabah-Fisch, Mary-Ann Lindsay
Provision of study materials or patients: Michael F. Press, Wolfgang Eiermann, Tadeusz Pienkowski, Miguel Martin, Nicholas Robert, John Crown, Kerry J. Flom, Giovanni Pauletti, Dennis J. Slamon
Collection and assembly of data: Michael F. Press, Guido Sauter, Marc Buyse, Roberta Guzman, Angela Santiago, Ivonne E. Villalobos, Wolfgang Eiermann, Nicholas Robert, Valerie Bee, Henry Taupin, Giovanni Pauletti
Data analysis and interpretation: Michael F. Press, Marc Buyse, Leslie Bernstein, Roberta Guzman, Angela Santiago, Miguel Martin, Valerie Bee, Henry Taupin, Kerry J. Flom, Giovanni Pauletti, Dennis J. Slamon
Manuscript writing: All authors
Final approval of manuscript: All authors

© 2010 by American Society of Clinical Oncology

REFERENCES

1. Clarke M, Collins R, Darby S, et al: Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 365:1687-1717, 2005

2. Doyle J, Neugut A, Jacobson J, et al: Chemotherapy and Cardiotoxicity in older breast cancer patients: A population-based study. J Clin Oncol 23:8597-8605, 2005

3. Hershman D, Neugut A, Jacobson J, et al: Acute myeloid leukemia or myelodysplastic syndrome following use of granulocyte colony-stimulating factors during breast cancer adjuvant chemotherapy. J Natl Cancer Inst 99:196-205, 2007

4. Pinder M, Duan Z, Goodwin J, et al: Congestive heart failure in older women treated with adjuvant anthracycline chemotherapy for breast cancer. J Clin Oncol 25:3808-3815, 2007

5. Muss H, Thor A, Berry D, et al: *cerb* B-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. N Engl J Med 330:1260-1266, 1994

6. 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 90:1361-1370, 1998

7. Piccart-Gebhart M: Anthracyclines and the tailoring of treatment for early breast cancer. N Engl J Med 354:2177-2179, 2006

8. Pritchard K, Shepherd L, O'Malley F, et al: HER2 and responsiveness of breast cancer to adjuvant chemotherapy. N Engl J Med 354:2103-2111, 2006

9. Thor A, Berry D, Budman D, et al: ErB-2, p53, and Efficacy of Adjuvant Therapy in Lymph Node-Positive Breast Cancer. J Natl Cancer Inst 90:1346-1360, 1998

10. Pegram M, Finn R, Arzoo K, et al: The effect of HER-2/neu overexpression on chemotherapeutic drug sensitivity in human breast and ovarian cancer cells. Oncogene 15:537-547, 1997

11. Bièche I, Tomasetto C, Régnier C, et al: Two distinct amplified regions at 17q11-q21 involved in human primary breast cancer. Cancer Res 56:3886-3889, 1996

12. Smith K, Houlbrook S, Greenall M, et al: Topoisomerase II co-amplification with erbB2 in human primary breast cancer, and breast cancer cell lines: Relationship to m-AMSA and mitoxantrone sensitivity. Oncogene 8:933-938, 1993

13. Tomasetto C, Regnier C, Moog-Lutz C, et al: Identification of four novel human genes amplified and overexpressed in breast carcinoma and located to the q11-q21.3 region of chromosome 17. Genomics 28:367-376, 1995

14. van de Vijver M, van de Bersselaar R, Devilee P, et al: Amplification of the neu (c-erbB-2) oncogene in human mammary tumors is relative frequent and is often accompanied by amplification of the linked c-erbA oncogene. Mol Cell Biol 7:2019-2023, 1987

15. Hoare S, Freeman C, Coutts J, et al: Identification of genetic changes associated with drug resistance by reverse in situ hybridization. Br J Cancer 75:275-282, 1997

16. Keith W, Douglas F, Wishart G, et al: Co-amplification of erbB2, topoisomerase II and retinoid acid receptor genes in breast cancer and allelic loss at topoisomerase I on chromosome 20. Eur J Cancer 29A:1469-1475, 1993

17. Murphy D, McHardy P, Coutts J, et al: Interphase cytogenetic analysis of erbB2 and topoII co-amplification in invasive breast cancer and polysomy of chromosome 17 in ductal carcinoma in situ. Int J Cancer 64:18-26, 1996

18. Park K, Kim J, Lim S, et al: Topoisomerase II-alpha (topoII) and HER2 amplification in breast cancers and response to preoperative doxorubicin chemotherapy. Eur J Cancer 39:631-634, 2003

19. Slamon D, Leyland-Jones B, Shak S, et al: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344:783-792, 2001

20. Eiermann W, Pienkowski T, Crown J, et al: Phase III study of doxorubicin / cyclophosphamide with concomitant versus sequential docetaxel as adjuvant treatment in patients with HER2-normal, node-positive breast cancer: Breast Cancer International Research Group (BCIRG)-005 Trial. J Clin Oncol in review 2010

21. Slamon D, Eiermann W, Robert N, et al: Efficacy and Safety of Adjuvant Trastuzumab in HER-2 Positive Breast Cancer. N Engl J Med in press 2010

22. Press M, Sauter G, Bernstein L, et al: Diagnostic Evaluation of HER-2/neu as a Molecular Therapeutic Target: Local Testing versus Centralized FISH Testing. Clinical Cancer Res 11:6598-6607, 2005

23. Mass R, Press M, Anderson S, et al: Evaluation of clinical outcomes according to HER2 detection by fluorescence in situ hybridization in women with metastatic breast cancer treated with trastuzumab. Clinical Breast Cancer 6:240-246, 2005

24. Sauter G, Lee J, Bartlett JM, et al: Guidelines for human epidermal growth factor receptor 2 testing: Biologic and methodologic considerations. J Clin Oncol 27:1323-1333, 2009

25. Bofin A, Ytterhus B, Hagmar B: Topo2A and HER-2 gene amplfication in fine needle aspirates of breast carcinomas. Cytopathology 14:314-321, 2003

26. Olsen K, Knudsen H, Rasmussen B, et al: Amplification of HER2 and TOP2A and deletion of TOP2A genes in breast cancer investigated by new FISH probes. Acta Oncol 43:35-42, 2004

27. Kaplan E, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457, 1958

28. Peto R, Pike M, Armitage P, et al: Design and analysis of randomized clinical trials requiring prolonged observation of each patient. I. Introduction and design. Br J Cancer 34:585-612, 1976

29. Chien KR: Herceptin and the heart–a molecular modifier of cardiac failure. N Engl J Med 354:789-790, 2006

30. Levine M: Trastuzumab cardiac side effects: Only time will tell. J Clin Oncol 23:7775-7776 2005

31. Tan-Chiu E, Yothers G, Romond E, et al: Assessment of cardiac dysfunction in a randomized trial comparing doxorubicin and cyclophosphamide followed by paclitaxel, with or without trastuzumab as adjuvant therapy in node-positive, human epidermal growth factor receptor 2-overexpressing breast cancer: NSABP B-31. J Clin Oncol 23:7811-7819, 2005

32. Dressler L, Berry D, 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

33. 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 92:1991-1998, 2000

34. Ravdin P, Green S, Albain K, et al: Initial report of the SWOG biological correlative study of c-erB-2 expression as a predictor of outcome in a trial comparing adjuvant CAF T with tamoxifen (T) alone. Proc Am Soc Clin Oncol 17:A374, 97a, 1998

35. Gennari A, Sormani M, Pronzato P, et al: HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: A pooled analysis of randomized trials. J Natl Cancer Inst 100:14-20 2008

36. O'Malley F, Chia S, Tu D, et al: Prognostic and predictive value of topoisomerase II alpha in a randomized trial comparing CMF to CEF in premenopausal women with node positive breast cancer (NCIC CTG MA. 5). J Clin Oncol Vol 24, No. 18S (June 20 Supplement) 533, 2006

37. O'Malley F, Chia S, Tu D, et al: Topoisomerase II alpha and Responsiveness of Breast Cancer to Adjuvant Chemotherapy. J Natl Cancer Inst 101: 644-650, 2009

38. Nielsen K, Ejlertsen B, Moller S, et al: The value of TOP2A gene copy number variation as a biomarker in breast cancer: Update of DBCG trial 89D Acta Oncologica 47:725-734, 2008

39. Ejlertsen B, Jensen MB, Nielsen KV, et al: HER2, TOP2A, and TIMP-1 and responsiveness to adjuvant anthracycline-containing chemotherapy in high-risk breast cancer patients. J Clin Oncol 28:984-990, 2010

40. Pritchard K, Messersmith H, Elavathil L, et al: HER-2 and topoisomerase II as predictors of response to chemotherapy. J Clin Oncol 26:736-744, 2008

41. Coon J, Marcus E, Gupta-Burt S, et al: Amplification and overexpression of topoisomerase IIalpha predict response to anthracycline-based therapy in locally advanced breast cancer. Clinical Cancer Res 8:1061-1067, 2002

42. Withoff S, Keith W, Knol A, et al: Selection of a subpopulation with fewer DNA topoisomerase II alpha gene copies in a doxorubicin-resistant cell line panel. Br J Cancer 74:502-507, 1996

43. Gudkov A, Zelnick C, Kazarov A, et al: Isolation of genetic suppressor elements, inducing resistance to topoisomerase II-interactive cytotoxic drugs, from human topoisomerase II cDNA. Proc Natl Acad Sci U S A 90:3231-3235, 1993

44. Zhou Z, Zwelling L, Kawakami Y, et al: Adenovirus-mediated human topoisomerase IIalpha gene transfer increases the sensitivity of etoposide-resistant human breast cancer cells. Cancer Res 59:4618-4624, 1999

45. Jarvinen T, Tanner M, Rantanen V, et al: Amplification and deletion of topoisomerase IIalpha associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. American J Pathol 156:839-847, 2000

46. Withoff S, De Jong S, De Vries E, et al: Human DNA topoisomerase II: Biochemistry and role in chemotherapy resistance. Anticancer Res 16:1867-1880, 1996

47. Durbecq V, Di Leo A, Cardoso F, et al: Comparison of topoisomerase-II alpha gene status between primary breast cancer and corresponding distant metastatic sites. Breast Cancer Res and Treatment 77:199-204, 2003

48. Jarvinen T, Tanner M, Barlund M, et al: Characterization of topoisomerase II-alpha gene amplification and deletion in breast cancer. Genes Chromosomes Cancer 26:142-150, 1999

 © 2010 by American Society of Clinical Oncology

49. Jarvinen T, Liu E: HER-2/neu and topoisomerase IIalpha in breast cancer. Breast Cancer Res Trt 78:299-311, 2003

50. Jarvinen T, Liu E: Topoisomerase IIalpha gene (TOP2A) amplification and deletion in cancer–more common than anticipated. Cytopathology 14:309-313, 2003

51. Di Leo A, Gancberg D, Larsimont D, et al: HER-2 amplification and topoisomerase IIalpha 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. Clinical Cancer Res 8:1107-1116, 2002

52. Di Leo A, Larsimont D, Gancberg D, et al: HER-2 and topo-isomerase IIalpha as predictive markers in a population of node-positive breast cancer patients randomly treated with adjuvant CMF or epirubicin plus cyclophosphamide. Ann Oncol 12:1081-1089, 2001

53. 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 Cooperative Group. J Clin Oncol 23:7483-7490, 2005

54. Press M, Slamon D, Flom K, et al: Evaluation of HER-2/*neu* Gene amplification and overexpression: Comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens. J Clin Oncol 20:3095-3105, 2002

55. Di Leo A, Isola J, Piette F, et al: A meta-analysis of phase III trials evaluating the predictive value of HER2 and topoisomerase II alpha in early breast cancer patients treated with CMF or anthracycline-based adjuvant therapy. Cancer Res 69, 2009 (suppl; abstr 705)

56. Harris LN, Broadwater G, Abu-Khalaf M, et al: 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. J Clin Oncol 27:3430-3436, 2009

57. Tubbs R, Barlow WE, Budd GT, et al: Outcome of patients with early-stage breast cancer treated with doxorubicin-based adjuvant chemotherapy as a function of HER2 and TOP2A status. J Clin Oncol 27:3881-3886, 2009

58. Pritchard KI: Are HER2 and TOP2A useful as prognostic or predictive biomarkers for anthracycline-based adjuvant chemotherapy for breast cancer? J Clin Oncol 27:3875-3876, 2009

59. Bartlett J, Munro A, Cameron D, et al: Type 1 receptor tyrosine kinase profiles identify patients with enhanced benefit from anthracyclines in the BR9601 adjuvant breast cancer chemotherapy trial. J Clin Oncol 26:5027-5035, 2008

60. Bartlett JMS, Munro AF, Dunn JA, et al: Predictive markers of anthracycline benefit: A prospectively planned analysis of the UK National Epirubicin Adjuvant Trial (NEAT/BR9601). Lancet Oncol 11:266-274, 2010

61. Levine MN: Trastuzumab cardiac side effects: Only time will tell. J Clin Oncol 23:7775-7776, 2005

62. Colozza M, Bisagni G, Mosconi A, et al: Epirubicin versus CMF as adjuvant therapy for stage I and II breast cancer: A prospective randomised study. Eur J Cancer 38:2279-2288, 2002

63. Colozza M, Sidoni A, Mosconi A, 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. Clinical Breast Cancer 6:253-259, 2005

64. De Placido S, Perrone F, Carlomagno C, et al: CMF vs alternating CMF/EV in the adjuvant treatment of operable breast cancer. A single centre randomised clinical trial (Naples GUN-3 study). Br J Cancer 71:1283-1287, 1995

65. Fisher B, Brown A, Dimitrov N, 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-nonresponsive tumors: Results from the National Surgical Adjuvant Breast and Bowel Project B-15. J Clin Oncol 8:1483-1496, 1990

66. Fisher B, Redmond C, Wickerham D, et al: Doxorubicin-containing regimens for the treatment of stage II breast cancer: The National Surgical Adjuvant Breast and Bowel Project experience. J Clin Oncol 7:572-582, 1989

67. Levine M, Bramwell V, Pritchard K, et al: Randomized trial of intensive cyclophosphamide, epirubicin, and fluorouracil chemotherapy compared with cyclophosphamide, methotrexate, and fluorouracil in premenopausal women with node-positive breast cancer. J Clin Oncol 16:2651-2658, 1998

68. Moliterni A, Menard S, Valagussa P, et al: HER2 overexpression and doxorubicin in adjuvant chemotherapy for resectable breast cancer. J Clin Oncol 21:458-462, 2003

© 2010 by American Society of Clinical Oncology