ORIGINAL RESEARCH

Positive response to neoadjuvant cyclophosphamide and doxorubicin in topoisomerase II nonamplified/HER2/neu negative/polysomy 17 absent breast cancer patients

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Purpose: Human epidermal growth factor receptor 2 (HER2)/neu, topoisomerase II alpha (TOP2A), and polysomy 17 may predict tumor responsiveness to doxorubicin (DOX) therapy.

Methods: We identified neoadjuvant DOX/cyclophosphamide treated breast cancer patients in our registry from 1997 to 2008 with sufficient tissue for testing (n = 34). Fluorescence in situ hybridization (FISH) testing was done on deparaffinized tissue sections pretreated using vendor's standard protocol modification, and incubated with US Food and Drug Administration approved Abbott Diagnostics Vysis PathVysion™ probe set, including Spectrum-Green-conjugated probe to α-satellite DNA located at the centromere of chromosome 17 (17p11.1–q11.1) and a Spectrum-Orange-conjugated probe to the TOP2A gene. Morphometric analysis was performed using a MetaSystems image analysis system. Manual counting was performed on all samples in which autofluorescence and/or artifact prevented the counting of sufficient numbers of cells. A ratio >2.0 was considered positive for TOP2A amplification. Polysomy 17 (PS17) presence was defined as signals of ≥2.5. Outcomes were pathological complete response (pCR), partial response (PR), and nonresponse (NR).

Results: Of 34 patients tested, one was TOP2A amplified (hormone receptor negative/HER2 negative, partial responder). The subset of TOP2A nonamplified, HER2 negative, and PS17 absent (n = 23) patients had treatment response: pCR = 2 (9%), PR = 14 (61%), and NR = 7 (30%). Including the two PS17 present and HER2-positive patients (n = 33), 76% of TOP2A nonamplified patients had pCR or PR.

Conclusions: We observed substantial treatment response in patients lacking three postulated predictors that would be difficult to attribute to cyclophosphamide alone. Patients who are HER2 negative and lack TOP2A amplification and PS17 should not be excluded from receiving DOX-containing regimens.

Keywords: neoadjuvant chemotherapy, response rates, anthracycline therapy

Introduction

Recent reports have proposed topoisomerase II alpha (TOP2A) biomarker status as a potential predictor of tumor responsiveness to doxorubicin (DOX) therapy.[1] These studies predominantly evaluated relapse-free survival in the adjuvant setting related to human epidermal growth factor receptor 2 (HER2)-neu and TOP2A status although more recent studies have evaluated direct response rates in the neoadjuvant setting.[1–6]

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Contradictory results have been reported for anthracycline response among HER2 amplified tumors, although in general anthracycline responsiveness has been correlated with HER2 positivity.[7,8] Konecny et al[1] have correlated anthracycline responsiveness with TOP2A amplification and deletion, though again there is no unanimity regarding this observation. Paradoxically, favorable response to anthracyline therapy, especially in the neoadjuvant setting, has been observed in patients with triple-negative breast cancer.[9]

The TOP2A gene product is a target of anthracyclines, and the gene is located in chromosome 17 (17q21–22) in close proximity to HER2. Polysomy 17 (PS17) has also been proposed as a predictive marker for anthracycline sensitivity although there is far less data available regarding this observation.[8] Our study aim is to evaluate tumor response in a retrospective cohort of breast cancer patients treated with neoadjuvant DOX and cyclophosphamide (C) in relation to HER2, topoisomerase II, and PS17 expression.

Materials and methods

We retrospectively identified all breast cancer patients in our community-based cancer clinic registry cohort treated with neoadjuvant DOX and cyclophosphamide (C) only prior to surgery. Hormone receptor (HR) and HER2 testing were completed as part of their initial clinical evaluation. Forty-five patients in our patient registry had neoadjuvant DOX/C treatment and were given no other neoadjuvant therapy. Thirty-four of these patients had sufficient tissue available for testing and adequate signal for measurement of TOP2A gene amplification. Choice of neoadjuvant treatment drug and number of cycles to be delivered was made by the treating physician.

Estrogen receptor and progesterone receptor status were assessed by immunohistochemistry (IHC) at various local laboratories; HER2 status was assessed by either IHC or fluorescent in situ hybridization (FISH) or both in the case of an equivocal IHC result (FISH). Breast biopsy tissue was preserved in paraffin blocks and stored at room temperature prior to testing. FISH testing was done on deparaffinized tissue sections pretreated using a modification of the vendor's standard protocol, and then incubated with the US Food and Drug Administration-approved Vysis PathVysion™ probe set, which includes Spectrum-Green-conjugated probe to the α-satellite DNA located at the centromere of chromosome 17 (17p11.1–q11.1) and a Spectrum-Orange-conjugated probe to the TOP2A gene (Abbott Diagnostics, Chicago, IL, USA).

For every batch of tumor sections, appropriate quality control sections were used for FISH testing. Morphometric analysis was performed using a MetaSystems image analysis system, incorporating the Metafer software with extended focus/tile sampling methodology (MetaSystems, Altlussheim, Germany). Manual counting was performed on all samples in which autofluorescence and/or artifact prevented the counting of sufficient numbers of cells. A ratio >2.0 is considered positive for TOP2A amplification. PS17 presence was defined as signal of ≥2.5. Figure 1 illustrates TOP2A present amplification.

Pretreatment tumor size was determined by imaging and physical examination and posttreatment size was determined from the pathological specimen and the pathologist's report with response measured postsurgery after receipt of DOX/C neoadjuvant therapy. The clinical end point was response to treatment and was measured as pathological complete response (pCR), partial response (PR), and nonresponse (NR) using RECIST criteria.[10] Response was assigned after surgery and pCR was assigned if there was no residual disease in the breast tissue or lymph nodes. PR was assigned if there was evidence of 30% or greater decrease in tumor size (PR) and no response (NR) if the tumor size reduction was less than 30%.

Statistical analysis was done using SPSS 17.0 software for univariate and bivariate analysis.[11] Pearson chi-square tests were done to identify significant differences between groups using a 2-sided test for probability using the Fisher's Exact test for small number comparisons.
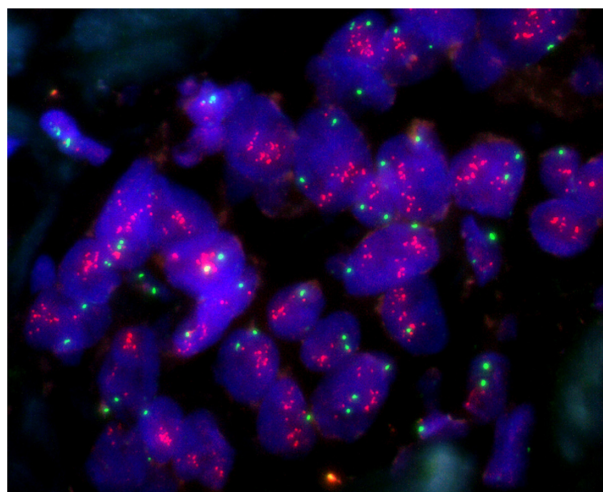

Figure 1 TOP2A amplified cells.

Results

The 34 patients tested for TOP2A were diagnosed between 1997 and 2008 with TNM stage II-IV breast cancer and were treated with neoadjuvant DOX and cyclophosphamide. Patients' age range was 29–66 years with a mean of 46.47 and a median age of 46. Seventy-six percent of the patients had clinically positive lymph node status. Clinical tumor size at the time of diagnosis was 1.6–11.0 cm with 5/34 (14.7%) equal to 1.6–2.5 cm, a mean of 6.13 cm and median of 6.0 cm. The most common histologic type was ductal carcinoma (79%) with 88% exhibiting high histologic grade and 71% with high nuclear grade. Out of 34 patients tested for topoisomerase II, one was TOP2A amplified (HR−/HER2−, partial responder). A total of 18 out of 34 were HR+ and 9 were HER2+. Data are presented in Table 1.

Twenty-four of the 34 patients were treated with 4 cycles of DOX/C, 3 of 34 had 5 cycles of DOX/C, and 4 of 34 had 6 cycles of DOX/C. One of 34 patients had 2 cycles and 1/34 had 3 cycles. One of the 34 patients had a weekly treatment protocol as part of a randomized clinical trial (RCT) receiving 15 weeks of DOX/C therapy. The 2 patients with less than 4 cycles of therapy had early substantial reduction in tumor size (PR) and went to surgery. Descriptives are presented in Table 1.

Among the subset of TOP2A normal patients (n = 33), treatment response was pCR = 4 (12.1%), PR = 21 (63.6%), and NR = 8 (24.2%) with the majority (75.7%) exhibiting significant responsiveness (pCR or PR) to DOX/C. Distribution by HR and HER2 status and therapy response for the subset of TOP2A nonamplified/PS17 absent patients (n = 31) was (1) HR−/HER2− (n = 9), 22% pCR, 56% PR, 22% NR; (2) HR+/HER2− (n = 14), 64% PR, 36% NR; (3) HR−/HER2+ (n = 6), 33% pCR, 67% PR; and (4) HR+/HER2+ (n = 2), 50% PR, 50% NR. For the subset of HER2 negative and TOP2A nonamplified patients (n = 24), the response rates were 8% pCR (2/24), 63% PR (15/24), and 29% NR (7/24). For the subset of HER2 negative, TOP2A nonamplified and PS17 absent patients (n = 23), treatment response were pCR = 2 (9%), PR = 14 (61%), and NR = 7 (30%). See Table 2 and Figure 2 for summary.

When response to treatment was analyzed for all cases (n = 34) by HR and HER2 status, the best response was among the HR−/HER2+ patients with 100% partial or complete response followed by the triple-negative group with 80% partial or complete response (Pearson χ^2 = 7.32, 6 degrees of freedom, P = 0.293; Table 3).

Table 1 Descriptors of patients tested for TOP2A (n = 34)

Variable	N (%)
Age in years	
Range	29–66
Mean	46.47
TNM stage	
II	11 (32%)
III	19 (56%)
IV	4 (12%)
Lymph node status	
Negative	8 (24%)
Positive	26 (76%)
Clinical tumor size, cm	
Range	1.6–11.0
Mean	6.13
Histologic type	
Ductal	27 (79%)
Lobular	3 (9%)
Other	4 (12%)
Histologic grade	
Intermediate	2 (9%)
High	30 (88%)
Nuclear grade	
Low to intermediate	9 (26%)
Intermediate	1 (3%)
High	24 (71%)
ER = positive	17 (50%)
PR = positive	11 (32%)
Her2 = positive	9 (27%)
TOP2A	
Normal	33 (97%)
Amplified	1 (3%)
PS17	
Not present	32 (94%)
Present	2 (6%)
Number of treatment cycles	
Weekly	1 (3%)
<4	2 (6%)
4	24 (71%)
5	3 (9%)
6	4 (12%)

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TOP2A, topoisomerase II alpha; PS17, polysomy 17.

Table 2 Summary of response to treatment by HER2 negative, TOP2A absent, and polysomy 17 absent status

HER2 status	TOP2A status	Polysomy 17 status	N	pCR, %	PR, %	NR, %
–	Nonamplified	–	33	12	64	24
–	Nonamplified	Absent	31	13	61	26
Negative	Nonamplified	–	24	8	63	29
Negative	Nonamplified	Absent	23	9	61	30

Abbreviations: HER2, human epidermal growth factor receptor 2; TOP2A, topoisomerase II alpha, pCR, pathological complete response; PR, partial response; NR, nonresponse.
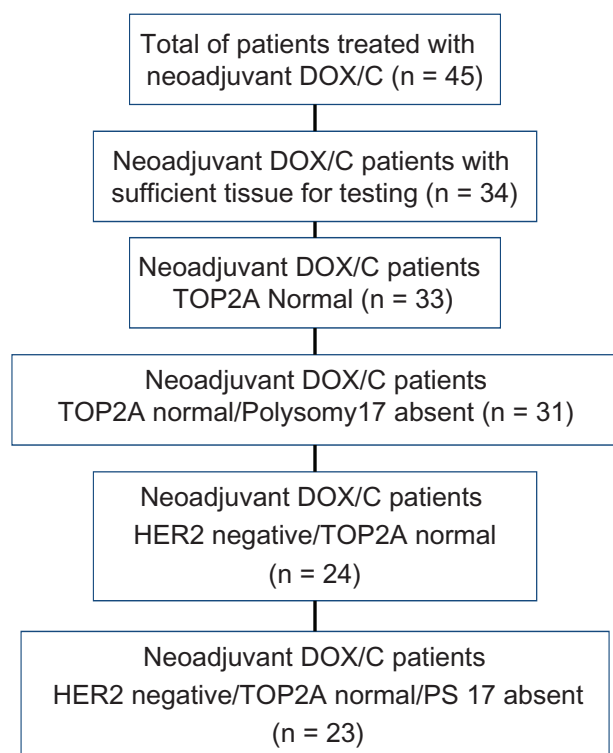
Figure 2 Diagram of neoadjuvant treatment study subjects.

Discussion

Topoisomerase is a known target for anthracycline cyto-toxicity and it has been proposed that alterations in TOP2A correlate with clinical antitumor activity making TOP2A a biomarker candidate for treatment selection decision making. This theory has been evaluated by retrospective review of TOP2A status and mortality outcomes of patients in random-ized controlled trials (RCTs) and cohort studies, and response to treatment in neoadjuvant studies with mixed results in all three types of studies.[5,9,10,12–14] Similarly, HER2 status has been correlated with anthracycline response to varying degrees, though reviews of the literature have led some investigators to conclude that anthracycline benefit may not be predicted by HER2 or TOP2A status.[15,16] Most recently, Bartlett et al[8] proposed that PS17 duplication (CH17CEP) is the strongest predictor of treatment benefit with anthracyclines.[8] They observe that the presence of HER2/TOP2A on chromosome 17 may explain the inconsistent results observed in regards to these biomarkers and anthracycline benefit.

To further assess these indicators, we reviewed the response of all patients who received neoadjuvant DOX/C alone at our institution. In particular, we examined the response of the patients in this group who lacked all three putative predictors of anthracycline responsiveness and also correlated response with HR status. The strength of our study is the use of neoadjuvant treatment tumor response as the outcome of interest instead of survival over time which has been the outcome measurement in RCT reviews. The weakness of our study is the absence of a sufficient number of TOP2A-amplified patients for a comparison group. It is and would be difficult to obtain a subset of TOP2A-amplified patients for the review of response to neoadjuvant therapy as neoadjuvant patients are more likely to be triple negative with a lower percentage of TOP2A amplification present. To assemble a suitably powerful cohort would require an initial sample size of approximately 200 patients not predominantly triple negative with at least 25% HER2 positive and 10% TOP2A-amplified patients (n = 20). A sample size of this magnitude would allow for an adequate observational report, but to evaluate the net benefit of TOP2A as a biomarker with the capacity to advise treatment selection an RCT has prefer-able strength. In lieu of these currently unobtainable options at our institution, we chose to conduct a study with results that can reassure clinicians of the effectiveness of anthra-cycline therapy previously proven by RCTs and currently called into question by underpowered studies with indirect end points, ie, disease specific and all cause mortality.

Among topoisomerase II normal patients, response was substantial with a 76% combined complete and PR rate. Both pCR and overall response rates were highest among HR-negative patients when the 34 cases were compared with HR and HER2 status, though we were unable to com-pare response with HR−/HER2+ nonresponders due to an absence of patients in that category. Patients who lacked HER-2/neu, PS17, and TOP2A amplification still exhibited a combined pCR/PR of 70% (pCR of 9%). By comparison, TOP2A-amplified patients had zero response to neoadju-vant therapy (n = 3) in the study by Kawachi et al[3] from a total sample of 14 patients and a pCR of 60% (3/5) was observed in TOP2A-amplified tumors that were estrogen and

Table 3 Response to treatment by hormone receptor and HER2 status (n = 34)[a]

Hormone receptor status	HER2 status	N	pCR, %	PR, %	NR, %
Negative	Negative	10	20	60	20
Positive	Negative	15	0	67	33
Negative	Positive	6	33	67	0
Positive	Positive	3	0	67	33
Total		34	12	65	24

[a]Pearson χ^2 = 7.32, 6 degrees of freedom, P = 0.293 (3 zero cells).
Abbreviations: HER2, human epidermal growth factor receptor 2; pCR, pathological complete response; PR, partial response; NR, nonresponse.

progesterone receptor negative and HER2 positive reported by Orlando et al[5] from a total sample size of 23 patients. Both of these studies had fewer patients, n = 14 and n = 23, than our current study but had more TOP2A-amplified patients, n = 3 and n = 5, respectively.

Conclusions

Initial drug trials in the 1960s using single-agent cyclophosphamide showed a 27% PR rate in the treatment of metastatic breast cancer.[17] Complete responses were rare. Although it may be possible that response rates for cyclophosphamide may be slightly higher than the rate of 27% reported from early trials, it is extremely unlikely that the partial and complete response rates observed in our patient population could be obtained by cyclophosphamide alone. Although a number of studies have suggested that HER2, TOP2A, and/or PS17 amplification may predict response to DOX, we observed substantial response to treatment in patients lacking all 3 postulated predictors of therapy that would be difficult to attribute to cyclophosphamide alone. Our results indicate HR and HER2 status are dominant indicators of response in the absence of TOP2A and PS17 amplification. Our results replicate the observations of others that triple-negative breast cancer (HR–/HER2–) patients have a high response rate to anthracyclines.[18] HER2-negative patients have previously been shown to have a very low incidence of TOP2A amplification or deletion so triple-negative patients likely lack TOP2A amplification or deletion as well.[19]

In general, there is a strong correlation between pCR from neoadjuvant chemotherapy and overall survival, but poor long-term survival has been observed in triple-negative disease despite positive response to initial therapy.[18] Factors other than those examined here may be involved in the initial response to treatment and those factors may change during the natural history of the disease. Tenari et al[20] has reported that TOP2A levels change over time and with treatment. Ejlertsen et al[21] has recently reported a correlation between anthracycline responsiveness and tissue inhibitor of matrix metalloproteinases 1(TIMP1), suggesting another molecular target that requires further analysis.

We found a clear utility of anthracycline/cyclophosphamide combined therapy in a cohort of patients treated with neoadjuvant therapy prior to surgery. Future studies may find a link or new marker that will correlate with and/or predict response to anthracycline therapy, but at this time there is insufficient evidence to exclude patients from receiving anthracycline therapy based on HER2, TOP2A, and/or PS17 expression.

Disclosure

The authors report no conflicts of interest.

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