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CLINICAL TRIAL

Genomic predictors of response to doxorubicin versus docetaxel in primary breast cancer

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Abstract Taxanes and anthracyclines improve the outcome of early breast cancer, although the benefit is limited to a small proportion of patients and are toxic. We prospectively looked for predictors of response to these drugs. Experimental design: Four cycles of doxorubicin (75 mg/m^2) or docetaxel (100 mg/m^2) were compared as presurgical chemotherapy for breast cancer. Biomarkers were determined by immunohistochemistry and fluorescent in situ hybridization using prechemotherapy core biopsies. Tumors were also classified into one of the molecular intrinsic subtypes using an immunohistochemical panel of five biomarkers and genomic profiles. Single genes and intrinsic subtypes were correlated with response to doxorubicin versus docetaxel. Among the 204 evaluable patients, significant predictors of sensitivity in multivariate analysis were low topo2a expression and ER-negative status for doxorubicin and small tumor size and ER-negative status for docetaxel. Predictors of resistance in multivariate analysis were triple-negative status (ER/PgR/HER2 negative by IHC/FISH) for doxorubicin, and high TNM stage for docetaxel. Triple-negative tumors were associated with topo2a overexpression more than the other subtypes. In 94 patients with gene expression profiles, docetaxel was superior to doxorubicin in the basal-like subtype (good pathological response rate − PCR + class I of 56 vs. 0%; $P = 0.034$); no significant differences were observed in the other subtypes when comparing these two drugs. Low topo2a expression and ER-negative status were predictors of response to doxorubicin, while small tumor size and ER-negative status predicted response to docetaxel. Docetaxel was superior to doxorubicin in triple-negative/basal-like tumors, while no significant differences were seen in the remaining intrinsic subtypes.

Keywords Breast cancer · Doxorubicin · Docetaxel · Genomic subtypes

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Introduction

The initial trials of adjuvant chemotherapy (CMF-like regimens) showed that these regimens could improve the outcomes of operable breast cancer patients [1]. Later, anthracycline combinations were found to be slightly but significantly more effective than the CMF regimen [2]. More recently, several adjuvant phase III trials have shown that taxane regimens further reduce the likelihood of recurrence and death, compared to anthracycline-containing combinations [3–10], but the absolute benefit was again small, and some trials were unable to show any advantage

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for the taxane arm [11, 12]. Taxanes and anthracyclines can induce some acute and chronic toxicities of considerable clinical concern. Hence there is a pressing need to identify predictors for anthracycline and taxane response in breast cancer treatment.

The aforementioned adjuvant trials were conducted using unselected populations of breast cancer patients. Genomic studies performed in the early 2000s have revealed that breast cancer is a molecularly heterogeneous disease consisting of at least four to five different tumor subtypes [13–16]. In neoadjuvant trials using combinations of anthracycline and taxane, the sensitivity of the different molecular subtypes to chemotherapy varies; the basal-like and Her2-enriched subtypes showing the best response [17, 18]. However, the relative contributions of each of these two classes of agents to response is still unknown.

To identify predictors of response, we performed this comparative, randomized neoadjuvant study comparing single agent docetaxel versus single agent doxorubicin in patients with locally advanced breast cancer.

Patients and methods

Women eligible were aged between 18 and 79 years with clinical stage IIB, IIIA or IIIB breast cancer not amenable to breast preserving surgery. The study was approved by the institutional review board of the institutions (identifier code: NCT 00123929). All patients signed an informed consent form before being enrolled in the trial. A complete staging workup, including bilateral mammography and MRI, sonography of the affected breast, body CT scan, and bone scan, was carried-out prior to recruitment into the study.

Study objectives

The primary study goal was to identify differential molecular/genomic predictors of response and resistance to single agent docetaxel versus doxorubicin.

Study procedures

Core biopsies of the tumor were obtained following the patient's informed consent for participation in the trial. Eligible patients were then randomly assigned to receive four cycles of either doxorubicin (75 mg/m^2 body-surface area) or docetaxel (100 mg/m^2 body-surface area) every 3 weeks followed by surgery. After surgery, patient treatment assignment was crossed-over to receive four cycles of the opposite drug, plus radiation therapy. Patients whose tumors were positive for hormone receptors received tamoxifen, or aromatase inhibitors, or a sequence of both for at least 5 years. From May 2005 onwards, patients with her2-amplified tumors received trastuzumab after surgery.

Evaluation of response

Clinical response was evaluated according to RECIST criteria comparing pre and post-chemotherapy MRI breast assessments.

Pathological response was evaluated in the surgery specimen (either lumpectomy or mastectomy; both with additional axillary clearance) according to the residual cancer burden (RCB) classification of Symmans et al. [19]. Patients with PCR and class I were considered as having a good pathological response (good PathResp) since both have an equivalent good prognosis, while those patients with class III were considered as resistant to therapy. This classification also provides a continuous variable (RCB) which measures the amount of residual tumor burden, which could provide additional information.

Molecular and genomic studies

IHC and FISH techniques

Paraffin-embedded tumor samples from core biopsies were evaluated by IHC analysis for p27 protein (mouse monoclonal antibody NCL-p27, Clone 1B4, 1:30 Leica Microsystems); topoisomerase IIα-topo2a-mouse monoclonal antibody NCL-Clone 3F6, 1:40 Leica Microsystems); BCL-2 (mouse monoclonal anti-human BCL-2 Oncoprotein, Clone 124, 1:100. Dako Cytomation), tau protein (Polyclonal Rabbit Anti-Human Tau dilution 1:200), estrogen receptor-ER (Dako Cytomation Clone 1D5, 1:35), progesterone receptor-PR (Dako Cytomation, Clone PgR 636, 1:50), epidermal growth factor receptor (EGFR, clone EGFRr.25, 1:50. Leica Microsystems), cytokeratins 5/6 (CK 5/6, clone D5/16B4, prediluted, Master Diagnostica) and Ki67 (Dako Cytomation, Clone MIB-1, 1:75). After incubation with the primary antibodies, the Bond Polymer Refine Detection with the Vision Biosystems Bondmax for immunostaining was applied.

Tau protein positivity was defined as $\geq$20% of stained cells, since the staining in our normal control breast tissue was always below that figure. The same cut-off point was used for Ki67. Since there is no standard cut-off for topoisomerase II alpha positivity, both means (20% of stained cells, pre-specified cut-off point in the protocol) and median values (10% of stained cells) were used for repeated analysis. Positivity for p27, BCL-2, EGFR, and CK 5/6 was defined as any degree of positive staining. The cut-off value for ER and PR positivity was established at

≥10% of stained cells in the original protocol but, again, the univariate and multivariate analysis were also repeated using a cut-off point of 1%.

HER2 and *TOP2A* gene amplification were evaluated by fluorescence in situ hybridization (FISH) (*HER2* FISH 30-161060 Path Vysion HER-2 DNA and the *TOPII* α FISH 32-190095 Vysis LSI *TOPII* α, respectively) and to centromere 17 according to manufacturer's instructions. Tumors were considered positive for *HER2* if amplification ratio more than 2.2 and for *TOP2A* if amplification ratio more than 2. The cut-off for the deletion positivity was established as a *TOP2A* genes to chromosome 17 ratio of less than 0.5. All the cut-off points were predefined before the correlations with response were performed. In all the determinations, the pathologist was blinded from patient's outcome and treatments.

Tumors were classified into molecular intrinsic subtypes based either on IHC/FISH parameters or using gene expression profiles [20–22]. The first method was based on an immunopanel of four biomarkers previously described by Hugh et al. [22] that includes 4 subtypes (luminal A and B, HER2, and triple negative):

- Luminal A: ER^+ and/or PR^+, $HER2^-$ (FISH), KI67 ≤ 13%
- Luminal B: ER^+ and/or PR^+, and either $HER2^+$ (FISH) or KI67 equal or superior to 14%
- *HER2*: ER^- and PR^-, $HER2^+$ (FISH)
- Triple-negative: ER^-, PR^-, and $HER2^-$ (FISH)

The second method was based on Agilent Human oligonucleotide microarrays. 94 fresh frozen core biopsies were assayed on customized 44,000 feature Agilent Human oligonucleotide microarrays. Total RNA purification and microarrays hybridization were done as previously described in Parker et al. [20]. The primary microarray data presented in this study is available in the GEO under accession number GSE21997. Tumors were classified into an intrinsic subtype using the PAM50 50-gene assays [20]. In addition, a recent identified subtype, namely Claudin-low, was also scored for using a centroid based predictor for this subtype [21]; in total, these two predictors result in a six subtype classification system that is Luminal A, Luminal B, Basal-like, HER2-enriched, Claudin-low, and Normal-like.

Statistical analysis

An empirical sample size of 100 evaluable patients in each treatment arm was established based on an assumption that if powerful predictors to response for these two drugs did exist, this sample size would be sufficient to detect a difference, and large enough to rule out the weak association of less important molecular markers.

The significant asassociation between categorical variables was tested by either chi-squared or Fisher exact test when appropriate. The association between the RBC index scores and other clinicopathological variables was assessed by *U* Mann–Whitney/*t* student test. Variables with clear or borderline statistical significance response to treatment in univariate analysis were included in a multivariable stepwise logistic regression model. Likelihood ratio test were used if the variables add significance to the predictive model.

Finally, a logistic regression analysis was performed for PathResp involving the overall population (204 patients). The covariates used in the analysis were those previously described as well as the presence/absence of interaction between the molecular predictors of response to either drug (top2A and ER expression) and the chemotherapy treatment. Adjustments were made for multiple comparisons. The statistical analysis was performed using SPSS 15.0/strata 10 package. All tests were two-tailed and P values <0.05 were considered significant.

Results

226 patients were initially registered; 211 were eligible and randomized to docetaxel ($n = 104$) or doxorubicin ($n = 107$); 204 patients (100 in the docetaxel arm and 104 in the doxorubicin arm) were fully evaluable for the statistical analyses of biomarker predictors and pathological response (see Appendix). The clinical and demographic characteristics of the patients are shown in Table 1. Genomic profiling was successfully performed in 94 patients (46% of total), whose characteristics were not significantly different from the overall sample (data not shown).

Anti-tumor activity

Clinical objective response rates (RECIST criteria) were 67% for doxorubicin and 77% for docetaxel ($P = 0.12$). The rates of good PathResp were 19% in the doxorubicin arm and 20% in the docetaxel arm ($P = 0.89$).

Prediction of anti-tumor activity: single biomarker model

Prediction of chemo-sensitivity (good PathResp; Symmans class 0 + I) Table 2 summarized the results of univariate analysis by treatment arm. The multivariable logistic regression analysis (Table 3) showed that topo2a and ER expression were the two independent significant molecular markers to response to doxorubicin. ER status provided significant additional information over topo2a (Wald likelihood-ratio test; $P = 0.0105$). Tumor size (Tsize) and ER

Table 1 Characteristics of the evaluable patients

Variable	Doxorubicin N	Docetaxel N
Number of evaluable patients	104	100
Median age; years (range)	52 (26–79)	51 (27–77)
Tumor size (cm); median (range)	6 (2–15)	6 (2–15)
Patients with tumors > 5 cm	60	55
Histology type		
Ductal	83	78
Lobular	18	16
Others	3	6
UICC stage		
II	37	37
IIIA	35	31
IIIB	32	33
Tumor grade		
3	39	37
1–2	65	63

status were the two independent predictors to response to docetaxel. Again, ER status provided additional information over Tsize (Wald likelihood-ratio test; $P = 0.0004$).

The results were remarkably similar when a cut point of 10% (topo2a) and 1% (hormone receptors status) were used to define positivity (data not shown).

To investigate if there was an interaction between the significant variables and treatments, an additional multivariable logistic model was built by combining the doxorubicin-treated and docetaxel-treated patients. The interaction terms, topo2a expression/treatment and ER status/treatment interactions were included as covariates. In this multivariable model, there was a significant interaction between topo2a expression and treatments ($P = 0.048$), but there was no significant interaction between ER status and treatments (Table 3e in Supplementary material). Figure 1 shows the correlation between topo2a protein expression and good PathRes in both arms.

Prediction of chemo-resistance (poor PathResp, RCB class III) Among patients treated with doxorubicin, no significant variables predicting chemoresistance (as defined as being RCB class III) were found (Table 4e in Supplementary material). Tumor size and tumor stage were associated with a significantly higher Symmans class III in univariate analysis on patients treated with docetaxel. In multivariable logistic regression analysis, tumor stage was the only independent predictor of chemo-resistance to docetaxel. The adjusted odds ratios for poor PathResp to docetaxel for Stage IIIA and IIIB tumors relative to Stage II tumors were 4.77 (95% CI: 1.33–17.11; $P = 0.016$) and 8.76 (95% CI: 2.53–30.353; $P = 0.001$), respectively.

Predicting anti-tumor activity by intrinsic breast cancer subtypes

IHC-FISH-based classification The differential response of intrinsic tumor subtypes to doxorubicin versus docetaxel is shown in Table 4. No selective differences in activity between doxorubicin and docetaxel were seen in the luminal and her2 subtypes. However, triple-negative patients treated with doxorubicin had a significantly higher mean RCB (3.255 vs. 2.238; $P = 0.025$) and a significantly higher proportion of poor PathResp (70 vs. 32%, $P = 0.010$) than those treated with docetaxel. The good PathResp rate was also numerically superior with docetaxel (10 vs. 29%), although this difference did not reach statistical significance ($P = 0.160$).

In view of these findings, we included a binary variable defined as triple-negative vs. not to the multivariable models predicting good and bad PathRes, respectively. In the model predicting good PathResp, triple-negative phenotype was not an independent predictor of response to either docetaxel or doxorubicin. On the other hand, in the model predicting bad PathResp, triple-negative phenotype was the only independent predictor of poor response to doxorubicin. Triple-negative patients treated with doxorubicin had an odds ratio for bad PathResp of 4.42 (95% CI 1.53–12.73, $P = 0.006$) with respect to non-triple- negative patients treated.

Gene expression profiles-based classification of molecular intrinsic subtypes A subset of evaluable patients (94/204) were also expression profiled on 44,000 feature full genome Agilent microarrays. Basal-like breast cancer was the only subtype that showed selective benefit for docetaxel over doxorubicin in terms of good PathRes (56 vs. 0%, $P = 0.029$), and mean RCB (1.626 vs. 3.245, $P = 0.039$) (Table 5).

Correlation between variables Figure 2 shows the relationship between topo2a protein overexpression and intrinsic subtype based on IHC/FISH. The rate of topo2a protein overexpression was different among subtypes ($P = 0.006$), with the triple-negative subset showing the higher rate (50%).

Discussion

In our trial, doxorubicin and docetaxel had similar antitumor activity in primary breast cancer. The few neoadjuvant studies conducted to-date with single agent doxorubicin or docetaxel reported a very similar activity [23–25]. In our trial, ER-negative status was a strong but unspecific predictor of sensitivy to either docetaxel and

Table 2 Prediction of good PathResp (PCR + class I): Univariate analysis by treatment arm

Variable	Doxorubicin Number of patients with good pathological response/number of determinations (%)	*P*	Docetaxel Number of patients with good pathological response/ number of determinations (%)	*P*
ER negative	9/33 (27)	0.156	16/48 (33)	0.002
ER positive	11/71 (15)		4/52 (8)	
PR negative	11/44 (25)	0.201	14/53 (26)	0.089
PR positive	9/60 (15)		6/47 (13)	
HER2 positive	8/29 (28)	0.179	7/26 (27)	0.305
HER2 negative	12/75 (16)		13/74 (18)	
Topo IIα IHC high	2/33 (6)	0.016	4/26 (15)	0.769
Topo IIα IHC normal	17/64 (27)		13/64 (20)	
Topo IIα FISH normal	16/64 (25)	0.541	16/69 (23)	0.444
Topo IIα FISH abnormal	3/20 (15)		1/12 (8)	
Topo IIα FISH amplified	3/20 (15)	0.749	0/8 (0)	0.193
Topo IIα FISH normal	16/64 (25)		16/69 (23)	
BCL-2 negative	8/34 (23)	0.194	7/43 (16)	0.611
BCL-2 positive	8/61 (13)		10/49 (20)	
TAU negative	10/47 (21)	0.721	9/49 (18)	0.977
TAU positive	9/49 (18)		8/43 (19)	
P27 negative	7/33 (21)	0.643	4/26 (15)	0.772
P27 positive	12/69 (17)		14/70 (20)	
KI67 low	4/27 (15)	0.582	3/31 (10)	0.108
KI67 high	16/77 (21)		17/69 (25)	
EGFR positive	4/24 (17)	1	4/28 (14)	0.570
EGFR negative	14/73 (19)		13/63 (21)	
Cytokeratin 5/6 positive	4/31 (13)	0.412	4/23 (17)	1
Cytokeratin 5/6 negative	14/67 (21)		14/67 (21)	
Tumor size $>$ 5 cm	11/60 (18)	0.786	3/55 (5)	$<$0.0001
Tumor size $\leq$ 5 cm	9/44 (20)		17/45 (38)	
Stage II	9/37 (24)	0.611	14/37 (38)	0.002
Stage IIIA	6/35 (17)		4/30 (13)	
Stage IIIB	5/32 (16)		2/33 (6)	
Grade 3	7/39 (18)	0.797	9/38 (24)	0.471
Grade 1 + 2	13/65 (20)		11/62 (18)	
Ductal	17/83 (20)	0.588	16/78 (20)	0.537
Lobular	3/17 (18)		2/16 (12)	
Others	0/4 (0)		2/6 (33)	

Table 3 Multivariate analysis (logistic regression) of factors predictive of good PathResp

Treatment arm	Variables	Adjusted OR	95% CI	*P*
Doxorubicin	Topo2a expression (IHC)	10.38	1.95–55.15	0.006
	ER status	4.64	1.40–15.31	0.012
Docetaxel	Tumor size	13.38	3.42–56.29	$<$0.001
	ER status	8.32	2.25–30.73	0.001

doxorubicin. Low topo2a protein expression was an independent predictor of response to doxorubicin, while small tumor size predicts the response to docetaxel. On the other hand, tumors with triple-negative status (by IHC/FISH) and basal-like subtype tumors defined by gene expression profiles were sensitive to docetaxel,

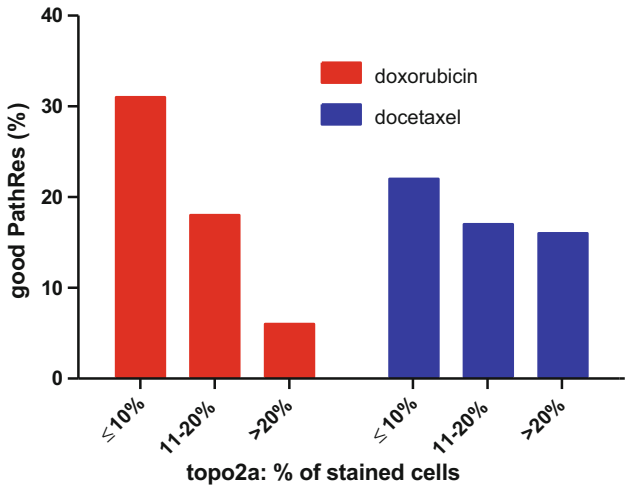

Fig. 1 Correlation between topo2a expression and response to doxorubicin and docetaxel. The differences among categories are statistically significant in the case of doxorubicin ($P = 0.017$), but not in the case of docetaxel ($P = 0.778$; Pearson $\chi 2$ test)

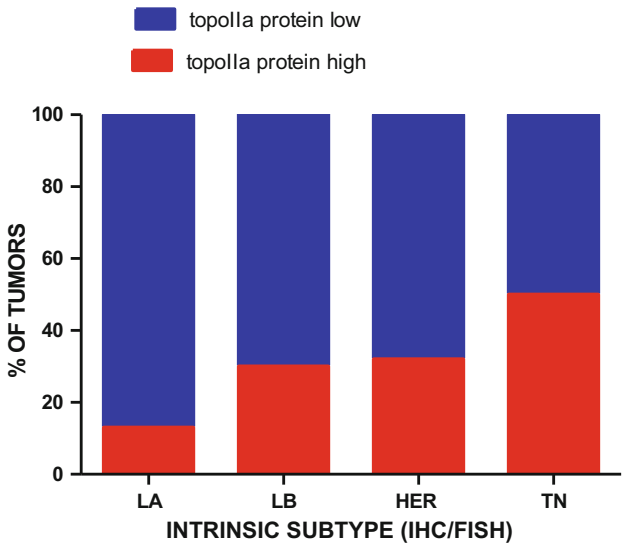

Fig. 2 Correlation between topo2a protein expression and intrinsic subtype

but appears to be resistant to doxorubicin. There were no significant differences among the other genomic subtypes.

With respect to doxorubicin, several studies have tried to identify single gene/protein biomarkers to predict anti-tumor activity. Both *HER2* and *TOPA2* gene status/protein expression have been reported as predictors of response in the adjuvant setting [26, 27]. A pooled analysis of trials published in the literature [28] has suggested that *HER2* overexpression/amplification predicts response to adjuvant anthracycline combinations. The final conclusions remains debatable as there might be a selection bias in these pub-

Table 4 Anti-tumor activity of doxorubicin and docetaxel in intrinsic subtypes based on IHC/FISH

	Good PathRes (pCR + class I)			Poor PathRes (class III)			Residual Cancer Burden (mean ± SEM)		
	Doxorubicin (%)	Docetaxel (%)	*P* value	Doxorubicin (%)	Docetaxel (%)	*P* value	Doxorubicin	Docetaxel	*P* value
Luminal A	3/18 (17)	2/18 (11)	1.000	7/18 (39)	7/18 (3)	1.000	2.622 ± 0.3667	2.572 ± 0.2915	0.680
Luminal B	9/55 (16)	6/42 (14)	0.779	20/55 (36)	13/42 (31)	0.709	2.593 ± 0.1776	2.621 ± 0.1689	0.878
Her2	6/11 (55)	4/12 (33)	0.414	2/11 (18)	3/12 (27)	1.000	1.809 ± 0.477	2.075 ± 0.3568	0.557
Triple-negative	2/20 (10)	8/28 (29)	0.160	14/20 (70)	9/28 (32)	0.010	3.255 ± 0.3291	2.232 ± 0.2913	0.030

Table 5 Anti-tumor activity of doxorubicin and docetaxel in intrinsic subtypes based on Agilent cDNA microarrays

	Good PathRes (pCR + class I)			Poor PathRes (class III)			Residual cancer burden (mean ± SEM)		
	Doxorubicin (%)	Docetaxel (%)	*P* value	Doxorubicin (%)	Docetaxel (%)	*P* value	Doxorubicin	Docetaxel	*P* value
Luminal A	1/12 (8)	0/5 (0)	NS	8/12 (67)	2/5 (40)	0.593	3.386 ± 0.343	2.839 ± 0.345	NS
Luminal B	2/13 (15)	0/11 (0)	NS	2/13 (15)	4/11 (36)	0.357	2.220 ± 0.334	2.806 ± 0.274	NS
Her2-enriched	1/6 (17)	2/5 (40)	NS	4/6 (67)	1/5 (20)	0.242	3.088 ± 0.674	1.670 ± 0.792	NS
Basal-like	0/8 (0)	5/9 (56)	0.029	5/8 (62)	2/9 (22)	0.153	3.245 ± 0.483	1.626 ± 0.499	0.039
Claudin-low	3/11 (27)	1/5 (20)	NS	6/11 (54)	2/5 (40)	1	2.626 ± 0.535	2.538 ± 0.728	NS
Normal	1/4 (25)	2/5 (40)	NS	1/4 (25)	3/5 (60)	0.524	2.183 ± 0.766	2.821 ± 0.639	NS

lications. In patients with advanced tumors, the results of the trials evaluating relationships between *HER2* and response to doxorubicin are contradictory [29–34], but most studies had been retrospective and too underpowered to show clinically relevant correlations. Our study did not observe a significant relationship between *HER2* gene amplification (as measured by FISH) and response to doxorubicin although, numerically, the absolute response rate for *HER2* amplified tumors was higher than for *HER2*-normal tumors (28 vs. 16%).

The association between *HER2* status and sensitivity to doxorubicin could be related to the close topographical location of the *HER2* and *TOP2A* genes [26]; TOP2A amplification almost always occurs within the context of simultaneous HER2 amplification.

The correlation between *TOP2A* gene status/topo2a protein expression and response to anthracyclines has been addressed by a legion of studies [23, 26, 30, 35–47] and are currently a matter of considerable debate. Most studies were essentially retrospective, and included breast cancer patients treated with anthracycline combinations rather than single agent anthracycline and, therefore, the activity of the other drugs of the combination could obscure any existing relationship. Finally, many of the studies were too underpowered to show clinically relevant relationships. A prospective, single arm neoadjuvant study, the TOP trial [48] has addressed the factors predictive of anti-tumor efficacy of epirubicin (100 mg/m^2 every 2 or 3 weeks) in 149 breast cancer patients carrying exclusively ER$^-$ tumors. *TOP2A* copy number alterations were highly predictive of pCR ($P = 0.0002$). However, the target of doxorubicin is the topo2a protein rather than the gene, and there is a lack of correlation between gene status and protein expression [39, 49, 50], probably because of a post-transcriptional regulation of the topo2a protein. As with the *TOP2A* gene status, the results of the trials that evaluated the correlation of topo2a protein expression and the response to anthracyclines are contradictory, but these studies presented the same methodological caveats previously mentioned [34, 51, 52]. In our study, we found that the overexpression of topo2a protein was the stronger predictor of resistance to doxorubicin in a multivariate analyses. Further, this relationship was specific for doxorubicin, as shown by the significant topo2a protein expression–treatment interaction found in the multivariate analysis in which the whole study sample was included. The value of our findings is strengthened by the prospective and planned nature of the study, as well as the study design (comparative, single drug arms). In our study, an excess of the target enzyme/protein is detrimental in the anti-tumor activity of doxorubicin. As Esteva and Hortobagyi [35] have highlighted in an Editorial discussing the relationship of topo2a and anthracycline responsiveness, increased concentration of the target enzyme does predict reduced activity of other anti-tumor drugs (methotrexate, for example); this explanation fits well with our model.

With respect to docetaxel, a number of single genes or gene products have been proposed as predictors of response (ER, tau protein, class I, II or III β-tubulin, bcl-2, ki-67, p53, among others) [53, 54]. As in the case of doxorubicin, most published studies contain significant weaknesses (retrospective and unplanned nature, and small sample size) and therefore, the real predictive values of HER2 [55, 56], microtubule-associated parameters [57–59], and ki-67 [60, 61], remain undefined. In our study, ER status was a strong predictor of docetaxel activity, while the remaining markers (PR, HER2, *TOP2A* gene alterations or topo2a protein expression, tau protein, p27, Ki67) were not.

The ability of single genes or single gene products to predict response to cytotoxic drugs is likely a limited approach. This is not surprising since these agents do not have a single target. Anthracyclines, for instance, have many mechanisms of action other than topo2A inhibition (such as intercalation into DNA leading to inhibited synthesis of macromolecules, generation of free radicals, DNA binding and alkylation, DNA cross-linking, etc.) [62]. Multiple gene models might predict response to these agents more accurately than single gene predictors.

The intrinsic subtype classification, an approach that integrates multiple individual biomarkers together to identify biologically based groups, have previously shown to exhibit prognostic value [14, 15, 20], besides, it could be useful in predicting response to chemotherapy. A retrospective subtype analysis of patients from the Canadian MA5 study suggested that CEF is better than CMF in patients with HER-2 overexpressing tumors, but worse than CMF in the basal-like subtype; CEF and CMF were similar in ER-positive, HER-2 negative luminal subtypes [63]. In the BCIRG 001 adjuvant study, an improved 3-year DFS with TAC versus FAC treatment schemes was shown in the luminal B group ($P = 0.025$), with a marginal trend in the triple negatives ($P = 0.051$) and HER2 ($P = 0.068$) subtypes. No DFS advantage was seen in the luminal A population [22]. Similarly, the GEICAM 9906 trial comparing FEC to FEC followed by weekly paclitaxel, found that the paclitaxel benefit was mainly concentrated in the triple-negative/basal-like patient population [64]. However, all these studies have the disadvantage of having compared multi-drug regimens and, as such, attributing benefit to any of drug individually becomes very difficult. Our study comparing head-to-head single agent doxorubicin with single agent docetaxel showed a

significant difference between doxorubicin and docetaxel: in the triple-negative/basal-like tumors, doxorubicin was associated with a lower response rate and higher RCB score relative to docetaxel. Indeed, the triple-negative status, as defined by IHC/FISH, was the only independent predictor of resistance (poor PathResp) to doxorubicin in a logistic regression analysis. The claudin-low subtype showed some sensitivity to chemotherapy. Both doxorubicin and docetaxel induced good pathological responses in 20–27% of patients. In our study, 40% of tumors classified as claudin-low expressed the triple-negative phenotype while 44% were ER-positive, her-2-negative. The claudin-low subtype seems to be related to the mammary epithelial stem cell (21) and was initially considered to be resistant to chemotherapy [65]. A recent study, however, has challenged this concept [66].

In summary, this prospective study has shown that, although doxorubicin and docetaxel are similarly effective overall as neoadjuvant therapy of breast cancer, docetaxel does seem to be more effective than doxorubicin in the triple-negative/basal-like subtype of patients. Triple-negative breast tumors currently are one of the biggest challenges in the breast cancer clinic. A new class of drugs, the inhibitors of poly(ADP-ribose) polymerase-1 (PARP-1), an enzyme involved in DNA repair, could open a window of hope. PARP-1 expression is often increased in triple-negative tumors. Several PARP-1 inhibitors are currently under development for use in combination with chemotherapy in triple-negative breast cancer; the optimum chemotherapy regimen for the combination remains to be established. Preclinical data suggests that DNA damaging agents (i.e. platinum salts) are the best partners for PARP-1 inhibitors, however, our results may also suggest that adding a taxane to PARP-1 inhibitors and platinum salts could be beneficial to basal-like/triple-negative patients.

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Appendix

See Fig. 3.

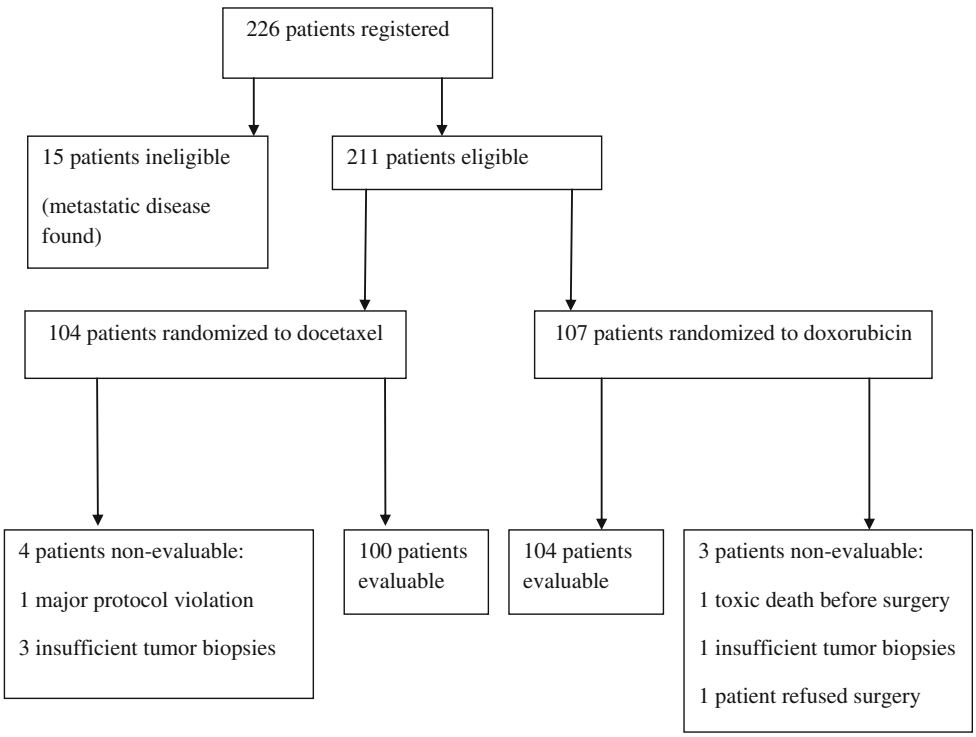

Fig. 3 Consort diagram

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