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CLINICAL TRIAL

BRCA1-like profile is not significantly associated with survival benefit of non-myeloablative intensified chemotherapy in the GAIN randomized controlled trial

A. G. J. van Rossum[1] · P. C. Schouten[1] · K. E. Weber[2] · V. Nekljudova[2] · C. Denkert[3] · C. Solbach[4] · C. H. Köhne[5] · C. Thomssen[6] · H. Forstbauer[7] · G. Hoffmann[8] · A. Kohls[9] · S. Schmatloch[10] · C. Schem[11] · G. von Minckwitz[2] · T. Karn[4] · V. J. Möbus[4] · S. C. Linn[1,12] · S. Loibl[2] · F. Marmé[13]

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Abstract

Purpose The *BRCA1*-like profile identifies tumors with a defect in homologous recombination due to inactivation of BRCA1. This profile has been shown to predict which stage III breast cancer patients benefit from myeloablative, DNA double-strand-break-inducing chemotherapy. We tested the predictive potential of the *BRCA1*-like profile for adjuvant non-myeloablative, intensified dose-dense chemotherapy in the GAIN trial.

Methods Lymph node positive breast cancer patients were randomized to 3 × 3 dose-dense cycles of intensified epirubicin, paclitaxel, and cyclophosphamide (ETC) or 4 cycles concurrent epirubicin and cyclophosphamide followed by 10 cycles of weekly paclitaxel combined with 4 cycles capecitabine (EC-TX). Only triple negative breast cancer patients (TNBC) for whom tissue was available were included in these planned analyses. *BRCA1*-like or non-*BRCA1*-like copy number profiles were derived from low coverage sequencing data.

Results 119 out of 163 TNBC patients (73%) had a *BRCA1*-like profile. After median follow-up of 83 months, disease free survival (DFS) was not significantly different between *BRCA1*-like and non-*BRCA1*-like patients [adjusted hazard ratio (adj.HR) 1.02; 95% confidence interval (CI) 0.55–1.86], neither was overall survival (OS; adj.HR 1.26; 95% CI 0.58–2.71). When split by *BRCA1*-like status, DFS and OS were not significantly different between treatments. However, EC-TX seemed to result in a trend to an improvement in DFS in patients with a *BRCA1*-like tumor, while the reverse accounted for ETC treatment in patients with a non-*BRCA1*-like tumor (p for interaction = 0.094).

Conclusions The *BRCA1*-like profile is not associated with survival benefit for a non-myeloablative, intensified regimen in this study population. Considering the limited cohort size, capecitabine might have additional benefit for TNBC patients.

Keywords *BRCA1*-like · Profile · Non-myeloablative · Intensified · Chemotherapy

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✉ F. Marmé
frederikmarme@me.com

[1] Netherlands Cancer Institute, Amsterdam, The Netherlands

[2] German Breast Group, Neu-Isenburg, Germany

[3] Charité - Universitätsmedizin, Berlin, Germany

[4] University Women's Hospital, Frankfurt, Germany

[5] University Clinic for Internal Medicine Oncology and Hematology, Oldenburg, Germany

[6] University Halle Wittenberg, Halle, Germany

[7] Gesellschaft für onkologische Studien, Troisdorf, Germany

[8] St. Josefs-Hospital, Wiesbaden, Germany

[9] Ev. Krankenhaus, Ludwigsfelde-Teltow, Germany

[10] Elisabeth Krankenhaus, Kassel, Germany

[11] Women's Hospital, University of Kiel, Kiel, Germany

[12] University Medical Center, Utrecht, The Netherlands

[13] Center for Gynecological Oncology at University Women's Hospital, Im Neuenheimer Feld 460, 69120 Heidelberg, Germany

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Introduction

Carriers of inactivating *germline BRCA1 (gBRCA1)* mutations are known to have an increased incidence of breast cancer with a life time risk of 45–60% [1–3]. *gBRCA1* mutations can result in inactivation of the BRCA1 protein. In an active state, this protein plays a pivotal role in the repair of DNA double strand breaks (DSBs) via the error-free process of homologous recombination (HR). In an inactive state however, the cell will use more error-prone mechanisms of DSB repair, such as non-homologous end joining (NHEJ). This results in genetic instability, which in turn, when abundant enough, impairs cell viability [4].

Inactivation of the BRCA1 protein can originate from germline mutations as well as from somatic mutations, hypermethylation of the promotor, or from still unknown mechanisms [5]. The genetic instability that arises from an inactive BRCA1 protein leads to a characteristic copy number (CN) profile [6–8]. Breast tumors can be classified in tumors that display this characteristic CN profile (*BRCA1*-like) and tumors that do not (non-*BRCA1*-like) [9]. Identifying tumors with inactivated homologous recombination may allow targeting the defect with different classes of drugs, like bifunctional alkylators, platinum, or PARP1 inhibitors. The *BRCA1*-like classifier has shown its predictive value for benefit of high dose alkylating chemotherapy previously [10–12].

Vollebergh et al. showed that 41 patients with a *BRCA1*-like profile receiving adjuvant myeloablative, high dose, platinum-based chemotherapy with stem-cell transplantation had an eightfold lower risk of recurrence than patients who received conventional anthracycline-based chemotherapy (test for interaction $p = 0.006$) [10]. Moreover, a disease free survival (DFS) and overall survival (OS) benefit was observed in 16 *BRCA1*-like patients when they were treated with a different myeloablative, high dose, alkylating chemotherapy regimen instead of conventionally dosed chemotherapy (hazard ratio 0.05, $p = 0.003$) [11]. Recently, the predictive capacity of the *BRCA1*-like profile was confirmed in 26 patients receiving tandem high dose chemotherapy with epirubicin, thiotepa, and cyclophosphamide [12]. Interestingly, all three studies have shown that *BRCA1*-like profile is associated with triple negative (TN) status. In the cohort of Vollebergh et al., up to 56% of the TN patients (34/60) had a *BRCA1*-like profile.

TN breast cancer (TNBC) has proven to be a difficult to treat subtype, partly due to its heterogeneity [13]. Taxanes, platinum compounds, alkylating agents, and several targeted agents (bevacizumab, cetuximab) have been investigated. Only taxanes provided a consistent survival benefit [14–17]. Although the value of capecitabine for TNBC patients is still unsettled [18–20], there is evidence that capecitabine might be effective [21, 22]. Clearly, predictive markers to optimize tailoring of treatment are warranted. Since the *BRCA1*-like profile is found in a substantial proportion of TNBC patients, this classifier might particularly be useful in this subgroup.

Although the survival benefit was striking, high dose chemotherapy treatment involved substantial toxicity. We therefore investigated the predictive value of the *BRCA1*-like classifier in patients treated with non-myeloablative intensified, dose-dense chemotherapy when compared to more conventional dose-dense chemotherapy in TNBC patients of the GAIN trial [23]. A previous study showed that the same intensified, dose-dense chemotherapy regimen improved survival compared to standard chemotherapy [24]. Our hypothesis was that *BRCA1*-like patients would derive a survival benefit when treated with the intensified chemotherapy regimen, since it contained high dose cyclophosphamide, a bifunctional alkylating agent. Since capecitabine was part of the conventional chemotherapy arm in the GAIN trial and not of the intensified chemotherapy treatment, we could also investigate what it would add in terms of efficacy.

Patients and methods

Patients

The German Adjuvant Intergroup Node-Positive (GAIN) study was an open label, phase III trial that was conducted between August 2004 and July 2008. Female patients biologically younger than 65 years of age with histologically confirmed invasive breast cancer, at least one positive axillary or internal mammary lymph node and no signs of distant metastases were considered eligible. Histologic complete resection (R0) of the primary tumor was required and patients needed to have an Eastern Cooperative Oncology Group (ECOG) performance score of < 2. Patient recruitment was described in detail previously [23]. The study protocol was approved by all involved ethical committees.

Treatment

The GAIN study (NCT00196872) had a 2×2 factorial design. First, patients were randomized between two chemotherapy regimens in a 1:1 ratio. The first arm consisted of three cycles of epirubicin 150 mg/m^2, three cycles of paclitaxel 225 mg/m^2, and three cycles of cyclophosphamide 2000 mg/m^2, sequentially given with a 2-week interval between cycles (ETC). The second treatment arm was four concurrent cycles of epirubicin 112.5 mg/m^2 and

cyclophosphamide 600 mg/m^2 given every 2 weeks followed by 10 weekly cycles of paclitaxel 67.5 mg/m^2 and capecitabine 2000 mg/m^2 administered on day 1–14, concurrently given in a three weekly schedule (EC-TX). During cyclophosphamide treatment, patients received prophylactic ciprofloxacine on day 5–12. Patients received growth factor support with pegfilgastrim, darbepoetin, or both for the complete duration of chemotherapy treatment. In a second randomization, patients were allocated to ibandronate (50 mg/day) for two years or observation in a 2:1 ratio.

Informed consent for study participation and biomaterial collection was obtained from all individual participants included in the study.

The REMARK criteria were followed (see appendix) [25].

DNA extraction, low coverage whole genome sequencing and *BRCA1*-like classification

From 421 TNBC patients within the GAIN trial, tissue was available from 199 patients.

Formalin-fixed paraffin-embedded (FFPE) tumor tissue blocks were revised and selected if they had a tumor cell percentage of 60% or more. Two unstained slides of 10 µm thickness of tissue were prepared at the Institute of Pathology, Charité – Universitätsmedizin in Berlin and sent to the Netherlands Cancer Institute in Amsterdam. DNA was extracted using the QiaAmp DNA mini kit (Qiagen, Venlo, the Netherlands) as described elsewhere [26].

Low coverage whole genome sequencing was performed as described previously [27]. Input for the reactions was 20–1000 ng of DNA. Libraries were prepared according to the TruSeq protocol. Ten to fifteen cycles of enrichment PCR were required to obtain enough yield for sequencing. Ten uniquely indexed samples were pooled equimolarly and sequenced using an Illumina HiSeq2000 machine to a coverage of $\times$0.5. This was done in one lane of a single-end 50 bp run according to manufacturer's instructions.

Reads were aligned to the reference genome (hg19) using the BWA backtrack algorithm [28]. Reads were subsequently counted in 20 kb non-overlapping bins and corrected for GC bias with a loess fit, and for mappability, by multiplying the mappability of a bin with the loess-corrected read count of the bin [29]. The loess and mappability corrected read counts were converted to log2 read counts. Subsequently, the log2 read counts were mapped to the original BAC clone locations, which were extended to 1 MB to capture a sufficient number of reads for every BAC clone. These BAC mapped profiles were subsequently used to classify samples as *BRCA1*-like or non-*BRCA1*-like. The *BRCA1*-like classification is a shrunken centroids classifier that assigns a probability that a new profile has similar amplifications and deletions to those found in *BRCA1*-mutated breast cancer. If a new profile shares many of these amplifications and deletions it is called *BRCA1*-like. If the profile better resembles amplifications and deletions found in cancers without *BRCA1* mutation it is called non-*BRCA1*-like. To classify a sample the algorithm uses a 371 genomic locations. Samples with a probability of being *BRCA1*-like >0.63 were called *BRCA1*-like. This threshold was obtained independently in previous work [10]. Details of the training of the classifier can be found in [6] and [10]. An R implementation of this classifier is available at http://ccb.nki.nl/software/nkibrca/. Classification of samples was done blinded to clinicopathological and outcome data.

Statistical analyses

We analyzed whether patients selected for these analyses have different characteristics compared to all TNBC patients. Relative total dose intensity (RTDI) is calculated as the ratio between the administered dose and the planned dose of the allocated treatment. Time to treatment (TTT) is the interval in days between surgery and the first cycle of the allocated chemotherapy. The categorical variables were compared using a Fisher's exact test or a χ^2 test; the continuous variables were compared using a Wilcoxon test.

Disease free survival (DFS) was defined as locoregional recurrence, distant recurrence, or death by any cause. Overall survival (OS) was defined as death by any cause. The Kaplan–Meier method was used to estimate survival in the *BRCA1*-like and the non-*BRCA1*-like subgroups. Survival was compared with log rank tests.

To ensure the robustness of multivariate Cox proportional hazards models we first tested all independent covariables in univariate models with respect to the endpoint and subgroup. Only covariables with a Wald p value <0.2 in their univariate model were included into the multivariate model. From these multivariate models adjusted hazard rates were derived. The predictive value of the *BRCA1*-like profile was evaluated by performing tests for interaction also based on Cox proportional hazards models.

All p values are two-sided, p values below 0.05 are considered significant. Confidence intervals (CI) are symmetric 95% confidence intervals. No corrections were made for multiple testing.

All analyses were performed according to the statistical analysis plan using SAS Enterprise Guide V4.3 (SAS Institute Inc., Cary, NC, USA).

Results

DNA extraction and library preparation was performed for 197 patients. A total of 34 samples were excluded: the quality of isolated DNA was insufficient, a library could not be constructed or data quality criteria were not met (Fig. 1). The clinicopathologic characteristics of patients who were included in the analyses were not significantly different from those of the other TNBC patients of the GAIN cohort (Table S1).

BRCA1-like profile was found in 119/163 patients (73%). *BRCA1*-like tumors had a higher Bloom-Richardson grade than non-*BRCA1*-like tumors ($p < 0.001$). No other correlations with clinicopathologic characteristics were observed (Table 1).

The median follow-up time of all included patients was 83.5 months. At the time of the analyses, 56 patients had a locoregional recurrence, distant recurrence, or died. In the total cohort, DFS was not significantly different between *BRCA1*-like patients and non-*BRCA1*-like patients [adjusted hazard ratio (adj. HR) 1.02; 95% confidence interval (CI) 0.55–1.86]. Similarly, there was no difference in OS (adj. HR 1.26; 95% CI 0.58–2.71). When split by *BRCA1*-like status (Fig. 2a, b), DFS was not significantly different in *BRCA1*-like patients when they were treated with EC-TX or ETC (unadj. HR 0.78; 95% CI 0.41–1.45). Neither was DFS in non-*BRCA1*-like patients (unadj. HR 2.20; 95% CI 0.71-6.86). However, a trend for interaction between *BRCA1*-like status and treatment was observed (unadj. $p = 0.094$; Fig. 3). Also in the multivariate model, EC-TX treatment seemed to result in a trend to an improvement in DFS in *BRCA1*-like patients (adj. HR 0.61; 95% CI 0.32–1.19, $p = 0.147$; data not shown), while ETC treatment showed an improvement for non-*BRCA1*-like patients (adj. HR 4.14; 95% CI 1.10–15.58, $p = 0.036$; data not shown). The same trends were observed for overall survival (unadj. HR 0.78; 95% CI 0.38–1.59 for *BRCA1*-like patients; unadj. HR 1.87; 95% CI 0.49–7.14 for non-*BRCA1*-like patients; Fig. 2c, d).

In a multivariate model, RTDI and TTT were significantly associated with DFS and lymph node status with DFS and OS (Tables 2, 3). When splitting the *BRCA1*-like subgroup according to lymph node (LN) status (Figure S2), patients with 10 or more positive LNs have a better DFS when they are treated with EC-TX compared to ETC (unadj. HR 0.33; 95% CI 0.11–0.94). However, OS was not significantly different between the treatment arms in these patients (unadj. HR 0.45; 95% CI 0.15–1.34). In non-*BRCA1*-like patients, neither DFS nor OS was significantly different between treatments in patients with 10 or more positive LNs (DFS: unadj. HR 0.86, 95% CI 0.10–7.52; OS: unadj. HR 0.93, 95% CI 0.11–8.09). However, subgroups in non-*BRCA1*-like patients were very small.

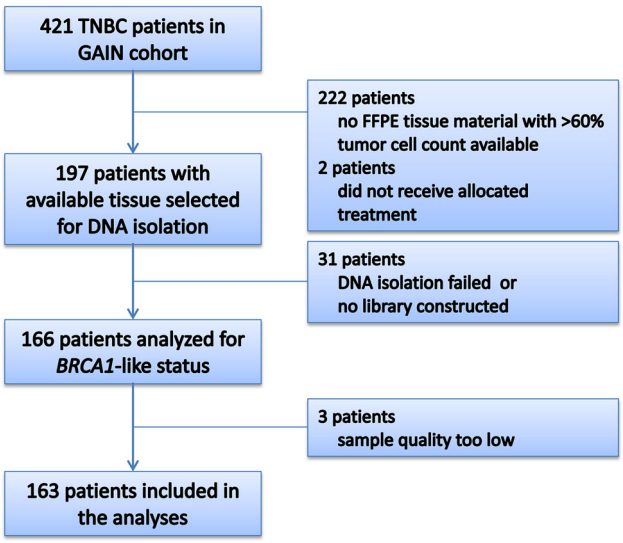

Fig. 1 Selection of TNBC patients for *BRCA1*-like analyses. *TNBC* triple negative breast cancer

Discussion

In this study, we investigated the predictive value of the *BRCA1*-like profile in non-myeloablative intensified, dose-dense chemotherapy and more conventional dose-dense chemotherapy with the addition of capecitabine. In a subset of 163 TNBC patients from the GAIN trial cohort, the *BRCA1*-like profile was not associated with treatment benefit of ETC or EC-TX.

Although both treatments were given in a dose-dense schedule, the differences between the treatments were sequential versus combination chemotherapy, the intensified doses of the ETC agents, and the addition of capecitabine in the EC-TX arm. While the cumulative dose of epirubicin and paclitaxel was the same for both regimens, the dose of the alkylating agent cyclophosphamide was 2.5 times higher in the ETC arm (6000 vs. 2400 mg/m^2). Previous research has shown that *BRCA1*-mutated tumors and tumors with molecular features of *BRCA1*-mutated tumors—called *BRCA*ness—are sensitive to drugs that form interstrand DNA cross links or drugs that stall the replication fork [4]. Cyclophosphamide is an alkylating agent with the ability to generate DNA cross links. Also, there is evidence of an association between dose intensity and treatment effect [30]. Therefore, we hypothesized that the intensified regimen would improve survival in *BRCA1*-like patients when compared to treatment with a more conventional schedule. We could not confirm the hypothesis in this trial. Moreover, the *BRCA1*-like subgroup seemed to benefit from treatment with EC-TX, whereas this

Table 1 Patient characteristics

Parameter	Category	*BRCA1*-like patients ($n = 119$)	Non-*BRCA1*-like patients ($n = 44$)	All patients ($n = 163$)	p value*
Menopausal status (%)	Pre- or perimenopausal	70 (58.8)	21 (48.8)	91 (56.2)	.285
	Postmenopausal	49 (41.2)	22 (51.2)	71 (43.8)	
	Missing	0	1	1	
Body mass index (%)	Normal weight	51 (42.9)	22 (50.0)	73 (44.8)	.300
	Underweight	0 (0.0)	1 (2.3)	1 (0.6)	
	Overweight	38 (31.9)	11 (25.0)	49 (30.1)	
	Obesity	30 (25.2)	10 (22.7)	40 (24.5)	
Surgery (%)	Breast conserving surgery	80 (67.2)	26 (59.1)	106 (65.0)	.359
	Mastectomy	39 (32.8)	18 (40.9)	57 (35.0)	
Tumor size (%)	pT1	30 (25.2)	13 (29.5)	43 (26.4)	.720
	pT2	76 (63.9)	24 (54.5)	100 (61.3)	
	pT3	11 (9.2)	6 (13.6)	17 (10.4)	
	pT4	2 (1.7)	1 (2.3)	3 (1.8)	
Nodal status (%)	pN0	0 (0.0)	0 (0.0)	0 (0.0)	.908
	pN1	53 (44.5)	18 (40.9)	71 (43.6)	
	pN2	37 (31.1)	15 (34.1)	52 (31.9)	
	pN3	29 (24.4)	11 (25.0)	40 (24.5)	
Histological type (%)	Ductal invasive	103 (86.6)	35 (79.5)	138 (84.7)	.083
	Lobular invasive	2 (1.7)	4 (9.1)	6 (3.7)	
	Other	14 (11.8)	5 (11.4)	19 (11.7)	
Bloom-Richardson grade (%)	I	0 (0.0)	0 (0.0)	0 (0.0)	<.001
	II	11 (9.3)	15 (34.1)	26 (16.0)	
	III	107 (90.7)	29 (65.9)	136 (84.0)	
	Missing	1	0	1	
Treatment arm (%)	ETC	63 (52.9)	19 (43.2)	82 (50.3)	.294
	EC-TX	56 (47.1)	25 (56.8)	81 (49.7)	
Ibandronate (%)	No	43 (36.1)	15 (34.1)	58 (35.6)	.856
	Yes	76 (63.9)	29 (65.9)	105 (64.4)	
Relative total dose intensity (%)	<80%	8 (8.7)	5 (11.1)	13 (9.5)	0.745
	80–90%	11 (12.0)	8 (17.8)	19 (13.9)	
	90–100%	51 (55.4)	23 (51.1)	74 (54.0)	
	≥100%	22 (23.9)	9 (20.0)	31 (22.6)	
	Missing	20	9	29	
Time to treatment (%)	≤21 days	23 (20.5)	8 (15.1)	31 (18.8)	0.307
	22–28 days	32 (28.6)	23 (43.4)	55 (33.3)	
	29–35 days	28 (25.0)	11 (20.8)	39 (23.6)	
	>35 days	29 (25.9)	11 (20.8)	40 (24.2)	
	Missing	1	0	1	

Patient characteristics of all triple negative breast cancer patients in the current study, split in patients classified as *BRCA1*-like and non-*BRCA1*-like

TNBC triple negative breast cancer, *E* epirubicin, *T* paclitaxel, *C* cyclophosphamide, *X* capecitabine; relative total dose intensity is the ratio between the administered dose and the planned dose of the allocated treatment; time to treatment is the interval in days between surgery and the first cycle of the allocated chemotherapy

* Fishers exact test for binary variables and χ^2 test for other variables (2-sided)

trend was observed for ETC treatment in non-*BRCA1*-like patients (p for interaction = 0.094).

There are three possible explanations. First, sequential treatment might provide a window of opportunity for the tumor to regrow. While a standard dose of epirubicin induces DNA damage only to a certain extent, *BRCA1*-like tumors might not benefit from the subsequent taxane treatment due to their relative resistance [31]. The three

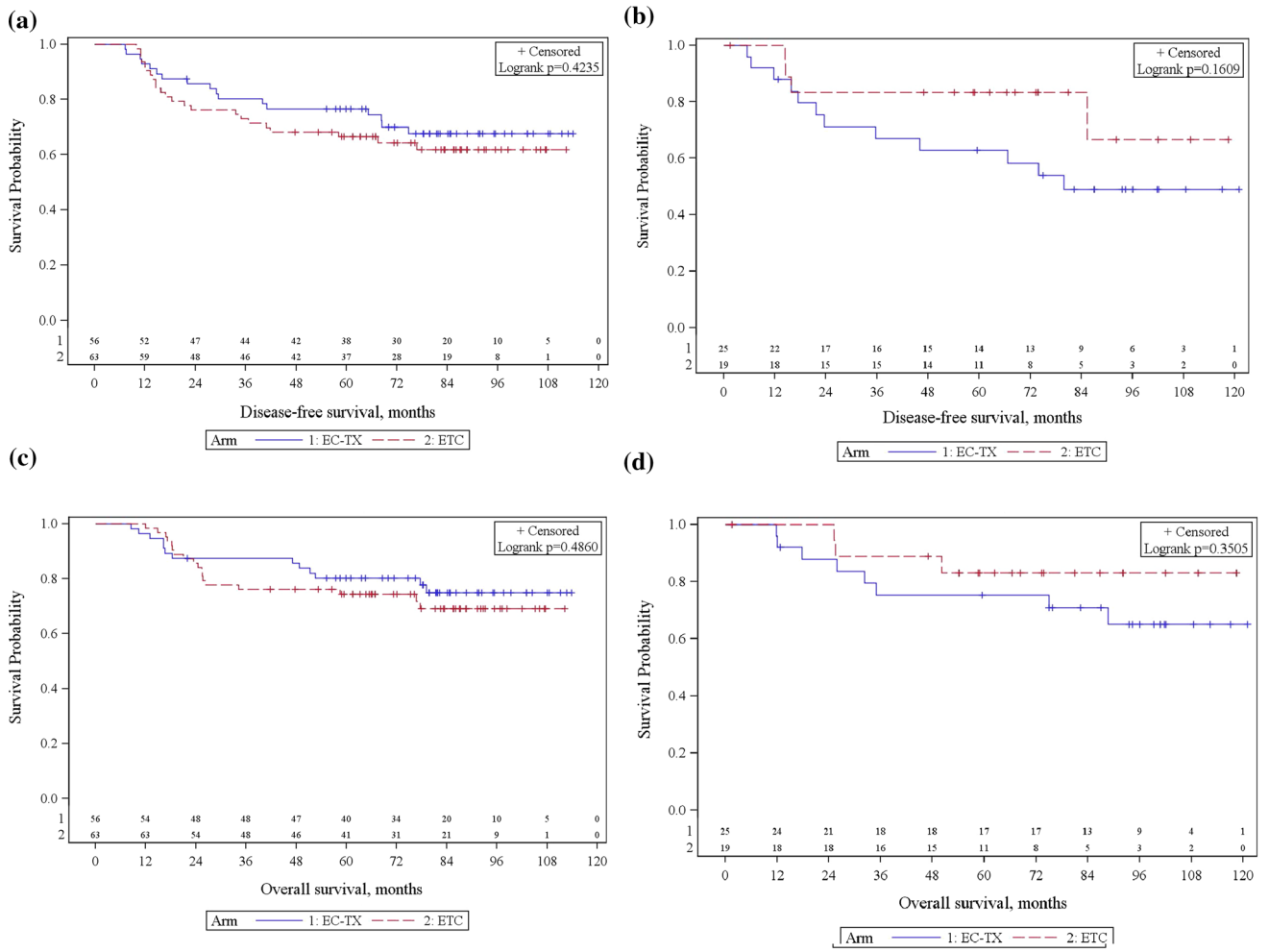

Fig. 2 Survival of *BRCA1*-like patients and non-*BRCA1*-like patients. Disease free survival in *BRCA1*-like patients (**a**) and non-*BRCA1*-like patients (**b**) when treated with ETC (*red line*) or EC-TX (*blue line*). Overall survival in *BRCA1*-like patients (**c**) and non-*BRCA1*-like patients (**d**) when treated with ETC (*red line*) or EC-TX (*blue line*). *E* epirubicin, *T* paclitaxel, *C* cyclophosphamide, *X* capecitabine

cycles of cyclophosphamide might be insufficient to effectively treat the disease. Secondly, the dose-increase of cyclophosphamide to more than standard might not result in greater efficacy. Two previously conducted clinical trials showed that an intensification and dose-escalation of cyclophosphamide when combined with doxorubicin did not result in improved disease free survival or overall survival, while toxicity did increase with dose [32, 33]. However, subgroup analyses were limited in these studies and it might be that a selected group of breast cancer patients would derive benefit from intensified and dose-increased cyclophosphamide. Thirdly, the addition of capecitabine to a combination regimen might have a greater effect than expected, especially in a subgroup of patients. In the recent 10 year survival update of the FinXX trial, Joensuu et al. showed that adding capecitabine to a taxane-anthracycline-based chemotherapy regimen improved recurrence free survival and breast-cancer specific survival compared to a capecitabine-free treatment regimen in TNBC patients [34]. Also, O'Shaughnessy et al. concluded that capecitabine results in a better DFS and OS in TNBC patients with a low Ki67 score (≤65%) [35]. From our study, it seems that TNBC patients with deficient HR, i.e., *BRCA1*-like patients, also might have a better survival when treated with a capecitabine-containing regimen. In an exploratory analysis, DFS of *BRCA1*-like patients with 10 or more positive lymph nodes treated with EC-TX was even significantly better than patients with the same characteristics treated with ETC.

Being an oral prodrug of 5-fluorouracil (5-FU), capecitabine is metabolized via three enzymes into 5-FU of which the last step is done by thymidine phosphorylase (TP). Intracellularly, 5-FU is converted into its active metabolites 5-fluoro-deoxyuridine monophosphate (fdUMP) and 5-fluorouridine triphosphate (fdUTP). These metabolites hamper RNA synthesis and interfere with the function of

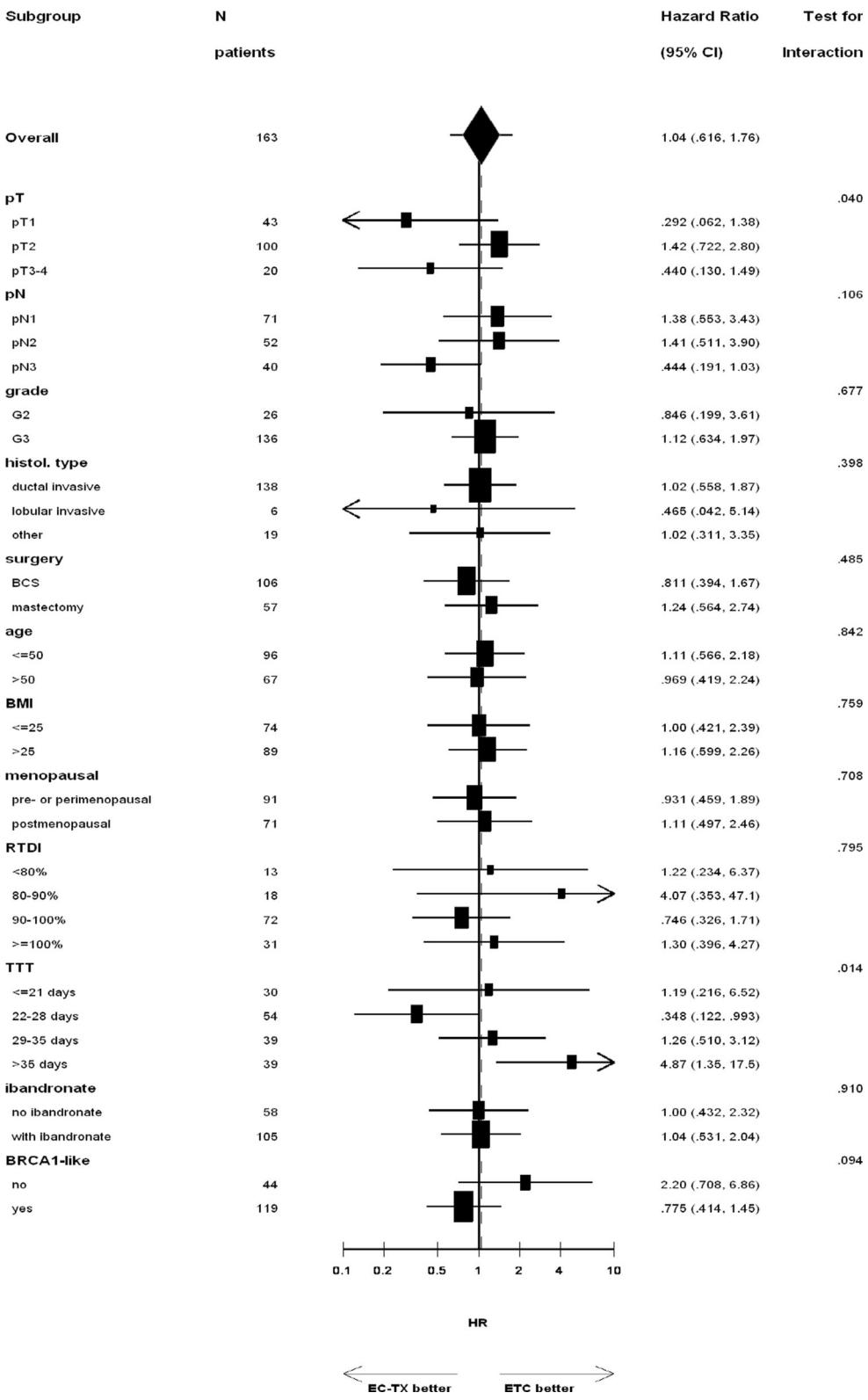

Fig. 3 Forest plot of hazard ratios (HR) for disease free survival by patient subgroup. Whereas the HR of *BRCA1*-like patients is in favor of EC-TX, ETC seems better in non-*BRCA1*-like patients (not significant). Grade is according to the Bloom-Richardson grading system; *BCS* breast conserving surgery, *BMI* body mass index, *RTDI* relative total dose intensity, *TTT* time to treatment

thymidylate synthase (TS). Forming a complex with fdUMP, TS is unable to convert deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). This causes imbalances in the deoxynucleotide (dNTP) pool, leading to DNA damage [36]. If a tumor cell is incapable of repairing DNA damage in an error-free manner, this will result in abundancy of DNA lesions, which affects cell viability. Therefore, it seems valid that

Table 2 Multivariate cox model for disease free survival (DFS)

Variable		Hazard ratio	Confidence interval		*p* value
			Lower	Upper	
Surgery	Mastectomy	1.39	0.63	3.09	0.421
	vs				
	Breast conserving surgery				
Tumor size	pT3-4	2.48	0.95	6.43	0.063
	vs				
	pT1-2				
Nodal status	pN3	2.06	0.91	4.66	0.049
	vs				
	pN2	0.69	0.30	1.58	
	vs				
	pN1				
Treatment	ETC	1.11	0.56	2.21	0.770
	vs				
	EC-TX				
BRCA1-like status	Yes	0.92	0.45	1.87	0.813
	vs				
	No				
Relative total dose intensity (%)	≥100%	0.45	0.16	1.25	0.027
	vs				
	90–100%	0.30	0.12	0.74	
	vs				
	80–90%	0.17	0.05	0.63	
	vs				
	<80%				
Time to treatment (%)	>35 days	1.86	0.64	5.41	0.004
	vs				
	29–35 days	5.36	1.88	15.24	
	vs				
	22–28 days	1.30	0.47	3.60	
	vs				
	≤21 days				

Only covariates that had a univariate Wald *p* value <0.2 were included in this model

adding capecitabine will improve survival in *BRCA1*-like patients, although the exact mechanism remains elusive at present.

Also, preclinical and clinical studies show that taxanes and capecitabine have a synergistic effect [37]. Tumor cells have a higher concentration of TP than normal cells. Moreover, taxanes cause an additional raise in TP levels in tumor cells, resulting in enhanced conversion of capecitabine into 5-FU and its subsequent active metabolites. This could clarify the seemingly enhanced efficacy of EC-TX in *BRCA1*-like patients, but not the moderate efficacy of this regimen in non-*BRCA1*-like patients. However, it is remarkable considering that tumors that harbor a *BRCA1* mutation or a *BRCA*ness signature are thought to be relatively resistant to taxanes or taxane-based combination regimens without capecitabine [31, 38, 39].

We investigated the predictive potential of the *BRCA1*-like classifier in a representative subset of TNBC patients of a randomized trial. The method that we used to classify patients as *BRCA1*-like or non-*BRCA1*-like is robust, as shown previously [27], and the investigators who performed the classification of samples were blinded for clinical outcome. However, the sample size of this predefined analysis is small. This might explain why we did not observe a significant treatment effect, despite the fact that

Table 3 Multivariate cox model for overall survival (OS)

Variable		Hazard ratio	Confidence interval		*p* value
			Upper	Lower	
Surgery	Mastectomy vs Breast conserving surgery	1.61	0.78	3.31	0.200
Tumor size	pT3-4 vs pT1-2	1.85	0.79	4.36	0.157
Nodal status	pN3 vs pN2	3.03	1.35	6.79	0.007
	pN2 vs pN1	1.11	0.46	2.67	
Histological type	Non-lobular vs Lobular	0.90	0.24	3.42	0.883
Treatment	ETC vs EC-TX	1.48	0.77	2.85	0.246
BRCA1-like status	Yes vs No	1.26	0.58	2.71	0.559

Only covariates that had a univariate Wald p value <0.2 were included in this model

the hazard rates for treatment in *BRCA1*-like patients and non-*BRCA1*-like patients are in opposite directions (HR 0.78 and HR 2.20 for DFS, resp.). Also, the univariate analysis showed a trend for interaction ($p = 0.094$). When the cohort is further divided by LN status, numbers of patients are very low, especially in the non-*BRCA1*-like groups. The preferred design to confirm the predictive potential of a biomarker would be a prospective, randomized trial. Currently, these trials are ongoing (NCT01898117; NCT01057069; NCT01646034). Alternatively, a matched case–control set up could be used [40].

In conclusion, we found no significant difference between treatment with non-myeloablative intensified, dose-dense ETC, or dose-dense EC-TX using the *BRCA1*-like classifier as predictive marker. However, the investigated cohort was small. Despite these low numbers, our results indicate that adding capecitabine to dose-dense chemotherapy might improve survival in *BRCA1*-like patients. Further research is warranted.

The study has been presented on a poster at the San Antonio Breast Cancer Symposium 2015.

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Compliance with ethical standards

Conflicts of interest All remaining authors have declared no conflicts of interest.

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Ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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