Adjuvant Capecitabine-Containing Chemotherapy Benefit and Homologous Recombination Deficiency Status Among Early-Stage TNBC Patients in the FinXX trial

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Abstract

Background: Recent data demonstrate that patients with early-stage triple negative breast cancer (TNBC) benefit from escalating adjuvant treatment with capecitabine. However, since a substantial proportion of patients does not benefit, predictive biomarkers to select those individuals upfront are needed. Over half of all TNBCs have a *BRCA1*-like DNA copy number signature similar to the profile found in germline *BRCA1*-mutated breast cancers and indicative for homologous recombination deficiency. We evaluate this signature as a predictive biomarker for capecitabine benefit in archived specimens of the randomized controlled FinXX trial. Additionally, we compared the concordance of our DNA-based *BRCA1*-like classifier with the RNA-based NanoString BRCAness signature.

Methods: Early-stage TNBC patients were randomized between adjuvant capecitabine-containing chemotherapy (TX+CEX: capecitabine plus docetaxel, followed by cyclophosphamide, epirubicin and capecitabine) and conventional adjuvant chemotherapy (T+CEF: docetaxel, followed by cyclophosphamide, epirubicin, and fluorouracil). Breast tumor *BRCA1*-like status was determined on low coverage, whole genome next-generation sequencing data using an established DNA comparative genomic hybridization algorithm. We used interaction analysis in proportional hazards models to evaluate whether benefit of adjuvant capecitabine-containing versus conventional chemotherapy differs between *BRCA1*-like and non-*BRCA1*-like tumors in early-stage TNBC patients.

Results: For 129 (63.9%) of the 202 TNBC patients the *BRCA1*-like status could be determined. Thirty-five recurrences and 32 deaths occurred during a median follow-up of 10.7 years. The capecitabine effect on recurrence-free survival did not significantly differ between the 68 patients (52.7%) with a *BRCA1*-like tumor (HR 0.66, 95% CI 0.24-1.81) and others (HR 0.23, 95% CI 0.08-0.70, P interaction = 0.17), also after adjustment for clinico-pathological variables.

Conclusions: In the FinXX trial, the *BRCA1*-like status was not associated with a differential benefit from capecitabine-containing adjuvant chemotherapy compared to conventional chemotherapy in the TNBC subgroup. Based on this study, it is unlikely that the *BRCA1*-like classifier can be used to distinguish patients who do and do not benefit from capecitabine-enriched standard adjuvant chemotherapy.

Background

Triple negative breast cancer (TNBC) accounts for 10–20% of all breast cancers and is associated with a high risk of early recurrence and poor survival once metastasized [1, 2]. Trials evaluating escalation of adjuvant treatment are emerging [3, 4], including trials on the addition of capecitabine for early-stage TNBC patients. This prodrug of 5-fluoruouracil belongs to the class of antimetabolites and shows cytotoxic activity through the inhibition of thymidylate synthase and the incorporation of its metabolites into DNA and RNA [5]. A recent meta-analysis including 3,854 early-stage TNBC patients showed that adjuvant capecitabine following or added to standard neoadjuvant anthracycline- and taxane-based therapy substantially improved disease-free survival (DFS) and overall survival (OS) [6, 7]. Hence, this

approach has been incorporated in national and international guidelines [8–10]. Although these results are promising, a substantial proportion of TNBC patients does not benefit from the addition of adjuvant capecitabine. This is, for instance, illustrated by the 8.5% absolute overall survival benefit at 5-years for TNBC patients who did not achieve a pathological complete remission on neoadjuvant chemotherapy with subsequently 6–8 courses of capecitabine versus those who did not receive capecitabine (78.8% versus 70.3%, respectively) [7]. To our knowledge, the only study exploring biomarkers that predict capecitabine benefit failed to identify a predictive marker in an 800-gene expression analysis [11]. Therefore, it remains unclear which early-stage TNBC patients have a high chance of benefitting from capecitabine.

Homologous recombination deficiency (HRD) may serve as a putative predictive biomarker to guide decisions on systemic therapy for patients with early-stage TNBC, especially for DNA damaging agents [12, 13]. In unselected TNBC patients, approximately 10% of the patients harbor a deleterious *BRCA1/2* mutation which results in tumors that are deficient in homologous recombination [14–18]. In TNBC patients without a germline *BRCA1/2* mutation, a significant number of tumors harbor HRD [12, 19, 20]. The array comparative genomic hybridization (aCGH) *BRCA1*-like classifier is an HRD-test that has been developed from the characteristic DNA copy number aberrations of *BRCA1*-mutated breast cancers [21]. This *BRCA1*-like classifier showed clinical validity and utility to predict benefit of intensified platinum-based chemotherapy for stage III HER2-negative breast cancer patients [22–25]. However, the predictive value of the *BRCA1*-like classifier for outcome after (neo)adjuvant treatment with other DNA damaging agent-containing regimens and/or dose-intensities is currently unknown.

In data from two studies contributing to the results of the meta-analysis of Van Mackelenbergh et al. [6], the GAIN trial and the CREATE-X trial, there are indications that patients with *BRCA1*-like TNBC tumors may benefit from capecitabine-containing treatment. First, Van Rossum et al. observed a trend for improved survival in the *BRCA1*-like TNBC patients of the GAIN trial treated with capecitabine-containing chemotherapy compared with intensified dose-dense chemotherapy [26]. Second, based on the CREATE-X trial, which was limited to patients with residual disease after neoadjuvant treatment, it seems that capecitabine could have a greater role in patients with tumors that are less sensitive or partially resistant to regimens containing anthracyclines and taxanes [7]. We hypothesize that the improved outcome of TNBC patients treated with capecitabine-containing chemotherapy in the CREATE-X and the FinXX trial were driven by patients with *BRCA1*-like tumors. Contrarily, recent findings of Asleh et al., using a RNA-based 800-gene panel in exploratory analyses, suggest that the BRCAness signature is not associated with the benefit of adjuvant capecitabine in early-stage TNBC [11]. Therefore, the predictive value of the DNA-based *BRCA1*-like status for the differential benefit from adjuvant capecitabine in early-stage TNBC needs further evaluation.

Our aim is to evaluate whether *BRCA1*-like status determines benefit of adjuvant capecitabine-containing systemic treatment in early-stage TNBC patients within the FinXX trial. The FinXX trial is a large phase III, randomized controlled trial comparing adjuvant conventional chemotherapy with adjuvant capecitabine-containing chemotherapy [27].

Methods

Patients

We studied early-stage TNBC patients who were included in the Finland Capecitabine (FinXX) trial; a large, multicenter, randomized controlled clinical trial conducted in Finland and Sweden between 2004 and 2007 [27, 28]. Eligibility criteria have been published previously [27]. In summary, the patients were younger than 65 years, had histologically confirmed invasive breast cancer with either regional lymph nodes containing cancer or node negative cancer with primary tumors of ≥ 20 mm diameter and negative progesterone receptor (PR) expression in immunohistochemistry, no distant metastases, and no prior neoadjuvant chemotherapy. TNBC was defined as estrogen (ER) and progesterone receptor (PR) negativity (< 10%), and no HER2 overexpression (determined either by immunohistochemistry or *in situ* hybridization). The study was approved by the Ethics Committee of the participating medical institutions and the National Agency for Medicines, Finland. Patients supplied written informed consent to allow the use of their tumor tissue for clinical study related research purposes. The Institutional Review Board at the Helsinki University Hospital, Finland, approved the use of archival tissue for the current translational study.

Treatment

Patients were randomized in a 1:1 ratio to either an adjuvant capecitabine(X)-containing chemotherapy regimen (TX + CEX: 3 cycles of capecitabine 900 mg/m² twice daily plus docetaxel 60 mg/m² 3-weekly, followed by 3 cycles of cyclophosphamide 600 mg/m², epirubicin 75 mg/m² and capecitabine 900 mg/m² twice daily, 3-weekly) or to adjuvant conventional chemotherapy (T + CEF: 3 cycles of docetaxel 80 mg/m² 3-weekly, followed by 3 cycles of cyclophosphamide 600 mg/m², epirubicin 75 mg/m², and fluorouracil 600 mg/m², 3-weekly). Patients received locoregional radiotherapy after completion of chemotherapy according to the local guidelines.

DNA extraction

Tumor DNA was isolated from two 10 µm whole slides of formalin-fixed paraffin-embedded (FFPE) tissue containing at least 50% tumor cells. A manual microdissection was carried out for slides containing ≤ 50% of representative tumor area to increase the percentage of neoplastic cells. Paraffin was removed with Qiagen's Deparaffinization Solution, and tissue was lysed using a mixture of 20 µL Proteinase K (20 mg/ml, included in the QIAsymphony DSP DNA kit) and 200 µL lysis buffer (0.05 M Tris-HCl ph 8.5, 0.04 mM EDTA, 0.5% Tween20) per sample at 56 °C overnight. DNA extraction was performed with QIAsymphony SP instrument using DSP DNA mini kit with 100 µL elution volume (Qiagen, Venlo, The Netherlands).

Low coverage whole genome sequencing and data processing

The amount of double-stranded DNA in the genomic DNA samples was quantified using the Qubit® dsDNA HS Assay Kit (Invitrogen, cat no Q32851). Up to 500 ng of double-stranded genomic DNA was fragmented using ultrasonicator shearing (Covaris.com, Massachusetts, USA) to obtain fragment sizes of 160–180 bp. Samples were purified using 1.8X Agencourt AMPure XP PCR Purification beads according to manufacturer's instructions (Beckman Coulter, cat no A63881). DNA library preparation for Illumina sequencing was performed using the KAPA Hyper Prep Kit (KAPA Biosystems, KK8504). During the ligation 144 unique adapter indices, manufactured by IDT (Integrated DNA Technologies IDT, Inc. Coralville, Iowa, USA), were used in a molarity of 15 μM. Six PCR cycles were used during library enrichment to obtain enough yield for sequencing. All DNA libraries were analyzed on the Caliper GX bioanalyzer (PerkinElmer) using the HT DNA High Sensitivity LabChip for determining the molarity. Up to 133 uniquely indexed samples were mixed together by equimolar pooling. The pools were analyzed on the Agilent Technologies 2100 Bioanalyzer and subsequently diluted to 10 nM. Each pool was subjected to sequencing in one lane of a single read 65 bp run, on an Illumina HiSeq2500 machine, according to manufacturer's instructions.

Reads were aligned to the reference genome GRCh38 using BWA-MEM algorithm (version 0.7.17) [29]. Per bin of 20 kb, using BEDTools [30], reads on autosomes were counted. Excluded were sites attracting excessive anomalous read mappings (ENCODE) [31] and bins that had a GRCh38 reference mappability below 0.2. Mappability is the fraction of 65 bp sequences, per bin, that aligns to itself. Local GC effects in samples were fitted with a non-linear loess, including a subset of reference mappabilities over 0.8, to correct sample bin counts. A line can be fitted through the origin and center of GC corrected counts per mappability density. The slope of this line is used to scale mappabilities to reference counts. Genomic profiles consist of log2 ratios of GC corrected bin counts divided by these scaled reference counts.

The sequencing data discussed in this publication have been deposited in NCBI's Sequence Read Archive (SRA) and are accessible through BioProject number PRJNA647428 [32].

BRCA1 -like classification

Genomic profiles were analyzed using a *BRCA1*-like classifier, which was originally developed using array comparative genomic hybridization (aCGH) data generated from breast cancers that were or were not associated with germline *BRCA1* mutations [21]. In brief, the *BRCA1*-like classifier is a shrunken centroid classifier that assigns a genomic profile to a *BRCA1*-like class using a probability score between 0 (non-*BRCA1*-like) and 1 (*BRCA1*-like). The threshold for assigning a breast tumor to the *BRCA1*-like group was set at ≥ 0.63 as obtained and validated in previous studies [22–25]. The *BRCA1*-like classifier can be used on genomic copy number variation (CNV) profiles obtained by low coverage whole genome sequencing [26, 33]. Recently, we implemented several updates in the processing of CNV sequencing (CNVseq) data and validated the *BRCA1*-like classification obtained with these data. A detailed description is provided in the Supplementary methods (Additional file 1: Supplementary methods). In brief, the *BRCA1*-like classification of copy number profiles can reliably be obtained with the updated CNVseq data with an accuracy of 85–93% when compared to the original BAC aCGH *BRCA1*-like

classifier (which is similar to previously established performance on low coverage, whole genome next-generation sequencing [33]).

Quality checks of the CNV profiles of the TNBC FinXX patients were performed blinded for *BRCA1*-like score and outcome. Samples with low quality were excluded from analyses.

Previously, the identification of BRCAness has been explored on the same dataset of early-stage TNBC patients using the RNA-based NanoString BRCAness signature [11]. Signature scores were calculated using prescribed algorithms developed by NanoString technologies [34]. In the present study, we additionally compared the concordance of our DNA-based CNV *BRCA1*-like classifier with the RNA-based NanoString BRCAness signature.

Statistical analyses

Characteristics of patients were compared by *BRCA1*-like status using Fisher's exact, Chi-square or linear-by-linear tests for categorical variables and Mann-Whitney U tests for continuous variables.

Recurrence-free survival (RFS) was defined as time from randomization to local or distant invasive breast cancer recurrence, death from any cause, or to the last date of follow-up, whichever occurred first. Overall survival (OS) was defined as time from randomization to death from any cause or the last date of follow-up. Median follow-up was calculated using the reversed Kaplan Meier estimator. Survival curves were computed with the Kaplan-Meier method. To evaluate whether benefit from adjuvant capecitabine-containing chemotherapy versus adjuvant conventional chemotherapy differs between *BRCA1*-like and non-*BRCA1*-like tumors, we applied Cox proportional hazards regressions that included the interaction term between treatment and *BRCA1*-like status. We estimated and compared interaction coefficients that were unadjusted and adjusted for the following variables: age at randomization, World Health Organization (WHO) performance status (0, 1), type of surgery (breast conserving, mastectomy), axillary surgery (dissection, sentinel node biopsy), T-stage (pT1, pT2, pT3), axillary nodal status (≤ 3 vs > 3 positive lymph nodes), histological type (ductal, lobular, other), and histological grade (1, 2, 3). Due to the relatively small number of events, interaction coefficients were adjusted for one covariate at a time. The prognostic effects of all covariates were also evaluated in separate models. The proportionality of hazards was checked using Schoenfeld residuals. A two-sided p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA) and Stata 16 (StataCorp., College Station, TX, USA).

To determine the concordance between our DNA-based CNV *BRCA1*-like classifier and the RNA-based NanoString BRCAness signature, we dichotomized the acquired continuous scores at the percentile of the established cut-off for the *BRCA1*-like classifier, as there is no predefined cut-off for the NanoString BRCAness signature.

Results

Patient characteristics

Of 202 TNBC patients included in the FinXX trial, we obtained *BRCA1*-like status for 129 (63.9%) patients (Fig. 1). Main reasons for failure were lack of available tumor tissue, low tumor percentage and insufficient amounts of isolated DNA. This subgroup did not differ substantially for the variables mentioned in Table 1 from those TNBC FinXX patients not included in the current analyses (Additional file 2: Table S1). Sixty-eight (52.7%) of the 129 tumors had a *BRCA1*-like profile (Table 1). As expected, patients with a *BRCA1*-like tumor had more frequently poorly differentiated tumors compared to patients with non-*BRCA1*-like tumors (P = 0.03) and had significantly more often less than four positive axillary lymph nodes (P = 0.047). Furthermore, *BRCA1*-like tumors had a higher T-stage (P = 0.03).

Table 1
Characteristics of TNBC patients with known *BRCA1*-like status

Characteristic	Total		Patients with a *BRCA1*-like profile		Patients with a non-*BRCA1*-like profile		P value
	N	(%)	N	(%)	N	(%)	
Total	129	(100)	68	(52.7)	61	(47.3)	
Median (IQR) age at study entry, y	53	(45–59)	52	(44–58)	54	(48–60)	0.11
WHO performance status							0.82
0	109	(84.5)	57	(83.8)	52	(85.2)	
1	20	(15.5)	11	(16.2)	9	(14.8)	
Median (IQR) tumor diameter, mm	25	(21–35)	28	(22–35)	25	(19–35)	0.07
T-stage							0.03
pT1	32	(24.8)	11	(16.2)	21	(34.4)	
pT2	87	(67.4)	51	(75.0)	36	(59.0)	
pT3	10	(7.8)	6	(8.8)	4	(6.6)	
Histological grade							0.03
1	1	(0.8)	0	(0)	1	(1.6)	
2	15	(11.6)	4	(5.9)	11	(18.0)	
3	113	(87.6)	64	(94.1)	49	(80.3)	
Histological type							0.48
Ductal	120	(93.0)	65	(95.6)	55	(90.2)	
Lobular	3	(2.3)	1	(1.5)	2	(3.3)	
Other	6	(4.7)	2	(2.9)	4	(6.6)	
Axillary nodal status							0.047
≤3	97	(75.2)	56	(82.4)	41	(67.2)	
>3	32	(24.8)	12	(17.6)	20	(32.8)	
Type of surgery							0.53
Breast conserving	43	(33.3)	21	(30.9)	22	(36.1)	
Mastectomy	86	(66.7)	47	(69.1)	39	(63.9)	

Characteristic	Total		Patients with a *BRCA1*-like profile		Patients with a non-*BRCA1*-like profile		P value
Axillary surgery							0.02
Dissection	111	(86.0)	54	(79.4)	57	(93.4)	
Sentinel node biopsy	18	(14.0)	14	(20.6)	4	(6.6)	
Treatment							0.82
T + CEF	69	(53.5)	37	(54.4)	32	(52.5)	
TX + CEX	60	(46.5)	31	(45.6)	29	(47.5)	

P values: patients with a *BRCA1*-like profile were compared with patients with a non-*BRCA1*-like profile. P values were calculated using Fisher's exact, Chi-square or linear-by-linear tests for categorical variables and Mann-Whitney U tests for continuous variables.

TNBC: triple negative breast cancer; *BRCA1*-like: *BRCA1*-like profile based on low coverage whole genome DNA next generation sequencing (lcNGS). Non-*BRCA1*-like: no *BRCA1*-like profile based on lcNGS. IQR: interquartile range; WHO: World Health Organization; T + CEF: 3 cycles of docetaxel 3-weekly, followed by 3 cycles of cyclophosphamide, epirubicin, and fluorouracil, 3-weekly; TX + CEX: 3 cycles of capecitabine plus docetaxel 3-weekly, followed by 3 cycles of cyclophosphamide, epirubicin and capecitabine, 3-weekly.

Association of BRCA1-like status with survival

The median follow-up was 10.7 years for all 129 patients, with 35 recurrences and 32 deaths, and with a total person time of 1,085 years. In this cohort, *BRCA1*-like status was not significantly associated with prognosis: unadjusted hazard rates (HRs) of RFS and OS for *BRCA1*-like patients when compared to non-*BRCA1*-like patients were 0.74 (95% CI 0.38–1.44) and 0.79 (95% CI 0.39–1.57), respectively (Table 2). A high number (> 3) of positive lymph nodes was significantly associated with an unfavorable RFS (HR 2.13; 95% CI 1.07–4.22), whereas T-stage (pT3 versus pT1 or pT2: HR 1.90; 95% CI 0.67–5.39) and histological grade was not (grade 3 versus grade 1 or 2: HR 0.79; 95% CI 0.31–2.04).

Table 2
Cox proportional hazards analyses of prognostic and predictive value of *BRCA1*-like status for RFS and OS

	RFS				OS			
Variable	**No. Events/ No. Patients**	**HR***	**95% CI***	**P value***	**No. Events/ No. Patients**	**HR***	**95% CI***	**P value***
DNA-based CNV pattern								
Non-*BRCA1*-like	19/61	1			17/61	1		
BRCA1-like	16/68	0.74	0.38–1.44	0.37	15/68	0.79	0.39–1.57	0.49
BRCA1-**like tumors**								
T + CEF	10/37	1			9/37	1		
TX + CEX	6/31	0.66	0.24–1.81	0.42	6/31	0.75	0.27–2.11	0.59
Non-BRCA1-**like tumors**								
T + CEF	15/32	1			14/32	1		
TX + CEX	4/29	0.23	0.08–0.70	< 0.01	3/29	0.19	0.05–0.66	< 0.01

Interaction test between *BRCA1*-like status and chemotherapy regimen: P = 0.17; P = 0.09. *All Cox proportional hazard analyses shown here were unadjusted for clinic-pathological variables. Similar results were obtained when adjusted for one covariate at the time (due to the relative small number of events).

RFS: recurrence-free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval; CNV: copy number variation; T + CEF: 3 cycles of docetaxel 3-weekly, followed by 3 cycles of cyclophosphamide, epirubicin, and fluorouracil, 3-weekly; TX + CEX: 3 cycles of capecitabine plus docetaxel 3-weekly, followed by 3 cycles of cyclophosphamide, epirubicin and capecitabine, 3-weekly.

Association of BRCA1-**like status with benefit of adjuvant capecitabine-containing chemotherapy**

Overall, adjuvant capecitabine-containing chemotherapy (TX + CEX) was more effective than the conventional chemotherapy (T + CEF) in our cohort of 129 TNBC patients (RFS: HR 0.39; 95% CI 0.19–0.82; P = 0.01). This is in line with the treatment effect in all 202 TNBC cases of the FinXX trial (RFS: HR 0.54; 95% CI 0.31–0.92; P = 0.02) [28]. While in non-*BRCA1*-like patients adjuvant capecitabine-containing

chemotherapy was significantly more effective than conventional chemotherapy (RFS: HR 0.23; 95% CI 0.08–0.70; P < 0.01), this was not observed in patients with a *BRCA1*-like tumor (RFS: HR 0.66; 95% CI 0.24–1.81; P = 0.42). However, the beneficial effect of the adjuvant capecitabine-containing regimen did not differ by *BRCA1*-like status (P interaction = 0.17) (Table 2, Fig. 2). Similar results were obtained after adjustment for each of the clinico-pathological variables (P-values ranging from 0.15 to 0.22). Furthermore, similar results were observed for OS (P interaction = 0.09) (Table 2, Additional file 3: Figure S1).

DNA-based CNV BRCA1**-like status versus RNA-based NanoString BRCAness signature**

Both scores of DNA-based CNV *BRCA1*-like classifier and the RNA-based NanoString BRCAness signature were available for 103/202 TNBC patients (Fig. 3). The established cut-off for the *BRCA1*-like classifier (0.63) occurred at the 42.7th percentile in this dataset. The BRCAness signature score does not have an established cut-off. We therefore dichotomized at the 42.7th percentile of the ranked BRCAness scores (6.18). For 78.6% (81/103) there is concordance in *BRCA1*-like/BRCAness classification. The 21.4% (22/103) disagreement is equal in both directions and the discordant patients are at an intermediate risk of recurrence between the concordant patients.

Discussion

In the present study we found no evidence that benefit from adjuvant capecitabine-containing chemotherapy compared to conventional chemotherapy is associated with *BRCA1*-like status in early-stage TNBC patients. Therefore, it is unlikely that the *BRCA1*-like classifier will help in selecting patients for adjuvant capecitabine-enriched chemotherapy.

Our hypothesis of differential treatment effects was based on previous observations made by Van Rossum et al. [26]. Their retrospective analysis of the randomized GAIN study suggested that patients with *BRCA1*-like tumors have a selective treatment benefit from a conventional dose-dense chemotherapy regimen, containing capecitabine, if compared with non-myeloablative, intensified dose-dense chemotherapy. There are three important differences between the GAIN study and the FinXX study. The main difference is that the FinXX study compared two regimens that only differed by the addition of capecitabine (yes/no) and a slightly lower dose of docetaxel in the capecitabine arm [27], while the GAIN study compared miscellaneous regimens that differed in drug dose and drug combinations [35]. Second, the GAIN study accrued only node-positive patients, while the FinXX study also recruited high risk node-negative patients. And lastly, the capecitabine dose and schedule differed between the studies (10 cycles of capecitabine 1000 mg/m^2 twice daily administered on day 1–14 in a three weekly schedule in the GAIN trial versus 6 cycles of capecitabine 900 mg/m² twice daily on day 1–15 every 3 weeks in the FinXX study). Interestingly, we observed a significant capecitabine benefit for the non-*BRCA1*-like group (RFS and OS P value < 0.01) and less evidence of benefit for the *BRCA1*-like group (RFS P value 0.42; OS P value 0.59), in contrast to the observations of Van Rossum et al. [26]. Whether or not these two observations represent inconsistent patterns is difficult to determine due to the small sample size in these

unplanned subgroup analyses. The patterns are actually reversed, so unless the direction of effects changes with more data, narrower confidence intervals will only make both studies less consistent with each other.

Two biological processes may explain why *BRCA1*-like status is not predictive for benefit of adjuvant capecitabine treatment. First, although capecitabine causes DNA-damage [5], it may not specifically result in DNA damage that is dependent on a proficient homologous recombination machinery resulting in error-free DNA repair [36]. In fact, capecitabine and its active form 5-fluorouracil lead to (1) DNA base pair mismatches which are repaired by the DNA mismatch repair (MMR) pathway [37] and (2) inhibition of DNA replication, leading to abasic sites that are repaired by base excision repair (BER) proteins [38]. Second, there are some data suggesting that capecitabine might result in better outcome in TNBC patients with grade 2 tumors, but not grade 3 [39]. Since *BRCA1*-like tumors are enriched for high-grade tumors, our findings of no predictive value of *BRCA1*-like status for benefit of capecitabine support these observations.

Our results are in line with previous preclinical findings of Quinn et al who observed no differential dose-response effect of capecitabine in *BRCA1*-mutated compared with wild-type *BRCA1* human BC cells [40], and with Alli et al who found a 5-fold lower sensitivity to 5-fluoro-uracil of *BRCA1*-deficient compared to wild-type *BRCA1* murine mammary epithelial cells [41]. Moreover, our findings are consistent with the recent observations of Asleh et al. [11]. In the same dataset, they found no significant association of improved outcome and signatures associated with DNA damage repair, including HRD and BRCAness, using an 800-gene panel in 111 early-stage TNBC patients treated with adjuvant capecitabine in the FinXX trial. Additionally, we demonstrated 78.6% concordance between our DNA-based CNV *BRCA1*-like classifier and the RNA-based NanoString BRCAness signature used by Asleh et al. [11]. Taken together, these data indicate that benefit of adjuvant capecitabine-containing chemotherapy could not be predicted by deficiency in homologous recombination. Currently, the NordicTrip (ClinicalTrials.gov Identifier: NCT04335669) is an ongoing translational clinical trial in early-stage TNBC prospectively comparing the effect on pathologic complete response (pCR) rate of adding capecitabine to epirubicin plus cyclophosphamide followed by carboplatin plus paclitaxel neoadjuvant chemotherapy, stratified for HRD positive versus HRD negative/ HRD-intermediate. Results of this study have to be awaited to further clarify the value of HRD as a predictive biomarker for benefit of capecitabine-containing chemotherapy in early-stage TNBC.

The main strength of our study is the study design, i.e., a prospective, randomized controlled trial with collection of archival material. This prospective-retrospective design is the first choice to assess a putative predictive biomarker in case a prospective randomized clinical trial is not feasible, because such trials require huge numbers of patients, are costly and take many years to complete [42]. An additional strength, in contrast to the exploratory analyses of Asleh et al. using an RNA 800-gene panel without predefined cutoff for the BRCAness signature [11], is that we evaluated a single biomarker with a predefined cutoff based on prior biological and empirical evidence [21, 23–25]. This confirmatory objective is required to establish the implementation of a predictive biomarker or to refute it [42].

A limitation of the present study is the small sample size, which is due to the fact that the FinXX trial was powered to evaluate the main effect of capecitabine among patients with any biological type of breast cancer rather than a treatment-marker interaction in the subgroup of TNBC patients. In addition, the number of patients was further reduced by the failure to obtain *BRCA1*-like status for all TNBC patients for several reasons. However, our patient group accounts for 129 (63.9%) of the 202 accrued TNBC patient in the FinXX trial, which is within the recommended range of sample size for a study to evaluate predictive biomarkers [42]. Furthermore, the included patients did not differ meaningfully from all accrued TNBC patients for the evaluated clinical variables. Lastly, whether BRCA1-like status has predictive value for capecitabine-containing chemotherapy only in individuals with residual disease after neoadjuvant chemotherapy is still an open question. Collaborate efforts to assess this in the CREATE-X trial are ongoing.

Our findings may have implications for treatment decisions in early-stage TNBC patients. Currently, the addition of the polyadenosine 5'diphosphoribose polymerase (PARP) inhibitors in the (neo) adjuvant treatment of these TNBC patients is an emerging area of investigation [43–46]. The OlympiA trial is one very important and large trial that evaluates the efficacy of adjuvant treatment with olaparib, a PARP inhibitor, compared to placebo in patients with non-metastatic, germline *BRCA* mutated (*gBRCAm*) high risk HER2-negative primary breast cancer (ClinicalTrials.gov Identifier: NCT02032823). An unanswered question remains what the added value of adjuvant treatment with PARP inhibitors would be compared to the current clinical practice of capecitabine-containing treatment. In our present study, we demonstrate that the efficacy of adjuvant capecitabine treatment in early-stage TNBC patients does not differ by *BRCA1*-like status. Therefore, the added value of PARP inhibition to capecitabine-containing adjuvant chemotherapy in g*BRCA*m-associated or *BRCA1*-like TNBC patients that did not achieve a pathological complete remission on standard neoadjuvant chemotherapy needs to be investigated.

Conclusions

Our data suggests that benefit from adjuvant capecitabine added to standard adjuvant chemotherapy in early-stage TNBC patients does not depend on *BRCA1*-like status. Identifying biomarkers that define the subgroup of early-stage TNBC patients who benefit from adjuvant capecitabine remains an urgent need in future research.

Abbreviations

(a)CGH: (array) comparative genomic hybridization; BER: base excision repair; CNV: copy number variation; DFS: disease-free survival; ER: estrogen receptor; FFPE: formalin-fixed paraffin-embedded; HRD: homologous recombination deficiency; MMR: mismatch repair; OS: overall survival; PARP: polyadenosine 5'diphosphoribose polymerase; pCR: pathologic complete response; PR: progesterone receptor; RFS: recurrence-free survival; SRA: Sequence Read Archive; TNBC: triple negative breast cancer; T+CEF: 3 cycles of docetaxel 80 mg/m² 3-weekly, followed by 3 cycles of cyclophosphamide 600 mg/m², epirubicin 75 mg/m², and fluorouracil 600 mg/m², 3-weekly; TX+CEX: 3 cycles of capecitabine 900

mg/m² twice daily plus docetaxel 60 mg/m² 3-weekly, followed by 3 cycles of cyclophosphamide 600 mg/m², epirubicin 75 mg/m² and capecitabine 900 mg/m² twice daily, 3-weekly; WHO: World Health Organization; X: capecitabine

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the participating medical institutions and the National Agency for Medicines, Finland. Patients supplied written informed consent to allow the use of their tumor tissue for clinical study related research purposes. The Institutional Review Boards at Helsinki University, Finland, approved the use of archival tissue for the current translational study.

Consent for publication

Not Applicable.

Availability of data and materials

The clinical datasets used and analyzed during the current study are available from Heikki Joensuu on reasonable request. The sequencing datasets generated and analyzed during the current study are available in the SRA repository, [https://www.ncbi.nlm.nih.gov/Traces/study1/?acc=PRJNA647428&].

Competing interests

HJ has ownership interest (including patents) at Sartar Therapeutics and is a board member, has a co-appointment at Orion Pharma and is employed by Orion Pharma, and has received fees from Neutron Therapeutics. SCL reports grants from ZonMw and A Sister's Hope during the conduct of the study. SCL is an advisory board member for AstraZeneca, Cergentis, IBM, Pfizer and Roche, and received institutional research grants from Agendia, AstraZeneca, Eurocept-pharmaceuticals and Pfizer. In addition, SCL received institutional research grants and institutional non-financial support from Genentech, Novartis, Roche, Tesaro and Immunomedics and other institutional support from AstraZeneca, Pfizer, Cergentis, Daiichi Sankyo, IBM and Bayer outside the submitted work. MK is an advisory board member for BMS, Roche, MSD and Daiichi and received institutional research support from AstraZeneca, BMS and Roche outside the submitted work.

The other authors declare that they have no competing interests.

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Authors' contributions

LWB performed the data acquisition and statistical analysis, interpreted the data, and drafted the manuscript. KJ performed statistical analyses, interpreted the data and critically revised the manuscript. HJ and SL performed the data acquisition and reviewed the manuscript. CS, WB, PCS and RJCK generated the sequencing data and critically revised the manuscript. MO and PMN were involved in acquiring the *BRCA1*-like status, interpreting the data, and critically revised the manuscript. HL and MK critically revised the manuscript. MH designed the study, analyzed and interpreted the data and critically revised the manuscript. SCL designed the study, interpreted the data, and critically revised the manuscript.

All authors read and approved the final manuscript.

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Figures

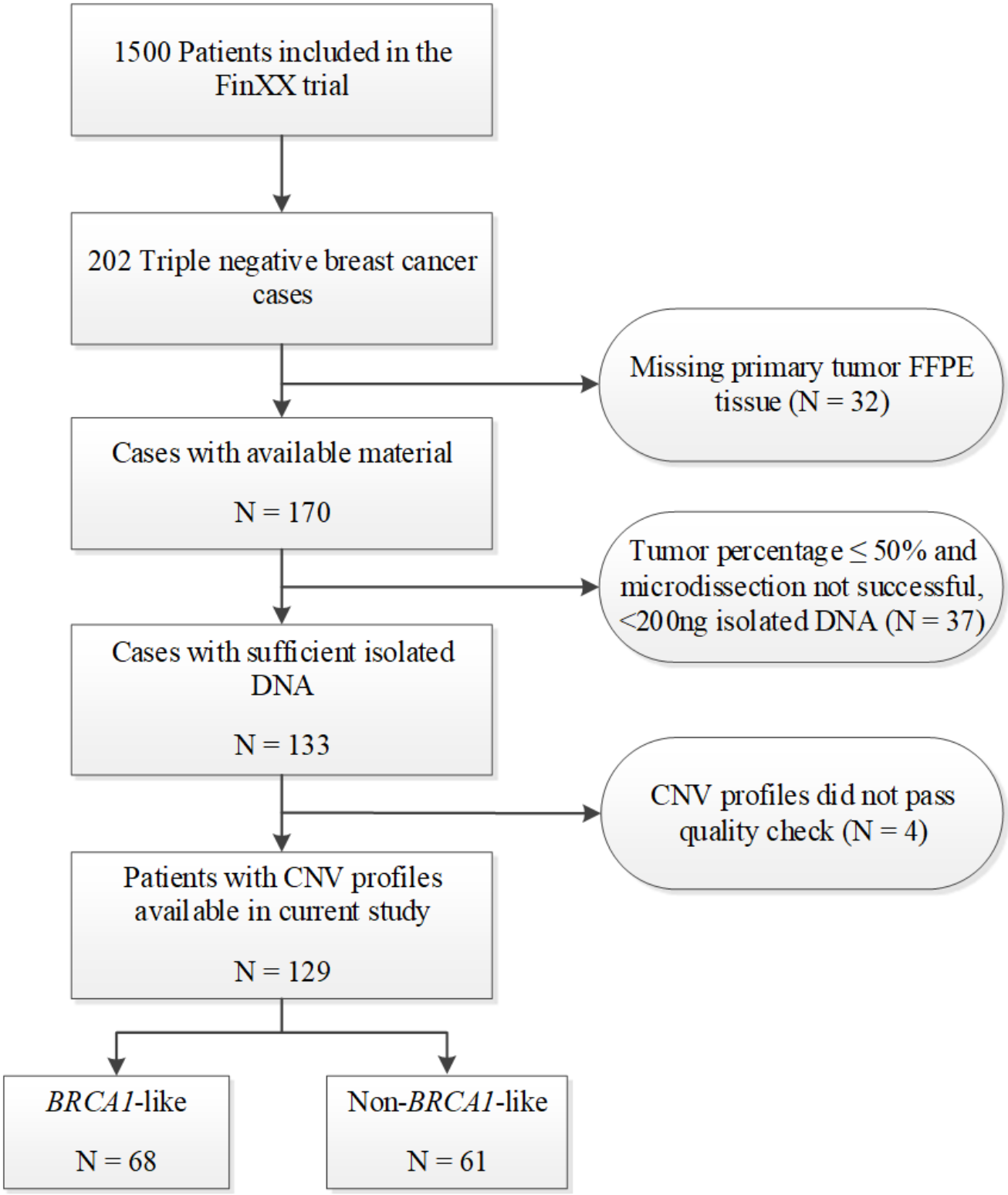

Figure 1

Flow diagram of patient selection in the current study Reasons for dropout are listed. Tumors of 129 patients could be evaluated for BRCA1-like status. Triple negative breast cancer was defined as estrogen (ER) and progesterone receptor (PR) negativity (<10%), and no HER2 overexpression. FFPE: formalin-fixed paraffin-embedded; CNV: copy number variation; BRCA1-like: BRCA1-like profile based on low coverage

whole genome DNA next generation sequencing (lcNGS). Non-BRCA1-like: no BRCA1-like profile based on lcNGS.

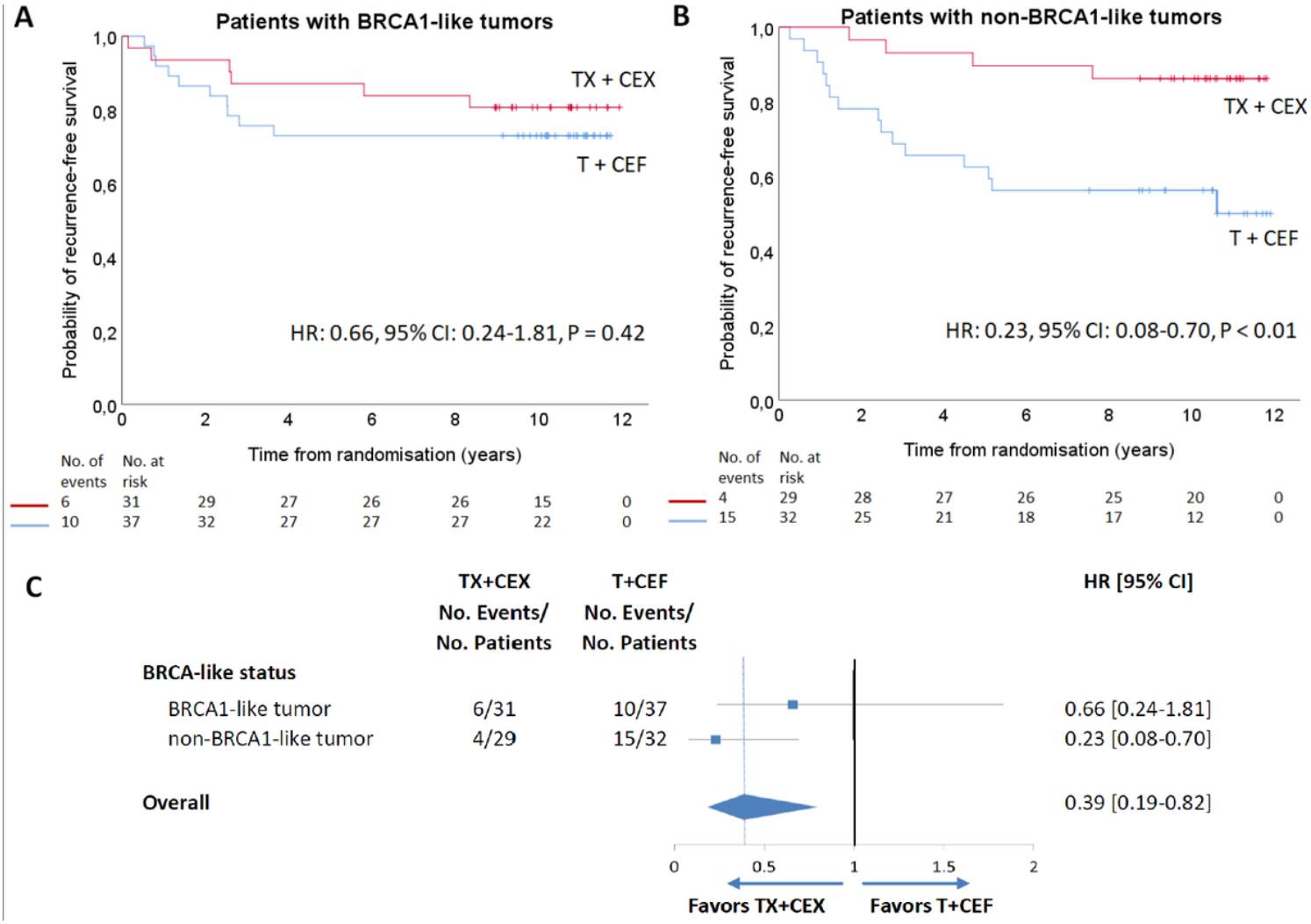

Figure 2

Recurrence-free survival for TNBC patients by BRCA1-like status and allocated adjuvant treatment Kaplan-Meier curves of RFS for TNBC patients with BRCA1-like (A) and non-BRCA1-like tumors (B) according to treatment. Number of events and patients at risk are reported below the figure. Unadjusted hazard ratios are derived from Cox regression models (A+B). Similar results were obtained when HRs were adjusted for clinico-pathological variables. (C) Forest plot of hazard ratios for recurrence-free survival according to BRCA1-like status and treatment. Patients had been randomly assigned between adjuvant TX+CEX or T+CEF. HR: hazard ratio; CI: confidence interval; RFS: recurrence free survival; TNBC: triple negative breast cancer; TX+CEX: 3 cycles of capecitabine plus docetaxel 3-weekly, followed by 3 cycles of cyclophosphamide, epirubicin and capecitabine, 3-weekly; T+CEF: 3 cycles of docetaxel 3-weekly, followed by 3 cycles of cyclophosphamide, epirubicin, and fluorouracil, 3-weekly.

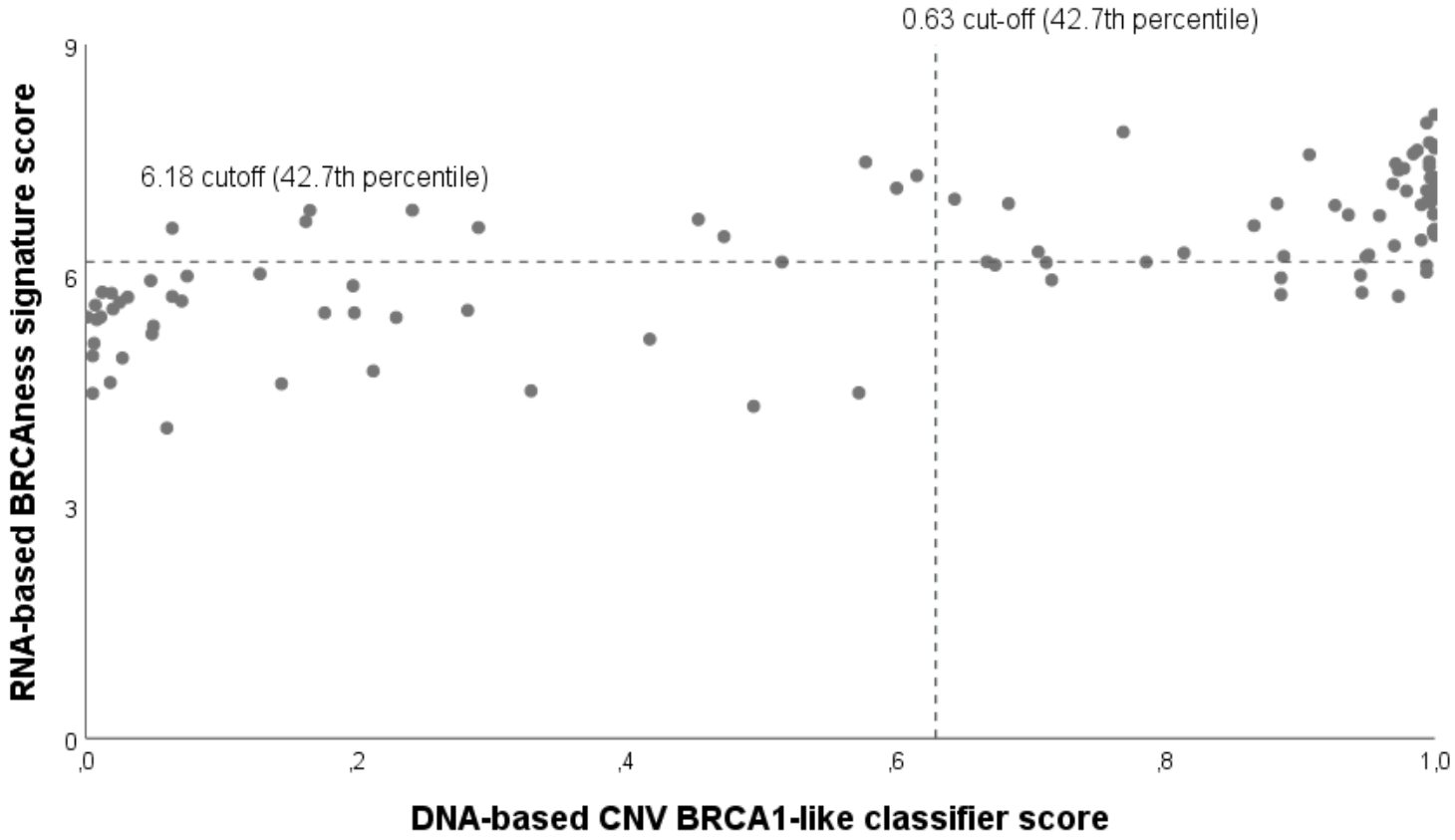

Figure 3

DNA-based CNV BRCA1-like classifier versus RNA-based BRCAness signature Scatterplot illustrating the corresponding DNA-based CNV BRCA1-like classifier score and RNA-based NanoString BRCAness signature score belonging to the same tumor. Both scores were available for 103/202 TNBC patients. The dashed lines illustrate the cut-off points when the scores are dichotomized at the percentile of the established cut-off for the BRCA1-like classifier (42.7th percentile).

Supplementary Files

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