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Prosigna Risk of Recurrence score and intrinsic subtypes are associated with adjuvant anthracycline chemotherapy benefit in high-risk breast cancer

Maj-Britt Jensen [1] ✉, Torsten O. Nielsen [2], John Bartlett [3], Anne-Vibeke Lænkholm [4], Lois Shepherd[5] & Bent Ejlertsen [1,6]

NCIC-CTG MA.5 and DBCG 89D are symmetrically designed randomized trials comparing adjuvant cyclophosphamide, epirubicin, and fluorouracil with cyclophosphamide, methotrexate, and fluorouracil in high-risk breast cancer patients. In a joint analysis we evaluate the predictive value in terms of anthracycline benefit of molecular subtyping by PAM50. A statistically significant interaction ($P = 0.008$) between continuous Risk of Recurrence (ROR) score and treatment regimen is evident, translating into a clear distinct treatment effect according to ROR score category with HR 0.51 for ROR score ≥ 72 and HR 1.10 for ROR score < 52 ($P_{interaction} = 0.004$). The analysis provides evidence of the benefit from anthracycline in HER2-enriched subtype; for patients with discordance of HER2 subtype and clinical HER2 status, HER2-enriched subtype was predictive of anthracycline benefit whereas clinical HER2 positive status was not. Anthracycline-based adjuvant chemotherapy may safely be withheld for patients with a low ROR score while the benefit increases with increasing ROR score.

While the meta-analyses conducted by the Early Breast Cancer Trialists Collaborative Group (EBCTCG)[1–3] demonstrated an overall benefit from adjuvant anthracycline chemotherapy, its use has persistently been restricted to breast cancer patients with a sufficiently high clinical risk or specific molecular characteristics[4,5]. The reluctance to recommend anthracyclines to all patients where chemotherapy is indicated is in part due to their adverse events including risks of heart failure and secondary leukemia. Furthermore, the heterogeneous results of individual trials have to some degree been associated with different patient selection criteria, motivating a search for predictive markers of benefit from anthracyclines in the National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) MA.5 and the Danish Breast Cancer Group (DBCG) 89D trials[6,7]. These independent trials had symmetrical designs comparing cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy to cyclophosphamide, epirubicin, and fluorouracil (CEF) in patients with early breast cancer. Trastuzumab was not available when these trials were performed, but *TOP2A* alterations were retrospectively linked to anthracycline sensitivity[8–10]. Other attempts to develop biomarkers or combinations have included HER2, CEP17 and TIMP1 but have not led to the adoption of markers in the clinical setting[11,12]. An individual-patient meta-analysis verified a moderate predictive value of HER2 positivity and *TOP2A* alterations but also highlighted the importance of subtypes, which are further investigated in the current study[13].

The predictive significance of the PAM50-based Prosigna assay for adjuvant chemotherapy with epirubicin and the discrepancies between PAM50 gene expression-based subtyping and immunohistochemical- or ISH-determined HER2 status were investigated in the NCIC-CTG MA.5 and DBCG 89D trials[14,15]. The results indicated that the effect is primarily driven by the PAM50 subtype, with the discordant groups following the intrinsic subtype, i.e. patients with HER2-negative tumors and a HER2-enriched subtype appeared to benefit from epirubicin, whereas patients with HER2-positive tumors but a non-HER2-enriched subtype received no benefit. Further, the benefit of epirubicin correlated with the Risk Of Recurrence (ROR) score, but without convincing statistical significance.

[1]Danish Breast Cancer Cooperative Group, Department of Oncology, Copenhagen University Hospital, Rigshospitalet Copenhagen, Denmark. [2]Department of Pathology and Laboratory Medicine, Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, Canada. [3]Cancer Research UK Scotland Centre, University of Edinburgh, Edinburgh, United Kingdom. [4]Department of Surgical Pathology, Zealand University Hospital, Roskilde, Denmark. [5]Canadian Cancer Trials Group, Queen's University, Kingston, ON, Canada. [6]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. ✉e-mail: maj-britt.raaby.jensen@regionh.dk

However, these observations in each separate trial were underpowered due to limited numbers and a combined analysis of the trials might provide the largest possible power to give robust estimates.

Results

Patient and tumor characteristics

Individual patient data on PAM50 was retrieved from 1140 (67%) of the eligible patients randomized and Table 1 describes patient and tumor characteristics reflecting that both trials selected high-risk patients. A majority of the patients was premenopausal, with a tumor size above 20 mm, high-grade malignancy and node-positive disease (Table 1). Both trials were performed before trastuzumab was introduced and HER2 status was established retrospectively. The MA.5 trial included a higher proportion of patients with luminal subtypes (ER-positive, HER2-negative), in accordance with the differential inclusion criteria. The treatment effect in this joint analysis was similar to the effect observed in the original study populations of 1696 patients in total; a HR favoring CEF for DRFS (adjusted HR 0.73; 95% CI 0.60 to 0.88) and OS (adjusted HR 0.89; 95% CI 0.74 to 1.06)[6,7,14,15].

ROR score

The association of the continuous ROR score with 10-year DR rate was not proportional across regimens over time and therefore analyzed separately (Fig. 1) for the early (0 to ≤ 2.5 years) and late periods (> 2.5 to 10 years). Treatment with CEF reduced the risk of recurrence as compared to CMF and the risk reduction increased with an increasing continuous ROR score. Thus, a statistically significant interaction ($P_{interaction}$ = 0.001) was observed between increase in ROR score and treatment group (Table 2). This translates into 10 year rates for distant recurrence of 49.0% and 57.6% for patients in the CMF arm having ROR score of 50 and 60, respectively, and a more moderate raise from 44.1% to 46.7% for patients in the CEF arm. The effect of increasing ROR score on the risk of distant recurrence is most pronounced in the first 2.5 years (Table 2; Fig. 1A, B). Further subdividing in patients with HER2-positive and patients with HER2-negative disease revealed a statistically significant interaction between ROR score and treatment regimen ($P_{interaction}$ = 0.009, including both early and late periods) and between ROR score and HER2 status ($P_{interaction}$ = 0.01 in unadjusted analysis) whereas the interaction between ROR score and treatment regimen is not heterogenous across HER2 status ($P_{interaction}$ = 0.46 and $P_{interaction}$ = 0.80 for early and late periods, respectively). The analysis of time to recurrence and overall survival as well as adjusted analyses provided similar results (Supplemental Table 1, Supplemental Table 2 and Table 2).

The ROR scores were, according to predefined cutpoints dived into three categories and 470 (41%) were in the low risk, 443 (39%) in the intermediate risk and 227 (20%) in the high-risk group, with a higher ROR score in patients with HER2-positive disease (Table 3). Figure 2 shows the cumulative incidence for distant recurrence according to the ROR score categories for each of the treatment regimens (Fig. 2A CMF and Fig. 2B CEF) and HER2 status. A clear distinction according to ROR score category within HER2-negative and HER2-positive patients is shown for the CMF but not for the CEF group. A statistically significant heterogeneity in treatment effect according to ROR score category is shown in the multivariable analysis (Fig. 3) for DR ($P_{interaction}$ = 0.004), and TR (Supplemental Fig. 1, $P_{interaction}$ = 0.002), but not for OS (Supplemental Fig. 2, $P_{interaction}$ = 0.20) although with a similar trend of better effect with higher ROR score. Univariable results are shown in Supplemental Fig. 3, 4 and 5. A subdivision of the patients according to HER2 status did not show a differential treatment versus ROR score category interaction comparing patients with HER2-negative disease and HER2-

Table 1 | Patient and tumor characteristics for the study population

		Treatment arm				Total	
		CMF		CEF			
		N	*Col%*	N	*Col%*	N	*Col%*
		595		545		1140	
Age	< 50	428	*72*	391	*72*	819	*72*
	≥ 50	167	*28*	154	*28*	321	*28*
Menopausal status	Premenopausal	486	*82*	444	*81*	929	*81*
	Postmenopausal	109	*18*	101	*19*	211	*19*
Tumor size	≤ 2 cm	240	*40*	199	*37*	439	*39*
	> 2, ≤ 5 cm	301	*51*	289	*53*	590	*52*
	> 5 cm	42	*7*	37	*7*	79	*7*
	Unknown	12	*2*	20	*4*	32	*3*
Nodal status	Negative	121	*20*	123	*23*	244	*21*
	1-3 positive LN	262	*44*	234	*43*	496	*44*
	≥ 4 positive LN	212	*36*	188	*35*	400	*35*
Malignancy Grade	I	47	*8*	34	*6*	81	*7*
	II	244	*41*	227	*42*	471	*41*
	III	274	*46*	260	*48*	534	*47*
	Not graded	30	*5*	24	*4*	54	*5*
ER status	Positive	221	*37*	202	*37*	423	*37*
	Negative	247	*42*	221	*41*	468	*41*
	Unknown	127	*21*	122	*22*	249	*22*
HER2 status	Positive	162	*27*	152	*28*	312	*27*
	Negative	433	*73*	393	*72*	826	*73*
Trial	MA.5	233	*39*	221	*41*	454	*40*
	DBCG 89D	362	*61*	324	*59*	686	*60*

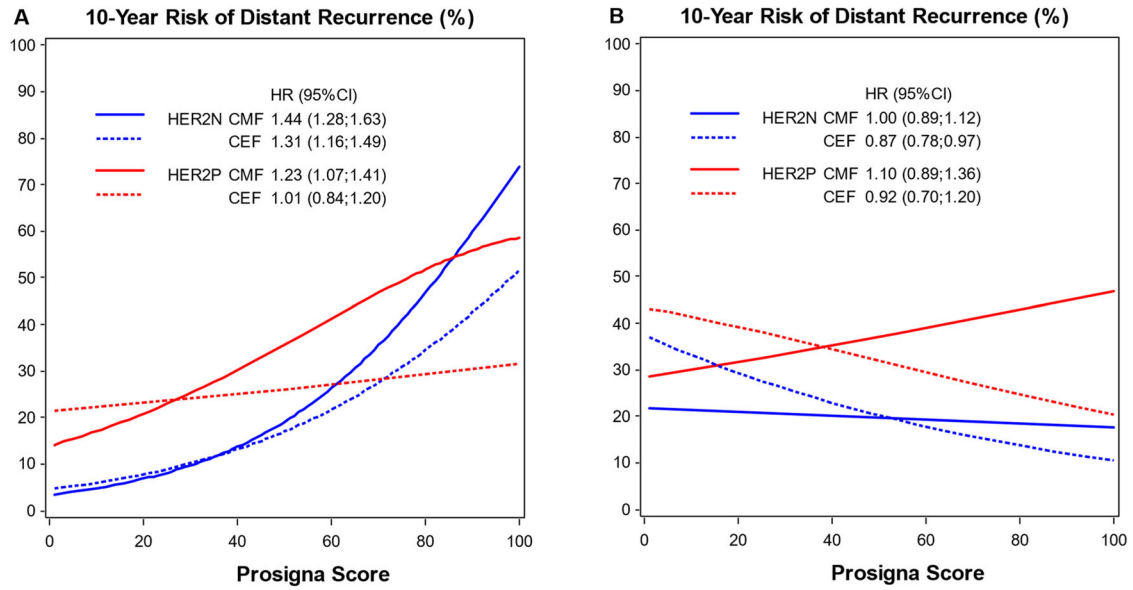

Fig. 1 | Distant recurrence rate by continuous ROR score for patients in the CMF regimen and patients in the CEF regimen and according to HER2 status. HER2N HER2-negative, HER2P HER2-positive. Hazard ratios and corresponding 95% CI for a 10-point difference in continuous ROR score are shown according to follow-up time; **A** Early (0–≤2.5 years) and **B** Late periods (>2.5–10 years).

Table 2 | Unadjusted and adjusted* results for ROR score continuous (10-point) on DR according to treatment group, divided according to follow-up time and HER2 status

	All			HER2-negative		HER2-positive		
	HR	(95% CI)	$P_{interaction}$	HR	(95% CI)	HR	(95% CI)	$P_{interaction}$
Unadjusted analysis								
			0.001					
CMF	1.27	1.19;1.36						
CEF	1.09	1.01;.1.17						
≤ 2.5 yrs			0.008					0.009
CMF	1.43	1.31;1.57		1.44	1.28;1.63	1.23	1.07;1.41	
CEF	1.25	1.13;1.38		1.31	1,16;1.49	1.01	0.84;1.20	
> 2.5 yrs								
CMF	1.08	0.97;1.19		1.00	0.89;1.12	1.10	0.89;1.36	
CEF	0.92	0.83;1.02		0.87	0.78;0.97	0.92	0.70;1.20	
Adjusted analysis								
			0.002					
CMF	1.16	1.08;1.26						
CEF	1.00	0.93;.1.09						
≤ 2.5 yrs			0.008					0.009
CMF	1.27	1.15;1.40		1.35	1.18;1.55	1.13	0.98;1.30	
CEF	1.12	1.02;1.24		1.25	1.10;1.42	0.95	0.82;1.10	
> 2.5 yrs								
CMF	1.02	0.92;1.14		1.01	0.89;1.14	1.05	0.84;1.31	
CEF	0.87	0.77;0.97		0.86	0.76;0.98	0.86	0.65;1.13	

CMF cyclophosphamide, methotrexate and fluorouracil; CEF cyclophosphamide, epirubicin and fluorouracil; ROR, risk of recurrence; DR, distant recurrence; HR, hazard ratio; 95% CI, 95% confidence interval; $P_{interaction}$, P derived from a Wald test for heterogeneity. *Adjusted for age, tumor size, nodal status, histological type and grade, estrogen receptor status and HER2 status.

positive disease; an increasing treatment effect with increasing ROR score category is present for both HER2-negative and HER2-positive disease, and a better treatment effect across all 3 ROR groups for HER2-positive (data not shown), following the results for ROR continuous score.

PAM50 subtypes

Among 826 patients with HER2-negative disease, 65 (8%) have a HER2-enriched PAM50 subtype, and among 314 patients with HER2-positive disease, 57 (18%) have a non-HER2-enriched subtype (Table 3).

For each of the four PAM50 subtypes, the DR rate over time is shown in Supplemental Fig. 6 according to treatment regimen. Patients with a HER2-enriched subtype had an improved outcome if treated with CEF as compared to CMF (Fig. 3, Supplemental Figs. 1 and 2), with a statistically significant interaction in treatment effect across the four subtypes ($P_{interaction}$ = 0.02, $P_{interaction}$ = 0.01 and $P_{interaction}$ = 0.03, for DR, TR and OS respectively), and with similar results for the HER2-enriched subtype compared with the non-HER2-enriched (Luminal A, Luminal B and Basal-like) and the HER2 status.

Among patients with a non-HER2-enriched subtype, the treatment effect does not differ significantly according to HER2 status. For patients with a HER2-enriched subtype, there was a clear differential outcome for the two treatment regimens, irrespective of HER2 status, with fewer events in the anthracycline (CEF) arm. The univariable analysis is presented in Supplemental Fig. 7 where the distinct pattern between HER2-enriched and non-HER2-enriched is evident.

Discussion

In breast cancer adjuvant chemotherapy regimens, benefit from exchanging methotrexate (in CMF) with an anthracycline (epirubicin, in CEF) has previously been demonstrated in the NCIC-CTG MA.5 and the DBCG 89D trials separately and is mirrored in this joint analysis of these two trials[6,7]. Furthermore, the risk of distant recurrence increased significantly with increasing ROR score. When broken down into predefined categories a differential benefit from anthracyclines was shown according to ROR score and intrinsic subtype.

The result of this MA.5 and DBCG 89D joint analysis highlights that breast cancer patients clinically classified as high-risk may not derive benefit from adjuvant anthracycline-based polychemotherapy if, by PAM50, they have a Luminal A subtype or a low ROR score. Notably, more than 80% of the patients included in this joint analysis were premenopausal, and almost 80% were node-positive (with 35% having ≥ 4 positive nodes). Further, the results from the DBCG 77B trial showing lack of benefit from CMF-based chemotherapy in high-risk predominantly node-positive patients whose breast tumors have a low ROR score or Luminal A subtype[16], might further support sparing these patients chemotherapy[16]. Furthermore, we also previously validated the prognostic clinical utility of PAM50 in patients with zero to three positive nodes; a low ROR score or a Luminal A subtype in patients who, without chemotherapy, were allocated to 5 years of adjuvant endocrine therapy was associated with a low risk of recurrence, as has also been demonstrated in a joint analysis of the ATAC and ABCSG-8 trials[17,18].

Treatment with anthracycline-containing adjuvant chemotherapy led to fewer recurrences in patients with an intermediate or high ROR score, although only those with a high ROR score obtained a significant survival benefit. While patients with a HER2-enriched subtype achieved a highly significant benefit from being assigned to CEF, this study cannot fully address to what extent those with a Basal-like or a Luminal B subtype benefitted. This study has some potential limitations. First, the two trials precede the era of HER2-directed therapies, and in the absence of trastuzumab a greater benefit from anthracycline-based adjuvant chemotherapy was observed in patients with HER2-positive than HER2-negative breast cancer. Second, results on the Prosigna assay were only available from 454 of the 716 participants in MA.5 and from 686 of the 980 Danish participants in 89D. Third, the subgroup analysis in our study was not adequately powered, and so moderate effects may not have been identified. Fourth, although the use of older mature clinical trial data has the advantage of providing long-term follow-up with many events, this comes with the limitation that extrapolation into the context of contemporary treatment practices (e.g. cdk4/6 inhibitors, immunotherapy) becomes more challenging. There was a

Table 3 | Distribution of ROR score and PAM50 subtype according to HER2 status

	Total		HER2-negative		HER2-positive	
	N	*Col %*	N	*Col %*	N	*Col %*
	1140		826		314	
ROR score						
≤ 51	470	*41*	404	*49*	66	*21*
52-71	443	*39*	322	*39*	121	*39*
≥ 72	227	*20*	100	*12*	127	*40*
PAM50 subtype						
HER2-enriched	322	*28*	65	*8*	257	*82*
Basal-like	353	*31*	338	*41*	15	*5*
Luminal B	188	*16*	171	*21*	17	*5*
Luminal A	277	*24*	252	*31*	25	*8*

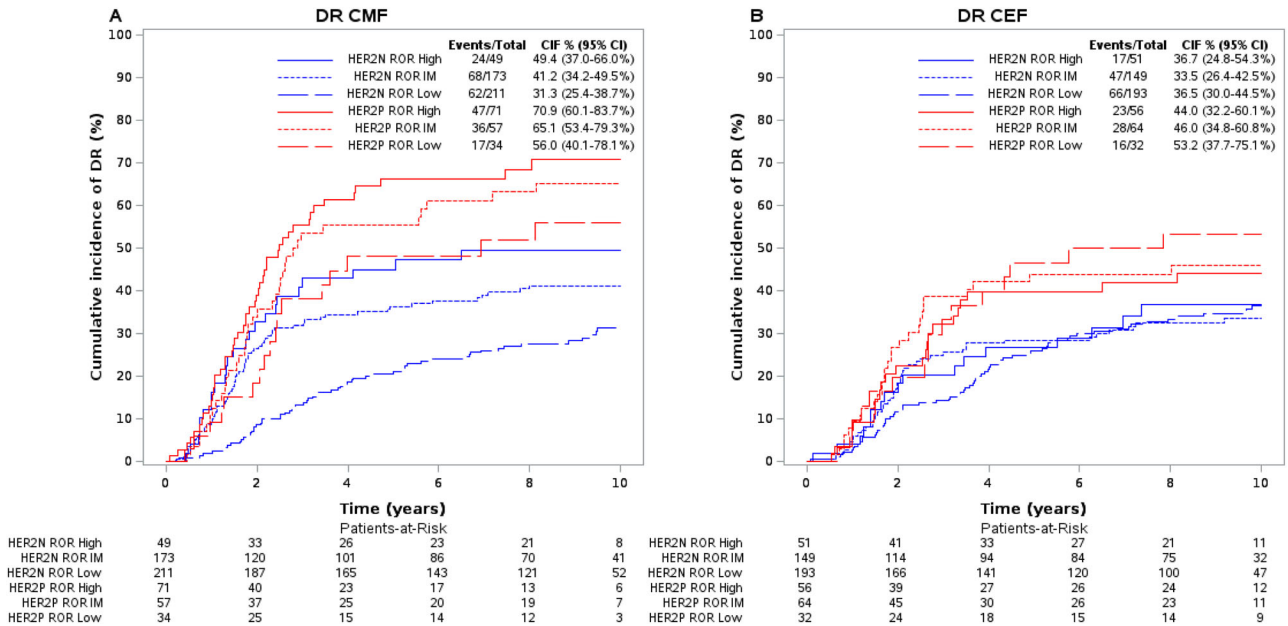

Fig. 2 | Estimates of distant recurrence (DR) rate according to Prosigna ROR ≤51 (Low), 52–71 (IM), and ≥72 (High), and HER2 status. HER2N HER2-negative, HER2P HER2-positive. 10-year estimates with 95% CI are included. **A** Patients in the CMF regimen and **B** Patients in the CEF regimen.

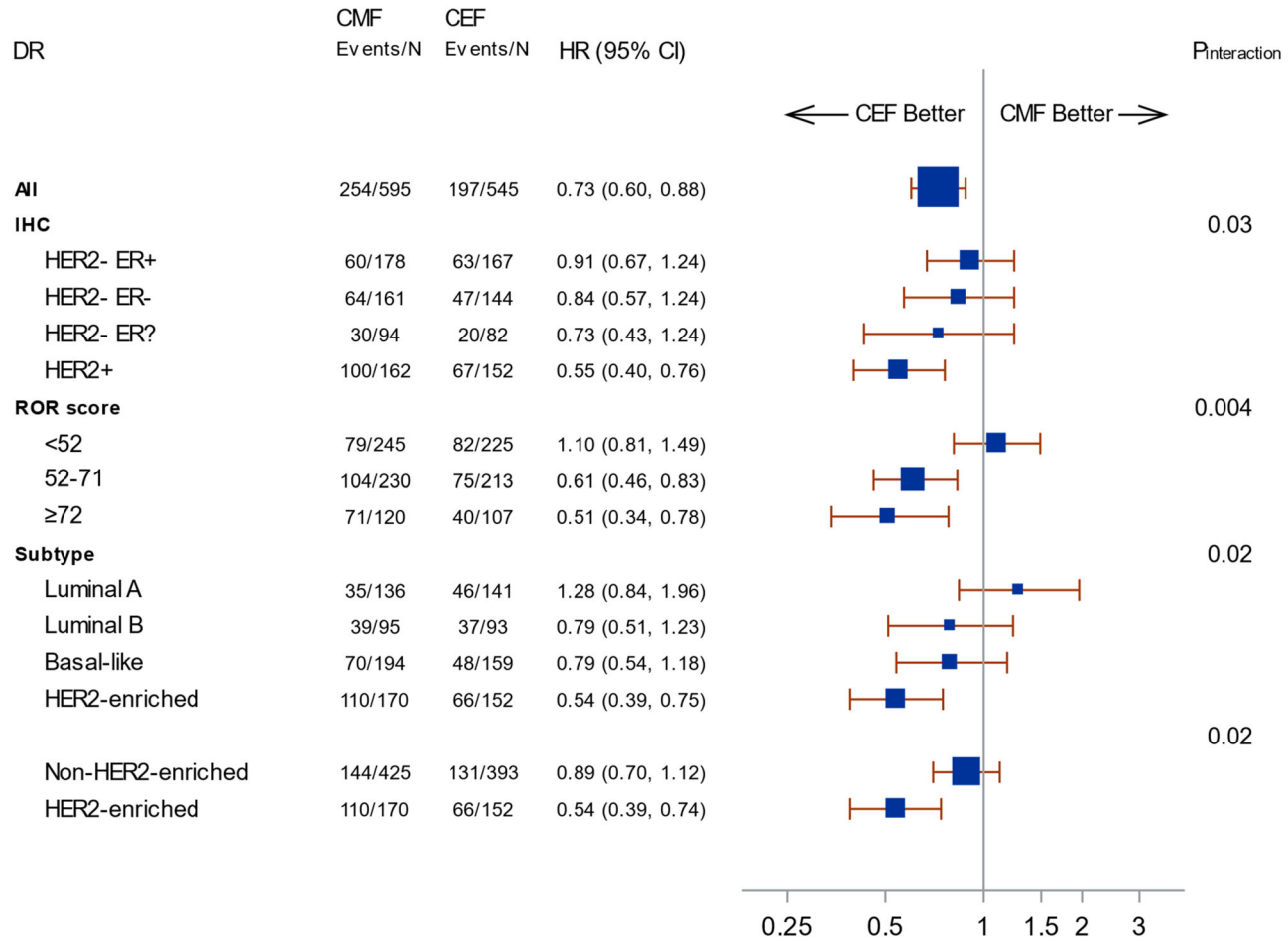

Fig. 3 | Forest plot illustrating subdistributional hazard models for distant recurrence (DR) according to immunohistochemistry (IHC) HER2 and ER status, Risk of reccurence (ROR) score and intrinsic subtype. - negative, + positive, ? unknown. Hazard ratios (HRs) refer to adjusted estimates obtained in multivariable analysis. Boxes represent the weight of data for each subgroup relative to the total data. $P_{interaction}$ derived from a Wald test for heterogeneity; for IHC: HER2 negative versus HER2 positive.

considerable overlap between having a HER2-enriched subtype and being HER2-positive; however, in those without concurrence a HER2-enriched gene expression subtype was predictive of benefit from anthracyclines whereas clinically HER2-positive disease was not. Anthracycline-containing chemotherapy therefore could be considered in patients with a PAM50 HER2-enriched tumor irrespective of HER2 status.

Responsiveness to anthracyclines has not been evaluated by other gene expression assays in comparable randomized trials or similar high-risk populations[19]. Although many patients who require adjuvant chemotherapy may avoid anthracyclines, no clear subsets based on clinical factors have been identified. While advancements in other therapies may have reduced the utility of anthracyclines, gene expression assays have proved useful to guide treatment with anthracyclines and it is still debated how this should affect treatment decisions. The evidence of this study suggest that anthracyclines improve outcomes especially for patients with high genomic risk. In conclusion, this joint analysis provides evidence that with increasing ROR score the benefit of including an anthracycline increases while patients with a low ROR score or a Luminal A subtype breast cancer do not obtain any incremental benefit and may be spared anthracyclines.

Methods

Study population

The NCIC.CTG MA.5 trial and the DBCG 89D trial were included in this pooled analysis. Both are open-labeled randomized phase 3 trials comparing CMF (cyclophosphamide, methotrexate, 5-fluorouracil) with an anthracycline-based regimen; CEF including epirubicin. Inclusion criteria, patient characteristics, trial results and several biomarker studies have been published[6–15]. In brief, the MA.5 trial included premenopausal women with node-positive breast cancer, and in DBCG 89D women with invasive early-stage breast cancer were eligible if they were (I) premenopausal with node-negative and grade II-III tumors, (II) premenopausal with node-positive and hormone receptor-negative tumors, or (III) postmenopausal with node-positive and hormone receptor-negative tumors. The trials were conducted in accordance with the Helsinki Declaration and approved by ethical committees with jurisdiction for the participating institutions[6,7]. Informed consent was obtained before randomisation following oral and written information. Meta-analyses do not require submission to an ethics committee/IRB review.

Assay and HER2 status

The Predictor Analysis of Microarray 50 (PAM50) gene expression algorithm characterizes invasive breast cancers by assigning a risk of recurrence (ROR) score and an intrinsic subtype[20]. Assessment of the intrinsic subtypes, the ROR score and the IHC/ISH-based HER2 status have been described in detail previously[8,9,13–15].

Study design

The study is a prospective-retrospective design following the REMARK criteria[21]. An analysis plan for the combined analysis was prespecified and agreed by the trial groups. Individual patient data previously published were used for this combined analysis[14,15].

Endpoints

The primary endpoint is distant recurrence-free survival (DRFS). This is defined as the interval from randomization until distant recurrence or death due to breast cancer. Contralateral breast cancer, other secondary cancer and death due to causes other than breast cancer were treated as competing events. Analyses was performed restricted to 10-year follow-up data. Secondary endpoints are overall survival, defined as the interval from randomization until death from any cause, and time to recurrence.

Statistical methods

Patient-level data were collected and analyzed at the DBCG statistical office. The PAM50 subtype, the PAM50 subtype in combination with clinical HER2 status and the ROR score both continuous and categorical were analyzed in separate models. Kaplan-Meier estimates were calculated for OS and estimates of cumulative incidence for recurrence. The prognostic and predictive values were analyzed using univariable and multivariable models. Competing-risk analysis using Fine and Gray's proportional subdistribution hazards model to evaluate the recurrence endpoints and the Cox proportional hazards regression modelling to evaluate the endpoint of OS were applied, all stratified for trial. The multivariable models included age (≤ 50, > 50), tumor size (≤ 20 mm, > 20 mm; ≤ 50 mm or unknown, > 50 mm), nodal status (ln(number of positive nodes)), histological type and grade (I,II and not graded, III), ER status (ER negative, ER-positive/unknown) HER2 status (negative, positive), treatment regimen (CMF, CEF) and the specific marker. PAM50 molecular subtype was included as four categories (Luminal A, Luminal B, Basal-like, HER2-enriched) and as two categories (HER2-enriched, non-HER2-enriched), the latter alone and in combination with clinical HER2 status. The ROR score was included as a continuous measure (10-point change) and in categorical risk groups (≤ 51, 52-71, ≥ 72), based on previous work[15,16]. Proportional hazards assumptions were assessed using Schoenfeld residuals and by including a time-dependent component for each covariate. Time-dependent components for time ≥ 5 years were included for ER, grade, ROR score and subtype, and at 2.5 years for ROR score for recurrence endpoints, to fulfill the model assumption. The Wald test was used to assess heterogeneity in treatment effect, and applied for ROR continuous score, ROR score categories, ROR score combined with HER2 status, PAM50 four subtypes, PAM50 HER2-enriched vs PAM50 Non-HER2-enriched, and the latter combined with clinical HER2-negative vs HER2-positive. P-values are 2-tailed, unadjusted for the number of comparisons. Statistical analyses used the SAS Enterprise Guide v8.3 software program package (SAS Institute, Cary, NC).

Data availability

Clinical data for the patients included in this study are not publicly available to protect patient privacy. Access to data used in this study can be made available to qualified researchers through application to the respective trial groups.

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Competing interests
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Correspondence and requests for materials should be addressed to Maj-Britt Jensen.