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CLINICAL TRIAL

Chromosome 17 centromere (CEP17) duplication as a predictor of anthracycline response: evidence from the NCIC Clinical Trials Group (NCIC CTG) MA.5 Trial

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Abstract *HER2* gene amplification and topoisomerase IIα gene (*TOP2A*) alteration have been associated with increased benefit from anthracycline compared to non-anthracycline containing adjuvant breast cancer chemotherapy in some but not other studies. Chromosome 17 centromere (CEP17) duplication was measured on TMAs from formalin-fixed paraffin-embedded specimens obtained from 639 of 716 premenopausal women with node positive breast cancer who received cyclophosphamide, epirubicin and fluorouracil (CEF) or cyclophosphamide, methotrexate and fluorouracil (CMF) in the randomized controlled mammary 5 (MA.5) adjuvant trial. The prognostic impact of CEP17 duplication and its interactions with treatment were studied for relapse-free survival (RFS) and overall survival (OS). Overall, CEP17 duplication was not significantly associated with RFS or OS in multivariate analysis. For patients whose tumours had normal CEP17 copy number there were no apparent benefits for CEF compared to CMF for RFS (HR 0.98; 95% CI 0.68–1.42) or OS (HR 1.10; 95% CI 0.72–1.69). For patients whose tumours had CEP17 duplication, there was significant benefit for CEF compared to CMF for RFS (HR 0.54; CI 0.33–0.89) and a trend towards significance for OS (HR 0.64; CI 0.37–1.09). The adjusted *P* values for interaction between treatment and CEP17 duplication were 0.09 for RFS and 0.13 for OS. This study suggests that CEP17 duplication has a borderline association with clinical responsiveness to anthracycline containing chemotherapy similar to previous results seen with *HER2* amplification and *TOP2A* alteration in MA.5. An appropriately powered meta-analysis is required to discriminate the predictive value of these three candidate markers.

Keywords Chromosome · Centromere · CEP17 · Duplication · Anthracycline

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Introduction

A number of previous studies associated both *HER2* overexpression and amplification with a favourable tumour response to anthracycline compared to non-anthracycline containing chemotherapy [1–6], although only two of these

analyses reached statistical significance [3, 6]. These observations were supported by the publication of three meta-analyses based on literature reports of published data [7–9]. However, inconsistency between studies relating to *HER2* led to a broader search for an appropriate biomarker. Subsequently, as a result of the proximity of *HER2* and topoisomerase IIα (*TOP2A*) on chromosome 17 [10, 11] it was hypothesized that *TOP2A* possibly represented the true target gene for anthracycline benefit. Several studies reported on association between *TOP2A* gene alterations and greater responsiveness to anthracyclines [10–12] again with mixed results. Knoop et al. [11] suggested that whilst *HER2* status gave no predictive information with respect to the benefit of anthracycline-containing chemotherapy, *TOP2A* deletion or amplification predicted benefit with borderline significance. A recent article by Pritchard et al. [13] reviewed the associations of *HER2* amplification/overexpression and *TOP2A* gene alterations from the published literature and found them inconsistent.

The potential role of *TOP2A* and *HER2* as predictive biomarkers for anthracycline benefit was most recently addressed in two linked UK studies [14, 15]. In an analysis of 1,870 breast cancers from the linked BR9601/NEAT studies, no consistent predictive value of either *HER2 or TOP2A* was shown [14, 15] and in a central, individual patient meta-analysis of four trials including 1,944 patients, Di Leo et al., showed a modest and only statistically borderline predictive value for either of these biomarkers [16, 17]. These studies raise significant questions and highlight controversy regarding the association between *HER2* or *TOP2A* gene alterations and the benefit of anthracycline-containing therapies in comparison to non-anthracycline-containing therapies in early breast cancer.

Previous studies have separately analysed, on the one hand, *HER2* amplification or overexpression or *TOP2A* gene amplification or deletion [1–18], topoisomerase II protein (topo2α) [19] and on the other, more conventional markers of response such as proliferation [20, 21]. The piecemeal publication of results relating to different biomarkers, often in under-powered patient cohorts, has hampered efforts to discriminate between the potential associations amongst *HER2* and *TOP2A* alterations and anthracycline therapy. This is particularly true since the majority of *TOP2A* amplified or deleted cases are also amplified for *HER2* when assayed by FISH. Interestingly, the recent analysis of the UK National Epirubicin Adjuvant Trial (NEAT)/BR9601 not only showed no interaction between *HER2* and *TOP2A* and an anthracycline versus non-anthracycline regimen but suggested that chromosome 17 centromere (CEP17) duplication was significantly associated with the benefit of anthracycline-containing regimens [15]. Thus, we here analyze data from the NCIC Clinical Trials Group (NCIC CTG) randomized Mammary 5 (MA.5) trial of cyclophosphamide, epirubicin and 5-fluorouracil (CEF) compared to cyclophosphamide, methotrexate and 5-fluorouracil (CMF) as adjuvant therapy for breast cancer [22, 23] to explore the association of CEP17 duplication with the efficacy of an anthracycline versus a non-anthracycline-containing regimen.

Subjects and methods

Patients

The MA.5 study randomized 716 premenopausal women with axillary lymph node-positive breast cancer (T1–T3a, N1–N2, M0) who had completed primary breast cancer surgery no more than ten weeks before random assignment [22, 23]. Patients were accrued between 1989 and 1993 at 35 centres in Canada. The MA.5 protocol was approved by the institutional review board at each participating centre and registered as NCI-V90-0027 on cancer.gov. Written informed consent was obtained from each woman before random assignment.

Treatment regimens

The adjuvant CEF regimen consisted of six cycles of epirubicin (Pharmorubicin; Pfizer, New York, NY) 60 mg/m^2 and 5-fluorouracil (5-FU) (Efudex; Valeant Pharm, Aliso Viejo, CA) 500 mg/m^2, both delivered intravenously on days 1 and 8, and oral cyclophosphamide (Cytoxan; Bristol Myers Squibb, New York, NY) 75 mg/m^2 daily on days 1–14. During this regimen, patients received antibiotic prophylaxis with trimethoprim–sulfamethoxazole (Septra; Glaxo, Philadelphia, PA; Merck, Whitehouse Station, NJ) 400 mg orally twice daily or ciprofloxacin (Cipro; Bayer, Berlin, Germany) 500 mg orally twice daily. The CMF regimen consisted of six cycles of methotrexate (Wyeth, formerly Lederle, Madison, NJ) 40 mg/m^2 and 5-FU 600 mg/m^2, both delivered intravenously on days 1 and 8, and oral cyclophosphamide 100 mg/m^2 daily on days 1–14.

Specimen collection

Representative formalin-fixed, paraffin-embedded (FFPE) tumour blocks from the primary surgical specimen were retrospectively requested for each woman enrolled in the study. For this analysis, pathologists were asked to submit a representative FFPE block of tumour tissue from each woman, or if tumour blocks were unavailable, 20.4-μm unstained sections, to the central office of the NCIC CTG. Paraffin blocks were stored at room temperature, and unstained sections were kept at 4°C. Samples were identified only by an identification number assigned to each patient at randomization. A stained section of each tumour sample was

prepared from blocks or slides to confirm the diagnosis and to identify representative tumour areas for microdissection. Further 4 μm sections were obtained for immunohistochemical analysis and fluorescence in situ hybridization (FISH). Assay results were reported to the NCIC CTG central office, where the statistical analysis was performed.

Measurements of HER2 and TOP2A amplifications and CH17 CEP centromere duplication

HER2 and *TOP2A* amplifications and deletions were measured by FISH as described in our previous studies [12]. The FISH probes used were the PathVysion *HER2* DNA probe kit and the Locus Specific Identifier (LSI) *TOP2A*/CEP17 probe kit (both from Vysis-Abbott, Downer's Grove, Illinois, USA). Slides were prepared according to the manufacturer's instructions for the paraffin sections. Sections were analysed using a Leica DMBRX epifluorescence microscope (Bannockburn, IL) equipped with filters for the separate detection of 4′,6-diamidino-2-phenylindole, spectrum green, and spectrum orange and with a triple bandpass filter for simultaneous detection of the three signals. The number of signals representing the gene of interest (*TOP2A or HER2)* and the number of chromosome 17 centromeres present in each cell were recorded for a minimum of 60 nuclei per case. Images were captured by a charge-coupled device camera using software from Applied Imaging (Santa Clara, CA). The ratio of the signal of interest per chromosome 17 centromere was calculated for each sample. A ratio of *HER2* to CEP17 of 2.0 or more was considered to indicate *HER2* amplification. A tumour was considered to have amplified *TOP2A* if the *TOP2A*:*CEP17P* ratio was 2.0 or greater; to have deleted *TOP2A* if the ratio was 0.8 or less; and to have normal *TOP2A* copy number if the ratio was between 0.8 and 2. "Altered" *TOP2A* was a combined category that included both patients with deleted and with amplified *TOP2A*. CEP17 duplication copy number results were collected for all cells with a minimum of two C17 signals/cell and CEP17 duplication defined as >2.25 mean observed copies/cell [24, 25] as this cut-off has been specifically validated for the scoring approach used here.

Statistics

Relapse-free survival (RFS), defined as time from randomization to first recurrence, and overall survival (OS), defined as time from randomization to death from any cause, were two outcomes of this study. The Kaplan–Meier method was used to estimate the RFS and OS at 5 years and associated confidence intervals. Univariate Cox proportional hazard model including only one single factor or multivariate Cox proportional hazard model adjusting for age (≥50, <50); number of positive nodes (≤3, ≥4); oestrogen receptor (ER) protein levels from the clinical data base of the MA.5 trial, i.e., local ER measurements from each centre (≥10, <10 fm protein/ml); surgical type (total vs. partial mastectomy); tumour size (T1, T2, T3), tumour grade (1, 2, 3, using the Elston & Ellis grading system [24], performed by central review on whole sections) and *HER2* (amplified or not amplified) and *TOP2A* (altered or normal) as measured by FISH was used to obtain hazard ratios for relapse or death.

Results

There were no apparent differences in patient characteristics between the overall trial populations and the populations included in this TMA study (Table 1) (consort diagram: Fig. 1). Of 628 patients included in this study, 332 (53%) relapsed and 250 (40%) died during the period of follow-up.

CEP17 duplication

CEP17 duplication as defined above was observed in 253/628 (40.3%) of cases included in the analysis. No significant association was observed between *HER2* gene amplification (observed in 24.4% of cases) and CEP17 duplication ($P = 0.95$). Cases with CEP17 duplication were equally distributed between *HER2* amplified (40.2%) and non-amplified (40.5%) cases. Only tumour grade was significantly associated with CEP17 duplication ($P = 0.006$; Table 1).

Association with RFS and OS

In univariate analysis, CEP17 duplication was not significantly associated with RFS (HR 1.19, 95% CI 0.96–1.49, $P = 0.12$; Fig. 1a) but its association with OS was significant (HR = 1.33, 95% CI 1.04–1.76 and $P = 0.03$; Fig. 1b) (Table 2). In Cox multiple regression models including the following covariates: CEP17 status; *HER2* status; *TOP2A* status; ER status; age; grade; nodal status; tumour size; surgery and treatment (CEF vs. CMF): tumour size, nodal status grade and surgery types were significantly associated with both RFS and OS. None of the three biomarkers (*HER2*, *TOP2A* or CEP17 status) was found significantly associated with either RFS or OS after adjusting for other variables (Table 3).

Treatment by marker interactions

For tumours with normal CEP17 copy number there were no apparent benefits for CEF compared to CMF for RFS

Table 1 Patient/tumour characteristics from the MA.5 trial and samples analysed for this study and their association with CEP17 status in univariate analysis

	All patients randomized	Samples analyzed for CEP17 centromere status			
		Total	Duplicated	Normal	*P* value*
Number	716	628	253	375	
Age in years median (range)	44.5 (23.4–57.2)	44.7 (23.4–57.2)	44.5 (27.6–55.9)	44.7 (23.4–57.2)	0.50
Treatment					
CEF	356 (49.7%)	312 (49.7%)	117 (46.3%)	195 (57.0%)	0.16
CMF	360 (50.3%)	316 (50.3%)	136 (53.7%)	180 (48.0%)	
Tumour size					
T1	279 (39.0%)	245 (39.0%)	104 (41.1%)	141 (37.6%)	0.62
T2	352 (49.2%)	310 (49.4%)	117 (46.3%)	193 (51.5%)	
T3	36 (5.0%)	31 (4.9%)	13 (5.1%)	18 (4.8%)	
Missing	49 (6.8%)	42 (6.7%)	19 (7.5%)	23 (6.1%)	
Nodes positive					
1–3	436 (60.9%)	382 (60.8%)	156 (61.7%)	226 (60.3%)	0.78
4–10	221 (30.9%)	200 (31.8%)	77 (30.4%)	123 (32.8%)	
>10	59 (8.2%)	46 (7.3%)	20 (7.9%)	26 (6.9%)	
Grade					
1	77 (10.8%)	73 (11.6%)	17 (6.7%)	56 (14.9%)	0.006
2	205 (28.6%)	199 (31.7%)	77 (30.4%)	122 (32.5%)	
3	344 (48.0%)	341 (54.3%)	151 (59.7%)	190 (50.7%)	
Missing	90 (12.6%)	15 (2.4%)	8 (3.2%)	7 (1.9%)	
ER level					
Negative	201 (28.1%)	176 (28.0%)	68 (26.9%)	108 (28.8%)	0.26
Positive	428 (59.8%)	384 (61.1%)	163 (64.4%)	221 (58.9%)	
Missing	87 (12.2%)	68 (10.8%)	22 (8.7%)	46 (12.3%)	
Surgery					
Lumpectomy	351 (49.0%)	312 (49.7%)	117 (46.3%)	195 (52.0%)	0.16
Mastectomy	365 (51.0%)	316 (50.3%)	136 (53.7%)	180 (48.0%)	
Her2 status					
Amplified	153 (21.4%)	153 (24.4%)	62 (24.5%)	91 (24.3%)	0.95
Not amplified	475 (66.3%)	475 (75.6%)	191 (75.5%)	284 (75.7%)	
Missing	88 (12.3%)	0 (0%)	0 (0%)	0 (0%)	
TOP2A status					
Amplified	54 (7.5%)	53 (8.4%)	24 (9.5%)	29 (7.7%)	0.15
Deleted	26 (3.6%)	26 (4.1%)	15 (5.9%)	11 (92.9%)	
Normal	358 (50.0%)	351 (55.9%)	131 (51.8%)	220 (58.7%)	
Missing	278 (38.8%)	198 (31.5%)	83 (32.8%)	115 (30.7)	

* For comparison between duplicated and normal CEP17 centromere from Wilcoxon test for age and Chi-square test for all other variables

CEP17 chromosome 17 centromere

(HR 0.98 with 95% CI 0.68–1.42, $P = 0.93$; Fig. 1c) or OS (HR 1.10 with 95% CI 0.72–1.69, $P = 0.66$; Fig. 1e). Conversely, for tumours exhibiting CEP17 duplication, the relative risks of relapse and death for patients receiving CEF rather than CMF were 0.54 (95% CI 0.33–0.89; $P = 0.02$, Fig. 1d) and 0.64 (95% CI 0.37–1.09, $P = 0.10$; Fig. 1f), respectively (Table 4).

In univariate analysis, CEP17 duplication was associated with a significantly increased benefit from CEF compared to CMF (Table 5) for both RFS and OS with treatment by marker interactions (HR 0.59 with 95% CI 0.37–0.92, $P = 0.02$) and (HR 0.61 with 95% CI 0.37–1.01, $P = 0.05$), respectively. Multivariate Cox regression analyses adjusting for age, nodal status, ER status, grade, tumour size, surgery type, *HER2* status and *TOP2A* status, showed a non-significant trend for a treatment by CEP17 interaction with respect to RFS (HR 0.59 with 95% CI 0.32–1.08, $P = 0.09$) but not with OS (HR 0.60 with 95% CI 0.31–1.12, $P = 0.13$) (Table 5). In contrast, in multivariate regression analyses using the Cox

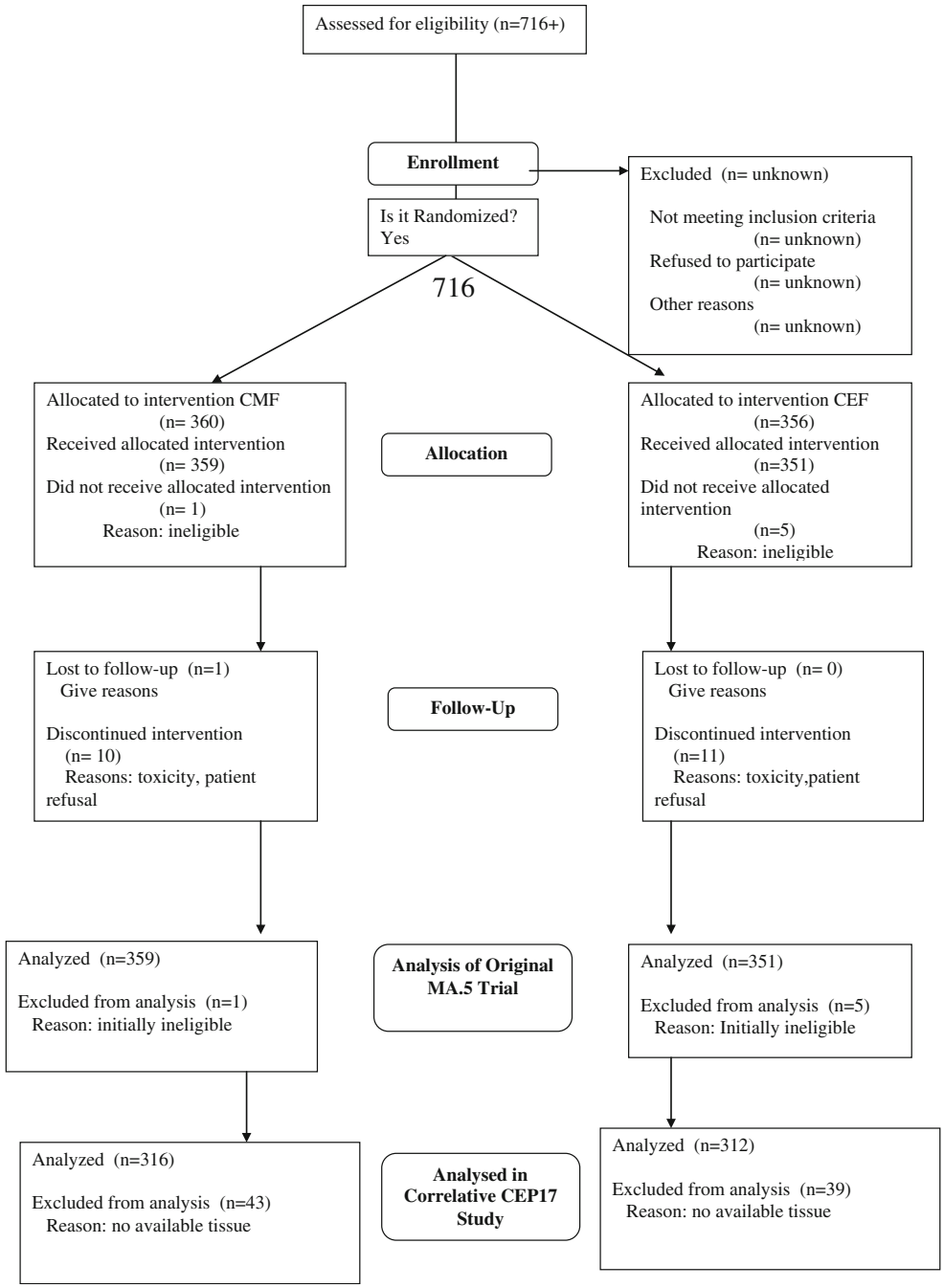

Fig. 1 The consort e-flowchart

model, treatment by *HER2* interaction was borderline significant for both RFS (HR 0.58 with 95% CI 0.33–0.99, $P = 0.05$) and OS (HR 0.57 with 95% CI 0.31–1.02, $P = 0.06$) and treatment by *TOP2A* interaction showed a trend towards significance for RFS (HR 0.53 with 95% CI 0.26–1.09, $P = 0.09$) and significant interaction with OS (HR 0.38 with 95% CI 0.17–0.85, $P = 0.02$) (Table 5).

HER2, *TOP2A* and CEP17 duplication sub-group analysis

Exploratory sub-group analyses were performed by combining *HER2* amplification status or *TOP2A* alteration status with CEP17 duplication status (Table 6). All cases with CEP17 duplication exhibited improved outcome from CEF compared to CMF (RFS or OS). Some evidence of an

Table 2 Association of *CEP17, HER2* and *TOP2A* Status with RFS and OS

Genetic characteristic	*N*	RFS			OS		
		5-year RFS (%) (95% CI)	Hazard ratio* (95% CI)	*P* value*	5-year OS (%) (95% CI)	Hazard ratio* (95% CI)	*P* value*
CEP17							
Duplicated	253	55 (48–61)	1.19 (0.96–1.49)	0.12	69 (63–75)	1.33 (1.04–1.70)	0.03
Normal	375	62 (57–67)			76 (72–80)		
HER2							
Amplified	153	47 (39–55)	1.46 (1.14–1.86)	0.0002	57 (50–65)	1.79 (1.37–2.33)	<0.0001
Not amplified	475	63 (59–67)			79 (75–82)		
TOP2A							
Altered (amplified or deleted)	79	52 (41–63)	1.20 (0.87–1.68)	0.27	57 (46–68)	1.41 (0.98–2.02)	0.06
Normal	351	62 (56–67)			76 (72–81)		

CEP17 chromosome 17 centromere, *HER2* human epidermal growth factor receptor-2, *TOP2A* topoisomerase 2A, *RFS* relapse-free survival, *OS* overall survival, *CI* confidence interval

* From univariate Cox model

Table 3 Multivariate analysis for all prognostic variables

Variables	Relapse-free survival		Overall survival	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
CEP 17 (duplicated vs. normal)	1.15 (0.86–1.54)	0.34	1.39 (0.90–2.15)	0.14
HER2 (amplified vs. not amplified)	1.19 (0.84–1.70)	0.33	1.29 (0.88–1.90)	0.20
TOP2A (altered vs. normal)	0.95 (0.63–1.42)	0.79	1.04 (0.67–1.62)	0.87
Treatment (CEF vs. CMF)	0.81 (0.61–1.08)	0.14	1.11 (0.73–1.70)	0.62
Age (<50 vs. ≥50 years)	1.17 (0.79, 1.72)	0.44	1.04 (0.68–1.59)	0.87
Positive nodes (≤3 vs. >3)	0.57 (0.43–0.76)	0.0001	0.58 (0.42–0.80)	0.0008
Tumour grade (1 vs. 2 vs. 3)	1.38 (1.04–1.83)	0.03	1.72 (1.25–2.37)	0.0009
Tumour size (T1 vs. T2 vs. T3)	1.51 (1.16–1.97)	0.002	1.50 (1.12–2.01)	0.007
Oestrogen receptor (positive vs. negative vs. unknown)	1.14 (0.90–1.44)	0.29	0.94 (0.72–1.24)	0.66
Surgery (mastectomy vs. lumpectomy)	1.48 (1.10–1.99)	0.01	1.77 (1.26–2.49)	0.001

effect of *HER2* or *TOP2A* was seen in the exploratory analyses. Cases with none of *HER2* amplification, *TOP2A* alteration or CEP17 duplication trended towards increased benefit from CMF rather than CEF.

Discussion

We have previously reported conflicting results on the role of type I receptor kinases as biomarkers for anthracycline response in two separate trials in early breast cancer (MA5 and NEAT/BR9601) [3, 12, 14, 15]. In MA5, *HER2*-amplified tumours and *TOP2A* gene-altered tumours were characterised by increased benefit from inclusion of anthracyclines [3, 12], whilst in NEAT/BR9601 *HER2* non-amplified (and HER1/3 low expressing) tumours appeared to derive enhanced benefit from anthracycline-based chemotherapy [14, 15]. In spite of differences in chemotherapy regimens and patient characteristics between these two studies, similar molecular methods were employed in both centres for the analysis of patient samples. Since, the observed differences between these studies provided no rational explanation for differences in results, we explored alternative scientific hypotheses which might unify the results for these two studies.

Following an observation by Reinholz et al. [25] suggesting paradoxically good outcome for patients with CEP17 duplication in tumours treated with AC-T we examined the relationship between CEP17 duplication and anthracycline response in each of our studies. Within the MA.5 trial, patients whose tumours exhibit CEP17 duplication showed a greater than 46% reduction in risk of relapse (either local or distant) and 36% in risk of death when treated with CEF instead of CMF (Table 4, Fig. 1c)

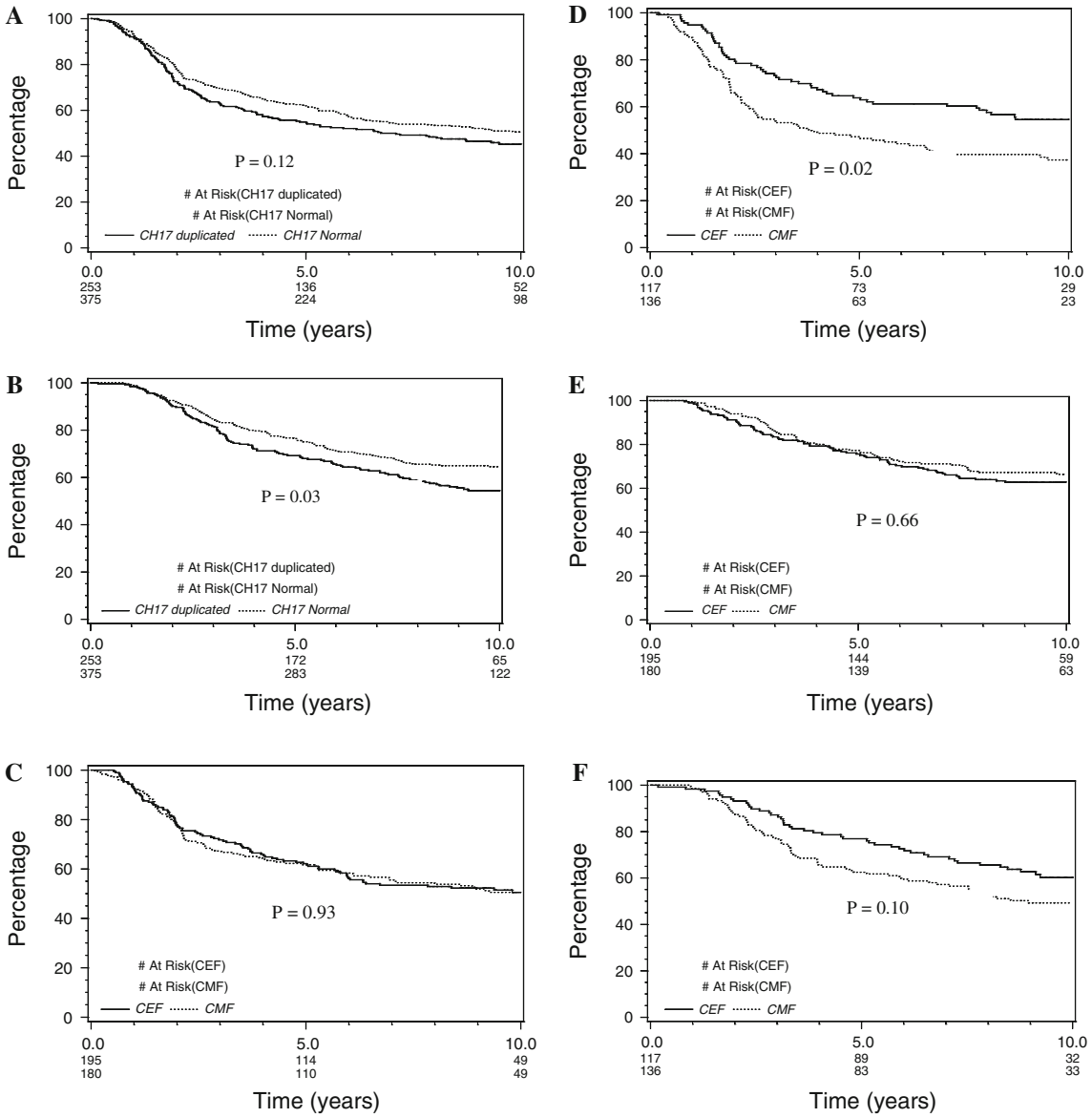

Fig. 2 **a** RFS by CEP17 status, **b** overall survival by CEP17 status, **c** RFS by treatment for those with normal CEP17 copy number, **d** RFS by treatment for those with CEP17 duplication, **e** overall survival by treatment for those with normal CEP17 copy number and **f** overall survival by treatment for those with CEP17 duplication

consistent with results from the NEAT/BR9601 study [15]. No apparent benefit from treatment with anthracyclines was observed in tumours with normal CEP17 copy numbers (Fig. 1c). A significant treatment by marker interaction for both RFS ($P = 0.02$) and OS ($P = 0.05$) was observed in univariate analysis, although only a trend towards significance was observed for RFS after adjustment for other variables.

These results, linking CEP17 duplication to benefit from CEF compared to CMF show similarity to those obtained from an analysis of the two UK trials BR9601 and *NEAT* [14, 15] suggesting that the measurement of CEP17 copy numbers may have provided a unifying biomarker for the prediction of benefit from anthracycline containing versus non-anthracycline-containing regimens. The NCIC CTG, UK (BR9601) and Scottish trial (NEAT) groups plan to use this marker in an individual patient meta-analysis with the same Danish and Belgian groups with whom we have previously examined the role of *HER2* amplification and *TOP2A* gene alterations [16, 17].

Attempts to explore the interaction of *HER2* amplification status, *TOP2A* alteration status and CEP17 duplication status with outcome in the two treatment groups were problematic mainly because of ever-diminishing sample

Table 4 Treatment difference by *CEP17*, *TOP2A* and *HER2* status

Relapse-free survival							Overall survival					
Genetic characteristic	CEF		CMF		Hazard ratio† (95% CI)	*P* value‡	CEF		CMF		Hazard ratio† (95% CI)	*P* value‡
	N	5 year RFS (%) (95% CI)	*N*	5 year RFS (%) (9(95"% CI) (95% CI))			*N*	5 year OS (%) (95% CI)	*N*	5 year OS (%) (95% CI)		
CEP17												
Duplicated	117	64 (54–72)	136	47 (39–55)	0.54 (0.33–0.89)	0.02	117	77 (68–84)	136	62 (54–70)	0.64 (0.37–1.09)	0.10
Normal	195	62 (55–69)	180	62 (54–68)	0.98 (0.68–1.42)	0.93	195	76 (69–81)	180	77 (70–83)	1.10 (0.72–1.69)	0.66
HER2												
Amplified	86	56 (43–67)	67	40 (29–50)	0.41 (0.22–0.74)	0.003	86	62 (49–73)	67	54 (42–64)	0.47 (0.25–0.88)	0.02
Not amplified	245	65 (58–70)	230	61 (55–67)	1.01 (0.72 to1.42)	0.96	245	80 (74–84)	230	77 (71–82)	1.17 (0.79–1.75)	0.43
TOP2A												
Altered (amplified or deleted)	42	67 (50–79)	37	35 (20–50)	0.34 (0.17–0.71)	0.004	42	69 (53–81)	37	43 (27–58)	0.30 (0.13–0.68)	0.004
Normal	165	63 (55–70)	186	61 (53–67)	0.91 (0.67–1.25)	0.57	165	75 (68–81)	186	77 (70–82)	1.12 (0.79–1.61)	0.52

CEP17 chromosome 17 centromere, *TOP2A* topoisomerase 2A, *HER2* human epidermal growth factor receptor-2, *CEF* cyclophosphamide, epirubicin, 5-fluorouracil, *CMF* cyclophosphamide, methotrexate, 5-fluorouracil. *RFS* relapse-free survival, *OS* overall survival, *CI* confidence interval

† Hazard ratio compares CEF over CMF, and has been adjusted for age, nodal status, tumour grade, ER, surgical procedure, tumour size, *CH17*, *HER2* and *TOP2A* status

‡ *P* values were derived from multivariate Cox model. All statistical tests were two-sided

Table 5 Hazard ratios for interactions between treatments and CEP17 duplication or amplification of HER2

From multivariate Cox models						
Results	CEP 17		*HER2*		*TOP2A*	
	Hazard ratio	*P* value	Hazard ratio	*P* value	Hazard ratio	*P* value
Unadjusted results						
RFS	0.59 (0.37–0.92)	0.02	0.62 (0.37–1.02)	0.06	0.48 (0.24–0.93)	0.03
OS	0.61 (0.37–1.01)	0.05	0.66 (0.38–1.14)	0.14	0.37 (0.18–0.76)	0.007
Adjusted results						
RFS	0.59 (0.32–1.08)	0.09	0.58 (0.33–0.99)	0.05	0.53 (0.26–1.09)	0.09
OS	0.60 (0.31–1.12)	0.13	0.57 (0.31–1.02)	0.06	0.38 (0.17–0.85)	0.02

Table 6 Treatment effects for the combination of *HER2* status and *TOP2A* FISH measurements with CEP17 status

Her2 FISH	Adjusted hazard ratio (95% CI) of CEF to CMF [*P* value]†			
	RFS		OS	
	Amplified	Not amplified	Amplified	Not amplified
(a) *HER2* FISH measurements with CEP17 status				
CEP17				
Duplicated	0.25 (0.07–0.96) [0.04]	0.65 (0.37–1.14) [0.13]	0.21 (0.05–0.89) [0.03]	0.82 (0.44–1.52) [0.52]
Normal	0.51 (0.25–1.02) [0.06]	1.36 (0.87–2.14) [0.18]	0.65 (0.31–1.36) [0.25]	1.55 (0.89–2.70) [0.12]
TOP2A FISH	Adjusted hazard ratio (95% CI) of CEF to CMF [*P* value]†			
	RFS		OS	
	Altered	Normal	Altered	Normal
(b) *TOP2A* FISH measurements with CEP17 status*				
CEP17				
Duplicated	0.08 (0.02–0.31) [0.0003]	0.71 (0.41–1.24) [0.23]	0.04 (0.01–0.26) [0.0007]	0.88 (0.49–1.60) [0.68]
Normal	0.54 (0.18–1.63) [0.27]	1.07 (0.71–1.59) [0.76]	0.46 (0.14–1.44) [0.18]	1.33 (0.83–2.15) [0.24]

* *CEP17* chromosome 17 centromere, *HER2* human epidermal growth factor receptor-2, *TOP2A* topoisomerase-2A, *FISH* fluorescence in situ hybridization, *CEF* cyclophosphamide, epirubicin, 5-fluorouracil, *CMF* cyclophosphamide, methotrexate, 5-fluorouracil. *RFS* recurrence-free survival, *OS* overall survival, *CI* confidence interval

† *P* values were determined from multivariate Cox model

size. Of the 639 patients studied in this CEP17 correlative analysis only 395 were available to deal with a multivariate analysis when all three potentially predictive factors were included, mainly because *TOP2A* data was available on only 438 women.

The rationale for an association between CEP17 duplication and anthracycline benefit is not intuitively obvious. The way in which we have measured CEP17 duplication may indicate changes including imbalanced translocations, sub-chromosomal amplification or deletion or whole chromosome genome duplication. Most previous studies using FISH interpreted duplication of CEP17 as chromosomal polysomy. The CEP17 marker however does not identify polysomy, therefore we have used the more accurate description of CEP17 duplication to describe this finding. Our approach does not invalidate previous

definitions of HER2 amplification since these definitions are also based on the ratio of the HER2 gene and the CEP17 centromere irrespective of the underlying chromosomal defect.

Although, our observation that CEP17 duplication is associated with benefit from anthracycline may offer a practical clinical approach to the selection of patients for such therapy, it is not clear what insight it provides into the possible mechanisms of action. Recent articles [26, 27] suggest that chromosomal 17 polysomy is far less common in early breast cancer than was previously suggested and that CEP17 duplication does not simply indicate polysomy or tumour anuploidy but also identifies cancers with subchromosomal duplication or amplification of the CEP17 region which is close to the HER2 amplicon. We cannot tell at the moment which of these different gene abnormalities may be associated with the underlying mechanism of anthracycline sensitivity. Further research with comparative genomic hybridization (CGH) or expression array analysis may be helpful in clarifying the potential mechanisms underlying these clinical/pathogenic observations. These mechanistic explanations should be explored to prepare more informed approaches to the future use of DNA damaging agents in populations who may be resistant to anthracyclines as well as guiding anthracycline usage.

In any case, we conclude that, since some other trials did not find *HER2* amplification or *TOP2A* alteration to be predictive of a differential benefit from CEF compared to CMF, CEP17 duplication may be a more consistent marker. Results from other trials and from our planned individual patient meta-analysis may clarify this matter if enough patients and their tumour markers can be included.

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