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The frequency and clinical significance of *centromere enumeration probe 17* alterations in human epidermal growth factor receptor 2 immunohistochemistry-equivocal invasive breast cancer

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The frequency and clinical significance of *centromere enumeration probe 17* alterations in human epidermal growth factor receptor 2 immunohistochemistry-equivocal invasive breast cancer

Background and aims: Chromosome 17 alterations affect the assessment of *HER2* gene amplification in breast cancer (BC), but its clinical significance remains unclear. This study aimed to identify the prevalence of centromere enumeration probe 17 (CEP17) alterations, and its correlation with response to neoadjuvant therapy (NAT) in BC patients with human epidermal growth factor receptor 2 (*HER2*) immunohistochemistry-equivocal score.

Methods and results: A large BC cohort ($n = 6049$) with *HER2* immunohistochemistry score 2+ and fluorescent *in-situ* hybridisation (FISH) results was included to assess the prevalence of CEP17

alterations. Another cohort ($n = 885$) with available clinicopathological data was used to evaluate the effect of CEP17 in the setting of NAT. *HER2*-amplified tumours with monosomy 17 (CEP17 copy number < 1.5 per nucleus), normal 17 (CEP17 $1.5 - < 3.0$) and polysomy 17 (CEP17 ≥ 3.0) were observed in 16, 59 and 25%, respectively, compared with 3, 74 and 23%, respectively, in *HER2*-non-amplified tumours. There was no significant relationship between CEP17 alterations and pathological complete response (pCR) rate in both *HER2*-amplified and *HER2*-non-amplified tumours. The independent predictors of pCR were

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oestrogen (ER) negativity in *HER2*-amplified tumours [ER negative versus positive; odds ratio (OR) = 11.80; 95% confidence interval (CI) = 1.37–102.00; $P = 0.02$], and histological grade 3 in *HER2* non-amplified tumours (3 versus 1, 2; OR = 5.54; 95% CI = 1.61–19.00; $P = 0.007$).

Keywords: alterations, breast cancer, *CEP17*, chromosome 17, *HER2*

Introduction

Human epidermal growth factor receptor 2 (*HER2*) protein overexpression and/or gene amplification occurs in approximately 15% of invasive breast cancer (BC).^{1,2} Patients with *HER2*-positive BC are often treated with a combination of sequential chemotherapy and *HER2*-targeted therapy in the neoadjuvant and/or adjuvant setting.³ *HER2* status is determined by immunohistochemistry (IHC) and *in-situ* hybridisation (ISH). The current American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines and United Kingdom (UK) recommendations for *HER2* testing recommend a two-tiered system using IHC and ISH testing if required. BC with *HER2* IHC score 2+ are considered equivocal and require the assessment of *HER2* gene amplification status using dual-probe ISH.^{4–6} In the dual-probe ISH assay, *HER2* gene copy number is reported relative to the centromere enumeration probe 17 (*CEP17*) nuclear signal as an internal control, and hence the *HER2/CEP17* ratio is influenced not only by average *HER2* copy number but also by *CEP17* alterations.

Previous studies have shown that the *HER2/CEP17* ratio and *HER2* gene copy number are predictive for neoadjuvant trastuzumab and chemotherapy response in *HER2*-positive BC.^{7–10} Our previous study demonstrated that the maximum benefit of neoadjuvant anti-*HER2* therapy is observed in the subgroup of patients with tumours that are *HER2* IHC score 3+, histological grade 3 or *HER2* IHC score 2+/*HER2*-amplified co-existing with oestrogen receptor (ER) negativity in *HER2*-positive BC patients.¹¹ Although *CEP17* alterations are common, their significance has received less attention in clinical practice.

Chromosome 17 (Ch17) alterations include whole chromosome gains or losses, gene copy number anomalies, allelic losses and structural rearrangements.^{12,13} Whether the number of *CEP17* signals by ISH reflects true polysomy or monosomy of Ch17 has been examined, and recent data have shown that an increased *CEP17* copy number is usually related to focal peri-centromeric gains rather than to true

Conclusion: The impacts of *CEP17* alterations are not as strong as those of *HER2/CEP17* ratio and *HER2* copy number. The hormonal receptors status and tumour histological grade are more useful to identify BC patients with a *HER2* immunohistochemistry-equivocal score who would benefit from NAT.

polysomy.^{12,14} However, most previous studies have interpreted *CEP17* copy number by ISH as whole Ch17 alterations.^{13,15} *CEP17* copy number was reported to affect *HER2* status assessment, and is associated with the clinical outcome and tumour grade.¹⁵ However, the definition of *CEP17* alterations (monosomy, duplication or polysomy), BC cohorts (early BC, metastatic BC, *HER2*-positive BC or *HER2*-amplified/non-amplified BC) and type of chemotherapy and anti-*HER2* therapy have varied among studies, leading to conflicting results. Some earlier reports observed that *CEP17* copy number gains correlated with poor prognosis, whereas other studies did not demonstrate any prognostic significance.^{15,16} Based on the retrospectively assessed clinical trials, the N9831 adjuvant trastuzumab trial suggests a treatment benefit independent of *CEP17* alterations in patients with *HER2*-positive tumours,¹⁷ while the NEAT/ER9601 adjuvant epirubicin trial suggests that *CEP17* duplication predicts benefit from anthracyclines in early stage BC.¹⁸

In clinical practice, ISH testing is performed in IHC *HER2* equivocal tumours. As this subgroup of tumours comprises a relatively low proportion of BCs and includes both *HER2*-positive (*HER2*-amplified) and *HER2*-negative (*HER2*-non-amplified) BCs, a large cohort of *HER2* tested BCs is required to analyse the clinical significance of centromere alterations adequately. Herein, we hypothesise that patients with IHC *HER2*-equivocal BC with different *CEP17* alterations show variable responses to therapy. This study included two large cohorts of BCs with IHC *HER2*-equivocal for which *HER2* fluorescent *in-situ* hybridisation (FISH) data were available and aimed to identify the prevalence of *CEP17* alterations and to assess their clinical impacts in the setting of neoadjuvant treatment (NAT).

Materials and methods

STUDY COHORT

The initial cohort included 6049 BCs with *HER2* IHC 2+ and available *HER2* FISH data from a single

Table 1. HER2 status and CEP17 alterations

Term	Definition	
HER2-amplified	HER2/CEP17 ratio \geq 2.0 or HER2 CN \geq 6.0	
HER2-non-amplified	HER2/CEP17 ratio $<$ 2.0 and HER2 CN $<$ 6.0	
CEP17 alterations	CEP17 monosomy (monosomy 17)	CEP17 signal number $<$ 1.5 per nucleus
	CEP17 normal (normal 17)	CEP17 signal number 1.5–3.0 per nucleus
	CEP17 polysomy (polysomy 17)	CEP17 signal number \geq 3.0 per nucleus

CEP17, centromere enumeration probe 17; HER2, human epidermal growth factor receptor 2; FISH, fluorescent *in situ* hybridisation; CN, copy number.

centre, the University Hospitals Birmingham NHS Foundation Trust, as a large, unselected patient cohort. This cohort was mainly used to assess the prevalence of CEP17 alteration in BC. Detailed clinicopathological and treatment data were available in the second multicentre cohort ($n = 885$). The majority of patients were treated at Nottingham University Hospitals NHS Trust, Nottingham ($n = 395$), with additional patients from Addenbrookes Hospital, Cambridge; University Hospitals of Leicester NHS Trust; St Vincent's University Hospital, Dublin; University Hospital Galway, Galway; Burney Breast Unit, St Helens and Knowsley Teaching Hospital NHS Trust, Liverpool; Guy's and St Thomas' NHS Foundation Trust, London; Ninewells Hospital, Dundee; and University of Turin, Turin Italy.

Patients were considered eligible for anti-HER2 therapies if their tumours showed a HER2/CEP17 ratio \geq 2.0 regardless of the HER2 gene copy number or if the HER2 gene copy number was \geq 6.⁶ Patients were divided into three groups: NAT received including chemotherapy alone; chemotherapy with trastuzumab; and chemotherapy with dual anti-HER2 agents (i.e. trastuzumab with either pertuzumab or lapatinib). The type of chemotherapy included anthracycline and taxane, anthracycline without taxane and non-anthracycline regimens. Pathological complete response (pCR) was defined as no residual invasive carcinoma in both breast and axillary lymph nodes regardless of the presence of residual ductal carcinoma *in situ* (DCIS) (ypT0/Tis ypN0).¹⁹ All data used in the analysis were derived from the original pathology reports.

IMMUNOHISTOCHEMISTRY AND FISH ASSAY

IHC for ER and progesterone receptor (PR) and both IHC and FISH for HER2 on biopsy specimens were assessed according to UK guidelines.^{6,20} For ER and

PR, tumours were classified as positive when there was \geq 1% nuclear staining in invasive tumour cells.²⁰ HER2 IHC was scored as positive (3+), equivocal (2+) or negative (1+/0). IHC score 2+ tumours were tested for HER2 gene amplification by FISH.⁶ As defined by the current ASCO/CAP guidelines, HER2 ISH status was assigned to one of five groups (Table 1).^{4,5} HER2 amplification was defined as HER2/CEP17 ratio \geq 2.0 or HER2 copy number \geq 6.0.⁶ As there is no optimal cut-off value of CEP17 copy number for classifying centromere alterations, we selected the commonly adopted threshold: monosomy (CEP17 copy number $<$ 1.5 per nucleus), normal (CEP17 1.5–3.0) and polysomy (CEP17 \geq 3.0) by FISH (Table 1 and Figure 1).^{13,16,18,21}

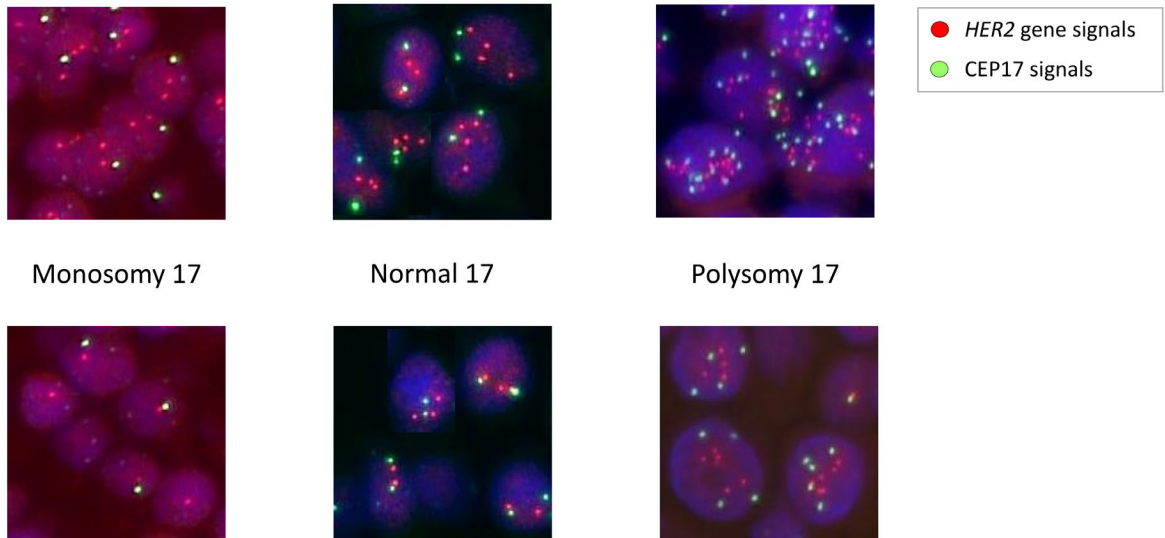
STATISTICAL ANALYSIS

Statistical analysis was performed using EZR software (Saitama Medical Center Jichi Medical University; <http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html>), which is a graphical user interface for R (the R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0).²² Associations between CEP17 alterations and clinicopathological variables or pCR were examined with Fisher's exact tests or Pearson's χ^2 test, as appropriate. A logistic regression model was applied to evaluate the effect of covariates on pCR. If a variable remained at a level of P value \leq 0.15, it was incorporated into the final multivariable model.²³ A P -value $<$ 0.05 was considered statistically significant.

Ethical approval and consent to participate

This study was approved by the Nottingham Research Tissue Bank Access Committee under the IRAS Project ID: 184265. All patients included were consented to participate in research. Data collected were fully anonymised. The study was performed in accordance with the Declaration of Helsinki.

HER2 amplified tumours



HER2 non-amplified tumours

Figure 1. The examples of human epidermal growth factor receptor 2 (HER2) fluorescent *in-situ* hybridisation (FISH) result categories focusing on centromere enumeration probe 17 (CEP17) alterations.

Results

THE PREVALENCE OF CEP17 ALTERATIONS

Fourteen per cent of tumours with IHC score 2+ were HER2-amplified (HER2-positive) and 86% were non-amplified (HER2-negative). Of the amplified group,

87% of cases were defined based on a HER2/CEP17 ratio of ≥ 2.0 , while 13% of cases were defined based on the mean HER2 copy number alone (≥ 6.0). Figure 2 shows the distribution of CEP17 alterations (cut-off of 1.5 CEP17 copy number for monosomy and 3.0 CEP17 copy number for polysomy). HER2-amplified

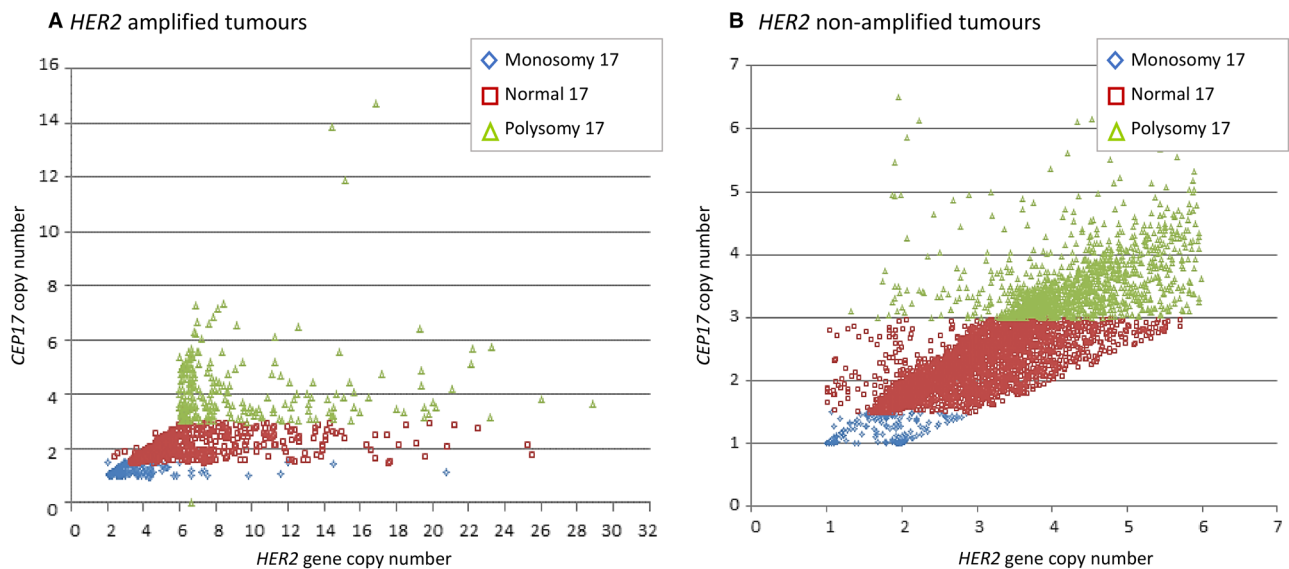


Figure 2. Scatter plot showing the prevalence of centromere enumeration probe 17 (CEP17) alterations in the initial cohort ($n = 6049$). A, HER2-amplified tumours ($n = 877$); B, HER2-non-amplified tumours ($n = 5172$).

tumours with monosomy, normal and polysomy of CEP17 were observed in 16, 59 and 25% of cases, respectively, and in *HER2*-non-amplified tumours in 3, 74 and 23%, respectively ($P < 0.05$).

THE RELATIONSHIP BETWEEN CEP17 ALTERATIONS AND CLINICOPATHOLOGICAL FEATURES

In the second cohort ($n = 885$), 369 cases showed *HER2* gene amplification while 516 tumours did not show evidence of *HER2* gene amplification. The median CEP17 signal number was 2.1 (range = 1.0–12.6) and *HER2* gene copy numbers was 4.1 (range = 1.4–42.4). *HER2*-amplified tumours with monosomy, normal and polysomy of CEP17 were observed in 20, 62 and 18% of cases, respectively, while *HER2*-non-amplified tumours with monosomy, normal and polysomy of CEP17 were observed in 7, 66 and 27%, respectively (Table 2). In *HER2*-non-amplified tumours, polysomy 17 was associated with higher tumour grade ($P < 0.001$). There was no significant relationship between CEP17 alterations and histological type or hormone receptor status.

THE RELATIONSHIP BETWEEN CEP17 ALTERATIONS AND PCR

Full data on NAT were available in 39% of patients ($n = 345$ of 885), including 165 with *HER2*-amplified and 180 with *HER2*-non-amplified tumours. pCR rate was calculated in tumours classified according to *HER2* status and CEP17 alterations, taking account of treatment regimens (Table 3). There was no significant relationship between CEP17 alterations and pCR rate in patients with *HER2*-amplified tumours and *HER2*-non-amplified tumours. The association between clinicopathological parameters and the attainment of a pCR was examined in both *HER2*-amplified tumours and *HER2*-non-amplified tumours by univariate and multivariate stepwise regression models (Table 4). In *HER2*-amplified tumours, ER negativity was identified as an independent predictor of pCR [ER negative versus positive; odds ratio (OR) = 11.80; 95% confidence interval (CI) = 1.37–102.00; $P = 0.02$]. In *HER2*-non-amplified tumours, histological grade 3 was an independent predictor of pCR (3 versus 1, 2; OR = 5.54; 95% CI = 1.61–19.00; $P = 0.007$).

Discussion

Focusing on BC patients with a *HER2* immunohistochemistry-equivocal score, we revealed the

prevalence of CEP17 alterations and their association with response to NAT. Our results showed that polysomy 17, defined by increased CEP17 copy number by ISH, was observed in 24% of *HER2* IHC-equivocal BC and was associated with an increasing *HER2* copy number. In addition, we observed that polysomy 17 was little more frequent in *HER2*-non-amplified tumours than *HER2*-amplified, as shown in a previous study.²⁴ Although Ch17 alterations have been estimated by counting CEP17 copy number by ISH in most previous studies, the definition of CEP17 alterations and the patient cohorts vary among studies, leading to conflicting results.^{13,15} Our cut-off for polysomy 17 (mean of ≥ 3 CEP17 signals per nucleus) is a commonly adopted threshold.^{13,15} Merola *et al.*²⁵ reported a 46% prevalence of polysomy 17 in *HER2* IHC-equivocal BC using a similar definition. Although a higher rate of polysomy 17 in *HER2* IHC-equivocal BC was reported by Merola *et al.*,²⁵ their cohort was smaller than our current series and the clinicopathological parameters were not clearly defined. Moreover, we emphasise that ISH analysis is routinely evaluated in *HER2* IHC-equivocal BC, so the clinical significance of CEP17 alterations needs to be analysed in a large cohort of *HER2* IHC-equivocal BC patients.

Others have reported monosomy 17 in 4% of *HER2*-positive cases.¹⁷ In our cohort, which comprised *HER2* IHC-equivocal BC only, monosomy 17 was present in 16% of *HER2*-amplified tumours compared to 3% of *HER2*-non-amplified tumours. These findings indicate that some cases with a low *HER2* copy number were classified as *HER2*-amplified because of low CEP17 when positivity is defined using *HER2*/CEP17 ratio alone.⁶ Mathematically, the lower the value of denominator (as in cases of Ch17 monosomy), the higher value of the overall ratio, even with a fixed numerator. Biologically, in cells with normal genetic content (2N), there is usually one active allele for each gene while the other copy is dormant. In monosomy 17, the single CEP17 signal binding would result in a higher *HER2*/CEP17 ratio ($> = 2.0$) even with lower levels of amplification of the active *HER2* allele. Although the response rate of these *HER2* IHC 2+ cases with monosomy 17 and low levels of *HER2* gene amplification classified as *HER2* positive based on *HER2*/CEP17 ratio to anti-*HER2* therapy is uncertain, there is no evidence that their response is significantly lower than other *HER2* IHC 2+ with *HER2* gene copy number > 6.0 .¹¹ Emerging new anti-*HER2* antibody drug conjugates (ADC) for 'HER2-low expression groups' would also help to refine the definition of *HER2* positivity for therapeutic purposes.²⁶

Table 2. Clinicopathological characteristics according to CEP17 alterations in both *HER2*-amplified and *HER2*-non-amplified tumours

Characteristics	Total number (%)	<i>HER2</i> -amplified			<i>P</i> -value	<i>HER2</i> -non-amplified			<i>P</i> -value
		Monosomy 17, no. (%)	Normal 17, no. (%)	Polysomy 17, no. (%)		Monosomy 17, no. (%)	Normal 17, no. (%)	Polysomy 17, no. (%)	
Total number	885 (100)	75 (20.3)	227 (61.5)	67 (18.2)		36 (7.0)	339 (65.7)	141 (27.3)	
CEP17 copy number (median [range])	2.1 [1.0–12.6]	1.3 [1.0–1.5]	1.9 [1.5–2.9]	4.2 [3.0–6.7]		1.4 [1.2–1.5]	2.1 [1.5–3.0]	3.60 [3.00–12.60]	
<i>HER2</i> copy number (median [range])	4.1 [1.4–42.4]	3.0 [2.1–42.4]	4.6 [3.0–38.4]	6.8 [1.7–31.2]		2.0 [1.4–2.8]	3.2 [1.6–5.4]	4.5 [1.4–5.9]	
Age (median [range])	59 [23–96]	54 [31–84]	56 [26–96]	59 [36–80]		54 [26–76]	60 [23–95]	62 [28–92]	
Histology type									
No special type	804 (91.0)	70 (94.6)	208 (91.6)	66 (98.5)	0.36	31 (86.1)	298 (87.9)	131 (93.6)	0.08
Lobular	50 (5.7)	3 (4.1)	13 (5.7)	1 (1.5)		1 (2.8)	26 (7.7)	6 (4.3)	
Mixed (no special type and lobular)	29 (3.3)	1 (1.3)	6 (2.7)	0		4 (11.1)	15 (4.4)	3 (2.1)	
Tumour grade									
1	82 (9.4)	5 (6.8)	13 (5.8)	2 (3.0)	0.34	9 (25.7)	42 (12.5)	11 (7.9)	< 0.001
2	577 (65.9)	54 (74.0)	142 (63.7)	45 (67.2)		19 (54.3)	240 (71.2)	77 (55.0)	
3	216 (24.7)	14 (19.2)	68 (30.5)	20 (29.8)		7 (20.0)	55 (16.3)	52 (37.1)	
ER									
Negative	121 (14.8)	8 (13.8)	36 (18.1)	8 (17.4)	0.75	7 (19.4)	47 (13.9)	15 (10.6)	0.35
Positive	698 (85.2)	50 (86.2)	163 (81.9)	38 (82.6)		29 (80.6)	292 (86.1)	126 (89.4)	
PR									
Negative	209 (27.5)	17 (32.7)	55 (32.5)	11 (24.4)	0.56	9 (26.5)	87 (26.9)	30 (21.9)	0.52
Positive	551 (72.5)	35 (67.3)	114 (67.5)	34 (75.6)		25 (73.5)	236 (73.1)	107 (78.1)	

CEP17, centromere enumeration probe 17; *HER2*, human epidermal growth factor receptor 2; ER, oestrogen receptor; PR, progesterone receptor.

Significant *P*-values are shown in bold type.

There are limited studies showing the relationship between CEP17 alterations and treatment response in the neoadjuvant setting, but multiple studies have investigated pathological biomarkers predicting response or resistance to NAT. *HER2* amplification level determined by FISH was associated with pCR, and the

pCR rate was significantly higher in highly amplified tumours.²⁷ Hormone receptor status, especially ER negativity, and high histological grade have been shown to predict response to neoadjuvant chemotherapy alone, and combined chemotherapy and anti-*HER2* therapy in BC.^{11,28–30} In our current cohort of

Table 3. Correlation between CEP17 alterations and pCR

Treatment	Total number	Monosomy 17 pCR/non-pCR, no. (%)	Normal 17 pCR/non-pCR, no. (%)	Polysomy 17 pCR/non-pCR, no. (%)	P-value
<i>HER2</i> -amplified tumours					
Chemotherapy and anti- <i>HER2</i> therapy	165	5/20 (20.0)	25/98 (20.3)	2/15 (11.8)	0.70
Anti- <i>HER2</i> therapy given (single)	146	5/18 (21.7)	22/86 (20.4)	1/14 (6.7)	0.43
Anti- <i>HER2</i> therapy given (dual)	19	0/2 (0)	3/12 (20.0)	1/1 (50.0)	0.46
Chemotherapy containing anthracycline given	110	2/18 (10.0)	16/64 (20.0)	1/9 (10.0)	0.47
<i>HER2</i> -non-amplified tumours					
Chemotherapy	180	1/15 (6.3)	13/105 (11.0)	7/39 (15.2)	0.59
Chemotherapy containing anthracycline given	165	0/15 (0)	11/102 (9.7)	5/32 (13.5)	0.33

CEP17, centromere enumeration probe 17; pCR, pathological complete response; *HER2*, human epidermal growth factor receptor 2.

Table 4. Univariate and multivariate logistic regression model for pCR in both *HER2*-amplified and *HER2*-non-amplified tumours

Parameters	Risk/reference	Univariate analysis			Multivariate analysis				
		pCR/non-pCR, no. (%)	pCR/non-pCR, no. (%)	OR 95% CI P-value	OR 95% CI P-value				
<i>HER2</i> -amplified tumour									
CEP17	Mono, poly/ normal	7/35 (16.7)	25/98 (20.3)	0.78 0.31–1.97 0.61	– – –				
ER	Negative/positive	14/22 (38.9)	18/111 (14.0)	3.92 1.70–9.04 0.001	11.80 1.37–102.00 0.02				
PR	Negative/positive	14/36 (28.0)	13/65 (16.7)	1.94 0.83–4.58 0.13	0.26 0.03–2.10 0.20				
Histological grade	3/1, 2	22/54 (28.9)	10/74 (11.9)	3.01 1.32–6.88 0.009	2.04 0.79–5.25 0.14				
<i>HER2</i> -non-amplified tumour									
CEP17	Mono, poly/ normal	8/54 (12.9)	13/105 (11.0)	1.20 0.47–3.06 0.71	– – –				
ER	Negative/positive	10/31 (24.4)	11/128 (7.9)	3.75 1.46–9.63 0.006	1.46 0.29–7.32 0.64				
PR	Negative/positive	12/48 (20.0)	6/92 (6.1)	3.83 1.35–10.80 0.01	2.67 0.53–13.50 0.24				
Histological grade	3/1, 2	16/54 (22.9)	4/103 (3.7)	7.63 2.43–24.00 < 0.001	5.54 1.61–19.00 0.007				

CEP17, centromere enumeration probe 17; CI, confidence interval; ER, oestrogen receptor; *HER2*, human epidermal growth factor receptor 2; OR, odds ratio; pCR, pathological complete response; PR, progesterone receptor. Significant *P*-values are shown in bold type.

HER2 IHC-equivocal BC within both *HER2*-amplified and *HER2*-non-amplified tumours, there was no significant relationship between CEP17 alterations and pCR rate. Similar to previous studies, hormone

receptor status and histological grade highlighted subgroups showing higher pCR rates. Page *et al.*¹⁶ provided evidence that patients with *HER2*-amplified monosomy 17 in early-stage BC benefited from

trastuzumab, supporting that pCR is not related to CEP17 alterations.

The NEAT/ER9601 adjuvant epirubicin trial¹⁸ showed CEP17 duplication, determined as CEP17 signal number > 1.86, predicted benefit from anthracyclines in early BC, and Tibau *et al.*²¹ reported that CEP17 duplication predicted pCR to primary anthracycline-based chemotherapy in BC. However, our cohort showed no difference in the rate of pCR among the different CEP17 in patients who received anthracycline-based NAT.

The strengths of the study include the large sample size of the cohort, including more uncommon FISH patterns (ASCO/CAP FISH group 2, 3 and 4), reliable well-validated FISH assessment in regional testing centres and the inclusion of a large single-centre cohort consisting of unselected patients. However, our study has some limitations. This was a retrospective non-randomised study and our samples were collected from multiple institutions, which may have some selection bias effect. Also, we have not technically determined if the cases in our series have true monosomy or loss of a portion of the chromosome and true polysomy or gain of a portion of the chromosome.

In conclusion, our findings suggest that the clinicopathological significance of CEP17 alterations is not as strong as that of the *HER2*/CEP17 ratio and *HER2* gene copy number. The hormonal receptor status and tumour histological grade are more useful to identify patients who will benefit from NAT in *HER2* IHC-equivocal BC.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Data availability statement

The authors confirm the data that has been used is available on reasonable request.

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