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TOP2A gene copy number change in breast cancer

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ABSTRACT

Aims The clinical significance of *TOP2A* as a prognostic marker has not been clarified. The aims of this study were to investigate the frequency of *TOP2A* copy number change; to correlate *TOP2A* with *HER2* status, hormone receptor (HR) status and molecular subtype, and further to explore differences in breast cancer-specific survival according to *TOP2A* and *HER2*.

Methods In this study, *TOP2A*, *HER2* and chromosome 17 copy number were assessed in 670 cases of breast cancer using in situ hybridisation techniques. Gene to chromosome ratios ≥ 2 were classified as amplification. *TOP2A* deletion (gene to chromosome ratio ≤ 0.8) or monosomy (only one signal for both gene and chromosome in more than 75% of nuclei) were classified as gene loss.

Results A strong association between *TOP2A* change and HR and *HER2* status was found. During the first 5 years after diagnosis, the risk of death from breast cancer was significantly higher for cases with *HER2* amplification irrespective of *TOP2A* status.

Conclusions *TOP2A* copy number change was strongly associated with HR and *HER2* status and as a prognostic marker *TOP2A* is probably of limited value.

with high histopathological grade³ and high proliferation,⁴ but the clinical significance of *TOP2A* and its relationship to *HER2* have not been clarified.

The aims of this study were to investigate the frequency of *TOP2A* copy number change in a well-characterised cohort of women with breast cancer⁵ and to correlate *TOP2A* with *HER2* status, hormone receptor (HR) status and molecular subtype. A further objective was to explore differences in breast cancer-specific survival (BCSS) according to *TOP2A* and *HER2*.

MATERIALS AND METHODS

Study population

A screening programme for early diagnosis of breast cancer was conducted by the Norwegian Cancer Registry between 1956 and 1959. The patients developed breast cancer in a time period with limited access to adjuvant treatment. None were treated with anthracyclines or trastuzumab. According to the guidelines at the time of diagnosis, 30.7% patients may have qualified for treatment with tamoxifen. The population has been described in detail previously.^{5–7} A total of 1393 women in the underlying population developed breast cancer in the follow-up period from 1961 to the end of 2008. Of these, 945 had tissue samples available at the Department of Pathology and Medical Genetics, St. Olav's Hospital, Trondheim, Norway, and 670 were suitable for assessment of *TOP2A* and *HER2* copy number. Survival data were generated after linkage between the Cause of Death Registry of Norway and the Norwegian Cancer Registry.

Specimen characteristics

All cases in this study have previously been classified according to histopathological type and grade and reclassified in molecular subtypes according to figure 1⁵ using oestrogen receptor (ER),

INTRODUCTION

The *HER2* gene has a well-established biological and clinical role in breast cancer, and the *HER2* amplicon on chromosome 17 harbours a number of genes involved in breast cancer pathophysiology. Copy number change among these genes is frequently observed though their significance remains to be clarified.¹

TOP2A is one of the genes close to *HER2* and its protein product, topoisomerase II α , is the molecular target of anthracycline treatment. *TOP2A* amplification status has been thought to be linked to response to treatment. However, data are conflicting and, as yet, unresolved.² *HER2* and *TOP2A* are associated

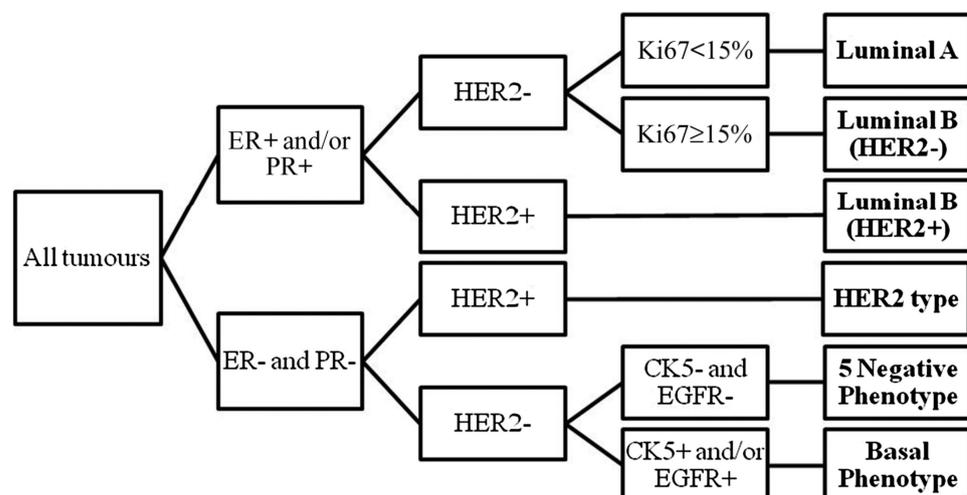


Figure 1 Classification algorithm for molecular subtyping.



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Table 1 Descriptive statistics of the 670 breast cancer cases

	<i>TOP2A</i> normal	<i>TOP2A</i> amplified	<i>TOP2A</i> loss	<i>HER2</i> normal	<i>HER2</i> amplified	Co-amplified	<i>TOP2A</i> loss, <i>HER2</i> amplified	<i>TOP2A</i> amplified, <i>HER2</i> normal	<i>TOP2A</i> loss, <i>HER2</i> normal	Normal <i>TOP2A</i> and <i>HER2</i>	Total
Number (%)	604 (90.2)	41 (6.1)	25 (3.7)	560 (83.6)	110 (16.4)	32 (4.8)	6 (0.9)	9 (1.3)	19 (2.8)	532 (79.4)	670
Mean age at diagnosis (SD)	73.4 (9.7)	69.5 (9.6)	72.0 (12.5)	74.0 (9.3)	68.3 (11.0)	68.2 (10.1)	69.2 (13.6)	74.3 (5.6)	72.8 (12.3)	74.0 (9.2)	73.1 (9.8)
Median years of follow-up after diagnosis (IQR)	6.7 (9.4)	5.8 (11.9)	6.4 (5.9)	7.1 (9.1)	4.5 (10.6)	5.1 (12.6)	5.0 (7.9)	6.0 (10.0)	6.7 (8.1)	7.1 (9.2)	6.6 (9.4)
Tumour grade (%)											
1	71 (11.8)	1 (2.4)	0	71 (12.7)	1 (0.9)	0	0	1 (11.1)	0	70 (13.2)	72 (10.8)
2	319 (52.8)	16 (39.0)	16 (64.0)	318 (56.8)	33 (30.0)	11 (34.4)	2 (33.3)	5 (55.6)	14 (73.7)	299 (56.2)	351 (52.4)
3	214 (35.4)	24 (58.5)	9 (36.0)	171 (30.5)	76 (69.1)	21 (65.6)	4 (66.7)	3 (33.3)	5 (26.3)	163 (30.6)	247 (36.9)
Tumour size (%)											
<2	136 (22.5)	8 (19.5)	5 (20.0)	135 (24.1)	14 (12.7)	5 (15.6)	0	3 (33.3)	5 (26.3)	127 (23.9)	149 (22.2)
2-5	292 (48.3)	15 (36.6)	7 (28.0)	270 (48.2)	44 (40.0)	14 (43.8)	3 (50.0)	1 (11.1)	4 (21.1)	265 (49.8)	314 (46.9)
>5	41 (6.8)	3 (7.3)	5 (20.0)	33 (5.9)	16 (14.6)	2 (6.3)	1 (16.7)	1 (11.1)	4 (21.1)	28 (5.3)	49 (7.3)
Uncertain	135 (22.4)	15 (36.6)	8 (32.0)	122 (21.8)	36 (32.7)	11 (34.4)	2 (33.3)	4 (44.4)	6 (31.6)	112 (21.1)	158 (23.6)
Molecular subtypes (%)											
Luminal A	300 (49.7)	7 (17.1)	10 (40.0)	317 (56.6)	0	0	0	7 (77.8)	10 (52.6)	300 (56.4)	317 (47.3)
Luminal B (HER2-)	166 (27.5)	1 (2.4)	6 (24.0)	173 (30.9)	0	0	0	1 (11.1)	6 (31.6)	166 (31.2)	173 (25.8)
Luminal B (HER2+)	37 (6.1)	23 (56.1)	1 (4.0)	0	61 (55.5)	23 (71.9)	1 (16.7)	0	0	0	61 (9.1)
HER2 type	35 (5.8)	8 (19.5)	5 (20.0)	0	49 (44.6)	9 (28.1)	5 (83.3)	0	0	0	49 (7.3)
Five negative phenotype	22 (3.6)	0	0	22 (3.9)	0	0	0	0	0	22 (4.1)	22 (3.3)
Basal phenotype	44 (7.3)	2 (4.9)	3 (12.0)	48 (8.6)	0	0	0	1 (11.1)	3 (15.8)	44 (8.3)	48 (7.2)
Hormone receptor											
Positive	503 (83.3)	31 (75.6)	17 (68.0)	490 (87.5)	61 (55.5)	23 (71.9)	1 (16.7)	8 (88.9)	16 (84.2)	466 (87.6)	551 (82.2)
Negative	101 (16.7)	10 (24.4)	8 (32.0)	70 (12.5)	49 (44.5)	9 (28.1)	5 (83.3)	1 (11.1)	3 (15.8)	66 (12.4)	119 (17.8)

progesterone receptor (PR), Ki67, cytokeratin 5 and epithelial growth factor receptor (EGFR) 1 as surrogate markers for gene expression. *HER2* status was assessed using chromogenic in situ hybridisation (CISH).

Assay methods

For the present study, fluorescence in situ hybridisation (FISH) was employed for detection of *TOP2A* and chromosome 17 according to the manufacturer's guidelines. Pretreatment was done using Histology FISH Accessory Kit, code K5799 (Dako). The probe mix (VYSIS *TOP2A*/CEP 17 FISH Probe Kit, code 03N89-020 Abbott Molecular Inc) was applied and denatured at 73°C for 5 min before hybridisation at 37°C overnight. For *HER2* and chromosome 17, the *HER2* CISH pharmDx Kit, code 109 (Dako), was used and immunostaining for ER (ER SP1 Cell Marque 33 mg/mL 1:100) and PR (PR 16 Novocastra 360 mg/mL 1:400) was done in a DakoCytomation Autostainer Plus (Dako) using Dako REAL EnVision Detection System with Peroxidase/DAB+, Rabbit/Mouse, code K5007, as previously described.⁵

Scoring and reporting

TOP2A gene copy number was evaluated under a fluorescence microscope (Nikon Eclipse 90i) and *HER2* gene under a bright field microscope (Nikon Eclipse 80i) by three of the authors (AMB, BY and MJE). A minimum of 20 non-overlapping tumour cell nuclei with signals for both chromosome and gene were counted in each case. Gene to chromosome ratios ≥ 2 were classified as amplification.^{8–11} *TOP2A* was considered to be deleted when the gene to chromosome ratio was ≤ 0.8 .^{9–12} Cases with only one signal for both gene and chromosome in more than 75% of all nuclei were recorded as monosomy. In the analyses, deletion and monosomy were grouped together. ER and PR were classified as positive when $\geq 1\%$ of the tumour cells showed positive nuclear staining.

Statistical analyses

Follow-up was from breast cancer diagnosis to death from breast cancer, death from any other cause or to December 31, 2010, whichever occurred first. BCSS was estimated using the Kaplan–Meier method, and Cox proportional hazards models were used to estimate risk of death from breast cancer. HRs were calculated with 95% CIs using Stata V.12.1 IC for Windows (Stata Corp).

RESULTS

Description of breast cancer cases

Of the 670 cases, 251 (37.5%) died of breast cancer, 314 (46.9%) died of other causes, and at the end of the observation period, 105 (15.6%) were still alive. Mean age at diagnosis was 73.1 years (SD 9.8; range 41–96 years), and median follow-up was 6.6 years (IQR 9.42 years). Histopathological grade, tumour size and molecular subtypes are given in table 1.

Amplification and deletion

Table 2 shows amplification of *TOP2A* was found in 41 cases (6.1%) and monosomy or deletion in 25 (3.7%). *HER2* was amplified in 110 cases (16.4%) and co-amplified with *TOP2A* in 32 cases (4.8%). Of the 25 cases with *TOP2A* loss, 6 were amplified for *HER2*. The majority with *TOP2A* amplification (78.1%) were co-amplified with *HER2*, whereas 34.5% of the *HER2* amplified tumours were either *TOP2A* amplified or showed *TOP2A* loss. The proportion of HR+ tumours was higher among cases with *TOP2A* amplification (75.6%) and

Table 2 Number of positive and negative cases for each marker

IHC (%)	<i>TOP2A</i> normal	<i>TOP2A</i> amplified	<i>TOP2A</i> loss	Total
HER2+	72 (11.9)	32 (78.1)	6 (24.0)	110 (16.4)
HER2–	532 (88.1)	9 (21.9)	19 (76.0)	560 (83.6)
ER+	500 (82.8)	31 (75.6)	17 (68.0)	548 (81.8)
ER–	102 (16.9)	10 (24.4)	8 (32.0)	120 (17.9)
PR+	361 (59.8)	19 (46.3)	5 (20.0)	385 (57.5)
PR–	243 (40.2)	22 (53.7)	20 (80.0)	285 (42.5)
Ki67 >15%	270 (44.7)	24 (58.5)	13 (52.0)	307 (45.8)
Ki67 >15%	333 (55.1)	17 (41.5)	12 (48.0)	362 (54.0)
CK5+	115 (19.0)	9 (21.9)	5 (20.0)	129 (19.3)
CK5–	489 (81.0)	32 (78.1)	20 (80.0)	541 (80.8)
EGFR+	46 (7.6)	1 (2.4)	3 (12.0)	50 (7.5)
EGFR–	558 (92.4)	40 (97.6)	22 (88.0)	620 (92.5)
Total	604 (90.2)	41 (6.1)	25 (3.7)	670 (100.0)

ER, oestrogen receptor; PR, progesterone receptor.

TOP2A loss (68.0%) compared with *HER2* amplification (55.5%).

Amplification and loss according to molecular subtypes

With the exception of 5N₁, *TOP2A* copy number aberrations were found in all subtypes and were associated with both HR and *HER2* status. A majority of 56.1% of *TOP2A* amplified cases were Luminal B (*HER2*+). Loss of *TOP2A* was found among the HR+ and *HER2* negative subtypes (Luminal A and Luminal B (*HER2*–)) (64.0%) or *HER2* subtype (20.0%). One of four *TOP2A* deleted case was Luminal B (*HER2*+).

BCSS, *TOP2A* and *HER2*

The Kaplan–Meier plots in figures 2 and 3 show BCSS according to *TOP2A* and *HER2*, respectively, and in figure 4 the BCSS according to the status of both genes. Loss of *TOP2A* in the absence of *HER2* amplification did not affect BCSS. The Kaplan–Meier plots show poorest survival in *HER2*-amplified cases and *TOP2A* aberrations did not affect this.

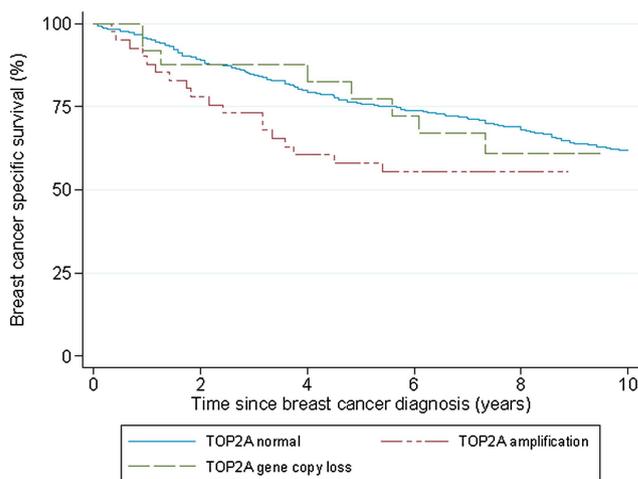


Figure 2 Kaplan–Meier plot. Breast cancer-specific survival (BCSS) according to *TOP2A*. p Value from log-rank test of differences in BCSS first 5 years after diagnosis was 0.02. After 5 years, the p value was 0.4.

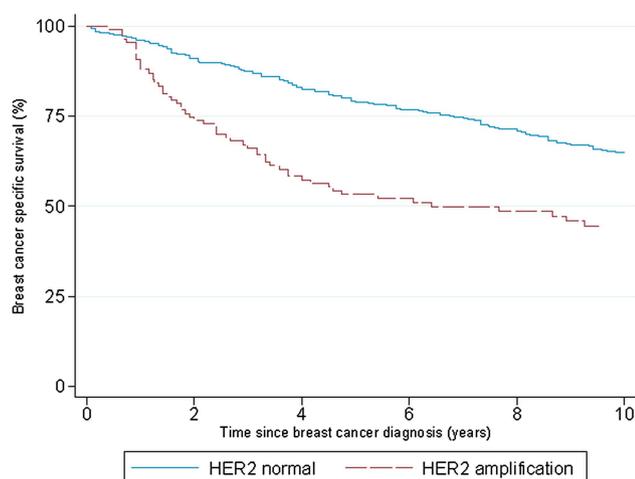


Figure 3 Kaplan–Meier plot. Breast cancer-specific survival (BCSS) according to *HER2*. p Value from log-rank test of differences in BCSS first 5 years after diagnosis was <0.0001. After 5 years, the p value was 0.9.

Risk of death from breast cancer, *TOP2A*, *HER2* and HR status

During the first 5 years, risk of death from breast cancer appears to be significantly higher in cases with amplification of *TOP2A*

and *HER2* when analysed separately. When compared with no amplification for *TOP2A* and *HER2*, respectively, the HR for *TOP2A* amplification was 2.03 (95% CI 1.22 to 3.360) and for *HER2* was 2.77 (95% CI 1.97 to 3.89). Adjusting for age and stage did not change the results. For those who survived the first 5 years after diagnosis, there were no statistically significant differences in survival according to gene amplification status.

However, as shown in table 3 and figure 4, *TOP2A* did not exert an independent effect on prognosis. Adjusting for HR status in the Cox proportional hazards model did not change the results (data not shown). During the first 5 years after diagnosis, the risk of death from breast cancer was significantly higher for HR+ cases with *HER2* amplification irrespective of *TOP2A* status. Among the HR– cases, the numbers in each category were low and the results must be interpreted with caution.

DISCUSSION

TOP2A gene copy number change in breast cancer is an infrequent finding and its significance has been difficult to establish. In this study of 670 cases of breast cancer with long-term follow-up, the number of cases with *TOP2A* amplification or loss was far lower than the number of *HER2*-positive cases. However, there was a large proportion of co-amplification. In contrast to others who have found that amplification of one or both genes entails a poorer prognosis compared with cases with no amplification,^{11 13 14} this study demonstrates that

Table 3 Risk of death from breast cancer according to *TOP2A* and *HER2* amplification

	Number of cases	Deaths from breast cancer	Hazard ratio 95% CI unadjusted		Hazard ratio 95% CI adjusted for age		Hazard ratio 95% CI adjusted for stage	
<i>TOP2A</i>								
Follow-up first 5 years after diagnosis	604	132	1.00		1.00		1.00	
Not amplified	41	17	2.03	1.22 to 3.36	2.07	1.24 to 3.47	2.11	1.27 to 3.50
Amplified	25	5	0.91	0.37 to 2.21	0.82	0.33 to 2.01	0.70	0.29 to 1.73
Loss	670	154						
<i>TOP2A</i>								
Follow-up from 5 years after diagnosis*	359	87	1.00		1.00		1.00	
Not amplified	22	5	0.75	0.30 to 1.85	0.74	0.30 to 1.86	1.02	0.41 to 2.54
Amplified	15	5	1.63	0.66 to 4.03	1.93	0.77 to 4.84	1.41	0.56 to 3.52
Loss	396	97						
<i>HER2</i>								
Follow-up first 5 years after diagnosis	560	105	1.00		1.00		1.00	
Not amplified	110	49	2.77	1.97 to 3.89	2.81	1.95 to 4.04	2.66	1.89 to 3.75
Amplified	670	154						
<i>HER2</i>								
Follow-up from 5 years after diagnosis*	346	83	1.00		1.00		1.00	
Not amplified	50	14	0.95	0.54 to 1.67	0.95	0.52 to 1.73	1.04	0.60 to 1.86
Amplified	396	97						
<i>HER2 and TOP2A</i>								
Follow-up first 5 years after diagnosis	532	100	1.00		1.00		1.00	
Normal <i>TOP2A</i> and <i>HER2</i>	38	17	2.61	1.56 to 4.36	2.76	1.63 to 4.69	2.68	1.60 to 4.51
<i>TOP2A</i> change and <i>HER2</i> amplification	28	5	0.96	0.39 to 2.37	0.89	0.36 to 2.21	0.77	0.31 to 1.90
<i>TOP2A</i> change and <i>HER2</i> normal	72	32	2.86	1.92 to 4.26	2.81	1.84 to 4.29	2.59	1.74 to 3.87
Amplified <i>HER2</i> , <i>TOP2A</i> normal	670	154						
<i>HER2 and TOP2A</i>								
Follow-up from 5 years after diagnosis*	328	79	1.00		1.00		1.00	
Normal <i>TOP2A</i> and <i>HER2</i>	19	6	0.99	0.43 to 2.28	0.97	0.41 to 2.28	1.44	0.62 to 3.37
<i>TOP2A</i> change and <i>HER2</i> amplification	18	4	1.07	0.39 to 2.94	1.26	0.45 to 3.50	0.91	0.33 to 2.51
<i>TOP2A</i> change and <i>HER2</i> normal	31	8	0.92	0.45 to 1.91	0.95	0.44 to 2.04	0.84	0.40 to 1.79
Amplified <i>HER2</i> , <i>TOP2A</i> normal	396	97						

*Conditional on surviving the first 5 years CI.

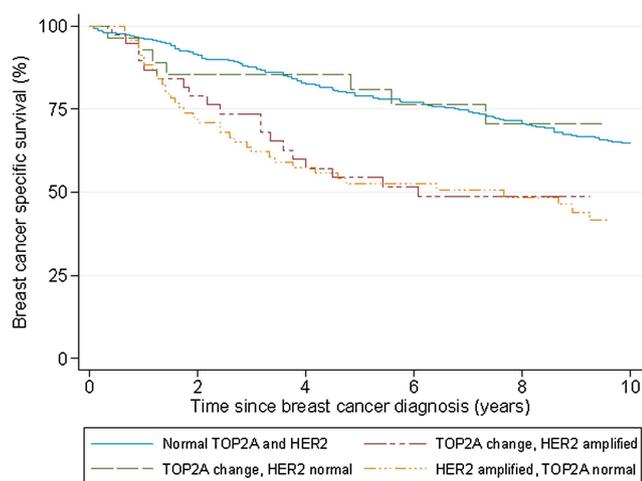


Figure 4 Kaplan–Meier plot. Breast cancer-specific survival (BCSS) according to *TOP2A* and *HER2*. p Value from log-rank test of differences in BCSS first 5 years after diagnosis was <0.0001. After 5 years, the p value was 1.0.

associations between BCSS and *TOP2A* copy number change are not independent of *HER2* and HR status.

The most important finding in this study is the strong association between *TOP2A* copy number change and HR and *HER2* status. These markers are well established as prognostic and predictive factors, and are to a high degree decisive for treatment after surgery. To the best of our knowledge, few studies have been designed to examine the prognostic value of *TOP2A*, though it has been shown that *TOP2A* amplification affects BCSS and risk of death from breast cancer¹⁵ and that *TOP2A* may be a prognostic marker in ER+ breast cancer.^{14 16} However, when the analyses include HR and *HER2* status, the present study shows that *TOP2A* has no independent prognostic impact. *TOP2A* may still have some modulating effects on prognostication, but this is probably of limited benefit in clinical practice.

Twenty of twenty-five cases with *TOP2A* loss were PR–, and of these, 12 were ER+. PR negativeness is a predictor of poor prognosis and appears to be associated with *TOP2A* loss. However, in this study, survival tended to be better in PR– cases with loss of *TOP2A* compared with cases with normal or amplified *TOP2A* (data not shown).

The proportion of amplification and co-amplification of *TOP2A* and *HER2* in breast cancer varies between studies. *HER2* amplification is reported to be around 15%.² For *TOP2A*, amplification varies from 5% to 19%.^{3 17 18} In *HER2*-positive breast cancer, amplification of *TOP2A* varies from 25% to 42%.^{1 19} Both amplification and deletion of *TOP2A* in the absence of *HER2* amplification have been demonstrated.^{3 20} In the present study, 29.1% of the *HER2*-amplified cases were co-amplified with *TOP2A*. The proportion of *TOP2A* positive tumours in this study was lower than in other studies.² However, the frequency of *HER2* amplification is comparable with others, and this weighs against methodological problems. Furthermore, a short DNA probe for *TOP2A* was used to avoid overlap with *HER2*.²¹ This may in part account for the low number of *TOP2A*-amplified cases in this study compared with previous studies and may reflect the true frequency of this finding.

Assessment of loss should be carried out with caution in histopathological sections because nuclear truncation may lead

to a falsely low estimation of copy number. The cut-off for amplification is usually set at a gene/chromosome ratio of ≥ 2.0 , and for deletion the cut-off level ranges from 0.5 to 1.0.²¹ It is possible that monosomy may have an impact similar to loss of individual genes, but this is uncertain. In this study, only four cases showed deletion and monosomy and deletion were grouped together.

HER2-positive breast cancer has been shown to be more aggressive than *HER2*-negative breast cancer. Co-amplification with other genes, such as *STARD3* and *GRB7*, may contribute to and possibly strengthen this aggressive behaviour.² The proportion of amplification and co-amplification of *TOP2A* and *HER2* in breast cancer is low, and even in a series of 670 patients, the numbers are too low to draw reliable conclusions. As a prognostic marker, *TOP2A* is probably of limited value. *TOP2A* aberrations are strongly associated with HR and *HER2* status, and the importance of these markers in prognostication is still unchallenged.

Take-home messages

- ▶ *TOP2A* gene copy number change is an infrequent finding in breast cancer.
- ▶ There is a strong association between *TOP2A* copy number change and hormone receptor and *HER2* status.
- ▶ As a prognostic marker, *TOP2A* is probably of limited value, and hormone receptor and *HER2* status remain unchallenged.

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Contributors MJE contributed to interpretation of in situ markers, carried out statistical analyses, interpretation of the results and drafted the manuscript. BY participated in planning and performing the laboratory work, contributed to interpretation of in situ markers and to discussion and review of the manuscript. LJV contributed to discussion of the study and review of the manuscript. SO participated in acquisition of data and tissue blocks, discussion of the statistical analyses and reviewed the manuscript. AMB contributed to conception and design of the study, interpretation of the in situ markers, interpretation and analyses of the data, and draft and review of the manuscript. All authors read and approved the final manuscript.

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Competing interests None.

Ethics approval Approval of the study and dispensation from the requirement of patient consent was granted by the Regional Committee for Medical and Health Sciences Research Ethics (REK, Midt-Norge, ref. nr: 836/2009).

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