HER2 and *TOP2A* as predictive markers for anthracycline-containing chemotherapy regimens as adjuvant treatment of breast cancer: a meta-analysis of individual patient data

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Summary

Background **Prediction of response to anthracycline-based therapy for breast cancer is challenging. We aimed to assess the value of *HER2* and *TOP2A* as predictive markers of response to anthracycline-based adjuvant therapy in patients with early breast cancer.**

Methods **We did a meta-analysis of individual patient data from five randomised adjuvant trials that compared anthracycline-based regimens with cyclophosphamide, methotrexate, and fluorouracil (CMF) regimens. We assessed the status of *HER2* and *TOP2A* genes with fluorescent in-situ hybridisation. Tumour samples were submitted to an external laboratory for validation. We calculated hazard ratios (HR) to compare event-free survival (EFS) and overall survival in patients receiving anthracycline-based treatment with those receiving CMF in two *HER2* cohorts (*HER2* amplified and non-amplified tumours) and in three *TOP2A* cohorts (normal, amplified, and deleted tumours).**

Findings **We analysed data for 3452 patients for *HER2* and 3102 patients for *TOP2A*. For EFS, HRs were 0·89 (95% CI 0·79–1·01) for *HER2* non-amplified patients and 0·71 (0·58–0·86) for *HER2*-amplified patients ($p_{interaction}$=0·0485); for overall survival, HRs were 0·91 (95% CI 0·79–1·05) for *HER2* non-amplified patients and 0·73 (0·59–0·89) for *HER2*-amplified patients ($p_{interaction}$=0·0718). In analysis of *TOP2A* status, HRs for EFS were 0·88 (0·78–1·00) for normal, 0·63 (0·46–0·87) for deleted, and 0·62 (0·43–0·90) for amplified ($p_{interaction}$=0·0513); HRs for overall survival were 0·89 (0·78–1·03) for normal, 0·68 (0·49–0·95) for deleted, and 0·67 (0·46–0·98) for amplified ($p_{interaction}$=0·1608). When patients with *TOP2A*-deleted and *TOP2A*-amplified tumours were grouped together (altered cohort) and compared with data from patients with normal *TOP2A* tumours, HRs for EFS were 0·64 (0·50–0·81) for altered and 0·88 (0·78–1·00) for normal ($p_{interaction}$=0·0183); HRs for overall survival were 0·67 (0·52–0·86) for altered and 0·89 (0·78–1·03) for normal ($p_{interaction}$=0·0455).**

Interpretation **Although *HER2* amplification and combined *TOP2A* amplification and deletion may have some value in the prediction of responsiveness to anthracycline-based chemotherapy, our findings do not support the use of anthracyclines only in patients with *HER2*-amplified or *TOP2A*-aberrated tumours.**

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Introduction

Findings from several retrospective analyses of randomised trials have suggested that anthracycline-containing adjuvant therapy might be beneficial to only those patients with breast cancer who have *HER2* gene amplification or protein overexpression,[1–4] but this effect cannot be explained by any biological rationale. One of the intracellular targets of anthracycline is the topoisomerase IIα protein—the gene for which, *TOP2A*, is on chromosome 17q12-q21.[5] Other retrospective analyses have suggested that anthracycline-containing adjuvant therapy might be most effective in patients whose tumours carry amplified *TOP2A*.[6–8] However, this association was not seen in a study reported in 2008.[9] Two studies suggested that *TOP2A* gene deletion might also confer increased sensitivity to anthracyclines,[10,11] although, as with *HER2*, this effect cannot be explained by any biological rationale.[5]

These studies have lent support to the idea of a tailored approach to the use of anthracyclines. Nevertheless, none of them alone could safely lead to firm conclusions for daily practice, because small study sample sizes have necessitated caution in application to routine care of patients. Thus, in this meta-analysis we aimed to corroborate or reject individual study findings. We analysed data from five adjuvant trials in which patients were randomly allocated to treatment with cyclophosphamide, methotrexate, and fluorouracil (CMF) or anthracycline-based therapy. In December, 2008, we presented preliminary results with data from 1944 patients.[12]

Here we report the final results with data from 3452 patients to assess the predictive value of *HER2* and 3102 patients to assess the predictive value of *TOP2A*.

Methods

Trial eligibility

Phase 3 trials were eligible for inclusion in our meta-analysis if they were a randomised comparison in the adjuvant setting between anthracycline-based therapy and CMF, if we had access to data from individual patients, and if we had access to archival primary tumour samples. Five trials met these criteria; detailed results of each have been reported previously.[13–16] The webappendix (p 1) shows details of treatment groups for each trial. One study[14] had three study groups, comparing CMF with two anthracycline-based treatments. For the purpose of this meta-analysis the two anthracycline-based groups were pooled. Data from individual patients from every trial were centralised at an independent statistical office (International Drug Development Institute, Louvain-la-Neuve, Belgium). The original files were converted into a SAS database (SAS version 9.1).

National laboratories quality control

An external laboratory (Laboratory of Cancer Biology, University of Tampere, Tampere, Finland) was originally going to do the central assessment of *HER2* and *TOP2A* for all tumour specimens with sections cut from tissue microarrays. However, in December, 2006, the protocol was amended because preliminary data showed suboptimum concordance between results from the external laboratory and those from the four national laboratories that did the assessments for the original studies when the external laboratory tested *HER2* and *TOP2A* on tissue microarray sections. Better concordance rates between the external and the national laboratories were reported when the external laboratory tested *HER2* and *TOP2A* on whole tumour sections. Therefore, the external laboratory retested *HER2* and *TOP2A* on whole tumour sections from a randomly selected sample of cases from each trial. Testing for both markers at the four national laboratories was done by fluorescent in-situ hybridisation (FISH), as described in the individual publications.[4,6,9–11] In the external laboratory, testing was done by FISH with three probes for *HER2*, *TOP2A*, and the centromere of chromosome 17 (Abbott Laboratories, Abbott Park, IL, USA).

Investigators at both the external and national laboratories were masked to clinical outcome of each individual patient and to the result of that sample from the other laboratory. For this analysis, a tumour was defined as *HER2* amplified or *TOP2A* amplified if the ratio between *HER2* or *TOP2A* gene copy number and number of copies of chromosome 17 centromere was two or more; we regarded *TOP2A* gene to be deleted if the ratio was 0·8 or lower and to be *TOP2A* normal if the ratio was greater than 0·8 but lower than two. We estimated concordance in *HER2* and *TOP2A* scores between the external and the four national laboratories by calculating the proportion of cases with the same definition of gene status (ie, amplified or non-amplified for *HER2*, and amplified, deleted, or normal for *TOP2A*).

During on-site monitoring visits, local data, sample flow, and FISH protocols were collected and verified for at least 50 randomly selected patients in each national laboratory. Level of compliance to randomised interventions was verified for each individual trial.

Subgroup analysis

We prospectively defined four biologically homogeneous cohorts: (1) highly hormone-sensitive tumours, defined as oestrogen-receptor and progesterone-receptor positive (≥10% of immunostained cells), *HER2* non-amplified, and grade 1–2; (2) moderately hormone-sensitive tumours, defined as oestrogen-receptor positive and progesterone-receptor negative independent of grade and *HER2* gene status, or oestrogen-receptor and progesterone-receptor positive and grade 3, or *HER2* gene amplified; (3) *HER2*-amplified tumours which were oestrogen-receptor and progesterone-receptor negative; and (4) triple-negative tumours, defined as oestrogen-receptor and progesterone-receptor negative and *HER2* non-amplified. We identified these four cohorts on the basis of gene expression signature studies reported in the past decade.[17] Oestrogen-receptor, progesterone-receptor, and histological grading were assessed at either local pathology units (Piccart and colleagues,[14] Ejlersten and colleagues,[15] and Poole and colleagues [NEAT and BR9601 trials][16]) or the national laboratory (Levine and colleagues[13]). In Poole and colleagues' trials,[16] no progesterone-receptor testing was done. Accordingly, all cases from the NEAT and BR9601 trials[16] defined as oestrogen-receptor positive were assumed to be also progesterone-receptor positive. Considering this assumption, we ran the analysis by molecular subgroups twice—ie, with and without the data from the NEAT and BR9601 trials.[16] The two analyses gave very similar results, so the molecular subgroup analyses include data from the NEAT and BR9601 trials.[16]

Statistical analysis

The primary study endpoint was the comparison in terms of EFS and overall survival between patients who received anthracycline-based treatment and those who received CMF in the two *HER2* cohorts (*HER2* amplified and non-amplified tumours) and in the three *TOP2A* cohorts (*TOP2A* normal, amplified, and deleted tumours). We used a statistical significance level of 0·05, but individual p values should be interpreted cautiously in view of the many comparisons done. All additional analyses presented here were prospectively defined in the statistical analysis plan and should be regarded as exploratory analyses. EFS was defined as time from date of randomisation to date of first relapse, secondary tumour, or date of death without relapse (whichever

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See Online for webappendix

	Country	Number of patients	Median age (years)	Node-positive (n [%])	Oestrogen-receptor-positive (n [%])	Receiving hormone therapy (n [%])	Receiving radiotherapy (n [%])	Median follow-up (years [95% CI])	Patients assessed for HER2 (n [%])	Patients assessed for *TOP2A* (n [%])
Piccart et al[14]	Belgium	804	49	804 (100%)	539 (67%)	346 (43%)	651 (81%)	12 (11·7–12·4)	353 (44%)	89 (11%)†
Levine et al[13]	Canada	716	44·5	716 (100%)	487 (68%)	0	712 (99%)	9·9 (9·8–10·0)	626 (87%)	437 (61%)
Ejlertsen et al[15]	Danish	980	47	627 (64%)	206 (21%)	NA*	392 (40%)	11·4 (10·9–11·6)	670 (68%)	773 (79%)
Poole et al (NEAT)[16]	UK	1684	48	1162 (69%)	1010 (60%)	NA	NA	7·5 (7·4–7·6)	1508 (89%)	1508 (89%)
Poole et al (BR9601)[16]	UK	374	51·1	325 (87%)	236 (63%)	269 (72%)	318 (85%)	6·9 (6·7–7·0)	295 (79%)	295 (79%)

TOP2A=topoisomerase IIα. NA=not available. *Use of hormone therapy not recommended. †*TOP2A* assessed in HER2 amplified cases only.

Table 1: **Main characteristics in the five trials**

	Patients assessed		*HER2* status		*TOP2A* status			Concordance with external laboratory (number assessed in external laboratory / number of concordant cases [% concordance])	
	HER2(n)	*TOP2A* (n)	Amplified (n [%])	Non-amplified (n [%])	Amplified (n [%])	Normal (n [%])	Deleted (n [%])	*HER2*	*TOP2A*
Piccart et al[14]	353	89	74 (21%)	279 (79%)	23 (26%)	58 (65%)	8 (9%)	61/56 (92%)	50/29 (58%)
Levine et al[13]	626	437	152 (24%)	474 (76%)	54 (12%)	356 (82%)	27 (6%)	17/15 (88%)	14/10 (71%)
Ejlertsen et al[15]	670	773	245 (36%)	425 (64%)	92 (12%)	594 (77%)	87 (11%)	28/28 (100%)	28/20 (71%)
Poole et al (NEAT)[16]	1508	1508	310 (20%)	1198 (80%)	90 (6%)	1274 (84%)	144 (10%)	31*/30 (97%)	31*/26 (84%)
Poole et al (BR9601)[16]	295	295	62 (21%)	233 (79%)	16 (5%)	229 (78%)	50 (17%)	31*/30 (97%)	31*/26 (84%)
Total	3452	3102	843 (24%)	2609 (76%)	275 (9%)	2511 (81%)	316 (10%)	137/129 (94%)	123/85 (69%)

TOP2A=topoisomerase IIα. *The same national laboratory assessed NEAT and BR9601 cases.

Table 2: ***HER2*** **and** ***TOP2A*** **gene status by trial and rate of concordance with the external laboratory**

occurred first). Overall survival was defined as time from date of randomisation to date of death from any cause. Patients who were alive (for overall survival) and disease-free (for EFS) at the time of the analysis were censored at their date of last contact. We used the log-rank test to compare treatment groups. In a secondary EFS and overall survival analysis, the log-rank test was adjusted by the main prognostic factors—pathological tumour size (≤2 cm or >2 cm) and number of ipsilateral positive axillary nodes (node-negative, 1–3 positive nodes, or ≥4 positive nodes). The predictive value of *HER2* and *TOP2A* for the effect of anthracyclines on EFS and overall survival was assessed through interaction tests. The χ^2 test for interaction had two degrees of freedom when patients were divided by *TOP2A* status into three cohorts (ie, normal, amplified, or deleted) and one degree of freedom when patients were allocated in two cohorts (ie, normal or aberrated). We used forest plots to show EFS and overall survival data by treatment and by *HER2* or *TOP2A* status. We used Kaplan-Meier estimates to produce EFS and overall survival curves by treatment group and by *HER2* or *TOP2A* gene status. We used SAS version 9.1 and SPLUS version 7 for statistical analyses.

Role of funding sources

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

1003 (22%) of 4558 of patients could not be assessed in the meta-analysis because of an absence of data for *HER2* or *TOP2A* gene status (table 1). When EFS curves from patients who participated in the meta-analysis were compared with those from patients who did not, within each trial and by treatment group, we recorded no statistically significant differences (data not shown).

Table 2 shows *HER2* and *TOP2A* gene status by trial and rates of concordance for *HER2* and *TOP2A* status between each of the four national and the external laboratories. In Piccart and colleagues' trial,[14] *TOP2A* gene status was assessed only within the *HER2* amplified cohort, which might explain why the rate of *TOP2A* gene amplification is more than double than that in the other trials. Likewise, the high proportion of *HER2* gene amplification reported in the Ejlertsen and colleagues' trial[15] might be explained by most patients having oestrogen-receptor-negative disease. The quality control study showed a high concordance between the four national laboratories and the external laboratory in their definition of *HER2* gene status; concordance in *TOP2A* definition was lower than it was for *HER2* (table 2).

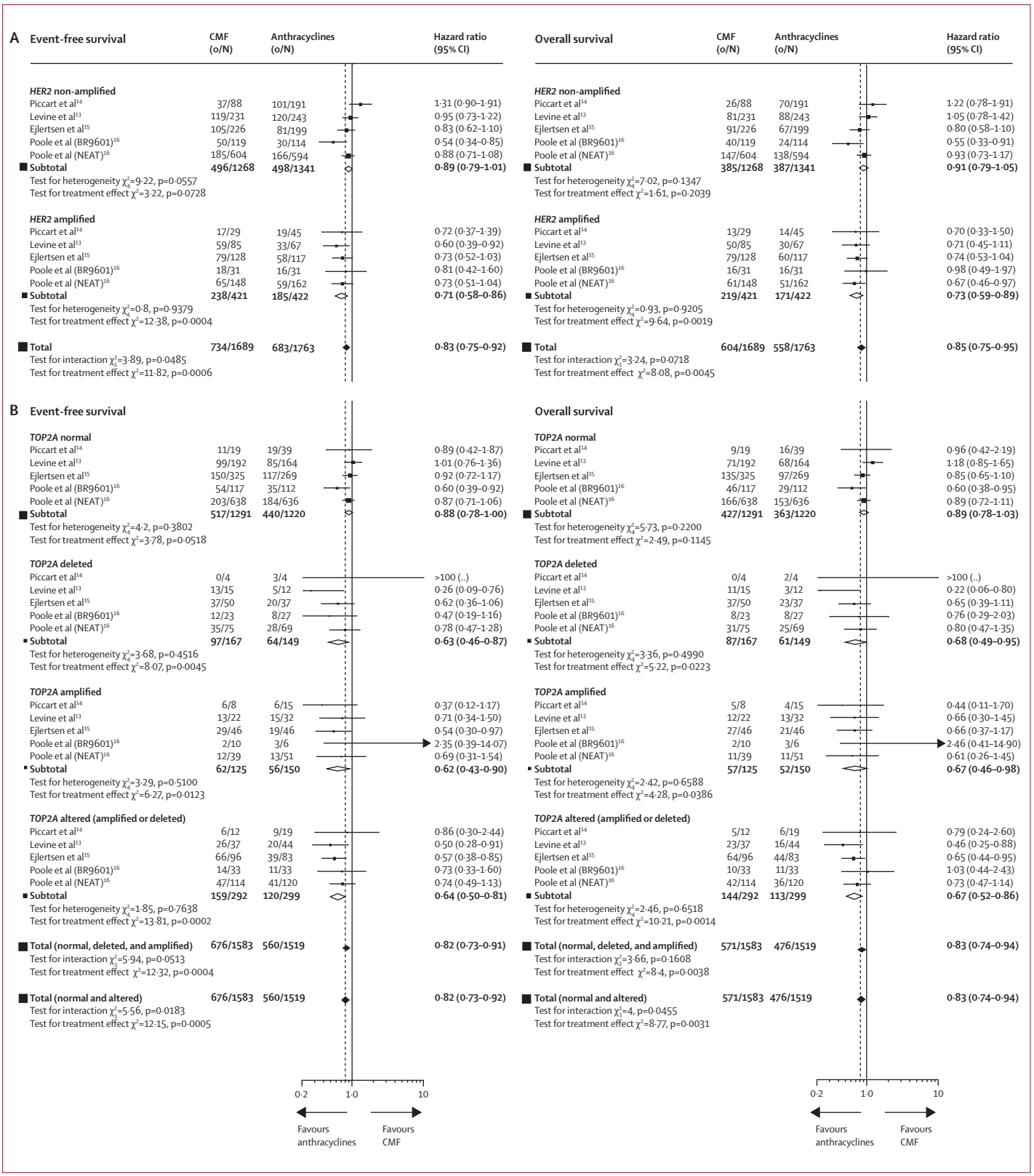

Figure 1: **Interaction between gene status and treatment effect, by survival analysis**
Survival by (A) HER2 status and (B) *TOP2A* status. CMF=cyclophosphamide, methotrexate, and fluorouracil. o=observed events.

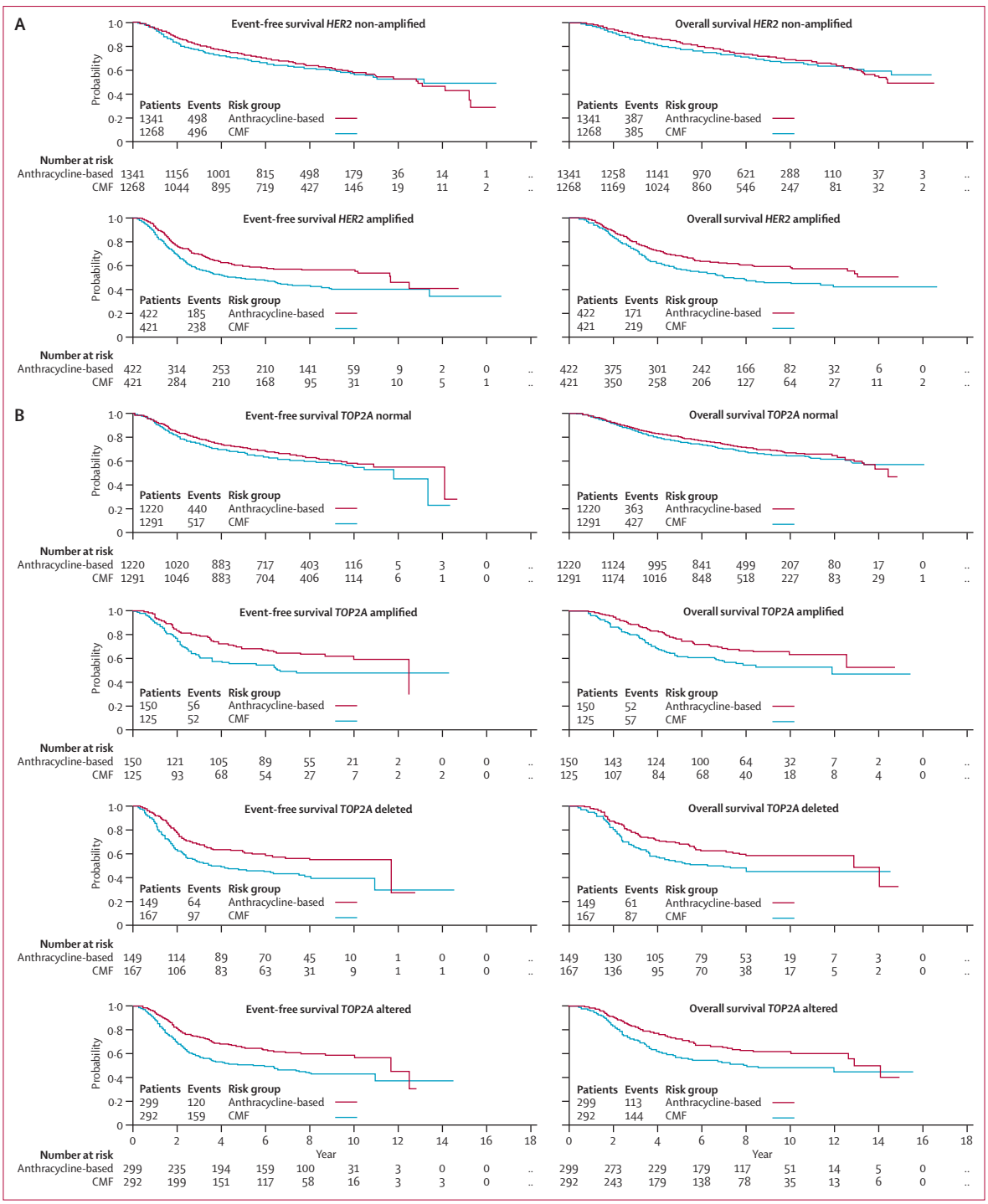

Figure 2: **Event-free survival and overall survival, by gene status**
Survival by (A) HER2 status (B) *TOP2A* status. CMF=cyclophosphamide, methotrexate, and fluorouracil.

The benefit of treatment with anthracyclines over treatment with CMF was greater for individuals with *HER2* gene amplification than it was for individuals without *HER2* gene amplification when analysing EFS ($p_{interaction}$=0·0485), but not when analysing overall survival ($p_{interaction}$=0·0718; figure 1 and figure 2). We recorded no significant difference in the benefit of treatment with anthracyclines over treatment with CMF when assessing the three separate *TOP2A* cohorts in terms of either EFS ($p_{interaction}$=0·0513) or overall survival ($p_{interaction}$=0·1608;

figure 1 and figure 2). However, when *TOP2A* amplifications and deletions were combined (altered cohort), we recorded a significant difference in the benefit of treatment with anthracyclines over treatment with CMF between patients with normal *TOP2A* status and those with altered *TOP2A* status when analysing both EFS ($p_{interaction}$=0·0183) and overall survival ($p_{interaction}$=0·0455; figure 1 and figure 2).

When adjusted according to the main prognostic factors (ie, pathological tumour size and number of positive ipsilateral axillary nodes), HRs for the effect of anthracyclines versus CMF in patients with *HER2* gene amplification were 0·70 (95% CI 0·57–0·85; p=0·0004) for EFS and 0·73 (0·59–0·90; p=0·003) for overall survival, and, for patients without *HER2* gene amplification, were 0·85 (0·75–0·96; p=0·012) for EFS and 0·87 (0·75–1·01; p=0·061) for overall survival. After adjustment, the benefit of treatment with anthracyclines over treatment with CMF was not statistically different between individuals with *HER2* amplification and those without *HER2* amplification in either EFS ($p_{interaction}$=0·10) or overall survival ($p_{interaction}$=0·17).

For *TOP2A*, adjusted HRs were 0·62 (0·42–0·92; p=0·019) for EFS and 0·68 (0·45–1·02; p=0·060) for overall survival for patients with *TOP2A* amplification, 0·57 (0·41–0·81; p=0·002) for EFS and 0·64 (0·45–0·92; p=0·016) for overall survival for patients with *TOP2A* deletion, and 0·86 (0·76–0·98; p=0·024) for EFS and 0·87 (0·75– 1·00; p=0·057) for overall survival for patients with *TOP2A* normal. After adjustment, the benefit of treatment with anthracyclines over treatment with CMF differed significantly between the three groups in terms of EFS ($p_{interaction}$=0·04) but not in overall survival ($p_{interaction}$=0·19). When *TOP2A* amplifications and deletions were combined, adjusted HRs were 0·60 (0·47–0·77; p=0·0001) for EFS and 0·63 (0·48–0·82; p=0·0005) for overall survival in patients with *TOP2A* alterations, and 0·86 (0·76–0·98; p=0·024) for EFS and 0·87 (0·75–1·00; p=0·056) for overall survival in patients with *TOP2A*-normal tumours. The benefit of treatment with anthracyclines over treatment with CMF differed significantly between the two groups in terms of both EFS ($p_{interaction}$=0·013) and overall survival ($p_{interaction}$=0·033).

Of 2588 patients with data that could be assessed, 740 (29%) were defined as highly hormone-sensitive, 878 (34%) as moderately hormone-sensitive, 311 (12%) as *HER2* amplified and oestrogen-receptor and progesterone-receptor negative, and 659 (25%) as triple negative (figure 3 and figure 4). We recorded no significant difference in the treatment effect of anthracyclines or CMF between molecular subgroups, but individuals with *HER2*-amplified tumours seemed to respond better to anthracyclines and those with highly hormone-sensitive tumours seemed to respond better to CMF (figure 3). EFS HRs for *HER2* amplified and *HER2* non-amplified moderately hormone-sensitive tumours were 0·78 (95% CI 0·55–1·11; p=0·17) and 0·79 (0·59–1·05; p=0·11), respectively. We compared treatment groups by *TOP2A* gene status within the *HER2* positive molecular subgroup, and, in all three cohorts, anthracycline-based therapy seemed to be more effective than CMF (webappendix p 2).

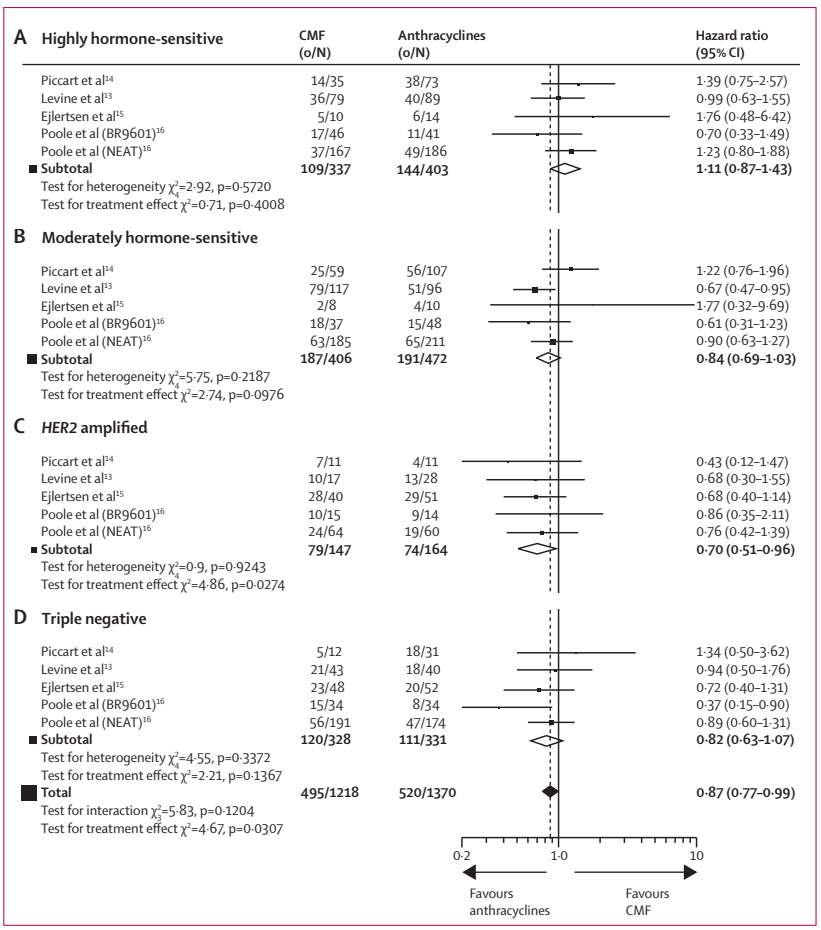

Figure 3: **Interaction between molecular subgroup and treatment effect (event-free survival analysis)**
CMF=cyclophosphamide, methotrexate, and fluorouracil. o=observed events.

Discussion

Our findings show a greater benefit from anthracycline-based adjuvant therapy in patients with *HER2* gene amplification than in patients without such amplification and in patients with *TOP2A* gene alterations than in patients with normal *TOP2A* status. However, our study also shows that patients with *HER2* non-amplified or *TOP2A* normal tumours might have some additional benefit from treatment with anthracyclines, which suggests a quantitative rather than a qualitative interaction between anthracycline activity and *HER2* or *TOP2A* status.

In this meta-analysis we included trials with differences in the type of anthracycline-based regimens or in the CMF schedules used (webappendix p 1), which

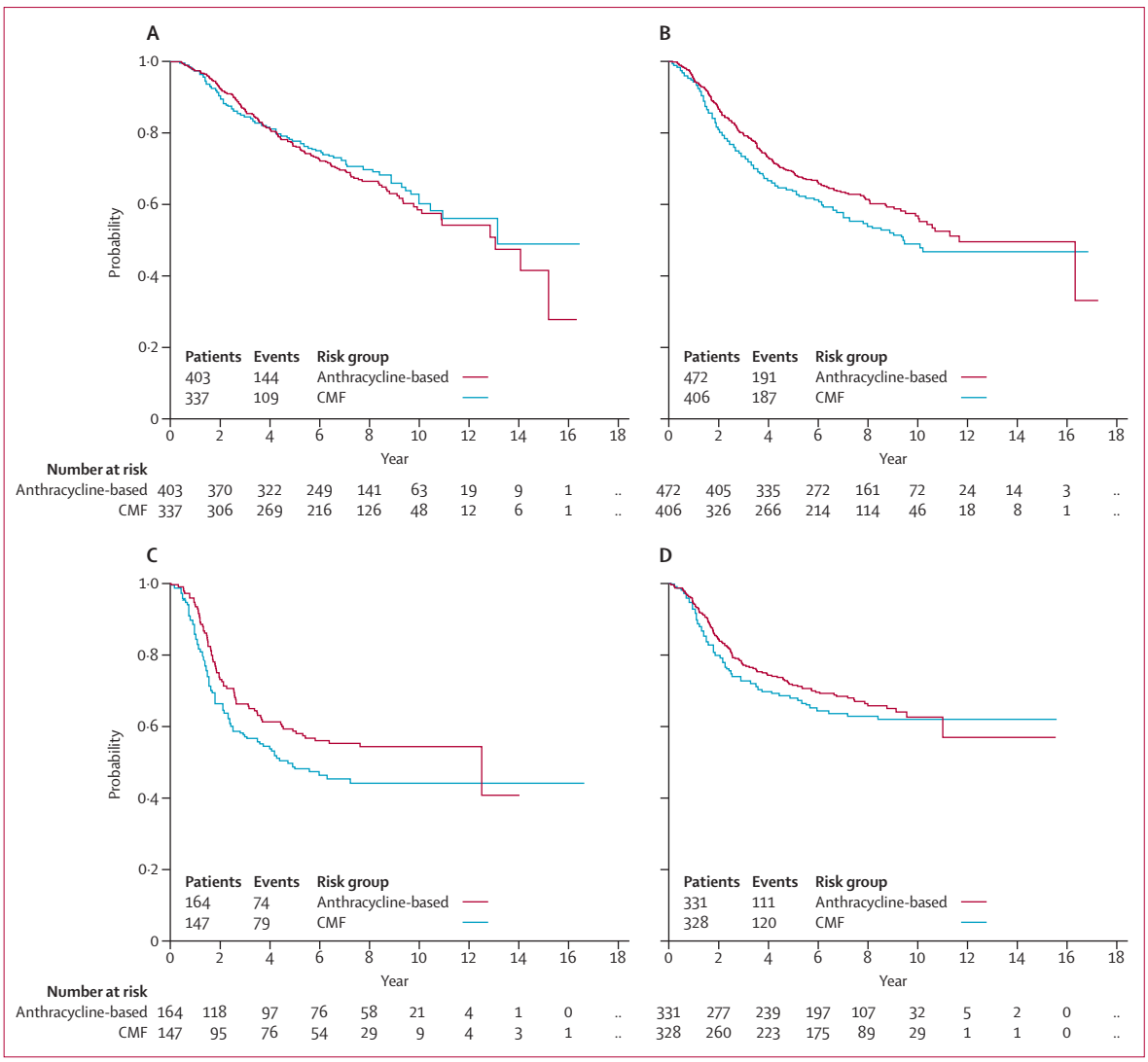

Figure 4: **Event-free survival analysis, by molecular subgroups**
Survival in patients with (A) highly hormone-sensitive tumours, (B) moderately hormone-sensitive tumours, (C) HER2-amplified tumours, and (D) triple-negative tumours.

is a potential limitation in the interpretation of the study results, although we recorded similar associations in the interaction between the activity of treatments and *HER2* or *TOP2A* status in each individual trial. Two pooled analyses of previously published data have investigated *HER2*, but not *TOP2A*, in the same setting with similar results.[18,19] Neither of these studies did an external quality control substudy for the testing of *HER2* or *TOP2A*. Our external *TOP2A*-testing quality control substudy suggests that procedures for *TOP2A* testing by FISH need increased standardisation to achieve better reproducibility. Our study design chose the external laboratory as the gold standard, but because tumour samples assessed in the external laboratory were not tested in another laboratory, and because samples from the four national laboratories were not cross-compared, we cannot identify the reason behind the recorded discordance.

A unique aspect of this study is the planned exploratory analysis in which patients were grouped into one of four molecularly defined cohorts. In view of the retrospective nature of this assessment and the restricted sample size, the results of this subgroup analysis have to be regarded as merely hypothesis-generating and should not lead to changes in clinical practice. This analysis, prompted by the known molecular and clinical heterogeneity of breast cancer,[17] suggests that, in contradiction to previously reported studies that analysed the *HER2* non-amplified cohort as a homogeneous group,[1–4,6,9,10,18,19] differential benefit from anthracyclines might exist within the *HER2* non-amplified cohort. In our analysis individuals with triple-negative or moderately hormone-sensitive tumours

seemed to respond better to treatment with anthracyclines than to treatment with CMF. Because all triple-negative tumours and almost 90% of moderately hormone-sensitive tumours from this study did not carry *TOP2A* gene amplification, other mechanisms of increased sensitivity to anthracycline might exist. We defined triple-negative tumours as such if oestrogen-receptor and progesterone-receptor immunostaining was less than 10%, and not less than 1% as suggested in international guidelines.[20] However we regard this discrepancy as irrelevant with regard to the suggested benefit from anthracyclines in patients who do not carry *TOP2A* gene amplification. Triple-negative tumours and moderately hormone-sensitive tumours are often characterised by high proliferation rates.[21–23] Proliferation signals can lead to topoisomerase II α protein over-expression independently of *TOP2A* gene status.[24,25] Indeed, data reported elsewhere[26–27] draw attention to the absence of concordance between *TOP2A* gene status and protein concentrations within the same tumour. Ideally, quantification of nuclear concentrations of the topoisomerase II α protein (ie, the active protein isoform) might be the most appropriate way to investigate its predictive value.[28] Other biological factors not related to *TOP2A* have been investigated as potential markers of sensitivity to anthracyclines. Among those, polysomy of chromosome 17, which could be a marker of genomic instability and DNA repair dysfunction, might play a part.[29] Moreover, factors involved in the regulation of the stroma–tumour interaction or in the immune response against a tumour might also be involved.[30–32] Future studies looking at molecular markers to predict response to anthracyclines will have to take into account the fact that probably only a multifactorial system will predict responsiveness to anthracyclines.

In conclusion, our findings do not justify routine use of *HER2* and *TOP2A* as molecular markers to predict anthracycline activity, because women with non-*HER2* amplified and non-*TOP2A* altered tumours seem to derive benefits from treatment with anthracyclines, and because problems exist with the reproducibility of *TOP2A* gene status assessment by FISH.

Contributors

ADL, JMSB, BE, KIP, CP, JI, HM, FPO'M, DC, MJP, and MB had the idea for and designed the study. CD, FP, LS, and MB collected the data. ADL, CD, JMSB, FP, BE, KIP, DL, CP, JI, HE, HM, FC, MT, AM, CJT, CS, DC, MJP, and MB analysed and interpreted the data. CD, JMSB, BE, KIP, DL, CP, HE, HM, FPO'M, FC, AM, CJT, CS, LS, DC, and MJP provided study materials.

Conflicts of interest

ADL has a consultancy and advisory role for and has received honoraria from Schering-Plough. JMSB has a consultancy and advisory role for DAKO and Abbott K. KIP has a consultancy and advisory role for and has received honoraria from Roche, Abraxis, AstraZeneca, Pfizer, Novartis, and Amgen, and has given expert testimony for Novartis and AstraZeneca. CP and DC have received honoraria from Pfizer. MB is founder and chairman of the International Drug Development Institute. CP and DC have received funding from Pfizer. BE has an immediate family member who works for DAKO A/S. All other authors declare that they have no conflicts of interest.

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