Neoadjuvant chemotherapy with or without anthracyclines in the presence of dual HER2 blockade for HER2-positive breast cancer (TRAIN-2): a multicentre, open-label, randomised, phase 3 trial

Mette S van Ramshorst, Anna van der Voort, Erik D van Werkhoven, Ingrid A Mandjes, Inge Kemper, Vincent O Dezentjé, Irma M Oving, Aafke H Honkoop, Lidwine W Tick, Agnes J van de Wouw, Caroline M Mandigers, Laurence J van Warmerdam, Jelle Wesseling, Marie-Jeanne T Vrancken Peeters, Sabine C Linn, Gabe S Sonke, on behalf of the Dutch Breast Cancer Research Group (BOOG)

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Department of Medical Oncology (M S van Ramshorst MD, A van der Voort MD, I Kemper MANP, V O Dezentjé MD, Prof S C Linn MD, G S Sonke MD), **Department of Biometrics** (E D van Werkhoven MSc, I A Mandjes MSc), **Department of Pathology** (Prof J Wesseling MD), **and Department of Surgery** (M-J T Vrancken Peeters MD), **Netherlands Cancer Institute, Amsterdam, Netherlands; Department of Medical Oncology, Reinier de Graaf Gasthuis, Delft, Netherlands** (V O Dezentjé); **Department of Medical Oncology, Ziekenhuisgroep Twente, Almelo, Netherlands** (I M Oving MD); **Department of Medical Oncology, Isala, Zwolle, Netherlands** (A H Honkoop MD); **Department of Medical Oncology, Maxima Medical Center, Eindhoven, Netherlands** (L W Tick MD); **Department of Medical Oncology, VieCuri Medical Center, Venlo, Netherlands** (A J van de Wouw MD); **Department of Medical Oncology, Canisius Wilhelmina Hospital, Nijmegen, Netherlands** (C M Mandigers MD); **Department of Medical Oncology, Catharina Cancer Centre, Eindhoven, Netherlands** (L J van Warmerdam MD); **and Department of Pathology, University Medical Centre, Utrecht, Netherlands** (Prof S C Linn)

Summary

Background The optimal chemotherapy backbone for dual HER2 blockade in the neoadjuvant setting for early breast cancer is unknown. We investigated whether the addition of anthracyclines would improve pathological complete response compared with a carboplatin–taxane regimen, when given in combination with the HER2-targeted agents trastuzumab and pertuzumab.

Methods The TRAIN-2 study is an open-label, randomised, controlled, phase 3 trial being done in 37 hospitals in the Netherlands. We recruited patients aged 18 years or older with previously untreated, histologically confirmed stage II–III HER2-positive breast cancer. Patients were randomly allocated using central randomisation software (1:1 ratio) with minimisation without a random component, stratified by tumour stage, nodal stage, oestrogen receptor status, and age, to receive 5-fluorouracil (500 mg/m²), epirubicin (90 mg/m²), and cyclophosphamide (500 mg/m²) every 3 weeks for three cycles followed by paclitaxel (80 mg/m² on days 1 and 8) and carboplatin (area under the concentration–time curve [AUC] 6 mg/mL per min on day 1 or optionally, as per hospital preference, AUC 3 mg/mL per min on days 1 and 8) every 3 weeks for six cycles, or to receive nine cycles of paclitaxel and carboplatin at the same dose and schedule as in the anthracycline group. Patients in both study groups received trastuzumab (6 mg/kg, loading dose 8 mg/kg) and pertuzumab (420 mg, loading dose 840 mg) concurrently with all chemotherapy cycles. The primary endpoint was the proportion of patients who achieved a pathological complete response in breast and axilla (ypT0/is ypN0) in the intention-to-treat population. Safety was analysed in patients who received at least one treatment cycle according to actual treatment received. This trial is registered with ClinicalTrials.gov, number NCT01996267, and follow-up for long-term outcome is ongoing.

Findings Between Dec 9, 2013, and Jan 14, 2016, 438 patients were enrolled and randomly assigned to the two treatment groups (219 patients to each group), of whom 418 were evaluable for the primary endpoint (212 in the anthracycline group and 206 in the non-anthracycline group). The median follow-up for all patients was 19 months (IQR 16–23 months). A pathological complete response was recorded in 141 (67%, 95% CI 60–73) of 212 patients in the anthracycline group and in 140 (68%, 61–74) of 206 in the non-anthracycline group (p=0·95). One patient randomly allocated to the non-anthracycline group did receive anthracyclines and was thus included in the anthracycline group for safety analyses; therefore, for the safety analyses there were 220 patients in the anthracycline group and 218 in the non-anthracycline group. Serious adverse events were reported in 61 (28%) of 220 patients in the anthracycline group and in 49 (22%) of 218 in the non-anthracycline group. The most common adverse events of any cause were grade 3 or worse neutropenia (in 131 [60%] of 220 patients in the anthracycline group *vs* 118 [54%] of 218 in the non-anthracycline group), grade 3 or worse diarrhoea (26 [12%] *vs* 37 [18%]), and grade 2 or worse peripheral neuropathy (66 [30%] *vs* 68 [31%]), with no substantial differences between the groups. Grade 3 or worse febrile neutropenia was more common in the anthracycline group than in the non-anthracycline group (23 [10%] *vs* three [1%], p<0·0001). Symptomatic left ventricular systolic dysfunction was rare in both groups (two [1%] of 220 *vs* 0 of 218). One patient in the anthracycline group died because of a pulmonary embolism, which was possibly treatment related.

Interpretation In view of the high proportion of pathological complete responses recorded in both groups and the fact that febrile neutropenia was more frequent in the anthracycline group, omitting anthracyclines from neoadjuvant treatment regimens might be a preferred approach in the presence of dual HER2 blockade in patients with early HER2-positive breast cancer. Long-term follow-up is required to confirm these results.

Funding Roche Netherlands.

Research in context

Evidence before this study

We searched PubMed on April 2, 2018, with the terms "breast cancer", "neoadjuvant treatment", "pertuzumab", "trastuzumab", and "anthracyclines" in several combinations for articles written in English with no restrictions in publication date. The published literature showed that in the presence of single HER2 blockade with trastuzumab, a carboplatin–taxane regimen has similar efficacy and reduced toxicity compared with an anthracycline–taxane regimen in the adjuvant setting. Furthermore, dual HER2 blockade with trastuzumab and pertuzumab as neoadjuvant treatment has improved pathological complete responses and progression-free survival at 5 years of patients with HER2-positive breast cancer. One study reported cardiac safety of an anthracycline-free and an anthracycline-containing regimen in the presence of dual HER2 blockade, but this non-comparative study was not designed to assess efficacy. Therefore, the optimal chemotherapy backbone in combination with dual HER2 blockade in patients with HER2-positive breast cancer remains unknown.

Added value of this study

To the best of our knowledge, the TRAIN-2 study was the first randomised controlled trial to compare the proportion of patients achieving a pathological complete response after treatment with an anthracycline-containing versus an anthracycline-free regimen, both combined with dual HER2 blockade, as neoadjuvant treatment for HER2-positive breast cancer. Our results show similar and high proportions of patients achieving pathological complete responses in both study groups, with more febrile neutropenia recorded in the anthracycline group than in the non-anthracycline group.

Implications of all the available evidence

The results of this study, along with previously published studies, indicate that anthracycline-free chemotherapy consisting of carboplatin and weekly paclitaxel is as effective but has fewer side-effects than anthracycline-containing regimens, and is therefore a preferred chemotherapy backbone to combine with dual HER2 blockade to treat patients with early HER2-positive breast cancer.

Correspondence to:
Dr Gabe S Sonke, Department of Medical Oncology, Netherlands Cancer Institute, 1066 CX Amsterdam, Netherlands
g.sonke@nki.nl

Introduction

Neoadjuvant polychemotherapy plus trastuzumab and pertuzumab results in high numbers of treated patients achieving pathological complete responses in HER2-positive breast cancer, but this treatment is not without toxicity.[1,2] The optimal chemotherapy backbone with respect to both efficacy and safety has not been identified. Because of the overlap in cardiotoxicity of anthracyclines and trastuzumab, anthracycline-free regimens have been assessed.[3–5] However, trials that directly compare anthracycline-containing regimens and anthracycline-free regimens are rare. In the presence of a single HER2 blockade, docetaxel, carboplatin, and trastuzumab showed similar disease-free and overall survival outcomes but reduced acute and long-term toxicity compared with a sequential anthracycline–taxane regimen plus trastuzumab in the randomised adjuvant BCIRG-006 trial.[3] This trial was not designed to show a difference between the two trastuzumab-containing regimens, and the anthracycline-free group had a shorter duration of treatment than the anthracycline group, which might have affected its efficacy.[6] By contrast with the BCIRG-006 results, an observational study from the MD Anderson Cancer Centre (Houston, TX, USA) noted significantly improved pathological complete responses and 3-year recurrence-free survival with a neoadjuvant sequential anthracycline–taxane plus trastuzumab regimen compared with docetaxel, carboplatin, and trastuzumab.[7] In the presence of dual HER2 blockade, one non-comparative trial (TRYPHAENA)[2] assessed neoadjuvant regimens with and without anthracyclines, but the non-comparative study design precludes definite statements about the relative efficacy and safety of both regimens. Therefore, the role of anthracyclines in the era of dual HER2 blockade is unknown.

In the single group TRAIN study,[8] 46 (43%) of 108 patients achieved pathological complete response, 5 (<5%) of 108 patients experienced febrile neutropenia, and no symptomatic left ventricular systolic dysfunction was observed with a neoadjuvant anthracycline-free regimen consisting of weekly paclitaxel, trastuzumab, and carboplatin. The TRAIN-2 study was designed to directly compare the efficacy and safety of an anthracycline-containing chemotherapy regimen (three cycles of 5-fluorouracil, epirubicin, and cyclophosphamide followed by six cycles of paclitaxel, trastuzumab, and carboplatin) with an anthracycline-free regimen of the same duration (nine cycles of paclitaxel, trastuzumab, and carboplatin) in combination with trastuzumab and pertuzumab in both groups. The paclitaxel, trastuzumab, and carboplatin schedule was designed to improve the docetaxel, carboplatin, and trastuzumab schedule used in the BCIRG-006 trial. We extended the duration of treatment to nine cycles to allow comparison with an anthracycline-containing regimen of equal duration and with the aim to further improve the proportion of patients achieving a pathological complete response.[3,8] Additionally, the use of weekly paclitaxel might improve efficacy and reduce haematological toxicity compared with taxane administration once every 3 weeks.[9,10] Safety results of the first 110 patients have been reported previously;[11] here, we report the efficacy and safety results of all included patients.

Methods

Study design and participants

See Online for appendix

The TRAIN-2 study is a randomised, open-label, multicentre trial done in 37 hospitals in the Netherlands. The study design is summarised in the appendix (p 2). Treatment-naive patients with histologically confirmed stage II–III HER2-positive breast cancer were eligible. Other key eligibility criteria were age 18 years or older, WHO performance status of 0–1, left ventricular ejection fraction of at least 50%, and adequate organ function based on local laboratory assessment of absolute neutrophil count, platelet count, aspartate transaminase, alanine transaminase, total bilirubin, and creatinine clearance. Patients were ineligible if they were pregnant, breastfeeding, had second primary breast cancer or other malignancy except carcinoma in situ and basal-cell and squamous cell carcinoma of the skin, unless the other malignancy was treated at least 5 years ago with curative intent without the use of chemotherapy or radiotherapy, and if they had any other medical condition that would place the patient at unusual risk. All included patients provided written informed consent. The medical ethics committee of the Netherlands Cancer Institute approved the study protocol (appendix p 10) and all amendments thereof.

Randomisation and masking

Patients were enrolled by medical oncologists who were involved in the study in the participating hospitals, and were randomly assigned (1:1) to the anthracycline group or the non-anthracycline group. Treatment was allocated by computerised central randomisation with minimisation (without a random component), with randomisation stratified by primary tumour stage (T0–2 *vs* T3–4), nodal stage (negative *vs* positive), oestrogen receptor status (<10% *vs* ≥10%), and age (<50 years *vs* ≥50 years). In this open-label study, patients, investigators, and the study team were not masked to study treatment. Pathologists assessing the amount of residual tumour after neoadjuvant treatment were not part of the study and were not informed about the treatment allocation.

Procedures

Patients in the anthracycline group received three cycles of 5-fluorouracil (500 mg/m²), epirubicin (90 mg/m²), and cyclophosphamide (500 mg/m²) intravenously, once every 3 weeks, followed by six cycles of paclitaxel (80 mg/m² on days 1 and 8) and carboplatin (area under the concentration–time curve [AUC] 6 mg/mL per min on day 1 or optionally AUC 3 mg/mL per min on days 1 and 8, as per the preference within the hospital) intravenously, once every 3 weeks. Patients in the non-anthracycline group received nine cycles of paclitaxel and carboplatin at the same dose and schedule as in the anthracycline group. Trastuzumab (6 mg/kg, intravenously or subcutaneously, as per hospital preference, day 1 of each 3-week cycle, loading dose 8 mg/kg, first day of neoadjuvant treatment only) and pertuzumab (420 mg, intravenously, day 1 of each 3-week cycle, loading dose 840 mg, first day of neoadjuvant treatment only) were administered every 3 weeks concurrently with all chemotherapy cycles in both groups.

At baseline, core biopsies of the primary tumour were taken for histological diagnosis, including local assessment of oestrogen receptor, progesterone receptor, and HER2 status. Tumours with oestrogen receptor or progesterone receptor expression of 10% or more were defined as hormone receptor positive. HER2 status was regarded as positive if the immunohistochemistry result was 3+ or 2+ and confirmed by positive in-situ hybridisation. HER2, oestrogen receptor, and progesterone receptor status were not centrally reviewed. A marker (either iodine seeds or twist markers) was placed at the primary tumour site before the start of treatment in all patients. Baseline nodal status was assessed by physical examination and ultrasound assessment with fine-needle aspiration of clinically suspicious lymph nodes. In patients with clinically node-negative disease, a sentinel node procedure was done, either before or after neoadjuvant therapy, according to local practice. All patients were screened for distant metastases at baseline.

Radiological response was assessed by MRI at baseline, after three cycles, and after nine cycles of neoadjuvant chemotherapy. Each chemotherapy cycle was preceded by an assessment of toxicity, including laboratory results for haematology and biochemistry. Left ventricular ejection fraction was measured once every 3 months during trastuzumab therapy or more frequently if indicated.

Laboratory monitoring including assessment of haematology and serum chemistry profile was done before the start of each treatment cycle and adverse events were assessed with each cycle of treatment according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Dose adjustment criteria have been previously described.[11] In brief, chemotherapy was delayed and secondary granulocyte-colony-stimulating factor (G-CSF, outsourced by each hospital and adminstered as per local protocols) prophylaxis was initiated in patients with isolated grade 2 or worse neutropenia or febrile neutropenia and was continued throughout all cycles of treatment. In patients with combined thrombocytopenia and neutropenia, dose reduction was warranted without G-CSF support. Chemotherapy was postponed with a subsequent 25% dose reduction of all or one specific agent (eg, carboplatin for thrombocytopenia and paclitaxel for neuropathy) in case of (febrile) neutropenia grade 2 or worse despite G-CSF, thrombocytopenia grade 2 or worse, non-haematological toxicities grade 2 or worse, or neuropathy grade 2 or worse. Trastuzumab and pertuzumab were temporarily or permanently discontinued if left ventricular ejection fraction decreased by more than 15 percentage points from baseline or if it decreased by 10 percentage points or more with left ventricular ejection fraction below the lower limit of normal.[11]

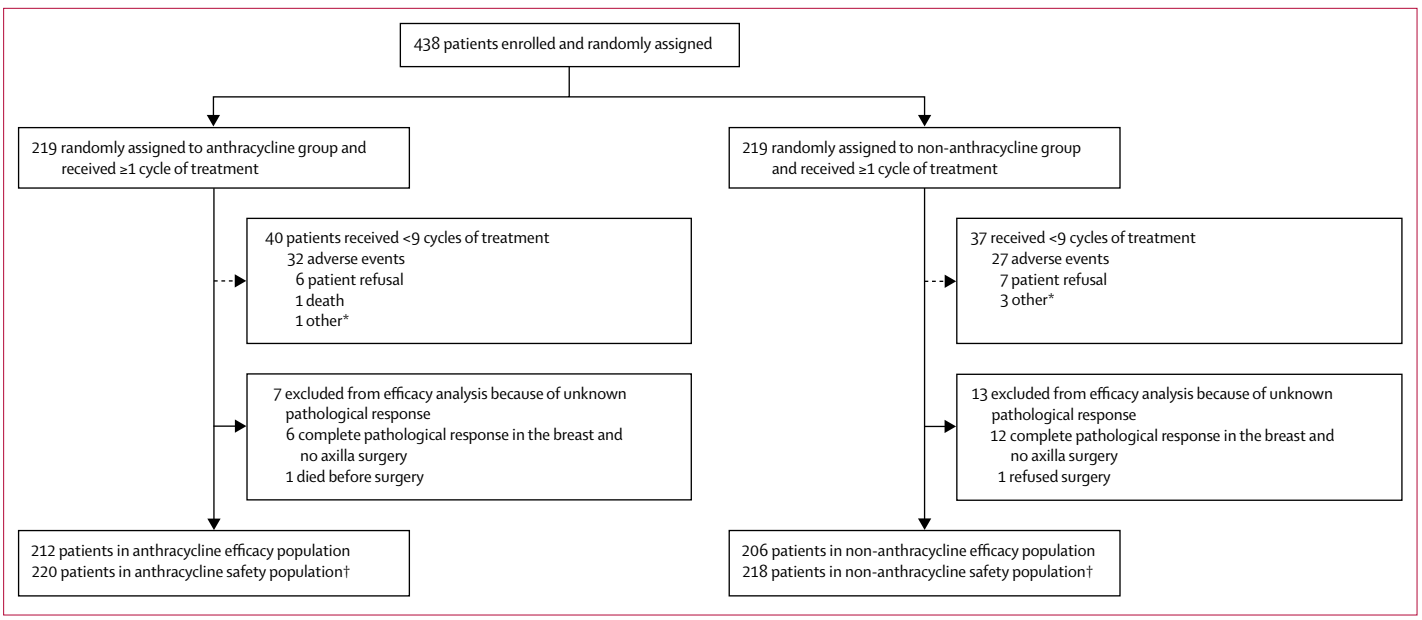

Figure 1: **Trial profile**
*Other reasons for receiving fewer than nine cycles of treatment were: decrease in performance score in the anthracycline group, and the presence of HER2-negative tumour with change of treatment, comorbidity, and second primary thyroid cancer requiring early surgery in the non-anthracycline group. †One patient allocated to the non-anthracycline group received anthracycline and was therefore included in the anthracycline group (and omitted from the non-anthracycline group) for safety analyses.

Patients underwent surgery within 6 weeks after their final dose of chemotherapy. The choice between breast-conserving and ablative surgery depended on tumour and patient characteristics, and patients' preferences. Axillary staging in patients with clinically node-negative disease involved a sentinel node procedure. In patients with clinically node-positive disease, the study protocol allowed axillary lymph node dissection, sentinel node procedure, or selective removal of the initially positive and marked lymph node. Postoperative radiotherapy was given according to local guidelines. Adjuvant trastuzumab was continued to complete 1 year of treatment and endocrine therapy was prescribed for patients with hormone receptor-positive tumours, according to Dutch national guidelines.[12] All enrolled and randomised patients remained in the study and follow-up unless consent was withdrawn.

Outcomes

The primary endpoint was the percentage of pathological complete responses, defined as the absence of invasive tumour cells in the breast and axilla (ypT0/is ypN0). Pathological response was locally assessed according to Dutch national guidelines[12] and was not centrally reviewed. Secondary endpoints were the percentage of patients experiencing grade 3–5 adverse events or grade 2 or worse peripheral neuropathy according to CTCAE version 4.03, the percentage of patients who underwent breast-conserving surgery, recurrence-free survival (time from randomisation to disease recurrence or death from any cause), distant metastasis-free survival (time from randomisation to distant recurrence or death from any cause), breast cancer-specific survival (time from randomisation to breast cancer-related death), and overall survival (time from randomisation to death from any cause). Primary and secondary objectives and endpoints are prespecified in the protocol (p 21).

Statistical analysis

We hypothesised that the anthracycline-containing regimen would improve the proportion of patients with a pathological complete response from 43% to 61% compared with the non-anthracycline containing regimen. These expectations were based on the 18% increase in pathological complete responses with anthracycline use described by Bayraktar and colleagues.[7] The expected proportion of pathological complete responses in the anthracycline-free group was derived from our experience with a weekly paclitaxel, trastuzumab, and carboplatin regimen and supported by similar pathological complete responses in the first neoadjuvant trials with a taxane plus dual HER2 blockade.[8,13,14] A sample size of 394 patients was sufficient for 80% power with a two-sided significance level of 0·05. To account for non-evaluable patients, we planned to enrol 438 patients.

Efficacy analyses (cutoff date June 1, 2017) were done according to allocated treatment and included all randomly assigned patients, including those with protocol violations, unless the primary endpoint could not be assessed (patient did not receive surgery according to protocol). Analyses were done by modified intention to treat. The proportions of patients with pathological complete responses were estimated and reported with 95% CIs obtained by the Clopper-Pearson method. Differences between the groups were tested using Fisher's exact test, except for the test

	Anthracycline group (n=219)	Non-anthracycline group (n=219)
Age (years)	49 (43–55)	48 (43–56)
ECOG performance status		
0	202 (92%)	204 (93%)
1	17 (8%)	14 (6%)
Unknown	0	1 (<1%)
Menopausal status		
Premenopausal	117 (53%)	119 (54%)
Peri-menopausal/ post-menopausal	99 (45%)	96 (44%)
Unknown	3 (1%)	4 (2%)
Clinical tumour stage		
0–2	148 (68%)	154 (70%)
3–4	71 (32%)	65 (30%)
Clinical nodal stage		
Negative	82 (37%)	76 (35%)
Positive	137 (63%)	143 (65%)
Disease stage		
I	0	1 (<1%)
II	139 (63%)	150 (68%)
III	79 (36%)	68 (31%)
IV	1 (<1%)	0
Hormone receptor status		
ER-negative and PR-negative	90 (41%)	93 (42%)
ER-positive and/or PR-positive	129 (59%)	126 (58%)
Tumour grade (biopsy)		
1	7 (3%)	12 (5%)
2	99 (45%)	100 (46%)
3	101 (46%)	95 (43%)
Unknown	12 (5%)	12 (5%)
Histology (biopsy)		
Ductal	196 (89%)	199 (91%)
Lobular	11 (5%)	6 (3%)
Other	12 (5%)	14 (6%)

Data are median (IQR) or n (%). ECOG=Eastern Cooperative Oncology Group. ER=oestrogen receptor. PR=progesterone receptor. Percentage totals might not sum up to 100% because of rounding.

***Table 1*: Baseline characteristics of the modified intention-to-treat population**

of the primary endpoint, which was done using the Cochran-Mantel-Haenszel test to take the stratification factors into account. Subgroup analyses were prespecified for the stratification factors of hormone receptor status, age, nodal status, and clinical tumour stage. Post-hoc subgroups were defined according to tumour grade and disease stage. Subgroup analyses were done using logistic regression. Safety analyses included all patients who received at least one treatment cycle and were done according to actual treatment received. An interim analysis for safety was performed after 110 patients were assessed, with no need to adjust the efficacy analysis.[11] Statistical significance for comparisons between the treatment groups was defined as $p<0·05$. All statistical analyses were done using R (version 3.5.0). A data monitoring committee did not oversee the study.

This study is registered with ClinicalTrials.gov, number NCT01996267.

Role of the funding source

The funder 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

Between Dec 9, 2013, and Jan 14, 2016, 438 patients were enrolled from 37 centres in the Netherlands (appendix p 9), with 219 patients randomly assigned to each treatment group (figure 1). Six protocol violations regarding eligibility were identified after randomisation (one stage I disease, one stage IV disease, one HER-2 negative tumour after re-testing, two concurrent contralateral breast cancer, 1 concurrent second primary tumour), but these patients were still included in the analyses (modified intention to treat. Baseline characteristics are presented by treatment group in table 1. Breast surgery was done in 431 patients (217 in the anthracycline group, 214 in the non-anthracycline group). Axillary surgery or staging was done in 421 patients (211 in the anthracycline group, 210 in the non-anthracycline group).

In the anthracycline group, seven patients were excluded from the primary endpoint analysis because of unknown pathological responses. One patient died before surgery and six patients had pathological complete response of the breast but no axilla surgery. In the non-anthracycline group, 13 patients were not included in the analysis for efficacy analysis. One patient refused surgery and 12 had pathological complete responses of the breast but no axilla surgery (figure 1).

In total, 418 patients were evaluable for the primary endpoint and were included in the efficacy population: 212 in the anthracycline group and 206 in the non-anthracycline group. The median follow-up for all patients was 19 months (IQR 16–23 months). In the anthracycline treatment group 141 (67%; 95% CI 60–73) of 212 patients achieved a pathological complete response, compared with 140 (68%; 95% CI 61–74) of 206 patients in the non-anthracycline group; thus, the difference in pathological complete response between the groups was –1·5%, (95% CI –11 to 8, p=0·95; figure 2A). If all non-evaluable patients were classified as having no pathological complete response, similar results were observed after a post-hoc sensitivity analysis (141 [64%] of 219 *vs* 140 [64%] of 219). In a post-hoc analysis of pathological complete responses according to hormone receptor status, more patients with hormone-receptor negative tumours achieved a pathological complete response than did those with hormone receptor-positive

tumours, regardless of treatment group (77 [89%] of 87 patients with hormone receptor-negative tumours *vs* 64 [51%] of 125 with hormone receptor-positive tumours in the anthracycline group; 76 [84%] of 90 *vs* 64 [55%] of 116, respectively, in the non-anthracycline group; figure 2b). The test for the interaction between treatment group and hormone receptor status was non-significant (p=0·32). Further analyses showed internal consistency of the primary endpoint across prespecified and post-hoc subgroups based on known prognostic factors (figure 3).

In the anthracycline group, 123 (56%) of 219 patients underwent breast-conserving surgery, 94 (43%) of 219 underwent mastectomy, and two (1%) of 219 did not have breast surgery. In the non-anthracycline group, 132 (60%) of 219 patients underwent breast-conserving surgery, 82 (37%) of 219 underwent mastectomy and five (2%) of 219 did not have breast surgery. There was no difference in the proportions of patients undergoing breast-conserving surgery between the treatment groups (p=0·33). 68 (31%) patients in each group underwent axillary lymph node dissection. Data for the other secondary endpoints of recurrence-free survival, distant metastases-free survival, breast cancer-specific survival, and overall survival were immature at the time of the primary analysis and will be presented in the future.

Patients in both groups received a median of nine treatment cycles (IQR 9–9 in both groups). The median number of cycles and relative total dose intensity per drug are summarised in the appendix (p 3).

Adverse events were the most common reason for early treatment discontinuation (figure 1), occurring in 32 (15%) of 219 patients in the anthracycline group and 27 (12%) of 219 in the non-anthracycline group. Other reasons for treatment discontinuation were patient refusal (six [3%] of 219 in the anthracycline group *vs* seven [3%] of 219 in the non-anthracycline group), death (one [<1%] of 219 *vs* 0 of 219) and other (one [<1%] of 219 *vs* three [1%] of 219). At least one dose reduction was implemented in 132 (60%) of 219 patients in the anthracycline group and in 150 (68%) of 219 patients in the non-anthracycline group (appendix p 4).

All randomly allocated patients received at least one treatment cycle and were included in the safety analyses (figure 1). One patient randomly allocated to the non-anthracycline group did receive anthracyclines and was therefore assessed according to the anthracycline group for safety analyses; therefore, for the safety analyses there were 220 patients in the anthracycline group and 218 in the non-anthracycline group. Serious adverse events were reported in 61 (28%) of 220 patients in the anthracycline group and in 49 (22%) of 218 in the non-anthracycline group (appendix p 7). In the anthracycline group, one patient died because of a pulmonary embolism during treatment (possibly treatment-related) and another was treated successfully for acute myeloid leukaemia 120 days after the last dose of chemotherapy (possibly treatment-related).

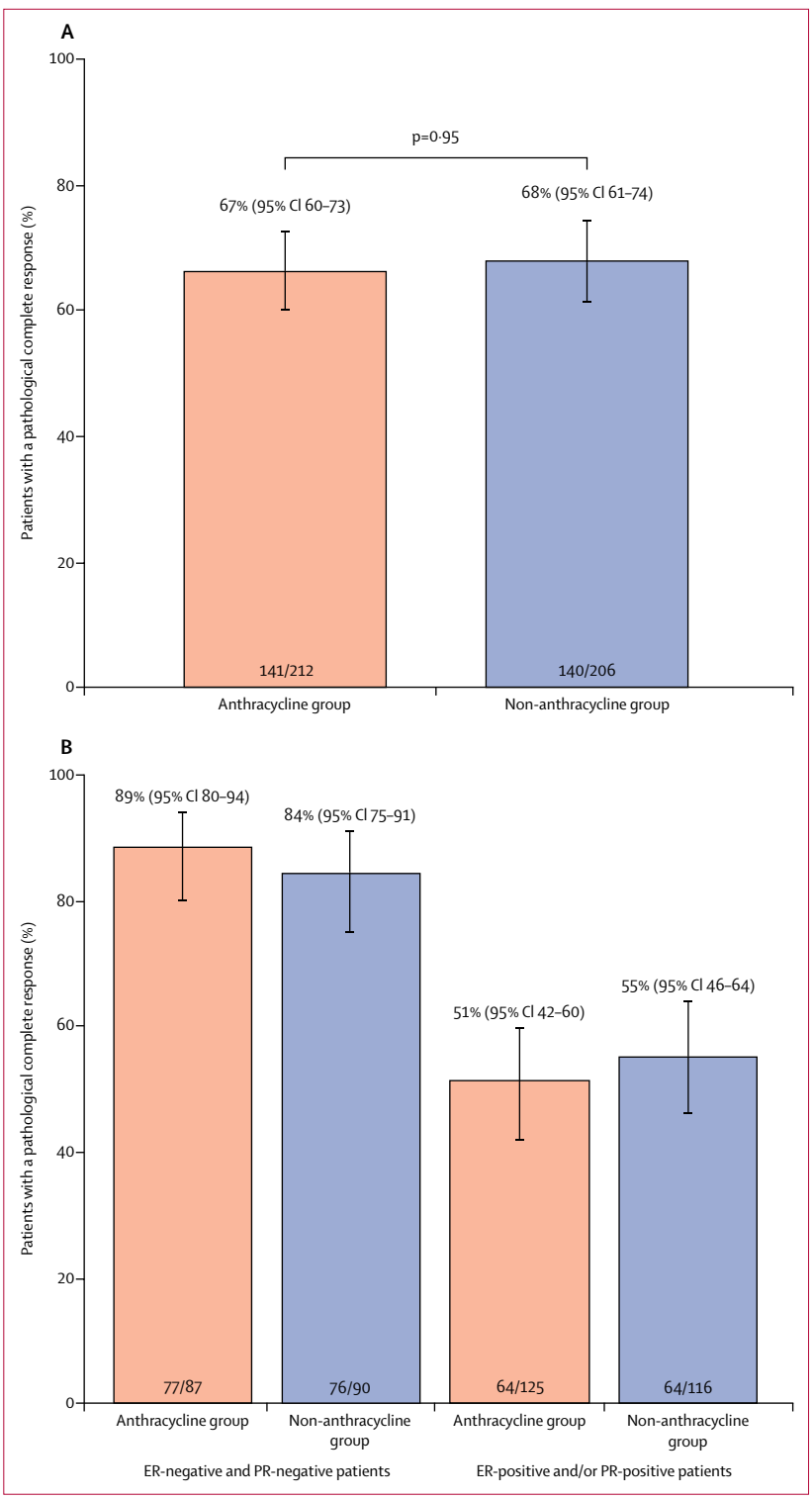

Figure 2: **Pathological complete responses**
(A) Pathological complete responses according to treatment group. (B) Pathological complete responses according to treatment group and by hormone receptor status. ER=oestrogen receptor. PR=progesterone receptor.

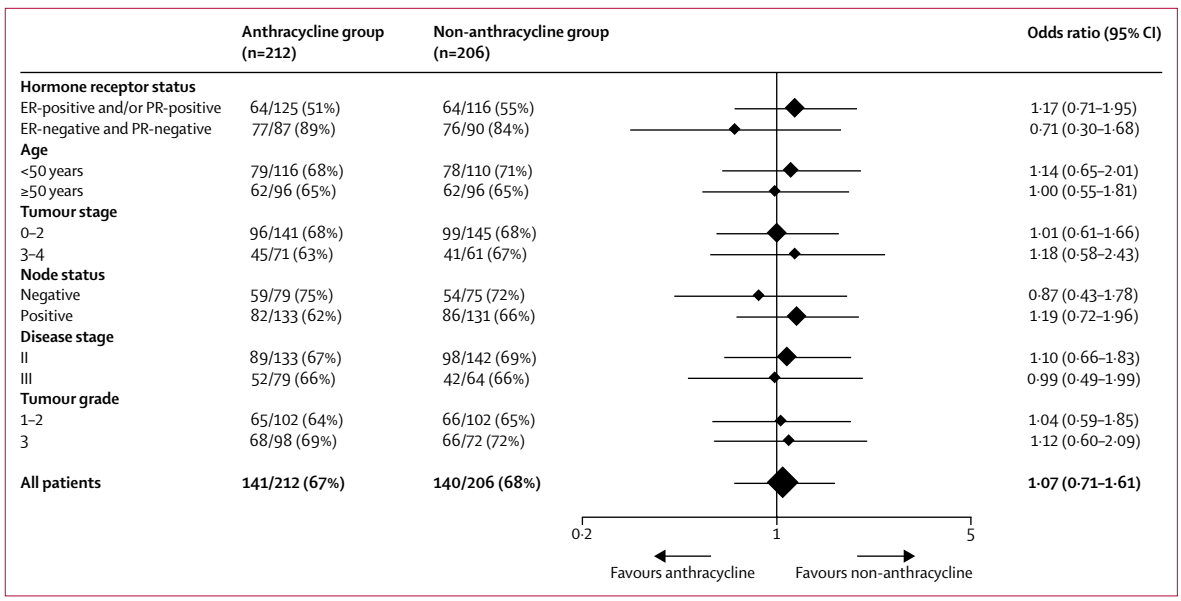

Figure 3: **Pathological complete responses by subgroup**
Disease stage II includes one patient with stage I disease in the non-anthracycline group. Disease stage III includes one patient with stage IV disease in the anthracycline group. Tumour grade and disease stage were post-hoc analyses. All other subgroup analyses were prespecified as stratification factors. ER=oestrogen receptor. PR=progesterone receptor.

	Anthracycline group (n=220)		Non-anthracycline group (n=218)	
	Grade 3	Grade 4	Grade 3	Grade 4
Neutropenia	92 (42%)	39 (18%)	104 (48%)	14 (6%)
Anaemia	43 (20%)	1 (<1%)	46 (21%)	0
Thrombocytopenia	31 (14%)	7 (3%)	35 (16%)	7 (3%)
Diarrhoea	26 (12%)	0	36 (17%)	1 (<1%)
Febrile neutropenia*	22 (10%)	1 (<1%)	3 (1%)	0
Hypokalaemia	18 (8%)	1 (<1%)	8 (4%)	0
Peripheral sensory neuropathy†	12 (5%)	0	15 (7%)	0
Alanine aminotransferase increased	11 (5%)	0	8 (4%)	0
Fatigue	9 (4%)	0	12 (6%)	0
Stomatitis	7 (3%)	0	2 (1%)	0
γ-glutamyltransferase increased	6 (3%)	0	6 (3%)	1 (<1%)
Dehydration	5 (2%)	0	1 (<1%)	0
Hypertension	5 (2%)	0	1 (<1%)	0
Anorexia	4 (2%)	0	3 (1%)	0
Ejection fraction decreased	4 (2%)	0	1 (<1%)	0
Aspartate aminotransferase increased	3 (1%)	0	1 (<1%)	0
Nausea	3 (1%)	0	4 (2%)	0
Syncope	3 (1%)	0	9 (4%)	0
Allergic reaction	2 (1%)	0	3 (1%)	0
Dizziness	2 (1%)	0	0	0
Left ventricular systolic dysfunction	2 (1%)	0	0	0
Thromboembolic event‡	2 (1%)	2 (1%)	0	0
Upper respiratory infection	2 (1%)	0	4 (2%)	0
Urinary tract infection	2 (1%)	0	2 (1%)	0
Vomiting	2 (1%)	0	6 (3%)	0

(Table 2 continues on next page)

Left ventricular ejection fraction decrease according to the commonly reported (non-CTCAE) definition of left ventricular ejection fraction decline of 10% or more and left ventricular ejection fraction below 50% was observed in ten (5%) of 220 patients in the anthracycline group versus six (3%) of 218 patients in the non-anthracycline group (p=0·32). Symptomatic left ventricular systolic dysfunction was reported in two patients in the anthracycline group. One patient in the non-anthracycline group developed heart failure after experiencing a myocardial infarction and then a stroke 1 month later.[11] All cardiac adverse events that occurred during neoadjuvant treatment are summarised in the appendix (p 5). Left ventricular ejection fraction grade 2 or worse decline according to CTCAE version 4.03 (left ventricular ejection fraction decline ≥10% or to <50%) was observed more frequently in the anthracycline group (64 patients [29%]) than in the non-anthracycline group (37 patients [17%]), and this decline did not recover during the follow-up period of the trial in about a third of these patients (appendix p 6).

All grade 3 or worse adverse events in both treatment groups are summarised in table 2. The most common adverse event was neutropenia, the incidence of which was similar in both groups (131 [60%] of 220 patients *vs* 118 [54%] of 218, p=0·29). Grade 4 neutropenia was more common in the anthracycline group than in the non-anthracycline group (39 [18%] of 220 *vs* 14 (6%) of 218, p=0·0004), as was grade 3 or 4 febrile neutropenia (23 [10%] of 220 *vs* three [1%] of 218, p<0·0001). Most neutropenic episodes occurred during the first three cycles of treatment (data not shown). Secondary G-CSF

prophylaxis was initiated in 66 (30%) patients in the anthracycline group and in 68 (31%) of those in the non-anthracycline group. The incidence of grade 3 or worse thrombocytopenia was similar in both groups: 38 (17%) of 220 patients in the anthracycline group and 42 (19%) of 218 in the non-anthracycline group (p=0·62), and occurred almost exclusively during the paclitaxel, trastuzumab, and carboplatin cycles (data not shown). One patient in the anthracycline group died from a thromboembolic event (pulmonary embolism) during treatment.

The most common grade 3 or worse non-haematological toxicity was diarrhoea, which occurred at a similar incidence in both groups overall (table 2). Grade 2 or worse peripheral neuropathy was reported in 66 (30%) of 220 patients in the anthracycline group and in 68 (31%) of 218 in the non-anthracycline group in those without anthracyclines (p=0·84).

Discussion

In the TRAIN-2 study, we did not record a significant increase in the proportion of patients achieving a pathological complete response with the use of anthracyclines in the presence of dual HER2 blockade. Instead, similarly high proportions of pathological complete responses were achieved with anthracyclines (67%) and without anthracyclines (68%). The toxicity profile of the two regimens differed, however, with more cases of febrile neutropenia in the anthracycline group than in the non-anthracycline group.

A comparison of chemotherapy regimens with and without anthracyclines in the presence of dual HER2 blockade has not been the primary aim of previous randomised trials in early HER2-positive breast cancer. Nevertheless, several studies support our results that anthracycline-free regimens can be considered in these patients. Similar to our study, the BCIRG-006 trial[3] reported similar 10-year disease-free and overall survival with a carboplatin–docetaxel regimen compared with an anthracycline-based regimen with single HER2 blockade with trastuzumab. A small, randomised Chinese study[15] found no difference in pathological complete responses with and without anthracyclines, and concluded that the regimens were similar, despite an unexpected large effect of multivariable adjustment. In the non-comparative TRYPHAENA trial, pathological complete responses achieved with trastuzumab plus pertuzumab in the non-anthracycline group and anthracycline groups were within the same range.[2] In the adjuvant Aphinity trial,[16] an anthracycline-free chemotherapy backbone was allowed according to the physician's choice and 535 (22%) of 2400 patients received an anthracycline-free regimen in combination with dual HER2 blockade. 3-year invasive disease-free survival was similar with and without anthracyclines.[16]

The finding of similar proportions of patients achieving pathological complete responses between treatment groups in our study was consistent across various patient

	Anthracycline group (n=220)		Non-anthracycline group (n=218)	
	Grade 3	Grade 4	Grade 3	Grade 4
(Continued from previous page)				
Aspiration	1 (<1%)	0	0	0
Bronchial infection	1 (<1%)	0	0	0
Chest pain	1 (<1%)	0	0	0
Chronic kidney injury	1 (<1%)	0	0	0
Cough	1 (<1%)	0	0	0
Device related infection	1 (<1%)	0	1 (<1%)	0
Dyspepsia	1 (<1%)	0	1 (<1%)	0
Fever	1 (<1%)	0	6 (3%)	1 (<1%)
Gastritis	1 (<1%)	0	0	0
Headache	1 (<1%)	0	1 (<1%)	0
Haemorrhoids	1 (<1%)	0	0	0
Hypophosphataemia	1 (<1%)	0	0	1 (<1%)
Hypotension	1 (<1%)	0	0	0
Infection	1 (<1%)	0	2 (1%)	0
Infusion site extravasation	1 (<1%)	0	0	0
Lung infection	1 (<1%)	0	1 (<1%)	0
Mania	1 (<1%)	0	0	0
Medullary thyroid carcinoma	1 (<1%)	0	0	0
Non-cardiac chest pain	1 (<1%)	0	0	0
Otitis media	1 (<1%)	0	0	0
Paronychia	1 (<1%)	0	0	0
Rash acneiform	1 (<1%)	0	2 (1%)	0
Rash maculopapular	1 (<1%)	0	0	0
Rectal haemorrhage	1 (<1%)	0	0	0
Renal and urinary tract disorders (other)	1 (<1%)	0	0	0
Thrombophlebitis	1 (<1%)	0	0	0
Tooth infection	1 (<1%)	0	0	0
Vasovagal reaction	1 (<1%)	0	0	0
Abdominal pain	0	0	3 (1%)	0
Acute coronary syndrome	0	0	1 (<1%)	0
Acute kidney injury	0	0	2 (1%)	0
Cataract	0	0	1 (<1%)	0
Cholecystitis	0	0	0	1 (<1%)
Constipation	0	0	1 (<1%)	0
Dysgeusia	0	0	1 (<1%)	0
Dyspnoea	0	1 (<1%)	1 (<1%)	0
Influenza-like symptoms	0	0	2 (1%)	0
Heart failure	0	0	1 (<1%)	0
Hypernatraemia	0	1 (<1%)	2 (1%)	0
Hypocalcaemia	0	0	2 (1%)	0
Hypomagnesaemia	0	2 (1%)	3 (1%)	1 (<1%)
Immune system disorder	0	0	0	1 (<1%)
Intraoperative gastrointestinal injury	0	0	1 (<1%)	0
Myocardial infarction	0	0	1 (<1%)	0
Palmar-plantar erythrodysthesia syndrome	0	0	1 (<1%)	0
Pneumonitis	0	0	1 (<1%)	0
Pneumothorax	0	0	2 (1%)	0
Rash, unspecified	0	0	1 (<1%)	0

(Table 2 continues on next page)

	Anthracycline group (n=220)		Non-anthracycline group (n=218)	
	Grade 3	Grade 4	Grade 3	Grade 4
(Continued from previous page)				
Renal calculi	0	0	1 (<1%)	0
Seizure	0	2 (1%)	0	0
Sepsis	0	0	0	1 (<1%)
Supraventricular tachycardia	0	0	1 (<1%)	0
Wrist fracture	0	0	1 (<1%)	0

Data are n (%). Grade 1–2 adverse events were not routinely recorded. Data are all grade 3–4 adverse events between randomisation and 30 days after the last neo-adjuvant treatment administration. One patient randomly allocated to the non-anthracycline group received treatment of the anthracycline group and was analysed accordingly for safety. *Febrile neutropenia (grade ≥3) was significantly more common in the anthracycline group (p<0·0001). †Grade 2 neuropathy was recorded and occurred in 54 (25%) patients in the anthracycline group and in 53 (24%) in the non-anthracycline group. ‡One patient died of a thromboembolic event in the anthracycline group (grade 5).

***Table 2*: Any-cause adverse events in the safety population**

subgroups, including subgroups defined by hormone receptor status. Ideally, markers that predict anthracycline benefit and cardiotoxicity would help to guide the decision of whether or not to administer these drugs, but none has been identified or robustly proven to have predictive value, precluding their use in clinical practice.[17–19]

The proportions of patients achieving pathological complete responses in our study were similar to those reported in other trials using polychemotherapy plus trastuzumab and pertuzumab, ranging from 55% to 69% for anthracycline-containing regimens[1,2] and from 56% to 66% for a carboplatin–docetaxel regimen.[2,20] The number of pertuzumab administrations in these trials ranged from three to eight, compared to nine in our study. At present, the optimal duration of pertuzumab treatment is unknown.

In our study, the anthracycline regimen was associated with more febrile neutropenia than was the non-anthracycline regimen. However, the proportion of patients who received G-CSF support was largely similar between the two treatment groups, because neutropenia was often observed in conjunction with thrombocytopenia in both groups (data not shown), in which case dose reduction and not G-CSF support was mandated according to the protocol. Clinically relevant left ventricular ejection fraction decline (decline ≥10% and left ventricular ejection fraction <50%) did not differ substantially between treatment groups, but we did observe more asymptomatic grade 2 ejection fraction decline in the anthracycline group than in the non-anthracycline group, which had not recovered during follow-up in about a third of the patients. The long-term clinical consequence of low grade cardiac toxicity is not well known and deserves further study. Similarly, the BCIRG-006 trial showed significantly more acute and long-term toxicity with the anthracycline regimen than with the non-anthracycline regimen, including secondary leukaemia and cardiotoxicity.[3,20] Cardiotoxicity and febrile neutropenia in the TRYPHAENA trial did not differ substantially between the groups.[2]

Although the non-anthracycline regimen had a more favourable toxicity profile than the anthracycline regimen, both regimens still had undesirable levels of toxicity. Grade 2 or worse neuropathy was seen in a substantial proportion of patients in both groups. Neuropathy is an important and challenging toxicity of taxanes and platinum salts, which requires early recognition and treatment adaptations. Moreover, diarrhoea is a common and cumbersome toxicity of pertuzumab and seems to be more pronounced when given concurrently with a taxane–carboplatin regimen. Secondary antidiarrhoeal prophylaxis with loperamide was advised in our study protocol and other strategies, including primary prophylaxis, are being studied to overcome pertuzumab-related diarrhoea.[21]

Strengths of this study include its prospective design, its aim to address the question of the optimal chemotherapy backbone for dual targeted HER2 therapy, and the use of regimens of equal duration, which removes the duration of treatment variable from the interpretation.

One of the limitations of our study is that neither of the two chemotherapy regimens assessed are currently standard regimens and therefore are not commonly used in clinical practice. When designing the TRAIN-2 trial, we hypothesised that weekly paclitaxel would be the optimal mode of taxane delivery. We also considered that despite a shorter treatment duration, docetaxel, carboplatin, and trastuzumab seemed to have similar activity to doxorubicin plus cyclophosphamide followed by docetaxel plus trastuzumab in BCIRG-006.[3] Therefore, to optimise the activity of our carboplatin–paclitaxel regimen, we increased the number of cycles to closely match the duration of the more familiar doxorubicin plus cyclophosphamide followed by docetaxel plus trastuzumab regimen, resulting in high cumulative doses of paclitaxel in our regimen. Whether or not our results can be extrapolated to anthracycline-free regimens with lower cumulative doses of taxanes remains unknown. The non-comparative TRYPHAENA study, however, did report similar outcome after six cycles of docetaxel compared with three cycles of 5-fluorouracil, epirubicin, and cyclophosphamide followed by three cycles of docetaxel.[2]

Another potential limitation of our study is the choice of pathological complete response as our primary endpoint, which we used as a surrogate marker for long-term outcome. However, although a meta-analysis by Cortazar and colleagues[22] concluded that comparing breast cancer regimens on the basis of pathological complete response does not reliably predict potential differences in long-term outcome, Broglio and colleagues[23] showed that in HER2-positive disease, the odds ratios for pathological complete response and hazard ratios for event-free survival and overall survival are correlated in randomised controlled trials. Long-term follow-up of our results remains important, however, to confirm the observed activity of our paclitaxel, trastuzumab, and carboplatin plus pertuzumab regimen in terms of event-free and overall survival. We plan to

publish an update of our results with long-term outcome data at a median follow-up of 3 years. The lack of collection of quality-of-life endpoints is another limitation of the study.

Another potential limitation of this study is the absence of central pathology review of HER2 status and hormone receptor status. However, Dekker and colleagues[24] previously showed excellent concordance rates for HER2 and oestrogen receptor status between regional pathology laboratories and central review in a study in more than 1000 Dutch patients. Additionally, quality control and auditing are in place in the Netherlands to maintain a high quality of pathology laboratories. We therefore do not expect the lack of central review to have a large effect on our results. Central review is planned as part of the translational side study in patients from whom study biopsies are available and these results will be reported in the future.

The observed proportions of patients achieving a pathological complete response were higher than anticipated in both treatment groups and the study was not powered to detect a subtle significant difference in pathological complete responses. Since the study was designed as a superiority trial, non-inferiority of the non-anthracycline regimen cannot be claimed. Nevertheless, the almost equal proportions of pathological complete responses achieved in the two groups, the favourable toxicity profile of the non-anthracycline group, and the results of previous non-comparative studies in this field support the use of a non-anthracycline regimen as an attractive option in the neoadjuvant treatment of early HER2-positive breast cancer in the presence of dual HER2 blockade.[2] Furthermore, weekly paclitaxel could be used as an alternative to docetaxel once every 3 weeks in the docetaxel, carboplatin, and trastuzumab regimen.

Given the observed high proportion of pathological complete responses in this trial and substantial treatment-related toxicity with existing polychemotherapy regimens, the question arises of whether or not we can reduce chemotherapy while maintaining efficacy. The GIM-2 study[25] showed no disease-free and overall survival benefit of adding 5-fluorouracil to epirubicin and cyclophosphamide in adjuvant treatment of patients with node-positive breast cancer. The GeparSixto trial[26] indicated that the addition of carboplatin to an anthracycline-based regimen does not increase anti-tumour activity in HER2-positive breast cancer. In anthracycline-free regimens, one could also question the need for carboplatin in all patients. Excellent survival is seen with weekly paclitaxel plus trastuzumab in low-risk patients with mainly stage I disease.[27] In patients with stage II–III disease, a taxane-only regimen plus dual HER2 blockade results in 39–52% patients achieving a pathological complete response.[13,14,28] Pathological complete responses have also been recorded with dual HER2 blockade without chemotherapy (with or without endocrine therapy).[14,29–31] These findings suggest substantial overtreatment with existing regimens in many patients, and studies that assess selective treatment de-escalation are eagerly awaited in this era of highly effective dual HER2 blockade. To this end, blood and tissue collection is part of a translational side study of our protocol that aims to study specific hypotheses based on *TOP2A* aberrations, PI3K pathway activation, and tumour-infiltrating lymphocytes in addition to exploratory analyses based on somatic mutation profiling, gene-expression profiling, and CNVseq analysis. These analyses could potentially identify patients at very low risk of recurrence in whom treatment de-escalation is feasible. The results of these analyses will be published in future reports. In the absence of markers to select patients for less intensive therapies upfront, image guided treatment de-escalation might offer strategies to reduce chemotherapy exposure. The TRAIN-3 study will be a single-arm multicentre trial, in which the number of neoadjuvant chemotherapy cycles will be based on the radiological responses recorded during treatment in neoadjuvant treatment of HER2-positive breast cancer. In this future study, we hypothesise that the long-term event-free and overall survival of patients in complete remission after three cycles of chemotherapy will be similar to that in patients who achieve a complete remission after six or nine cycles. If our hypothesis is verified, the findings of the study will hopefully support a treatment approach in which patients with an early complete radiological remission can be referred for early surgery.

In conclusion, we found similar and high proportions achieving a pathological complete response both with and without anthracyclines in the presence of trastuzumab and pertuzumab in patients with early-stage HER2-positive breast cancer. Febrile neutropenia was more common in the anthracycline group than in the non-anthracycline group. Consequently, omitting anthracyclines might be an attractive approach for the treatment of HER2-positive breast cancer in the presence of dual HER2 blockade. Further follow-up and the overall survival data are needed to confirm the observed similar efficacy of both regimens used in this study.

Contributors

GSS contributed to the conception and design of the study. MSvR, AvdV, EDvW, and IAM provided administrative support. MSvR, IK, VOD, IMO, AHH, LWT, AJvdW, CMM, LJvW, JW, M-JTVP, SCL, and GSS contributed to the provision of study materials or patients. MSvR, EDvW, and IAM contributed to the collection and assembly of data. MSvR, AvdV, EDvW, and GSS contributed to the data analysis and interpretation. All authors contributed to the writing and had final approval of the manuscript.

Declaration of interests

SCL has received institutional research funding from AstraZeneca, Amgen, BMS, Roche, and Genentech and is an advisory board member for AstraZeneca. GSS has received institutional research funding from AstraZeneca, Merck, Novartis, and Roche. All other authors declare no competing interests.

Data sharing

The data collected for this study can be made available to others in de-identified form after all primary and secondary endpoints have been published and in the presence of a data transfer agreement. Requests for data sharing can be made to the corresponding author, including a proposal that must be approved by the trial's steering committee.

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References

1 Loibl S, Jackisch C, Schneeweiss A, et al. Dual HER2-blockade with pertuzumab and trastuzumab in HER2-positive early breast cancer: a subanalysis of data from the randomized phase III GeparSepto trial. *Ann Oncol* 2016; **28:** 497–504.

2 Schneeweiss A, Chia S, Hickish T, et al. Long-term efficacy analysis of the randomised, phase II TRYPHAENA cardiac safety study: evaluating pertuzumab and trastuzumab plus standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer. *Eur J Cancer* 2018; **89:** 27–35.

3 Slamon DJ, Eiermann W, Robert NJ, et al. Ten year follow-up of BCIRG-006 comparing doxorubicin plus cyclophosphamide followed by docetaxel (AC→T) with doxorubicin plus cyclophosphamide followed by docetaxel and trastuzumab (AC→TH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2+ early breast cancer. *Cancer Res* 2016; **76** (suppl 4)**:** S5–04 (abstr).

4 Mantarro S, Rossi M, Bonifazi M, et al. Risk of severe cardiotoxicity following treatment with trastuzumab: a meta-analysis of randomized and cohort studies of 29,000 women with breast cancer. *Intern Emerg Med* 2015; **11:** 123–40.

5 Jones SE, Collea R, Paul D, et al. Adjuvant docetaxel and cyclophosphamide plus trastuzumab in patients with HER2-amplified early stage breast cancer: a single-group, open-label, phase 2 study. *Lancet Oncol* 2013; **14:** 1121–28.

6 von Minckwitz G, Untch M, Nuesch E, et al. Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neo-adjuvant chemotherapy trials. *Breast Cancer Res Treat* 2011; **125:** 145–56.

7 Bayraktar S, Gonzalez-Angulo AM, Lei X, et al. Efficacy of neoadjuvant therapy with trastuzumab concurrent with anthracycline- and nonanthracycline-based regimens for HER2-positive breast cancer. *Cancer* 2012; **118:** 2385–93.

8 van Ramshorst MS, van Werkhoven E, Mandjes IA, et al. Trastuzumab in combination with weekly paclitaxel and carboplatin as neo-adjuvant treatment for HER2-positive breast cancer: the TRAIN-study. *Eur J Cancer* 2017; **74:** 47–54.

9 Sparano JA, Wang M, Martino S, et al. Weekly paclitaxel in the adjuvant treatment of breast cancer. *N Engl J Med* 2008; **358**: 1663–71.

10 Perez EA, Suman VJ, Rowland KM, et al. Two concurrent phase II trials of paclitaxel/carboplatin/trastuzumab (weekly or every-3-week schedule) as first-line therapy in women with HER2-overexpressing metastatic breast cancer: NCCTG study 983252. *Clin Breast Cancer* 2005; **6:** 425–32.

11 van Ramshorst MS, van Werkhoven E, Honkoop AH, et al. Toxicity of dual HER2-blockade with pertuzumab added to anthracycline versus non-anthracycline containing chemotherapy as neoadjuvant treatment in HER2-positive breast cancer: the TRAIN-2 study. *Breast* 2016; **29:** 153–39.

12 Oncoline. Borstkanker: landelijke richtlijn, versie: 1.0. https://www.oncoline.nl/borstkanker (accessed April 20, 2018)

13 Baselga J, Bradbury I, Eidtmann H, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet* 2012; **379:** 633–40.

14 Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol* 2012; **13:** 25–32.

15 Huang L, Chen S, Yang W, et al. Efficacy and safety analysis of trastuzumab and paclitaxel based regimen plus carboplatin or epirubicin as neoadjuvant therapy for clinical stage II-III, HER2-positive breast cancer patients: a phase 2, open-label, multicenter, randomized trial. *Oncotarget* 2015; **6:** 18683–92.

16 von Minckwitz G, Procter M, de Azambuja E, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. *N Engl J Med* 201; **377:** 122–31.

17 Press MF, Sauter G, Buyse M, et al. Alteration of topoisomerase II-alpha gene in human breast cancer: association with responsiveness to anthracycline-based chemotherapy. *J Clin Oncol* 2011; **29:** 859–67.

18 Bartlett JM, Campbell FM, Mallon EA. Determination of HER2 amplification by in situ hybridization: when should chromosome 17 also be determined? *Am J Clin Path* 2008; **130:** 920–26.

19 Linschoten M, Teske AJ, Cramer MJ, van der Wall E, Asselbergs FW. Chemotherapy-related cardiac dysfunction: a systematic review of genetic variants modulating individual risk. *Circ Genom Precis Med* 2018; **11:** e001753.

20 Hurvitz SA, Martin M, Symmans WF, et al. Neoadjuvant trastuzumab, pertuzumab, and chemotherapy versus trastuzumab emtansine plus pertuzumab in patients with HER2-positive breast cancer (KRISTINE): a randomised, open-label, multicentre, phase 3 trial. *Lancet Oncol* 2018; **19:** 115–26.

21 Gao JJ, Tan M, Pohlmann PR, Swain SM. HALT-D: a phase II evaluation of crofelemer for the prevention and prophylaxis of diarrhea in patients with breast cancer on pertuzumab-based regimens. *Clin Breast Cancer* 2016; **17:** 76–78.

22 Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* 2014; **384:** 164–72.

23 Broglio KR, Quintana M, Foster M, et al. Association of pathologic complete response to neoadjuvant therapy in HER2-positive breast cancer with long-term outcomes: a meta-analysis. *JAMA Oncol* 2016; **2:** 751–60.

24 Dekker TJ, Borg ST, Hooijer GK, et al. Determining sensitivity and specificity of HER2 testing in breast cancer using a tissue micro-array approach. *Breast Cancer Res* 2012; **14:** R93.

25 Del Mastro L, De Placido S, Bruzzi P, et al. Fluorouracil and dose-dense chemotherapy in adjuvant treatment of patients with early-stage breast cancer: an open-label, 2×2 factorial, randomised phase 3 trial. *Lancet* 2015; **385:** 1863–72.

26 von Minckwitz G, Schneeweiss A, Loibl S, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. *Lancet Oncol* 2014; **15:** 747–56.

27 Tolaney SM, Barry WT, Dang CT, et al. Adjuvant paclitaxel and trastuzumab for node-negative, HER2-positive breast cancer. *N Engl J Med* 2015; **372:** 134–41.

28 Carey LA, Berry DA, Cirrincione CT, et al. Molecular heterogeneity and response to neoadjuvant human epidermal growth factor receptor 2 targeting in CALGB 40601, a randomized phase III trial of paclitaxel plus trastuzumab with or without lapatinib. *J Clin Oncol* 2016; **34:** 542–49.

29 Nitz UA, Gluz O, Christgen M, et al. De-escalation strategies in HER2-positive early breast cancer (EBC): final analysis of the WSG-ADAPT HER2+/HR- phase II trial: efficacy, safety, and predictive markers for 12 weeks of neoadjuvant dual blockade with trastuzumab and pertuzumab +/- weekly paclitaxel. *Ann Oncol* 2017; **28:** 2768–72.

30 Rimawi MF, Mayer IA, Forero A, et al. Multicenter phase II study of neoadjuvant lapatinib and trastuzumab with hormonal therapy and without chemotherapy in patients with human epidermal growth factor receptor 2-overexpressing breast cancer: TBCRC 006. *J Clin Oncol* 2013; **31:** 1726–31.

31 Llombart-Cussac A, Cortes J, Pare L, et al. HER2-enriched subtype as a predictor of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast cancer (PAMELA): an open-label, single-group, multicentre, phase 2 trial. *Lancet Oncol* 2017; **18:** 545–54.