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Original article

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

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ABSTRACT

Background: The addition of pertuzumab to neoadjuvant trastuzumab-based chemotherapy improves pathologic complete response rates in HER2-positive breast cancer. However, increased toxicity has been reported with the addition of pertuzumab, and this may differ between various chemotherapy backbone regimens. We evaluated toxicities of pertuzumab when added to either FEC-T (5-fluorouracil, epirubicin, cyclophosphamide, trastuzumab) or weekly paclitaxel, trastuzumab, carboplatin (PTC).
Methods: The TRAIN-2 study is a neoadjuvant randomized controlled trial in stage II and III HER2-positive breast cancer (NCT01996267). Patients are randomly assigned to receive either three cycles of FEC-T plus pertuzumab or three cycles of PTC plus pertuzumab, followed by six cycles of PTC plus pertuzumab in both arms. Toxicities are described per treatment arm according to the Common Toxicity Criteria for Adverse Events version 4.03.
Results: This analysis includes 110 patients balanced over both treatment arms. Neutropenia was the most common hematologic toxicity, with grade 3–4 occurring in 53% in the FEC-T-arm and in 51% in the PTC-arm. Febrile neutropenia occurred in 9% in the FEC-T arm and did not occur in the PTC-arm. Secondary G-CSF prophylaxis was used in 35–40% of patients. Asymptomatic ejection fraction decrease grade 2 was observed in 24% in the FEC-T-arm and 11% in the PTC-arm. The most common grade 3–4 non-hematologic toxicity was diarrhea (5% in the FEC-T-arm and 18% in the PTC-arm).
Conclusions: Pertuzumab in combination with FEC-T mostly causes neutropenia, and when added to PTC mostly causes diarrhea. Significant cardiac toxicity is rare with both regimens, and toxicity is overall well manageable.

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Introduction

Trastuzumab-based (neo)adjuvant chemotherapy is the standard of care for patients with stage II and III human epidermal growth factor receptor 2 (HER2) positive breast cancer [1]. The addition of pertuzumab to trastuzumab-based chemotherapy almost doubles the pathologic complete response (pCR) rate (from 22% to 39%) and has recently been registered for use in the neoadjuvant setting [1,2]. However, important toxicity has been reported as well, which may differ between various chemotherapy regimens. Thus, the optimal chemotherapy backbone for

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dual HER2-blockade is unknown, both from an efficacy and from a toxicity point-of-view. In particular, it is uncertain whether anthracyclines and cyclophosphamide should be part of the optimal chemotherapy regimen. Anthracyclines and cyclophosphamide are associated with severe long-term toxicities including cardiotoxicity, secondary malignancies, and infertility [3–5]. Therefore, regimens without these two agents might be preferable if the same efficacy can be achieved [3,6]. In the TRAIN-study we evaluated a trastuzumab-containing regimen with weekly paclitaxel and carboplatin and this regimen showed a promising high pCR rate (*van Ramshorst* et al. *submitted)*, within the same range as observed with regimens including dual HER2-blockade [2,7]. Subsequently, the TRAIN-2 study was designed to compare the efficacy and safety of six cycles of weekly paclitaxel, carboplatin, trastuzumab plus pertuzumab preceded by three cycles of weekly paclitaxel, carboplatin, trastuzumab plus pertuzumab or three cycles of 5-fluorouracil, epirubicin, cyclophosphamide, trastuzumab plus pertuzumab. Here, we report the toxicity analyses of the first 110 patients treated in the TRAIN-2 study.

Methods

Study design and patients

The TRAIN-2 study is a randomized, open-label, multicenter trial designed to compare the efficacy and safety of dual HER2-blockade with trastuzumab plus pertuzumab in combination with anthracycline-containing combination chemotherapy versus a non-anthracycline chemotherapy regimen in newly diagnosed stage II and III HER2-positive breast cancer patients (NCT01996267). Eligible patients were 18 years or older, treatment-naïve, and had no history of prior malignancy. Other eligibility criteria included a WHO performance status 0–1, a baseline left ventricular ejection fraction (LVEF) of $\geq$50%, adequate liver, renal, and bone marrow function, and no current pregnancy or breastfeeding. All patients gave written informed consent. The medical ethics committee of the Netherlands Cancer Institute approved the study protocol and any modifications thereof. The primary endpoint of the study is the percentage pCR. Safety was a key secondary endpoint, defined as the percentage patients with grade $\geq$3 toxicity, and grade $\geq$2 for cardiotoxicity and neuropathy. Toxicities were recorded according to Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03. This pre-specified safety analysis was performed after the first 110 patients had completed neoadjuvant treatment and had undergone surgery, which comprises 25% of the total planned accrual (n = 437).

Treatment schedule

Patients were randomly assigned to receive three cycles of three-weekly paclitaxel (80 mg/m^2 day 1 and 8), trastuzumab (6 mg/kg, loading dose 8 mg/kg), carboplatin (AUC = 6 mg·min/ml) [PTC] plus pertuzumab (420 mg, loading dose 840 mg) or three cycles of three-weekly 5-fluoruoracil (500 mg/m^2), epirubicin (90 mg/m^2), cyclophosphamide (500 mg/m^2) and trastuzumab [FEC-T] plus pertuzumab, followed by six additional cycles of three-weekly PTC plus pertuzumab in both arms (Supplementary Fig. 1). Physical examination, hematologic and biochemical investigations, and toxicity evaluation were completed before every chemotherapy cycle. LVEF measurements were repeated every three months or more often if indicated. Criteria for dose adjustments are summarized in Table 1.

Surgery was performed within six weeks of last chemotherapy and pathologic response was evaluated according to Dutch national guidelines, with pCR defined as the absence of any residual invasive tumor cell in the breast and axilla. After surgery adjuvant trastuzumab was continued to complete one year of treatment. Further adjuvant treatment was applied according to local guidelines.

Statistical analysis

Descriptive statistics of baseline, treatment, and toxicity data are reported according to treatment arm. Differences in treatment intensity and toxicity between the arms were tested for significance using the Wilcoxon rank sum test for continuous variables and the two-sided Fisher's exact test for categorical variables. All statistical calculations were made using R (www.r-project.org) and statistical significance was defined as $p < 0.05$.

Results

Between December 2013 and November 2014, 110 patients were included. All patients were female and median age was 47 years (range 32–73). Sixty-three percent of patients had stage II disease, and 63% had a hormone-receptor positive tumor. Baseline characteristics are summarized in Table 2.

Treatment intensity

Eighty-two percent of patients in the FEC-T-arm received all nine courses of treatment compared with 80% in the PTC-arm (Supplementary Fig. 2 contains a consort flow diagram). Chemotherapy dose was reduced in five patients (9%) in each arm during the first three treatment courses. During the subsequent six courses at least one dose reduction was implemented in 45% (25/55) of patients in the FEC-T-arm and in 56% (30/54) in the PTC-arm. The median number of received courses per drug and the administered cumulative dose as a percentage of the expected cumulative dose per drug are shown in Table 3.

Hematological adverse events

Grade 3–4 neutropenia occurred at a similar rate in both arms (53% vs 51%), although grade 4 neutropenia was more common with FEC-T (16% vs 4%; p = 0.06; Table 4). Febrile neutropenia was seen in five patients (9%) in the FEC-T-arm and in none in the PTC-arm (p = 0.06). G-CSF support was initiated following grade $\geq$3 neutropenia according to protocol in 40% of patients in the FEC-T-arm and in 35% of patients in the PTC-arm (p = 0.69), and prevented further grade $\geq$3 neutropenia in all but four patients. As described in Table 1, G-CSF support was not indicated if neutropenia occurred simultaneously with thrombocytopenia in the FEC-T arm.

Grade 3–4 thrombocytopenia was seen in ten patients in the FEC-T-arm and in seven in the PTC-arm, but was exclusively seen during the PTC cycles in both arms.

One patient was diagnosed with acute myeloid leukemia (AML) after three cycles FEC-T plus pertuzumab and two cycles PTC plus pertuzumab, which was considered possibly related to treatment, although the interval since start of chemotherapy was very short. However, cytogenetic analysis revealed a chromosome 16 inversion, which is described in association with topoisomerase II inhibitors like anthracyclines [8].

Cardiac toxicity

Cardiac toxicity was rare in both treatment arms. The lowest measured LVEF per patient ranged between 45% and 76% in the

Table 1
Toxicity-based dose adjustments.

Toxicity	Action
Hematological toxicity	*Delay treatment cycle until recovery[a], with a maximum of 2 weeks[b]*
• Isolated neutropenia (absolute neutrophil count <1.0 × 10^9/L)	• Start G-CSF support (continue until last course) • During G-CSF support: 25% dose reduction of chemotherapy
• Isolated thrombocytopenia (platelet count <75 × 10^9/L)	• FECT-arm: 25% dose reduction of FEC • PTC-arm: 25% dose reduction of carboplatin
• Neutropenia and thrombocytopenia	• FECT-arm: 25% dose reduction of FEC • PTC-arm: start G-CSF support and 25% dose reduction of carboplatin
Non-hematological toxicity	*Delay treatment cycle until recovery[c], with a maximum of 2 weeks[b]*
• Non-hematological toxicity ≥ grade 3	• FECT-arm: 25% dose reduction of FEC[d] • PTC-arm: 25% dose reduction of paclitaxel and carboplatin[d]
• Polyneuropathy ≥ grade 2	• FECT-arm: 25% dose reduction of FEC[d] • PTC-arm: 25% dose reduction of paclitaxel and carboplatin[d]
Cardiotoxicity	
• LVEF decrease of >15% points from baseline • LVEF decrease of ≥10% points with LVEF below LLN	• Retain trastuzumab and pertuzumab and repeat LVEF after 3 weeks[e] (resume if criteria for continuation are met)

G-CSF, granulocyte-colony stimulating factor; FECT, 5-fluorouracil, epirubicin, cyclophosphamide, trastuzumab.
PTC, paclitaxel, trastuzumab, carboplatin; LVEF, left ventricular ejection fraction; LLN, lower limit of normal.
[a] Hematological recovery is defined as absolute neutrophil count ≥1.0 × 10^9/L and platelet count ≥75 × 10^9/L.
[b] In case of a treatment delay of > 2 weeks (i.e. consecutive or segregated) discuss individual patient management with study team.
[c] Non-hematological recovery is defined as toxicity of maximum grade 2, and for polyneuropathy of maximum grade 1.
[d] Unless the toxicity can unambiguously be related to a specific agent, then specific dose adjustments may be applied.
[e] Permanently discontinue trastuzumab and pertuzumab if 2 consecutive holds or a total of 3 holds occur.

Table 2
Baseline characteristics according to treatment arm.

	FECT-Ptz (n=55)	PTC-Ptz (n=55)	Total (n=110)
	n (%)	n (%)	n (%)
Age			
Median (range)	48 (32-65)	47 (32-73)	47 (32-73)
Primary tumor size			
Median (range)	39 (10-95)	37 (12-120)	38 (10-120)
Performance status			
WHO 0	51 (93)	49 (89)	100 (91)
WHO 1	3 (5)	6 (11)	9 (8)
Unknown	1 (2)	0 (0)	1 (1)
Clinical N-stage			
Negative	19 (35)	16 (29)	35 (32)
Positive	36 (65)	39 (71)	75 (68)
Disease stage			
II	23 (60)	36 (65)	69 (63)
III	28 (40)	19 (34)	41 (37)
Grade			
1	0 (0)	2 (4)	2 (2)
2	15 (27)	13 (24)	28 (25)
3	22 (40)	18 (33)	40 (36)
Unknown	18 (33)	22 (40)	40 (36)
Histology			
Ductal	45 (82)	51 (93)	96 (87)
Lobular	4 (7)	2 (4)	6 (5)
Mixed	3 (5)	1 (2)	4 (4)
Other	3 (5)	1 (2)	4 (4)
Hormone receptor status			
ER- and PR-	20 (36)	21(38)	41 (37)
ER+ and/or PR+	35 (64)	34 (62)	69 (63)

Table 3
Treatment intensity according to treatment arm per drug (median number of received courses and administered cumulative dose as percentage of the expected cumulative dose).

	FEC-T-Ptz (n = 55)	PTC-Ptz (n = 55)
	Median (range)	Median (range)
Trastuzumab		
Number of courses	9 (4–9)	9 (2–9)
Received cumulative dose of expected (%)	100 (47–100)	99 (27–100)
Pertuzumab		
Number of courses	9 (4–9)	9 (2–9)
Received cumulative dose of expected (%)	100 (50–100)	100 (30–100)
Paclitaxel		
Number of courses	6 (1–6)	9 (2–9)
Received cumulative dose of expected (%)	94 (8–100)	93 (16–100)
Carboplatin[a]		
Number of courses	6 (0–6)	9 (2–9)
Received cumulative dose of expected (%)	90 (0–100)	89 (43–100)
5-Fluorouracil[b]		
Number of courses	3 (2–3)	–
Received cumulative dose of expected (%)	99 (60–100)	–
Epirubicin[b]		
Number of courses	3 (2–3)	–
Received cumulative dose of expected (%)	99 (50–100)	–
Cyclophosphamide[b]		
Number of courses	3 (2–3)	–
Received cumulative dose of expected (%)	99 (58–100)	–

[a] One patient in the FEC-T arm did not receive carboplatin because of ototoxicity after FEC-T.
[b] One patient in the FEC-T arm received only 2 cycles FEC-T because of elevated transaminases, but did receive the subsequent PTC-Ptz cycles.

FEC-T-arm and 32%–72% in the PTC-arm. One patient with multiple pre-existing cardiovascular risk factors in the PTC-arm suffered an acute myocardial infarction with subsequent drop in LVEF to 32% (grade 3 ejection fraction decrease). One month later the same patient suffered a stroke. Further neoadjuvant treatment was discontinued and early surgery was performed. This was the only patient experiencing symptomatic left ventricular dysfunction, and no other grade ≥3 cardiotoxicity was observed. Twenty-four percent (95%CI 13–37%) of patients in the FEC-T-arm and 11% (95%CI 4–22%) in the PTC-arm (p = 0.21) experienced asymptomatic grade 2 LVEF decrease (LVEF 50–40% or LVEF decrease 10–19%). Of the seven patients with an LVEF below 50% during treatment, three remained below 50% at the last LVEF measurement before surgery (LVEF of 46% in two patients, and LVEF of 32% in one patient who experienced a myocardial infarction). One patient, in the PTC-arm, attended the hospital because of palpitations with a supraventricular tachycardia, which converted to a sinus rhythm after adenosine. This episode was reported to be unlikely related to study drugs.

Table 4
Hematological toxicities grade ≥3.

	FEC-T-Ptz (n = 55)		PTC-Ptz (n = 55)		p^*
	Grade 3	Grade 4	Grade 3	Grade 4	
	n (%)	n (%)	n (%)	n (%)	
Overall					
Anemia	14 (25)	0 (0)	7 (13)	0 (0)	*0.14*
Neutropenia	20 (36)	9 (16)	26 (47)	2 (4)	*1.00*
Thrombocytopenia	7 (13)	3 (5)	6 (11)	1 (2)	*0.60*
Febrile neutropenia	5 (9)	0 (0)	0 (0)	0 (0)	*0.06*
During cycle 1–3					
Anemia	0 (0)	0 (0)	1 (2)	0 (0)	*1.00*
Neutropenia	11 (20)	7 (13)	7 (13)	1 (2)	*0.04*
Thrombocytopenia	0 (0)	0 (0)	2 (4)	0 (0)	*0.50*
Febrile neutropenia	4 (7)	0 (0)	0 (0)	0 (0)	*0.12*
During cycle 4–9[a]					
Anemia	14 (25)	0 (0)	7 (13)	0 (0)	*0.14*
Neutropenia	21 (38)	2 (4)	25 (46)	1 (2)	*0.57*
Thrombocytopenia	7 (13)	3 (5)	5 (9)	1 (2)	*0.42*
Febrile neutropenia	1 (2)	0 (0)	0 (0)	0 (0)	*1.00*

*p-value: difference in incidence of grade ≥3 toxicity between both arms.
Statistical significance is defined as p-value <0.05 are in italics.
[a] In the PTC-arm only 54 patients started the 4th cycle.

Non-hematological adverse events

Diarrhea was the most common grade 3–4 non-hematological adverse event, occurring in 5% of patients in the FEC-T-arm and 18% in the PTC-arm (p = 0.07). Diarrhea occurred early during treatment with 5% of patients experiencing grade≥ 3 diarrhea after the first course. Diarrhea grade ≥3 did not result in less administered therapy, as both patients with and without diarrhea received similar doses of therapy (data not shown). Dehydration was reported in one patient in each arm, both grade 3. Other non-hematological toxicities grade ≥3 observed in at least two patients in the same treatment arm are summarized in Table 5. Grade 3 peripheral neuropathy was reported in two patients (4%) in the FEC-T-arm and in three patients (5%) in the PTC-arm, and grade 2 was described in 14 patients (25%) and in 15 patients (27%) respectively. Neuropathy was almost exclusively seen in a later phase of the treatment. In total 26 patients (24%) experienced one or more serious adverse events (SAEs) requiring hospital admission, and were well balanced between both treatment arms. Most SAEs were related to febrile neutropenia or diarrhea.

Discussion

In this safety analysis of the TRAIN-2 study, pertuzumab in combination with FEC-T and PTC was well tolerated as neoadjuvant treatment in patients with stage II-III HER2-positive breast cancer. Over 80% of patients in both arms completed all planned treatment cycles. The most common grade 3–4 toxicity was neutropenia, occurring at similar rates in both arms. Febrile neutropenia was very rare (<5%), with secondary G-CSF prophylaxis used in 35–40% of patients. Cardiac tolerability of both regimens was good, and only one patient (<1%), with pre-existing cardiovascular risk factors, experienced left ventricular dysfunction. Most common non-hematological toxicity was diarrhea and was well manageable in almost all patients.

Previous studies reported neutropenia rates similar to what we found [2,3,9]. In our study, the severity of neutropenia was worse with FEC-T compared with PTC, with more grade 4 neutropenia and more febrile neutropenia, possibly attributable to the use of three instead of two chemotherapeutics in the FEC-T-arm and the particular myelosuppressive effect of anthracyclines and cyclophosphamide [10]. The TRAIN-2 study was designed before the publication of the GIM-2 study results, which show similar efficacy of FEC versus EC. Therefore, 5-fluorouracil was incorporated in our study regimen, and may have contributed to additional toxicity, which can be avoided in the future by using EC [11]. In contrast to our results, similar rates were reported in both arms receiving six cycles of pertuzumab in the Tryphaena trial: in the arm receiving three times FEC-T plus pertuzumab followed by docetaxel/trastuzumab plus pertuzumab, and the arm receiving six cycles TCH plus pertuzumab neutropenia grade ≥3 was reported in 47% and 46% respectively, and febrile neutropenia in 18% and 17% respectively [9]. In the Neosphere study neutropenia grade ≥3 occurred in 45% and febrile neutropenia in 8% of patients receiving dual HER2-blockade with trastuzumab plus pertuzumab combined with docetaxel [2]. In a retrospective study of 70 patients treated with TCH plus pertuzumab and who received primary G-CSF prophylaxis, febrile neutropenia was observed in 3% of patients [12]. The results of the BCIRG-006 trial, the first study comparing trastuzumab-based chemotherapy with and without the anthracycline/cyclophosphamide combination, are in line with our findings with significantly more grade ≥3 neutropenia in the anthracycline-containing arm with four cycles of doxorubicin/cyclophosphamide followed by four cycles of docetaxel/trastuzumab ([AC-TH], 72%) compared to the anthracycline-free arm with six cycles of docetaxel/carboplatin/trastuzumab ([TCH], 66%) [3]. In addition, a more recent neoadjuvant trial of 100 patients randomized to either paclitaxel/carboplatin/trastuzumab or 5-

Table 5
Non-hematological toxicities grade ≥3 occurring in at least two patients in the same treatment arm.

	FEC-T-Ptz (n = 55)		PTC-Ptz (n = 55)		p^*
	Grade 3	Grade 4	Grade 3	Grade 4	
	n (%)	n (%)	n (%)	n (%)	
Overall					
ALAT/ASAT elevated	3 (5)	0 (0)	5 (9)	0 (0)	*0.72*
Allergic reaction	0 (0)	0 (0)	2 (4)	0 (0)	*0.50*
Diarrhea	3 (5)	0 (0)	10 (18)	0 (0)	*0.07*
Ejection fraction decreased[a]	0 (0)	0 (0)	1 (2)	0 (0)	*1.00*
Electrolyte disturbances[b]	1 (2)	0 (0)	3 (5)	1 (2)	*0.36*
Fatigue	2 (4)	0 (0)	4 (7)	0 (0)	*0.68*
Infection (any site)[c]	4 (7)	0 (0)	2 (4)	0 (0)	*0.68*
Peripheral neuropathy	2 (4)	0 (0)	3 (5)	0 (0)	*1.00*
Syncope	0 (0)	0 (0)	4 (7)	0 (0)	*0.12*
During cycle 1–3					
ALAT/ASAT elevated	1 (2)	0 (0)	3 (5)	0 (0)	*0.62*
Allergic reaction	0 (0)	0 (0)	2 (4)	0 (0)	*0.50*
Diarrhea	3 (5)	0 (0)	6 (11)	0 (0)	*0.49*
Electrolyte disturbances[b]	1 (2)	0 (0)	0 (0)	0 (0)	*1.00*
Fatigue	1 (2)	0 (0)	2 (4)	0 (0)	*1.00*
Infection (any site)[c]	2 (4)	0 (0)	0 (0)	0 (0)	*0.50*
Peripheral neuropathy	0 (0)	0 (0)	0 (0)	0 (0)	*1.00*
Syncope	0 (0)	0 (0)	0 (0)	0 (0)	*1.00*
During cycle 4–9[d]					
ALAT/ASAT elevated	2 (4)	0 (0)	2 (4)	0 (0)	*1.00*
Allergic reaction	0 (0)	0 (0)	0 (0)	0 (0)	*1.00*
Diarrhea	1 (2)	0 (0)	5 (9)	0 (0)	*0.11*
Electrolyte disturbances[b]	0 (0)	0 (0)	3 (6)	1 (2)	*0.06*
Fatigue	1 (2)	0 (0)	4 (7)	0 (0)	*0.21*
Infection (any site)[c]	2 (4)	0 (0)	1 (2)	0 (0)	*1.00*
Peripheral neuropathy	2 (4)	0 (0)	3 (6)	0 (0)	*0.68*
Syncope	0 (0)	0 (0)	4 (7)	0 (0)	*0.06*

*p-value: difference in incidence of grade ≥3 toxicity between both arms.
Statistical significance is defined as p-value <0.05 are in italics.
[a] Ejection fraction was measured every three months during therapy, thus first time was during the 4–9th cycle unless indicated earlier.
[b] Electrolyte disturbances consisting of: 1 hypocalcemia, 1 hypokalemia, 1 hypomagnesemia, 1 hyponatremia, 1 hypophosphatemia.
[c] Infection sites: 1 lower respiratory tract, 1 upper respiratory tract, 1 tooth, 1 urinary tract, 1 infection with unspecified site.
[d] In the PTC-arm only 54 patients started the 4th cycle.

fluorouacil,/epribucin/trastuzumab found neutropenia grade ≥3 neutropenia in 56% and 70% respectively, and febrile neutropenia in 8% and 14% respectively [13]. However, both these studies were performed before the pertuzumab era. G-CSF was not administered as primary prophylaxis in our study, but was prescribed according to protocol at first occurrence of grade ≥3 neutropenia or febrile neutropenia for all subsequent cycles, but without implementation of a dose reduction at first instance. After initiation of G-CSF only four more instances of neutropenia occurred. Primary G-CSF prophylaxis does not seem warranted based on the observed febrile neutropenia rate below 10%, and secondary prophylaxis should be implemented according to protocol or supportive treatment guidelines [14]. Thrombocytopenia was seen at a similar rate in both arms (18% versus 13%), but occurred exclusively during the PTC-cycles of the treatment, probably related to the known toxicity profile of carboplatin [3,15]. Grade ≥3 thrombocytopenia occurred in 6% of patients treated with TCH in the BCIRG-006 trial, and in 12% of patients treated with TCH plus pertuzumab in the Tryphaena trial [3,9].

Asymptomatic grade 2 LVEF decrease, defined as LVEF 40%–50% or an absolute decline of 10–19% compared to baseline, was observed in 24% of patients in the FEC-T-arm and in 11% of patients in the PTC-arm. In the Tryphaena trial, 5% of patients receiving anthracyclines and 4% of patients on an anthracycline-free regimen experienced an LVEF-decline of ≥10% from baseline to <50%. LVEF had recovered to ≥50% at data cut-off in all these patients. In our study, one patient (2%) in the FEC-T-arm and two patients (4%) in the PTC-arm experienced an LVEF-decline of ≥10% from baseline to <50%. In the BCIRG-006 trial a sustained LVEF-decrease of >10% at last follow-up evaluation (48 months since randomization) was seen in 19% in the AC-TH-group and in 9% in the TCH-group [3]. This LVEF decline continued to exist with ten year follow-up [16]. Longer follow-up is required to evaluate sustained cardiac toxicity in the TRAIN-2 study. Symptomatic cardiotoxicity occurred in 2% in the AC-TH-group and in 0.4% in the TCH-group of the BCIRG-006 trial, and in 3% in one of the anthracycline-containing arms of the Tryphaena study [3,9]. A recently published large retrospective study confirmed the highest cumulative incidence of symptomatic cardiotoxicity in patients treated with anthracyclines plus trastuzumab, but reported a considerable higher rate (6.6%; event rate 22.1 per 1000 patient-years) than found in clinical trials. The incidence was also substantial in patients treated with trastuzumab-based chemotherapy without anthracyclines (5.1%; event rate 17.0 per 1000 patient-years) [17]. This discrepancy in results might be related to a different baseline cardiovascular risk in patients in clinical trials compared to patients in daily clinical practice. One patient in our study, receiving PTC-pertuzumab, developed clinically manifest heart failure subsequent to a myocardial infarction. This patient exhibited several pre-existing cardiovascular risk factors, but was considered eligible for study participation. Cardiac ischemia is not associated with trastuzumab, but is occasionally described in relation to paclitaxel and platinum drugs [18]. Lastly, the addition of pertuzumab to trastuzumab does not increase cardiotoxicity [19].

The most common non-hematological toxicity was diarrhea, which is a known side-effect of pertuzumab [2,20–23]. Grade 3 diarrhea was more frequently seen in the PTC-arm (18%) than in the FEC-T-arm (5%), and grade 4 diarrhea was not observed. The higher incidence of diarrhea in the PTC-arm could be related to cumulative gastro-intestinal toxicity of pertuzumab, a taxane and carboplatin [9,15,24]. Similar results were found in the Tryphaena trial, which reported grade ≥3 diarrhea in 4–5% of patients in the FEC-T-arms and 12% in the TCH-arm [9]. In the Neosphere study, grade ≥3 diarrhea was only seen in patients receiving docetaxel, but the incidence was substantially lower (1–2%) than seen in our study, possibly due to the addition of carboplatin in the PTC-regimen as mentioned before [2]. In concordance with other studies, diarrhea occurred early during treatment and one could speculate that it may be related to the loading dose of pertuzumab at the first cycle [21,23,25]. However, the dose-finding studies of pertuzumab monotherapy do not support this hypothesis, as no increase in diarrhea incidence was seen with escalating doses [26,27]. No prophylactic anti-diarrheal therapy was used in our study, but loperamide treatment was advised if diarrhea occurred. Anti-diarrheal therapy should be initiated early during therapy in combination with adequate fluid intake according to supportive therapy guidelines to prevent dehydration and possible subsequent renal toxicity [28].

Neuropathy is an important and potentially irreversible, dose-dependent side-effect of paclitaxel and carboplatin [29,30]. Grade ≥2 neuropathy was reported in 29% and 33% of patients in the FEC-T-arm and PTC-arm respectively. Remarkably, neuropathy was not described in the Tryphaena and the primary publication of the Neosphere trial [2,9]. In the recent update of the Neosphere trial, sensory neuropathy grade ≥2 was reported in 10–15% of patients across the four arms [31]. In the BCIRG-006 study sensory neuropathy, any grade, was reported in 36% in the TCH-arm receiving six courses and in 49% in the anthracycline-containing arms with four cycles docetaxel [3]. Nevertheless, in the absence of prevention and treatment modalities it is important to recognize polyneuropathy early and adapt treatment accordingly [29,32].

Besides neutropenia and diarrhea, rash has also been described with the addition of pertuzumab [21,23,33]. The incidence of rash grade ≥2 was seven percent in the FEC-T-arm and 13% in the PTC-arm, with only one grade 3 and no grade 4 rash reported. This is similar to rates observed in other pertuzumab trials, with rash (any grade) seen in 11–37% of patients [2,33].

In conclusion, both regimens in the TRAIN-2 study are associated with expected and manageable bone marrow toxicity and are well tolerable with dose adjustments and G-CSF support. A regimen consisting of nine cycles PTC plus pertuzumab has a more favorable hematological toxicity profile than a regimen with three cycles of FEC-T plus pertuzumab followed by six cycles of PTC plus pertuzumab, but is associated with more diarrhea. Clinical manifest cardiotoxicity was very rare with both regimens. Pending the efficacy results of the TRAIN-2 study, these toxicity data may help patients and physicians when choosing between one of these regimens.

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Conflict of interest statement

None of the authors report conflicting interests. Relevant financial activities outside the submitted work and other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing: SL is an advisory board member for Cergentis, Novartis, Roche, and Philips health BV. SL received institutional research support funding from AstraZeneca, Genentech, and Roche.

Ethical approval

The medical ethics committee of the Netherlands Cancer Institute approved the study protocol and any modifications thereof. All patients gave written informed consent.

Authors' contributions

Study concepts: GS, SL.
Study design: GS, MVR.
Data acquisition: AH, CS, GS, IK, IM, IO, JS, SL, VD.
Quality control of data and algorithms: EvW, GS, IM, MvR.
Data analysis and interpretation: EvW, GS, MvR.
Statistical analyses: EvW, GS, MvR.
Manuscript preparation: GS, MvR.
Manuscript editing: AH, EvW, CS, GS, IK, IM, IO, JS, MvR, SL, VD.
Manuscript review: AH, EvW, CS, GS, IK, IM, IO, JS, MvR, SL, VD.

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List of abbreviations

AC-TH	Doxorubicin/Cyclophosphamide – Docetaxel/Trastuzumab
AML	Acute Myeloid Leukemia
CTCAE	Common Toxicity Criteria for Adverse Events
ER	Estrogen Receptor
FEC-T	5-Fluorouracil/Epirubicin/Cyclophosphamide/Trastuzumab
G-CSF	Granulocyte Colony Stimulating Factor
HER2	Human Epidermal Growth factor Receptor 2
LVEF	Left Ventricular Ejection Fraction
MRI	Magnetic Resonance Imaging
PCR	Pathologic Complete Response
PET	Positron Emission Tomography
PTC	Paclitaxel/Carboplatin/Trastuzumab
PR	Progesterone receptor
SAE	Serious Adverse Event
TCH	Docetaxel/Carboplatin/Trastuzumab
WHO	World Health Organization

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.breast.2016.07.017.

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