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# **Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neo-adjuvant chemotherapy trials**

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## **Abstract**

### **Purpose**

Pathological complete response (pCR) to neoadjuvant treatment correlates with outcome in breast cancer. We determined whether characteristics of neoadjuvant therapy are associated with pCR.

### **Methods**

We used multi-level models, which accounted for heterogeneity in pCR across trials and trial arms, to analyze individual patient data from 3332 women included in 7 German neoadjuvant trials with uniform protocols.

### **Results**

PCR was associated with an increase in number of chemotherapy cycles (odds ratio [OR] 1.2 for every two additional cycles;  $p=0.009$ ), with higher cumulative anthracycline doses (OR 1.6;  $p=0.002$ ), higher cumulative taxane doses (OR 1.6;  $p=0.009$ ), and with capecitabine containing regimens (OR 1.62;  $p=0.022$ ). Association of pCR with increase in number of cycles appeared more pronounced in hormone receptor (HR)-positive tumors (OR 1.35) than in HR-negative tumors (OR 1.04;  $p$  for interaction=0.046). Effect of anthracycline dose was particularly pronounced in HER2-negative tumors (OR 1.61), compared to HER2-positive tumors (OR 0.83;  $p$  for interaction=0.14). Simultaneous trastuzumab treatment in HER2-positive tumors increased odds of pCR 3.2fold ( $p<0.001$ ). No association of pCR and number of trastuzumab cycles was found (OR 1.20,  $p=0.39$ ).

### **Conclusion**

Dosing characteristics appear important for successful treatment of breast cancer. Longer treatment, higher cumulative doses of anthracyclines and taxanes, and the addition of capecitabine and trastuzumab are associated with better response. Tailoring according to breast cancer phenotype might be possible: longer treatment in HR-positive tumors, higher cumulative anthracycline doses for HER2-negative tumors, shorter treatment at higher cumulative doses for triple-negative tumors, and limited number of preoperative trastuzumab cycles in HER2-positive tumors.

## **Introduction**

Several anthracycline and taxane-based chemotherapy regimens are established for treatment of early breast cancer [1,2,3]. Specific dosing characteristics of regimens, including the cumulative dose of agents and the number of cycles, is largely dependent on local guidelines or preference of individual physicians. A recent guideline on the diagnosis and treatment of early and locally advanced breast cancer published by the UK National Collaborating Centre for Cancer on behalf of NICE [4], for example, did not provide any recommendation on the dosages and numbers of cycles, and may contribute to the already existing, considerable variation in treatment standards across regions and countries. Similarly, collaborative analyses of randomized trials [1,2,3] were forced to ignore the issue of dosing and mainly reported average effects across many different regimens with considerable heterogeneity in dosing characteristics.

Neoadjuvant regimens are considered equivalent to adjuvant regimens with identical dosing schemes [5,6,7]. However, in contrast to adjuvant therapy the neoadjuvant approach allows to immediately ascertain the effects of chemotherapy based on the histopathological assessment of surgically removed tissue. Pathologically confirmed complete response (pCR) after chemotherapy highly correlates with favorable long-term outcome and was therefore suggested as a surrogate marker for survival [5,8]. Between 1998 and 2006, the German Breast Group (GBG) and the Arbeitsgemeinschaft Gynäkologische Onkologie (AGO) “Breast”, conducted a series of neoadjuvant trials. To address the current uncertainty surrounding dosing characteristics, we performed a pooled analysis of these trials and determined whether variations in cumulative dose of taxanes, anthracyclines and trastuzumab, number of administered cycles and administration of additional drugs, including tamoxifen and capecitabine, are associated with treatment response overall and across different breast cancer phenotypes.

## Methods

### *Patients*

Between 1998 and 2006, a total of eight major prospective neoadjuvant trials in 6625 breast cancer patients were conducted in Germany [9,10,11,12,13,14,15,16,17,18]. Details of design and conduct of individual trial were previously reported (Table 1). Seven trials were phase III randomized parallel-group trials (6408 patients) [9-17] and one was a phase II case series (217 patients) [18]. In two trials (2357 patients), allocation to intervention groups depended on an intermediate clinical assessment of tumor response after two cycles of TAC (docetaxel [Taxotere®], doxorubicin [adriamycin], cyclophosphamide): in the GeparTrio pilot trial [11] the 74 non-responders were randomly allocated to 4 further cycles of TAC (41 patients) or to 4 cycles of NX (vinorelbine [Navelbine®] and capecitabine [Xeloda®], 33 patients). The 211 responders all received a further four cycles of TAC. In the GeparTrio main trial [12,13], the 622 non-responders were randomized to 4 further cycles of TAC (321 patients) or 4 cycles of NX (301 patients), whereas the 1390 responders were allocated to 4 or 6 further cycles of TAC (704 and 686 patients, respectively). To avoid confounding by treatment selection according to intermediate clinical assessment of tumor response, we excluded the 334 patients allocated to NX, a proportional random sample of 95 responders, who received a total of 6 cycles of TAC in the GeparTrio pilot trial [11], and the 686 responders, who received a total of 8 cycles of TAC in the GeparTrio main trial [12,13]. 134 patients included in the run-in period of GeparTrio [12,13] and GeparQuattro [14,15] who dropped out before randomization because of progression, malcompliance, or side-effects, were analyzed in actual treatment arms as determined by computer-generated random allocation. Characterization of tumor subtypes by human epidermal growth factor receptor 2 (HER2) was not protocol mandated in the early trials. We therefore excluded one trial, [16], in which none of the 668 randomized patients had undergone HER2 testing. Of the remaining 4813 patients we excluded another 1114 patients with unknown HER2 status and 367 patients with other unknown baseline parameters. Our analysis is therefore based on patient-level data from the remaining 3332 patients from 7 trials with 12 treatment arms. Baseline characteristics of the 3332 analyzed patients and the 3293 excluded patients were similar (Supplemental Table).

All trials used uniform definitions for patient selection: diagnosis of invasive breast cancer had to be histologically confirmed by core biopsy. Patients needed to have measurable tumor dimensions either by palpation, sonography or mammography. Locally advanced (cT4a-d) and inflammatory breast cancers were eligible for all trials except GeparDo [9] and GeparDuo [10]. Patients were eligible irrespective of hormone receptor and HER2 receptor status, except in Techno [18], in which only HER2-positive patients were allowed. All trials were approved by the respective ethics committees.

Tumor size was determined before start of systemic treatment by palpation or sonography. Histological type, tumor grade, and estrogen-, progesterone, and HER2 receptor status were determined locally on pre-treatment core-biopsies.

To calculate cumulative anthracycline doses we converted epirubicin doses into doxorubicin equivalence doses based on a pre-specified ratio of 3:2, assuming, e.g., 90mg/m<sup>2</sup> epirubicin to be equivalent to 60mg/m<sup>2</sup> doxorubicin. For taxanes, we converted paclitaxel doses to docetaxel equivalence doses based on a pre-specified ratio of 7:4, 175mg/m<sup>2</sup> paclitaxel, e.g., were considered equivalent to 100 mg/m<sup>2</sup> docetaxel. We calculated cumulative equivalence doses administered across all cycles as pre-specified in individual trial protocols and considered  $\geq 300\text{mg/m}^2$  cumulative doxorubicin equivalents as a higher cumulative anthracycline dose, and  $\geq 400\text{mg/m}^2$  cumulative docetaxel equivalents as a higher cumulative taxane dose. A standardized definition of pCR was used for all trials: undetectable invasive residual tumor in the excised tissue of breast and axillary lymph nodes, corresponding to ypT0/is ypN0 [19].

#### *Statistical methods*

We used multi-level logistic regression models with random effects at the level of trials and trial arms. All analyses were performed according to the intention-to-treat as specified in the protocol. In univariable analyses, we compared baseline characteristics of patients and tumors and treatment parameters between patients with and without pCR. The primary analysis in all 3332 patients was a multivariable analysis including all baseline characteristics and treatment parameters to determine the association of pCR with number of cycles of chemotherapy (per 2 cycles), cumulative dose of taxanes and anthracyclines (high versus low dose), administration of capecitabine, tamoxifen and trastuzumab (yes versus no). The effect of trastuzumab was estimated for HER2-positive patients only. Secondary analyses were stratified according to HR status, HER2 status, and biological subtype (HER2-negative/HR-positive, HER2-positive/HR-positive, HER2-positive/HR-negative, HER2-negative/HR-negative), with P-values for interaction between effect of the treatment variable and stratum derived from appropriate interaction terms in the multi-level model. Finally, we determined the association of the cumulative number of trastuzumab cycles (4 versus 8 to 12), overall and stratified according to age (<40 versus  $\geq 40$  years), tumor stage (stages 1 to 2 versus 3 to 4), grading (grade 1 to 2 versus 3), and HR status. All p-values are two-sided. Statistical analyses were performed using Stata Version 10.1 (Stata Corporation, College Station, Texas).

## Results

In included trials, number of cycles ranged from 4 to 12, cumulative anthracycline dose from 200 to 400mg/m<sup>2</sup>, and cumulative taxane dose from 300 to 450mg/m<sup>2</sup>. Capecitabine was administered in two treatment arms (966 patients), tamoxifen in 3 arms (215 patients), and trastuzumab in 4 arms (614 HER2-positive patients, see Table 1). Table 2 shows the association of baseline characteristics of patients and tumors and treatment parameters with pCR in univariable analyses. Higher pCR rates were observed in younger patients, lower stages, ductal-invasive carcinomas, less differentiated tumors, HR-negative and HER2-positive tumors (p for trend or interaction all  $\leq 0.001$ ). There was an association between pCR and number of cycles (p=0.005), but little evidence for differences in treatment response according to cumulative anthracycline or taxane dose, use of capecitabine or tamoxifen in the univariable analysis. The test for trend was formally positive for the association between number of trastuzumab cycles and pCR, but this was driven by the difference in pCR between patients with and without trastuzumab (p for difference < 0.001), but not by an association with number of trastuzumab cycles: the odds ratios comparing 4 cycles with no trastuzumab (2.84, 95%-CI 1.93-4.18), and 8 to 12 cycles with no trastuzumab (3.14, 95%-CI 2.28-4.32) were similar and confidence intervals overlapped widely.

Figure 1 presents results from multivariable analysis of treatment characteristics after adjustment for all potential confounders. pCR became more likely with an increase in the number of cycles: the odds of a pCR increased approximately 1.2fold with every two additional treatment cycles (p=0.009). The odds of pCR were approximately 1.6fold higher in regimens with higher cumulative anthracycline doses (p=0.002), regimens with higher cumulative taxane doses (p=0.009), and capecitabine containing regimens (p=0.022). There was little evidence that concurrent tamoxifen use impacted on pCR (p=0.69). Simultaneous trastuzumab treatment in patients with HER2 positive tumors, however, increased the odds of pCR 3.2fold (p<0.001).

Figure 2 presents stratified analyses of the association of pCR with treatment characteristics by receptor status. Stratification by HR status (Panel A) indicated that the association of pCR with number of cycles was particularly pronounced in patients with HR-positive tumors, as compared with HR-negative tumors, with a positive test for interaction between treatment effect and HR status (p=0.039). The effect of trastuzumab appeared more pronounced in patients with HR-negative tumors, but confidence intervals were wide and a test for interaction negative (p for interaction=0.20). There was little evidence that the association of pCR with remaining treatment characteristics varied according to HR status (p for interaction  $\geq 0.44$ ). Figure 2 (Panel B) presents results from analyses stratified by HER2 status. The effects of increasing numbers of cycles, cumulative taxane dose and use of capecitabine appeared not to vary according to HER2 status (p for interaction  $\geq 0.45$ ). The estimated odds ratios of pCR for tamoxifen were below one in patients with HER2-positive tumors and above

one in patients with HER2-negative tumors, but confidence intervals were wide, overlapped the line of no difference at 1.0 and a test for interaction was negative ( $p$  for interaction=0.28). The association of pCR with cumulative anthracycline dose was pronounced in patients with HER2-negative tumors ( $p=0.004$ ), whereas no association was apparent in patients with HER2-positive tumors ( $p=0.66$ ), confidence intervals of estimates were wide and overlapped again between strata and the test for interaction did not reach significance ( $p$  for interaction=0.15).

Figure 3 shows the association of pCR with treatment characteristics in biological breast cancer subtypes. In general, the patterns observed for the 4 subtypes were in concordance with analyses stratified according to HR and HER2 status even though tests for interaction were negative. The effect of number of cycles was pronounced in HR-positive tumors, irrespective of HER2 status. The effect of the cumulative anthracycline dose was pronounced HER2-negative tumors irrespective of HR status. No clear pattern evolved for cumulative taxane dose, administration of capecitabine, tamoxifen and trastuzumab, with little evidence to suggest variation across subgroups.

Figure 4 presents analyses of the association of pCR with number of trastuzumab cycles (4 versus 8 to 12 cycles). Overall, we found no evidence for an association of pCR with number of cycles (OR 1.20, 95%-CI 0.79 to 1.81,  $P=0.39$ ). In stratified analyses, there was no evidence that the effects of the number of cycles varied according to age, stage and grade ( $p$  for interaction  $\geq 0.24$ ). However, the effect of number of cycles appeared to be more pronounced in HR-negative compared to HR-positive tumors ( $p$  for interaction=0.023).



## Discussion

In this pooled analysis of German neoadjuvant breast cancer trials, we found that higher numbers of cycles and higher cumulative doses of both, anthracyclines and taxanes, and use of capecitabine and trastuzumab were associated with a clinically relevant increase in pCR. When stratifying according to hormone receptor and HER2 status, we found number of cycles to be particularly relevant in patients with HR-positive tumors, and cumulative anthracycline dose in patients with HER2-negative tumors. Further stratification by biological breast cancer subtype revealed imprecise results, but mainly confirmed effects found in overall analyses and those stratified according to HR and HER2 status. There was no evidence that the benefit of higher cumulative taxane doses and capecitabine varied according to biological subtypes. In the triple-negative subgroup, higher cumulative doses of anthracycline and taxane and administration of capecitabine, but not more treatment cycles appeared beneficial, but it remained unclear whether this subgroup also benefits from an increase in the number of cycles. Finally, in an analysis restricted to patients with HER2-positive tumors receiving trastuzumab, we found little evidence to suggest that an increasing number of trastuzumab cycles (from 4 to 8-12) is associated with an advantage regarding pCR in HER2-positive patients.

Our statistical model allowed a coherent analysis of the association of pCR with treatment characteristics, implicitly assigned more weight to randomized within-trial comparisons than to comparisons of treatment characteristics between trials, and fully accounted for the heterogeneity in pCR between trials. However, our analysis is observational in nature, and we cannot exclude confounding as a potential explanation of observed results. Confounding by indication may be important. To minimize this source of systematic error, we implemented two measures which led to the exclusion of almost 50% of trial participants. First, our ascertainment of treatment characteristics was based on the pre-specified, protocol-defined “intention to treat”, not on the treatment actually received. Second, we omitted treatment arms, in which confounding by indication was induced by treatment selection depending on an intermediate clinical assessment of tumor response at the end of a run-in period after administration of an initial series of chemotherapy cycles. Confounding by indication introduced by an association between treatment selection and prognosis is therefore unlikely. HER2 status is of a major prognostic importance in breast cancer. In some of the earlier trials, HER2 status was not determined. To avoid confounding by this unobserved tumor characteristic, we restricted the analysis to trials with available HER2 status [16]. Since availability of HER2 status is a mere function of time and not associated with prognostically important patient or tumor characteristics (Table 2), this restriction is unlikely to have introduced bias. Other unmeasured confounders might be tumor characteristics associated with time, however median tumor size and nodal status in the early Gepardo trial [9] was similar to the most recent GeparQuattro [14] trial.

It cannot be fully excluded that the observed higher pCR rate with the longer therapies in the HR-positive subset, which are in general more slowly proliferating, may be in part due to a longer time interval from chemotherapy initiation to breast resection with the longer treatments.

Dividing regimens into lower and higher according to their cumulative dose may be considered arbitrary. However, according to the results achieved, regimens with a lower cumulative dose have to be considered as underdosed, whereas those with a higher cumulative dose are to be considered as adequately dosed. It has to be recognized, that many regimen recommended today as standard options do not qualify for such an adequate cumulative dose of anthracyclines, taxanes, or both. Sufficiently powered non-inferiority trials are warranted before such lower doses should be used in routine care of patients.

Equivalence doses for paclitaxel and docetaxel were chosen according to labels. However, a post-hoc sensitivity analysis assuming 240mg/m<sup>2</sup> paclitaxel to be equivalent to 100mg/m<sup>2</sup> docetaxel yielded nearly identical overall results (data available on request). The final, but most important limitation of this analysis is the use of pCR (the primary endpoint of all 7 trials) as a surrogate outcome. Even though pCR was found to be associated with prognosis [5,6,7], the associations with pCR observed in this analysis may not necessarily translate into associations of treatment characteristics with recurrence-free or overall survival.

Previous analyses of neoadjuvant trials compared neoadjuvant to adjuvant chemotherapy [6,7] or taxane-containing versus non-taxane containing neoadjuvant treatments [20] and were based on aggregate level data only. Two recent pooled analyses explored the interaction between treatment and HER2 status. Gennari et al [21] analyzed 8 randomized adjuvant trials comparing anthracycline-based with non-anthracycline-based regimens in early breast cancer, which reported clinical effectiveness according to HER2 status. Anthracyclines appeared beneficial in patients with HER2-positive, but not in patients with HER2-negative tumors, with positive tests for interaction for both, disease-free and overall survival. Dhesy-Thind et al [22] included trials of tamoxifen, anthracyclines, and taxanes, and found no evidence for a treatment by HER2 status interaction for tamoxifen, but positive interactions for anthracyclines and taxanes regarding disease-free survival, with both types of drugs being more effective in HER2-positive tumors. In an additional analysis of 3 trials comparing higher with lower cumulative anthracycline doses, the authors found some evidence that higher anthracycline doses were more beneficial regarding disease-free survival only for HER2-positive tumors, but a test for interaction did not reach statistical significance. These analyses of anthracycline effects [20,21] were performed in trials without concomitant taxane administration and cannot necessarily be generalized to current anthracycline-taxane combinations, whereas the analysis of taxane effects<sup>21</sup> did not address the impact of different cumulative doses.

The observed effect of capecitabine is in opposite to the results of the individual study (GeparQuattro) [14], which showed no differences regarding pCR rates for the arms containing capecitabine and the control arm. However, the cumulative taxane dose was 300mg/m<sup>2</sup> in the capecitabine arms and 400mg/m<sup>2</sup> in the control arm, so capecitabine may have only compensated for the loss of taxane effectiveness. Other trials, which were not confounded by a lower taxane dose, showed a benefit for the addition of capecitabine [23,24]. The simultaneous use of trastuzumab during neoadjuvant chemotherapy has consistently shown higher pCR rates in HER2-positive breast cancer [25,26]. In how far our observation regarding the lack of additional benefit for more than 4 trastuzumab cycles, with respect to tumor response, translates into similar results for clinical long term outcome will be answered by several adjuvant trials comparing different durations of trastuzumab in the coming years [27,28,29].

In conclusion, dosing characteristics appear important for the successful treatment of breast cancer. Higher (or adequate) cumulative doses of anthracyclines and taxanes and longer treatment are associated with better response. Our results lend support to the concept of tailoring chemotherapy regimens according to biological tumor type. In contrast to current practice, we submit that tumors with assumed partial resistance to chemotherapy (e.g. HR-positive tumors) require more cycles of adequately dosed chemotherapy to achieve an optimal effect. Less aggressive treatment may be sufficient in highly sensitive tumors: HER2-positive cancers might achieve optimal response already at a low cumulative dose of anthracyclines and at a low number of trastuzumab cycles, and HR-negative tumors may not need long treatment durations.

## Figure Legends

### Figure 1:

Multivariable analysis of the association of pathological complete response (pCR) with treatment characteristics, after adjustment for age, tumor stage, grade, type, HR and HER2 status in 3332 patients. §Effect as estimated for HER2-positive patients.

### Figure 2:

Multivariable analysis of the association of pathological complete response (pCR) with treatment characteristics stratified by hormone receptor (HR) status (Panel A referring to N=1182 patients with HR-positive and N=2150 patients with HR-negative tumors) and HER2 status (Panel B referring to N=1015 patients with HER2-positive and N=2317 patients with HER2-negative tumors), after adjustment for age, tumor stage, grade, type, HR and HER2 status. §Effect as estimated for HER2-positive patients.

### Figure 3:

Multivariable analysis of the association of pathological complete response (pCR) with treatment characteristics stratified by subgroups according to hormone receptor (HR) status and HER2 status, after adjustment for age, tumor stage, grade, type, HR and HER2 status as applicable.

### Figure 4:

Multivariable analysis of the association of pathological complete response (pCR) with tumor characteristics in N=2317 with HER2-negative tumors not treated with trastuzumab and N=1015 patients with HER2-positive tumors treated with 4 or 8 to 12 cycles trastuzumab, after adjustment for age, tumor stage, grade, type, and HR status. \*Analysis not adjusted for tamoxifen use because of collinearity problems.

Figure 1

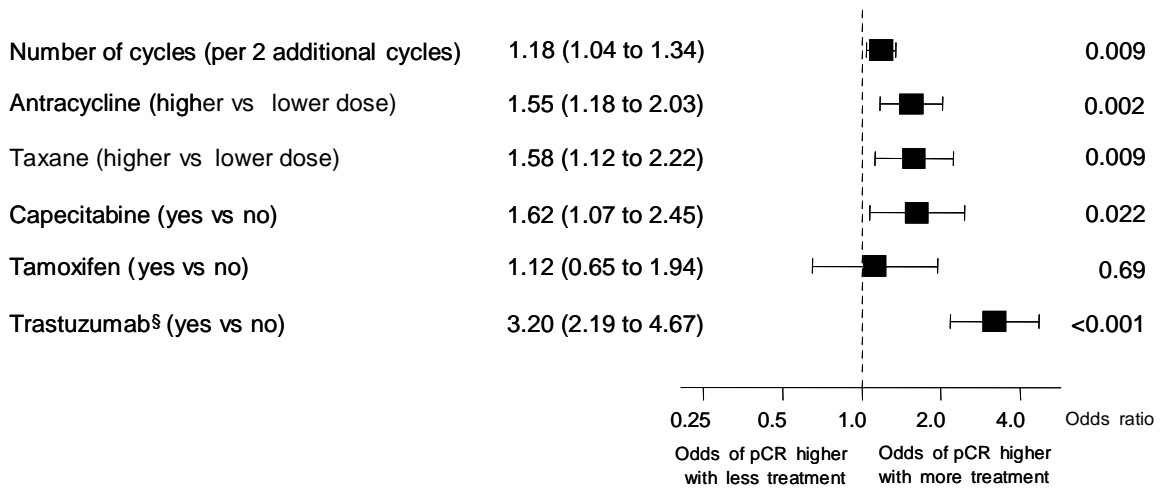
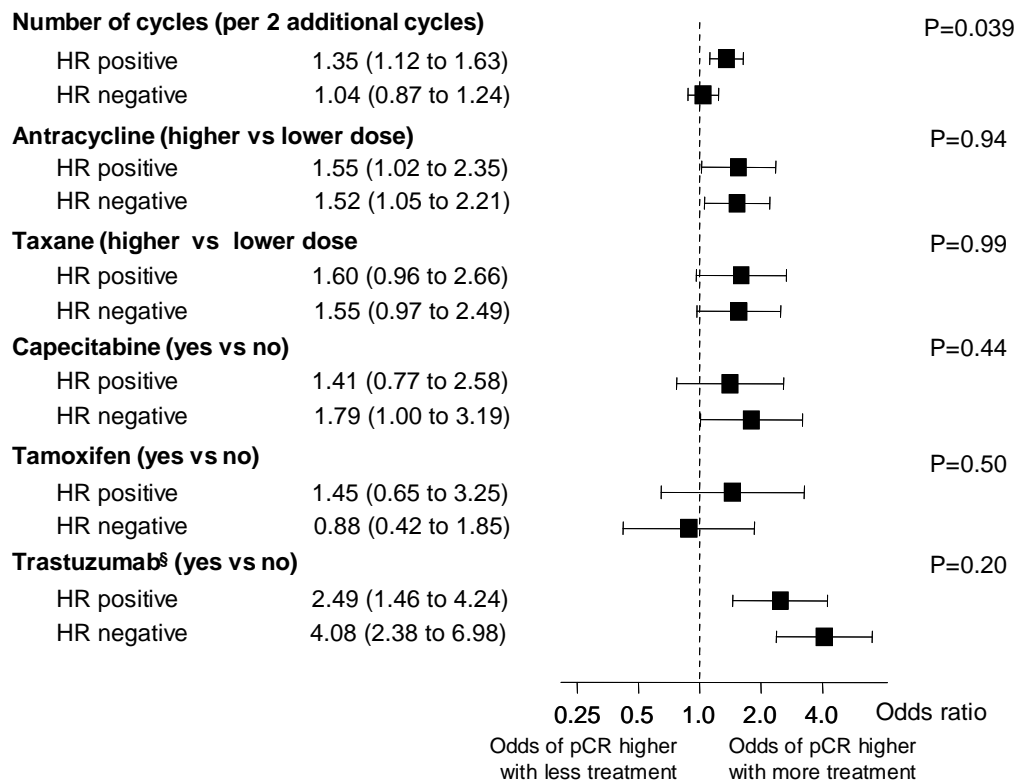


Figure 2

Panel A



Panel B

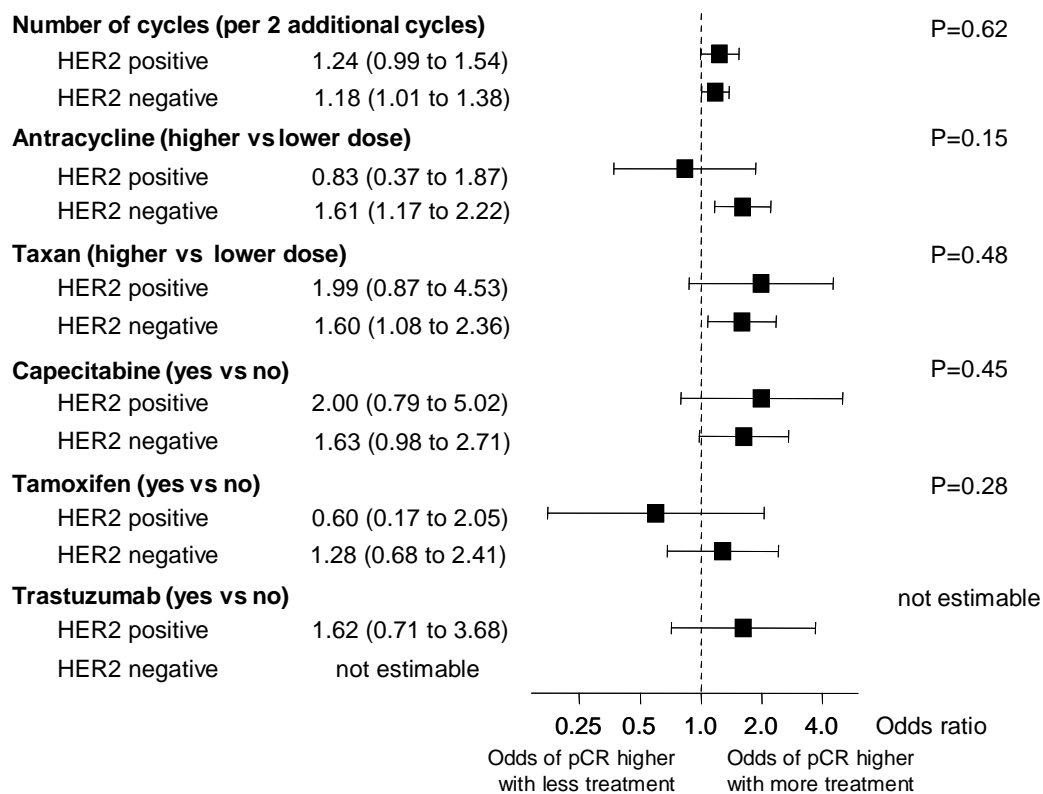
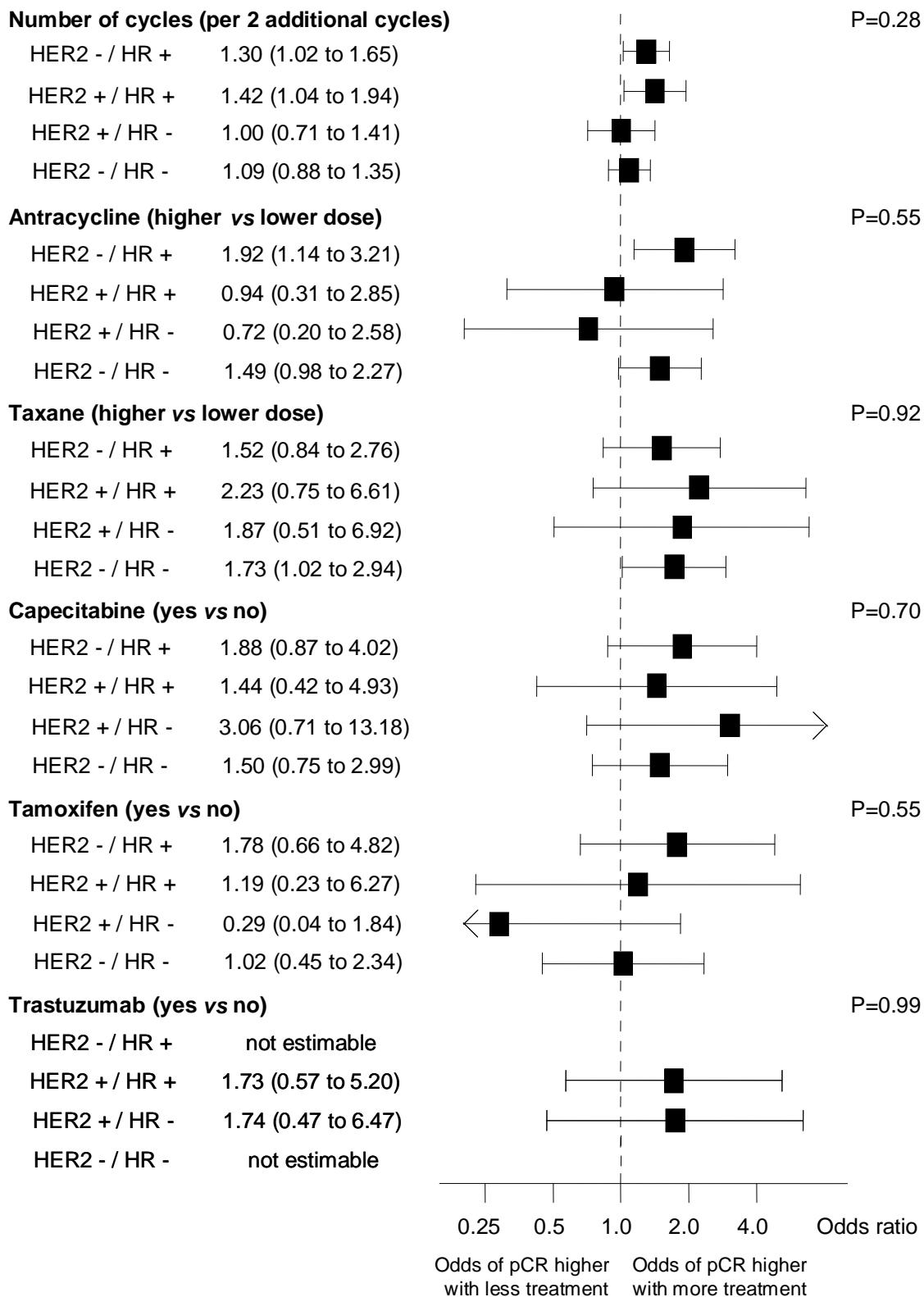
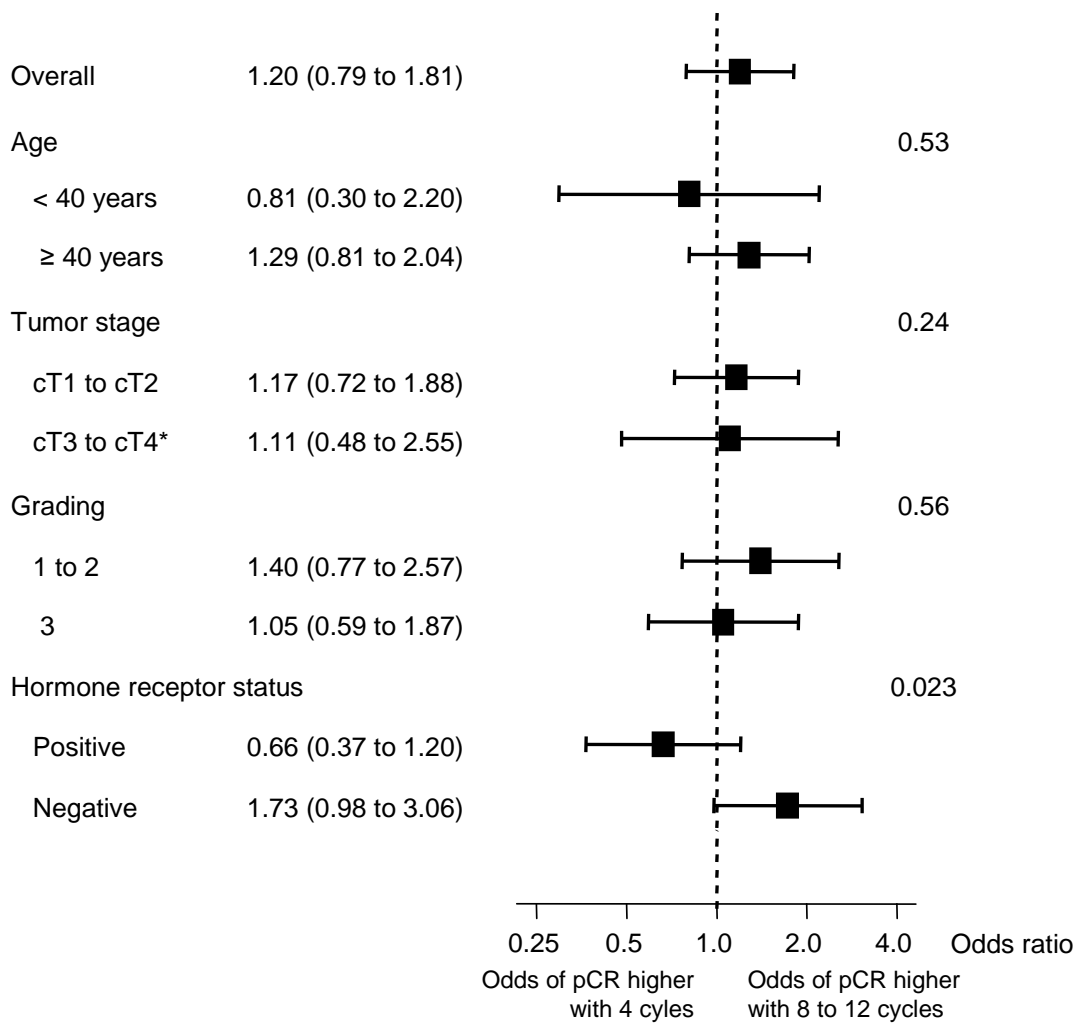


Figure 3



**Figure 4**



*Table 1: Overview of the German neo-adjuvant breast cancer studies including reasons for exclusion of patients from analysis.*



Study Time span	Design	Intervention (all patients)	Intervention group	Comparison	Reason for excluding patients from analysis
<b>GeparDo<sup>9</sup></b> <b>NCT00543829</b>	Randomized phase IIb	doxorubicin (50 mg/m <sup>2</sup> , day 1) plus docetaxel (75 mg/m <sup>2</sup> , day 1) every 2 weeks (ddAD) for 4 cycles with G- CSF	Tamoxifen	no tamoxifen	N=52 unknown HER2 status
1998-1999	Recruited 248, included in analysis: 196				
<b>GeparDuo<sup>10</sup></b> <b>NCT00793377</b>	Randomized phase III	tamoxifen 20 mg daily	doxorubicin (50 mg/m <sup>2</sup> , day 1) plus docetaxel (75 mg/m <sup>2</sup> , day 1) every 2 weeks (ddAD) for 4 cycles with G- CSF	doxorubicin (60 mg/m <sup>2</sup> , day 1) plus cyclophosphamide (600 mg/m <sup>2</sup> , day 1) given every 3 weeks for 4 cycles followed by docetaxel (100 mg/m <sup>2</sup> , i.v., day 1) given every 3 weeks for 4 cycles (AC-D)	N=787 unknown HER2 status N=4 unknown other baseline characteristics
1999-2001	Recruited 907, included in analysis: 116				
<b>GeparTrio Pilot<sup>11</sup></b> <b>NCT00544765</b>	Randomized phase IIb	docetaxel (75 mg/m <sup>2</sup> , day 1) plus doxorubicin (50 mg/m <sup>2</sup> , day 1) plus cyclophosphamide (500 mg/m <sup>2</sup> , day 1) (TAC) given every 3 weeks for 2 cycles. Response was assessed by palpation for stratification of patients. Patients with a complete or partial response received further 4 cycles TAC. Only patients with no change in tumor size were randomized.	vinorelbine (25 mg/m <sup>2</sup> , days 1+8) plus capecitabine (2000 mg/m <sup>2</sup> , days 1-14) every 3 weeks for 4 cycles (NX).	4 further cycles TAC	N=33 non- responders randomized to NX N=95 responders randomized to TAC N=13 unknown HER2 status N=10 unknown other baseline characteristics
2001-2002	Recruited 285, included in analysis: 134				
<b>AGO 1<sup>16</sup></b> <b>[not registered]</b>	Randomized phase III		epirubicin (90 mg/m <sup>2</sup> , i.v., day 1) plus paclitaxel (175 mg/m <sup>2</sup> , day 1) every 3 weeks for 4 cycles (EP)	epirubicin (150 mg/m <sup>2</sup> , day 1) every 2 weeks for 3 cycles followed by paclitaxel (250 mg/m <sup>2</sup> , day 1) every 2 weeks for 3 cycles (dDE- ddP).	N=668 unknown HER2 status
1998-2002	Recruited 668, included in analysis: 0				

<b>Prepare</b> <sup>17</sup> <b>NCT00544232</b>	Randomized phase III with 2 randomizations patients had predominantly HER2-negative tumors.	epirubicin (150 mg/m <sup>2</sup> , day 1) every 2 weeks for 3 cycles followed by paclitaxel (225 mg/m <sup>2</sup> , day 1) every 2 weeks for 3 cycles followed by cyclophosphamide (500 mg/m <sup>2</sup> , days 1+8) plus methotrexate (40 mg/m <sup>2</sup> , days 1+8) plus 5-fluorouracil (600 mg/m <sup>2</sup> , days 1+8) every 4 weeks for 3 cycles (ddE-ddP-CMF).	epirubicin (90 mg/m <sup>2</sup> , day 1) plus cyclophosphamide 600 mg/m <sup>2</sup> , day 1) every 3 weeks for 4 cycles followed by paclitaxel (175 mg/m <sup>2</sup> , day 1) every 3 weeks for 4 cycles (EC-P)	N=86 unknown HER2 status N=206 unknown other baseline characteristics
2002-2005	Recruited 733, included in analysis: 441	In addition, patients were randomized to: 2-weekly darbepoetin alpha simultaneously to chemotherapy	no darbepoetin alpha	
<b>Techno</b> <sup>18</sup> <b>NCT00795899</b>	non-randomized phase II patients with HER2-positive tumors only	Epirubicin (90 mg/m <sup>2</sup> , day 1) plus cyclophosphamide 600 mg/m <sup>2</sup> , day 1) every 3 weeks for 4 cycles followed by paclitaxel (175 mg/m <sup>2</sup> , day 1) every 3 weeks for 4 cycles (EC-P) plus trastuzumab (2 (8) mg/kg body weight, day 1) in all P cycles.		N=26: unknown baseline characteristics
2003-2005	Recruited 217, included in analysis: 191			
<b>GeparTrio</b> <sup>12,13</sup> <b>NCT00544765</b>	Phase III with 2 randomizations	TAC at same dose as in GeparTrio pilot study for 2 cycles, followed by sonographically assessment of response.	responding patients: 6 further cycles of TAC	responding patients: 4 further cycles TAC.
				N=301 non-responders randomized to NX N=29 initially non-randomized, but then artificially allocated to NX N=686 responders randomized to TAC N=176 unknown HER2 status N=71 unknown other baseline characteristics
2003-2005	Recruited 2072, included	non-responding patients: 4 cycles	non-responding patients: 4 further	

	in analysis: 809		NX.	cycles TAC	
<b>GeparQuattro</b> <sup>14,15</sup> <b>NCT00288002</b>	Randomized phase III	epirubicin (90 mg/m <sup>2</sup> , day 1) plus cyclophosphamide (600 mg/m <sup>2</sup> , day 1) every 3 weeks for 4 cycles followed by docetaxel (100 or 75 mg/m <sup>2</sup> , day 1) every 3 weeks for 4 cycles (EC-D)	1. capecitabine (1800 mg/m <sup>2</sup> , days 1-14) given concomitantly with docetaxel (75 mg/m <sup>2</sup> )	-	N=50 unknown baseline characteristics
2005-2006	Recruited 1495, included in analysis: 1445	patients with HER2 positive tumors received trastuzumab (2(8) mg/kg body weight, day 1) every 3 weeks with all cycles	2. capecitabine (1800 mg/m <sup>2</sup> , days 1-14) given after docetaxel (100 mg/m <sup>2</sup> )		

*Supplemental Table: Comparison of baseline characteristics of all trial participants included or excluded from this analysis.*

<b>Characteristic</b>	<b>Included population (N=3332)</b>	<b>Excluded population (N=3293)</b>
<b>Age</b>		
<35	208 (6%)	215 (7%)
35-39	325 (10%)	341 (10%)
40-49	1158 (35%)	1038 (32%)
50-59	979 (29%)	997 (30%)
>=60	662 (20%)	702 (21%)
<b>Tumor stage</b>		
cT1	107 (3%)	109 (3%)
cT2	2323 (70%)	2115 (66%)
cT3	478 (14%)	633 (20%)
cT4a-c	244 (7%)	221 (7%)
cT4d	180 (5%)	114 (4%)
<b>Histological type</b>		
Ductal invasive	2707 (81%)	2399 (77%)
Lobular invasive	416 (12%)	460 (15%)
Other types	209 (6%)	262 (8%)
<b>Grading</b>		
1	101 (3%)	131 (5%)
2	1843 (55%)	1598 (56%)
3	1388 (42%)	1113 (39%)
<b>Hormone receptor (HR) status</b>		
HR +	1182 (35%)	891 (30%)
HR -	2150 (65%)	2053 (70%)

Table 2: Univariable analysis on the association of baseline characteristics of patients and tumors and treatment parameters with pathological complete response (pCR) in the 3332 patients included in the analysis

Characteristics	N without pCR (%)		N with pCR (%)		Crude OR (95% CI)	P value
<b>Age</b>						0.001
< 35 years	151	(73%)	57	(27%)	1.00 (reference)	
35-39 years	243	(75%)	82	(25%)	0.88 (0.59 to 1.31)	
40-49 years	902	(78%)	256	(22%)	0.75 (0.53 to 1.06)	
50-59 years	787	(80%)	192	(20%)	0.64 (0.45 to 0.91)	
≥ 60 years	538	(81%)	124	(19%)	0.60 (0.42 to 0.87)	
<b>Tumor stage</b>						<0.001
cT1	74	(69%)	33	(31%)	1.00 (reference)	
cT2	1771	(76%)	552	(24%)	0.72 (0.47 to 1.11)	
cT3	418	(87%)	60	(13%)	0.34 (0.20 to 0.55)	
cT4a-c	207	(85%)	37	(15%)	0.34 (0.20 to 0.59)	
cT4d	151	(84%)	29	(16%)	0.41 (0.23 to 0.73)	
<b>Histological type</b>						<0.001
Ductal invasive	2079	(77%)	628	(23%)	1.00 (reference)	
Lobular invasive	380	(91%)	36	(9%)	0.31 (0.22 to 0.45)	
Other types	162	(78%)	47	(22%)	0.91 (0.65 to 1.28)	
<b>Grading</b>						<0.001
1	94	(93%)	7	(7%)	1.00 (reference)	
2	1558	(85%)	285	(15%)	2.37 (1.08 to 5.17)	
3	969	(70%)	419	(30%)	5.77 (2.65 to 12.59)	
<b>Hormone receptor (HR) status</b>						<0.001
HR + <sup>#</sup>	1870	(87%)	280	(13%)	1.00 (reference)	
HR -	751	(64%)	431	(36%)	3.79 (3.18 to 4.52)	
<b>HER2 status</b>						<0.001
HER2 + <sup>§*</sup>	694	(68%)	321	(32%)	1.00 (reference)	
HER2 -	1927	(83%)	390	(17%)	0.48 (0.39 to 0.57)	
<b>HER2/HR status</b>						<0.001
HER2-/HR+	1435	(91%)	140	(9%)	1.00 (reference)	
HER2+/HR+*	435	(76%)	140	(24%)	3.14 (2.40 to 4.09)	
HER2+/HR-*	259	(59%)	181	(41%)	6.78 (5.16 to 8.90)	
HER2-/HR-	492	(66%)	250	(34%)	5.33 (4.23 to 6.73)	
<b>Number of cycles</b>						0.005
4 cycles	237	(92%)	20	(8%)	1.00 (reference)	

6 cycles	768	(81%)	175	(19%)	2.58 (1.48 to 4.51)	
8 cycles	1087	(75%)	353	(25%)	3.79 (2.15 to 6.68)	
9 to 12 cycles	529	(76%)	163	(24%)	4.22 (2.28 to 7.79)	
<b>Anthracycline dose</b>						0.15
Low	1687	(77%)	497	(23%)	1.00 (reference)	
High	934	(81%)	214	(19%)	1.39 (0.89 to 2.18)	
<b>Taxane dose</b>						0.47
Low	1132	(79%)	296	(21%)	1.00 (reference)	
High	1489	(78%)	415	(22%)	1.10 (0.85 to 1.43)	
<b>Capecitabine</b>						0.75
No	1892	(80%)	474	(20%)	1.00 (reference)	
Yes	729	(75%)	237	(25%)	0.95 (0.71 to 1.28)	
<b>Tamoxifen</b>						0.23
No	2431	(78%)	686	(22%)	1.00 (reference)	
Yes	190	(88%)	25	(12%)	0.66 (0.33 to 1.30)	
<b>Trastuzumab**</b>						<0.001
None	328	(82%)	73	(18%)	1.00 (reference)	
4 cycles	117	(61%)	74	(39%)	2.84 (1.93 to 4.18)	
8 to 12 cycles	249	(59%)	174	(41%)	3.14 (2.28 to 4.32)	

<sup>#</sup>≥10% of cells stained positive for estrogen and/or progesterone receptor <sup>§</sup>positive if either local immunohistochemical staining was 3 or fluorescent in-situ hybridization was positive \*Note that the analysis of HER2-positive tumors includes patients with and without trastuzumab therapy. \*\*Refers to patients with HER2-positive tumors only.

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