Effect of neoadjuvant anthracycline–taxane-based chemotherapy in different biological breast cancer phenotypes: overall results from the GeparTrio study

Jens Huober, Gunter Minckwitz, Carsten Denkert, Hans Tesch, Erich Weiss, Dirk Michael Zahm, Antje Belau, Fariba Khandan, Maik Hauschild, Christoph Thomssen, et al.

Effect of neoadjuvant anthracycline-taxane based chemotherapy in different biological breast cancer phenotypes – Overall results from the GeparTrio study

Jens Huober[1], Gunter von Minckwitz[2], Carsten Denkert[3], Hans Tesch[4], Erich Weiss[5], Dirk Michael Zahm[6], Antje Belau[7], Fariba Khandan[8], Maik Hauschild[9], Christoph Thomssen[10], Bernhard Högel[11], Silvia Darb-Esfahani[3], Keyur Mehta[2], Sibylle Loibl[2]

[1]Brustzentrum Kantonsspital St. Gallen, Switzerland and University Tübingen, Dep of Gynaecology, Germany

[2]German Breast Group, Neu-Isenburg, Germany

[3]Charité, Institute of Pathology, Translational Tumorpathology Unit, Berlin, Germany

[4]Onkologische Gemeinschaftspraxis am Bethanien Krankenhaus, Frankfurt am Main, Germany

[5]Hospital Sindelfingen-Böblingen, Böblingen, Germany

[6]SRH Waldkliniken, Gera, Germany

[7]University Greifswald, Dep. Of Gynaecology, Greifswald, Germany

[8]St. Markus Hospital, Frankfurt am Main, Germany

[9]Hospital Rheinfelden, Rheinfelden, Germany

[10]Martin Luther University, Dep. of Gynaecology, Halle, Germany

[11]Frauenklinik vom Roten Kreuz, München, Germany

Corresponding Author:

Sibylle Loibl, MD

German Breast Group

Martin-Behaim Str. 12

63263 Neu-Isenburg

+49-6102-7480426

+49-6102-7480126

Email: Sibylle.loibl@germanbreastgroup.de

Abstract

Purpose: To explore the effect of neoadjuvant chemotherapy (NACT) on clinical mid-course and pathological complete response (pCR) at surgery in different biological breast cancer subtypes.

Methods: The GeparTrio study included 2072 patients with operable or locally advanced breast cancer. After 2 cycles with docetaxel, doxorubicin and cyclophosphamide (TAC) patients were randomized according to their clinical response. Clinical and biological factors were assessed for predicting clinically mid-course response and pCR at surgery.

Results: The overall pCR rate, defined as no invasive residuals in breast and axilla, was 20.5 %. The highest pCR rate of 57% was observed in patients below 40 years of age with triple negative or grade 3 tumors. Independent factors for mid-course response and pCR were: young age, non-T4 tumors, high grade, and hormone receptor status, the strongest single predictive factor. Within the biological subtypes grading was an independent factor to predict pCR for luminal tumors, clinical tumor stage for the HER2 like tumors and age for the triple negative ones. Grading gave independent information for mid-course response within the triple negative group. No factor predicted mid-course response within the other groups.

Conclusion: Grading and age can identify subgroups within the luminal and triple negative patients who have an increased benefit from neoadjuvant chemotherapy.

Key words: neoadjuvant chemotherapy, breast cancer, predictive factors, lobular histology, age,

Introduction:

Hormone receptor (HR) status is long known as independent predictor for chemotherapy response. Recently we and others showed that the rate of pathological complete remissions (pCR) differs between biologic phenotypes. [1-5] In a smaller subset of the GeparDuo trial [6] patients with a HR+/HER2- had a very low chance of achieving a pCR but had still an excellent prognosis. Whereas patients with HR-/HER2+ tumors have only an excellent prognosis when achieving a pCR. [1]

The identification of patients with a high likelihood of achieving a pCR using the biological phenotype along with age and grading is still of interest. Even in less chemosensitive tumors, a subgroup might still benefit from an anthracycline/taxane containing chemotherapy regimen, depending on age and grading.

A secondary endpoint of the GeparTrio study was to examine the correlations between different clinical and established biological markers and pCR to NACT and to identify potential biological groups who will benefit from TAC chemotherapy. Since there is a relation between clinical (sonography or physical examination) mid-course response and pathological remission at surgery, the aim of the study was also to look for markers predicting mid-course response and whether these factors were different from those predicting a pathological complete remission [7,8].

Methods:

The GeparTrio trial was a multicenter, prospective, randomized, phase III trial with the primary goal of evaluating clinical activity of 6-8 cycles of docetaxel, doxorubicin and cyclophosphamide (TAC) or 2 cycles of TAC followed by 4 cycles of vinorelbine and capecitabine (NX). One of the secondary endpoints of the study was to investigate clinical and biological markers that may predict early as well as late response to NACT. From July 2002 until December 2005, 2090 patients were registered for participation. Eligibility criteria were described elsewhere [7,8]

All patients started treatment with two cycles of TAC (docetaxel 75 mg/m^2, doxorubicin 50 mg/m^2, cyclophosphamide 500 mg/m^2, on day 1, every 3 weeks). Clinical response was determined preferably by sonography or other clinical methods if sonography was considered as inappropriate by the investigator. Response was assessed by palpation (caliper) when the breast tumor could not be measured by sonography or in case of inflammatory disease by measurement of the skin lesion in case a palpable mass was missing. Patients with an early response were randomized to proceed with either 4 or 6 cycles of TAC whereas patients without response were randomized either to continue with 4 cycles of TAC or to 4 cycles of NX (vinorelbine 25mg/m^2 on days 1 and 8 plus capecitabine 1000 mg/m^2 orally twice a day on days 1–14, every 3 weeks). None of the HER2+ patients received trastuzumab during neoadjuvant treatment. Within 21 days after completion of chemotherapy and after overall assessment of response, patients had to undergo surgery and postoperative treatment according to standard recommendations. Patients with progression were excluded from randomization and treated at the discretion of the investigator. In patients with disease progression during further preoperative therapy immediate surgery was recommended.

Marker selection:

The clinico-pathological markers available at baseline (see Supplementary Table 1a) were evaluated for response prediction.

Definition of response to neoadjuvant therapy:

Response to NACT was evaluated twice. Mid-course response after TACx2 was defined as a decrease of the product of the two largest perpendicular tumor diameters by at least 50%. Pathological response at surgery was based on the modified regression grading system by Sinn et al. [7-9]. For a better understanding, regression grade 3 (only non invasive residuals left in the breast) was divided into group 3a (without lymph node involvement) and 3b (with involved lymph nodes). Pathological complete remission (pCR) was defined as a histopathological complete remission of all invasive tumor cells from the breast and axillary tissue removed at surgery (ypT0,ypTis, ypN0 = regression grade 3a and 5). (Supplmentary Table 1b)

The histological tumor type was defined according to the WHO definitions. The pathological examinations were performed by local pathologists of the participating sites; however, all pathology reports were centrally reviewed for pCR at the German Breast Group headquarters, Neu-Isenburg, Germany.

ER, PgR and HER2 were measured locally at each participating centre on tissue sections of the true/core-cut-biopsies obtained at the time of primary diagnosis before treatment. Steroid hormone receptor positivity or negativity by immunohistochemistry was not further quantified. HER2 overexpression required either immunohistochemical staining of 3+ or positivity by fluorescence in situ hybridization (FISH) technique. In case of a 2+ score by IHC confirmatory FISH testing was required. Missing test results were imputed from the central IHC measurement for HER2, ER and PgR status at the Charité Pathology Department, Berlin. The following antibodies were used: rabbit monoclonal antibody against human ERα (clone SP1, Neomarkers, 1:50); mouse monoclonal antibody against human progesterone PgR (clone PgR 636, Dako, 1:50); rabbit

polyclonal antibody against human HER2 (HercepTest™ antibody, Dako, 1:500); ER and PgR immunohistochemistry was scored positive if at least 10% of tumor cell nuclei showed a staining signal. In case of conflicting results the central measurement has been taken.

Biological sub classification using ER, PgR, and HER2 were performed. Luminal A was defined as ER+; PgR+ HER2-; Luminal B tumors were defined as ER+PgR-HER2-; ER+PgR-HER2+; ER-PgR+HER2-; ER+PgR+HER2+; HER2 like tumors were defined as ER-PgR+HER2+, ER-PgR-HER2+; and triple negative tumors as ER-; PgR-; HER2 negative.

Statistics:

Descriptive analyses were performed. Results were compared using the chi square and Fisher´s exact test. A binary logistic regression for uni- and multivariate (full model) analysis was performed. All tests were two-sided with significance levels set at 0.05. With regard to the exploratory nature of this analysis no adjustments were made for multiple comparisons. Statistical computations were performed in SPSS version 14.0.

Results:

Of the 2090 patients registered, 2072 patients were randomized. For more detailed information on patients´ availability please refer to the consort statement (Fig1). Except for the HER2 status, which was missing in 17% of the patients at primary diagnosis, all other factors were known in approximately 95% of the patients. Response after 2 cycles TAC was observed in 1400 of 2034 patients (68.8 %) evaluated for mid-course response. The investigated baseline markers of the GeparTrio study and their impact on mid-course as well as pCR is outlined in Table 1. The overall pCR rate, defined as no invasive tumor residuals in the breast and no involved lymph nodes at surgery (ypT0/ypTis, ypN0= RG5 +RG3a) was 20.5%. A regression grade 5 defined as no invasive and no non-invasive residuals (ypT0, ypN0= RG 5) could be seen in 16.7% of the patients. (Table 1b)

Predictors of mid-course response and pCR at surgery in univariate analysis including baseline clinico-pathological factors.

Single factors, significantly associated with the achievement of mid-course response as well as a pCR at surgery in the univariate analysis were: age below 40 years, a non-T4 tumor, high tumor grade (G3), ER and PgR negative. Tumor stage, nodal involvement and HER2 status neither had an impact on mid-course response nor on pCR (Table 2). Non-lobular tumor type had a significant impact only on pCR.

Impact of several factors on pCR rates in different age groups according to histological subtypes and HER2 expression

Age at diagnosis:

17.4 % of the patients were below the age of 40 years with a pCR rate of 31.0%. Pathological complete remission was significantly higher in patients under the age of 40

years compared to those 40 years or older (Fig 2a). The highest pCR rate could be detected for those under 40 years with an ER/PgR negative (p=0.001) or an undifferentiated (p =0.001) tumor. When a tumor was triple negative pCR rates almost doubled and were as high as 57% in the < 40 years population compared to 34 % in the patients ≥ 40 years (p<0.0001).

Histological tumor type:

The pCR rate for lobular-invasive tumors (n=277) was significantly lower than for the ductal-invasive tumors (9.4% versus 22.2%; p=0.0001). (Fig.2b) Histological tumor type proofed to be an independent predictive factor for pCR (OR2.29 [95% CI 1.28-4.08]; p=0.0052). (Table 2)

HER2 and ER/PgR status - biological subgroups:

For the entire study population over-expression of HER2 was neither predictive for mid-course response after 2 cycles TAC (HER2 positive vs. negative 71.4% vs. 68.4%; p=0.223; OR 1.15 [95%CI: 0.92-1.45]) nor for pCR at surgery (HER2 positive vs. negative: 23.6% vs. 20.0%; p=0.099; OR1.23 [95%CI 0.96-1.58]) in the uni- and multivariable model. However the pCR rates for HER2-positive and -negative patients varied in the different subgroups (Fig. 2c).

In the multivariate analysis age, grading and hormone receptor status were independent predictive factors for mid-course response after two cycles of TAC (Table 2). Young age (OR: 1.623; p=0.0043), non-T4 tumors (OR: 1.81; p=0.0139); grade 3 tumors (OR: 1.93; p<0.0001), a non-lobular histology (OR: 2.29;p= 0.0052),and negative hormone receptor status (OR: 3.08; p<0.001) predicted significantly, independently a pCR at surgery. A negative hormone receptor status was the strongest single predictive factor (Table 2).

The ROC curve for the multivariable logistic regression model shows an area under the curve of 0.727. (Figure 3)

HER2 status and ER and PgR were used to further subclassify the tumors (methods section). The rate of mid-course response and pCR rate at surgery of the four biological subgroups is given in Supplementary Table 1. The significant predictors for the overall cohort (age, grading, tumor stage and histological subtype) were further tested within the biological subgroups. Patients with a luminal A tumor had only a probability to have a pCR rate above 10% with grade 3 tumors. The multivariable analysis revealed only grading as an independent factor within the luminal A and B subgroup. Within the HER2 like group the clinical tumor stage at baseline was an independent factor. Age was the only independent factor within the triple negative subgroup (Table 3). The histological subtype gave no independent information in any of the biological subgroups. Besides the triple negative subgroup pCR rates were lower in the lobular than in the ductal group but results did not reach statistical significance. Within the triple negative group age (84.8% age <40 vs 73.6% age ≥ 40; p= 0.039) and grading were significant predictors to reach mid-course response after TACx2 in the univariable analysis. Grade 3 remained an independent significant predictive factor (80.5% vs 67.9%; p=0.01; OR1.93 [95%CI 1.17-3.18]). No factor could be found to determine mid-course response within the other biological subgroups.

<u>Early Responders and Non-Responders</u>

Within the Non-Responder group no subgroup could be identified which benefitted from the more intense therapy with 6xTAC neither by classical factors nor by biological subtyping.

In the Responder group patients with a HER2 like tumor seemed to have a higher pCR rate with TAC x8 (27.4% TACx6 vs 44.2% TAC x8; p=0.026). (Supplementary Table 2)

Discussion:

In this analysis it could be demonstrated that biological factors combined with clinical and pathological information can be used both to predict pathological as well as mid-course response to neoadjuvant TAC chemotherapy in patients with primary breast cancer. In univariate testing there was a high concordance of factors predicting an early clinical response after 2 cycles TAC and those that predict a pCR at surgery. Only histological type was exclusively predictive for pCR at surgery. In multivariate testing all these factors provided independent information for predicting a pCR at surgery.

In contrast to some other trials, HER2 as a single marker had neither predictive value for mid-course response nor for pCR [3,10]. None of the HER2-positive patients received trastuzumab at that time. Therefore this trial has the capability to evaluate biomarkers predicting response to chemotherapy alone in a HER2-positive group of patients.

Nevertheless, HER2 status gave different predictive information depending on the ER/PgR status of the tumor, reflecting the biological phenotypes. In patients with luminal tumors the pCR in the luminal B group including also HER2 positive cases, was higher than in the luminal A group. Similar results were seen by Guarneri et al. [11] in a pooled analysis of multiple neoadjuvant trials with different chemotherapy regimens. The pCR rate in the HR+ and HER2 positive tumors was significantly higher than for those with an HR+ and HER2 negative tumor (15% vs. 6%). These results are matching those from the Geparduo trial in a smaller set of 116 patients with a pCR rate of only 1.8% for the HR+/HER2- compared to 23.1% for the HR+/HER2+ group.[1] Absolute values might differ between different analyses but should not be overestimated.

Using the Sorlie classification, several breast cancer subtypes with distinct gene expression patterns and different prognoses can be identified [12]. Immunohistochemically, this classification is so far mainly based on the ER, PgR and HER2status. Patients with tumor features suggestive for the luminal subtype have in

general a low pCR rate, and grading was of utmost importance in our investigation for selecting those patients with an expected pCR rate >10%. However, one would like to have integrated those parameters as well as molecular information into one test. Several strategies have been undertaken to select those luminal patients with a higher probability for a pCR e.g. using the Genomic Grade Index (GGI) or the Neoadjuvant Luminal Response Score (NLRS) [13, 14]. The GGI compared to conventional parameters adds only modest but still independent predictive information. The NLRS will be able to select those luminal tumors which had a chance for pCR above 10% to prevent overtreatment.

Our study had several strengths and limitations. Above all, one strength was the sample size which provided robust evidence and allowed to draw conclusions for the smaller subgroups of patients. This analysis has prospectively been planned for all markers included. Most identified factors were predictive not only for a pCR at surgery but also for a mid-course response. The study was a good model for evaluating biomarkers also in HER2 positive patients because a combination chemotherapy alone without trastuzumab was used.

In the present analysis, for predictive factors, we used here the more recent and now generally accepted pCR definition that requires a tumor-free axilla, but allowed remaining DCIS (ypT0/ypTis ypN0), taking into account that non-invasive residuals do not negatively influence the long term outcome [15]. In the separate analysis of the responding and non-responding patients, the definition of pCR was absence of all invasive and non-invasive tumor cells in the removed breast tissue irrespective of the axillary involvement. Even though the numbers were slightly different when using these different definitions, the predictive power of the examined factors did not change (data not shown).

One limitation might be that mid-course responders and non responders did not receive the same therapy. We considered this procedure as acceptable since in those patients who did not respond after 2 cycles of TAC, the response to NX was not significantly inferior to 4 additional cycles of TAC. Informations regarding the biological factors could not be obtained from all patients. However, except for the HER2 status, which was missing in 17% of the patients at primary diagnosis (still available for a total of 1717 patients), all other factors were known for more than 90 % of the study population.

Our study shows, that even in the time of gene array analyses, pCR can be reliably predicted by clinical and standard histopathological parameters in addition to the biological phenotype [16]. Additionally, we could identify clinico-biological marker combinations which significantly predicted a mid-course response after 2 cycles of TAC as well as a pCR at surgery. Grading was of great importance to select the patients within the luminal subtype of the tumors who would have a higher pCR rate. Age added valuable information in the chemosensitive groups e.g. those with a triple negative phenotype which underlines the fact that the triple negative is a mixed population of different tumor types, where those without pCR have an extremely poor prognosis [17, 18]. A different biological behavior between invasive lobular and invasive ductal breast carcinomas has been described for the adjuvant and neoadjuvant setting [19-21]. In our study a total of 277 patients with lobular histology were evaluated and confirmed that this subtype is less sensitive to chemotherapy and will only benefit if other biomarkers defining aggressive behavior as grade 3 or ER/PgR negativity were apparent.

This analysis might help to guide the clinician in the decision making process for neoadjuvant therapy. Those patient populations with a pCR rate of less than 10% might not be the adequate candidates for NACT with the regimen used in this study. Our results may help to further sharpen the profile of the eligible patient population for future

trials evaluating new tools like gene expression signature arrays in order to identify predictive marker sets for neoadjuvant chemotherapy in the specific subgroups (22).

References:

1. Darb-Esfahani S, Loibl S, Müller BM, et al. Identification of biology-based breast cancer types with distinct predictive and prognostic features: role of steroid hormone and HER2 receptor expression in patients treated with neoadjuvant anthracycline/taxane-based chemotherapy. Breast Cancer Res 2009; 11:R69

2. von Minckwitz G, Sinn HP, Raab G, et al. Clinical response after two cycles compared to HER2, Ki-67, p53, and bcl-2 in independently predicting a pathological complete response after preoperative chemotherapy in patients with operable carcinoma of the breast. Breast Cancer Res 2008; 10(2) R30

3. Rouzier R, Perou CM, Symmans WF, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 2005; 11:5678-5685

4. Colleoni M, Viale G, Zahrieh D, et al. Expression of ER, PgR, HER1, HER2 and response: a study of preoperative chemotherapy. Ann Oncol 2008; 19:465-472

5. Colleoni M, Bagnardi V, Rotmensz N, et al. Increasing steroid hormone receptors expression defines breast cancer subtypes non responsive to preoperative chemotherapy. Breast Cancer Res Treat 2009;116: 359-369

6. von Minckwitz G, Raab G, Caputo A, et al. Doxorubicin with cyclophosphamide followed by docetaxel every 21 days compared with doxorubicin and docetaxel every 14 days as preoperative treatment in operable breast cancer: the Geparduo study of the German Breast Group. J Clin Oncol 2005; 23:2676-2685

7. von Minckwitz G, Kuemmel S, Vogel P, et al. Intensified neoadjuvant chemotherapy in early-responding breast cancer: phase III randomized GeparTrio study. J Natl Cancer Inst 2008; 100:552-562

8. von Minckwitz G, Kümmel S, Vogel P, et al. Neoadjuvant vinorelbine-capecitabine versus docetaxel-doxorubicin-cyclophosphamide in early nonresponsive breast cancer: a phase III randomized GeparTrio trial. J Natl Cancer Inst 2008; 100:542-551

9. Sinn HP, Schmid H, Junkermann H, et al. Histological regression of breast cancer after primary (neoadjuvant) chemotherapy. Geburtsh u Frauenheilk 1994; 54: 552-558

10. Loibl S, Müller B, Roller M, et al. Local versus central HER2 immunohistochemistry correlates with kinetic RT-PCR but only central immunohistochemistry and RT-PCR predict pathological complete response: results from the neoadjuvant multicenter Gepar-Trio trial. Cancer Res 2008; 69: (suppl) (2): Abstract 1070

11. Guarneri V, Broglio K, Kau S-W, et al. Prognostic value of pathological complete response after primary chemotherapy in relation to hormone receptor status and other factors. J Clin Oncol 2006; 24:1037-1044

12. Sorlie T, Perou CM, Tibshirani R et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 2001; 98: 10869-10874

13. Liedtke C, Hatzis C, Symmans FW, et al. Genomic Grade Index is associated with response to chemotherapy in patients with breast cancer. J Clin Oncol 2009; 27: 3185-3191.

14. von Minckwitz G, Budczies J, Loibl S, et al. Validated 3 gene signature predicts response to neo-adjuvant chemotherapy in luminal breast cancer – Results from GeparTrio and GeparQuattro study. Cancer Res 2009, 69 (suppl) (24): Abstract nr 2132

15. Mazoumi C, Peintinger F, Wan-Kau S, et al. Residual carcinoma in situ in patients with complete eradication of invasive breast cancer after neoadjuvant chemotherapy does not adversely affect patient outcome. J Clin Oncol 2007; 25:2650-2655

16. Goldstein NS, Decker D, Severson D et al. Molecular classification system identifies invasive breast carcinoma patients who are most likely and those who are least likely to achieve a complete pathologic response after neoadjuvant chemotherapy. Cancer 2007; 110: 1687-166

17. Rakha EA, Ellis IO: Triple-negative/basal-like breast cancer: review.
Pathology 2009; 41:40-47

18. Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant chemotherapy and long term-survival in patients with triple negative breast cancer. J Clin Oncol 2008; 26:1275-1281

19. Cristofanilli M, Gonzalez-Angulo A, Sneige N, et al. Invasive lobular carcinoma classic type: response to primary chemotherapy and survival outcomes. J Clin Oncol 2005; 23:41-48

20. Katz A, Saad EA, Porter P, et al. Primary systemic chemotherapy of invasive lobular carcinoma of the breast. Lancet Oncol 2007; 8:55-62

21. Pestalozzi BC, Zahrieh D, Mallon E, et al. Distinct clinical and prognostic features of infiltrating lobular carcinoma of the breast: combined results of 15 international Breast Cancer Study Group clinical trials. J Clin Oncol 2008; 18:3006-3014

22. Bonnefoi H, Potti A, Delorenzi M, et al. Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00-01 clinical trial. Lancet Oncol 2007; 8:1071-1078

Table 1: Univariable analysis of factors in association with mid-course response after TACx2 and pathological complete response at surgery

Factor	Mid-course response after TACx2					Response at surgery (pCR)				
	Response (cCR/cPR)				Wald Chi-Square (p-value)	pCR				Wald Chi-Square (p-value)
	N	(%)	OR	95% CI		N	(%)	OR	95% CI	
All						423	20.5			
Age (years):										
< 40	272	76.4%	1.580	1.212-2.059	0.0007	112	31.0%	2.023	1.569-2.610	<0.0001
≥ 40	1127	67.2%				311	18.2%			
Clinical tumor stage:										
cT1-cT3	1201	69.9%	1.463	1.123-1.908	0.0049	380	21.7%	2.041	1.394-2.987	0.0002
cT4	167	61.4%				33	12.0%			
Histological tumor type:										
ductal / other	1218	69.5%	1.215	0.928-1.590	0.1558	396	22.2%	2.748	1.807-4.178	<0.0001
lobular	178	65.2%				26	9.4%			
Tumor grade:										
III	545	74.0%	1.574	1.285-1.929	<0.0001	211	28.1%	2.612	2.072-3.293	<0.0001
I+II	763	64.4%				156	13.0%			
Hormone receptor status:										
ER and PgR negative	503	76.3%	1.731	1.400-2.142	<0.0001	238	35.6%	4.369	3.463-5.514	<0.0001
ER and/or PgR positive	849	65.1%				149	11.2%			
HER2 status:										
HER2 positive	357	71.4%	1.153	0.917-1.449	0.2229	118	23.6%	1.234	0.961-1.584	0.0993
HER2 negative	825	68.4%				243	20.0%			
ER/PgR/HER2 status:										
triple negative	285	76.0%	1.534	1.178-1.997	0.0015	198	38.9%	3.551	2.749-4.588	<0.0001
not triple negative	873	67.4%				147	15.2%			

Table 2: Factors associated with mid-course response after TACx2 and response at surgery (pCR) in a multivariable analysis only showing variables which have been significant in the univariable model.

Factor	Mid-course response after TACx2 Multivariate analysis (N=1503)				Response at surgery (pCR) Multivariate analysis (N=1511)			
	cCR/cPR (%)	Odds ratio	(95% CI)	p-value	pCR (%)	Odds ratio	(95% CI)	p-value
Age (years)								
< 40	76.2	1.533	1.113-2.111	0.0088	28.5	1.623	1.164-2.263	0.0043
≥ 40	66.7				17.5			
Clinical tumor stage								
cT1-cT3	69.1	1.301	0.943-1.796	0.109	20.5	1.807	1.128-2.895	0.0139
cT4	62.6				11.3			
Histological tumor type								
ductal	68.9	1.090	0.783-1.517	0.6801	20.9	2.286	1.280-4.082	0.0052
lobular	63.6				7.5			
Tumor grade								
III	74.7	1.460	1.143-1.864	0.0024	29.6	1.903	1.436-2.522	<0.0001
I+II	64.4				13.4			
Hormone receptor status								
ER and PgR negative	74.9	1.396	1.084-1.797	0.0098	34.2	3.084	2.325-4.092	<0.001
ER and/or PgR positive	65.0				11.5			

Table 3: Multivariable analysis for pCR at surgery within the biological subgroups using the covariates (age, grading, histological tumor type and stage) which were significant in the whole group.

Factor	Luminal A pCR at surgery (n=562)				Luminal B pCR at surgery (n=462)				HER2 like pCR at surgery (n=193)				Triple negative pCR at surgery (n=351)			
	pCR (%)	Odds ratio	(95% CI)	p-value	pCR (%)	Odds ratio	(95% CI)	p-value	pCR (%)	Odds ratio	(95% CI)	p-value	pCR (%)	Odds ratio	(95% CI)	p-valu
Age (years)																
< 40	10.1	1.69	0.68-4.17	0.256	24.1	1.53	0.842-2.76	0.163	33.3	1.35	0.63-2.87	0.441	**57.0**	**2.03**	**1.18-3.50**	**0.01**
≥ 40	6.6				17.8				28.2				**34.1**			
Tumor grade																
III	**16.2**	**5.2**	**2.56-10.55**	**<0.0001**	**24.3**	**1.81**	**1.11-2.98**	**0.018**	31.3	1.27	0.67-2.42	0.164	39.5	1.42	0.89-2.27	0.137
I+II	**3.8**				**14.7**				25.3				30.5			
Clinical tumor stage																
cT1-cT3	7.3	2.20	0.64-7.52	0.209	19.8	1.62	0.76-3.44	0.209	**32.4**	**5.29**	**1.19-23.56**	**0.028**	39.7	1.35	0.62-2.93	0.446
cT4	3.4				14.3				**11.1**				30.8			
Histological tumor type																
ductal/others	7.8	1.64	0.55-4.85	0.376	20.0	2.78	0.97-8.0	0.058	31.0	4.42	0.55-35.7	0.164	38.9	1.22	0.44-3.34	0.702
lobular	4.3				11.9				7.7				39.1			

Supplementary Tables and Figures:

Supplementary Table 1a: Mid-course response and pCR (ypT0/ypTis, ypN0) according to factors investigated further from the overall GeparTrio trial.

	all patients		mid-course response after TACx2 N=2034		response at surgery (pCR) n=2072	
	N	%	N	%	N	%
All patients	**2072**		**1400**	**68.8**	**423**	**20.5**
Age [years]						
< 35	139	6.7	102	74.5	54	38.8
35 - < 40	222	10.7	170	77.6	58	26.1
40 - < 50	683	33.0	470	70.0	150	22.0
50 - < 60	580	28.0	399	69.6	97	16.7
60+	448	21.6	259	59.7	64	14.3
Clinical Tumor Stage						
cT1	25	1.2	14	56.0	6	23.1
cT2	1315	63.5	931	70.8	314	23.4
cT3	378	18.2	257	68.0	60	15.5
cT4	272	13.1	167	61.4	33	12.0
missing	44					
Tumor Size						
< 40mm	792	64.1	534	68.6	175	22.1
>=40mm	1255	32.8	850	69.0	242	19.3
missing	25					
Nodal Status						
cN0	896	43.2	616	69.9	194	21.7
cN+	1094	52.8	725	67.6	214	19.6
not assessed	82					
Histological tumor Type						
ductal	1620	77.9	1100	69.3	351	21.7
other	173	8.4	120	70.2	45	26.0
lobular	278	13.4	179	65.3	26	9.4
not assessed	7	0.34				
Tumor Grade						
I	81	3.9	48	60.0	6	7.4
II	1119	54.0	716	64.8	150	13.4
III	751	36.2	545	74.0	211	28.1
not assessed	121	5.8				
ER /PgR						
neg neg	670	32.3	504	76.4	238	35.5
neg pos	89	4.3	65	74.7	19	21.3
pos neg	296	14.3	1204	69.6	55	18.6
pos pos	943	45.5	580	67.7	75	8.0
not assessed	74	3.6				
HER2 Status						
pos	501	24.2	357	71.4	118	23.6
neg	1216	58.7	825	68.4	243	20.0
not assessed	355	17.1				
Biological subgroups						
Luminal A	592	28.6	367	62.4	42	7.1
Luminal B	499	24.1	345	69.6	94	18.8

HER2 like	212	10.2	161	75.9	62	29.2
Triple negative	378	18.2	285	76.0	147	38.9
unknown	391	18.9				

Supplementary Table 1b

Regression grade at surgery (n=2072). pCR is defined as regression grade 5 and 3a (ypT0/ypTis; ypN0).

Regression grade at surgery	n	%
Total	2072	100
RG 5 (ypT0&ypN0)	346	16.7
RG 4 (ypT0&ypN+/?)	39	1.9
RG 3a (ypTis&ypN0)	79	3.8
RG 3b (ypTis&ypN+)	23	1.1

Supplementary Table 2: Rate of pathological complete response according to predictive factors in the group of patients with (responder) and without (non-responder) mid-course response (univariable analysis; Qui square test)

	Responder					Non-Responder				
	TACx6		TACx8		p-value	TACx6		TAC-NX		p-value
	pCR		pCR			pCR		pCR		
	N	%	N	%		N	%	N	%	
age < 40 yrs	48	35.8	54	39.0	0.617	4	10.3	6	13.6	0.743
age ≥40 yrs	126	22.1	147	26.5	0.094	21	7.4	15	5.8	0.493
cT1-3	158	25.9	177	30.3	0.107	22	8.5	19	7.6	0.747
cT4	12	14.8	16	19.8	0.409	3	5.5	2	4.3	1.0
grade1/2	63	16.8	74	19.2	0.397	10	5.1	8	3.7	0.631
grade 3	88	30.6	97	38.5	0.057	13	11.7	10	13.5	0.821
ductal / other	163	26.7	189	31.2	0.099	21	7.7	21	8.2	0.873
lobular	11	11.7	11	13.3	0.822	21	8.5	0	0.0	0.117
Luminal A	175	8.5	186	11.8	0.291	102	3.9	115	0.9	0.125
Luminal B	176	20.5	168	25.6	0.257	84	14.3	65	4.6	0.052
HER2 like	84	27.4	77	44.2	**0.026**	29	6.9	20	15.0	0.357
Triple negative	149	50.3	135	46.7	0.537	48	12.5	41	4.9	0.210

Figure Legends

Fig. 1: Consort statement

Fig 2: Impact of grade, hormone receptor and HER2 expression of pCR in different subgroups

* significant difference ≤ 0.05; ** significant difference ≤ 0.001

Fig 2a : Impact of grade, steroid hormone receptor, and HER 2 expression on pCR rates in different age groups

< 40 years: ; ≥ 40 years:

Fig. 2b: Impact of age, grade, steroid hormone receptor, and HER 2 expression on pCR rates in tumors with different histological type

ductal/other: ; lobular:

Fig 2c: Impact of grade and steroid hormone receptor status on pCR rates in tumors with different expression of HER2

HER2 negative: ; HER2 positive:

Fig 3: Receiver operating characteristic curve (ROC) for the pathologic complete response to TAC based chemotherapy using the factors which gave significantly independent information for pCR.

The area under the ROC curve including age, grading, ER/PgR status, histological tumor type and clinical T stadium is 0.727.

Figure 1: Consort Statement

Confirmed eligibility	2090			
			withdrawal of consent	18
Started chemotherapy with TAC	**2072**			
Baseline characteristics complete		missing values		
			Stage	44
			size	25
			Nodal status	82
			Histological type	7
			Grade	121
			ER/PgR	74
			HER2	355
Clinical assessement after 2 cycles TAC	2034	not done		38
Randomized after 2 cycles TAC	2012	not done		60
Surgery	2004	unknown		68
Type of surgery	1981			91
Pathological response assessment	1989	not available		83
All predictive factors and efficacy variable available	1511			

Fig 2a : Impact of grade, steroid hormone receptor, and Her 2 expression on pCR rates in different age groups

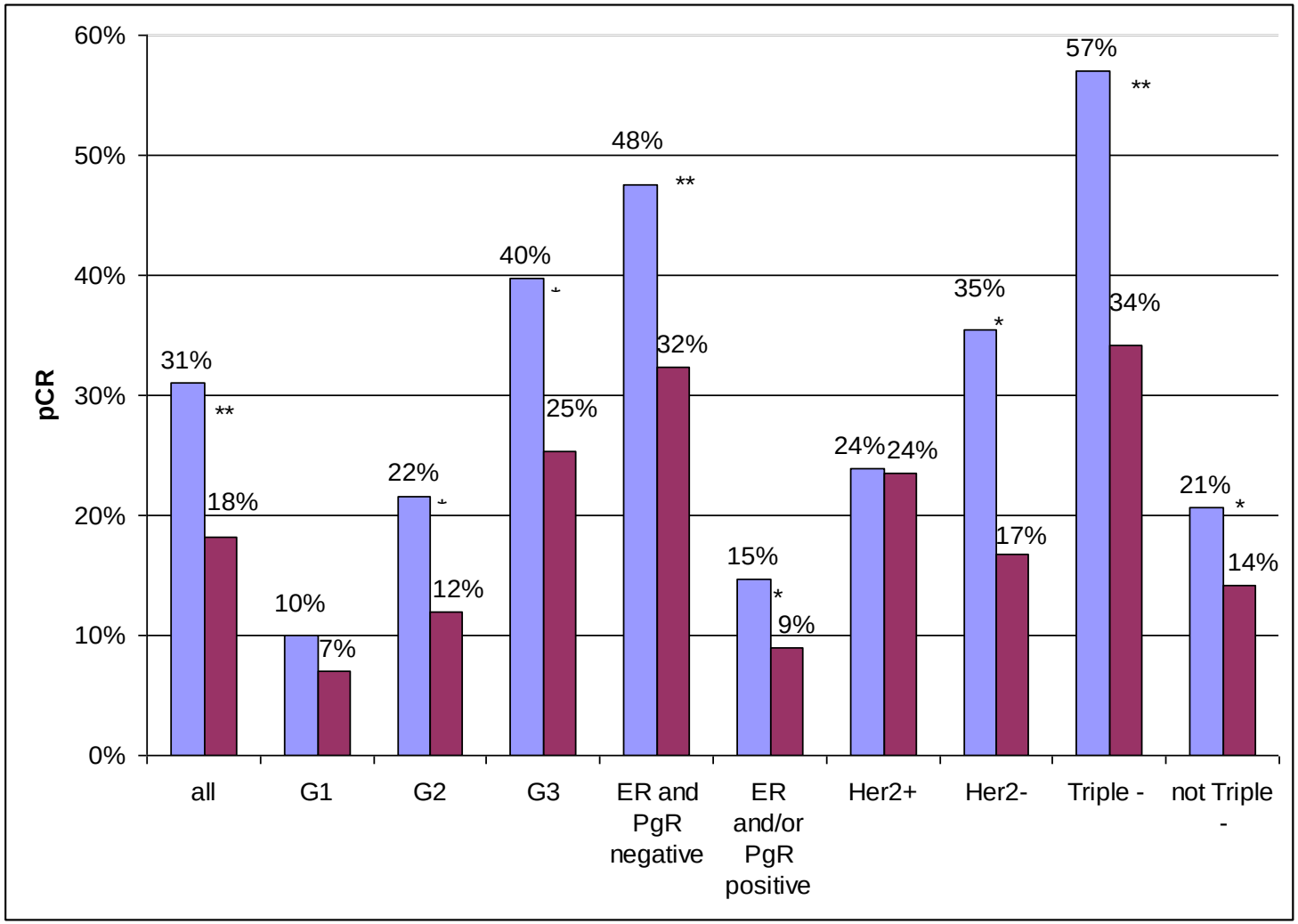

Fig. 2b: Impact of age, grade, steroid hormone receptor, and HER2 expression on pCR rates in tumors with different histological type

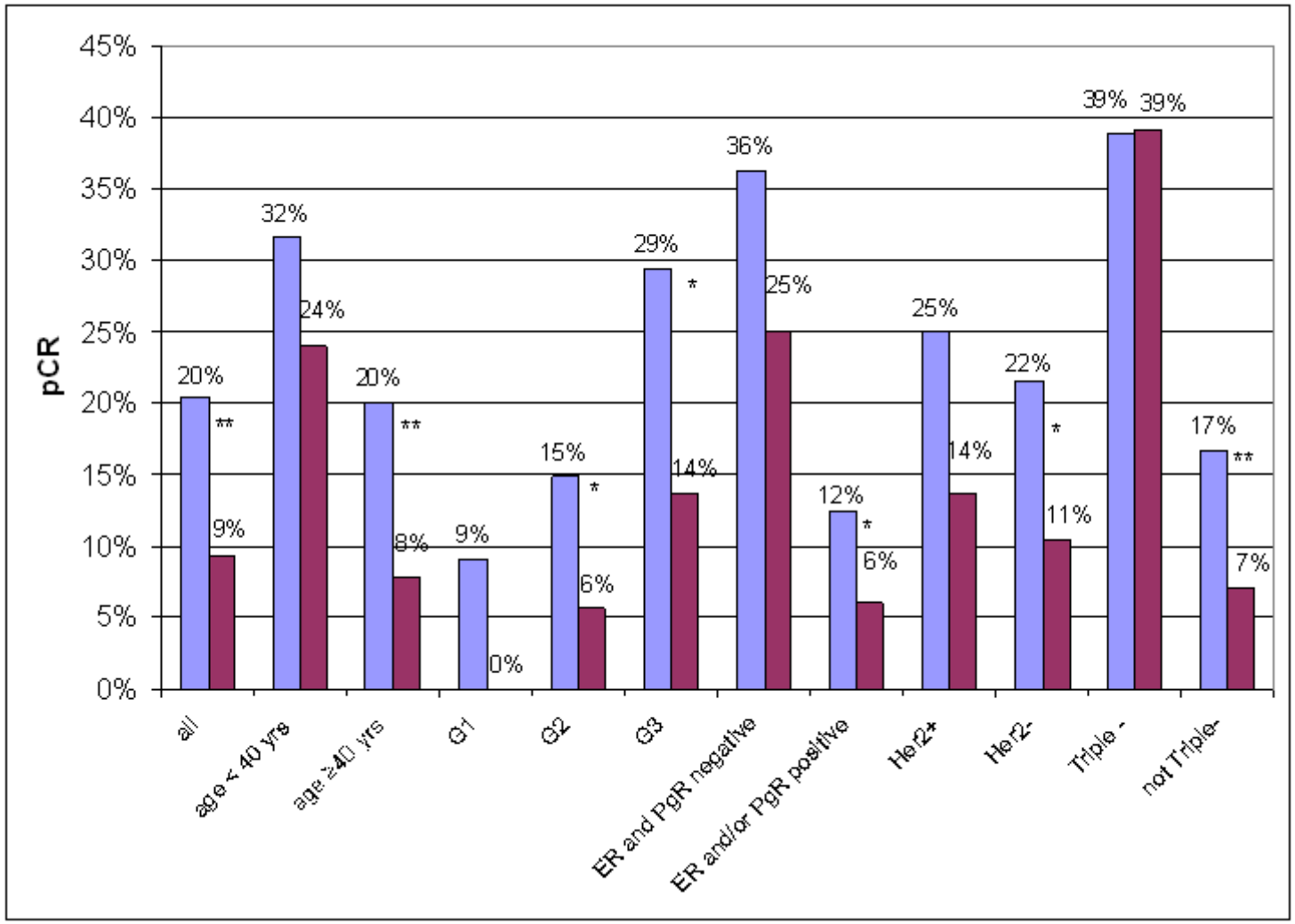

Fig 2c: Impact of grade and hormone receptor status on pCR rates in tumors with different expression of HER2

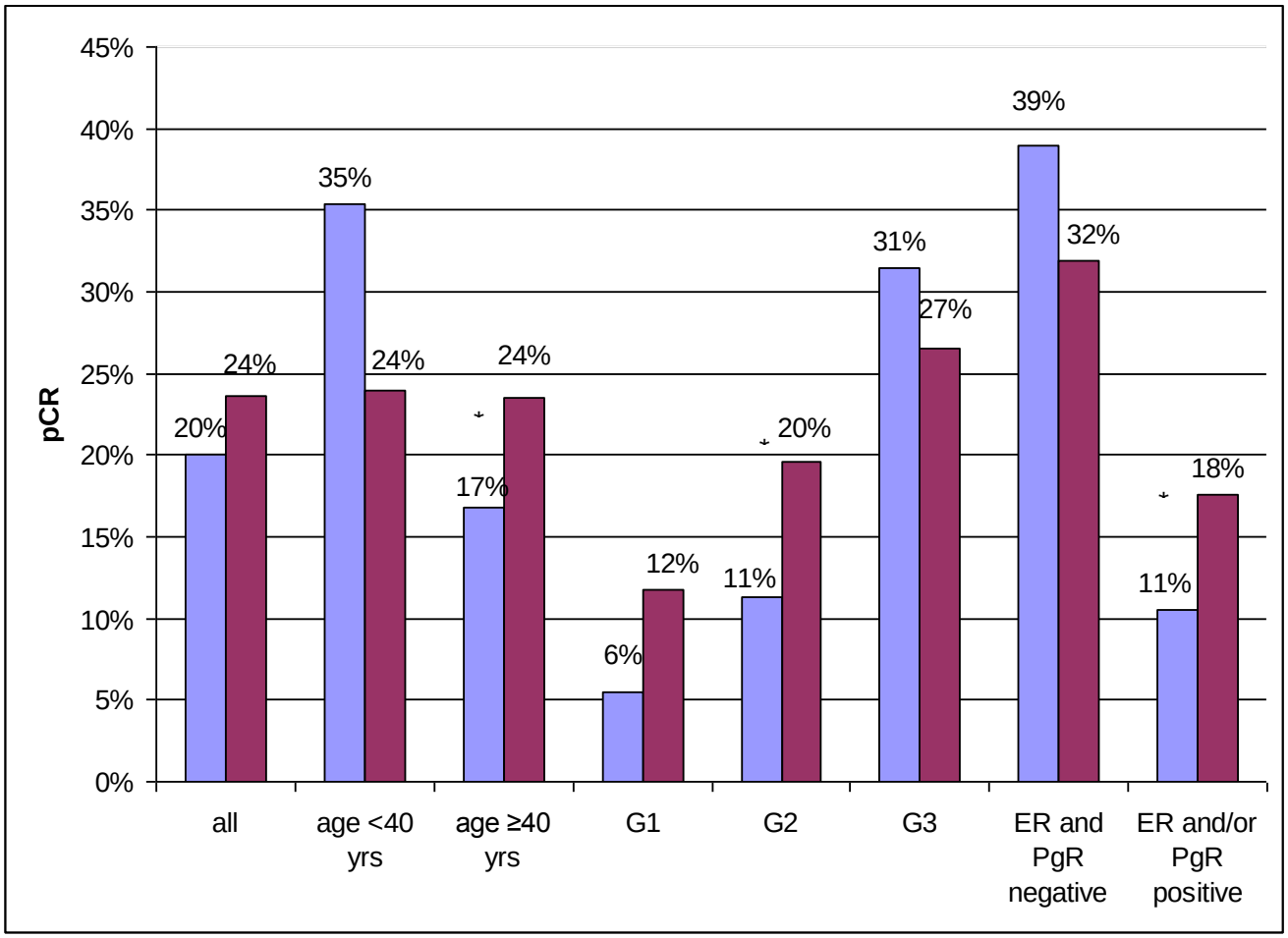

Fig 3: ROC curve