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CLINICAL TRIAL

# Loss of HER2 after HER2-targeted treatment

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## Abstract

**Purpose** HER2 expression has been reported to be discordant between primary tumor and metastatic tissue.

**Patients and methods** HER2 discordance and relation to HER2-targeted treatment was investigated in 227 patients with primary breast cancer.

**Results** HER2 discordance between primary biopsy and second biopsy after neoadjuvant or adjuvant treatment was observed in 20.7%. This discordance was related only to the use of HER2-targeted treatment: 30 of 33 (90.9%) women with downgraded HER2 expression underwent a HER2-targeted therapy, whereas in the group of patients with concordant HER2 expression, only 32 of 180 (17.8%) received HER2-targeted treatment ($p < 0.0001$). HER2 discordance was associated with reduced disease-free survival but not overall survival. In a second cohort, including patients with HER2 overexpressing tumors, trastuzumab treatment was associated with change of HER2 expression from positive to negative in 47.3% of cases. Addition of pertuzumab increased the rate of HER2 loss up to 63.2%. Notably, the interval between last HER2-targeted treatment and the time of surgical excision of the tumor after neoadjuvant chemotherapy (NACT) or the biopsy of the metachronous metastasis was associated with a significant change in HER2 expression. The median time between NACT and the time of surgical excision was 23 days (range 5–81 days) for tumors with decreased HER2 expression and 51 days (range 10–179 days) for tumors with concordant HER2 expression. Furthermore, median time between the end of adjuvant treatment and second histology of the metachronous metastases accounted for 15 days (range 2–165 days) and 478 days (range 7–2739 days) was observed in the group of patients with decreased or unchanged HER2 expression, respectively.

**Conclusion** The interval between anti-HER2 treatment and the determination of HER2 in second histology is strongly associated with HER2 expression.

**Keywords** Trastuzumab · Breast cancer · HER2

## Introduction

*HER2* gene is overexpressed in approximately 15% of primary breast cancer and HER2-targeted therapies are associated with significantly improved survival [1]. Discordance of HER2 status has been reported between primary tumor (PT) and metastatic specimen as well as between primary tumor and the remaining tumor after neoadjuvant chemotherapy (NACT) [2–4]. It has been suggested that chemotherapy and especially trastuzumab treatment might be responsible for the loss of HER2 overexpression [2, 3, 5, 6]. In contrast, early studies have not demonstrated any significant changes of HER2 status particularly in the absence of treatment [7–9]. Nevertheless, the exact mechanisms of HER2 discordance remain unknown and further investigations are needed.

In this study, we aimed to investigate the influence of HER2-targeted treatment on HER2 receptor status. The interval between end of last HER2-targeted treatment and the surgical excision of the tumor after neoadjuvant chemotherapy (NACT) or the biopsy of the metachronous metastasis was assessed and correlated with HER2 expression.

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## Patients and methods

The data of all patients with primary invasive non-metastatic breast cancer, who had been admitted to the Department of Gynecology and Obstetrics, Otto-von-Guericke University, Magdeburg, Germany, from January 2005 to December 2016, were selected by retrospective analysis. HER2 expression was compared between PT and the remaining tumor tissue after NACT, or between PT and the metachronous metastatic tissue. The tissue collected after NACT or the metastatic tissue was called "second biopsy." Of 776 women assessed for analysis, 549 were excluded because they had no second biopsy ($n=506$) or had a pathological complete response after NACT with no tumor tissue in the second biopsy ($n=43$) (Fig. 1). Cohort I consisted of 227 patients eligible for analysis. HER2 expression in core needle biopsy of the PT was compared with HER2 expression in surgically excised breast cancer tissue after NACT ($n=132$; 58.1%) or with HER2 expression in metachronous metastatic tissue ($n=95$; 41.9%). In a second cohort consisting of 296 patients with HER2 overexpressing tumors, we aimed to investigate the influence of HER2-targeted treatment on HER2 receptor expression. After exclusion of 72 women because of pathological complete response after NACT ($n=59$) or missing second biopsy ($n=13$), 205 women were eligible for analysis and 167 (81.5%) underwent a HER2-targeted treatment (Fig. 1).

### HER2 status and trastuzumab treatment

The HER2 status was assessed as already described using the HercepTest™ according to the manufacturer's instructions [10, 11]. The immunohistochemical (IHC) staining of HER2 is based on staining intensity and distribution in accordance with the ASCO/CAP guidelines [12]. The HER2 status is grouped as follows: 0, no staining; 1+, faint/barely perceptible membrane staining detected in > 10% of the tumor cells; 2+, strong, circumferential membrane staining in < 10% of tumor cells or weak-moderate circumferential membrane staining > 10% of the tumor cells; 3+, strong, circumferential membrane staining in > 10% of tumor cells. Fluorescence in situ hybridization (FISH) testing is routinely used to assess *HER2* gene amplification in cases with moderate HER2 2+ expression. Tumors evaluated for HER2 expression before 2013 were retrospectively re-evaluated using the ASCO/CAP guidelines after 2013. Based on HER2 overexpression, the patients were divided into two main groups: HER2-negative and HER2-positive (Fig. 1).

The study was prepared in accordance with the STROBE statement criteria [13]. Informed consent regarding breast cancer treatment was obtained from all individual participants included in the study before treatment. According to the statement of Research and Ethical Committee, Otto-von-Guericke University, Magdeburg, Germany, an additional individual consent was not required for this analysis. Before analysis, patient data underwent a pseudonymization, by which the personal data of the patients were translated into a specific code, allowing the future re-identification of the patients if necessary.

### Statistical analysis

The statistical calculations were performed using SPSS Version 22.0 (SPSS, Chicago, IL, USA). An association between HER2 expression and the tumor and patients' variables was evaluated using the $\chi^2$ test or Fisher's exact test. For statistical analysis, HER2 expression was considered

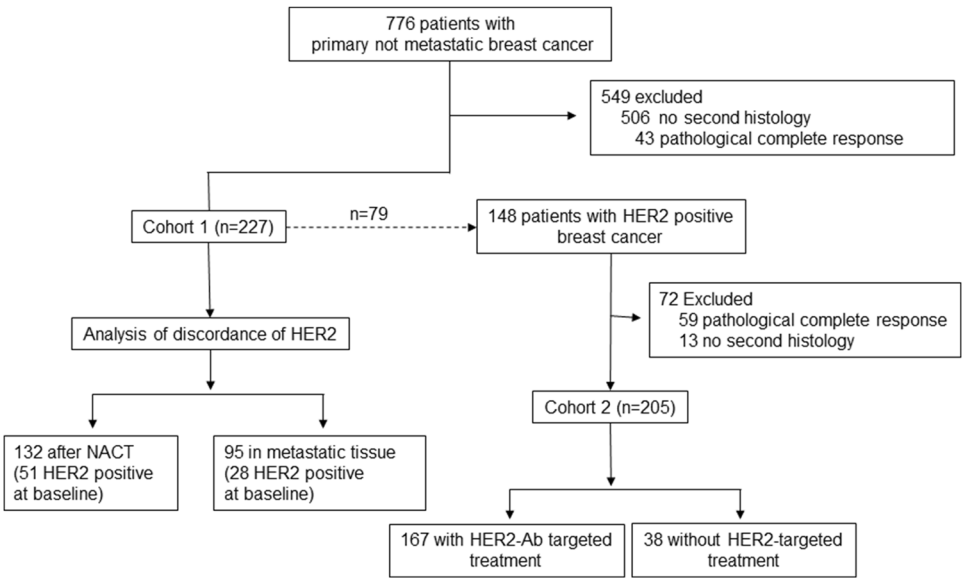


**Fig. 1** Study design

negative (HER2 expression by IHC 0, 1, and 2 with FISH ratio < 2) or positive (IHC 3 or 2 with FISH score > 2). The interval between end of the therapy and the second histological examination of HER2 was estimated after the last documented treatment and the date of the surgical excision of the tumor or the core needle biopsy of the metastasis. Disease-free survival (DFS) was defined as the period from the date of diagnosis to local and/or regional recurrence, distant metastases or death from disease, whichever occurred first. The follow-up either ends with the patient's death, the last follow-up at 15.09.2014 or the last available information in the tumor registry. The overall survival (OS) was defined as the time between primary diagnosis and death from any cause. Survival probability distribution was studied using the Kaplan–Meier method. The equality of survival curves was tested using the log-rank test. Univariate Cox proportional hazards regression analysis was used to identify significant prognostic factors and then the prognostic significance was evaluated using multivariate analysis. The statistical analyses were two-sided, and $p$ values < 0.05 were considered statistically significant.

## Results

A total of 776 patients with primary non-metastatic breast cancer were analyzed. HER2 discordance was investigated in 132 patients who received NACT and in 95 patients who developed a metachronous metastasis. In the first case, HER2 expression was compared between core needle biopsy of PT and the remaining surgically excided tumor tissue after NACT. In the case of metachronous metastasis, HER2 expression was compared between core needle biopsy of PT and the obtained metastatic tissue after adjuvant treatment. Most of the tumors ($n = 180$, 79.3%) remained concordant regarding their HER2 expression and 47 of them (26.1%) were HER2 positive (Table 1). Fourteen (6.2%) patients who were HER2 negative before treatment were HER2 positive after treatment. Another 33 (14.5%) tumors lost their HER2 positivity during treatment. In the group of 47 tumors with HER2 discordance, IHC score in the primary tumor was 0 in 12 (25.5%), 1 in 3 (6.4%), 2 (FISH negative) in 0%, 2 (FISH positive) in 8 (17.0%), and 3 in 24 (51.1%).

Next, we evaluated whether HER2 discordance is related to different clinical and pathological variables (Table 1). Patient age, tumor size, tumor type, tumor grade and Ki-67

**Table 1** Clinical and pathological characteristics of cohort I

| Variable | HER2 expression | | | | | | $p$ value |
|---|---|---|---|---|---|---|---|
| | Negative to positive | | Constant | | Positive to negative | | |
| | $n$ | % | $n$ | % | $n$ | % | |
| Total | 14 | 6.2 | 180 | 79.3 | 33 | 14.5 | |
| Age, years | 58.7 (35–76) | | 52.3 (32–88) | | 58.1 (44–91) | | 0.066 |
| Tumor size | | | | | | | |
| < 2 cm | 2 | 18.2 | 33 | 21.3 | 7 | 24.1 | 0.907 |
| > 2 cm | 9 | 81.8 | 122 | 78.7 | 22 | 75.9 | |
| LN status | | | | | | | |
| Negative | 6 | 54.5 | 67 | 45.6 | 9 | 33.3 | 0.39 |
| Positive | 5 | 45.5 | 80 | 54.4 | 18 | 66.7 | |
| Histology | | | | | | | |
| Ductal | 13 | 100 | 161 | 94.7 | 31 | 96.9 | 0.619 |
| Lobular | 0 | 0 | 9 | 5.3 | 1 | 3.1 | |
| Grade | | | | | | | |
| Low | 2 | 15.4 | 5 | 3.3 | 1 | 3.3 | 0.157 |
| Intermediate | 4 | 30.8 | 74 | 49.3 | 18 | 60 | |
| High | 7 | 53.8 | 71 | 47.3 | 11 | 36.7 | |
| Ki-67, % | 22.5 (10–35) | | 30.0 (5–90) | | 32 (10–70) | | 0.681 |
| Chemotherapy | | | | | | | |
| No | 3 | 21.4 | 38 | 21.1 | 3 | 9.1 | 0.27 |
| Yes | 11 | 78.6 | 142 | 78.9 | 30 | 90.9 | |
| HER2-directed treatment | | | | | | | |
| No | 14 | 100 | 148 | 82.2 | 3 | 9.1 | < 0.0001 |
| Yes | 0 | 0 | 32 | 17.8 | 30 | 90.9 | |

expression, LN status, and use of chemotherapy (neoadjuvant or adjuvant) were not significantly associated with change of HER2 expression. Interestingly, HER2-targeted treatment was significantly associated with HER2 receptor discordance. Thirty of 32 (93.7%) women with downgraded HER2 expression underwent a HER2-targeted therapy, whereas in the group of patients with concordant HER2 expression, 32 of 47 (68.1%) HER2 positive tumors received HER2-targeted treatment (Table 1, $p < 0.0001$).

To investigate the influence of HER2-targeted treatment on HER2 overexpression, we used a second cohort which consisted of patients with HER2 overexpressing tumors.

**Table 2** Clinical and pathological characteristics of cohort II

| | HER2 expression | | | | *p* value |
|---|---|---|---|---|---|
| | Constant | | Positive to negative | | |
| | *n* | % | *n* | % | |
| Total | 119 | 58 | 86 | 42 | |
| Age, years | 54 (26–85) | | 56 (33–96) | | 0.255 |
| Tumor size | | | | | |
| < 2 cm | 57 | 50 | 35 | 43.8 | 0.465 |
| > 2 cm | 57 | 50 | 45 | 56.3 | |
| LN status | | | | | |
| Negative | 61 | 54 | 42 | 53.8 | 1 |
| Positive | 52 | 46 | 36 | 46.2 | |
| Histology | | | | | |
| Ductal | 108 | 91.5 | 77 | 90.6 | 0.653 |
| Lobular | 7 | 5.9 | 7 | 8.2 | |
| Other | 3 | 2.5 | 1 | 1.2 | |
| Grade | | | | | |
| Low | 2 | 1.9 | 2 | 2.5 | 0.866 |
| Intermediate | 57 | 54.3 | 40 | 50.6 | |
| High | 46 | 43.8 | 37 | 46.8 | |
| Ki-67, % | 40 (5–90) | | 30 (10–80) | | 0.272 |
| Chemotherapy | | | | | |
| No | 17 | 14.3 | 5 | 5.8 | 0.067 |
| Yes | 102 | 85.7 | 81 | 94.2 | |
| HER2-directed treatment | | | | | |
| No | 34 | 28.6 | 4 | 4.7 | < 0.0001 |
| Yes | 85 | 71.4 | 82 | 95.3 | |

In this cohort, HER2 overexpression remained concordant during the treatment in 119 (58%) cases and decreased in 86 (42%) cases (Table 2). Again, only HER2-targeted treatment was associated with change of HER2 expression from positive to negative ($p < 0.0001$). Interestingly, the combination of trastuzumab and pertuzumab was associated with significant decrease in HER2 expression. Nearly two-thirds (63.2%) of HER2 positive patients who received dual blockade showed HER2 decrease (Table 3), whereas trastuzumab alone was associated with HER2 decrease in 47.3% of cases. Because of the small number of patients treated with trastuzumab and pertuzumab ($n = 19$), these results should be interpreted with caution. Only 21 (10.2%) of the 205 patients received Chemotherapy.

We were interested, whether the interval between last HER2-targeted treatment and the second histology are associated with any significant change in HER2 expression. The analysis of the interval was possible in 99 patients who underwent NACT and in 68 patients who received adjuvant treatment. The median interval between NACT and determination of HER2 expression in second histology was 23 days (range 5–81 days) for tumors with decreased HER2 expression and was significantly shorter than the interval of 51 days (range 10–179 days) for tumors with concordant HER2 expression (Table 4). Similar results were obtained for patients treated with adjuvant HER2-targeted therapy. A median interval of 15 days (range 2–165 days) between the end of adjuvant treatment and second histology was observed in the group of patients with a decrease of HER2 expression. This interval estimated 478 days (range 7–2739 days) for the group of patients with unchanged HER2 expression (Table 4). As demonstrated in Fig. 2, the rate of HER2 change from positive to negative is most common in the first 30 days after the end of HER2-targeted therapy. After this period, the rate of HER2 decrease in expression is relatively negligible (< 5%).

Notably, these data were similar for patients with HER2 IHC score of 3 or HER2 IHC score of 2 and positive FISH score.

Next, we compared patient outcome depending on HER2 concordance. Among the patients eligible for analysis with HER2 concordance, the 5-year DFS was 74.4% and was significantly lower in comparison with 5-year DFS of patients

**Table 3** HER2 concordance depend on HER2-targeted treatment

| HER2-directed treatment | Total (*n*) | HER2 expression | | | | *p* value |
|---|---|---|---|---|---|---|
| | | Constant | | Positive to negative | | |
| | | *n* | % | *n* | % | |
| No | 38 | 34 | 89.5 | 4 | 10.5 | < 0.0001 |
| Trastuzumab | 148 | 78 | 52.7 | 70 | 47.3 | |
| Trastuzumab and pertuzumab | 19 | 7 | 36.8 | 12 | 63.2 | |

**Table 4** HER2 discordance depends on Interval between end of HER2-targeted treatment and second histology

| HER2 discordance | Total (*n*) | HER2 expression | | *p* value |
|---|---|---|---|---|
| | | Constant | Positive to negative | |
| Days after HER2-directed neoadjuvant treatment | 99 | 51 (10–179) | 23 (5–81) | < 0.0001 |
| Days after HER2-directed adjuvant treatment | 68 | 478 (7–2739) | 15 (2–165) | < 0.0001 |

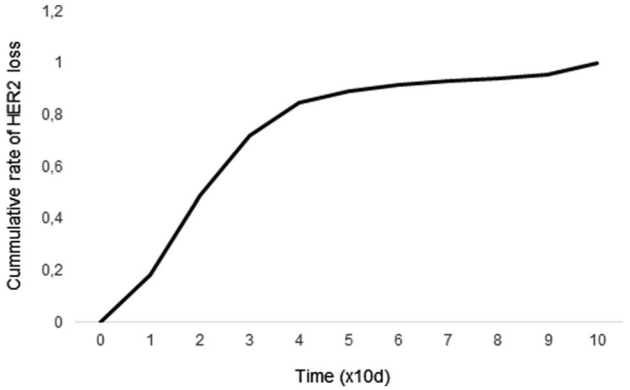


**Fig. 2** Cumulative incidence of HER2 discordance

with HER2 discordant status (59.6%) (Fig. 3a, $p = 0.030$). The 5-year OS of the patients based on the HER2 status was as follows: 82.2% for HER2 concordant patients and 80.9% for HER2 discordant patients (Fig. 3b, $p = 0.447$). This difference was statistically not significant. After adjustment for interval between anti-HER2 treatment and second biopsy, age, hormone receptor status, tumor size, lymph node status, and tumor grade, multivariate analysis regarding DFS (Table 5) revealed significantly increased hazard due to HER2 discordance (hazard ratio (HR) = 2.73; 95% CI 1.43–5.20, $p = 0.002$). Positive lymph nodes and low grade of differentiation remained unfavorable factors for DFS (HR 3.34; 95% CI 1.72–6.49, $p = 0.0001$) and (HR 3.87; 95% CI 2.02–7.39, $p = 0.0001$), respectively (Table 5). Regarding OS only, lymph node metastases and low grading were associated with unfavorable prognosis (HR 4.65; 95% CI 1.72–12.60, $p = 0.002$) and (HR 3.13; 95% CI 1.23–7.93, $p = 0.016$), respectively (Table 5). HER2 discordance was not associated with OS.

## Discussion

In this large retrospective study, we found that HER2-targeted treatment is the most important factor responsible for HER2 discordance between PT and metastatic tissue or tissue after NACT. In general, we observed a low rate of HER2 discordance. The rate of HER2 gain or loss was observed in 6.5% and 14.2%, respectively. This is in agreement with early studies who have demonstrated a high rate of HER2 concordance [7–9]. Loss of HER2 expression has been most commonly observed than gain of HER2 expression [4]. The median discordance rates of HER2 between PT and their paired metastatic lesions were estimated to be 10% and range between 0 and 60% [2, 4] and is in a high agreement with our data. The changes in tumor expression of HER2 were not associated with any clinical and pathological characteristics and have been confirmed by others [4, 7].

Nevertheless, there is a growing body of evidence suggesting that NACT and especially anti-HER2 treatment may significantly influence the loss of HER2 expression [2, 3, 5, 6]. Mittendorf et al. have found that 32% post-treatment tumors lost their HER2 positivity after such treatment [6]. In a retrospective cohort study with 21 755 Japanese patients

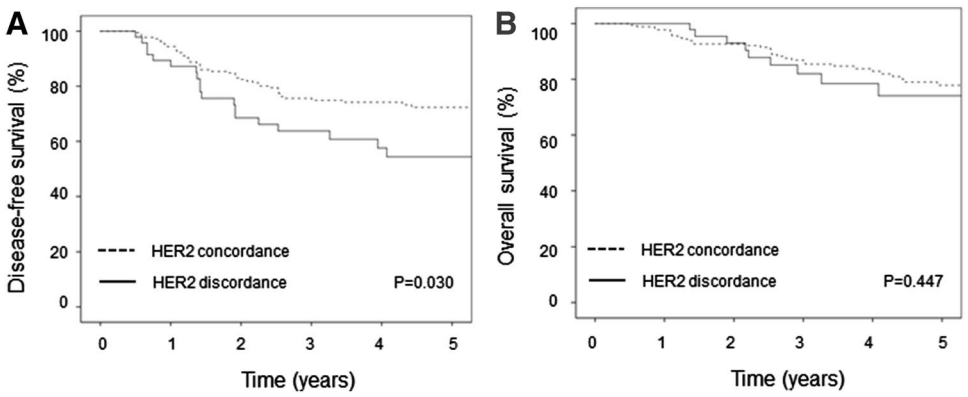


**Fig. 3** Kaplan–Meier analysis of disease-free survival (**a**) and overall survival (**b**) of patients with breast cancer depend on HER2 concordance

**Table 5** Multivariate analysis of prognostic factors for disease-free and overall survival for patients with breast cancer

| Variable | DFS | | OS | |
|---|---|---|---|---|
| | Hazard ratio for events (95% CI) | *p* value | Hazard ratio for events (95% CI) | *p* value |
| HER2 discordance | | 0.002 | | 0.136 |
| No | 1 | | 1 | |
| Yes | 2.73 (1.43–5.20) | | 2.02 (0.80–5.06) | |
| Interval between HER2-targeted therapy and biopsy | 1 | 0.083 | 1 | 0.06 |
| | 1.70 (0.93–1.30) | | 2.15 (0.97–7.78) | |
| Age at diagnosis (years) | 1 | 0.471 | 1 | 0.586 |
| | 1.22 (0.71–2.10) | | 0.82 (0.39–1.70) | |
| Tumor size (mm) | 1 | 0.177 | 1 | 0.364 |
| ≤ 20 | 0.56 (0.24–1.30) | | 0.592 (0.19–1.84) | |
| > 20 | | | | |
| Lymph node status | 1 | 0.0001 | 1 | 0.002 |
| Negative | 3.34 (1.72–6.49) | | 4.65 (1.72–12.60) | |
| Positive | | | | |
| Histological grade | 1 | 0.0001 | 1 | 0.016 |
| 1, 2 | 3.87 (2.02–7.39) | | 3.13 (1.23–7.93) | |
| 3 | | | | |
| Hormone receptor status | 1 | 0.753 | 1 | 0.24 |
| Negative | 0.91 (0.51–1.64) | | 0.62 (0.28–1.38) | |
| Positive | | | | |

with breast cancer, loss of HER2 positivity was observed in 20.4% after NACT [2] and was confirmed by us, showing that trastuzumab treatment alone changes HER2 positivity in 47.3% of cases, whereas treatment with trastuzumab and pertuzumab was associated with loss of HER2 positivity in 63.2% of HER2 positive patients. The data regarding HER2 loss and dual blockade are to the best of our knowledge the first in the literature and should be confirmed by future investigation. Notably, the NACT alone without HER2-targeted treatment was not significantly associated with decreased HER2 positivity. In a recent report, only NACT was associated with a significant loss of HER2 expression, but not the anti-HER2 treatment [14, 15]. The direct comparison with these results is difficult due to the missing data regarding the interval between anti-HER2 treatment and second histology.

In the present study, only 85.1% of HER2-positive patients received anti-HER2 treatment, due to increased patients' age, comorbidity, lack of recommendation, and/or refusal by the patients or their relatives. However, we were able to demonstrate for the first time the importance of this interval for change of HER2 expression. The median interval between NACT and determination of HER2 expression in surgical excided specimen was 23 days (range 5–81 days) for tumors with decreased HER2 expression and was significantly shorter than the interval of 51 days (range 10–179 days) in the group of tumors with concordant HER2 expression. This observation was confirmed for tumors adjuvantly treated with anti-HER2 therapy. The median interval between the end of adjuvant HER-targeted therapy and the determination of HER2 in a metastatic tissue was estimated to be 15 days (range 2–165 days) and was significantly shorter than the interval of 478 days (range 7–2739 days) for the group of patients with unchanged HER2 expression. The exact mechanisms of HER2 receptor downregulation are very poorly understood yet. It has been reported that trastuzumab may induce internalization and degradation of HER2 [16, 17]. Nevertheless, new reports suggest that HER2-targeted treatment including trastuzumab and pertuzumab only in limited extent reduced HER2 expression by inducing internalization [18–20]. More possibly, there are further not well-understood mechanisms of HER2 trafficking [21] and they need more investigations. However, the combination of trastuzumab and pertuzumab is associated with synergistic increase of HER2 internalization and degradation [18, 19] and was confirmed by us. In this context, the most accurate method to determine HER2 expression after NACT may be the gene amplification using FISH test. Notably, FISH testing is associated with less discordance than IHC method [2, 22]. In this way, changes in HER2 expression influenced by HER2 internalization and degradation may be avoided. Another possible mechanism of HER2 loss is the clonal selection of the primary tumor. It has been suggested that an intratumoral heterogeneity of HER2 expression exists in breast [23] and other HER2-expressing cancers [24]. Thus, treatment with HER2-targeted therapy might be associated with intratumoral selection of HER2-negative tumor cells,

leading to advantageous growth of HER2-negative cells and their superiority to the HER2-positive cells. "HER2 tumor heterogeneity was investigated in 25 of our patients (data not shown). Tumor heterogeneity was observed in only 2 (8%) of tumors. However, the importance of HER2 heterogeneity for HER2 loss should be further investigated."

Nevertheless, our data suggest that the interval between the end of neo- and/or adjuvant treatment with anti-HER2 agents and HER2 evaluation in a post-treatment tumor should be always considered. In a case of discrepancy in HER2 expression between PT and metastatic tissue, the actual guidelines recommend the use of HER2 status in metastatic tissue [4]. Moreover, HER2 discordance is associated with poor survival in comparison with patients who retained their HER2 status [2, 6]. These findings were confirmed in our study. However, HER2 discrepancy was associated with decreased median DFS but not OS. Thus, in a case of loss of HER2 positivity in metastatic specimens, we recommend a prolonged interval and re-biopsy instead of avoiding further HER2-targeted therapy. A biopsy in the first 30 days after HER2-targeted therapy is associated with high level of discordance and should be considered by the physicians. Once again, beside the intratumoral heterogeneity, selection of different resistance clones, and inconsistencies in IHC methods [4], treatment interval after HER2-targeted therapy seems to be one of the most important factors determining discordant HER2 expression. The prolongation of this interval may be associated with a re-expression of HER2.

One limitation of our study is its retrospective character with all associated bias. Other limitation is the lack of central pathological review regarding HER2 status. Furthermore, in a case of strong HER2 expression (3+), FISH test was not performed to confirm a *HER2* overexpression.

The strengths of the present study are (i) large sample size; (ii) high level of external validity as trastuzumab treatment was investigated under real clinical conditions (e.g., the study population was similar to the general population, and the exclusion criteria were kept to a minimum); (iii) during the study period, HER2 expression was determined under similar conditions including fixation, slides preparation, and observer analysis, thus excluding performance bias [4].

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## Compliance with ethical standards

**Conflict of interest** The authors have no conflicts of interest.

**Ethical approval** The experiments comply with the current laws of Germany and were performed according to the good clinical practice (GCP) guidelines.

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