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Discordances in ER, PR and HER2 receptors after neoadjuvant chemotherapy in breast cancer

Cancer Treatment Reviews 2011; 37(6): 422-430

Introduction

Neoadjuvant systemic treatment is increasingly used for breast cancer and there is a trend for tailored therapies currently based on the presence of estrogen (ER), progesterone (PR) and Human epidermal growth factor receptor 2 (HER2)-receptor in the tumor. Little is known about the influence of neoadjuvant chemotherapy (NAC) on those receptors and the possible consequences for subsequent adjuvant systemic therapy.

ER, PR and HER2 were the first biomarkers recommended for routine clinical use [1]. They have shown mixed prognostic and predictive values, depending on the treatment given [2]. Although ER expression is known to be a strong predictor of response to endocrine therapy [3], it is of negative predictive value with respect to response to chemotherapy resulting in lower pathological complete response (pCR) rates after NAC [4-7]. HER2 is overexpressed in 10-25% of breast cancers and is associated with a more aggressive form of cancer [8]. HER2 amplification is recognized to be of strong predictive value in the treatment with HER2 blocking agents; patients with HER2 positive breast cancer have better responses and higher pCR rates are reported when trastuzumab is added to NAC [9-12].

Information about the ER, PR and HER2 is traditionally obtained by means of immunohistochemistry (IHC) as a quantitative measurement of receptor expression. The ER, PR and HER2 status is dichotomized as positive or negative based on a cutoff point in the percentage of tumor cells stained by this method (quantitative expression). According to current international guidelines, endocrine treatment is indicated in all patients with a positive hormone receptor (HR) status, which is defined as ER positive and/or PR positive [13]. Despite the importance of establishing the receptor status of tumors and the widespread use of IHC for their assessment, standardization has not yet been achieved and a wide variety of scoring systems and cut-off points have been used. Since a few years, determination of the HER2 amplification using in situ hybridization methods (ISH) is the golden standard for HER2 determination [14]. Most commonly used methods for the gene amplification are Fluorescent in Situ Hybridization (FISH), Chromogenic In Situ Hybridization (CISH) and Silver In Situ Hybridization (SISH).

The accuracy of the core needle biopsy (CNB) for determination of the receptor status in breast cancer patients has been extensively studied and can be used with confidence for ER and HER2 determination. The results for PR are more variable and need to be used with caution [15]. Adjuvant treatment is mainly based on the
immunohistochemical findings of the HR and HER2 status on the core needle biopsy before any treatment is given. With the growing use of NAC and trastuzumab, it is important to know whether these therapies modulate these markers. A change in HR or HER2 status would have important therapeutic, prognostic and financial consequences for both patients and health care providers.

Data on the influence of NAC and trastuzumab on the expression status of ER, PR and HER2 are limited and an overview of the results and therapeutic consequences is lacking. In this article, all published data available on these important issues are reviewed.

Materials and methods

In May 2010 a systemic literature search in PubMed was performed using the following terms: ‘neoadjuvant therapy’ OR ‘neoadjuvant chemotherapy’ OR ‘primary systemic therapy’ AND ‘trastuzumab’ OR ‘herceptin’ AND ‘breast cancer’ AND ‘steroid receptors’ OR ‘hormonal receptors’ OR ‘hormonal status’ OR ‘biological receptors’ OR ‘HER2’ OR ‘biomarkers’ OR ‘pathology’ AND ‘change’ OR ‘comparison’ or ‘concordance’ and MESH terms ‘neoadjuvant therapy’ AND ‘trastuzumab’ AND ‘breast neoplasms’ AND ‘biological markers’ OR ‘molecular markers’ OR ‘receptors’, ‘steroid’ OR ‘receptor’, ‘erbB-2’ OR ‘genes’, ‘erbB-2’ AND ‘biopsy’, ‘fine-needle’ AND ‘change’ OR ‘modulation’ OR ‘comparison’. Additional literature was found by means of references in previous found articles, or by using the link “related articles” in PubMed. Only articles from which full text could be obtained were included for reviewing.

Studies were included in which patients received NAC with or without trastuzumab, underwent a core needle biopsy prior to treatment and an operation afterwards. Studies with neoadjuvant endocrine therapy were excluded. We extracted data regarding type of neoadjuvant chemotherapy, study design (retrospective or prospective), study size and number of tissues available for final pathologic assessment after neoadjuvant treatment, pathological methods and ER/PR/HER2 measurement results of the core needle biopsy and operation material. We rated trials primary by study design (prospective vs. retrospective) and secondary by sample size. We distinguished results for changes in receptor expression and/or status, depending on how the results were presented by the author. Regarding the HER2 receptor, we also distinguished between results obtained by IHC and/or FISH. Cut off points for positive receptor status, study design, sample size and chemotherapy schedules of the references are described in the tables.
Results

Initial search led to 139 (regular)-241 (MESH) references. When combining these search terms with ‘change’ OR ‘comparison’, the total number of results decreased to 30 and 24, respectively. By using the link related articles in PubMed, an additional number of 101 articles were found. Five trials were included that were found as a reference in other articles. Trials that did not compare the ER and/or PR and/or HER2 receptors of the biopsy pre-NAC with the resection material after NAC were excluded for this review, remaining 32 relevant studies. When the search was extended for the effects of NAC combined with trastuzumab, seven trials were obtained. Two of those were excluded because of the trial design (pilot study), one article was in Italian and one was excluded because of the methodology of HER2 measurement (in serum instead of tissue).

Concordance of the ER and PR receptors after neoadjuvant chemotherapy (NAC)

We reviewed the influence of NAC on ER and PR receptors expression and status (table 1A and table 1B). ER expression levels before and after NAC were investigated in 10 trials [6, 16, 17-24]. Trials that reported a change in the expression or status of ER and/or PR are depicted in table 1A. Four trials detected a change in ER expression [6, 17-19]. In a large retrospective study reported a 9% change in ER expression in 399 patients, decrease and increase in expression equally divided. In a small prospective study ER expression changed in 46% of 31 patients, consisting of a 42% decrease [19]. Table 1B summarizes the results of all trials that did not found changes in ER and/or PR expression or status after NAC. Six trials concluded that ER expression had not been altered after NAC, of which three were prospectively performed with smaller patient numbers (N = 25-56 pts) [16, 20-24].

NAC induced change in ER status was studied in 15 trials of which eight reported a change after NAC (Table 1A) [6], [25-31]. In the largest prospective study, a significant (P = 0.02) switch to an ER negative status occurred in 14% of the 214 patients [25]. Results of other large studies ranged between a 2.5% change in ER status in a prospective trial (N = 118) to a 14.9% change in a retrospective trial (N = 459) [27, 29]. Small retrospective studies reported discordances in ER status between 7% and 17% [6, 28, 30-31]. Seven studies concluded that NAC does not affect the ER status (table 1B) [21-22, 32-36]. Two studies reported no change at all, [22, 35] whereas five small prospective trials reported non-significant changes in ER receptor status after NAC [21, 32, 34-36].
PR expression has been retested after NAC in seven trials [6, 16-17, 19, 22-24]. Change in PR expression was reported in four of these trials (table 1A), with a 58% decrease in PR expression being the highest reported change in the only prospective trial (N = 31). 19 Larger retrospective trials found discordances between 26% and 42%, mainly consisting of a decrease in PR expression [6, 17]. A significant decrease (P = 0.007) in PR expression was also reported in a retrospective trial with 86 patients, results were not further specified [16]. Three other trials depicted in Table 1B concluded a good concordance in PR expression after NAC [22-24].

The influence of NAC on the PR status was tested in 13 trials, of which eight trials reported a discordant PR status after NAC (table 1B) [6, 25-31]. Four of them were large prospective trials (N = 73, 118, 173 and 191) and changes in PR status of 22%, 5.9%, 15.6% and 51.7%, respectively were found [25-28]. Five trials reported no significant change in PR status after NAC, of which four trials were prospectively designed (table 1B) [21-22, 33-34 and 36]. All of these trials were performed with a small number of patients (N = 23-56) compared to the prospective trials that concluded that PR status does change after NAC.

Reported changes in ER or PR receptors consisted mostly of a decrease in ER/PR expression or a switch to a negative status. Overall, studies that concluded NAC modulates the content of receptor expression or status consisted of larger sample sizes compared to those which did not.

Eight out 24 trials tested the influence of NAC on the HR status (ER and/or PR positive status). Four out of eight studies conclude that HR status changes after NAC (table 1A) [27, 29, 31, 37]. Reported discordancess in HR status range between 8% in a prospective cohort of 118 patients to 33% in a small retrospective study (N = 18) [27, 31]. The two largest retrospective trials consisting of 459 and 420 patients report a significant change in HR status of 16% and 23%, respectively. [29, 37] Four studies conclude that NAC does not change the HR status (table 1B). Three of those studies are prospective trials, although all with small sample sizes of 23-56 patients [17, 21-22 and 34]. The reported changes in HR status after NAC can equally be distributed between a positive and negative switch in HR status.

**Concordance of the HER2 receptor after neoadjuvant chemotherapy**

Seven out of 19 studies verified their IHC assessments by means of FISH. Six of these trials found no significant change in HER2 amplification after chemotherapy (table 2B) [22, 26, 28, 34, 38-39]. Only one large, retrospective study (N = 368) described
a change in the HER2 status in 9.5% of the patients; in 6% of the patients there was a HER2 positive switch and in 3.5% a negative switch of the HER2 status [29].

The influence of NAC plus trastuzumab on HER2 has been investigated in three studies (table 3). All used FISH to determine the HER2 status, two showed a loss of HER2 amplification in 32% (N = 25) and 43% (N = 23) of the patients with residual disease [40-41]. The third study reported a 12% change in HER2 status by IHC but these changes were not confirmed with FISH [42].

Discussion

With NAC becoming more common as primary treatment for breast cancer patients, questions about the stability of the ER, PR and HER2 receptors status after NAC need to be addressed to optimize future tailored adjuvant therapy. This paper reviews 32 trials that investigated the influence of NAC with or without trastuzumab on the ER, PR and HER2 receptors in breast cancer.

Concordance of ER, PR and HER2 without NAC

Without NAC, little discordance in ER (concordance 98.2%), PR (concordance 85%) and HER2 (concordance 98.8%) status between core needle biopsy and resection material have been reported probably caused by the following confounders. Firstly, it is generally known that breast cancer is a heterogeneous disease with intratumoral heterogeneity. Especially PR tends to be concentrated more diversely in the tumor [43]. Secondly, immunohistochemical procedures can be modulated by variation in tissue processing and fixation [37, 44-45]. A tendency for upgrading scoring of ER expression in core needle biopsies has been described and may be due to better fixation of the CNB compared to the surgical specimen [15]. Thirdly, intra- and inter-observer variability can result in differences in receptor status between CNB and the resection material. Comparison of centrally (pathology review) versus locally assessed ER, PR and HER2 receptors revealed discordant results in a substantial proportion of patients [46-47].

Further efforts in defining reproducibility and accuracy are an important priority. An updated guideline from the American Society of Clinical Oncology (ASCO) in order to improve the accuracy of IHC ER and PR testing in breast cancer is recently published [48]. This ASCO guideline recommends a cut off value of ≥1% ER- or PR-positive tumor cells, while most institutes currently use a cut off value of ≥10% ER- or PR-positive tumor cells to predict a response to hormonal therapy.
Concordance and discordance of ER, PR and HER2 after NAC

With NAC, we found that discordance in ER, PR and HER2 between core needle biopsy and resection material are more evident than the reported discordance in patients not treated with NAC. Although the studies reviewed are quite heterogeneous regarding study method, study design and outcome measurements, we believe that these discordances can only partly be explained by the above-mentioned confounders and are most likely due to the direct effect of the chemotherapy.

A change of 8.33% in HR status after NAC was reported [27, 29, 31 and 37]. Studies that reported a good concordance of the HR status after NAC were performed with relatively lower number of patients (N = 26-56) compared to the studies that found significant changes (N = 18-459). This might have prevented their results to become statistical significant. PR receptor was more discordant compared to the ER receptor. These results do indicate though, that the HR status of the core biopsy pre-NAC cannot reliably be used for further adjuvant systemic treatment decisions.

HER2 seems to be more stable than the hormone receptors during NAC. Discordance was only reported in one of the seven trials that tested HER2 using FISH [39]. In contrast, if NAC was combined with trastuzumab, up to 43% of the patients showed no HER2 amplification in the residual tumor [40, 41]. Since this is a relatively new treatment modality and the number of studies is low, more studies are needed to confirm these results. Nevertheless, the high percentage of 43% indicates that retesting of HER2 status in the residual tumor cells should be considered if trastuzumab was used in the neoadjuvant setting. It cannot be excluded that dose intensity, cumulative dose and each individual cytostatic agent might contribute to changes in receptor status. However, these changes are difficult to evaluate, since various combination and schedules have been used in the reviewed trials.

Explanations for discordances

Possible mechanisms for a change in receptor status or expression in breast cancer cells caused by chemotherapy are:

(1) Targeting chemosensitive tumor cells leaves insensitive tumor cells with different biology behind in the residual disease.

(2) Change in receptor status and biology as a survival mechanism of tumor cells, leading to resistance of a specific therapy.
(3) Regression to a positive hormone receptor status under the influence of chemotherapy, since all cells are originally derived from well differentiated hormone receptor positive breast cancer cells [37].

(4) Lower circulating levels of estrogens caused by ovarian insufficiency during or after chemotherapy in premenopausal women [49] might cause downregulation of the estrogen and/or progesterone receptor of the tumor leading to estrogen-independent growth. Postmenopausal women may also obtain some additional endocrine effect from suppression of the adrenal glands due to chemotherapy and steroids [50].

As the expression of ER, PR and HER2 are highly dependent on each other, modulating one receptor with NAC can change the expression of other receptors as well [51, 52]. Except for one [26], all authors that tested both hormone and HER2 receptors after NAC concluded that either chemotherapy does change both hormone and HER2 receptors, [22, 24, 29, 32, 34, 36] or it does not change these receptors at all [16-17, 27-30]. As expected, these studies demonstrated that in case of an increase in ER expression, HER2 expression decreased and the other way around. Loss of HER2 amplification was associated with an increased ER status in patients treated with NAC and trastuzumab as well [40]. These combined changes support the theory that receptor changes after NAC are induced by NAC and not random changes due to heterogeneity, laboratory procedures or observer variability.

**Predictive and prognostic value of a changed receptor**

Little is known about the predictive or prognostic value of a changed receptor status. A few investigators tried to correlate changes to treatment response, but discordant conclusions were drawn [18, 26-27]. A positive switch of the HR-status could be an indicator for a better outcome and indeed was significantly correlated with better OS and DFS in patients that were treated with adjuvant endocrine therapy compared to those with a positive switch who were not [29, 37]. The value of further adjuvant treatment with trastuzumab should also be evaluated in patients treated with neoadjuvant trastuzumab.

**Discordances after neoadjuvant hormonal therapy and in metastatic lesions**

Neoadjuvant endocrine therapy is an upcoming treatment modality, especially for elderly breast cancer patients with a high level of hormone receptor expression. Neoadjuvant treatment with both tamoxifen and aromatase inhibitors has been
associated with a decreased ER and PR expression \[20, 54-55\]. Downregulation of the HER2 receptor status in as many as 41\% of the HER2 positive patients treated with an aromatase inhibitor without a HER2 blocking agent has been described, which was related to treatment response \[56\]. Discordance of the ER and PR receptors status between metastatic lesions and the primary tumor has been reported in 15-40\% of patients \[57\]. Discordance of the HER2 receptor status is less common, but changes between 5\% and 13.6\% have been reported \[44, 57-58\]. Patients with a change in receptor status of metastatic lesions seem to have a poor survival, possibly due to inappropriate use of targeted therapies or tumor dedifferentiation \[58\]. Re-biopsy of metastatic lesions to optimize therapy was recently recommended by investigators at the latest ASCO meeting in June 2010 based on discordant results in their trials \[59, 60-61\].

**Summary**

Chemotherapy seems to affect the receptor status of the primary tumor. Reported changes in ER, PR and HER2 receptors are more evident in patients treated with neoadjuvant chemotherapy and trastuzumab compared to those who are not. Chemotherapy might directly or indirectly change the biology of tumor cells, or might cause a selection of resistant tumor cells in the residual disease. A change in receptor status might have important clinical consequences for adjuvant systemic treatment. We recommend that retesting of the receptor status in residual disease after NAC should be considered in situations where this might be of clinical relevance. Particularly, ER/PR negative tumors since these are more sensitive to NAC and therefore theoretically most changes can be expected. Furthermore, initially ER or PR weak positive tumors, because any change in the tumor cells due to chemotherapy can easily modulate the hormone receptor status. Finally, we also recommend retesting the HER2 status with ISH in patients who have been treated with NAC and trastuzumab. Our findings underscore the importance of a central pathology review in multicenter studies.

In conclusion, NAC seems able to change ER and PR receptors expression and status. HER2 amplification appears to be more stable but might be modified when trastuzumab is added to NAC. Until more comparable studies are done, retesting of the hormone and HER2 receptors should be considered in certain situations to optimize adjuvant systemic therapy.
References

15. Arnedos M et al.: Discordance between core needle biopsy (CNB) and excisional biopsy (EB) for estrogen receptor (ER), progesterone receptor (PgR) and HER2 status in early breast cancer (EBC). Ann Oncol, 20 (2009), pp. 1948–1952


Part I: Reliability and optimization of prognostic factor evaluation in breast cancer

Discordances in ER, PR and HER2 receptors after neoadjuvant chemotherapy in breast cancer

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44. Amir E, Clemons M. Should a biopsy be recommended to confirm metastatic disease in women with breast cancer? Lancet Oncol, 10 (2009), pp. 933–935
45. Gwon AM. Current issues in ER and HER2 testing by IHC in breast cancer. Mod Pathol, 21 (Suppl. 2) (2008), pp. S8–S15
Table 1. Overview of studies that concluded NAC changes hormone receptors.

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>ER</th>
<th>PR</th>
<th>HR status</th>
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<tr>
<td>prospective trials</td>
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<tr>
<td>Taucher et al. (2003)</td>
<td>$N = 191$ pts</td>
<td>14% decrease in ER pos status ($P = 0.02$)</td>
<td>517% decrease in PR pos status ($P = 0.0005$)</td>
<td>Change: Pos to Neg</td>
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<td></td>
<td>Control group: 236 pts</td>
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<td>Change: Pos to Neg</td>
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<td></td>
<td>Cut off value: $\geq 10%$</td>
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<td></td>
<td>NAC: CMF or FEC</td>
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<tr>
<td>Kasami et al. (2008)</td>
<td>$N = 173$ pts</td>
<td>11% change in ER status vs. 6.8% change in control group ($P = 0.234$)</td>
<td>15.6% change in PR status vs. 77% change in control group ($P = 0.045$)</td>
<td>Change: Pos to Neg</td>
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<td></td>
<td>Control group: 117 pts</td>
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<td>Change: Pos to Neg</td>
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<td></td>
<td>Cut off value: $\geq 10%$</td>
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<td></td>
<td>NAC: 78% EC</td>
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<tr>
<td>Burcombe et al. (2005)</td>
<td>$N = 118$ pts</td>
<td>2.5% change in ER status</td>
<td>5.9% change in PR status</td>
<td>8% change in HR status</td>
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<td></td>
<td>No control group</td>
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<td></td>
<td>Cut off value: Allred score $\geq 3$</td>
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<tr>
<td></td>
<td>NAC: 6× anthracycline</td>
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<tr>
<td>Shet et al. (2007)</td>
<td>$N = 73$ pts</td>
<td>13% change in status</td>
<td>22% change in status</td>
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<td></td>
<td>No control group</td>
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<td></td>
<td>Cut off value: $\geq 5%$</td>
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<tr>
<td></td>
<td>NAC: 3–6× anthracycline</td>
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<tr>
<td>Makris et al. (1999)</td>
<td>$N = 31$ pts</td>
<td>46% change in expression ($P = 0.04$)</td>
<td>58% change in expression</td>
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<tr>
<td></td>
<td>Control group: $N = 20$ pts</td>
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<td></td>
<td>Cut off value: Allred score $\geq 3$</td>
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<td></td>
<td>NAC: Mitomycin + mitoxantrone</td>
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<tr>
<td>Retrospective trials</td>
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<tr>
<td>Hirata et al. (2009)</td>
<td>$N = 459$ pts</td>
<td>14.9% change in status</td>
<td>29.1% change in status</td>
<td>16% change in HR status</td>
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<tr>
<td></td>
<td>No control group</td>
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<td></td>
<td>Change: 8.2% Pos to Neg, 7.9% Neg to Pos</td>
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<td></td>
<td>Cut off value: $\geq 10%$</td>
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<tr>
<td></td>
<td>NAC: anthracyclines + taxane</td>
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<tr>
<td>Study</td>
<td>Methods</td>
<td>ER</td>
<td>PR</td>
<td>HR status</td>
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</tbody>
</table>
| Tacca et al. (2007)<sup>1</sup> | N = 420 pts  
Control group: N = 100 pts  
Cut off value: ≥ 10%  
NAC: different schedules | 23% change in HR status compared  
to 3% change in control group  
Change: 42% Neg to Pos, 13% Pos to Neg  |
| Colleoni et al. (2004)<sup>6</sup> | N = 255 pts  
No control group  
Cut off value: ≥ 10%  
NAC: anthracycline + taxane | Premenopausal pts: 9% change in expression  
Postmenopausal pts: 9% change in expression  
8% change in status  
Change: 4% Pos to Neg, 3% Neg to Pos  
7% change in status  
Change: 4% Pos to Neg, 3% Neg to Pos  | Premenopausal pts: 26% change in expression  
24% change in status  
Change: 17% Pos to Neg, 7% Neg to Pos  
Postmenopausal pts: 30% change in expression  
23% change in status  
Change: 4% Neg to Pos, 4% Neg to Pos  |
| Neubauer et al. (2008)<sup>20</sup> | N = 87 pts  
No control group  
Cut off value: ≥ 10%  
NAC: anthracycline or taxane | Change: 43% Neg to Pos, 57% Pos to Neg  | Change: 19% Neg to Pos, 81% Pos to Neg  |
| MacGrogan et al. (1996)<sup>9</sup> | N = 86 pts  
No control group  
Cut off value: ≥ 10%  
NAC: 3× EVM + 3× MTV | No significant difference in expression  
Change in expression (P = 0.007)  | Change: decrease  |
| Piper et al. (2004)<sup>15</sup> | N = 35 pts  
Control group: N = 35 pts  
Cut off value: unknown  
NAC: 4× AC or 4× TAC | 33% change in expression  
42% change in expression  | 5.7% change vs. 5.7% change in control group  |
| Jain et al. (1996)<sup>11</sup> | N = 18 pts  
No control group  
Cut off value: ≥ 10%  
NAC: anthracyclines | 17% change status  
Change: 67% Neg to Pos, 33% Pos to Neg  | 22% change in status  
Change: all Pos to Neg  | 33% change in HR status  
Change: equally divided  |
Table 1B. Overview of studies that concluded NAC does not change hormone receptors.

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>ER</th>
<th>PR</th>
<th>HR status</th>
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</thead>
<tbody>
<tr>
<td><strong>Prospective trials</strong></td>
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<tr>
<td>Makris et al. (1997)</td>
<td>N = 128 pts</td>
<td>91.5% concordance</td>
<td>75.5% concordance</td>
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<tr>
<td>No control group</td>
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<tr>
<td>Cut off value: Allred score $\geq 3$</td>
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<tr>
<td>NAC: not specified</td>
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<tr>
<td>Hawkins et al. (1990)</td>
<td>N = 62 pts</td>
<td>No change in expression</td>
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<tr>
<td>No control group</td>
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<tr>
<td>NAC: 4× CHOP</td>
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<tr>
<td>Lee et al. (2003)</td>
<td>N = 56 pts</td>
<td>61% change in ER or PR expression, compared to 48% in the control group</td>
<td></td>
<td>5% change compared to 5% change HR status in control group</td>
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<tr>
<td>Control group: N = 56</td>
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<tr>
<td>Control group: N = 56</td>
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<tr>
<td>Cut off value: expression of 1–3</td>
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<tr>
<td>NAC: AC or T</td>
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<tr>
<td>Faneyte et al. (2003)</td>
<td>N = 49 pts</td>
<td>29% change in status (14/49 pts)</td>
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<tr>
<td>No control group</td>
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<tr>
<td>Cut off value: $\geq 10%$</td>
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<tr>
<td>NAC: 3× FEC</td>
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<tr>
<td>Bottini et al. (1996)</td>
<td>N = 41–44 pts</td>
<td>0.6% change in status</td>
<td>4.5% change in status</td>
<td></td>
</tr>
<tr>
<td>No control group</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Cut off value: HSCORE $&gt; 75$</td>
<td></td>
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</tr>
<tr>
<td>NAC: CMF + tamoxifen</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Study</td>
<td>Methods</td>
<td>ER</td>
<td>PR</td>
<td>HR status</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------------</td>
<td>--------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>Schneider et al. (2000)</td>
<td>N = 37 pts</td>
<td>No significant change in expression (P = 0.8394)</td>
<td>No significant change in expression (P = 0.1881)</td>
<td>Change: 42% Pos to Neg</td>
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<tr>
<td></td>
<td>No control group</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Cut off value: &gt;10%</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>NAC: 3–6× CAF</td>
<td></td>
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</tr>
<tr>
<td>Arens et al. (2004)</td>
<td>N = 25 pts</td>
<td>20% Change in expression</td>
<td>20% Change in expression</td>
<td>No change in HR status</td>
</tr>
<tr>
<td></td>
<td>Control group: N = 30 pts</td>
<td>Change: 12% increase, 8% decrease</td>
<td>Change: 8% increase, 12% decrease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: unknown</td>
<td>No change in ER status</td>
<td>No change in PR status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: 4× AT or 4× AC + T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varga et al. (2005)</td>
<td>N = 23 pts</td>
<td>9% change in status (P = 0.5)</td>
<td>21% change in status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td>Change: Pos to Neg</td>
<td>Change: 9% Neg to Pos, 13% Pos to Neg</td>
<td>3% change in HR status</td>
</tr>
<tr>
<td></td>
<td>Cut off value: &gt;10%</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>NAC: anthracycline</td>
<td></td>
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<tr>
<td>Retrospective trials</td>
<td>Penault-Llorca et al. (2003)</td>
<td>N = 91 pts</td>
<td>No significant change in expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Cut off value: &gt;10%</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>NAC: 6× anthracycline</td>
<td></td>
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</tr>
<tr>
<td>Migietta et al. (2009)</td>
<td>N = 52 pts</td>
<td>No change in ER status</td>
<td>77% change in status, P = NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td></td>
<td>Change: Pos to Neg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: &gt;20%</td>
<td></td>
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<tr>
<td></td>
<td>NAC: anthracycline + taxane</td>
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</tr>
<tr>
<td>Adams et al. (2008)</td>
<td>N = 26 pts</td>
<td>77% change in status, P = NS</td>
<td>19.2% change in status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td>Change: Pos to Neg</td>
<td>Change: Pos to Neg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: &gt;10%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: 4× taxane + anthracycline</td>
<td></td>
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</tbody>
</table>
Table 2B. Overview of studies that concluded NAC does not change the HER2 receptor.

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>IHC</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prospective</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kasami et al. (2008)</td>
<td>N = 173 pts</td>
<td>2.3% change in status</td>
<td>No change (P = 1)</td>
</tr>
<tr>
<td>Control group: N = 117 pts</td>
<td>Change: 1.7% Pos to Neg, 6% Neg to Pos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FISH when IHC 2+</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NAC: 78% EC</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Taucher et al. (2003)</td>
<td>N = 85 pts</td>
<td>4.6% change in status (P = NS)</td>
<td>2% change in status: Neg to Pos</td>
</tr>
<tr>
<td>No control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut-off value: 3+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAC: CMF/FEC</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bottini et al. (1996)</td>
<td>N = 77 pts</td>
<td>3.1% change in status (P = NS)</td>
<td></td>
</tr>
<tr>
<td>No control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut-off value: unknown</td>
<td></td>
<td></td>
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<tr>
<td>NAC: CMF + tamoxifen</td>
<td></td>
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<tr>
<td>Faneyte et al. (2003)</td>
<td>N = 50 pts</td>
<td>6% change in status (P = NS)</td>
<td></td>
</tr>
<tr>
<td>No control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut-off value: 2+</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NAC: 3× FEC</td>
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<tr>
<td>Quddus et al. (2009)</td>
<td>N = 39 pts</td>
<td>28.5% decrease in expression vs. 11.7% in control group (P &lt; 0.013).</td>
<td></td>
</tr>
<tr>
<td>Control group: N = 60 pts</td>
<td>12.5% change in status vs. 3.3% in the control group (P = 0.104)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut-off value: 3+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAC: not specified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vargais-Roig et al. (1999)</td>
<td>N = 37 pts</td>
<td>29% change in status (P = 0.8)</td>
<td></td>
</tr>
<tr>
<td>No control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut-off value: ≥1%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAC: 3–4× FAC or FEC</td>
<td></td>
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<td></td>
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</tbody>
</table>
### Study Methods IHC FISH

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>IHC</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arens et al. (2004)</td>
<td>N = 25 pts</td>
<td>20% change in expression</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>Control group: N = 30 pts</td>
<td>Change: 8% decrease, 12% increase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut-off value: 3+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: 4× AT or 4× AC+T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varga et al. (2005)</td>
<td>N = 23 pts</td>
<td>35% change in status (P = 0.289)</td>
<td>13% change: 8% Pos to Neg</td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut-off value: 2+</td>
<td></td>
<td>5% Neg to Pos</td>
</tr>
<tr>
<td></td>
<td>NAC: 2–6× FE or TC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrospective</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penault-Llorca et al. (2003)</td>
<td>N = 105 pts</td>
<td>No significant difference in expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: ≥10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: 6× anthracycline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vincent-Salomon et al. (2002)</td>
<td>N = 42 pts</td>
<td>4.8% change in status</td>
<td>4.8% change in status</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change: 4% Pos to Neg</td>
<td></td>
</tr>
</tbody>
</table>
Table 2A. Overview of studies that concluded NAC does change the HER2 receptor.

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>IHC</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prospective trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burcombe et al. (2005)</td>
<td>N = 118 pts</td>
<td>76% change in status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td>Change: 4.2% Neg to Pos, 3.4% Pos to Neg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: 3+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: 6× anthracycline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shet et al. (2007)</td>
<td>N = 73 pts</td>
<td>15% change in expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td>1.3% change in status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: ≥ 10% membrane staining (score 2 and 3 Hercept test)</td>
<td>Change: Pos to Neg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: 3–6× anthracyline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rasbridge et al. (1994)</td>
<td>N = 30 pts</td>
<td>30% change in status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td>Change: 10% Pos to Neg, 20% Neg to Pos</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: strong staining in ≥75% cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: anthracycline + alkylating agent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrospective trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hirata et al. (2009)</td>
<td>N = 368 pts</td>
<td>9.5% change in HER2 status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td>Change: 6% Pos to Neg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: ≥ 1%</td>
<td>3.5% Neg to Pos</td>
<td></td>
</tr>
<tr>
<td>MacGrogan et al. (1996)</td>
<td>N = 125</td>
<td>Decrease in ‘expression’ (P = 0.008)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td>Change: Decrease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: ≥ 1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neubauer et al. (2008)</td>
<td>N = 87 pts</td>
<td>15% change in status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td>Change: 85% Pos to Neg, 15% Neg to Pos</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: 3+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: 6× anthracycline/taxane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Methods</td>
<td>IHC</td>
<td>FISH</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------------------------------------</td>
<td>----------------------------------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Piper et al. (2004)</td>
<td>$N = 35$ pts</td>
<td>25% change in HER 2 status</td>
<td>No change in control group</td>
</tr>
<tr>
<td></td>
<td>Control group $N = 35$</td>
<td></td>
<td>Change: 67% Neg to Pos, 33% Pos to Neg</td>
</tr>
<tr>
<td></td>
<td>Cut off value: unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: $4 \times AC$ or $4 \times AC + T$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adams et al. (2008)</td>
<td>$N = 26$ pts</td>
<td>23% change in expression ($P = 0.027$)</td>
<td>Change: increase</td>
</tr>
<tr>
<td></td>
<td>No control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cut off value: 3+</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAC: $4 \times$ anthracycline</td>
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</table>
Table 3. Overview of the trials that investigated the influence of NAC combined with trastuzumab on the ER, PR and HER2 receptors.

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>HER2-IHC</th>
<th>HER2-FISH</th>
<th>ER status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mittendorf et al. (2009)</td>
<td>Prospective</td>
<td>32% loss of HER2</td>
<td>20% change: Neg to Pos</td>
<td></td>
</tr>
<tr>
<td>Hurley et al. (2006)</td>
<td>Prospective</td>
<td>43% loss of HER2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harris et al. (2007)</td>
<td>Prospective</td>
<td>12% change: Pos to Neg</td>
<td>No change</td>
<td>37% change: Pos to Neg (P = NS)</td>
</tr>
</tbody>
</table>

N = number of patients
NAC: neoadjuvant chemotherapy