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Original article

# Topoisomerase IIα gene status and prediction of pathological complete remission after anthracycline-based neoadjuvant chemotherapy in endocrine non-responsive Her2/neu-positive breast cancer

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**Abstract**

*Purpose*: Topoisomerase IIα (Topo II) is a potential marker of responsiveness to anthracycline-based therapy. We analyzed the role of Topo II gene status in the prediction of pathological complete remission (pCR) after primary anthracycline-based chemotherapy in non- endocrine responsive breast cancers overexpressing Her2/neu.
*Methods*: Twenty-three patients, with T2–T4, ER and PgR absent, overexpressing Her2/neu breast cancers treated with anthracycline-based chemotherapy were evaluated. Topo II gene status was assessed by FISH in pre-treatment tumor specimens and the results were correlated to pathological and clinical responses.
*Results*: Overall, six patients had a pCR (26%). Topo II was amplified in 5 (22%) of the tumors. In all patients with Topo II amplification, Her2/neu gene amplification was also detected. Among patients without amplification, one had polysomia of chromosome (Cr) 17 and four patients had deletion of the Topo II gene. A higher probability of pCR was observed when Topo II amplification and Cr 17 polysomy were present: pCR was reported in 3 of 5 amplified tumors (60%), in the polysomic tumor (amplified plus polysomic 67%) and in only 2 out of 13 tumors without alteration of Topo II status (15%). If we compare the frequency of pCR in tumors with amplification or polysomy versus the frequency of tumors with not amplification (deletion or no modification), a significant difference was detected ($p = 0.02$). One progressive disease (PD) was reported in one tumor with Topo II deletion (1/4, 25%) and one in tumor without any modification of Topo II gene status (1/13, 8%).
*Conclusions*: In patients with endocrine unresponsive and Her2 overexpressing tumors, Topo II amplification or the presence of chromosome 17 polysomy correlate with a significantly high probability of achieving pCR after neoadjuvant, anthracycline-based chemotherapy. Further prospective studies in order to more clearly define the predictive role of Topo II status in this subgroup of patients are warranted.


*Keywords:* Predictive markers; Topoisomerase II; Preoperative chemotherapy; Breast cancer

## Introduction

Preoperative treatment is indicated for patients with operable breast cancer for whom a reduction of primary tumor size may allow breast conservation. A clear benefit of preoperative chemotherapy in terms of treatment effects is the achievement

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of sufficient tumor shrinkage to allow breast-saving surgery in some of these patients. Several randomized trials comparing primary chemotherapy with traditional adjuvant treatment have been published, and similar disease-free survival (DFS) or overall survival (OS) were observed.[1–3]

Preoperative chemotherapy might be advantageous for patients with breast cancer in several ways in addition to allowing breast conservation surgery in some patients. In fact, the response to the primary treatment may be used as a prognostic marker. In particular the degree of response [pathological complete remission (pCR)] predicts overall outcome in terms of DFS.[2]

The identification of markers able to predict whether or not a chosen drug would be likely to work in a specific patient is of crucial importance. Previously published studies indicated that both the pCR rate and the frequency of node- negative status at final surgery were significantly higher following preoperative chemotherapy for patients whose tumors did not express ER and PgR, compared with the receptor positive cohort.[4–6] Despite the higher probability of response, the rate of pCR uncommonly exceeds 30%. Therefore, it seems reasonable to study in these population further markers predictive of response (pCR).

Topoisomerase IIα (Topo II) is an enzyme involved in cellular transcription, replication and repair processes, by twisting and supercoiling specific regions of DNA.[7,8] Topo II is a crucial target of anthracyclines and has been proposed as a chemosensitivity marker of anthracycline-containing therapies by in vitro and in vivo studies.[9,10] In tumor cells with high nuclear Topo II expression, doxorubicin exerted a potent antitumor activity while in tumor cells lacking Topo II expression, the antitumor activity was decreased. Moreover some retrospective analyses of adjuvant and neoadjuvant chemotherapy for breast cancer have found a correlation between levels of Topo II expression or Topo II amplification and clinical response to different anthracycline schedules.[11,12] Based on the hypothesis that co-amplification of Topo II is responsible for anthracyclines sensitivity and for a high chance to achieve pCR after anthracycline-based chemotherapy, we retrospectively analyzed, among patients with endocrine non-responsive and Her2/neu-overexpressing locally advanced breast cancers, the correlation between Topo II gene amplification and pCR rates after primary anthracycline-based chemotherapy.

## Materials and methods

From 1997 to 2006 286 breast-cancer patients, staged T2–T4, were treated with anthracycline-based neoadjuvant chemotherapy at the European Institute of Oncology. Among these, we selected all patients with endocrine non-responsive disease [Estrogen receptor (ER) and Progesterone receptor (PgR) absent]. We subsequently identified those patients with Her2/neu-overexpression at the biopsy performed before treatment. Clinical examination, hematological and biochemical analysis and radiological procedures, including chest X-ray, abdominal ultrasound and bone scan, were performed to rule out metastatic disease. Mammography and breast ultrasound were performed at the beginning and after every three cycles of chemotherapy for response evaluation. Response was classified according to WHO criteria. Patients who achieved a complete response (CR), partial response (PR) or stable disease (SD) continued treatment to a total of six courses. Pathological complete response (pCR) was considered as the absence of invasive tumor in the breast and axilla.

In tumors considered resectable after primary chemotherapy, total mastectomy or breast-conserving surgery (BCS) were performed. Radiotherapy was proposed after BCS or after mastectomy when indicated. Two hundred and eighty patients received infusional chemotherapy with ECF regimen (epirubicin 50 mg/m$^2$ day 1, cisplatin 60 mg/m$^2$ day 1 and 5-fluorouracil 200 mg/m$^2$ daily as continuous infusion, every 3 weeks), 5 received TEF regimen (taxotere 35 mg/m$^2$ weekly, epirubicin 40 mg/m$^2$ day 1 and day 8, and 5-fluorouracil 200 mg/m$^2$ as continuous infusion for 2 weeks, every 3 weeks), and 1 received TAX regimen (taxotere 30–35 mg/m$^2$ weekly, doxorubicin 20–30 mg/m$^2$ day 1 and day 8, capecitabine 1650–2000 mg/m$^2$ daily, every 3 weeks).

### *Immunohistochemistry and FISH test evaluation for Her2/neu*

Her2/neu overexpression was assessed on routinely processed, formalin-fixed, paraffin-embedded tissues obtained by tumor biopsy, before the start of treatment, by immunohistochemical investigations using an automated immunostainer (TechMate 500; Dako, Glostrup, Denmark) and a peroxidase-based detection system in kit form (ChemMate; Dako) according to the manufacturer's instructions. The primary specific monoclonal antibody used was clone CB11; Biogenex. FISH test was performed on routinely processed, formalin-fixed, paraffin-embedded tissues using PathVision Her2 DNA Probe Kit (Vysis Inc., Downers Grove, IL, and Inform Her2 Gene Detection System, Ventana Medical Systems Inc., Tucson, AZ). Tumor grade was evaluated according to Elston and Ellis and peritumoral vascular invasion (PVI) was assessed according to Rosen. Estrogen (ER) and progesterone receptor (PgR) status, and Ki-67 labeling index (assessed with the MIB 1 monoclonal antibody), were evaluated immunohistochemically as previously reported.[6] The threshold for ER and PgR positivity was 1% and for Mib1 positivity was 20%, as previously published. The threshold for Her2/neu was assessed as described: no staining at all, or membrane staining in $<10\%$ of the observed tumor cells was considered negative (0). A faint/barely perceptible membrane staining in $>10\%$ of tumor cells or staining of part of their membrane was scored as negative (1+). A weak to moderate staining of the entire membrane in $>10\%$ of the tumor cells was considered weakly positive (2+). A moderate to strong staining of the entire membrane in $>10\%$ of the tumor cells was scored as strongly positive (3+).

### *Dual-color FISH test (TOP2A)*

A commercially available dual color FISH assay (LSI TOP2A spectrum Orange/CEP 17 Spectrum Green, Vysis,

Downers Grove, IL) was used to simultaneously evaluate TOP2A gene and chromosome 17 copy number according to the manufacturer's instructions. The locus-specific identifier TOP2A DNA probe is a 160 kb unique sequence probe, directly labeled with spectrum Orange, hybridizing to the 17q21–22 region containing the TOP2A gene. The chromosome enumeration probe is labeled with spectrum Green and hybridizes to the alpha satellite DNA located at the centromere of chromosome 17 (17p11.1–q11.1). At least 100 cells in the tumor component were evaluated for gene amplification. The ratio was calculated as the number of signals for the gene probe divided by the number of signals for centromere 17. Cases were scored as TOP2A FISH amplified when the ratio was >2.

A TOP2A deletion is considered present when the ratio was less than 0.8 based on the work of Di Leo and coworkers.[13] Polysomy was defined as the occurrence of three or more copy numbers of centromeres for chromosome 17 per cell.

Digital images were obtained using a Leica DMRB epifluorescence microscope equipped with a Leica digital camera DC250 (Leica imaging Systems Ltd., Cambridge, UK). FITC, Cy3, and DAPI fluorescent signals were detected using specific filters. The images were recorded, pseudocolored and merged using the QFluoro software (Leica Inc., Deerfield, IL).

### *Statistical methods*

Summary statistics and frequency table data are given whenever appropriate. Fisher's exact test was used to assess the association between pathological response and the Topo II gene amplification or polysomy. Two-sided *p* values are presented. The statistical analysis was performed with the Statistical Analysis System Version 8.2 (SAS Institute, Cary, NC).

## Results

Among 286 patients treated with anthracycline-based preoperative chemotherapy, we found 78 patients with non-endocrine responsive tumors. Among these, 40 patients were identified with Her2/neu positive tumors at the pretreatment core biopsy. Of these, 24 breast cancers had histological materials available. The characteristics of the patients are listed in Table 1. One patient was considered not evaluable for response because she had received only two courses of chemotherapy due to neurological toxicity. Therefore, 23 patients represent the subject of the present analysis. The median age at diagnosis was 48 years (range 30–62). Ten were cT2, 4 cT3 and 9 cT4 tumors (seven inflammatory tumors, cT4d). In 20 patients Ki-67 was ≥20% (87%). Twenty-one patients received the infusional regimen ECF, one received TEF and one the TAX regimen. Median number of courses was 6 (range 2–6).

We found Topo II amplification in 5 of the 23 tumors (22%) while in 18 tumors (78%) it was not amplified. Among non-amplified tumors, 4 had gene deletion and 1 had Cr 17 polysomy. All tumors with Topo II amplification also demonstrated co-amplification of Her2/neu.

Table 1
Characteristics of patients

| Patients | No. |
|---|---|
| Evaluable | 23 |
| Median age, years (range) | 48 (range 30–62) |
| Menopausal status | |
| premenopausal | 17 |
| postmenopausal | 6 |
| Type of chemotherapy | |
| ECF | 21 |
| TEF | 1 |
| TAX | 1 |
| Median number of courses | 6 (range 2–6) |
| cT | |
| cT2 | 10 (43%) |
| cT3 | 4 (17%) |
| any cT4 | 9 (39) |
| cT4d | 7 (30%) |
| cN | |
| cN0 | 2 (9%) |
| cN1 | 20 (87%) |
| cN2 | 1 (4%) |
| ER and PgR negative | 23 (100%) |
| Ki-67 | |
| <20% | 3 (13%) |
| ≥20% | 20 (87%) |
| Her2/Neu | |
| +++ | 23 (100%) |
| Grading | |
| G2 | 14 (61%) |
| G3 | 9 (39%) |

We studied the relationship between Topo II status and response to therapy in terms of pathological and clinical response. Results are shown in Table 2. Overall, six patients had a pCR (26%). We observed 3 pCRs in the 5 tumors presenting amplification of Topo II and 3 pCRs in 18 tumors without amplification of Topo II (60% and 17% respectively). The pCR was obtained in 4 cT2, 1 cT3 and 1 cT4d tumor. When we considered the polysomic and deleted tumors separately from the tumors without any modification of Topo II status, one pCR was observed in the polysomic tumor (1/1), two pCRs were reported in breast cancers without any modification of Topo II status (2/13, 15%), while no pCR was achieved in the deleted tumors (0/4). When the presence of Topo II gene amplification and the polysomy were considered together (as synonymous of increased Topo II gene copies) (Table 3), pCR was observed in 4 of the 6 tumors (67%). If we compare the frequency of pCR in tumors with amplification or polysomy versus the frequency of pCR in tumors with non-amplification (normal Topo II status plus gene deletion, 2/17, 12%), we can see that there is a significant difference ($p = 0.02$).

Clinical response rate did not differ between the two cohorts of amplified and non-amplified tumors: objective responses (CR plus PR) were 5 in Topo II amplified tumors and in the tumor with polysomy (83%), 9 in tumors with normal Topo II status (69%) and 3 in the deleted tumors (75%). Two clinical PD were observed, one in a tumor with Topo II deletion (1 /4, 25%) and one in a tumor without modification of Topo II status.

Table 2
Pathological and clinical response to primary chemotherapy according to Topo II status

| Best response | Amplification ($n = 5$) | Polysomia ($n = 1$) | Deletion ($n = 4$) | No modification ($n = 13$) |
|---|---|---|---|---|
| pCR | 3 (60%) | 1 (100%) | 0 | 2 (15%) |
| CR[a] | 0 | 0 | 0 | 1 (8%) |
| PR[a] | 4 (80%) | 1 (100%) | 3 (75%) | 8 (61%) |
| SD[a] | 1 (20%) | 0 | 0 | 3 (23%) |
| PD[a] | 0 | 0 | 1 (25%) | 1 (8%) |

[a] Clinical.

Table 3
Pathological and clinical response to primary chemotherapy according to Topo II status (association of amplification with polysomy versus deletion plus no modification)

| Best response | Amplification + polysomia ($n = 6$) | Deletion + no modification ($n = 17$) | $p$ |
|---|---|---|---|
| pCR | 4 (67%) | 2 (12%) | 0.02 |
| CR plus PR | 5 (83%) | 12 (70%) | n.s. |

n.s., not significant.

## Discussion

The preoperative setting offers a unique tool to detect reliable markers of response to the therapy. Since the degree of response [pathological complete remission (pCR)] predicts overall outcome in terms of DFS and OS,[2] the identification of predictive factors for pCR appears to be crucial. In the present study, we retrospectively analyzed the response to anthracycline-based neoadjuvant chemotherapy in a highly selected subgroup of patients with breast cancer. In fact, in the present analysis we included patients with both chemoresponsive tumors (ER and PgR absent) and with Her2/neu overexpression, and therefore with a high chance of response to neoadjuvant chemotherapy which includes anthracyclines. In fact, the pCR rate achieved (26%) was in keeping with previously published data in this subgroup of patients.[4–6] However, the results of the present study indicated that the presence of Topo II amplification or polysomy of CR 17 predicted a significantly higher chance of obtaining a pCR (67%) after anthracycline-based neoadjuvant chemotherapy.

Previous studies were conducted in order to find molecular and biological markers predictive of response to chemotherapy. A significant correlation between response and decay of high expression of Ki-67 due to primary chemotherapy[14,15] was observed. The results of immunohistochemical assessment of p53, Her2/neu, glutathione *S*-transferase, Ki-67, pS2, and ER/PR in baseline core biopsy samples from 134 patients in the primary chemotherapy group from the Bordeaux randomized trial have shown that estrogen receptor-negative status and high Ki-67 were correlated with the clinical tumor response.[4] The largest analysis of predictive factors was performed on 493 patients in the NSABP B-18 trial showing that poor nuclear grade significantly predicted pCR.[16]

More recently, data suggested a role of gene expression profiles in predicting the response to chemotherapy in women treated with preoperative chemotherapy although they failed to predict the response to a specific systemic regimen.[17–19] All these studies, through the use of a pattern of candidate genes (mainly ER-related gene cluster, proliferation-related gene cluster and immune-related gene cluster) indicated that transcriptional profiling has the potential to identify a gene expression pattern significantly correlated with pCR achievement, with a sensitivity ranging from 43% to 92%. With the support of gene expression profiles, usually a predictive value of 60% is considered of clinical value due to its ability to double the probability of observing pCR in unselected patients.[17]

Although the present data should be handled with caution due to the limited sample size which does not permit enough power to be obtained to make reliable tests on all the interesting comparisons, in our series of highly selected patients we were able to detect a 60% pCR in Topo II amplified tumors (5 patients).

In our series, all patients were selected also according to Her2 overexpression. In fact Her2 overexpression has been correlated with increased sensitivity to doxorubicin therapy, and its role as predictor of response to anthracyclines has been shown in large retrospective analyses in the adjuvant setting.[20–22] On the basis of preclinical studies, the predictive value of Her2 overexpression could be explained by co-amplification with Topo II,[23,24] sited closely to the Her2/neu gene on Cr 17. In our series, we observed co-amplification of Topo II in 22% of tumors. In previous analyses, Topo II co-amplification was identified in 37–84% of Her2/neu amplified breast cancers,[25] while Topo II amplification in the absence of Her2 positivity has been reported in the range of 1.7–10.9%.[12] The wide range observed in the rate of co-amplification could be explained with the different criteria utilized to defined Topo II activity: immunohistochemistry for detecting overexpression, fluorescent in situ hybridization for gene amplification, and quantitative real-time polymerase chain reaction for gene copy number analysis.[26] This supports the need for a standardized method to measure levels of Topo II.

Previous trials investigated the role of Topo II gene amplification as predictive markers of response to primary anthracycline-containing regimens. McGrogan and colleagues have demonstrated a significant direct correlation between the level of Topo II expression and breast cancer response to anthracycline-based chemotherapy.[27] Another study has confirmed a higher rate of objective responses in patients whose primary tumors had Topo II overexpression, thus supporting the link between Topo II and anthracycline activity.[28] Coon and coworkers analyzed the correlation between coamplification of Her2 and Topo II genes and found a significant association with favorable clinical response.[29] However, unlike the above-mentioned trials evaluating the correlation of Topo II with objective responses, the present study focused on the correlation with pCR, a major end-point in primary therapy.[2] A previous evaluation of the role of gene expression profiling in predicting pathological tumor response did not find a correlation between Topo II and pCR in patients treated with anthracycline and taxane neoadjuvant chemotherapy.[30] Similarly, in patients

treated with FEC neoadjuvant chemotherapy, overexpression of Her2 or Topo II amplification were not associated with pCR.[31] These results are not easy to explain and the different methods to detect Topo II activity (FISH or polymerase chain reaction) can only partially explain the controversial data.

In our series, one patient with polysomy of chromosome 17 achieved pCR. In breast cancer, aneusomy of chromosome 17, either monosomy or polysomy, is frequently observed by conventional cytogenetics and FISH. The impact of polysomy 17 on Her2/neu has been analyzed: polysomy 17, in the absence of Her2/neu gene amplification, results in a modest increase of Her2/neu gene copies in tumor cells, and in some cases, also in an increased Her2/neu protein production.[32] There are at present no data on the correlation between polysomy and Topo II status although the presence of increased Topo II gene copies cannot be excluded.

Finally, we failed to observe pCR in tumors with deletion of Topo II gene (4 tumors). Knoop and coworkers have reported that patients with Topo II amplification as well as patients with Topo II deletion had an increased recurrence-free survival and overall survival (HR, 0.63 and HR, 0.56 respectively) if treated with CEF chemotherapy when compared with CMF in the adjuvant setting.[12] The clinical significance of Topo II deletion has not been analyzed in depth in previous studies due to the low frequency of this gene aberration. Deletions of Topo II gene could predict anthracycline resistance rather than increased sensitivity as shown by our results. However, the involvement of Topo II gene is more complex and other hypotheses should be mentioned. Experimental data have suggested that the type of genetic defects at the Topo II locus may account for both sensitivity and resistance to anthracyclines, so deletions in different loci could exert a sensitizer of inhibitory effect.[33]

## Conclusion

Topo II gene amplification might correlate with increased pCR rate after anthracycline-based chemotherapy. The results of the present study indicate a possible role for Topo II gene amplification and for chromosome 17 polysomy in the identification of a subgroup of Her2/neu positive, endocrine-unresponsive tumors most likely to benefit from neoadjuvant, anthracycline-containing chemotherapy. These results should be regarded as hypothesis-generating and further prospective studies on a larger sample size to properly define the predictive role of Topo II status are required.

## Conflict of interest statement

None declared.

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