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Predictive molecular markers of anthracycline effectiveness in early breast cancer

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ABSTRACT

The past decade has seen extensive research into potential markers of responsiveness to anthracycline therapy in breast cancer patients; however, such markers have remained elusive. The status of both human epidermal growth factor receptor-2 (*HER2*) and topoisomerase II alpha (*TOP2A*) genes has been investigated in this context, but neither can be considered a clinically reliable predictor of response to anthracyclines. Expression of the TOP2A protein is affected by a number of factors not always related to gene copy number, and these might provide future potential markers. Recent studies have suggested that stroma-related signatures could predict anthracycline resistance, and increased expression of tissue inhibitor of metalloproteinases 1 (*TIMP1*) has also been implicated in resistance. Deficiency of the *BRCA1* and *BRCA2* genes may render cells more sensitive to topoisomerase inhibitors such as anthracyclines by disrupting repair of damaged DNA. Chromosome 17 polysomy has also been associated with increased responsiveness to anthracyclines. Given the complexity of topoisomerases and DNA repair pathways, it may be that a multifactorial approach, rather than reliance on a single biomarker, is needed to identify anthracycline-sensitive patients.

1. Introduction

The past decade has seen extensive research aimed at identifying specific biomarkers that are predictive of anthracycline efficacy.[1] Tools for prediction of efficacy would be valuable in guiding choice of this class of antiblastic agents, and would spare patients in whom they would be ineffective, with potential associated toxicities. Such individualised prescription is especially pertinent in the adjuvant setting, in which long-term treatment-related adverse effects such as cardiomyopathy and secondary leukaemia must be considered. The research for promising predictive biomarkers has been driven largely by studies suggesting that anthracyclines are most effective in breast cancer patients with tumours that over-express human epidermal growth factor receptor-2 (HER2),[2] or topoisomerase II alpha (TOP2A).[3–8] However, it is now clear that multiple factors determine the response to anthracycline therapy, and this will need to be considered in attempts to identify patients who could derive the greatest benefit from such treatment.

2. Anthracycline responsiveness and HER2 status

The relationship between anthracycline responsiveness and HER2 status was investigated in a meta-analysis

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based on data abstracted by Gennari et al., which included eight trials of adjuvant chemotherapy in patients with early breast cancer.[2] These trials involved a total of 6564 patients, with information on HER2 status available in 5354. In HER2-positive patients (n = 1536), anthracycline-based regimens were significantly more effective than non-anthracycline regimens in terms of both disease-free and overall survival; the pooled hazard ratio (HR) for relapse in anthracycline-treated patients was 0.71 (95% confidence interval [CI] 0.61–0.83, P < 0.001), while the corresponding HR for death from any cause was 0.73 (95% CI 0.62–0.85, P < 0.001). By contrast, in HER2-negative patients (n = 3818), there was no significant difference between patients receiving anthracycline-based and non-anthracycline regimens in either disease-free survival (HR 1.00, 95% CI 0.90–1.11, P = 0.75) or overall survival (HR 1.03, 95% CI 0.92–1.16, P = 0.60).[2]

Although these findings suggest that the benefits of anthracycline therapy in early breast cancer are confined to women whose tumours over-express HER2, this conclusion must be treated with caution. The doses of alkylating agents were not the same in the anthracycline- versus non-anthracycline-based regimens; thus, the lack of difference between the two regimens in HER2-negative disease may be attributable to either the omission of anthracycline and/or the reduced dose of alkylating agent. Furthermore, meta-analyses and retrospective subgroup analyses may be hypothesis-generating, but are generally underpowered to provide robust conclusions about subgroup efficacy and biomarker predictive value, as these considerations were not incorporated in the statistical trial design and calculation of sample size.

It is critical to point out that HER2-negative breast cancer is a heterogeneous condition, and it is unlikely that all such patients are equally unresponsive to anthracyclines. Several patient subgroups can be identified: HER2-negative disease encompasses highly endocrine-sensitive, moderately hormone-sensitive and triple-negative breast cancers. Furthermore, biological heterogeneity within these subgroups, particularly for features such as proliferation, highlight the lack of biological rationale for grouping all HER2-negative tumours together. Moreover, the studies included in the meta-analysis of Gennari et al. used various methods to measure HER2 expression and differing definitions of HER2-positivity, and there was no independent confirmation of HER2 status.[2] It should also be noted that there is no biological rationale for increased responsiveness to anthracyclines in HER2-positive tumours. This latter concern has led to a focus on TOP2A as a potential marker of anthracycline responsiveness.

3. TOP2A as a predictor of anthracycline response

Anthracyclines act by inhibiting the TOP2A enzyme.[9] Topoisomerases relieve torsional stresses during DNA transcription and replication, and inhibition of TOP2A by anthracyclines results in double-stranded DNA breaks, which ultimately may lead to cell death.[1,9] The *HER2* and *TOP2A* genes both lie on the long arm of chromosome 17, and co-amplification of the two genes is common; approximately 30–70% of breast cancers with *HER2* amplification also show *TOP2A* gene copy number aberrations (i.e. amplification or deletion), and in approximately 40% of *HER2*-amplified tumours there is co-amplification.[10] In '*in vitro*' studies in breast cancer cell lines, amplification of the *TOP2A* genes was associated with increased tumour sensitivity to anthracyclines, whereas cells with *TOP2A* deletions showed anthracycline resistance.[11]

The potential value of TOP2A as a predictor of anthracycline response was first investigated in a study in which *HER2* and *TOP2A* amplification were measured in breast tumour samples from patients participating in a phase III trial of adjuvant chemotherapy with epirubicin plus cyclophosphamide or cyclophosphamide, methotrexate and fluorouracil (CMF).[3] Gene amplification was measured by fluorescence *in situ* hybridisation (FISH); *HER2* amplification was present in 21% of samples, whereas *TOP2A* amplification was present in 38% of *HER2*-amplified tumours. In HER2-positive patients, event-free survival tended to be longer with anthracycline-based therapy than with CMF, whereas the two treatments were comparable in efficacy in HER2-negative patients. However, among HER2-positive patients, the superiority of anthracycline therapy appeared to be confined to TOP2A-positive patients (Fig. 1). Similar results were obtained in a subgroup analysis of the Breast Cancer International Research Group (BCIRG) 006 study, in which approximately one third of the patients showed co-amplification of *HER2* and *TOP2A*.[5] In these patients, the anthracycline-based arm without trastuzumab (i.e. the control arm) seemed to be as effective as the two trastuzumab-based arms. By contrast, in HER2-positive patients without co-amplification of *TOP2A*, the anthracycline-based arm without trastuzumab seemed to be less effective than the trastuzumab-based arms. These findings might suggest that the apparent predictive value of HER2 over-expression for anthracycline activity is related to co-amplification of *TOP2A*.

A number of other studies have investigated the predictive value of *TOP2A* amplification, with conflicting results.[4,6–8,12–14] In a recent meta-analysis of five trials in which data on *HER2* amplification were available in 3452 patients and *TOP2A* data in 3102 patients, both genes had only modest predictive value for anthracycline responsiveness, which was of borderline statistical significance.[15] In patients with *HER2* amplification, the HR for disease recurrence in anthracycline-treated patients, compared with those receiving CMF, was 0.71 (95% CI 0.58–0.86), whereas in patients without

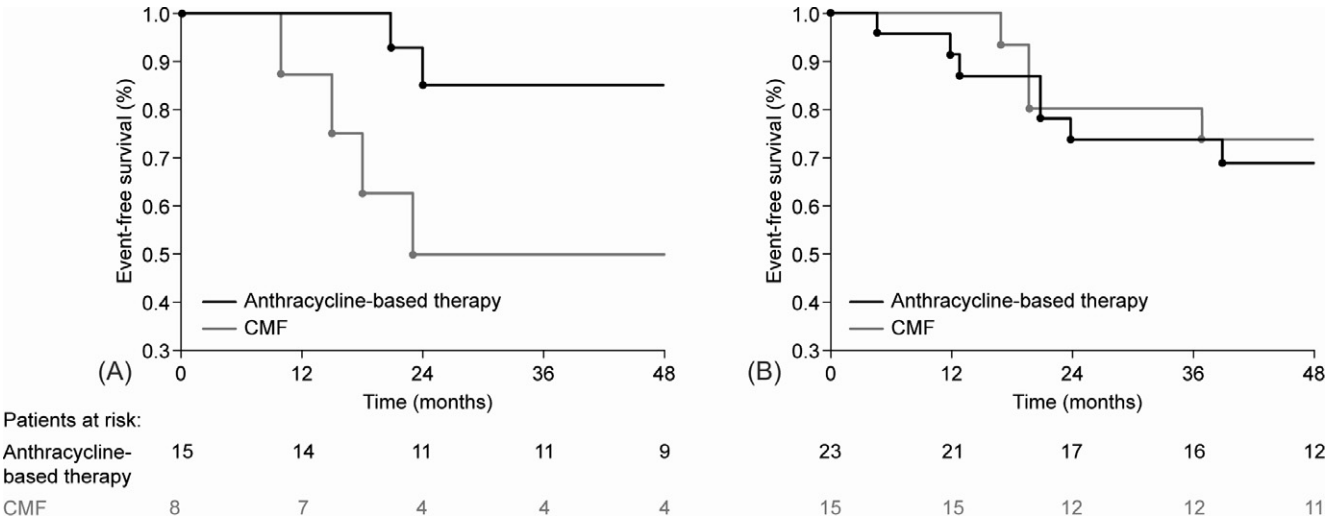

Fig. 1 – Event-free survival in human epidermal growth factor receptor-2 (HER2)-positive breast cancer patients receiving adjuvant therapy with anthracycline-based regimens or cyclophosphamide, methotrexate and fluorouracil (CMF).[3] (A) Patients with topoisomerase II alpha (*TOP2A*) amplification; (B) patients without *TOP2A* amplification. Reproduced from Di Leo A, et al. HER-2 amplification and topoisomerase IIα gene aberrations as predictive markers in node-positive breast cancer patients randomly treated either with an anthracycline-based therapy or with cyclophosphamide, methotrexate, and 5-fluorouracil. *Clin Cancer Res* 2002;8:1107–16.

HER2 amplification the corresponding value was 0.89 (95% CI 0.79–1.01). Similarly, in patients with *TOP2A* amplification, the HR for disease recurrence in patients receiving anthracyclines was 0.62 (95% CI 0.43–0.90), compared with 0.88 (95% CI 0.78–1.00) in patients with a *TOP2A* normal status and 0.63 (95% CI 0.46–0.87) in those with *TOP2A* deletion. Similar trends were seen for overall survival.[15]

This analysis also explored the comparison between anthracyclines and CMF in different patient subgroups defined by molecular phenotypes: high or moderate hormone sensitivity; HER2-positivity (with negative hormone-receptor status); and the triple-negative phenotype.[15] With the exception of highly hormone-sensitive patients, disease-free survival in these subgroups tended to be better in anthracycline-treated patients than in those receiving CMF. The finding that anthracyclines were associated with improved outcome in patients with the triple-negative and moderately hormone-sensitive phenotypes would suggest that, contrary to the findings of previous studies,[2] the benefits of anthracycline therapy are not confined to HER2-positive patients.

Most studies of the impact of HER2 and TOP2A expression on anthracycline responsiveness have focused on gene amplification, as measured by FISH. It is important to recognise, however, that protein expression is not related to levels of gene activity alone (Fig. 2).[16] *TOP2A* gene transcription can be enhanced by proliferation signals independently of gene aberrations, and levels of *TOP2A* mRNA fluctuate markedly during the cell cycle.[17,18] Furthermore, the half-life of *TOP2A* mRNA appears to be controlled by redox-sensitive protein complexes that bind to the untranslated 3′-region, resulting in stabilisation of the mRNA molecule.[18] In addition, splicing of *TOP2A* mRNA results in the formation of different protein isoforms, of which the cytoplasmic isoforms appear to be functionally inactive.[19] The expression of TOP2A is thus dependent on multiple factors that could potentially affect the responsiveness to anthracyclines.

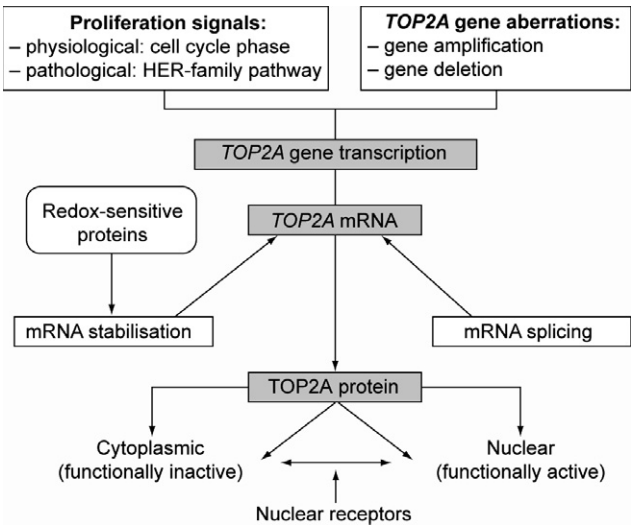

Fig. 2 – Factors regulating topoisomerase II alpha (TOP2A) protein expression.[16] HER, human epidermal growth factor receptor. Reprinted with permission from Di Leo A, Moretti E. Anthracyclines: the first generation of cytotoxic targeted agents? A possible dream. *J Clin Oncol* 2008;26(31): 5011–3. Copyright ©2008 American Society of Clinical Oncology. All rights reserved.

The correlation between responsiveness to anthracyclines and gene amplification or protein over-expression was investigated in the neoadjuvant Trial of Principal (TOP) study, in which 149 patients with oestrogen receptor-negative tumours were treated with epirubicin monotherapy.[20] *TOP2A* gene status was measured by FISH, TOP2A protein was determined by immunohistochemistry, and gene expression profiles were generated. A pathological complete response (pCR) was obtained in 14% of the evaluable patients, and *TOP2A* amplification was significantly associated with pCR ($P \leqslant 0.001$): six of the 10 patients whose tumours carried *TOP2A* amplification achieved pCR. However, *TOP2A* deletion or protein over-expression was not associated with pCR. As isolated markers may be insufficient to predict response or resistance to treatment, a gene expression signature called the A-Score, which integrates the *TOP2A* signature and two previously developed gene expression signatures (the stromal and immune response signatures), was developed in this study to help to identify patients who do not benefit from anthracyclines. This score was found to have a high negative predictive value overall and in the HER2-positive and HER2-negative subpopulations. The authors concluded that the A-Score could, if further validated, become a useful clinical tool to identify those patients who do not benefit from anthracyclines and could therefore be spared the potential adverse effects of this therapy.

4. Chromosome 17, DNA repair and potential future markers of anthracycline responsiveness

Chromosome 17 harbours important genes involved in the pathogenesis of breast cancer, such as *HER2* and *TOP2A*, and important tumour suppressors, such as *TP53* and *BRCA1*. *TP53* is involved in several cellular processes including DNA repair and apoptosis. Studies using differing drug regimens and analysing different tumour subtypes for *TP53* mutations and p53 protein expression as surrogates of mutations have given conflicting results on anthracycline response.[21] Studies suggest that *TP53* mutations confer resistance to anthracyclines,[22,23] whereas others have shown the opposite, reporting an association between mutations and increased sensitivity.[24,25] The conflicting results may be treatment-related, as the latter studies showing increased sensitivity used high-dose alkylating agents.[24,25] Thus, *TP53*-mutant tumours may have a poor outcome when treated with conventionally dosed chemotherapies, but may be highly responsive to dose intensification.[25]

BRCA1 has numerous roles in human cells, including maintenance of genome integrity, regulation of cell cycle checkpoints, and transcriptional regulation.[26] *BRCA1* mutations are associated with a form of hereditary cancer, which include breast cancer. The majority of studies have shown that absence of BRCA1 leads to hypersensitivity to DNA-damaging agents. Patients with *BRCA1* mutations have shown an improvement in response to treatment with anthracycline-containing chemotherapies.[27,28] Additionally, preclinical models have confirmed that deficiency of BRCA1, and also BRCA2, renders cells more sensitive to topoisomerase inhibitors, apparently by disrupting the repair of damaged DNA.[29]

Chromosomal imbalances may also influence the response to anthracycline therapy. In particular, regions at chromosome 17q22–24 of breast tumours are often affected by gains or small losses of genetic material. This chromosomal region contains important genes involved in DNA repair and the cell cycle, thus causing a genetic instability of tumour cells.[30,31] In a recent analysis from a trial of adjuvant epirubicin therapy, duplication of chromosome 17 centromere enumeration probe (*Ch17CEP*) was associated with significantly longer relapse-free and overall survival in anthracycline-treated patients, compared with those receiving CMF.[12]

5. Tumour microenvironment, immune cellular effects and anthracycline treatment in breast cancer

The growing recognition that multiple factors are likely to determine the response to anthracycline therapy has led to attempts to identify new potential markers.[1] Recent evidence suggests that stromal and immune elements within the tumour may mediate response to chemotherapy.[32,33] One such recent study has investigated the predictive value of stromal gene expression in patients receiving neoadjuvant therapy with fluorouracil, epirubicin and cyclophosphamide (FEC).[34] A tumour stroma-related 50-gene signature was found to be highly predictive of resistance to FEC: this relationship was seen in two independent cohorts of treated patients, but not in an untreated control group, indicating that the signature is predictive rather than prognostic.[34] A different study has shown that the over-expression of a single stromal gene, the tissue inhibitor of metalloproteinases 1 (*TIMP1*), may be associated with resistance to anthracyclines: in patients with TIMP1-negative tumours, the HR for disease progression in FEC-treated patients, compared with those receiving CMF, was 0.51 (95% CI 0.31–0.84, $P = 0.0085$), whereas there was no significant difference in disease-free survival between the two treatments in patients with TIMP1-positive tumours (HR 0.88, 95% CI 0.68–1.13, $P = 0.32$).[35] Further studies that specifically link these biological factors to response to anthracyclines, or other antineoplastic agents, are however needed.

6. Conclusions

Despite the extensive research into potential markers of anthracycline responsiveness during the last decade,

reliable markers remain elusive. Neither *HER2* nor *TOP2A* gene status can be considered a clinically reliable predictor of anthracycline response. TOP2A protein expression is independent of gene status, and correlates with proliferation signals. Quantification of TOP2A protein, and determination of the subcellular localisation of this protein, might provide clinically relevant markers in the future. In addition, the fact that high sensitivity to anthracyclines can be observed in the absence of gene amplification and protein expression suggests that other factors, such as markers of stroma function or dysregulation of DNA repair, may also provide useful measures of anthracycline sensitivity. Given the complexity of topoisomerases and DNA repair pathways, it may be that a multifactorial approach, rather than reliance on a single biomarker, is needed to identify anthracycline-sensitive patients.

7. Conflict of interest statement

None declared.

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