

# Predictive biomarkers for anthracycline benefit in early breast cancer: When do we act?

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With cost and toxicity posing important stumbling blocks for more rapid deployment of cancer therapies, interest is growing in biomarkers that can identify which patients will and which will not likely benefit from costly and potentially toxic treatments. Estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status are well-established key biomarkers for selecting which breast cancer patients receive hormonal therapies and trastuzumab, respectively, and both have recently been associated with predictive value for chemotherapy effectiveness. Multiple biomarkers that combine signals of multiple genes (as determined by cDNA techniques) are also used to predict chemotherapy effect, for example the Oncotype 21 gene assay in North America and the 70-gene MammaPrint (Amsterdam) assay in Europe. Emerging potentially useful markers include the combination of tissue inhibitor of metalloproteinase-1 (TIMP1) and topoisomerase II-alpha (TOP2A), described recently by Danish researchers Ejlertsen et al at the 2008 San Antonio Breast Cancer Symposium (SABCS)<sup>1</sup> and discussed below.

## HER2 AND TOP2A

A product of the TOP2A gene, the TOP2A protein is a key enzyme in unwinding the DNA double helix associated with DNA replication, translocation and repair, and is the molecular target for the class of agents known as TOP2 inhibitors. The TOP2A gene is located close to the HER2 proto-oncogene at chromosome band 17q12-q21. Because of this physical proximity, TOP2A gene abnormalities often coincide with HER2 amplification, and the other way around.<sup>2</sup> Experimental and subsequently human studies confirmed that the TOP2A-inhibiting anthracyclines and taxanes are more effective if abnormal TOP2A has rendered the DNA repair mechanism defective. In their pivotal 1988 report of TOP2A and HER2 interaction in 57 HER2-amplified primary breast carcinomas,<sup>3</sup> Jarvinen et al reported that a total of 86% had a TOP2A abnormality, of which 25 (44%) had TOP2A amplification and 42% had a physical deletion of the TOP2A gene. Importantly, no TOP2A copy number aberrations were found in 40 primary tumours without HER2 amplification (i.e. HER2-negative cases). The finding of a large proportion of TOP2A aberrations may explain the increased chemosensitivity to TOP2A inhibitors reported in HER2-amplified

breast cancers. Some studies subsequently confirmed the association of TOP2 aberrations and anthracycline effectiveness in breast cancer<sup>4,5</sup> but others did not<sup>6,7</sup> and a recent editorial advises caution.<sup>8</sup>

Better evidence for HER2 is available, with a recent meta-analysis of seven studies confirming a significant interaction ( $p < 0.001$ ) for anthracycline impact and HER2 status among 1536 HER2-positive patients for both disease-free survival (DFS) and overall survival (OS).<sup>11</sup> Further, patients with amplified HER2 — but not those with normal HER2 — have been shown to benefit from regimens intensified by adding four cycles of paclitaxel after four cycles of doxorubicin and cyclophosphamide.<sup>10</sup>

## THE 2T PROFILE BIOMARKER

In their presentation at the December 2008 SABCS, Ejlertsen et al showed that combined assessment of TOP2A and TIMP1 may be more effective than previous biomarkers in selecting breast cancer patients for anthracycline chemotherapy.<sup>1</sup> TIMP1 activates a pathway that allows cells to escape the effect of anthracyclines in destroying TOP2A. Ejlertsen et al postulated that poor DNA repair due to abnormal TOP2A, together with more robust chemotherapy-related apoptosis due to normal TIMP1, would enhance the effect of chemotherapy. Thus, they defined a constellation of abnormal TOP2A (deleted or amplified) plus normal TIMP1 as the ideal chemosensitive state, and named the double biomarker the 2T profile: 2T-responsive indicates TOP2A-abnormal and/or TIMP1-negative and 2T-nonresponsive indicates TOP2A-normal and TIMP1-positive.

The analysis used data from the Danish Breast Cancer Cooperative Group (DBCG) trial 89D,<sup>11</sup> which randomized 980 women with high-risk early breast cancer to nine treatment cycles with cyclophosphamide + methotrexate + fluorouracil (CMF) or cyclophosphamide + epirubicin + fluorouracil (CEF) chemotherapy, without endocrine therapy. Results showed superior DFS and OS for the anthracycline-containing CEF regimen, which, while more effective overall, was more costly and toxic than the CMF regimen. The same percentage, 43%, of both CMF- and CEF-treated patients had the 2T-responsive profile. Cox regression analysis showed that CEF provided superior DFS and OS in the 2T-responsive patients (adjusted HR of 0.48 for DFS and 0.54 for OS,  $p < 0.001$ ), but in the 2T-nonresponsive patients CEF actually led to 18% more recurrences compared to CMF (Table 1, page 4). No individual marker, including ER, HER2, TIMP1 and TOP2A, predicted for anthracycline effect.

Identifying multiple biomarkers, each with strong predictive value, highlights their molecular associations through multiple mechanisms for therapeutic activity. Thus, when

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HER2 is amplified, an ER-negative phenotype is likely to be expressed, and both are associated with a higher S-phase fraction and higher growth fraction — all factors associated with a higher chemotherapy cell kill.

The Danish results are potentially important, but need to be validated and reproduced, with central retesting and international coordination of laboratories (as with ER and HER2 receptor measurement) to confirm quality control. They also need to be confirmed in multivariate analysis with other more established predictive markers (HER2, ER status, grade, etc.) to assess their overall cost-benefit and the need to do them all. Hopefully, full publication of Ejlersten et al's 2T biomarker study will address this issue. As well, new trials that prospectively evaluate biomarkers by multivariate analysis are needed.

## CHALLENGES TO RAPID IMPLEMENTATION

Once confirmed, further challenges need to be overcome to implement use of biomarkers such as the 2T profile into routine clinical practice.


**Tissue availability:** It is becoming increasingly difficult to access tumour tissue from patients in a given trial to retest a preliminary observation. One reason is that tumour samples are too small for the many additional tests per sample now being contemplated. All cancer research centres will need to adopt techniques such as tissue microarrays that enable assessment of multiple biomarkers on a small tumour sample — a large but surmountable logistic challenge.

**Quality control:** High quality control for reliability, quality and reproducibility of techniques to identify individual biomarkers is essential. Poor quality control of biomarker testing causes errors in giving or withholding a certain treatment that can affect both survival and quality of life, in addition to overall societal cost — as recently experienced in various regions with falsely negative ER tests and inaccurate immunohistochemistry tests for HER2.

**Fiscal issues:** Funding is a major issue for biomarker research and clinical implementation. Cost-benefit evaluations

need to take into account both the upfront costs and the downstream savings from implementing treatment choices based on biomarker information. Estimates from prior breast cancer biomarker research (e.g. HER2 expression, ER status and possibly TOP2) and from the Danish group's work on the 2T profile indicate that millions of dollars could potentially be saved annually, by restricting costly anthracycline and/or taxane therapies only to patients who stand to really benefit, based on biomarker testing. These saved costs would far offset the upfront expenditures to implement their routine testing.

## A BALANCING ACT

For ethical and potentially for legal reasons, we need to find a balance between acting prematurely on newly identified biomarkers vs acting too slowly, and thus failing to optimize the costs and benefits of cancer therapeutics in an appropriate time-frame. A focused and well-funded biomarker research initiative could substantially facilitate optimum outcomes, saving more lives in the long term at much lower cost.<sup>12</sup> 

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**TABLE 1. Disease-free survival and overall survival by biomarker subgroups in patients receiving CEF vs CMF chemotherapy in the DBCG 89D breast cancer trial<sup>1</sup>**

marker	disease-free survival hazard ratio (95% CI)			overall survival hazard ratio (95% CI)		
	positive	negative	p-value for interaction	positive	negative	p-value for interaction
ER	0.69 (0.52–0.92)	1.07 (0.69–1.66)	0.10	0.73 (0.55–0.94)	0.94 (0.60–1.49)	0.36
HER2	0.66 (0.44–0.99)	0.86 (0.64–1.15)	0.29	0.63 (0.42–0.96)	0.88 (0.65–1.11)	0.19
TIMP1	0.52 (0.32–0.87)	0.88 (0.68–1.15)	0.07	0.57 (0.34–0.96)	0.85 (0.65–1.11)	0.18
TOP2A	0.45 (0.29–0.71)	0.98 (0.74–1.28)	0.004	0.52 (0.33–0.81)	0.93 (0.70–1.23)	0.03
2T	0.48 (0.34–0.69)	1.18 (0.87–1.60)	< 0.0001	0.54 (0.38–0.77)	1.07 (0.78–1.47)	0.03