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Journals Club

Review of: Comparison of gene expression profiles predicting progression in breast cancer patients treated with tamoxifen

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Citation of original article:
Kok *et al.* ***Breast Cancer Research and Treatment*** 4 March 2008 [Epub ahead of print].

Abstract of the original article:
Background: Molecular signatures that predict outcome in tamoxifen-treated breast cancer patients have been identified. For the first time, we compared these response profiles in an independent cohort of (neo)adjuvant systemic treatment naïve breast cancer patients treated with first-line tamoxifen for metastatic disease. **Methods:** From a consecutive series of 246 estrogen receptor (ER) positive primary tumors, gene expression profiling was performed on available frozen tumors using 44K oligoarrays ($n = 69$). A 78-gene tamoxifen response profile (formerly consisting of 81 cDNA clones), a 21-gene set (microarray-based Recurrence Score), as well as the HOXB13-IL17BR ratio (Two-Gene Index, RT-PCR) were analyzed. Performance of signatures in relation to time to progression (TTP) was compared with standard immunohistochemical (IHC) markers: ER, progesterone receptor (PgR) and HER-2. **Results:** In univariate analyses, the 78-gene tamoxifen response profile, the 21-gene set and the HOXB13-IL17BR ratio were all significantly associated with TTP with hazard ratios of 2.2 (95% CI 1.3–3.7, $P = 0.005$), 2.3 (95% CI 1.3–4.0, $P = 0.003$) and 4.2 (95% CI 1.4–12.3, $P = 0.009$), respectively. The concordance among the three classifiers was relatively low, they classified only 45–61% of patients in the same category. In multivariate analyses, the association remained significant for the 78-gene profile and the 21-gene set after adjusting for ER and PgR. **Conclusion:** The 78-gene tamoxifen response profile, the 21-gene set and the HOXB13–IL17BR ratio were all significantly associated with TTP in an independent patient series treated with tamoxifen. The addition of multigene assays to ER (IHC) improves the prediction of outcome in tamoxifen-treated patients and deserves incorporation in future clinical studies.

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Review

Summary

Kok *et al.* present a comparison of three-gene expression profiles reported to be able to predict progression in breast cancer patients treated with tamoxifen. These are a 78-gene profile [1], a 21-gene set (21-gene recurrence score [2]) and a two-gene ratio [3]. The ability of these signatures to detect

recurrence was compared in an independent dataset (independent of the original set of patients on whom the gene signatures were developed) of 246 estrogen receptor positive (ER+) breast cancer patients who were treated with tamoxifen in the metastatic setting and had not received any systemic treatment in the adjuvant setting. The results were compared to ER, progesterone receptor (PgR) and HER-2 by immunohistochemistry and other prognostic markers.

They found that all the gene signatures were significantly associated with response in the independent dataset. Interestingly, the 78-gene profile appeared to be more predictive of tamoxifen response, whilst the other two seemed to be both prognostic and predictive, in that they were able to predict poor outcome in women in both the primary post-diagnosis adjuvant setting and whilst on tamoxifen in the metastatic setting. The latter seemed to be only able to predict time to relapse whilst the patients were being treated with tamoxifen.

Interestingly, concordance between all three classifiers was low – around 50% – suggesting that the genes are capturing different aspects of tumor biology that relate to a poor outcome in ER+ breast cancer. The authors also found that in a multivariable Cox analysis, the three-gene signatures could provide further information other than currently used clinico-prognostic indicators – ER, PGR, HER-2 and histologic grade.

Analysis of study

This study compares three previously reported gene signatures developed using tamoxifen-treated patients. For further information on the development and validation of these signatures, please refer to reference [4]. These signatures were developed in slightly different ways, using different technological platforms, and have undergone various external validations. The 21-gene recurrence score is currently being actively marketed in the US as a clinico-diagnostic tool and is also the subject of a large clinical trial assessing its ability to predict response to chemotherapy [5].

This study addresses three important issues regarding the relevance of gene expression signatures to the clinic.

1. *Prognosis vs. prediction*: The differences in determining whether a gene set predicts differential response to tamoxifen or whether it is ascertaining prognosis, that is the tumor would do poorly regardless of treatment.
   (a) This is attempted by analyzing the dataset in two different ways – the first by using first occurrence of metastatic disease known as 'disease-free interval (DFI)' as an endpoint compared with 'time to progression (TTP)', which was defined as time on tamoxifen after diagnosis of metastatic disease until it was ceased due to progression of disease. Because the patients received no adjuvant systemic therapy, one can assume that we are really seeing the disease's natural history, or namely its prognosis when using DFI as an endpoint. Similarly, we can truly ascertain if a patient has responded to tamoxifen by using TTP as an endpoint. In this way the authors could determine whether the gene signature predicts response to tamoxifen.

   These are quite important distinctions as clinicians ideally would like to know whether their patients will respond to the treatment given, rather than knowing whether a patient is going to have a poor outcome, irrespective of the treatment accorded to them. The use of biomarkers or gene expression signatures that can predict response to treatments may be more useful in the clinical setting, whereas those that predict poor prognosis may be more applicable for selecting patients for clinical trials evaluating new treatments.
2. *Concordance of classification*: The concordance of the signatures relates to how the gene signatures group the patients. This is of interest as the genes contained in these gene expression signatures rarely overlap, but they may be tracking the same biology if they classify patients in the same way. The lack of gene overlap is most likely due to the different patient populations and different microarray platforms the signatures were developed on – that is Affymetrix has short oligonucelotides probe sets whilst Agilent uses long oligonucleotides. Fan *et al.* [6] in a previous study looked at the same issue with gene expression profiles developed to ascertain prognosis and found high concordance amongst classifiers, suggesting that all of the studied signatures, despite the different genes, were tracking similar biological pathways. However, in this report there was poor concordance, even though all signatures were statistically significant in the survival analysis. This perhaps suggests that the biology of tamoxifen response is complicated and that these gene sets may be useful in different ways or in combination. Of note, the two-gene ratio has recently been combined with a molecular grade index to improve its prognostic ability [7].
3. Finally, what is the extra value of a gene signature compared with current clinico-pathologic factors. Can these new tools really help clinicians individualize treatment for their patients? This is often

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assessed by using a multivariable Cox model, such as in this paper, by comparing the gene signatures performance to that of ER, histologic grade, tumor size and nodal status.

In other papers, this has been analyzed by comparing the gene signature's performance to that of other prognostic clinical tools such as Adjuvant! On-line or the Nottingham Prognostic Index [8,9]. These comparisons may be a better indication than a multivariable Cox model of a gene signature's clinical relevance. Cox models may give unstable results if any of the analyzed factors have high correlations with each other such as, for example, histologic grade. However, these comparisons were not appropriate in this instance as this paper was primarily assessing a gene signatures' ability to predict tamoxifen response.

Future directions

Independent validation studies like these are useful as they can tell us whether reported gene signatures are truly robust at predicting clinical outcome in different datasets, using different patients and different array platforms from those on which they were developed. However, despite the demonstration of their ability to predict outcome, gene signatures still remain difficult to implement in the clinic as there are many of them, which claim to do similar things, and we do not understand fully the biological information they are portraying. Furthermore, the technology is complicated to implement and is expensive; hence, their accessibility for everyday patients remains elusive. It is also difficult at present to reconcile the different signatures available and how they fit in with the previously observed molecular subtypes of breast cancer [10]. These issues will need to be addressed prior to any real effort to begin clinical implementation of any gene expression signature as a diagnostic tool or predictive marker in breast cancer.

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