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Review of:
Classification of human breast cancer using gene expression profiling as a component of the survival predictor algorithm

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Abstract of the original article

Purpose: Selection of treatment options with the highest likelihood of successful outcome for individual breast cancer patients is based on a large degree on accurate classification into subgroups with poor and good prognosis reflecting a different probability of disease recurrence and survival after therapy. Here we propose a breast cancer classification algorithm taking into account three main prognostic features determined at the time of diagnosis: oestrogen receptor (ER) status; lymph node (LN) status; and gene expression signatures associated with distinct therapy outcome.

Experimental design: Using microarray expression profiling and quantitative reverse transcription-polymerase chain reaction (Q-RT-PCR) analyses, we compared expression profiles of the 70-gene breast cancer survival signature in established breast cancer cell lines and primary breast carcinomas from cancer patients. We classified 295 breast cancer patients using 14-, 13-, 6-, and 4-gene survival predictor signatures into subgroups having statistically distinct probability of therapy failure ($P < 0.0001$). We evaluated the prognostic power of breast cancer survival predictor signatures alone and in combination with ER and LN status using Kaplan–Meier analysis.

Results: The breast cancer survival predictor algorithm allowed highly accurate classification into subgroups with dramatically distinct 5- and 10-year survival after therapy of a large cohort of 295 breast cancer patients with either ER-positive or ER-negative tumours as well as LN-positive or LN-negative disease ($P < 0.0001$, log-rank test).

Conclusions: Our data imply that quantitative laboratory tests measuring expression profiles of a limited set of identified small gene clusters may be useful in stratification of breast cancer patients at the time of diagnosis into subgroups with statistically distinct probability of positive outcome after therapy and assisting in selection of optimal treatment strategies. The estimated increase in survival due to the optimization of treatment protocols may reach many thousands of breast cancer survivors every year at the 10-year follow-up checkpoint.

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Review

Adjuvant systemic chemotherapy, with or without endocrine therapy, is the current standard treatment for patients with node-positive breast cancer, and for a large portion of those with node-negative breast

cancer, a group where approximately 22–33% of patients develop recurrence of their disease after a 10-year follow-up [1]. The absolute clinical benefit of this therapy depends, however, on the initial risk of cancer recurrence and death, which is determined currently by a combination of variables including age, oestrogen-receptor (ER) status, lymph node (LN) status, tumour grade and stage. The accuracy of these conventional markers of outcome is imperfect, so that a number of women who would benefit from adjuvant therapy do not receive it, and others are exposed to the side effects unnecessarily [2]. Hence, an important step to optimizing current treatment is to improve the understanding of individual cancer and patient characteristics that influence treatment selection and outcome.

A contemporary approach is to utilize microarray analysis to define gene expression profiles that are associated with poor clinical outcome in order to provide more sensitive prognostic tools [3–7]. The most advanced published data addressing the utility of such an assay is currently in breast cancer where the Netherlands Cancer Institute and Antoni van Leeuwenhoek Hospital have pioneered the use of microarray profile analysis in conjunction with conventional prognostic tests to determine which women will receive adjuvant treatment after surgery [8]. This gene expression signature based on 70 genes is a powerful predictor of the outcome of disease in young patients [3]. Importantly, this gene expression profile has been tested in 295 breast cancer patients with either LN-negative or LN-positive breast cancer to assign patients into good and bad prognosis groups [4]. While this study has provided an important impetus for the development of microarray-based prognostic tools, it is clear that it has also raised several questions. First, the applicability of the prognostic profile to the full spectrum of breast cancer patients given that the study was based on expression profiling of cancers from patients less than 55 years of age and with small cancers [2]. Secondly, the finding that 97% of the cancers in the 'good prognosis' group were ER positive based on ER transcript levels detected by microarray, raises the possibility that the 70-gene signature is not suitable for stratifying ER-negative patients into good and poor prognosis groups [9]. Also, the limitations associated with microarray-based tools at present, in particular, the current reliance on frozen tissue, lead to questions about its applicability to a routine laboratory environment.

A recently published paper by Glinsky *et al*. (2004) has taken steps to develop a clinically useful predictive tool based on the results of the Netherlands Cancer Institute microarray analyses [9], by addressing some of these issues. In this study, they measured gene expression levels of the 70 genes identified by van't Veer and co-workers, in seven established human breast cancer cell lines and normal human breast epithelial cells using quantitative reverse transcription-polymerase chain reaction (Q-RT-PCR). Sets of genes were then identified that exhibited differential expression in both the cancer vs. 'normal' cell lines, and the good vs. poor prognosis groups identified by van't Veer and co-workers. Although they do not report the total number of such sets or 'clusters' of genes that they identified from the seven breast cancer cell lines they examined, the authors present four 'top-performing' clusters (4-gene, 6-gene, 13-gene, 14-gene) that have the ability to classify the 78 patients presented originally by van't Veer *et al*. into prognostic groups. They then utilized data from the validation study of 295 patients by van de Vijver *et al*. to confirm the ability of some of these clusters to segregate patients by prognosis. A combination of all four clusters representing 25 of the 70 genes in the original prognosis profile, performed the best in patient classification with 51% of patients with 4 poor prognosis signatures dying within 10 years of initiation of adjuvant therapy compared with 98% of patients with 4 good prognosis signatures remaining alive at 10 years after therapy. In addition, they determined a 5- and 3-gene signature that was able to stratify the ER-negative/LN-negative and ER-negative/LN-positive breast cancers within the large cohort of 295 patients into distinct prognostic groups.

Several important conclusions can be made from this study. The first is that small clusters of genes may be useful in stratifying breast cancer patients into prognostic groups. The data support the proposal that identification of breast cancer patients who might benefit from adjuvant therapy may require the measurement of only 15–25 genes and that this geneset may indeed perform better than both? the 70-gene profile identified previously and conventional markers of breast cancer outcome. The second is evidence for the existence of a gene profile that has the potential to classify accurately ER-negative/LN-positive and ER-negative/LN-negative patients into prognostic subgroups, which was not possible with the 70-gene profile. The third is the possibility of translation of a microarray-based assay to quantitative platforms such as Q-RT-PCR, available in the conventional pathology laboratory. A significant advantage of Q-RT-PCR over microarray currently is its ability to utilize formalin-fixed paraffin-embedded tissue (FFPE), a critical step to the practical application of such a tool.

A major shortcoming of this study is that the data were tested in the same patient cohort as reported by van't Veer *et al*. and hence there remains a critical

need to test both the 70-gene profile and subsets of genes as proposed by Glinsky *et al*. in other large cohorts of breast cancer patients that represent the full gamut of the disease. They also failed to test the applicability of the predictor algorithm to a Q-RT-PCR using RNA derived from FFPE patient tissue. Nonetheless, Glinksy and co-workers are asking the right questions and this study will undoubtedly lead to further insight into the ability to utilize gene expression profiles of breast cancers as predictive tools for identifying patients with aggressive disease beyond the scope of conventional markers alone.

Acknowledgement

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