Gene-Expression Profiling and the Future of Adjuvant Therapy

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Explain how clinical experience influences treatment selection and how this is likely to be refined by molecular diagnosis in the future.

2. Describe the potential of gene profiling in personalized cancer medicine.

3. Discuss some of the limitations of clinical medicine as it relates to the rapid application of gene-expression profiling.

ABSTRACT

Gene-expression profiling can help distinguish between patients at high risk and those at low risk for developing distant metastases, and so identify patients for adjuvant therapy. For several years, the Netherlands Cancer Institute has been working on gene-expression profiling of breast cancer using a microarray platform containing 25,000 genes. Using supervised classification, a prognostic classifier consisting of 70 genes could be identified. In addition to providing prognostic information, gene profiling should also enable us to detect patients who are likely to respond to particular adjuvant interventions. Well-known predictors for response to systemic therapy include estrogen receptor status HER-2 status, c-kit mutation, and epidermal growth factor receptor mutation. Because of the long periods required for predicting responsiveness in the adjuvant setting, neoadjuvant trials promise far quicker results. Several neoadjuvant studies are under way or planned to investigate gene-expression profiling as a means of predicting the therapeutic response to docetaxel (Taxotere®; Aventis Pharmaceuticals Inc., Bridgewater, NJ, http://www.aventispharma-us.com), paclitaxel (Taxol®; Bristol-Myers Squibb, Princeton, NJ, http://www.bms.com), cyclophosphamide, and doxorubicin (Adriamycin®; Bedford Laboratories, Bedford, OH, http://www.bedfordlabs.com) in breast cancer patients. It is expected that in the coming years an increasing number of novel prognostic and predictive tests will help in guiding the adjuvant systemic treatment of breast cancer and other malignancies. The Oncologist 2005;10(suppl 2):30–34

INTRODUCTION

In breast cancer in the adjuvant setting, as with other tumors, the first step toward individualizing therapy is to distinguish those patients who need treatment from those who do not. Bear in mind, for example, that even poor-prognosis patients, as defined by clinicopathological factors, with no adjuvant therapy have a 50%–60% risk for developing distant metastases, indicating that 40%–50% of these patients will remain free of disease without any adjuvant systemic therapy [1–3]. The second step is to tailor treatment to the characteristics of the specific tumor, given that certain cancers respond better to one form of adjuvant therapy and others to a different form.
Although still at an early stage of development, gene-expression profiling is now contributing both to the identification of patients at greatest risk for relapse and to the selection of treatment regimens according to the likely sensitivity of the tumor.

Several years ago, the Netherlands Cancer Institute (NKI) in collaboration with Roseatta Inpharmatics (Seattle, WA, http://www.rii.com) began working with a 25,000-gene microarray. This array requires fresh-frozen tumor rather than paraffin-embedded specimens. The array is hybridized with fluorescently labeled cRNA isolated from the tumor and reference cDNA, giving a readout of the expression of 25,000 genes [4,5].

While it is theoretically possible that a handful of hitherto undiscovered genes are highly prognostic, or predictive of response to a specific therapy, this has not been the case to date. We find that 50–100 genes need to be assayed and that the differences in expression between tumors with good prognosis and those with poor prognosis is relatively subtle.

**Gene-Expression Profiling and Prognosis**

In an initial study, tumor tissue was obtained from 78 breast tumors [6]. All patients were aged under 55 years. Tumors were <5 cm diameter, and no patient had lymph node involvement. Patients were all treated at the NKI, and outcome over 5 or more years was known.

From the initial pool of 25,000 genes, a system of supervised classification (Fig. 1) identified a group of 70 genes that were associated with poor prognosis; that is, different levels of expression tended to be found in the 34 patients who developed distant metastases within 5 years compared with the 44 patients with no recurrence in this period. The threshold was set at 10% for false negatives (Fig. 2). A subsequent validation study investigated the prognostic power of these 70 genes in 295 patients, some of whom were lymph node positive (but all aged under 53 years) [7]. Figure 3 shows that patients with a good prognosis signature had a <15% risk for developing distant metastases over 10 years and a <10% risk for dying. Patients with tumor genes associated with a poor prognosis had a 50% risk for distant metastases and a 50% mortality rate. Further work, conducted in collaboration with Stanford University, showed that the prognostic value of the 70-gene assay could be refined [8]. This was achieved by the inclusion of genes associated with a wound response in fibroblasts, which the Stanford group had previously shown to be predictive of outcome in breast cancer [9].

![Figure 1. Supervised classification for prognosis. From [6], with permission.](http://theoncologist.alphamedpress.org/)

![Figure 2. Supervised classification prognosis: “Leave-one-out” crossvalidation. From [6], with permission.](http://theoncologist.alphamedpress.org/)

![Figure 3. Validation study investigating the prognostic power of the 70 genes in stage I and II patients (n = 295). From van de Vijver MJ, He YD, van’t Veer LJ et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002; 347:1999–2009. ©2002 Massachusetts Medical Society. All rights reserved. Adapted with permission, 2005.](http://theoncologist.alphamedpress.org/)
Incorporation of the “wound genes” could not distinguish among patients within the good-prognosis group, as defined by the 70-gene signature. However, within the poor-prognosis group, patients with a quiescent wound signature had an outcome that was significantly less bad than those with an activated wound signature (Fig. 4).

Wang et al. [10] in Rotterdam, another group with access to a large bank of tumor specimens, have also made appreciable progress using gene-expression profiling to predict the occurrence of distant metastases in lymph node–negative primary breast cancer. Supervised classification of tumor genes from 286 cases (none undergoing adjuvant therapy) identified a 60-gene expression profile in estrogen receptor–positive patients and 16 genes forming a prognosis profile in receptor-negative patients.

Interestingly, genes identified by that group, who used an Affymetrix platform, showed very little overlap with those identified at the NKI in Amsterdam; only three were common to the two expression profiles. However, survival data showed the Rotterdam gene profile to have much the same prognostic value as the profile we had developed. A greater degree of overlap would have enhanced confidence in our identification of the exact set of genes needed to predict outcome. Nevertheless, we are confident that the two datasets, ultimately, will prove compatible.

**Gene Profiles as Predictors of Response to Therapy**

Clearly, several factors predicting response to therapy of various kinds have already been established. Thus, estrogen receptor positivity predicts response to hormonal therapy, HER-2 positivity predicts response to trastuzumab (Herceptin®; Genentech, Inc., South San Francisco, CA, http://www.gene.com), presence of c-kit mutation predicts response to imatinib (Gleevec®; Novartis Pharmaceuticals Corporation, East Hanover, NJ, http://www.pharma.us.novartis.com), and presence of epidermal growth factor receptor mutation predicts response to gefitinib (Iressa®; AstraZeneca Pharmaceuticals, Wilmington, DE, http://www.astrazeneca-us.com). However, we are still far from being able to individualize therapy with any great degree of confidence.

Two groups have attempted to predict responsiveness to tamoxifen (Nolvadex®; AstraZeneca Pharmaceuticals). Jansen et al. [11] produced a molecular classification of tamoxifen-resistant breast carcinomas by gene-expression profiling, and Ma et al. [12] reported that a two-gene expression ratio predicted clinical outcome in breast cancer patients treated with tamoxifen.

However, attempts to predict responsiveness in the adjuvant setting inevitably require very long periods of follow-up to generate outcome data. Neoadjuvant trials promise far quicker results, but data published so far relate only to small series of patients.

The Baylor group of Chang et al. [13] investigated gene-expression profiling as a means of predicting the therapeutic response to docetaxel (Taxotere®; Aventis Pharmaceuticals Inc., Bridgewater, NJ, http://www.aventispharma-us.com) in breast cancer patients. There were 24 cases in the training set and six for validation. At MD Anderson Cancer Center, Ayers et al. [14] looked at gene-expression profiles in a similar number of patients as a means of predicting pathological complete response to neoadjuvant paclitaxel (Taxol®; Bristol-Myers Squibb, Princeton, NJ, http://www.bms.com) and fluorouracil, doxorubicin (Adriamycin®; Bedford Laboratories, Bedford, OH, http://www.bedfordlabs.com), and cyclophosphamide. Many centers are now investigating the predictive value of the gene profiling of biopsied tumor in larger groups of patients.

At the NKI in Amsterdam, we have concluded a small study in 49 patients with locally advanced breast cancer randomized to neoadjuvant chemotherapy with either doxorubicin plus cyclophosphamide or docetaxel plus cyclophosphamide (each for six cycles) prior to surgery and/or radiotherapy and tamoxifen (Fig. 5) [15]. To date, it has not been possible to identify a gene profile in initial biopsy material that predicts responsiveness to one or the other of the alternative regimens. This will very likely require the study of a large number of patients. However, there is little doubt that genes predictive of response will eventually be found.

Figure 4. Combining gene-expression signatures. From [8], with permission.
**Gene Profiling in Routine Clinical Practice**

Given that prognostic and predictive gene profiles have or will be identified, the next question is: How do we incorporate them into routine clinical practice? To establish feasibility, we have embarked on the RASTER (microarray profiling in breast cancer) study, in which microarray technology was made available to eight hospitals, the majority being community based.

The aim was to look at T1 and T2, node-negative patients aged less than 60 years, with a view to establishing a 70-gene prognostic profile for these patients. The protocol required standardized acquisition of tissue using punch biopsy, with the specimen being placed in a commercially available reagent (RNAlater®; Qiagen, Venlo, The Netherlands, http://www1.qiagen.com/SelectCountry.aspx, conveyed by conventional mail, and frozen by the receiving center within 7 days of the specimen being taken. Extracted RNA is analyzed using a custom-made 70-gene microarray test, and patients are classified into good and poor prognosis groups.

In 60 weeks, 251 patients have been entered into the RASTER study, and 133 microarray tests have been conducted. Since the study is confined to lymph node–negative patients, and lymph node status is established only at subsequent surgery, the main reason for tissue not being analyzed (in 24% of cases) is that the patients were identified as node positive. Technical problems, such as the tumor being ≤50% of the sample, poor RNA quality, and poor tissue preservation, were minor considerations. Results of the study are awaited, but the initial phase should give us confidence that procedures for acquiring material for genetic profiling are robust.

**Prospective Versus Retrospective Validation**

Recently, a company called Genomic Health (Redwood City, CA, http://www.genomichealth.com) has developed a system that can establish gene profiles in material that has been paraffin-embedded [16]. Using this system, they have collaborated with the National Surgical Adjuvant Breast and Bowel Project (NSABP) to identify and validate a 21-gene prognostic classifier using retrospectively collected paraffin-embedded tumors from patients treated in NSABP randomized clinical trials. Such randomized controlled trials provide the ideal environment in which to assess the predictive value of gene profiles. The fact that the 70-gene assay requires frozen material may limit its use on archival samples.

The fact that the TRANSBIG validation study, using retrospectively collected material, found that the 70-gene profile was not a strong prognostic factor needs to be taken into account. Prospective clinical studies are under way to further test microarray-based prognostic tests. In the Microarray for Node-Negative Disease May Avoid Chemotherapy (MINDACT) trial, more than 5,000 patients will be randomized to treatment according to their 70-gene profile as well as clinical prognostic factors, and results are awaited with interest.

Gene-expression profiling and other high-throughput techniques are helping to discover novel prognostic tests and novel therapy response–predicting tests. These tests will help in guiding adjuvant systemic treatment.

**Disclosure of Potential Conflicts of Interest**

The author indicates no potential conflicts of interest.

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**References**


