

# ADVANCES IN ONCOLOGY

Current Developments in the Management of Solid Tumor Malignancies

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## The Use of Microarray Technology in the Management of Breast Cancer

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**H&O** How was microarray technology first developed and utilized in diagnostic medicine?

**LP** Over the past several decades it has become increasingly obvious that cellular functions are regulated by very complex networks of molecular interactions. At the same time, almost all of the available analytic tools could measure only one or a few molecules at a time. There was a desperate need to develop high throughput, parallel measurement technologies that could enable scientists to measure several hundred molecules simultaneously. This need has led to the development of several novel high throughput analytical methods, including gene expression profiling with DNA microarrays. This technology allows the semiquantitative measurement of several thousand mRNA species from a biologic specimen in a single experiment.

Advances in diagnostic medicine are often driven by advances in technology. Clinical investigators are keen to test new analytic technologies and examine if they could be developed into novel diagnostic tools. In the past 10 years, gene expression profiling using DNA microarrays has been studied in various disease settings. At first, it was believed that this technology would be used as a platform to identify potentially useful new markers of disease outcome, which would then be measured using more established techniques, such as immunohistochemistry at the protein level or reverse transcriptase polymerase chain reaction (RT-PCR) at the mRNA level. However, an increasing number of investigators have reported that microarray results are reproducible and reliable enough so that this technology itself may serve as the diagnostic

platform. Recently, a large collaborative study, the MAQC project, led by the US Food and Drug Administration (FDA) confirmed that microarray-based mRNA measurements are quite reproducible and robust.<sup>1</sup> Current investigations focus on examining the reproducibility and clinical value of gene signature–based prediction results.

**H&O** How could this technology assist in the clinical management of breast cancer?

**LP** It is hoped that microarray technology will improve medical decision-making. In breast cancer, the two most important diagnostic challenges are identifying prognosis at the time of diagnosis and identifying which adjuvant treatment to use if one is needed due to poor prognosis. Three different treatment modalities have been shown to improve survival after surgery for breast cancer: endocrine therapy, such as tamoxifen or aromatase inhibitors; trastuzumab (Herceptin, Genentech), the monoclonal antibody against HER2; and a variety of different chemotherapeutic regimens. Determination of estrogen receptor (ER) expression aids selection of patients for hormonal therapies, HER2 expression is used to select patients for trastuzumab therapy. However, none of these tests is perfect. The positive predictive value of ER expression is 50% or less, meaning that only half, or less, of patients who receive endocrine therapy may benefit from it. Gene signature–based tests may help to refine this prediction further. The same is true for HER2 expression; less than a third of patients who are HER2-positive benefit from trastuzumab. The situation is even worse for chemotherapy. There are several different regimens that are equally efficacious but with large differences in cost and substantial differences in toxicity. However, a model for rational selection of one regimen over another based on a patient's molecular profile does not exist. Rather, physicians choose regimens based on training or the influence of regional centers, for example. Again, the hope is that gene signatures may identify subsets of patients who are particularly sensitive to a given drug or regimen of drugs.

**H&O** How has prognosis typically been determined in breast cancer, and how can microarray technology be combined with this approach?

**LP** The current state of the art to predict the prognosis of patients with breast cancer is to use a clinical variable-based risk prediction model. Tumor size, histologic grade, age, ER status and the number of involved lymph nodes can be combined into a relatively accurate multivariable risk prediction model. One such freely available online prognostic tool is AdjuvantOnline ([www.adjuvantonline.com](http://www.adjuvantonline.com)). Clinical variable-based predictors are very useful, but not perfect, and further improvements in their accuracy would be welcome.

Two different groups in The Netherlands, with the help of industry partners, developed gene expression signatures that could predict individuals' prognosis. One is a 70-gene prognostic signature (MammaPrint, Agendia), which was approved by the US Food and Drug Administration for clinical use in February 2007, and the other is a 76-gene prognostic signature (Veridex). Both have been shown to separate patients with good prognosis from those whose disease is more likely to relapse. The important question is to what extent these genomic prognostic predictors are superior or complementary to the clinical variable-based models. One recent publication at the end of last year reported that the 70-gene signature could re-stratify patients by recurrence risk within a given clinical risk category.<sup>2</sup> For example, the genomic predictor could identify a subset of patients among those who were considered low risk (<8–12% risk of relapse) by clinical characteristics, who in fact had a recurrence rate closer to 20%. However, relatively few patients were included in this validation study and therefore the confidence intervals around the risk estimates are quite broad. We are still uncertain how precise these genomic prognostic estimates are. Based on the currently available results, a patient predicted to have good prognosis with the MammaPrint assay can have a recurrence rate between 5% and 19%. It is my impression that clinical and genomic prognostic predictors will be used as complementary risk assessment tools. Their combined use will likely yield better medical decisions than the use of either alone. I consider these emerging diagnostic tests to be analogous in their medical use to imaging studies. Mammograms, ultrasonogram, and magnetic resonance imaging (MRI) are complementary imaging tools to assess the breast. In many cases, a single imaging modality is sufficient for diagnosis, but in other cases, more than one imaging modality is needed in order to make the best medical decision.

### **H&O** What are other applications of gene expression profiling technology in breast cancer?

**LP** A multigene assay called Oncotype DX (Genomic Health) is increasingly used by physicians to identify ER-positive patients who may not need adjuvant chemo-

therapy because they have sufficiently good prognosis with adjuvant endocrine therapy alone. This assay is a 21-gene predictor of benefit from endocrine therapy for lymph node–negative, ER-positive patients.<sup>3</sup> It uses multiplex RT-PCR rather than microarray technology but the basic diagnostic principle is very similar.

My research group is interested in developing gene signature-based predictors of chemotherapy response. We recently reported that it is possible to predict who will and will not achieve a pathologic complete response to preoperative paclitaxel, 5-fluorouracil, doxorubicin, and cyclophosphamide (T/FAC) chemotherapy based on a 31-gene signature.<sup>4</sup> A corollary of this observation is that those who are unlikely to achieve this very favorable response, which is almost invariably associated with prolonged cancer-free survival, may be the best candidates for clinical trials that test novel therapies.

### **H&O** How was this trial conducted?

**LP** The trial included 133 patients with stage I–III breast cancer who all received preoperative chemotherapy with T/FAC. We prospectively collected fine needle biopsies of the cancer in the breast or in the lymph nodes for gene-expression profiling. We identified genes that differ between those who had a pathologic complete response and those who did not, and we combined these informative genes into a multivariable response prediction model. We used the first 82 cases to develop our predictor and assessed its accuracy on the remaining 51 independent cases. Our predictor showed significantly higher sensitivity (92% vs 61%) than a clinical variable–based predictor of response. The high sensitivity indicates that we correctly identified almost all of the patients (92%) who actually achieved pathologic complete response. The negative predictive value of the test was also high (96%), which indicates that less than 5% of test-negative patients (ie, predicted to have residual disease) achieved the favorable response. These performance statistics are similar or better than those seen with ER immunohistochemistry or HER2 gene amplification as predictive markers for response to endocrine or trastuzumab therapies, respectively. In the next 2–3 months, we will begin to use this and another endocrine sensitivity assay developed by our pharmacogenomic group to triage breast cancer patients into various preoperative therapies based on their gene-expression profile in the context of a new clinical trial. Those predicted to be highly sensitive to chemotherapy or endocrine therapy will receive these standard therapies, whereas those who are unlikely to benefit from the standard therapies will be enrolled in separate clinical trials with novel therapies.

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### **H&O** Could you further discuss methods of validation?

**LP** There is a consensus among clinical investigators and biostatisticians that the proper way to develop a new diagnostic assay is to define the reproducibility and robustness of the assay first; then identify the unmet diagnostic niche, obtain samples from the appropriate patient population to develop, and optimize the predictor assay; finalize all details (including thresholds to call a case “positive” or “negative”); and then assess the predictive accuracy of the new test.<sup>5</sup> Debate exists regarding what number of patients needs to be included in the discovery cohort in order to develop the best possible multivariable genomic prediction models. It is somewhat easier to define the number of patients needed to validate a model because this represents a simpler statistical problem.

A third piece of validation is also needed, which is to show that the predictor improves clinical outcome. Making a more accurate outcome prediction does not necessarily mean the patient’s outcome will also be improved. It is important to show that people who receive treatment based on novel test results will have a better outcome—for example, live longer or experience less toxicity—compared to those treated without using the novel test.

### **H&O** Regarding chemotherapeutic drug resistance, how does the use of combination regimens affect the predictive value of microarrays?

**LP** In the past, drug resistance was usually discussed in the context of an individual drug. However, for early-stage breast cancer, a combination of drugs is used in the clinic. It has been suggested that individual drug-specific response predictors could be developed and that these may be combined into a regimen-specific composite predictor. There have been some promising developments in this field of research; in particular, investigators at Duke University reported that they could develop drug-specific predictive signatures using cell lines as model systems. This research needs to be confirmed by others and will need to be tested in the clinic. Perhaps in the future it will be possible to formulate an individualized treatment cocktail based on predicted susceptibility to particular drugs.

### **H&O** Could you discuss the role of microarrays in the development of drugs for breast cancer?

**LP** Numerous companies are trying to develop patient selection methods in parallel with the development of a drug. This effort is inspired by the success story of trastuzumab and HER2 testing. It is widely held that if trastuzumab were tested in unselected patients in clinical trials, the drug would not have made it to the market because

the overall response rates would have been disappointingly low, 10% or less. Fortunately, patients were included in the trastuzumab studies only if they were HER2-positive, and therefore trastuzumab showed a 30% response rate in this molecularly selected population.

### **H&O** What are the challenges with research and study design for microarrays?

**LP** The major challenge with biomarker discovery studies is identifying a large enough number of cases from which a reliable predictor can be developed and, after that, to accrue a large enough patient population to validate the test and draw a definitive conclusion about its predictive accuracy. Archived specimens that are stored in tumor banks often represent a hodgepodge of different types of treatments and different stages of disease. They are rarely collected to address a particular diagnostic problem. The smaller the discovery and validation sample sizes are, the lower our confidence in the results. Oncologists and funding agencies are not yet attuned to prospective biomarker discovery and validation studies that could be very costly. Furthermore, diagnostic companies do not have the financial resources or experience that pharmaceutical companies have for conducting prospective clinical trials. Nevertheless, though these obstacles do exist, the clinical research community is tackling them with slow and steady success.

### **H&O** What do you believe is the future for microarray technology in breast cancer?

**LP** Multigene prognostic and predictive tests will become part of routine management. Not all patients may need these tests but some could benefit from the information that they provide. One of the important promises of high-throughput gene expression profiling is that material from a single needle biopsy could be used to provide multiple different prediction results, each based on a different set of genes. Thus, from a single test, one could determine ER and HER2 status, estimate the risk of relapse, and also gauge the endocrine and chemotherapy sensitivity of the tumor.

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