Clinical Utility of Comprehensive Microarray Testing in Early-stage Breast Cancer

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Abstract

Microarray gene expression profiling in the diagnostic setting offers the opportunity of reading out multiple profiles and genes from a single array. Several additional profiles have been added to the Agendia breast cancer suite called Symphony(TM), along with MammaPrint, a US Food and Drug Administration (FDA) cleared prognostic and predictive assay, by virtue of this technology. These additional profiles, TargetPrint, BluePrint, and TheraPrint, expand the clinical utility of the Symphony profile. They also demonstrate the versatility of gene expression profiling in the diagnostic setting, enabling the addition of many more clinically relevant profiles (such as drug response profiles) as experience grows.

Early detection and treatment of breast cancer have dramatically improved patient survival and favorably influenced the natural history of the disease. Clinicopathologic criteria such as tumor size and lymph-node involvement have historically been used to guide treatment, and earlier detection has now placed more emphasis on prognostic as well as predictive biomarkers that are useful in planning treatment for patients with early stage disease (i.e. T1–2, N0–1,M0). Traditional biomarkers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are combined with genomic profiling,[1,2] multiple gene or protein biomarkers,[3] and molecular subtyping[4,5] to improve treatment recommendations. In addition, gene expression read out for ER, PR and HER2 is offered to supplement conventional immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) testing;[6] expression profiling offers the technical advantages of being both quantitative and more objective than IHC.

Three assays have emerged commercially representing three different technologies: MammaPrint (microarray), Oncotype DX (realtime polymerase chain reaction [RT-PCR]), and Mammostrat (immunohistochemistry [IHC]).[1,3,7] All use differing technologies to identify and 'quantitate' gene (i.e. MammaPrint or Oncotype DX) or protein (i.e. Mammostrat) expression along with different algorithms to assess the risk of recurrence. Mammostrat looks at five proteins, Oncotype DX at 21 genes (16 target and five reference), and MammaPrint at 70 target genes with an additional 465 normalization and

536 control genes, whose simultaneous detection and measurement is facilitated by the scalability of the microarray platform. MammaPrint measures 70 genes found to be important in the prognosis of untreated women with early-stage breast cancer as determined by genome wide analysis. Currently, MammaPrint is the only US Food and Drug Administration (FDA) cleared prognostic and predictive assay for breast cancer. Several additional signatures have been added to the diagnostic array to enhance the clinical utility MammaPrint. These additional signatures include TargetPrint,[6] which measures the quantitative expression of ER, PR and HER2; BluePrint,[5] which uses an 80-gene profile to characterize the patient's tumor as luminal, basal, or ERBB2 (HER2) type; and TheraPrint, which looks at 56 genes that are the targets of a variety of antineoplastic agents or which have been shown to be involved in resistance or response.

MammaPrint

MammaPrint was developed at The Netherlands Cancer Institute (NKI) as a tool for clinicians to use to help determine which patients with early stage breast cancer would develop metastases and who would remain disease-free without any treatment following initial curative surgery and radiotherapy, as indicated (see *Figure 1*). MammaPrint provides an accurate assessment of recurrence risk independent of the patient's tumor size, ER status, or age, and has been cleared by the FDA. MammaPrint is the only breast cancer multigene assay to have achieved this metric. In fact, MammaPrint is prognostic in women with

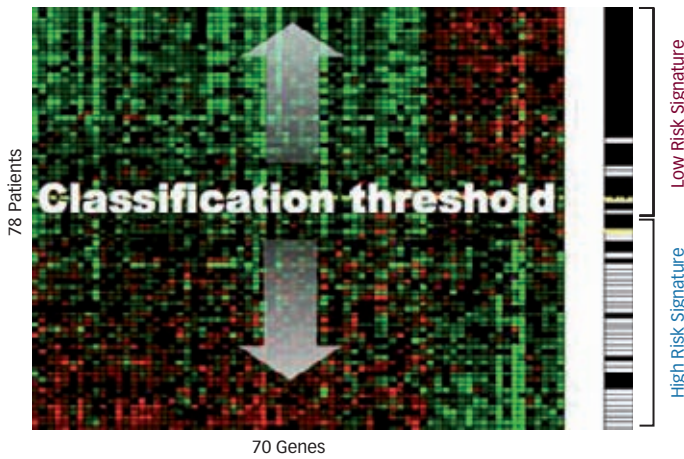**Figure 1: Heatmap of 70-gene profile MammaPrint of Patient Training Series**

Figure 2: TargetPrint Patient Result Form

known benefit of hormonal therapy in ER-positive patients (i.e. ~50 % risk reduction), these patients are at a sufficiently low risk for recurrence so that hormone therapy alone is optimal treatment.[9] Conversely, those patients with a 'high risk' result have a 30 % risk of recurrence and in addition, can be expected to benefit from both neo-adjuvant[10] and/or adjuvant[11] chemotherapy.

To further evaluate the benefits of the MammaPrint profile in clinical practice, two large prospective, randomized trials are under way. The Investigation of Serial Studies to Predict your Individual Response with Imaging and Molecular Analysis (I-SPY2) trial was recently launched at 20 major cancer centers across the US and is designed to accelerate Phase II drug development using a novel adaptive design sanctioned by the FDA, National Cancer Institute (NCI), and a number of pharmaceutical companies.[12]

The Microarray in Node-Negative Disease May Avoid Chemotherapy (MINDACT) trial is a much more ambitious undertaking that has been accruing patients for several years across the EU. It is a prospective, randomized trial to determine whether patients are better served by treatment prompted by the MammaPrint profile or by clinicopathologic assessment using the Adjuvant! Online tools.[13] It is designed to enroll 6,000 patients so that the number of patients on the discordant arm wherein genomic treatment assignment is compared with that prompted by Adjuvant! Online is sufficient to detect a clinically meaningful and statistically significant benefit. MINDACT finished enrollment in July 2011 and will be ready for evaluation by 2014.

TargetPrint

TargetPrint was developed as an independent and quantitative measure of ER, PR, and HER2 expression using messenger RNA (mRNA) detection and validated against IHC at a central reference lab. Concordance between the two techniques was high with values of 93, 83, and 96 %, respectively. The slightly lower concordance values for PR likely represents a reduced specificity, due to several species of the PR receptor detected by standard IHC immunostaining. The importance of accurate testing for ER, PR, and HER2 cannot be overemphasized in determining the best treatment for a given patient and documented variability from day-to-day and from institution to institution prompted the biomarker group of the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) to publish guidelines for ER and PR testing in 2010.[14]

These guidelines address a number of pre-analytic variables including fixation time which have been found to influence the intensity of the immunostains and, hence, the extent of positivity for ER and PR. Similar variability in HER2 detection has also been observed with IHC, prompting re-testing for patients who were strongly positive to FISH testing as the standard of care.[15] However, immunostaining is, by its very nature, a qualitative process that depends on the experience of the pathologist as well as all the pre-analytic and analytic variables mentioned in the guidelines. As such, a semi-quantitative measure using gene expression may have advantages with respect to precision and reproducibility unattainable by routine IHC. Indeed, TargetPrint

node negative and node positive disease with one to three positive lymph nodes.[8] In addition, as MammaPrint was both developed and validated on untreated patients, it is most consistent in newly diagnosed, untreated women who present to their surgeons and oncologists for treatment advice. Women with a 'low risk' result have a 10 % risk of developing distant metastases over the next 10 years without any adjuvant hormone or chemotherapy. Thus, given the

might be viewed by the pathologist or clinician as a 'second opinion' for IHC results. *Figure 2* shows the commercial TargetPrint result received by the physician.

BluePrint

The pioneering work of Sorlie and Perou[4] indicates that valuable prognostic and predictive information can be obtained by molecular subtyping of a patient's breast cancer using a genomic classification system based on microarray analysis of a large number of genes. This classification system was first reported in 2000 and since then has undergone several iterations with the key findings grouping breast cancer into four major subtypes: basal, luminal (A and B), and ERRB2 (HER2) type. Each of these subtypes behave differently clinically, and appear to benefit from differing therapeutic approaches.

Agendia has developed and commercialized an 80-gene microarray-based assay that mirrors the Perou classification and which is designed for use in concert with the other Symphony profiles, MammaPrint and TargetPrint, to provide a clinically meaningful classification system. Together they not only provide prognostic information, but can also be used to help guide therapy.[5] The schema for development of BluePrint is shown in *Figure 3*, samples that were analysed both by TargetPrint and IHC in case of concordant results the samples were used for BluePrint development. By using concordant results we ensure to have mRNA expression and protein expression of the ER, PR, and HER2 markers. Of particular interest is the capability of sub-classifying HER2-positive patients into high- and low-risk groups by MammaPrint, with very different recurrence risks. As the overall response rate to herceptin is ~30 %, treatment assignment as a function of genomic profiling most likely have utility in both improving the response rate and minimizing the cost and toxicity of treatment.[16]

Molecular subtyping is currently being used in clinical trials to help stratify patients, anticipating that such information will have a beneficial effect on patient outcomes.

TheraPrint

While clinical practice guidelines suggest therapeutic choices for neo-adjuvant, adjuvant, and first-line metastatic disease, advanced recurrent disease may prove to be a therapeutic conundrum for the clinician. Patients are usually extensively pre-treated and criteria for disease progression and drug resistance may vary among practitioners.

TheraPrint is a multigene assay that specifically measures the expression level of 56 genes that are the known factors in controlling the response to endocrine agents, chemotherapy drugs, small molecules and monoclonal antibodies. TheraPrint compares the levels of expression of these genes with those of an untreated, invasive breast cancer pool. The genes present in this panel are listed on a multiple page result form (*Figure 4* shows a sample first page) and represent the majority of identified genomic biomarkers associated with a defined therapy, which may be useful for patients with breast cancer. A Registry trial is being developed by Agendia to record how physicians will use TheraPrint assay and if its use can be shown to improve patient outcomes when compared to treatments chosen empirically.

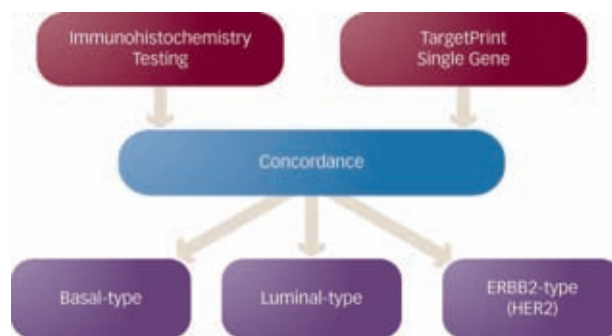**Figure 3: Rational Based Method of BluePrint Development**

Figure 4: TheraPrint Patient Result Form

Unigene Symbol	UniGene Cluster ID	Unigene Name	Absolute Expression (Intensity)	Reference Distribution 5% - 95%
AKT1	Hs.525622	V-akt murine thymoma viral oncogene homolog 1	9.8	9.1 - 11.4
AURKA	Hs.250822	Aurora kinase A	7.9	7.5 - 9.8
BCL2	Hs.150749	B-cell CLL/lymphoma 2	10.3	7.2 - 11.1
BRAF	Hs.550061	V-raf murine sarcoma viral oncogene homolog B1	7.5	7.0 - 8.6
BRCA1	Hs.194143	Breast cancer 1, early onset	7.5	6.6 - 8.3
BRCA2	Hs.34012	Breast cancer 2, early onset	6.1	5.9 - 6.8
C11orf30	Hs.352588	Chromosome 11 open reading frame 30	8.0	7.0 - 8.6
CCND1	Hs.523852	Cyclin D1	10.3	8.5 - 12.4
CCNE1	Hs.244723	Cyclin E1	9.4	8.4 - 10.7
CDH1	Hs.461086	Cadherin 1, type 1, E-cadherin (epithelial)	12.2	9.5 - 13.6
CDH3	Hs.554598	Cadherin 3, type 1, P-cadherin (placental)	8.2	7.7 - 11.7
CRYAB	Hs.408767	Crystallin, alpha B	8.9	8.4 - 13.3
CSK	Hs.77793	C-src tyrosine kinase	7.6	7.2 - 8.9
CXCL12	Hs.522891	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	8.6	7.2 - 10.8
CXCL14	Hs.483444	Chemokine (C-X-C motif) ligand 14	17.0	10.3 - 16.6
DHFR	Hs.83765	Dihydrofolate reductase	9.0	7.8 - 10.6
ECGF1	Hs.592212	Endothelial cell growth factor 1 (platelet-derived)	9.5	8.2 - 11.3
EGFR	Hs.488293	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	6.0	6.0 - 8.0
ERBB3	Hs.118681	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	10.4	8.6 - 11.4
ERBB4	Hs.390729	V-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	7.3	6.0 - 8.1
ESR2	Hs.660607	Estrogen receptor 2 (ER beta)	6.2	6.0 - 6.5
FANCF	Hs.651196	Fanconi anemia, complementation group F	8.9	8.4 - 12.6
FLT1	Hs.507621	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	7.7	7.4 - 9.5
FLT3	Hs.507590	Fms-related tyrosine kinase 3	7.8	6.0 - 8.0
FLT4	Hs.646917	Fms-related tyrosine kinase 4	7.8	6.3 - 7.8
FRAP1	Hs.338207	FK506 binding protein 12-rapamycin associated protein 1	7.8	7.2 - 8.5
GSDML	Hs.306777	Gasdermin-like	7.1	6.4 - 9.4

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Conclusions

Gene expression profiling such as the Agendia Symphony Breast Cancer Decision Suite (TargetPrint, MammaPrint, BluePrint, and TheraPrint) can be used for both prognosis and prediction and are important tools for the medical oncologist to use in both evaluating their patients and in choosing an appropriate neo-adjuvant or adjuvant treatment program. Further, all validated multigene assays should be included in guidelines (i.e. St Gallen, National Comprehensive Cancer Network [NCCN], and ASCO guidelines) and this would lend further credence to their growing importance in current practice. ■

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