

# State of the Art in the Use of Gene Expression Technologies for Breast Cancer Management

a report by

**Christine Desmedt**

*Translational Research Unit, Institut Jules Bordet, Université Libre de Bruxelles*

DOI: 10.17925/EOH.2008.04.1.66

## The Clinical Problem

Breast cancer is a global public health issue. It is the most frequently diagnosed malignancy in women in the western world and the most common cause of cancer death in European and American women. According to estimates, in 2002 there were 1,151,298 new cases of breast cancer diagnosed, 410,712 deaths caused by breast cancer and more than 4.4 million women living with breast cancer worldwide.<sup>1</sup> In Europe, one out of every eight to 10 women, depending on the country, will develop breast cancer during their lifetime.<sup>2</sup>

In current clinical practice, the majority of patients with early breast cancer receive some form of systemic adjuvant therapy (chemo-, endocrine and/or targeted therapy), which leads to an increased burden in terms of healthcare costs. Unfortunately, our current understanding of the optimal adjuvant therapy for the individual patient is still very limited, with many being over- or undertreated, or treated inefficiently. For these reasons, the identification of prognostic and predictive markers that will assist the clinician in selecting the most suitable form of medical therapy has become a very high priority, as well as a real challenge, in translational research.

## The Molecular Classification of Breast Cancer

Clinicians have long recognised that the diagnosis of breast cancer includes tumour types with different natural histories and responses to various treatments. Nevertheless, traditional histopathological characteristics have been unable to capture the biologic heterogeneity of breast tumours. Many early studies started to search for subgroups of the breast cancer tumours using non-supervised approaches, such as clustering or principal components analysis, where the structure of the data is studied without a specific *a priori* hypothesis.<sup>3-6</sup> These studies consistently showed that:

- oestrogen receptor (ER) status has the strongest association with gene expression, followed by histological grade;
- breast tumours can be grouped according to at least four individual subgroups: the basal-like and human epidermal growth factor receptor-2 (HER2) subgroups, which are predominantly ER-negative,

and two or more luminal subgroups, which are predominantly ER-positive; and

- each subgroup has a distinct clinical outcome and may therefore respond differently to various therapeutics.

Interestingly, these studies also revealed that clinically relevant variables such as menopausal status, tumour size and nodal status grade were not associated with distinct expression profiles. This class discovery has been very useful in highlighting that breast cancers are a heterogeneous group of diseases, and has helped to better understand

The molecular classification of breast cancer has highlighted that these tumours are a heterogeneous group of diseases, and this classification has already altered the way oncologists perceive the disease and design clinical trials.

breast tumour biology. However, at this stage it is difficult to use these results to tailor cancer treatment and improve the prognosis and response of cancer patients to commonly used drugs. Indeed, unsupervised methods often remain subjective since different partitions can be obtained when using different measuring similarities or aggregation algorithms. More importantly, although these methods have been effective at highlighting biological differences between tumours, they do not look for the differentially expressed genes with respect to the clinical outcome of a specific treatment. Thus, they are not suited, at least when used on their own, to identify reproducible prognostic or predictive markers.

## Gene Expression Profiles as a Prognostic Tool

Although different tools have been developed to assist clinicians in selecting patients who should receive adjuvant therapy, such as the St Gallen consensus criteria,<sup>7</sup> the National Institutes of Health (NIH) guidelines<sup>8</sup> or Adjuvant! Online,<sup>9</sup> it still remains a challenge to distinguish those patients who really need adjuvant systemic therapy from those who could be spared such a treatment. In order to develop a more accurate tool for breast cancer prognosis, several groups have conducted comprehensive genome-wide assessments of gene expression profiling and identified prognostic gene expression signatures. To this end, two different approaches have been used: the 'top-down' approach and the hypothesis-driven or 'bottom-up' approach (see *Figure 1*).



Christine Desmedt is a researcher in the Translational Unit of the Institut Jules Bordet, Université Libre de Bruxelles. Her research projects, conducted in collaboration with different international institutions, involve the identification and validation of prognostic and predictive markers in breast cancer, as well as a better characterisation of breast cancer development and metastasis. Dr Desmedt holds a PhD from the Université Libre de Bruxelles.

E: christine.desmedt@bordet.be

Examples of signatures that were developed using the first approach, i.e. by seeking gene expression profiles that are associated or correlated with clinical outcome without any biological assumption, are the 70- and 76-gene signatures developed by the National Cancer Institute in Amsterdam and Rosetta, and the Erasmus MC in Rotterdam together with Veridex, respectively.<sup>10,11</sup> Although these signatures were built using different microarray platforms, a feature common to both signatures is that they correctly identified high-risk patients while also identifying a higher number of low-risk patients not needing treatment compared with the clinically based risk classifications.

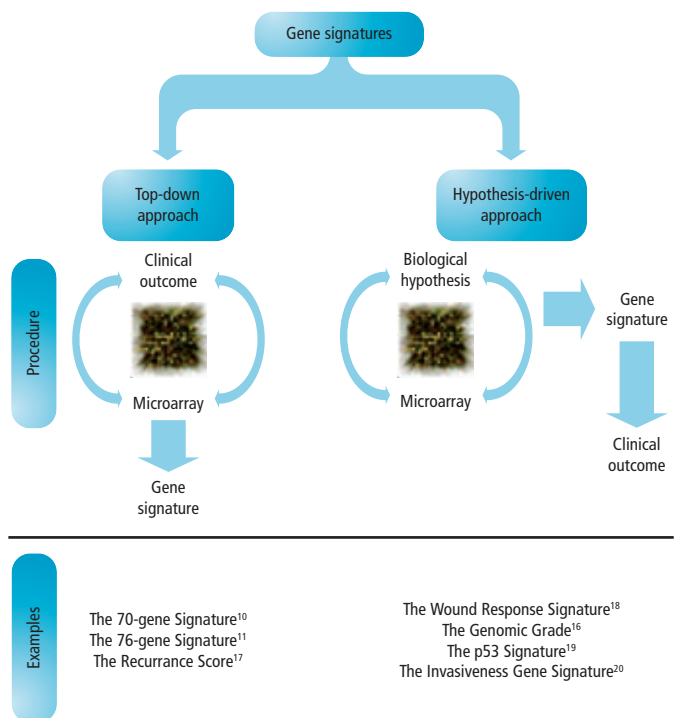
A major challenge for gene expression profiling studies, especially those with clear clinical implications, is independent validation. In this context, the Translational Research part of the Breast International Group (TRANSBIG) conducted an independent validation study of these two prognostic signatures.<sup>12,13</sup> Although there was only a three-gene overlap between these signatures, both were validated in this patient cohort, even after adjustment for clinical risk. In addition, a very interesting finding of this validation work was the heterogeneous behaviour of these gene signatures over time, which could be observed thanks to the unusually long follow-up (14 years) of the patients in this validation series. The signatures appeared to be strong predictors of the development of early distant metastases, while showing a decreased prognostic ability with increasing number of follow-up years. This finding, which was not observed for clinical risk, is not entirely unexpected since the signatures were built to identify patients with distant metastases within five years; however, it suggests that different mechanisms may be associated with the development of early and late distant metastases, as already proposed by Klein and his group.<sup>14,15</sup>

An example of deriving a prognostic gene expression signature using a hypothesis-driven approach was the study reported by our group that focused on histological grade, a well-established pathological parameter rooted in the cell biology of breast cancer. Indeed, clinicians face a huge problem with respect to patients who have intermediate-grade tumours (grade 2), as these tumours – which account for 30–60% of cases – are the major source of inter-observer discrepancy and may display intermediate phenotype and survival, making treatment decisions for these patients a great challenge, with subsequent under- or overtreatment. Performing a supervised analysis, we developed a Gene expression Grade Index (GGI) score based on 97 genes.<sup>16</sup> These genes were mainly involved in cell-cycle regulation and proliferation and were

Proliferation is the common driving force of several prognostic signatures and also the strongest parameter predicting clinical outcome in the ER-positive/HER2-negative subgroup of patients only.

consistently differentially expressed between low- and high-grade breast carcinomas. Interestingly, the GGI, which essentially quantifies the degree of similarity between the tumor expression pattern of these 97 genes and tumour grade, was able to reclassify patients with histological grade 2 tumours into two groups with distinct clinical outcomes, similar to those of histological grade 1 and 3, respectively.

**Figure 1: Schematic of the Two Different Approaches to the Development of Gene Expression Signatures**



The ‘top-down’ approach compares gene expression data from cohorts of cases with known clinical outcomes to identify genes that are associated with prognosis without any a priori biological assumption. The hypothesis-driven or ‘bottom-up’ approach first identifies gene expression patterns associated with a specific biological phenotype or a deregulated molecular pathway, then correlates these findings with clinical outcome.

In addition to the signatures described above, many other research groups have contributed gene expression signatures that are predictive of clinical outcome in breast cancer (see Figure 1).<sup>17–21</sup> However, the generation of these prognostic signatures has mainly been performed on global sets of breast cancer patients. Since it is clear that breast cancer is a molecular heterogeneous disease, with subgroups defined primarily by ER and HER2, we recently aimed at refining breast cancer prognosis according to these molecularly homogeneous subgroups of patients in the context of a meta-analysis involving publicly available gene expression data from more than 2,100 patients.<sup>22</sup> In that study, we showed that proliferation is the common driving force of several prognostic signatures and is also the strongest parameter predicting clinical outcome in the ER-positive/HER2-negative subgroup of patients only, whereas immune response and tumour invasion appear to be the main biological processes associated with prognosis in the ER-negative/HER2-negative and HER2-positive subgroups, respectively.

**Gene Expression Profiles to Predict Benefit of Treatment**

Although improving breast cancer prognosis is of critical importance to better identify those patients needing treatment, it is not sufficient since we also need to know which therapy will benefit the individual patient. Indeed, only a proportion of patients will respond to a particular treatment, whereas most will experience the adverse side effects. In addition, the current overtreatment of patients results in major expense for individuals and society – an expense that may not be indefinitely sustainable. Currently, the only accepted and recommended predictive markers for breast cancer are the hormonal receptors, which are used to select patients likely to respond to

**Figure 2: The Use of Expression Profiles as Biomarkers to Improve Breast Cancer Management Is Coming of Age**



**Table 1: Key Features of TAILORx and MINDACT Validation Trials**

	TAILORx	MINDACT
	Co-ordination: ECOG	Co-ordination: EORTC
Population	Node-negative, ER+	Node-negative, ER+/-
Molecular assay	OncotypeDX® (Q-RT-PCR)	Mammaprint® (Microarray)
Level of evidence (I–V)	II	III
Tissue	FFPE	Fresh/frozen
No.	~10,500	~6,000
No. randomised	4,390	1,920
Randomised group	RS 11–25 (40%)	Discordant risk between clinical defined by Adjuvant! Online and genomic (32%)
Randomisation	Treatment with hormonal therapy ± chemotherapy	Treatment by clinical versus genomic risk
Non-randomised groups	RS <11: hormonal therapy RS >25: chemotherapy + hormonal therapy	Both low-risk (13%): hormonal therapy; both high-risk (55%): chemotherapy ± hormonal therapy
Primary objectives	Is chemotherapy beneficial if the RS is 11–25?	Can chemotherapy be avoided with a low-risk 70-gene signature and in spite of a high clinical risk?
Secondary objectives	Create a specimen bank (FFPE + blood)	Create a specimen bank (frozen + FFPE + blood)
Risk groups	Risk of distant recurrence with hormonal therapy is about 12.5% for an RS of 11–25	Risk of death at 10 years around 12% for the low-risk group if untreated and <10% with endocrine therapy
Adjuvant therapy	Choice of hormonal and/or chemotherapy regimen	Hormonal therapy: randomisation between TAM 2y→AI 5y or AI 7y; chemotherapy: randomisation between anthracycline regimen or docetaxel–capecitabine

AI = aromatase inhibitor; ECOG = Eastern Co-operative Oncology Group; EORTC = European Organisation for Research and Treatment of Cancer; FFPE = formalin-fixed paraffin-embedded; ER = oestrogen receptor; RS = recurrence score; Q-RT-PCR = quantitative reverse transcription polymerase chain reaction; TAM = tamoxifen.

hormone therapy, and HER2, for selecting patients for treatment with the antibody trastuzumab. Otherwise, systemic treatment is not tailored to take into account the heterogeneity of the tumour biology and the response to treatment.

Several predictors have been identified for endocrine therapy, most of them using the ‘top-down’ approach. Using a small training set of 50 tumours from ER-positive women with advanced disease, researchers from Rotterdam identified 44 genes that predicted response to tamoxifen using a complementary DNA (cDNA) microarray platform.<sup>23</sup>

This set of genes was then applied to an independent validation set of 66 tumours and correctly predicted the outcome of response to tamoxifen treatment in 80% of cases. In another published study, an expression signature predictive of disease-free survival was developed from 60 patients treated with adjuvant tamoxifen. The signature was reduced to a two-gene expression ratio, HOXB13 versus IL17RB, transposed onto a polymerase chain reaction (PCR)-based technology and validated on an independent series using standard formalin-fixed paraffin-embedded tissue.<sup>24</sup> Similarly, Genomic Health developed a 16-gene assay on formalin-fixed paraffin-embedded samples, called the recurrence score (RS), which can predict the risk of recurrence in women receiving adjuvant tamoxifen.<sup>17</sup> However, it is difficult to dissect endocrine sensitivity from inherent prognosis. For example, it has been shown that the Genomic Grade and Recurrence Score, in which the proliferation set of genes has the highest ‘weight’ or co-efficient in the formula, was associated with clinical outcome in both systemically untreated and tamoxifen-treated populations.<sup>25,26</sup>

Recently, two separate publications also reported the two-gene expression ratio HOXB13/IL17BR to be a strong independent prognostic factor in ER-positive breast cancer patients, independent of tamoxifen therapy.<sup>27,28</sup> This suggests that adjuvant tamoxifen monotherapy does not alter the poor clinical outcome of the high-grade/high-RS subgroup/high two-gene ratio.

Interestingly, Kok et al. recently compared the performance of the tamoxifen signature identified by Jansen et al.,<sup>23</sup> the RS<sup>17</sup> and the two-gene expression ratio HOXB13/IL17BR<sup>24</sup> in an independent cohort of systemic-treatment-naïve breast cancer patients treated with first-line tamoxifen for metastatic disease.<sup>29</sup> They report that although the concordance between the three classifiers was low (they classified only 45–61% of patients in the same category), they were all significantly associated with time to progression in this independent patient series treated with tamoxifen, supporting the fact that the addition of multigene assays to ER improves the prediction of outcome in tamoxifen-treated patients and deserves to be incorporated in future clinical studies.

In the context of chemotherapy, few studies have been reported so far. The major reason for this is that most of these studies ideally require prospective sample collection in the context of a clinical trial. Several groups have identified genes associated with response to chemotherapy.<sup>30–36</sup> However, the statistical confidence of these studies remains low due to the small sample sizes and the unselected patient populations. Indeed, it has been shown in the literature that ER-negative and high-grade tumours were associated with a better response to chemotherapy. It is therefore not surprising that a number of genes reported to be predictive of response are in fact associated with ER, grade or proliferation genes.

Although the results of these ‘top-down’ studies are very encouraging and may even exceptionally lead to the identification of novel single markers with clear biological meaning – such as the discovery of microtubule-associated protein tau (MAPT), which mediates resistance to paclitaxel<sup>36</sup> – these signatures may not capture all of the relevant biology. Recently, using the ‘hypothesis-driven’ approach researchers at Duke identified several expression patterns associated with the deregulation of a variety of oncogenic pathways that could predict

**Table 2: Currently Available Commercial Molecular Assays to Predict Clinical Outcome in Breast Cancer**

	Mammaprint® 70-gene assay	OncotypeDX® 21-gene Recurrence Score	AviaraDx® 2-gene H/I Ratio	MapQuant Dx™ Genomic Grade
Provider	Agendia, Inc.	Genomic Health, Inc.	AviaraDx, Inc.	Ipsogen
Type of tissue sample	Fresh/frozen	FFPE	FFPE	Fresh/frozen
Technology	DNA microarrays	Q-RT-PCR	Q-RT-PCR	DNA microarrays
CLIA-certified laboratory	Yes	Yes	Yes	Yes
Indication	Stage I–II disease, tumour size 5cm, node-negative	Stage I–II disease, ER-positive, node-negative	Stage I–II disease, ER-positive, node-negative	Specifically indicated for invasive primary ER-positive grade 2 tumours
Level of evidence (I–V)	III	II	III	III
FDA clearance	Yes	No	No	No
Availability	Europe/US	Europe/US	Europe/US	Europe
Cost	~US\$3,200	~US\$3,500	~US\$3,000	Up to ~US\$3,200
Reimbursement by health insurance companies	No	Yes (only in US)	No	No
Website	www.agendia.com	www.genomichealth.com/oncotype/default.aspx	www.aviaradx.com	www.ipsogen.com

CLIA = Clinical Certification for Laboratory Services; FDA = US Food and Drug Administration; FFPE = formalin-fixed paraffin-embedded; Q-RT-PCR: quantitative reverse transcription polymerase chain reaction.

response to different therapeutic agents targeting specific deregulated pathways.<sup>37</sup> The same group, using publicly available drug sensitivity data derived from *in vitro* experiments, developed multiple classifiers of response to a variety of chemotherapy drugs and showed that a combination of these classifiers accurately predicted response to pre-operative multidrug regimen treatments derived from two breast cancer studies.<sup>38</sup> These investigators also provided proof-of-principle that combining these classifiers with predictors of oncogenic pathway deregulation could improve prediction accuracy.

Although promising, the true value of these predictors still has to be investigated in the different breast cancer molecular subgroups. Indeed, we can no longer ignore the fact that breast cancer is a collection of molecularly and biologically very distinct diseases and that distinct molecular classes show different degrees of chemosensitivity.<sup>39</sup> This is why we believe that the future lies in the development of molecular-subtype-dependent predictors.

### Concluding Remarks

Several gene expression studies have shown that a pattern of molecular markers might be more useful than individual markers and that multimarker predictors have great potential. Today, there is also significant evidence to support the fact that the use of expression profiles as biomarkers to improve breast cancer management is coming of age (see *Figure 2*).

First, some of the initial concerns regarding messenger RNA (mRNA) and microarray technology and procedures involved in the development and validation of prognostic/predictive classifiers have already been successfully addressed by the Microarray Quality Control (MAQC) initiative.<sup>40</sup> Indeed, the authors from this project, which included both academic and industry collaborators, found reliable intra-platform consistency across test sites as well as a high level of inter-platform concordance in terms of genes identified as differentially expressed. This study confirmed that microarray technology is technically robust if performed under stringent conditions and, importantly, that different laboratories and platforms were detecting similar gene changes. The disparity in results was comparable to the variability reported by pathology laboratories for immunohistochemical assessment of hormone receptors<sup>41</sup> and HER2.<sup>42,43</sup>

Second, two gene expression signatures have already entered the process of clinical validation, namely the 70-gene signature<sup>10</sup> currently being tested in the prospective Microarray for Node-negative Disease Avoids Chemotherapy Trial (MINDACT)<sup>44</sup> and the OncotypeDX® signature<sup>17</sup> being tested in the prospective Trial Assigning Individualized Options for Treatment Trial (Rx) (TAILORx).<sup>45</sup> MINDACT – which is partly sponsored by the European Commission, run under the auspices of the TRANSBIG consortium and co-ordinated by the European Organisation for Research and Treatment of Cancer (EORTC) – will prospectively evaluate the added value of the 70-gene prognostic gene signature over the clinical criteria Adjuvant! Online software. The primary objective of MINDACT is to confirm that patients with a ‘low-risk’ molecular prognosis and ‘high-risk’ clinical prognosis can be safely spared chemotherapy without affecting clinical outcome.

The final purpose of the different ‘-omic’ approaches should not be to neglect the commonly used clinico-pathological markers, but rather to try to find a way to make predictions more accurate by integrating different types of information.

TAILORx, which is sponsored by the US National Cancer Institute (NCI) and co-ordinated by the Eastern Co-operative Oncology Group (ECOG), is the first study that has been developed as a result of the NCI’s Program for the Assessment of Clinical Cancer Tests (PACCT). The study will enrol over 10,000 women at 900 sites in the US and Canada. Women recently diagnosed with ER- and/or progesterone-receptor-positive, HER2-negative breast cancer that has not yet spread to the lymph nodes are eligible for this study. Patients with a low RS will receive standard hormonal therapy, patients with a high RS will receive combination chemotherapy followed by hormonal therapy and patients presenting an intermediate RS (expected to represent 40% of the whole population) will be randomised to receive either chemotherapy followed by hormonal therapy alone or hormonal therapy alone.

Both studies, which are summarised in *Table 1*, will provide an excellent opportunity to address several issues related to tissue handling and shipping, reproducibility, quality control and standardisation of these 'new' molecular tools. These trials should then provide level I evidence about the clinical relevance of applying gene expression predictors to daily breast cancer patient management.

Third, four gene expression predictors, which are summarised in *Table 2*, are currently available as commercial assays: the Mammprint® 70-gene assay<sup>10</sup> (Agendia Inc.), the OncotypeDX 21-gene Recurrence Score<sup>17</sup> (Genomic Health, Inc.), the AviraDx® two-gene H/I Ratio<sup>24</sup> (Avira) and the MapQuant Dx™ Genomic Grade<sup>16</sup> (Ipsogen). However, the process of commercialisation is on its way for a number of additional multigene predictors (reviewed in reference 46).

To date, most prognostic and predictive studies have focused on tumour characteristics, but it is likely that pharmacogenetics, genetic variability in the metabolism of therapeutic agents and interactions between host and

tumour cells also play an important role. For example, the drug metabolism rate may also affect response to therapy, such as the association between adjuvant tamoxifen benefit and the genetic variants of CYP2D6, a cytochrome involved in the metabolism of tamoxifen.<sup>47</sup> Therefore, the application of the rapidly evolving high-throughput techniques for the realisation of genomic, proteomic and metabolomic profiles holds great promise for increasing our biological understanding of the disease.

Finally, the ultimate purpose of these different '-omic' approaches should not be to neglect the commonly used clinico-pathological markers, but rather to try to find a way to make predictions more accurate by integrating different types of information, thus providing oncologists with more accurate tools to facilitate treatment decision-making for individual patients. ■

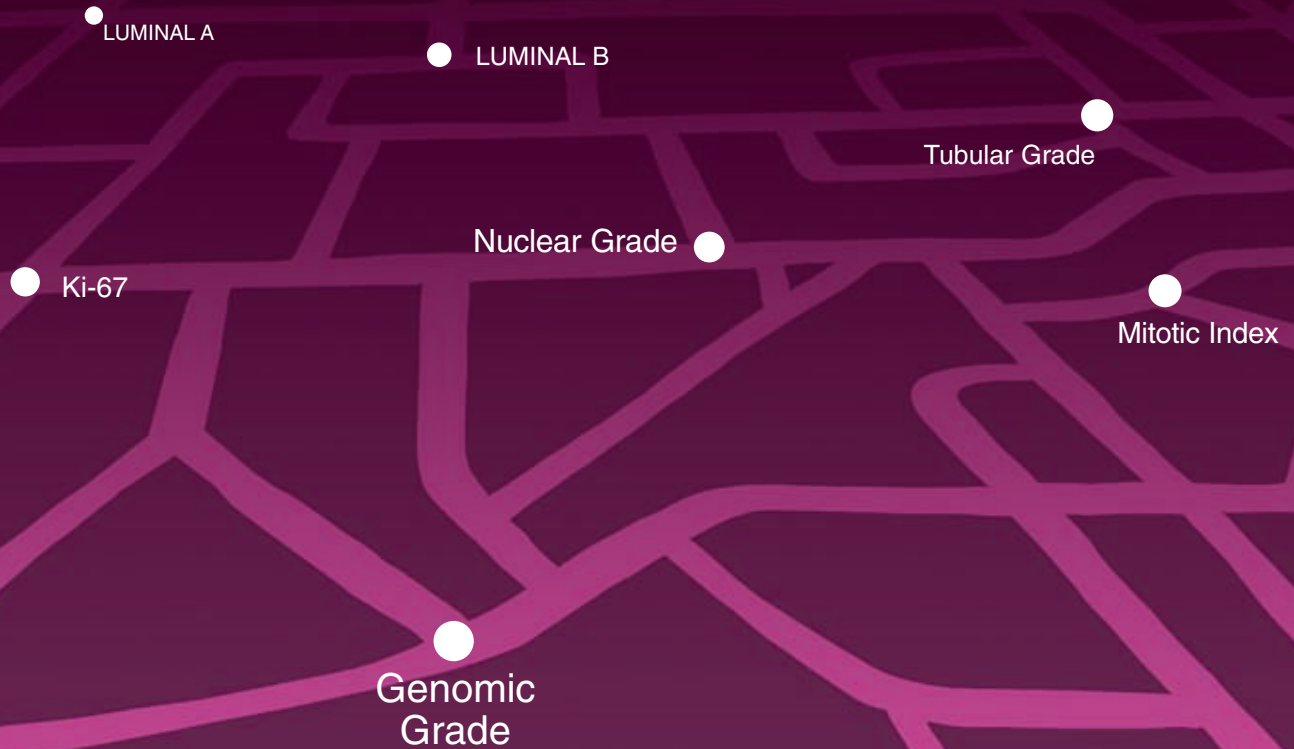
## Acknowledgements

The author would like to thank the Belgian National Foundation for Cancer Research (FNRS) for its support.

- Veronesi U, Boyle P, Goldhirsch A, et al., Breast cancer, *Lancet*, 2005;365:1727–41.
- Ferlay J, Bray F, Pisani P, Parkin DM, *Globocan 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0 IARC CancerBase No. 5*, Lyon, IARC Press, 2001.
- Sorlie T, Perou CM, Tibshirani R, et al., Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications, *Proc Natl Acad Sci U S A*, 2001;98:10869–74.
- Sorlie T, Tibshirani R, Parker J, et al., Repeated observation of breast tumor subtypes in independent gene expression data sets, *Proc Natl Acad Sci U S A*, 2003;100:8418–23.
- Sotiriou C, Neo SY, McShane LM, et al., Breast cancer classification and prognosis based on gene expression profiles from a population-based study, *Proc Natl Acad Sci U S A*, 2003;100:10393–8.
- Perou CM, Sorlie T, Eisen MB, et al., Molecular portraits of human breast tumors, *Nature*, 2000;406:747–52.
- Goldhirsch A, Glick JH, Gelber RD, et al., Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005, *Ann Oncol*, 2005;10:1569–83.
- Eifel P, Axelsson JA, Costa J, et al., National Institutes of Health Consensus Development Conference Statement: adjuvant therapy for breast cancer, November 1–3, 2000, *J Natl Cancer Inst*, 2001;13:979–89.
- Olivotto IA, Bajdik CD, Ravdin PM, et al., Population-based validation of the prognostic model ADJUVANT! for early breast cancer, *J Clin Oncol*, 2005;23:2716–25.
- van't Veer LJ, Dai H, van de Vijver MJ, et al., Gene expression profiling predicts clinical outcome of breast cancer, *Nature*, 2002;415:530–36.
- Wang Y, Klijn JG, Zhang Y, et al., Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer, *Lancet*, 2005;365:671–9.
- Buyse M, Loi S, van't Veer L, et al., Multi-centre independent validation of a 70-gene prognostic signature for women with node-negative breast cancer, *J Natl Cancer Inst*, 2006;98:1183–92.
- Desmedt C, Piette F, Loi S, et al., Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series, *Clin Cancer Res*, 2007;11:3207–14.
- Klein CA, Blankenstein TJ, Schmidt-Kittler O, et al., Genetic heterogeneity of single disseminated tumour cells in minimal residual cancer, *Lancet*, 2002;360:683–9.
- Schmidt-Kittler O, Ragg T, Daskalakis A, et al., From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression, *Proc Natl Acad Sci U S A*, 2003;100:7737–42.
- Sotiriou C, Wirapati P, Loi S, et al., Gene Expression Profiling in Breast Cancer: Understanding the Molecular Basis of Histologic Grade to Improve Prognosis, *J Natl Cancer Inst*, 2006;4:262–72.
- Paik S, Shak S, Tang G, et al., A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer, *N Engl J Med*, 2004;351:2817–26.
- Chang HY, Sneddon JB, Alizadeh AA, et al., Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds, *PLoS Biol*, 2004;2:E7.
- Miller LD, Smeds J, George J, et al., An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival, *Proc Natl Acad Sci U S A*, 2005;102:13550–55.
- Liu R, Wang X, Chen GY, et al., The prognostic role of a gene signature from tumorigenic breast-cancer cells, *N Engl J Med*, 2007;3:217–26.
- Sotiriou C, Piccart MJ, Taking gene-expression profiling to the clinic: when will molecular signatures become relevant to patient care?, *Nat Rev Cancer*, 2007;7:545–53.
- Desmedt C, Haibe-Kains B, Wirapati P, et al., Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes, *Clin Can Res*, in press.
- Jansen MP, Foekens JA, van Staveren IL, et al., Molecular classification of tamoxifen-resistant breast carcinomas by gene expression profiling, *J Clin Oncol*, 2005;23:732–40.
- Ma XJ, Wang Z, Ryan PD, et al., A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen, *Cancer Cell*, 2004;6:607–16.
- Loi S, Haibe-Kains B, Desmedt C, et al., Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade, *J Clin Oncol*, 2007;10:1239–46.
- Fan C, Oh DS, Wessels L, et al., Concordance among gene-expression-based predictors for breast cancer, *N Engl J Med*, 2006;355:560–69.
- Ma XJ, Hilsenbeck SG, Wang W, et al., The HOXB13:IL17BR expression index is a prognostic factor in early-stage breast cancer, *J Clin Oncol*, 2006;24:4611–19.
- Jansen MP, Sieuwerts AM, Look MP, et al., HOXB13-to-IL17BR expression ratio is related with tumor aggressiveness and response to tamoxifen of recurrent breast cancer: a retrospective study, *J Clin Oncol*, 2007;25:662–8.
- Kok M, Linn SC, Van Laar RK, et al., Comparison of gene expression profiles predicting progression in breast cancer patients treated with tamoxifen, *Breast Cancer Res Treat*, 2008 Mar 4 [Epub ahead of print].
- Ayers M, Symmans WF, Stec J, et al., Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer, *J Clin Oncol*, 2004;22:2284–93.
- Bertucci F, Finetti P, Rougemont J, et al., Gene expression profiling for molecular characterization of inflammatory breast cancer and prediction of response to chemotherapy, *Cancer Res*, 2004;64:8558–65.
- Chang JC, Wooten EC, Tsimelzon A, et al., Patterns of resistance and incomplete response to docetaxel by gene expression profiling in breast cancer patients, *J Clin Oncol*, 2005;20:1169–77.
- Folgueira MA, Carraro DM, Brentani H, et al., Gene expression profile associated with response to doxorubicin-based therapy in breast cancer, *Clin Cancer Res*, 2005;11:7434–43.
- Hannemann J, Oosterkamp HM, Bosch CA, et al., Changes in gene expression associated with response to neoadjuvant chemotherapy in breast cancer, *J Clin Oncol*, 2005;23:3331–42.
- Iwao-Koizumi K, Matoba R, Ueno N, et al., Prediction of docetaxel response in human breast cancer by gene expression profiling, *J Clin Oncol*, 2005;23:422–31.
- Rouzier R, Rajan R, Wagner P, et al., Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer, *Proc Natl Acad Sci U S A*, 2005;102:8315–20.
- Bild AH, Potti A, Nevins JR, Linking oncogenic pathways with therapeutic opportunities, *Nat Rev Cancer*, 2006;6:735–41.
- Potti A, Dressman HK, Bild A, et al., Genomic signatures to guide the use of chemotherapeutics, *Nat Med*, 2006;12:1294–1300.
- Rouzier R, Perou CM, Symmans WF, et al., Breast cancer molecular subtypes respond differently to preoperative chemotherapy, *Clin Cancer Res*, 2005;11:5678–85.
- MAQC Consortium, Shi L, Reid LH, et al., The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements, *Nat Biotechnol*, 2006;9:1151–61.
- Layfield LJ, Goldstein N, Perkinson KR, Proia AD, Interlaboratory variation in results from immunohistochemical assessment of estrogen receptor status, *Breast J*, 2003;9:257–9.
- Thomson TA, Hayes MM, Spinelli JJ, et al., HER-2/neu in breast cancer: interobserver variability and performance of immunohistochemistry with 4 antibodies compared with fluorescent in situ hybridization, *Mod Pathol*, 2001;14:1079–86.
- Wolff AC, Hammond ME, Schwartz JN, et al., American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer, *J Clin Oncol*, 2007;25:118–45.
- Cardoso F, Van't Veer L, Rutgers E, et al., Clinical application of the 70-gene profile: the MINDACT trial, *J Clin Oncol*, 2008;26:729–35.
- Sparano JA, Paik S, Development of the 21-gene assay and its application in clinical practice and clinical trials, *J Clin Oncol*, 2008;26:721–8.
- Ross JS, Hatzis C, Symmans WF, et al., Commercialized multigene predictors of clinical outcome for breast cancer, *Oncologist*, 2008;13:477–93.
- Schroth W, Antoniadou L, Fritz P, et al., Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes, *J Clin Oncol*, 2007;25:5187–93.

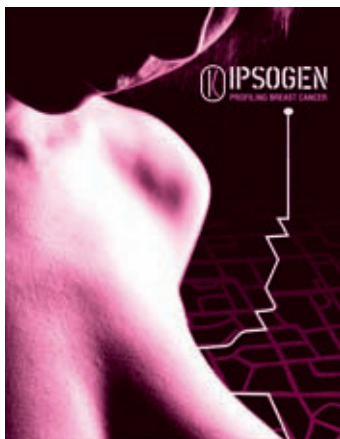
# MapQuant Dx™ Genomic Grade

BREAST CANCER



## IMPROVING THE CLINICAL VALUE OF TUMOR GRADING

PROFILING



A 97 gene index that solves uncertainty about histological grade 2 breast tumors.

MapQuant Dx™ Genomic Grade\* is the diagnostic test based on the Genomic Grade index (GGi).

\* GGi has been discovered by oncology researchers at the Institut Jules Bordet (Brussels, Belgium) and ISREC (Lausanne, Switzerland), two leading European cancer research institutes.

Ipsogen is the exclusive worldwide licensee of the GGi technologies.

MapQuant Dx™ Genomic Grade is available for diagnostic use in Europe only.

Find out more on MapQuant Dx™ Genomic Grade and Ipsogen's diagnostic tools for blood and breast cancers at: [www.ipsogen.com](http://www.ipsogen.com) or contact [infos@ipsogen.com](mailto:infos@ipsogen.com)

### MAPPING DISEASES, GUIDING DECISIONS

Ipsogen SA, Luminy Biotech Entreprises, Case 923, 163 avenue de Luminy  
13288 Marseille Cedex 9, FRANCE - Tel.: +33 (0)4 91 29 30 90 - Fax: +33 (0)4 91 29 30 99

