CANCER GENOMICS & PROTEOMICS *4*: 147-156 (2007)

Review

Individualization of Therapy Using Mammaprint®™: from Development to the MINDACT Trial

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Abstract. *To date, most treatment decisions for adjuvant chemotherapy in breast cancer are based on conventional clinicopathological criteria. Since breast cancer tumors with similar clinicopathological characteristics can have strikingly different outcomes, the current selection for adjuvant chemotherapy is far from accurate. Using high-throughput microarray analysis, a 70-gene signature was identified which can accurately select early stage breast cancer patients who are highly likely to develop distant metastases, and therefore, may benefit the most from adjuvant chemotherapy. This review describes the development of the 70-gene profile (Mammaprint®™), its retrospective validation and feasibility studies, and its prospective validation in the large adjuvant MINDACT (Microarray In Node-negative Disease may Avoid ChemoTherapy) clinical trial.*

The outcome of patients with breast cancer has improved in the last 20 years, due to both early detection and the amelioration of adjuvant systemic treatment. The majority of early stage breast cancer patients receive adjuvant systemic treatment, which may include chemotherapy, hormonal therapy, immunotherapy or a combination. Nowadays, patients who should receive chemotherapy are selected by using consensus guidelines like the St. Gallen, or the National Comprehensive Cancer Network (NCCN) guidelines which are based on the assessment of clinicopathological criteria such as age, tumor size and grade, hormonal receptor status and axillary lymph node involvement (1-3). However, breast cancer tumors with similar clinicopathological characteristics can have strikingly different outcomes, reflecting the heterogeneity of the disease. Consequently, the current adjuvant treatment decision-making process for breast cancer patients is far from accurate. The majority of early stage breast cancer patients, particularly those with lymph node-negative disease (60-70%), has a fairly good 10-year overall survival with locoregional treatment alone, with only 30-40% developing distant metastases (4). Notwithstanding these facts, most lymph node-negative breast cancer patients are offered chemotherapy, according to the currently used guidelines, causing an important proportion of overtreatment (1-3). This is justified largely by our inability to clearly identify those patients who will not relapse and hence do not need adjuvant chemotherapy. Since metastatic breast cancer is an incurable disease, the only chance for cure is in the adjuvant setting. However, overtreatment not only unnecessarily exposes women to potential toxicity and side-effects of this treatment, but also increases the economic burden of breast cancer on society. It is thus quite clear that robust and reliable prognostic markers to accurately select patients not requiring aggressive adjuvant therapy are urgently needed.

With the introduction of new high-throughput methods, such as gene expression microarray technologies, the expression level of tens of thousands of genes can be measured simultaneously. Using microarray techniques, several studies have recently classified breast tumors according to their gene expression profile and identified prognostic and predictive classifiers (5-14). Although these studies appear to be very promising, microarray analysis has some potential pitfalls. For example, the analysis of the large amount of data obtained through this technology can cause process errors and overfitting. Furthermore, retrospective studies using frozen tissue processed and stored many years ago could result in different levels of gene expression due to differences in tissue handling and pertain to patient populations which may be different from

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Key Words: Breast cancer, prognostic, chemotherapy, gene expression microarray, 70-gene profile, Mammaprint®™, validation, MINDACT, review.

those diagnosed today. Taking all this into account, validation studies, particularly prospective ones, are indispensable in assessing the reliability and reproducibility of the results and in identifying the true benefit of a classifier for clinical practice.

Here we provide an overview of the development of the 70-gene profile (Mammaprint®™) from discovery to application in clinical trials, including retrospective validation and feasibility studies, and its prospective validation in the large MINDACT (Microarray In Node-negative Disease may Avoid ChemoTherapy) clinical trial.

The Development of the 70-gene Signature

By using gene expression profiling, Van't Veer and colleagues developed a 70-gene classifier that accurately distinguished breast cancer patients who were likely to remain free of distant metastases (good profile) from breast cancer patients at high risk of developing distant metastases (poor profile) (8). To develop this 70-gene profile, 78 tumors from women with lymph node-negative breast cancer were studied. Patients were under 55 years of age at diagnosis, had a primary invasive breast carcinoma less than 5 cm in diameter, no previous malignancies and were treated at The Netherlands Cancer Institute (NKI). All patients were treated by modified radical mastectomy or breast conserving therapy. Five out of 78 patients received adjuvant systemic treatment, consisting of chemotherapy (n=3) or hormonal therapy (n=2); all 5 patients developed distant metastases within 5 years of diagnosis. Forty-four patients remained free of distant metastases for at least 5 years (good-prognosis group), whereas the remaining 34 patients did develop distant metastases within 5 years of diagnosis (poor-prognosis group). The mean follow-up of the good prognosis group was 8.7 years, the mean time to distant metastases was 2.5 years.

From all 78 frozen tumor samples, the percentage of tumor cells was determined in a haematoxylin and eosin stained section, before and after cutting sections for RNA isolation. Only tumor samples with at least 50% tumor cells were eligible. RNA was isolated and labeled with a fluorescent dye. An equal amount of RNA from all tumors was pooled and provided reference RNA. Both tumor RNA and reference RNA were hybridized on an oligonucleotide microarray platform containing approximately 25,000 genes, synthesized by inkjet technology (produced by Agilent) (15).

In a first step, using a statistical analysis method called "supervised classification", the expression of 231 genes appeared to be significantly correlated with disease outcome (distant metastases within 5 years). These 231 genes were ranked, based on their correlation coefficient with disease outcome; the top 70 of these were shown to most accurately classify tumors in either the good- or the poor-prognosis category.

All 78 tumors were ranked according to their correlation with the average expression of the 70 genes of the patients who did not develop a distant metastasis (good-outcome patients). Where the sensitivity was optimized by setting a threshold resulting in a misclassification of less than 10% of patients with a poor disease outcome. Consequently, 3 out of the 34 patients with a poor disease outcome would erroneously be withheld chemotherapy based on this new tool (9% misclassification).

This supervised classification strategy resulted in the 70-gene dichotomous risk classifier, using the 78 tumors as a training set. To initially validate the 70-gene profile, an additional set of 7 tumors from patients with a good clinical outcome (free from distant metastases for at least 5 years after diagnosis) and 12 tumors from patients with a poor clinical outcome (distant metastases within 5 years of diagnosis) were analyzed. The 70-gene profile accurately predicted disease outcome in 17 out of the 19 patients, thereby confirming the initial performance of the prognostic classifier.

Although the first results were very promising, one major comment on the development of the 70-gene profile was the small sample size of both the training and the test sets. Supervised analysis of a relatively small sample size, in combination with the enormous number of parameters (genes) can result in what is called "overfitting" (16). Since the classifier is developed and optimized to classify the tumors in the training set accurately, the model will fit this training set but could predict disease outcome imprecisely in an independent sample set. Therefore, well-designed validation studies were necessary to confirm these earlier findings.

First Retrospective Validation Series Confirms the Prognostic Value of the 70-gene Signature

The first validation of the 70-gene profile was performed by Van de Vijver and colleagues, on a consecutive series of 295 breast cancer tumors; 144 tumors from lymph node-positive and 151 tumors from lymph node-negative breast cancer patients (7). Sixty-one lymph node-negative patients were also part of the previous series used to develop the prognostic profile. All patients were diagnosed between 1984 and 1995 at the NKI and under the age of 53 at diagnosis. Tumors were primary invasive breast carcinomas less than 5 cm, treated with locoregional therapy alone (56%), or in combination with adjuvant systemic treatment (44%) consisting of chemotherapy alone (31%), hormonal therapy alone (7%), or a combination (7%). The median follow-up was 7.8 years for the 207 patients without metastasis as first event and the median time to distant metastases was 2.7 years. The median follow-up among all 295 patients was 6.7 years.

For all 234 samples that were not part of the previous study, the correlation coefficient of the average level of expression of the 70 genes with the previously established good profile was calculated. Tumors with a correlation coefficient above the previously determined threshold (above 0.4) were assigned to the good-profile group. For the 61 patients who were included in the previous study, a threshold of 0.55 was used to correct for overfitting.

The profile accurately distinguished a good-prognosis group (of 115 tumors) with a 10-year overall survival of 95% (±2.6%) from a poor-prognosis group (of 180 tumors) with a 10-year overall survival of 55% (±4.4%). The 70-gene profile was associated with established prognostic factors such as age, tumor grade and estrogen receptor (ER) status. Remarkably, the 70-gene profile did not seem to depend on the lymph node status, since the 144 tumors from lymph node-positive patients were equally distributed over the poor- and good-prognosis groups. In the multivariate analysis of the risk of distant metastases as first event, the poor-prognosis signature, large tumor size, presence of vascular invasion and no chemotherapy treatment were the only significant independent factors for the prediction of the likelihood of developing distant metastases. With an overall hazard ratio of 4.6, the 70-gene profile was by far the most powerful predictor of distant metastases (95% CI 2.3-9.2; $p<0.001$).

To assess the value of this new prognosticator in a clinical context, the 70-gene profile was compared to the St. Gallen and NIH criteria used at that time (17,18). The 70-gene profile assigned 40% of the patients in the good-prognosis or low-risk group, compared with only 15% according to the St. Gallen consensus guidelines and 7% according to the NIH criteria. Furthermore, patients identified as being at low risk (good prognosis) by the 70-gene profile were more likely to remain free of distant metastases, compared with patients classified as being at low risk according to the St. Gallen or the NIH criteria. On the other hand, patients identified as being at high risk (poor prognosis) by the 70-gene profile had a higher risk of developing distant metastases than the high-risk patients classified by the St. Gallen or NIH criteria. The misclassification of patients using the clinicopathological criteria is even more clearly perceptible when the high-risk group, according to the NIH (140 out of 151 lymph node-negative breast cancer patients), is subdivided using the 70-gene classifier. This NIH high-risk group includes 53 out of 140 patients with a good 70-gene prognosis and indeed a good clinical outcome, indicating a better prediction of disease outcome when using the 70-gene profile.

In this validation series, the 70-gene profile had a high negative predictive value in all subgroups; 97% for the new lymph node-negative patients; 96% for the lymph node-positive patients; and 96% for all new patients, respectively. Due to the setting of the threshold in the previous study, the profile was built to have a minimum of misclassified patients with a poor disease outcome. Consequently, the positive predictive value was only 38% for all new patients. Although this would still lead to overtreatment, the absolute number of patients unnecessarily exposed to chemotherapy would still be reduced by 25-30%, compared to treatment selection based on the clinicopathological criteria, since the total proportion of poor-prognosis patients identified by the 70-gene profile is much smaller than the proportion of high-risk patients according to the St. Gallen or NIH criteria. Moreover, the overall selection of patients who should receive chemotherapy and patients who can safely be spared this treatment seems to be far more accurate.

An important criticism of this first validation was that the series included 61 patients from the study on which the classifier was established. Although this validation already showed a significant prognostic value in patients that were not included in the previous study when analyzed separately (OR 15.3; 95% CI 1.8-127; $p=0.003$), this was further substantiated in a recently published independent validation study performed by the TRANSBIG research consortium (19). This independent validation also addressed the question whether the 70-gene profile, which was developed and so far validated on a selected group of patients (young patients with stage I or II tumors, from a single institution), could be applied to a larger proportion of breast cancer patients.

Independent Multi-center Validation Established the Prognostic Value of the 70-gene Profile

In the study from the TRANSBIG consortium, recently published in the JNCI, the 70-gene profile was independently validated in 302 patients from 5 different European hospitals (19). Patients were up to 61 years at diagnosis, diagnosed before 1999, with a lymph node-negative T1 or T2 breast carcinoma and had not received any adjuvant systemic therapy. The median follow-up was 13.6 years.

The frozen tumor samples were sent to Agendia, a spin-off company of the NKI in Amsterdam, where RNA was isolated and the microarray analysis was performed. The samples were hybridized on the Mammaprint®™, which is a custom-made microarray slide, assessing the mRNA expression of the previously identified 70 genes in triplicate. A tumor was classified as high risk if the correlation coefficient for the average expression of the 70-gene profile was under 0.4. Importantly, researchers at Agendia were blinded to the clinical data while performing the genomic test. Clinical data from these patients were collected, audited by two independent auditors and sent to an independent statistical partner in Brussels. The researchers

collecting the clinical data were blinded for the genomic test results. Furthermore, a central pathology review was performed in Milan to decrease the potential heterogeneity of results from different laboratories (ER status and grade were centrally assessed in 80% of samples). Only the independent statistical office had simultaneous access to both clinical and genomic data and performed the correlation analysis.

This independent validation confirmed that the 70-gene profile is a strong prognostic factor for overall survival and time to distant metastases, with hazard ratios of 2.79 (95% CI 1.60-4.87) and 2.32 (95% CI 1.35-4.0), respectively. The prognostic value of the 70-gene profile remained statistically significant after adjustment for other risk classifications, using clinicopathological criteria with known prognostic value, such as the St. Gallen consensus guidelines, the Nottingham Prognostic Index and the prognostic evaluation tool Adjuvant! Online. This last tool is a software program (www.adjuvantonline.com) which can calculate a 10-year survival probability based on the patient's age, co-morbidities, tumor size, grade and ER status (20). The prognostic model is constructed using the risk estimates based on the observed overall survival from thousands of breast cancer patients, recorded in the SEER database, and was recently validated on more than 4000 breast cancer patients from British Columbia (21). To distinguish a low-risk group from a high-risk group using Adjuvant!, the TRANSBIG consortium decided the following: a low-risk group would be defined as patients with a 10-year breast cancer survival of at least 88% for estrogen receptor (ER)-positive patients and at least 92% for ER-negative patients. The rationale for these 2 different cutoffs is the assumption that ER-positive patients would now all receive hormonal treatment (with an estimated average absolute 10-year survival benefit of 4%) and patients in this validation series were all untreated (19).

After adjustment for the clinical risk groups defined by Adjuvant!, the hazard ratios for overall survival and time to distant metastases given by the 70-gene profile were 2.13 (95% CI 1.19-3.82) and 2.63 (95% CI 1.45-4.79), respectively. Moreover, patients in the good-prognosis group according to the 70-gene profile had a 10-year survival rate of 88% and 89%, respectively, for low and high clinical risk as classified by Adjuvant!. On the other hand, patients in the poor-prognosis group defined by the 70-gene profile had a 10-year survival rate of 69%, for both low and high clinical risk defined by Adjuvant!. These findings suggest that the 70-gene profile predicts disease outcome independently of the clinicopathological criteria.

The median follow-up time in the original series was less than half that of this validation series (6.7 years versus 13.6 years, respectively). Therefore, the 70-gene profile hazard ratios were also calculated with arbitrary censoring of all observations at different time points. A strong time dependency of the 70-gene profile was observed, with adjusted HR of 4.68 and 16.99 at 5 years, and 3.5 and 3.46 at 10 years for time to distant metastases and overall survival, respectively, suggesting a better prediction of early distant metastases (*i.e.* occurring during the first five years) by the 70-gene profile. The different duration of follow-up could be a plausible explanation for the discrepancy in hazard ratios between the first validation series and this independent validation series.

The results of this independent validation strengthen the previous findings that the 70-gene profile is a strong independent prognostic marker in early stage breast cancer, also in patients up to the age of 61. The substantiation of the prognostic value in this independent validation study was a prerequisite for the initiation of a large prospective validation study, the MINDACT trial.

In the meantime, two other prognostic gene expression signatures were developed, using the Affymetrix microarray platform: the 76-gene Veridex/Rotterdam signatures (22) and the Genomic Grading Index (23).

To decide which signature would be the best tool to move forward with in the large, prospective MINDACT trial, the TRANSBIG consortium performed the retrospective validation of these two signatures using the same methodology and the same patient population as described for the 70-gene profile. The results have shown that the three signatures performed in quite a similar way, all being superior to the classical clinicopathological evaluation and all possess a strong time dependency (are better predictors of early relapse) (22). Since no significant differences were seen in the performances of the three signatures and the 70-gene profile test is robust, with a good interlaboratory reproducibility, and available for patient diagnostic testing, even as an FDA approved test (24), the TRANSBIG consortium has decided to move forward with this tool in the MINDACT trial (Table I).

Implementation of 70-gene Profile in Daily Clinical Practice Requires Adjustments to Standard Procedures

In addition to thorough validation studies, the implementation of a new test in daily clinical practice should also be feasible, before it can be applied in a prospective trial. One major obstacle for the implementation of microarray techniques such as the 70-gene profile, is the requirement for good quality RNA. Since RNA is very unstable, the tumor tissue must be preserved either by snap freezing or in a special preservation fluid based on RNAlater® (Qiagen), rather than in paraffin. The logistics for the collection of fresh frozen tissue is complex and varies from hospital to hospital. Therefore, performing microarray analysis,

Table I. *Summary of development and validation of the 70-gene profile.*

	Nature paper (8)	NEJM paper (7)	TRANSBIG paper (19)
Purpose	Development of breast cancer prognosis 70-gene profile	Validation of the 70-gene profile in consecutive series of breast cancer patients	Independent European validation of the 70-gene profile
Patient & tumor characteristics	n=78, <55 years, pT1-2, node-negative, 50% ER-positive	n=295, <53 years, pT1-2, node-negative/node-positive, 77% ER-positive	n=302, <61 years, pT1-2, node-negative, 70% ER-positive
Year of diagnosis	1983-1996	1984-1995	<1999
Adjuvant systemic treatment	Chemotherapy 4%; hormonal therapy 3%	Chemotherapy 31%; hormonal therapy 7%; both 7%	No adjuvant systemic treatment
Follow-up	8.7 years (mean) in the good-prognosis group	6.7 years (median)	13.6 years (median)
Good profile	35 (45%)	115 (39%)	111 (37%)
Comments	Multivariate OR of 18 (95% CI: 3.3-94; $p=1.4 * 10^{-4}$) for distant metastases <5 years	DMFS by 70-gene profile at 10 years: poor-prognosis profile 55% (±4.4), good-prognosis profile 95% (±2.6) Multivariate HR for distant metastases as first event 4.6 (95% CI: 2.3-9.2; $p<0.001$) (poor *versus* good profile)	DMFS by 70-gene profile at 10 years: poor-prognosis profile 69%, good-prognosis profile 88% Univariate HR for overall survival 2.79 (95% CI: 1.60-4.87; $p<0.001$) Strong time dependency

ER, estrogen receptor; OR, odds ratio; HR, hazard ratio; DMFS, distant metastases-free survival; CI, confidence interval.

especially on a real-time basis, can cause some logistical problems such as insufficient freezing procedures, or transport-related issues. Thus, close collaboration between pathologists, surgeons and oncologists is of paramount importance.

To investigate whether Mammaprint®™ could be implemented in daily clinical practice, the Netherlands Cancer Institute (with financial support from the Dutch Health Care Insurance Board) performed a multi-center feasibility study: the RASTER study (Figure 1). The first aim of this study was to assess the feasibility of collecting good quality tissue from several community hospitals in the Netherlands to be used to perform the 70-gene profile analysis (25). In this RASTER study, the so-called Constructive Technology Assessment (CTA) was used as a tool to facilitate the introduction of the 70-gene profile in daily clinical practice by evaluating aspects of the dynamics of its implementation, such as communication, logistics, juridical-ethical matters and cost effectiveness (26). The results of these evaluations will be used in decision-making concerning the large-scale application of the 70-gene profile in daily clinical practice and related guidelines. Other aims of the RASTER study were to determine the proportion of good- and poor-profile patients and to establish the concordance between the risk assessment defined by the 70-gene profile and the one defined by the Dutch breast cancer guidelines, which are based on common clinicopathological criteria (25).

Lymph node-negative breast cancer patients under the age of 61, with a T1 or T2 tumor, were eligible. A tumor sample from the excised specimen was obtained from all patients, using a 6 mm biopsy punch, and placed in the commercially available preservation fluid at room temperature. Subsequently, the sample was sent by conventional mail to the NKI, were it was frozen in liquid nitrogen and stored at –80˚C until processing. The Mammaprint®™ was performed at Agendia and patients were classified into good- and poor-prognosis groups. Preliminary results show the feasibility of collecting good quality tissue for microarray analysis from several community hospitals. A minority of the samples were lost due to processing errors, such as initial storage in formalin resulting in an insufficient RNA quality (25, 27). The study ended in December 2006 and the final results are expected in due course.

The Prospective Validation of the 70-gene Profile in a Large Randomized Clinical Trial: the MINDACT Study

The 70-gene profile has been extensively validated in a retrospective series. Furthermore, the logistics concerning

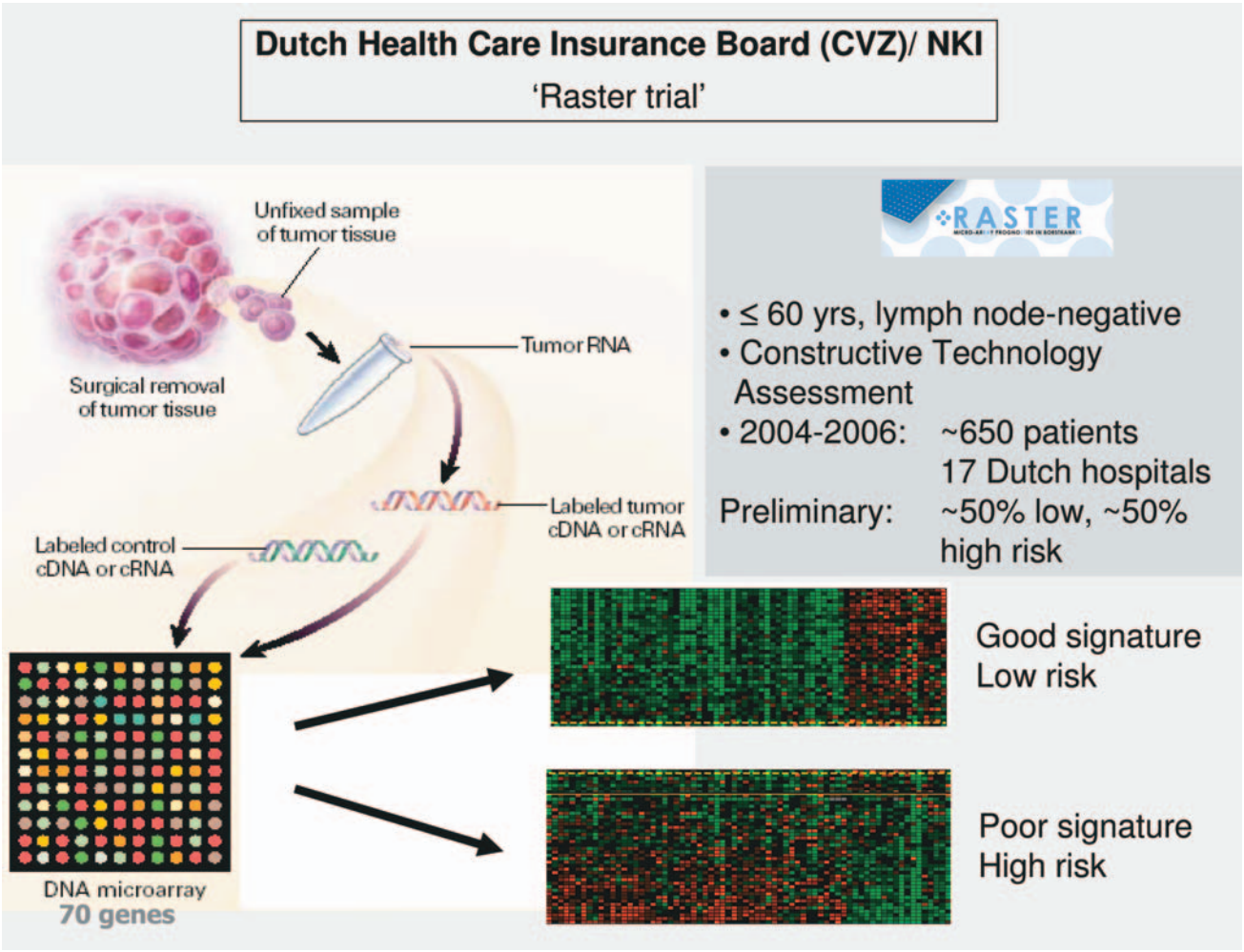

Figure 1. *RASTER study design. From Sauter G and Simon R. Predictive Molecular Pathology. N Engl J Med 347(25): 1995-1996, 2002. Copyright © 2002 Massachusetts Medical Society. All rights reserved. Adapted with permission, 2007.*

fresh frozen tissue collection were tested and adjusted where necessary. The final step before the implementation of the 70-gene profile in clinical practice is its prospective validation in the MINDACT trial. This trial is a multicentric large prospective randomized study, comparing the 70-gene profile with currently used tools for selecting lymph node-negative breast cancer patients for adjuvant systemic treatment. The primary aim of the study is to show that patients defined as at low risk using the 70-gene profile but who are at high risk according to the current clinicopathological criteria can be safely spared chemotherapy, without deterioration of the clinical outcome. The study will enroll 6,000 node-negative breast cancer patients who will have their risk assessed by both the 70-gene profile and the currently used clinicopathological criteria through an updated version of Adjuvant! Online. We estimate that about 55% will be classified as at high risk by both methods and these patients will be offered adjuvant chemotherapy; about 10% will be classified as at low risk by both methods and will not be offered adjuvant chemotherapy. The patients with a discordant risk assessment (approx. 35%), *i.e.* a high genomic risk (according to the 70-gene profile) and a low clinical risk (according to Adjuvant! software) or vice versa (low genomic risk and high clinical risk) will be randomized for the treatment decision tool. In other words, 50% of those patients will receive adjuvant treatment according to their genomic risk and 50% will be treated according to their clinical risk (Figure 2).

Other objectives of the study are related to the type of adjuvant systemic treatment. A second randomization will compare an anthracycline-based regimen to a docetaxel-capecitabine regimen, which might be associated with increased efficacy and reduced long-term side-effects,

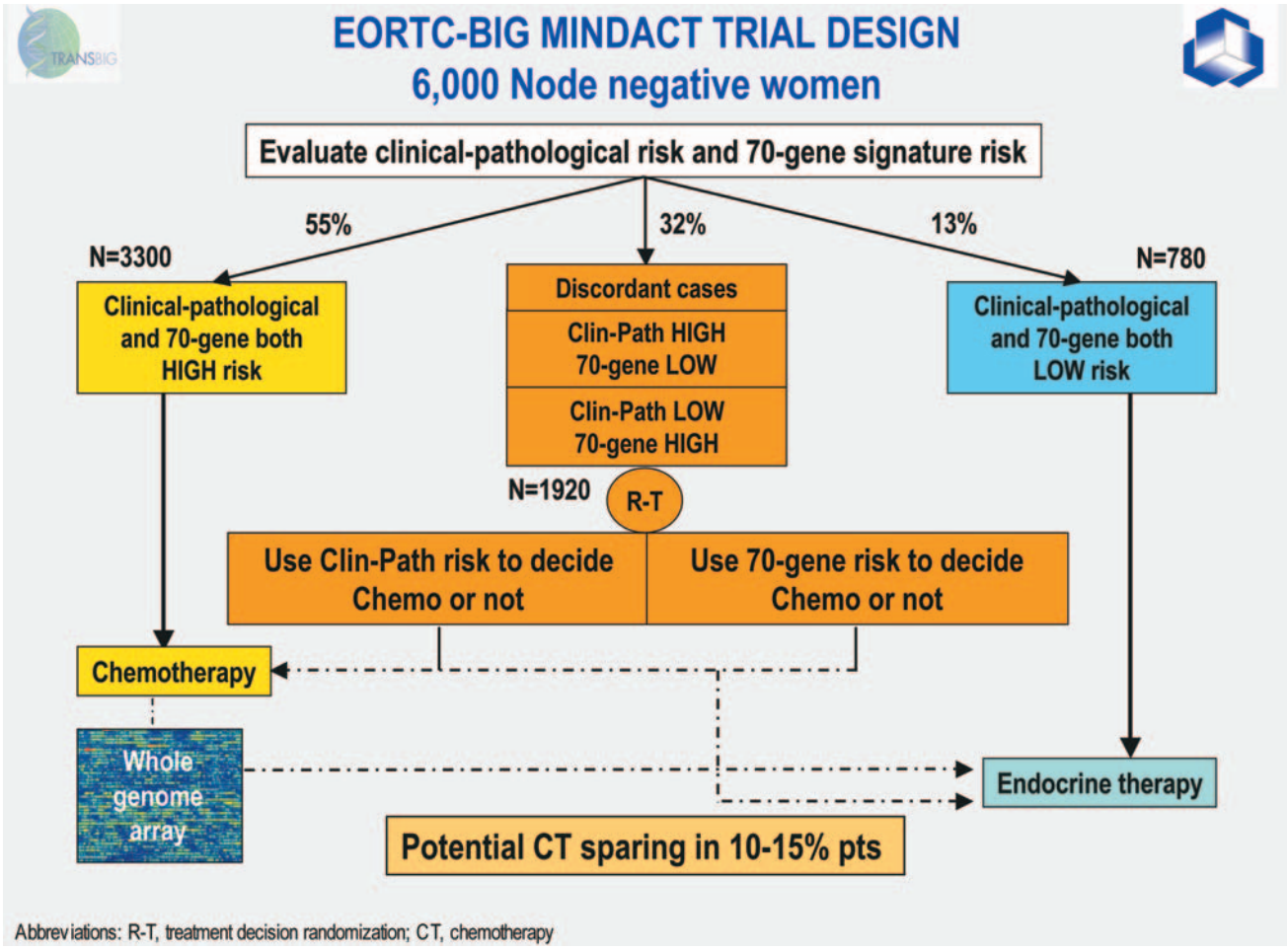

Figure 2. *MINDACT study design.*

particularly cardiotoxicity and leukemia. A third randomization, which will be offered to all postmenopausal hormone receptor-positive patients, will compare 2 years of tamoxifen followed by 5 years of letrozole to 7 years of letrozole upfront (Figure 2). Women aged 18-70 years with an operable invasive breast carcinoma and a negative sentinel node or axillary clearance are eligible. An overview of the MINDACT trial was recently published in Nature Clinical Practice of Oncology, explaining in detail the rationale behind the study design (28).

An additional and important effort has been made to perform not only the analysis of the 70-gene profile, but whole genome arrays for all 6,000 eligible patients. This will potentially allow for the discovery of new genomic profiles with prognostic or predictive value and eventually new drug targets. Fresh frozen tissue, paraffin-embedded tissue and blood samples from all 6,000 patients will be stored in an independent biobank repository, representing an invaluable resource for future research.

The Collection of Fresh Frozen Tissue for the MINDACT Trial

Although microarray experiments are becoming more and more standardized, operator and technical variability are well known to influence the measurement of gene expression levels. For all samples in the MINDACT trial, RNA isolation, quality controls and microarray analysis will be performed at Agendia, Amsterdam, to avoid the bias of potential interlaboratory reproducibility. Consequently, tumor samples from all over the world will be shipped to the Netherlands within a fixed timeframe. Additionally, since one of the aims of the MINDACT trial is the establishment of a biological material bank for future research, also in the field of proteomics, frozen tumor samples and blood samples will be collected from all patients. Preservation of the tumor samples in RNAlater® might influence several processes in the tissue, such as the level of proteins, therefore, temporary preservation in RNAlater®, as tested in the RASTER study,

is not suitable and material frozen in liquid nitrogen will be mandatory for the MINDACT trial.

To test the logistics of collection, freezing and shipment of the samples, the authors performed a logistics pilot study in 6 European hospitals. For this feasibility study, patients with early stage breast cancer under the age of 71 were eligible. All patients signed an informed consent to donate a piece of tumor tissue for research. The pathologist obtained a representative tumor sample within one hour of surgery, using a 6-mm biopsy punch, according to a standardized procedure. The tumor samples were snap frozen in liquid nitrogen and stored at –80˚C until shipment. All samples were shipped on dry ice by a specifically contracted courier specialized in transportation of frozen material at –80˚C. At Agendia, the percentage of tumor cells in the samples was determined as described by van't Veer *et al.* (8). When the sample was representative of the tumor (*i.e.* tumor cell ≥50%), RNA was isolated and, after measurement of its quality and quantity, the 70-gene profile was performed. The primary endpoint of this logistics pilot study was the success rate of hybridization. Preliminary results show that, in general, it is feasible to collect and ship good quality fresh frozen tumor samples from several locations throughout Europe to Amsterdam. The procedures (of tissue collection, freezing and transportation) tested in this study formed the basis of the standard operating procedures written for the MINDACT trial. The final results of this pilot study will be published in 2007.

Future Prospects

The MINDACT study will determine the clinical relevance of the 70-gene profile and its prognostic value compared to the currently available prognostic clinicopathological criteria. Moreover, as tumor and blood material and whole genome microarray data will be collected from all patients, a valuable bank of material will be established, providing an opportunity for the identification of predictive gene expression profiles and potential drug targets. Nowadays, the choice among treatment options is based upon patient and tumor characteristics, such as age and estrogen receptor status, but overall it is extrapolated from the percentage of risk reduction measured in a large population to the individual patient. In the future, we might be able to identify the genomic fingerprint of each individual tumor, telling us not only if a given patient needs adjuvant systemic treatment, but also which treatment will have the best response and which treatment should be avoided because of potentially serious side-effects.

Acknowledgements

The studies mentioned in this article were supported by the European Commission Framework Programme VI, the Center of Biomedical Genetics, the Dutch Health Care Insurance Board, the Dutch National Genomic Initiative - Cancer Genomics Program, the Breast Cancer Research Foundation and the European Organisation for Research and Treatment of Cancer (EORTC) - Breast Cancer Group. S. Mook was partially supported by the traineeship program of TRANSBIG network. The authors thank the numerous individuals who have contributed to the studies mentioned in this review, especially those from the TRANSBIG consortium and the EORTC, and to all the patients who have and still are participating in these studies.

Conflicts of Interest

Dr. L.J. van't Veer is a named inventor on a patent application for Mammaprint®™ and reports holding equity in Agendia BV.

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Received March 8, 2007
Revised April 25, 2007
Accepted April 26, 2007