

## Evolution of the 21-gene Assay Oncotype DX® from an Experimental Assay to an Instrument Assisting in Risk Prediction and Optimisation of Treatment Decision-making in Early Breast Cancer

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### Abstract

Decision-making for risk-adapted adjuvant systemic treatment has become a complex process in early breast cancer. In hormone-receptor-positive disease in particular, risk of recurrence and the potential benefit of additional chemotherapy have to be balanced. The 21-gene assay Oncotype DX® has been developed and validated systematically in retrospective studies. The Recurrence Score (RS) generated by the assay has proved to be a prognostic and predictive marker for patients with hormone-receptor-positive node-negative and node-positive disease, offering information beyond that provided by traditional prognostic markers. Recent data demonstrated that RS is also prognostic for patients receiving adjuvant treatment with an aromatase inhibitor. Ongoing prospective studies will further clarify its value in specific clinical settings. Oncotype DX has now been integrated into major international guidelines. The considerable body of evidence for its clinical utility from clinical studies and its use in clinical practice demonstrates that Oncotype DX has evolved to be an accepted tool in the decision-making process in early breast cancer.

### Keywords

Oncotype DX®, multigene assay, breast cancer, hormone-receptor-positive, node-negative, node-positive, adjuvant, tamoxifen, chemotherapy, aromatase inhibitor, prognostic marker, predictive marker

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In general, mortality due to breast cancer is declining almost everywhere in the world. However, one of the few published reports on the current epidemiology of cancer in Europe estimated that there were 370,100 new cases of breast cancer and 129,900 deaths from breast cancer in the EU in 2004.<sup>1</sup> The majority of newly diagnosed patients will present with node-negative, hormone-receptor (HR)-positive breast cancer. A significant proportion of these women will suffer recurrence even if treated with polychemotherapy according to international guidelines. By contrast, another substantial proportion will survive recurrence-free without any exposure to cytotoxic drugs.<sup>2</sup> It is accepted that both adjuvant hormonal therapy and chemotherapy significantly improve disease-free survival (DFS) and overall survival (OS) in pre-menopausal and post-menopausal women.<sup>2</sup> However, whether to treat early-stage breast cancer using adjuvant chemotherapy is far from clear-cut. One of the major challenges in patients with node-negative breast cancer is weighing the risk of recurrence against the expected benefit of additional chemotherapy, which brings considerable morbidity and cost.

Research into tumour biology and our evolving knowledge of molecular biologic tumour features have broadened our understanding of breast cancer as a heterogeneous disease. Researchers have taken this heterogeneity into account and have developed new molecular markers that complete or in some cases supersede the classic clinical, pathological and immunohistochemical parameters. However, few of these tests have undergone systematic validation, subsequent commercialisation and, most importantly, broad acceptance of clinical utility by experts and patients.

This article reviews the case of Oncotype DX®, a 21-gene assay that has been developed in close collaboration with the National Surgical Adjuvant Breast and Bowel Project (NSABP).

### Development of the 21-gene Assay Oncotype DX

To accommodate clinical practice, the logical and pragmatic approach is to develop a test that does not depend on fresh or snap-frozen tissue but can be performed on routinely processed and archived tumour blocks or, optimally, slides. Despite the increasing degradation

and fragmentation of RNA over time during storage, Cronin et al. managed to develop a robust, high-throughput, realtime, reverse-transcriptase polymerase chain reaction (RT-PCR) analysis of formalin-fixed paraffin-embedded (FFPE) tumour tissue that could also be used for archival tissue blocks.<sup>3,4</sup>

As a second step, 250 candidate genes were selected from published literature, genomic databases and experiments based on DNA arrays performed on fresh-frozen tissue. Expression of these genes was measured by RT-PCR in tumour samples of 447 patients with node-negative, oestrogen-receptor (ER)-positive breast cancer from three independent clinical studies. Individual clinical data and results of gene expression analyses were correlated to identify prognostic genes.<sup>5-7</sup> The 16 genes with the highest correlation to distant recurrence after 10 years were selected for final model building and validation.<sup>8</sup> The panel includes genes associated with proliferation, invasion, ER, the human epidermal growth factor HER2neu and three other genes (see Figure 1).<sup>9</sup> Relative expression of these genes is measured in relation to the average expression of five reference genes. An algorithm was designed to calculate a numerical Recurrence Score (RS) ranging between 1 and 100 (see Figure 1). A low RS value corresponds to a low probability of distant recurrence at 10 years, whereas a higher score is associated with a higher probability.

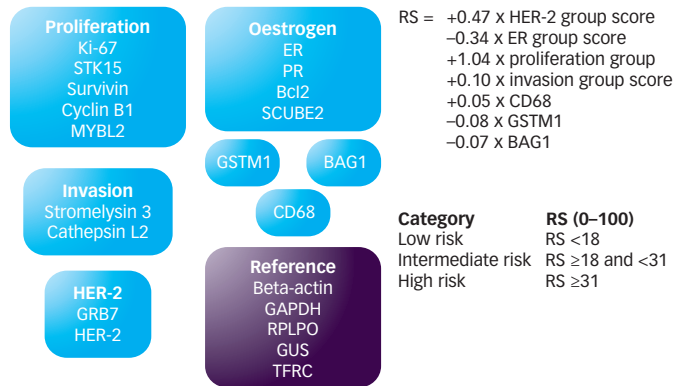
**Validation of the Recurrence Score in Node-negative, ER-positive Breast Cancer**

The Oncotype DX assay validation study was conducted utilising tumour specimens from patients who had been prospectively enrolled within the NSABP B-14 study.<sup>8</sup> The B-14 study included 2,617 women with node-negative, ER-positive breast cancer treated with tamoxifen for five years. RT-PCR was successfully performed in 668 of 675 cases. In these cases tumour blocks contained sufficient material to perform the test. The Kaplan-Meier estimate for distant recurrence at 10 years for the corresponding patients was 15%, indicating a highly representative node-negative, ER-positive patient population. Based on the results of previous studies, three risk groups were defined: a low risk of recurrence at 10 years after surgery for an RS <18, an intermediate risk for an RS of 18–30 and a high risk for an RS ≥31 (see Figure 1). The distribution of patients in these groups was 51, 22 and 27%, respectively. The RS proved to be a reliable predictor for the probability of distant recurrence at 10 years after surgery. Only 6.8% (95% confidence interval [CI] 4.0–9.6%) of patients with a low RS had distant recurrence at 10 years, 14.3% (95% CI 8.3–20.3%) of those in the intermediate group and 30.5% (95% CI 23.6–37.4%; p<0.001 compared with the low RS group) of those with a high RS. The proportion of patients without distant recurrence in the low RS group was 93% – significantly higher than the figure of 69.5% in the high-risk group. The RS was also significantly correlated with the relapse-free interval and OS (p<0.001 for both). In a multivariate Cox model, RS predicted distant recurrence independently of age and tumour size (p<0.001). RS is a continuous variable for predicting distant recurrence at 10 years (see Figure 2). The probability of distant recurrence at 10 years increases continuously with increasing scores. For RS >50, the likelihood of distant recurrence increases only slightly with further increases of the RS (see Figure 2).

**Population-based External Validation of the Prognostic Significance of the Recurrence Score**

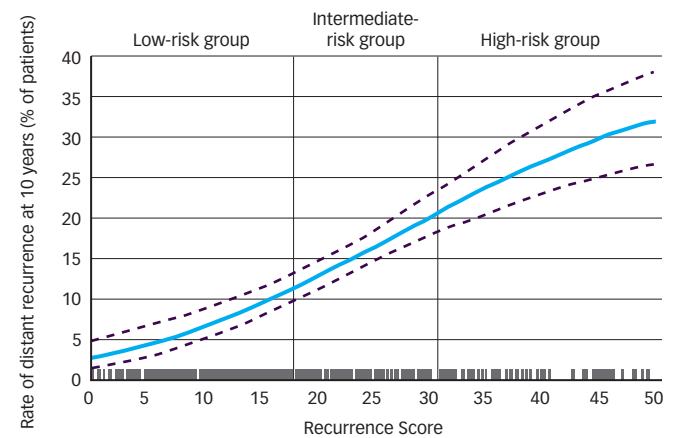
For further validation, a case-control study was performed in 4,964 patients diagnosed with node-negative breast cancer who did not

**Figure 1: Oncotype DX (Genomic Health, Redwood City, CA) Recurrence Score – Genes and Algorithm**



RS = Recurrence Score.  
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**Figure 2: Rate of Distant Recurrence as a Continuous Function of the Recurrence Score**



The dashed curves indicate the 95% confidence interval. The rug plot on top of the x-axis shows the Recurrence Score for individual patients in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 study.<sup>8</sup>

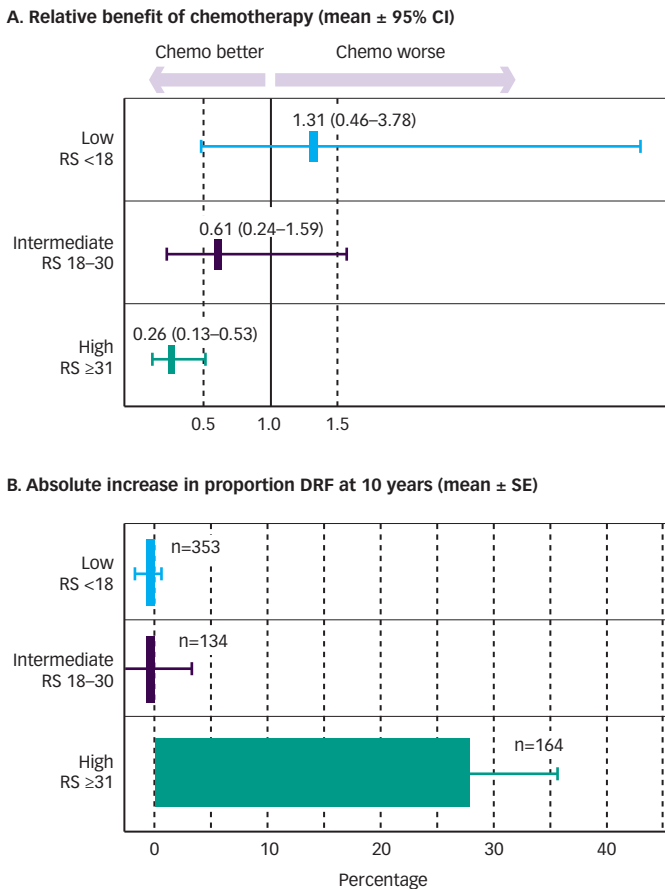
receive adjuvant chemotherapy.<sup>10</sup> The analysis included 220 cases and 570 individually matched controls alive at the date of death of their matched patients. After adjusting for tumour size and grade, the RS correlated with the risk of breast cancer death in ER-positive patients regardless of tamoxifen treatment. At 10 years after surgery, the risk of breast cancer death in ER-positive tamoxifen-treated patients was 2.8% (95% CI 1.7–3.9%), 10.7% (95% CI 6.3–14.9%) and 15.5% (95% CI 7.6–22.8%) in the low, intermediate and high RS groups, respectively.

The results of both the B-14 validation study and the external validation study were the basis for regulatory Clinical Laboratory Improvement Amendments (CLIA) approval of Oncotype DX as a diagnostic test for ER-positive, lymph-node-negative breast cancer treated with tamoxifen.

**Recurrence Score as a Predictor of the Additional Benefit of Chemotherapy**

The results of a neoadjuvant study hinted towards a correlation between RS and response to chemotherapy.<sup>11</sup> Patients with locally advanced breast cancer received chemotherapy with doxorubicin and paclitaxel

**Figure 3: Relative and Absolute Risks of Chemotherapy Benefit as a Function of Recurrence Score Risk Category**



CI = confidence interval; DRF = distant recurrence free; RS = recurrence score  
SE = standard error.<sup>12</sup>  
Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved. Paik S, et al., Gene Expression and Benefit of Chemotherapy in Women With Node-Negative, Estrogen Receptor-Positive Breast Cancer, *J Clin Oncol*, Vol. 24, No 23 (August 10), 2006: 3726–3734.

pre-operatively and were treated with adjuvant cytoxan, methotrexate and fluorouracil (CMF) after surgery. Of 93 patients with an available biopsy, RNA could be extracted for multigene analysis in 89 cases. The RS positively correlated with the probability of pathological complete response ( $p=0.005$ ). Patients at the highest risk of recurrence had a greater chance of benefiting from neoadjuvant chemotherapy.

To further investigate the correlation between RS and benefit from chemotherapy, tumour samples of patients included in NSABP B-20 study were analysed.<sup>12</sup> The B-20 study included 2,363 women with ER-positive, node-negative breast cancer. Tumour blocks of 651 patients were available, 227 of whom had received adjuvant tamoxifen and 424 adjuvant tamoxifen plus chemotherapy with CMF or MF. *Figure 3* illustrates the relative and absolute benefit of chemotherapy for each RS group. Patients with tumours that had a high RS received the highest benefit from chemotherapy (hazard ratio [HR] 0.26). The rate of distant recurrence 10 years after surgery could be increased by an absolute of 27.6%. For patients presenting with a tumour with an RS <18, no additional benefit of chemotherapy could be demonstrated. The test for interaction between chemotherapy treatment and RS was statistically significant ( $p<0.05$ ). When RS was examined as a continuous variable in a Cox model, the magnitude of the chemotherapy benefit appeared to increase continuously as the RS increased. Results for tumours with an intermediate RS were not conclusive. They did not appear to have a large

benefit (relative risk 0.61, 95% CI 0.24–1.59, 1.8% increase in absolute risk), but the uncertainty in the estimate could not exclude a clinically important benefit. As a consequence, this question will be further investigated in the ongoing prospective study Trial Assigning Individualized Options for treatment (Rx) (TAILORx; see *Figure 4A*).<sup>9</sup>

## Recurrence Score Offers Additional Information Over Established Prognostic Markers

The results of the validation study in NSABP B-14 demonstrated that the RS provided significant prognostic power independent of age and tumour size ( $p<0.001$ ).<sup>8</sup> RS predicted distant recurrence for all age categories and all categories of tumour size. Nevertheless, it is worthwhile to explicitly point out some findings illustrating how RS offers additional and sometimes rather unexpected information compared with standard prognostic markers. In the B-14 data set, 44 of 109 women with tumours <1cm in diameter had an RS ≥18 and an intermediate or high risk rather than the expected low risk of recurrence. The subset of patients with moderately differentiated tumours could be distinguished to be at low or high risk by the RS. A subgroup of patients with well differentiated tumours had a high RS and a high rate of distant recurrence, while another subset with poorly differentiated tumours had a low RS and a low rate of distant recurrence.<sup>8</sup>

The results from the NSABP B-20 study confirmed that the expected rather poor prognosis of some women under 40 years of age with large tumours or poor tumour grading could be revised when a low RS was found (see *Figure 5*). By contrast, some patients with tumours <1cm in diameter, those over 60 years of age or patients with a good tumour grading had to be classified as high-risk when an RS ≥31 was found (see *Figure 5*).<sup>12</sup>

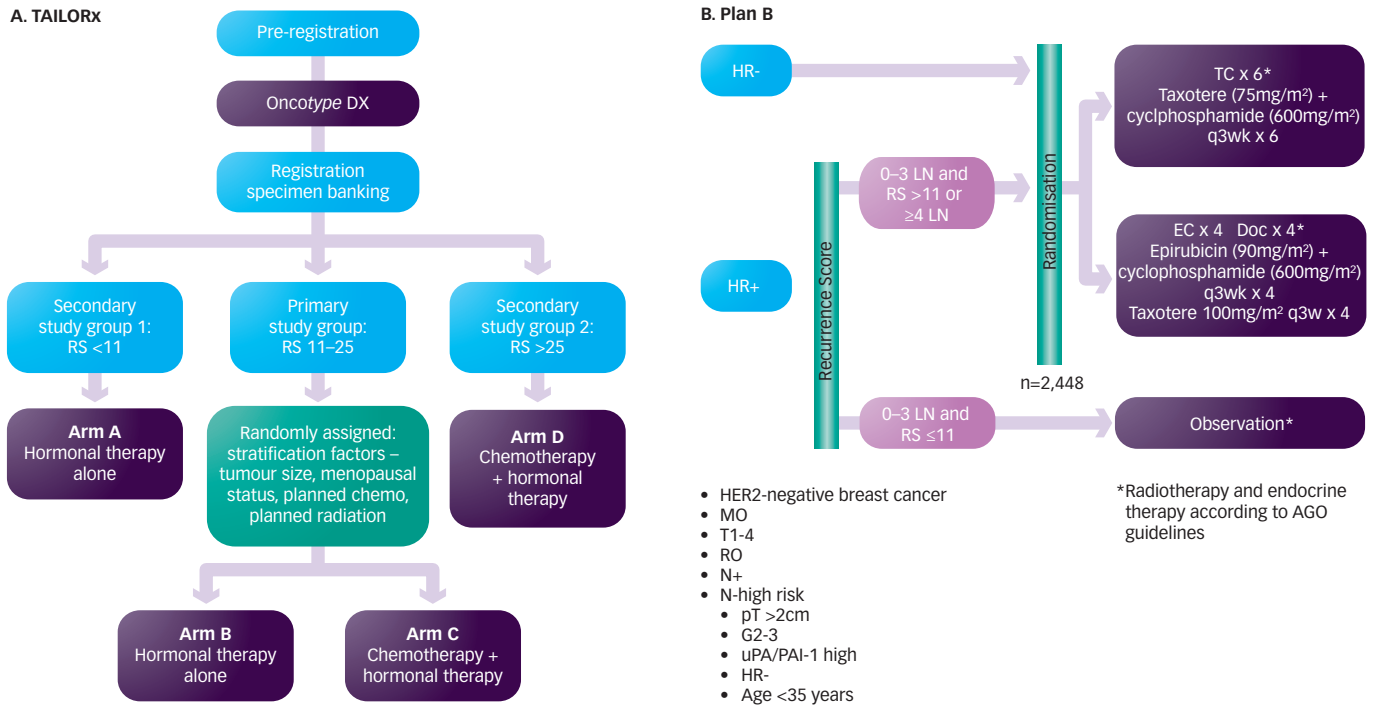
In Eastern Cooperative Oncology Group (ECOG) study E2197, a sample of 465 patients with HR-positive disease with zero to three positive lymph nodes with and without recurrence of disease had their tumour tissue evaluated using the Oncotype DX assay. Clinical variables were integrated by an algorithm modelled after Adjuvant! Online but adjusted to five-year outcomes, and RS predicted recurrence more accurately than the modified Adjuvant! Online integrator.<sup>13</sup>

In a study with 300 consecutively referred breast cancer patients, neither standard clinicopathological parameters nor commonly used assessment tools could predict the RS compared with a clinical trial population.<sup>14</sup>

## Recurrence Score as a Prognostic and Predictive Marker in Node-positive Breast Cancer

Oncotype DX was developed and validated in node-negative, ER-positive breast cancer. Analysis from a case-cohort sample of patients enrolled in ECOG study E2197 identified RS as a significant predictor of recurrence in those with HR-positive disease with zero to three positive lymph nodes, regardless of nodal status.<sup>15</sup> The study included 2,885 evaluable patients with zero to three positive nodes and operable breast cancer, who received chemotherapy with doxorubicin plus either cyclophosphamide or docetaxel if HR-negative or chemotherapy plus hormonal therapy if HR-positive. Tissue for RT-PCR was available from 776 patients, of whom 179 had developed a recurrence and 597 had not. In HR-positive patients, recurrence risk was significantly elevated for an intermediate RS (HR 2.96;  $p=0.0002$ ) or a high RS ( $n=108$ , HR 4.0;  $p=0.0001$ ) compared with patients with a

**Figure 4: Design of TAILORx (A), WSG Plan B (B) and Michelangelo (C) Studies**

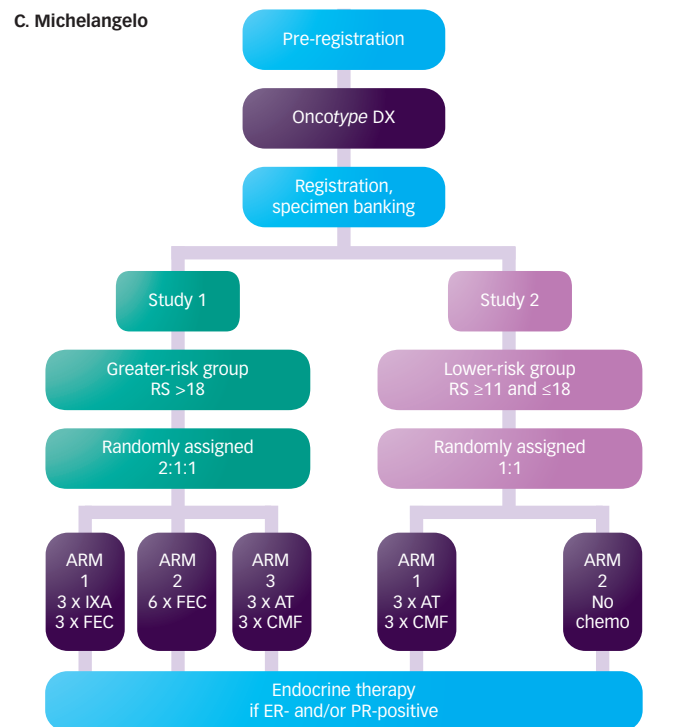


low RS. Patients with a low RS had excellent outcomes for five-year relapse-free interval, DFS and OS regardless of whether they were node-negative or node-positive.<sup>15</sup>

The prognostic and predictive value of the Oncotype DX recurrence score in node-positive disease was confirmed in an analysis of tumour samples from the phase III study S8814 of the Southwestern Oncology group.<sup>16</sup> S8814 demonstrated an added benefit for adjuvant chemotherapy with cyclophosphamide, doxorubicin and fluorouracil versus tamoxifen alone with respect to DFS and OS in post-menopausal patients with node-positive, ER-positive breast cancer. Due to optional specimen banking, the Oncotype DX assay could be performed in 367 patients. RS distribution was 40% for a low RS (<18), 28% for an intermediate RS (18–30) and 32% for a high RS (≥31). The RS proved to be a prognostic marker for DFS at 10 years in the tamoxifen-only patients (p=0.006). The effects were similar in the subsets presenting with one to three positive nodes or more than four positive nodes involved and for OS. The RS was also confirmed as a predictive marker for the additional benefit of chemotherapy. In patients with a high RS, DFS at 10 years was significantly longer if treated with chemotherapy. The Kaplan-Meier estimate of DFS at 10 years was 55% for patients treated with chemotherapy compared with 43% for women treated with tamoxifen only (p=0.03). Patients with a low or intermediate RS did not seem to derive an additional benefit from chemotherapy in this retrospective analysis.

### Prognostic Value of Recurrence Score Confirmed for Adjuvant Aromatase Inhibitor Treatment

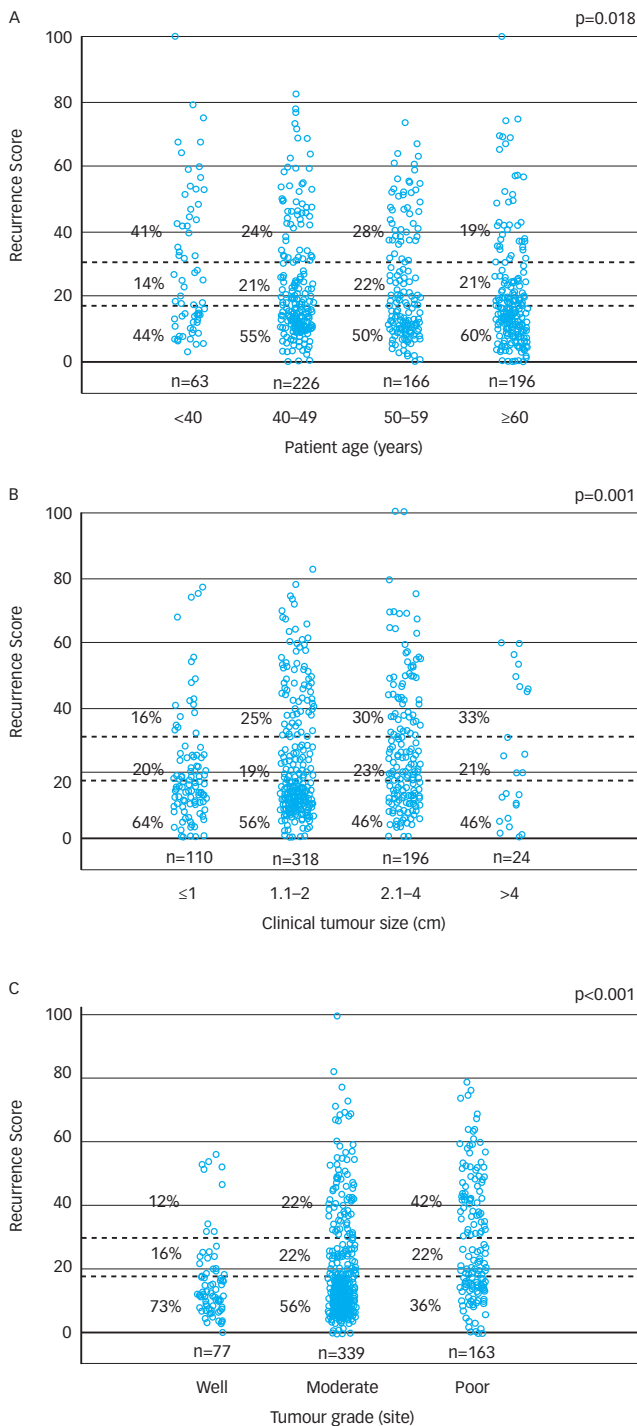
Recently, results from the TransATAC study demonstrated that RS is also an independent predictor of the risk of distant recurrence in node-negative and node-positive HR-positive patients treated with the aromatase inhibitor anastrozole.<sup>17</sup> The Arimidex, Tamoxifen Alone or in Combination (ATAC) trial initiated in 1996 compared adjuvant treatment with anastrozole alone, tamoxifen alone and the



A = doxorubicin; C = cyclophosphamide; E = epirubicin; F = fluorouracil; IX = ixabepilone; M = methotrexate; N = nodes; RS = Recurrence Score; T, Doc = docetaxel; TAILORx = Trial Assigning Individualized Options for treatment (Rx); WSG = West German Study Group. Figure 4A reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved. Sparano J, et al., Development of the 21-Gene Assay and Its Application in Clinical Practice and Clinical Trials, J Clin Oncol, Vol. 26, No 5 (February 10), 2008: 721–728.

combination of both in 9,366 post-menopausal women with early-stage operable breast cancer. In an effort to identify molecular characteristics of tumours, the TransATAC project was initiated to collect tumour blocks retrospectively from patients participating in ATAC.<sup>18</sup> For Oncotype DX analysis, tumour tissue of 1,231 HR-positive patients treated with either anastrozole or tamoxifen was available.<sup>17</sup>

**Figure 5: Distribution of Recurrence Score and Standard Prognostic Factors**



A: Recurrence Score (RS) versus age; B: RS versus clinical tumour size; C: RS by tumour grade (National Surgical Adjuvant Breast and Bowel Project [NSABP] site pathologist).<sup>17</sup> Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved. Paik S, et al., *Gene Expression and Benefit of Chemotherapy in Women With Node-Negative, Estrogen Receptor-Positive Breast Cancer*, *J Clin Oncol*, Vol. 24, No 23 (August 10), 2006: 3726-3734.

Of these, 872 women had node-negative and 306 node-positive disease, and in 53 cases nodal status was unknown. In a prospectively defined multivariate analysis, tumour size, grade and RS were each separately statistically significant in predicting time to distant recurrence in node-negative patients (p<0.01, 0.003 and <0.001). Similar results were seen in node-positive patients. For node-negative patients, distribution into the three risk categories was 59% for low,

26% for intermediate and 15% for high RS, and the nine-year rates of distant recurrence were 4, 12 and 25%, respectively. Both distribution of patients into the three RS categories and proportions of rates of distant recurrence were very similar to the patient distribution and rates of distant recurrence at 10 years reported by Paik in the NSABP B-14 validation set. For the node-positive subset, distribution into the low, intermediate and high RS groups were 52, 31 and 17%, respectively, with rates of distant recurrence at nine years of 17, 28 and 49%, respectively. RS showed statistically significant prognostic value beyond that provided in Adjuvant! Online in both node-negative (p<0.001) and node-positive patients (p=0.003). The data could not depict a differential benefit between anastrozole and tamoxifen, i.e. RS does not seem to be predictive for type of endocrine therapy.

## Adoption of Oncotype DX in International Treatment Guidelines

Several international scientific societies and study groups have evaluated multigene assays in their guidelines:

- In 2007, the American Society of Clinical Oncology (ASCO) updated its recommendations for the use of tumour markers in breast cancer. *Oncotype DX* is the only multigene assay recommended for use in newly diagnosed ER-positive, node-negative breast cancer patients to predict risk of recurrence. It is also recommended to identify patients who may be spared adjuvant chemotherapy.<sup>18</sup> Among the plethora of potential new prognostic factors, the only other prognostic factor recommended by ASCO for breast cancer decision-making was the urokinase plasminogen activator (uPA/plasminogen activator inhibitor 1 (PAI-1) assay).<sup>19</sup>
- The updated National Comprehensive Cancer Network guidelines include the option of using *Oncotype DX* to help guide chemotherapy treatment decisions within the systemic adjuvant treatment decision pathway for patients with node-negative or pN1mi HR-positive, HER-2-negative tumours that are 0.6-1cm in diameter and moderately/poorly differentiated, or with unfavourable features or >1cm in diameter.<sup>20</sup>
- In 2009 the St Gallen international expert consensus conference on the primary therapy of early breast cancer acknowledged the added value of validated multigene assays in the decision process for adjuvant chemotherapy of patients with ER-positive, HER-2-negative disease.<sup>21</sup>
- The 2010 guidelines of the German Arbeitsgruppe Gynaekologische Onkologie (AGO) consider *Oncotype DX* a prognostic marker for node-negative breast cancer (AGO±). To further clarify its prognostic and predictive value regarding adjuvant chemotherapy decision-making, the AGO recommends participation in clinical studies and using the assay as a predictive marker for decision-making for adjuvant chemotherapy only in individual cases outside clinical studies.<sup>22</sup>

## Impact of Recurrence Score on Clinical Decision-making

*Oncotype DX* has been used in more than 120.000 patients in over 50 countries.<sup>23</sup> An analysis of all ER-positive tumour specimens successfully examined in the Genomic Health Laboratory from June 2004 to December 2008 was performed.<sup>24</sup> There were 347 male and 82,434 female breast cancer patients included in the analysis. The distribution of female breast cancer patients showed 53.4, 36.3 and 10.3% in the low, intermediate and high RS groups, respectively. While the proportion of low-risk tumours was similar to those found in the

clinical study populations, the proportion of patients in the high RS groups was lower. This trend can be observed for most studies in actual clinical practice and probably reflects a selection bias by physicians prescribing the test in specific clinical situations only. The test has proved to be most useful in HER-2-negative disease, since 50 of the 55 HER-2-positive patients in the B-14 validation study presented with high RS.<sup>9</sup> Interestingly, it was also demonstrated that the distribution of RS in males was similar to that in females, with 53.6, 35.2 and 11.2% in the low, intermediate and high RS groups, respectively.<sup>24</sup>

Recently, a few studies<sup>25–31</sup> on the impact of RS on decision-making in early breast cancer have been published. The results reflect the fact that the assay has been adopted in clinical practice and knowledge of RS affects management of patients. In 21–44%<sup>25,28</sup> of cases in the reported studies, recommendations from clinicians changed with knowledge of the individual RS. The most common change was from chemo–hormonal to hormonal-only treatment. However, in some cases a high RS resulted in a recommendation for additional adjuvant chemotherapy. In situations where the recommendation or eventual treatment was not changed after RS knowledge was obtained, it was reported anecdotally by physicians and patients that the RS increased confidence and reassurance about the chosen treatment plan.<sup>25</sup> The patient perspective was formally investigated in one prospective multicentre study.<sup>30,31</sup> Patient satisfaction, anxiety and decisional conflict for adjuvant treatment selection of 89 patients were recorded pre- and post-RS knowledge with the help of validated questionnaires. RS resulted in changes in medical oncologist treatment recommendation in 31.5% of cases, 27% of patients had a change in treatment plan post-RS and 83% of patients reported that the assay had influenced their treatment choice. The results indicated reduced conflict over treatment decisions post-RS ( $p < 0.0001$ ), greater patient satisfaction and increased confidence with the choice of adjuvant therapy ( $p < 0.0001$ ).

Recent quality assurance data suggest that in cases of prior core biopsy, microdissection needs to be performed in order to prevent interference of post-biopsy tissue alterations with the Oncotype DX test results.<sup>32</sup>

### Health Economic Impact of Recurrence Score-guided Treatment Strategy

The list price for Oncotype DX is US\$3,975 per test. Health economic data on RS-guided therapy are still scarce. There is one US study, published in 2007.<sup>33</sup> The clinical impact of different treatment strategies was assessed by estimating the gain in life expectancy or life-years saved from NSABP B-20 and B-14. RS-guided therapy was estimated to provide net cost savings of US\$2,256 compared with chemotherapy plus tamoxifen. The estimated incremental costs associated with RS-guided therapy and chemotherapy plus tamoxifen were US\$4,272 and US\$6,527, respectively, compared with tamoxifen alone. The incremental cost-effectiveness ratio compared with tamoxifen alone favoured the RS-guided therapy strategy (US\$1,944/life-year saved) over the chemotherapy plus tamoxifen approach (US\$3,385/life-year saved). RS-guided therapy was found to be more costly for low-cost chemotherapy regimens not requiring additional supportive care, whereas a net cost saving of between US\$500 and US\$10,000 was estimated with RS-guided therapy for other commonly used adjuvant chemotherapy regimens, such as AC, AC-T, TAC, etc. However, the authors state that the estimated cost savings provided are probably underestimates as only the cost of drugs was considered, while other treatment-related direct and indirect costs – e.g. cost of administration, professional fees, laboratory testing, complications, transportation, etc. – were not.

### Alternative Prognostic and Predictive Tests

uPA and PAI-1 have been identified as prognostic markers for node-negative breast cancer independent of tumour size, grade and HER2 or hormone receptor status. The prognostic impact of the uPA/PAI-1 test has been validated at the highest level of evidence (LOE I) by a prospective clinical trial as well as a pooled analysis in over 8,000 patients.<sup>34,35</sup> The test requires fresh or snap-frozen tumour tissue. uPA and PAI-1 are components of the plasminogen activating system shown experimentally to be associated with invasion, angiogenesis and metastasis. Low levels of both are associated with a lower risk of recurrence.<sup>34,35</sup> Results of a prospective trial that stratified node-negative patients according to uPA and PAI-1 demonstrated that CMF-based chemotherapy contributed substantial benefit compared with observation alone in patients with a high risk of recurrence as determined by high levels of uPA/PAI-1.<sup>35</sup> This predictive impact was confirmed by combined analysis of two retrospective studies suggesting that patients with high uPA/PAI-1 derive enhanced benefit from adjuvant chemotherapy.<sup>36</sup> Enrolment into the second prospective trial, NNBC3-Europe, with patients assigned to one of two strategies for risk assessment and decision-making (either existing guidelines or determination of levels of uPA/PAI-1), has recently been completed at 4,150 patients.

Mammprint™ is a microarray-based assay assessing the expression of 70 genes focused primarily on proliferation, with additional genes associated with invasion, metastasis, stromal integrity and angiogenesis.<sup>37</sup> The test requires a sample of fresh or snap-frozen tissue that contains at least 30% malignant cells. The signature was found to be a prognostic marker for high or low risk of distant metastasis.<sup>38</sup> Retrospective data show its utility for node-negative patients up to 70 years of age. Recently, its independent prognostic value was also shown for patients with one to three lymph nodes involved.<sup>39</sup> The large international prospective Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy (MINDACT) trial is currently comparing the 70-gene signature with routine clinical–pathological assessment in selecting patients with zero to three lymph nodes involved for adjuvant chemotherapy.

### Evaluation of Oncotype DX in Current Clinical Trials

For patients with HR-positive, node-negative breast cancer and an intermediate RS, a large prospective US intergroup trial (TAILORx; see *Figure 4A*)<sup>9</sup> will clarify the additional benefit of adjuvant chemotherapy. Patients with an RS of 11–25 are randomised to receive either endocrine or chemo–endocrine therapy. Patients with an RS <11 or >25 are not randomised but receive endocrine therapy alone or chemo–endocrine therapy, respectively. RS cut-offs were modified to prevent potential undertreatment.

The Plan B trial of the West German Study Group (WSG) will randomise only HER-2-negative patients to either anthracycline-free chemotherapy or an anthracycline–taxane-based chemotherapy (see *Figure 4B*). Patients with high-risk node-negative and node-positive disease are eligible. Oncotype DX will be performed prospectively as a stratification parameter for all HR-positive patients with zero to three involved lymph nodes. HR-positive patients will receive chemotherapy only if they have more than four positive nodes or if their RS is >11. Moreover, risk assessment by Oncotype DX will be compared prospectively with risk assessment by uPA/PAI-1.

The Italian Michelangelo foundation has launched a phase III study of adjuvant systemic therapy in women with HER-2-negative conventionally high-risk tumours (node-positive and/or T2–T3), using *Oncotype DX* to select and differentially treat patients at greater risk as defined by an RS >18 and lower risk as defined by an RS ≤18 (see *Figure 4C*).

These are only three of several ongoing prospective studies incorporating *Oncotype DX*.

## Summary and Conclusions

*Oncotype DX* has taken the step from an experimental assay to an accepted instrument assisting in the clinical decision-making process in HR-positive early breast cancer. The 21-gene test was developed and validated systematically in close collaboration with a major clinical study group (NSABP). A particular advantage of this test is that no special care is required at the site where surgery is performed as it is validated on paraffin-embedded material. Therefore, the test can be performed whenever information on RS is needed. The RS has proved to be a prognostic and predictive marker for patients with HR-positive, node-negative disease treated with tamoxifen, offering insight beyond that provided by traditional

prognostic markers. For patients with a low RS (<18) or a high RS (≥31), the assay can predict the potential benefit of adjuvant chemotherapy. Its prognostic and predictive value was also retrospectively confirmed for node-positive disease. Recent data have established its prognostic value for node-negative and node-positive post-menopausal patients receiving adjuvant treatment with an aromatase inhibitor. Major scientific societies and study groups have acknowledged the evidence in their guidelines, and *Oncotype DX* has been widely adopted in clinical practice. More often, knowledge of RS results in patients being spared adjuvant chemotherapy. The pharmacoeconomic impact of such an RS-guided approach needs to be further elucidated. The results of ongoing prospective studies will add to the significant body of evidence. ■



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