Clinical Roundtable Monograph

Incorporating the Oncotype DX Breast Cancer Assay into Community Practice: An Expert Q&A and Case Study Sampling

Faculty

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Introduction

Advances in breast cancer research have confirmed that this malignancy is not a single disease, but rather a collection of genetically distinct diseases with different treatment requirements. In recent years, several studies have confirmed the clinical validity of the Oncotype DX breast cancer assay, not only as a way to predict recurrence but also as a tool for determining therapeutic benefit from adjuvant chemotherapy.

Recently, Drs. Terry Mamounas, G. Thomas Budd, and Kathy Miller answered questions about the Oncotype DX assay that are particularly relevant to routine clinical practice. This expert dialog provides a useful update and essential clinical insights about how, why, and when community oncologists may want to incorporate this multi-gene assay into their care of breast cancer patients. In addition, sample case studies offer tangible examples of the practical application of Oncotype DX.

The Onco*type* DX Breast Cancer Assay—An Expert Q&A and Case Study Sampling

What is the Onco*type* DX breast cancer assay and how was it developed?

Dr. Terry Mamounas The Onco*type* DX assay measures the expression profile of a specific set of 21 genes from primary breast tumor samples.[1] By measuring changes in the expression levels of these genes, the assay can be used as a prognostic tool for patients. Gene expression is measured in triplicate from formalin-fixed, paraffin-embedded breast tumor tissue removed during surgery.

The Onco*type* DX assay was developed using a diverse set of patients drawn from three separate independent clinical cohorts (136 node-negative/node-positive patients from Providence, St. Joseph's Hospital, Burbank, Calif., 78 node-positive patients with ≥10 nodes from Rush Presbyterian, Chicago, Ill., and 233 node-negative patients from the National Surgical Adjuvant Breast and Bowel Project [NSABP] B-20 trial). Patients from these three cohorts included those traditionally recognized as having low-risk or high-risk prognoses, as well as patients receiving hormonal therapy and/or chemotherapy. Currently, the Onco*type* DX test has only been validated in and approved for use in lymph node–negative, estrogen receptor (ER)-positive breast cancer patients treated with tamoxifen.

Dr. G. Thomas Budd The first step in assay development was to optimize a real-time reverse transcription polymerase chain reaction (RT-PCR) protocol for use with paraffin-embedded tissue samples.[2] Once optimized, this RT-PCR protocol was then used to determine the expression of 250 candidate genes in paraffin-embedded samples taken from 447 patients who had participated in three independent breast cancer clinical studies. Of the 250 candidate genes originally tested, 16 were found to be potentially significant for determining patient prognosis, evident by a relation between gene expression and breast cancer recurrence.[3,4]

How were the genes assayed for in the Onco*type* DX test selected?

GTB The original 250 candidate genes were selected by reviewing the microarray data and genomic databases available at the time of assay development, which suggested a number of genes that possibly could be prognostic. Additionally, the published literature was used to identify genes related to breast tumor biology.

Dr. Kathy Miller Of the 250 genes originally tested in the initial assay development, 16 were identified as being consistently statistically associated with distant breast cancer recurrence. Importantly, these 16 genes had the most prognostic power across all three of the patient populations initially studied.[5-7] In addition to these 16 cancer-related genes, 5 genes were selected to be used as references for normalizing the expression of the cancer-related genes (Table 1). These 5 genes were chosen based on their relatively consistent expression across patient samples.

TM Many of the cancer-related genes evaluated in the Onco*type* DX assay have historically been associated with cellular functions important for cancer cell growth and survival. For example, several genes encode proteins involved

Table 1. The 21 Genes Included in the Oncotype DX Assay

Genes	Group
Ki-67 STK15 Survivin Cyclin B1 MYBL2	Proliferation
ER PR Bcl2 SCUBE2	Estrogen
Stromelysin 3 Cathepsin L2	Invasion
GRB7 HER2	HER2
GSTM1 CD68 BAG1	Other
Beta-actin GAPDH RPLPO GUS TFRC	Reference (not cancer related)

in cell proliferation. When these genes are overexpressed the tumor cells multiply at a faster rate. Similarly, other genes are associated with estrogen and progesterone receptor expression, and their expression therefore affects response to hormonal therapy. Most of the remaining genes are involved in tumor cell invasion and metastasis, as well as inhibition of cell death.

What is the recurrence score and what does it tell clinicians?

TM The recurrence score is the result produced by the Oncotype DX assay. It is based on the pattern of gene expression in the breast tumor and ranges from 0 to 100. Lower recurrence scores are associated with a generally favorable prognosis, while higher recurrence scores are indicative of an unfavorable prognosis. Using the recurrence score, node-negative, ER-positive patients can be categorized into one of three groups, which are predictive of the risk of disease recurrence. Nearly half of these patients fall into the low-risk group, identified as having a recurrence score of less than 18. These patients are less likely to experience a disease recurrence and have a good prognosis. Approximately 25% of the patients are classified as having a high risk of recurrence, with a score of 31 or

greater. These patients have a particularly poor prognosis. The remaining 25% of patients fall into an intermediate category (recurrence score 18–30).

Recently, a population-based study of breast cancer patients evaluated the ability of the recurrence score produced by the Oncotype DX assay to predict prognosis.[8] This study included 790 cases (n=220) and controls (n=570) who either did or did not receive tamoxifen therapy. Significantly, the recurrence score was linked with the risk of breast cancer death in both tamoxifen-treated and -untreated ER-positive patients (P=.003 and P=.03, respectively).

GTB In addition to the prognostic ability of the test, the Oncotype DX assay can also predict which patients are likely to respond to chemotherapy, as was demonstrated in the NSABP B-20 trial.[9] In this clinical study, 651 patients were randomized to receive tamoxifen alone or tamoxifen plus chemotherapy. The recurrence score was found to be statistically associated with benefit from chemotherapy (P=.038). Patients with a high recurrence score experienced a large benefit from the addition of chemotherapy, with a mean absolute decrease in 10-year distant recurrence rate of 28% (relative risk [RR], 0.26; 95% confidence interval [CI], 0.13–0.53). Conversely, women with a low recurrence score received no significant benefit from chemotherapy, with a mean absolute decrease in distant recurrence rate at 10 years of -1.1%±2.2% (RR, 1.31; 95% CI, 0.46–3.78).

Based on the two pieces of information gleaned from the recurrence score—the prognosis of the patient and the prediction of whether the patient will benefit from chemotherapy—the clinician and patient together are able to make a more informed decision regarding the addition of chemotherapy to hormonal therapy.

KM However, there are a certain number of patients in whom the benefit of chemotherapy is uncertain. These patients fall into the intermediate-risk group. In the NSABP B-20 study population, the benefit of adding chemotherapy to tamoxifen in the intermediate group was estimated to be between 0 and 5% for the intermediate-risk group.[9] However, the degree of uncertainty regarding this estimate did not preclude the possibility that some patients do experience a benefit. To better estimate this benefit, a prospective clinical study is currently underway. The main objectives of the Trial Assigning Individualized Options for Treatment (Rx) (TAILORx) study is to explore the benefit of chemotherapy in a group of patients with uncertain chemotherapy benefit based on their recurrence score.[10] Patients enrolled in this study are assigned to a primary study group or secondary study group based on their recurrence score after an Oncotype DX assay. Of these, the primary study group will be the mid-range group, with recurrence scores of 11–25. These patients will be randomized to receive either hormone
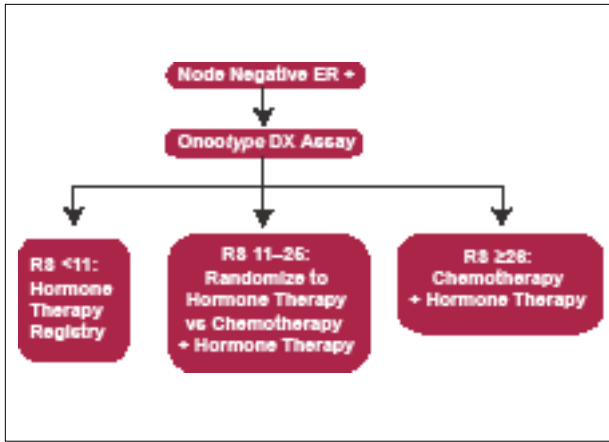
Figure 1. TAILORx schema.

ER=estrogen receptor; RS=recurrence score; TAILORx=Trial Assigning Individualized Options for Treatment.

Figure 2. Recurrence score (RS) as a continuous variable.

Adapted from Paik S, et al. *N Engl J Med*. 2004;351:2817-2826.

therapy alone or chemotherapy followed by hormonal therapy (Figure 1).

Another important objective of the TAILORx trial is to develop a tissue and specimen bank to enable further exploration of the biology of early-stage breast cancer, including the assessment of new cancer tests.

How is the recurrence score considered to be a continuous predictor?

TM As the recurrence score increases, so does the risk for the occurrence of distant recurrence (Figure 2). Therefore, within the prognostic categories of low, intermediate, and high risk, some patients have lower or higher risks of recurrence compared to others. For example, in the low-risk group of 0 to 17, a patient with a recurrence score of 5 has less risk of recurrence than does a patient with a recurrence score of 13 even though both of these patients are in the low-risk group. This is true in the intermediate- and high-risk groups as well. Because the recurrence score is a continuous predictor, the Onco*type* DX assay has a particular advantage over other prognostic tests, which only place patients in low- and high-risk groups with no further prognostic discrimination within the groups.

What impact do risk factors have on the recurrence score?

TM Compared to the traditional prognostic factors of age, tumor size, and tumor grade, the recurrence score is a better predictor of risk of recurrence. The recurrence score
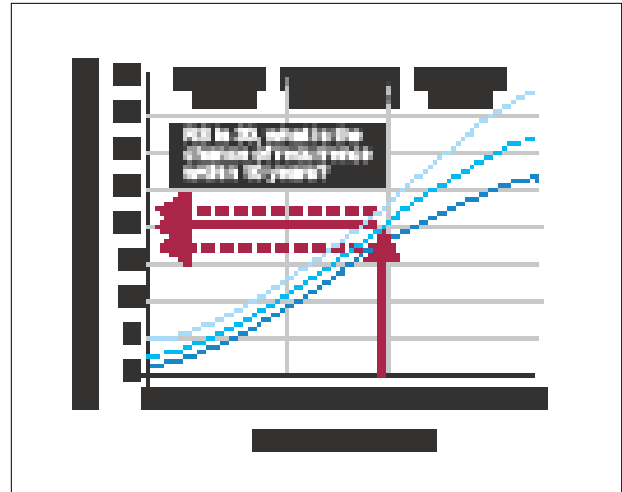
is prognostic irrespective of these traditional factors, and, importantly, menopausal status does not impact the recurrence score, making it just as useful in younger patients as in older patients.

How was the Onco*type* DX assay validated?

GTB Two aspects of the Onco*type* DX assay have been validated. First, the ability of the assay to determine prognosis in tamoxifen-treated women was shown in patients enrolled in the NSABP B-14 study.[3] From the 2,617 tamoxifen-treated patients involved in this study, 675 paraffin-embedded samples were available. Based on recurrence scores calculated for each patient, 51% of the population was classified as low-risk, 22% was identified as intermediate-risk, and 27% fell into the high-risk group. The recurrence score was found to statistically correlate with the 10-year incidence of distant recurrence-free survival (6.8% for low-risk, 14.3% for intermediate-risk, and 30.5% for high-risk; *P*<.00001 for comparison between low- and high-risk groups). Similar results were seen in a second validation study, the case-controlled Kaiser Permanente study discussed above.[8]

Second, the ability of the assay to serve as a predictor of patient response to chemotherapy has been recently validated. The NSABP B-20 trial showed that patients with higher recurrence scores benefited from the addition of chemotherapy, while those with lower scores did not.[9] In addition, recently reported results from the phase III S8814 study show that the recurrence score is also predictive of chemotherapeutic benefit in node-positive, ER-positive, postmenopausal women, validating the observations made

in patients treated on NSABP B-20.[11] This is some of the first evidence showing the reliability of the Onco*type* DX assay in lymph node–positive patients and in patients treated with anthracycline-based chemotherapy.

What advantages are there to using Onco*type* DX over conventional predictors of prognosis?

TM Historically, treatment decisions for patients with node-negative, ER-positive breast cancer were based on a combination of clinical and histological factors.[12] However, these factors are limited in their prognostic ability. Traditional prognostic factors, including tumor size and patient age, have a narrow predictive power while factors such as tumor grade offer poor reproducibility. Biological markers, including proliferation markers, are also often used to determine patient prognosis, but the immunohistochemical assays that are used to measure them are difficult to standardize.

Currently the standard therapy for this group of patients is to offer the majority of them adjuvant chemotherapy. Although most patients are offered the same therapy, relatively few actually benefit from it. Still other patients refuse therapy, deciding that the small potential benefit does not outweigh the toxic side effects associated with treatment. The result is that some patients are undertreated while others are overtreated.

KM Additionally, the RT-PCR technique used in the Onco*type* DX assay adds to its advantages. The RT-PCR has the ability to measure changes in gene expression that are highly quantitative over a large and dynamic range. Also, the use of multiple genes allows several pathways important in breast cancer cell biology to be evaluated simultaneously, adding to the power of the assay.

What key factors are important when considering whether a multigene assay, such as Onco*type* DX, is ready for routine clinical use?

TM First, the assay must be relatively simple to carry out. The Onco*type* DX assay is particularly easy to perform, as it uses paraffin-embedded tissue sampled at the time of a patient's surgery. Also, the assay must be validated, meaning that the score produced by the assay has been shown to be a true reflection of the prognosis of the patient. Well-validated assays, such as the Onco*type* DX, will have narrow confidence intervals, indicating a great degree of certainty surrounding the prediction of prognosis.

KM Additionally, there are several factors that a clinician would want to be aware of before routinely using a multigene assay. Key among these is whether the test has been validated in multiple independent data sets. This is important as it

will show that the assay is applicable for a broad range of patients and not just the particular patient set in which the assay was developed. Also, the assay must be reliable, meaning that the assay will yield similar results when repeated multiple times on the same patient sample. The Onco*type* DX has been found to be very reliable, with a coefficient of variation determined to be 3–6%. Finally, the most useful tests are those that can help to determine the most beneficial therapies to patients. Assays that are merely prognostic are not as useful as assays that have an additional predictive ability, such as the Onco*type* DX assay.

What is Adjuvant! Online?

TM Adjuvant! Online is a computer model used to estimate risk for recurrence based on traditional prognostic factors including age, tumor size, and receptor status, among others. An analysis of the Intergroup trial E2197 presented at the 2007 San Antonio Breast Cancer Symposium compared the prognostic ability of the Onco*type* DX assay with the Adjuvant! Online assay.[13] Although both assays provided independent prognostic information, the recurrence score provided by the Onco*type* DX assay was found to perform slightly better, meaning it can better discriminate a patient's risk. Therefore, there is a broader differentiation between patients in the low-risk versus high-risk groups using the recurrence score compared to the Adjuvant! Online assay. The recurrence score by Adjuvant! Online interaction term was not statistically significant, which confirms independent prognostic ability.

Recently, a population-based study found that although several prognostic factors used in the Adjuvant! Online assay correlated with the recurrence score, the Adjuvant! Online assay itself was unable to predict the recurrence score. It is important to note that neither assay was optimized for 5-year data. Specifically, Adjuvant! Online is based on 10-year event data and so would not be expected to perform optimally within a 5-year evaluation; study results should be considered accordingly.

GTB An important part of the Adjuvant! Online prognostic tool is the incorporation of comorbidity factors. Although this can be useful clinically, these factors may not be applicable for otherwise healthy breast cancer patients, such as those entered into clinical trials, reducing the usefulness of this component of Adjuvant! Online in the research setting.

Ultimately, the best results will stem from the incorporation of all of the information from both the Adjuvant! Online assay and the Onco*type* DX assay. Ideally, in the future the biologic information summarized in the recurrence score can be applied in the context of the patient's age and comorbidities.

In what situations is the Oncotype DX assay most useful?

TM Because the Oncotype DX assay was developed and validated in patients with node-negative and ER-positive breast tumors, this is the group of patients for whom it is currently indicated. Additionally, this assay is particularly useful for the subset of patients within this group who are HER2-negative, because having HER2-positive disease almost always results in either an intermediate or high recurrence score.

KM Recent studies have begun to assess the usefulness of the Oncotype DX assay in node-positive, ER-positive patients.[11] Although an initial trial in this group of patients showed that the assay was as useful as in node-negative patients, it was a small study with a limited number of patients.

GTB Most ER-negative patients have a high recurrence score and therefore the Oncotype DX assay is not particularly useful for this group of patients. In the future, it may be possible to derive a different algorithm using a different set of genes for these patients.

How might the Oncotype DX assay influence treatment decisions?

TM The assay has already started to be incorporated into national guidelines and routine clinical practice. Recently, the American Society of Clinical Oncology released a set of guidelines regarding the use of tumor markers.[14] These guidelines recommended that the Oncotype DX assay is the first and only multigene assay to be used in routine clinical practice for making therapeutic decisions based on its prognostic ability and its predictive ability of chemotherapy benefit. This was followed by an update of the National Comprehensive Cancer Network guidelines to also reflect the incorporation of the recurrence score into clinical practice.[15] At this point, over 40,000 Oncotype DX assays have been performed in clinical practice.

GTB The widespread use of multigene assays such as Oncotype DX will lead to more appropriate treatment decisions, enabling patients to receive the treatment from which they will gain the most benefit.

Several studies evaluating how the use of the Oncotype DX assay affects therapeutic decisions have found that use of this assay has changed treatment decisions in approximately one third of patients and has often led to less chemotherapy use.[16-19] By rationally choosing not to undergo chemotherapy if there is no clear benefit predicted, fewer patients will have to deal with the toxic effects and increased costs of that treatment.[20]

How are ER and PR expression measured by RT-PCR?

KM There are several methods with which ER and progesterone receptor (PR) expression can be measured. The conventional method, using immunohistochemistry, is very tricky in that it can produce both false positive and false negative results. False positive results are routinely caused by the binding of the ER- or PR-directed antibody to other proteins that are similar in sequence. False negative results can arise from a lack of sensitivity of the antibody. Although the RT-PCR technique used in the Oncotype DX assay can be biased by how well the messenger RNA is extracted from the tumor sample, this is corrected for by normalizing the expression of the 16 cancer-related genes against the 5 reference genes (see Table 1).

TM The expression of both the ER and PR is more accurately and quantitatively predicted by RT-PCR than with the conventional immunohistochemical technique.[21,22] Importantly, measuring the expression levels of ER by RT-PCR allows for the detection of a wider range of ER expression, estimated to be 3,000-fold overall. Even in ER-positive patients there is approximately a 200-fold difference in ER expression, which is not discernible using immunohistochemistry. Similarly, a 1,000-fold difference in expression can be observed in PR levels. Future studies directly comparing the two techniques will further elucidate if RT-PCR is a more effective and accurate method for determining receptor expression. The best way to test this would be to establish if the receptor expression by RT-PCR is more predictive of benefit to hormonal therapy compared to immunohistochemistry. The quantitative expression of ER and PR as measured by RT-PCR using Oncotype DX is now included on the second page of all Oncotype DX reports.

GTB Another important advantage to RT-PCR is that it is much more reproducible than the standard immunohistochemistry technique.

Do you foresee that a multigene test like the Oncotype DX assay will be able to be used to determine the best chemotherapy regimen for a specific patient?

KM Absolutely. We've come to recognize over the last several years that breast cancer is actually a collection of many different diseases that share in common the fact that they arise in the breast. Across patients there are a number of differences, which can often be traced back to the genetic biology of the tumor, that ultimately lead to variations in response to treatment and patient prognosis.

Current efforts are focused on improving on the Onco*type* DX assay for patient populations other than node-negative, ER-positive. As this assay is applied to other patient groups we will be able to gather information on the predictive ability of the assay for other chemotherapy regimens besides those used in the NSABP B-20 trial (predominantly CMF).

Case Studies

The panel was presented with several cases typically seen in clinical practice and asked how the Onco*type* DX assay might be utilized in each scenario.

Case 1: The patient is over 60 years old and postmenopausal, presenting with a breast tumor measuring 2 centimeters. The patient is node-negative and ER-positive.

GTB Prior to the development of the Onco*type* DX assay, chemotherapy would generally be recommended for this patient. However, the benefit of adding chemotherapy decreases with increasing age, so there would be many patients who would experience no benefit. I would tell this patient that my default recommendation is chemotherapy, but offer her the option of the Onco*type* DX assay. If the patient did decide to get the test, I would make my treatment decision largely based on the resulting recurrence score. If the patient had a low recurrence score I would recommend hormonal therapy alone, while a score indicating high risk would lead me to recommend adjuvant chemotherapy with the hormonal therapy. For an intermediate score I would usually recommend chemotherapy or participation in the PACCT-1 (TAILORx) trial.

KM This is exactly the type of patient for whom the Onco*type* DX assay was designed. This is a patient who, based on the NSABP B-20 trial, would have a small (but not zero) benefit from chemotherapy, meaning there is some benefit. The Onco*type* DX assay would allow those patients who would most benefit from therapy to be identified.

This makes what used to be a difficult decision for all patients a relatively easy decision for the approximately 75% of patients that have either a low or high recurrence score. I think also that the high-risk patients are more willing to accept the toxic effects of the chemotherapy because they know they are truly receiving a large benefit. Only the 25% of patients with an intermediate score still have an uncertain treatment decision.

Case 2: Patient is over 60 years old and postmenopausal, presenting with a breast tumor measuring less than 1 centimeter. The patient is node-negative and ER-positive.

TM Patients with tumors measuring <1 cm are generally considered to have a favorable prognosis. However, a select few individuals do experience a disease recurrence. These patients, therefore, could benefit from obtaining a recurrence score from the Onco*type* DX assay, to identify which ones should receive chemotherapy. By performing the Onco*type* DX assay in patients with small tumors, patients with a low recurrence score have an additional rationale to not receive chemotherapy. Conversely, the assay allows the identification of those patients with small tumors but high recurrence scores, who would benefit from chemotherapy.

GTB I would add that in the absence of the Onco*type* DX assay I would generally not recommend chemotherapy for this patient because only a limited number of patients would benefit. However, it is clear that some patients would, and in order to identify these, the Onco*type* DX assay is very useful.

Case 3: The patient is less than 40 years old, presenting with a breast tumor measuring less than 1 centimeter. The patient is node-negative and ER-positive.

KM I generally do not recommend the Onco*type* DX assay for patients with tumors measuring <1 cm, because these patients usually have little to no increased benefit from receiving chemotherapy. The exception would be for tumors of 8–9 mm in younger patients, where I would consider chemotherapy if the recurrence score were to come back as high.

Case 4: The patient is 45 years old, presenting with a breast tumor measuring more than 2 centimeters. The patient is node-negative and ER-positive.

TM Again, before the availability of the Onco*type* DX assay, I would definitely recommend chemotherapy for this patient. But when an Onco*type* DX assay is used, I am comfortable not recommending chemotherapy for this patient in the presence of a low recurrence score. And I think the Onco*type* DX assay would be useful in these patients up to a tumor size of 4 cm.

KM I will use the Onco*type* DX assay for tumors up to 5 cm, but there are relatively few patients with these large tumors who still remain lymph node–negative.

Case 5: The patient is 50 years old, presenting with a breast tumor measuring 1.5 centimeters. The patient is node-negative and ER-positive.

GTB Without the Onco*type* DX assay, I would recommend chemotherapy for this patient, with the understanding that a number of these patients will experience no benefit from this treatment. For this reason, the Onco*type* DX assay would be very useful in this situation, to eliminate the need for treatment in those patients who would not benefit.

Case 6: The patient is 50 years old, presenting with a breast tumor measuring 3 centimeters. The patient is node-negative and ER-positive.

GTB In patients with larger tumors, I am less inclined to recommend the Onco*type* DX, because tumor size is of prognostic value. For a tumor of 3 cm, I would still recommend the Onco*type* DX, but if the tumor is 4 cm or greater, I would generally go ahead and recommend chemotherapy in the absence of getting a recurrence score.

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