Original Article

Using Multigene Tests to Select Treatment for Early-Stage Breast Cancer

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Abstract

Oncotype DX, PAM50, and MammaPrint are multigene tests that are being used clinically for early-stage breast cancer to predict recurrence risk and guide adjuvant chemotherapy decisions. These tests have been validated in multiple retrospective studies, and prospective clinical trials are in progress. The TAILORx trial uses the Oncotype DX recurrence score to assign estrogen receptor–positive (ER+), node-negative patients to chemotherapy plus hormonal therapy versus hormonal therapy alone. The RxPONDER (SWOG S1007) trial uses Oncotype DX in a similar approach but on node-positive patients, and it includes the PAM50 test as a secondary analysis. The MINDACT trial uses MammaPrint and Adjuvant! Online for treatment arm assignments. MINDACT has very broad eligibility criteria and 2 secondary randomizations for selecting chemotherapy and hormonal therapy regimens. This article discusses how the latest results on cancer genome sequencing apply to early-stage breast cancer. Several hundred breast cancers have already undergone genome sequencing, and the somatic DNA changes found in the tumor, compared with the patient's normal DNA, have been identified. Higher rates of point mutations and chromosomal translocations are found in aromatase inhibitor–resistant ER+ cancers and in the basal-like and HER2-enriched breast cancer subtypes. Correlations of somatic mutations with neoadjuvant aromatase inhibitor response are discussed. Genome sequencing can potentially identify the molecular abnormalities that underlie the poor risk identified by multigene tests and provide potential new targets for therapy, but more clinical trials correlating clinical outcome and somatic DNA changes are needed. (*JNCCN* 2013;11:174–182)

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Describe the multigene tests that are being used clinically for early-stage breast cancer to predict recurrence risk and guide adjuvant chemotherapy decisions.
- Discuss the role of genome sequencing in breast cancer.

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Breast cancer is a heterogeneous disease with a wide range of outcomes that are not fully predicted by routine clinical and pathologic features. Over the past decade, major efforts have been made to develop predictors of recurrence and adjuvant chemotherapy benefit for patients with early-stage breast cancer. The first predictors were based on microarray tests used in research laboratories,[1–3] and multigene tests that can be easily ordered by clinicians were subsequently developed. Prospective clinical trials are underway to determine whether these multigene tests can effectively guide adjuvant chemotherapy decisions for early-stage breast cancer. These prospective trials include TAILORx and RxPONDER (SWOG S1007), which test Oncotype DX in patients with negative nodes (N0) and 1 to 3 positive nodes (N1), respectively, and MINDACT, that tests MammaPrint in both N0 and N1 patients. This article reviews the clinical data on 3 multigene tests: Oncotype DX, PAM50, and MammaPrint. Other multigene or multianalyte tests have been published, such as the Rotterdam 76-gene signature,[4] a 30-gene panel to predict response to neoadjuvant T/FAC chemotherapy,[5] the 3-gene SCMGENE panel,[6] and the IHC4 immunohistochemistry panel,[7] but they are not discussed because approved clinical assays are not currently available for them. Finally, how the latest results on cancer genome sequencing may apply to early-stage breast cancer is discussed.

Oncotype DX

Oncotype DX is a quantitative reverse transcriptase polymerase chain reaction (RT-PCR)–based test that measures 21 genes in formalin-fixed paraffin-embedded breast tumors (Table 1). Patients are classified into 3 categories based on their recurrence score (RS): low risk (RS<18), intermediate risk (RS 18–30), or high risk (RS≥31).[8] Retrospective studies showed that the Oncotype DX score predicted the likelihood of distant recurrence or breast cancer death when treated with hormonal therapy alone.[8,9] Archival samples from the NSABP B-14 trial, which tested the use of tamoxifen in patients with estrogen receptor–positive (ER+), node-negative breast cancer, showed a significant difference in the rate of distant recurrence at 10 years between the low- and high-risk groups (6.8% vs. 30.5%, respectively; $P<.001$).[8] Similarly, a retrospec-

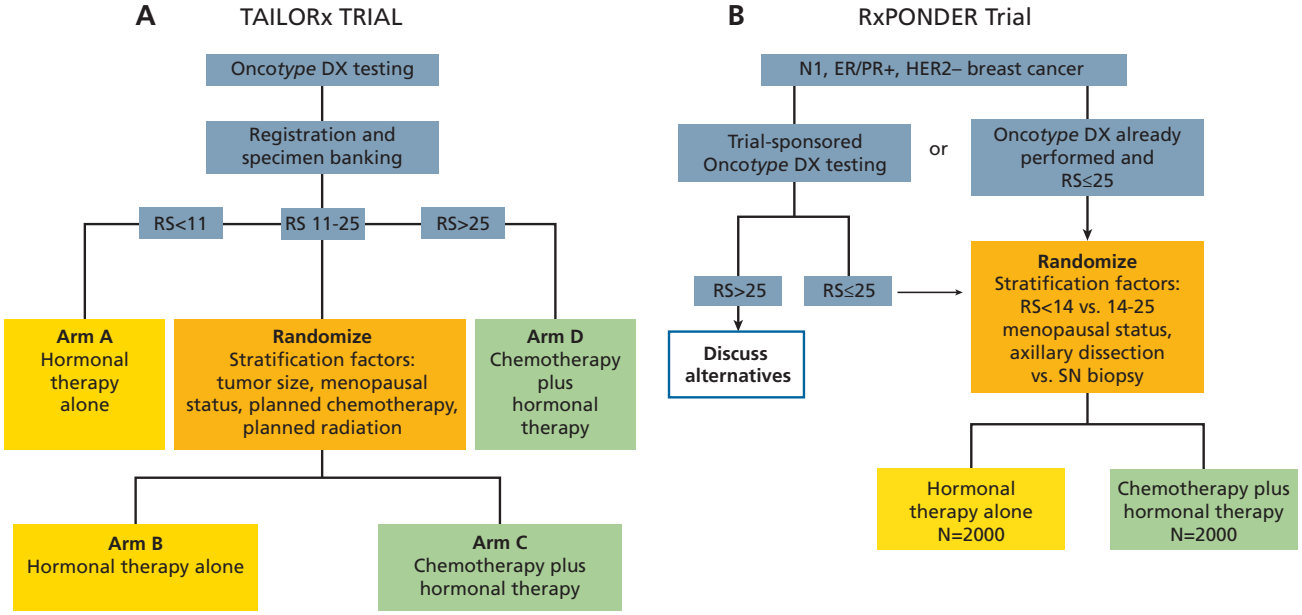

Figure 1 Schema for the (A) TAILORx and (B) RxPONDER clinical trials.
Abbreviations: ER, estrogen receptor; PR, progesterone receptor; RS, recurrence score; SN, sentinel node.
Sources: (Panel A) Adapted from Sparano JA. TAILORx: trial assigning individualized options for treatment (Rx). Clin Breast Cancer 2006;7:347–350, ©2013, with permission from Elsevier. (Panel B) Data from Gonzalez-Angulo AM, Barlow WE, Gralow JR, et al. A randomized phase III clinical trial of standard adjuvant endocrine therapy +/- chemotherapy in patients with 1-3 positive nodes, hormone receptor–positive and HER2-negative breast cancer with recurrence score (RS) of 25 or less: SWOG S1007 [abstract]. Presented at the 2011 San Antonio Breast Cancer Symposium; December 6–10, 2011; San Antonio, Texas. Abstract OT1-03-01. Figure courtesy of Ana M. Gonzalez-Angulo, MD, Houston, Texas.

tive case-control study conducted by Kaiser Permanente in the community hospital setting showed that the Oncotype risk categories were strongly associated with the 10-year rates of breast cancer death. In patients treated with tamoxifen, the death rate was 2.8% in those with low risk, 10.7% in those with intermediate risk, and 15.5% in those with high risk (*P*=.003).[9]

The ability of Oncotype DX to predict benefit from adjuvant chemotherapy was determined by retrospective analysis of the NSABP B-20 and SWOG-8814 trials.[10,11] NSABP B-20 randomized ER+, node-negative patients to either tamoxifen or CMF/MF chemotherapy (methotrexate, fluorouracil, +/–cyclophosphamide) plus tamoxifen. High-risk patients (RS≥31) experienced a significant benefit from chemotherapy (hazard ratio [HR], 0.26; 95% CI, 0.13–0.53), whereas low-risk patients had minimal or no benefit from adjuvant chemotherapy.[10] The SWOG-8814 trial extended this result to node-positive patients.[11] This trial randomized postmenopausal, ER+, node-positive patients to tamoxifen or CAF chemotherapy (cyclophosphamide, doxorubicin, fluorouracil) followed by tamoxifen. Patients with a high RS had improved disease-free survival (HR, 0.59; log rank *P*=.033) with the addition of CAF, but patients with a low RS did not benefit from adjuvant chemotherapy (HR, 1.02; log rank *P*=.97). In the low-risk patients, this suggests that Oncotype DX may identify women who will not benefit from chemotherapy despite the presence of positive lymph nodes.[11] Based on these findings, Oncotype DX has changed patient management in 30% to 40% of cases and has decreased adjuvant chemotherapy use in women with early-stage breast cancer.[12–14] Oncotype DX testing is incorporated into current NCCN and ASCO guidelines for breast cancer care (Table 1).

Prospective Clinical Trials of Oncotype DX: TAILORx and RxPONDER

The TAILORx trial has enrolled more than 11,000 patients with ER+, HER2–, node-negative breast cancer (Figure 1A). Patients with an RS less than 11 are assigned to hormonal therapy only (arm A), patients with an RS greater than 25 receive adjuvant chemotherapy plus hormonal therapy (arm D), and patients with an

Table 1 Comparison of Oncotype DX, PAM50, and MammaPrint Multigene Tests

	Oncotype DX	PAM50	MammaPrint
Number of genes	21	50 (+5 control genes)	70
Sample requirements	Formalin-fixed, paraffin-embedded tissue	Formalin-fixed, paraffin-embedded tissue	Fresh-frozen tissue
Technique	Quantitative PCR	Quantitative PCR and nCounter technology	DNA microarray
Study population used to develop the test	Patients with ER+, node-negative, breast cancer	Patients with stage I–III breast cancer	Women <61 years, with T1–T2,N0 disease
Features	Recurrence score predicts likelihood of recurrence at 10 years Identify low-risk patients who can be spared from adjuvant chemotherapy	Provides the intrinsic subtype classification Predicts distant relapse-free survival and likelihood of recurrence at 10 years in the setting of ER+ breast cancer treated with tamoxifen Identifies patients who would benefit from neoadjuvant endocrine therapy or chemotherapy	Stratifies patients into good or poor prognosis signatures
Guidelines	NCCN and ASCO CLIA assay, no formal regulatory approval	FDA and European approvals pending	FDA approved
Prospective clinical trials	TAILORx and RxPONDER	RxPONDER trial will compare PAM50 and Oncotype DX scores	MINDACT

Abbreviations: CLIA, Clinical Laboratory Improvement Amendments; ER, estrogen receptor; PCR, polymerase chain reaction.

RS of 11 through 25 are randomized to hormonal therapy only (arm B) versus chemotherapy plus hormonal therapy (arm C).[15] The choice of regimens for both hormonal treatment and chemotherapy are at the discretion of the treating physician but must be within one of several standard options described in the trial. The RS scores used as cutoffs between these groups are different from those reported in the NSABP studies[8,10] and this was done to minimize the potential for undertreating high-risk patients.[15] NSABP B-20 data was reanalyzed when designing the TAILORx trial, and this statistical analysis supported the use of an RS of 11 through 25 as the group for treatment randomization.[15] Use of these RS cutoffs would place 27% of the NSABP B-20 patients in arm A (RS<11), 30% in arm D (RS>25), and 43% in the group undergoing randomization (RS 11–25).[15] Trial enrollment completed in 2010 and trial results are not yet available.

In January 2011, the RxPONDER trial (SWOG S1007) was launched. The study population is patients with ER+, HER2– breast cancer with 1 to 3 positive nodes (N1). The trial plans to screen 9400 women through Oncotype DX testing and enroll 4000 women with an RS of 25 or less. Patients may enroll either by consenting to study-sponsored Oncotype DX testing or by having an RS of 25 or less already obtained through recent testing (Figure 1B). Patients are randomized 1:1 to either hormonal therapy or chemotherapy plus hormonal therapy, with 2000 patients per arm planned. The primary objective is to determine the effect of chemotherapy on patients with node-positive breast cancer who have an RS of 25 or less. Secondary objectives include comparison of Oncotype DX and PAM50 risk of relapse (ROR) scores (see next section) and measurement of quality-of-life effects. The design of this trial was strongly based on the SWOG-8814 trial of node-positive patients, and the cutoff of RS of 25 or less was chosen to match the TAILORx trial. Similar to TAILORx, the choice of regimens for both hormonal treatment and chemotherapy are at the discretion of the treating physician but must be within one of several standard options described in the trial.[16]

PAM50 ROR Score

The PAM50 test is based on biologic subtypes of breast cancers, termed the *intrinsic subtypes*, that were identified by Perou and colleagues.[1,2] Most of the studies on PAM50 use quantitative RT-PCR, but this test has been implemented on the nanotechnology-based nCounter digital gene expression platform,[17] which allows it to be performed in any pathology laboratory. PAM50 measures expression of 50 classifier genes and 5 control genes, categorizes tumors into the 4 intrinsic subtypes (luminal A, luminal B, HER2-enriched, and basal-like), and provides an ROR score to estimate the probability of relapse at 5 years.[18] PAM50 provides information that is independent of standard criteria (stage, grade, HER2, ER, and Adjuvant! Online), and in multivariate models of tamoxifen-treated, early-stage, ER+ breast cancer, only the PAM50 and stage remain significant.[19] The PAM50 was predictive of the benefit of tamoxifen in premenopausal women in the NCIC MA12 trial, whereas ER status alone had limited value.[20] The PAM50 has now been fully transferred to the nCounter system and comparisons to Oncotype DX are being reported. Dowsett et al[21] recently presented a TransATAC analysis that demonstrated that the nCounter PAM50 ROR score provided more prognostic information about 10-year distant recurrence than Oncotype DX, and fewer patients were assigned to the intermediate-risk category. These findings are mirrored by a recent publication from Kelly et al,[22] who used the RT-PCR version of PAM50 on a panel of 304 sequential treated patients and found that although good agreement was seen between PAM50 ROR and Oncotype DX, more patients were assigned to the low-risk category by PAM50.[22] In this study, approximately half of the patients in the intermediate-risk Oncotype DX group were classified into the low-risk PAM50 luminal A category. Prat et al[23] compared 6 different multigene tests in a breast cancer panel assembled from several publically available microarray datasets. They found that most of these multigene tests successfully predicted outcome for ER+ breast cancer, and suggested that combining several multigene tests may offer more accurate outcome prediction.[23] However, this conclusion was not based on validated clinical tests but was a research bioinformatics study in which multiple signatures were derived from a single microarray database. Prospective comparisons of the clinical nCounter version of the PAM50 ROR and Oncotype DX are needed, and this is being performed in the RxPONDER trial.[16]

MammaPrint and the MINDACT Prospective Clinical Trial

Using DNA microarrays, a 70-gene prognostic signature, MammaPrint, for node-negative breast cancer was developed in 2002.[3] This signature assigned patients to either being low- or high-risk for distant metastases at 5 years. The MammaPrint test has been validated in retrospective studies. Researchers at the Netherlands Cancer Institute tested it in both node-negative and node-positive patients, and the TRANSBIG consortium tested it in 307 node-negative patients.[24,25] Both of these studies showed that MammaPrint outperformed standard clinical and histologic predictors of patient prognosis. MammaPrint was approved by the FDA in 2007 for node-negative patients (Table 1).[26] Because MammaPrint is performed using a DNA microarray, it requires frozen breast cancer samples, which is difficult to obtain in many US cancer centers.

The MINDACT trial (Microarray In Node-negative and 1–3 Node-Positive Disease May Avoid ChemoTherapy) is a prospective randomized phase III clinical trial comparing the MammaPrint test with commonly used clinical criteria, as calculated in a modified version of Adjuvant! Online, for decision-making about adjuvant chemotherapy. The trial initially enrolled only node-negative patients but was amended in 2008 to include patients with 1 to 3 positive lymph nodes (N1 disease).[26,27] MINDACT had a predefined pilot phase in which the data and treatment decisions of the first 800 patients were analyzed, and those results were published in 2011.[26] MINDACT eligibility criteria include both ER+ and ER–, HER2+ and HER2–, and premenopausal and postmenopausal patients.[26] The patient's clinical risk from breast cancer is calculated using Adjuvant! Online, version 8.0, with the addition of HER2 status.[26] Patients with a 10-year breast cancer–specific survival rate of greater than 92% for ER– disease or greater than 88% for ER+ disease are defined as having low clinical risk (C-low; Figure 2). Genomic risk is calculated based on the MammaPrint result, and patients are determined to be high risk or low risk (G-high or G-low, respectively). Patients who are C-high and G-high (Figure 2) are assigned to receive adjuvant chemotherapy, whereas those who are C-low and G-low do not receive chemotherapy.[26] Patients with discordant clinical and genomic risk assessment results are randomized 1:1 to receive or not receive adjuvant chemotherapy. Analysis of the first 800 patients showed that approximately 25% of patients were C-high and G-high, 48% were C-low and G-low, and 27% had discordant results between the 2 risk calculation tests. Compliance with the assignments from randomization was very good, with greater than 92% of the discordant patients following the randomization assignments.[26]

The MINDACT trial has a complex design, with secondary randomizations for choice of chemotherapy and hormonal therapy regimens.[26,27] Patients assigned to chemotherapy are further randomized to receive either an anthracycline-containing regimen or docetaxel/capecitabine. Of the first 800 patients, approximately 50% of those assigned to chemotherapy underwent this randomization.[26] All ER+ patients are offered a nonmandatory randomization for hormonal therapy, with the 2 arms being: 1) a switching strategy of tamoxifen for 2 years, then letrozole for 5 years, versus 2) letrozole for 7 years.[26] Premenopausal patients are required to have ovarian ablation or suppression for the entire duration of hormonal therapy. Of the first 800 patients, approximately 84% are ER+ and 16% are ER–; 67% are 50 years of age or older and 33% are younger than 50 years; and 86% are HER2–, 11% are HER2+, and 2.9% are HER2 status unknown.[26] Other prospective clinical trials using MammaPrint are the neoadjuvant trials I-SPY1 and I-SPY2.[28,29]

Future Developments: Cancer Genome Sequencing

A revolution in DNA sequencing technology began in 2005 with the development of massively parallel, next-generation DNA sequencing methods, which has enabled genome sequencing studies on hundreds of individual human cancers.[30] Next-generation DNA sequencing has an output that is several orders of magnitude greater and many-fold cheaper than the traditional DNA sequencing method first developed by Nobel laureate Frederick Sanger.[30] Using next-generation DNA sequencing, the first individual breast cancer genomes were sequenced in 2009–2010 by researchers at Washington University School of Medicine in St. Louis, Missouri, and the Michael Smith Genome Sciences Centre in Vancouver, Canada.[31,32] Both studies sequenced the patient's normal DNA, the primary tumor DNA, and the DNA of the metastasis. Through comparing the DNA sequences of the primary tumor and the patient's normal DNA, the somatic DNA changes present in the cancer were identified. Comparison

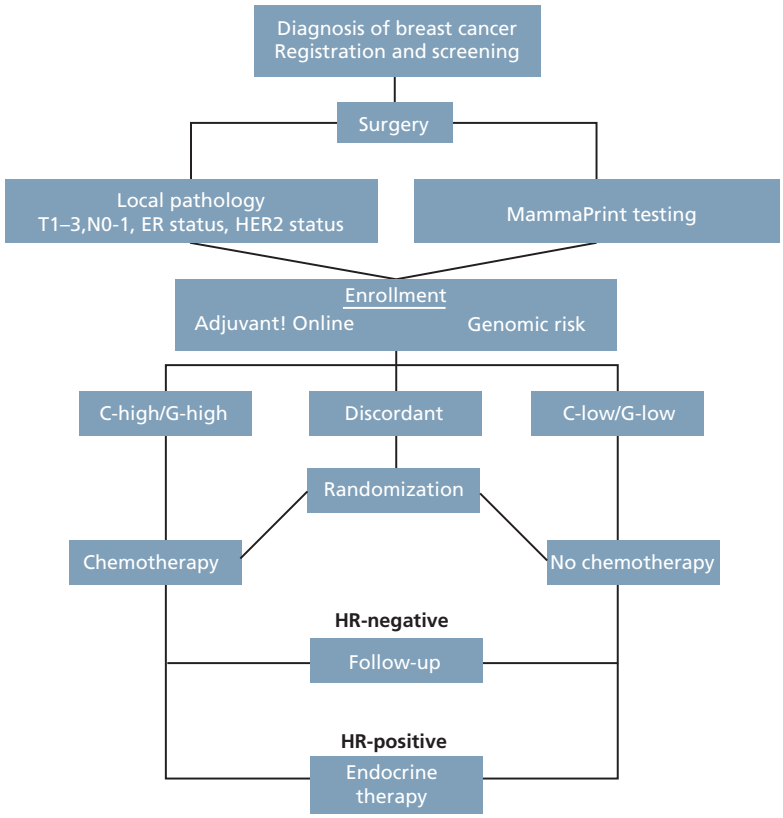

Figure 2 Schema for the MINDACT clinical trial.
Abbreviations: C-low/high, low/high clinical risk; ER, estrogen receptor; G-low/high, low/high genomic risk; HR, hormone receptor.
Source: Adapted from Rutgers E, Piccart-Gebhart MJ, Bogaerts J, et al. The EORTC 10041/BIG 03-04 MINDACT trial is feasible: results of the pilot phase. Eur J Cancer 2011;47:2742–2749, ©2013, with permission from Elsevier.

of the DNA of the primary tumor and the metastasis showed that although they share many of these somatic DNA changes, significant evolution in the cancer can occur during metastatic spread.

Four publications appearing in June 2012 collectively sequenced approximately 350 breast cancer cases,[33–36] and in October 2012, a publication by The Cancer Genome Atlas (TCGA) project provided results on over 500 breast cancer cases.[37] These studies have predominantly sequenced patients with stage I–III disease. This wealth of information is starting to create a picture of the types of alterations present in the different subtypes of breast cancer. For example, in luminal subtype breast cancers, a statistically significant difference (P=.02) is seen in the number of point mutations between cancers that are aromatase inhibitor (AI)–sensitive and AI-resistant.[33] Figure 3A shows a genome wheel in which the 23 chromosomes are represented by the multicolored outer ring. Point mutations are indicated by lettering pointing outward from the wheel; DNA copy number changes are indicated by the black line in the grey inner ring; and chromosomal translocations are indicated by the arcs or lines on the inside of the rings. Translocations can either be interchromosomal events (between 2 chromosomes, as indicated by the green lines) or intrachromosomal events (within 1 chromosome, as indicated by the blue lines in Figure 3A). The genomes of an AI-sensitive cancer and an AI-resistant cancer are shown in Figure 3A, and the striking differences in numbers of point mutations and chromosomal translocations can be readily seen.[33] Similarly, comparison of the genomes of basal-like, HER2-enriched, luminal A, and luminal B breast cancers, as defined by PAM50, shows that the number of translocations is much higher in basal-like and HER2-enriched cases (Figure 3B).[35] Weigman et al[38] recently reported similar findings after examining DNA copy number changes (amplifications and deletions) across the breast cancer subtypes. They found that basal-like cancers had the highest number of DNA copy number changes, and luminal A had the lowest.

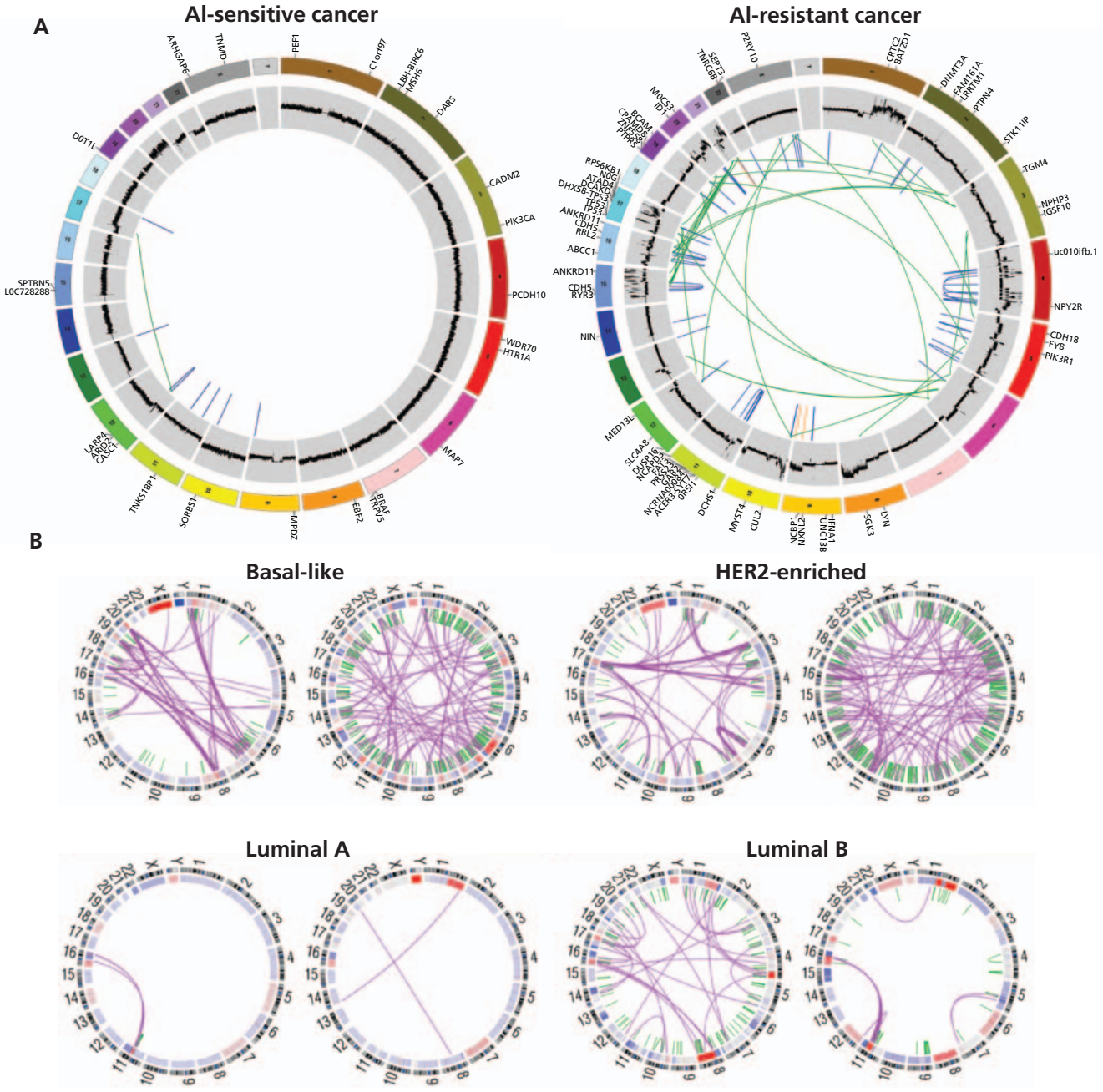

Figure 3 Breast cancer genome sequencing results. (A) The genome wheels show point mutations, copy number changes, and chromosomal translocations in aromatase inhibitor (AI)–sensitive and AI-resistant breast cancer cases. (B) Genome wheels showing chromosomal translocations in the 4 intrinsic subtypes of breast cancer. Interchromosomal and intrachromosomal translocations are indicated by purple and green arcs/lines, respectively.
Sources: (A) From Ellis MJ, Ding L, Shen D, et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. Nature 2012;486:353–360. (B) Data from Banerji S, Cibulskis K, Rangel-Escareno C, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature 2012;486:405–409.

Correlation of somatic DNA changes with clinical outcome is just beginning. Breast cancer samples from the neoadjuvant AI trial, ACOSOG Z1031, were sequenced and tumors with mutations in the p53 tumor suppressor gene (official gene symbol *TP53*) were found to have higher preoperative endocrine prognostic index scores and higher pretreatment and posttreatment proliferation indexes, as measured with immunostaining

for Ki-67.[33] In contrast, mutations in the transcription factor, GATA3, were associated with response to AI therapy because these mutations were associated with low post-AI treatment Ki-67, but not baseline Ki-67 staining. The MAP kinase gene, MAP3K1, was associated with both low pre– and post–AI treatment Ki-67 level (the opposite of p53), and therefore is correlated with good-prognosis breast cancer.[33] Several more years of research are required before genome sequencing can be applied in the clinic, but this is an area of great promise. Genome sequencing may ultimately identify the molecular abnormalities that underlie the poor risk identified by multigene tests and provide potential new targets for therapy that may improve the outcome for these patients. The authors encourage physicians to watch for clinically applicable results from future cancer genomics studies.

Conclusions

The use of Onco*type* DX and MammaPrint testing for early-stage breast cancer has improved adjuvant chemotherapy decision-making. The results of the ongoing prospective trials are eagerly awaited and will show whether these tests should be the standard of care for early-stage breast cancer. The PAM50 test, performed on the nanotechnology-based nCounter system, has the potential to be the first gene expression test that can be performed in any pathology laboratory, and FDA approval is currently pending. Dr. George Sledge, in his 2011 ASCO Presidential Address, said that "we are now, I would suggest, just beginning to enter…the era of genome-based therapy."[39] Genome sequencing is highly likely to play a growing role in the future, but more clinical trials correlating clinical outcome and somatic DNA changes are needed.

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Instructions for Completion

To participate in this journal CE activity: 1) review the learning objectives and author disclosures; 2) study the education content; 3) take the posttest with a 70% minimum passing score and complete the evaluation at **http://education.nccn.org/node/11346;** and 4) view/print certificate. After reading the article, you should be able to answer the following multiple-choice questions. Credit cannot be obtained for tests completed on paper. You must be a registered user on NCCN.org. If you are not registered on NCCN.org, click on "New Member? Sign up here" link on the left hand side of the Web site to register. Only one answer is correct for each question. Once you successfully answer all posttest questions you will be able to view and/or print your certificate. Software requirements: Internet.

Posttest Questions

1. True or False: The MINDACT trial (Microarray In Node-negative and 1–3 Node-Positive Disease May Avoid Chemo-Therapy) is a prospective randomized phase III clinical trial comparing the MammaPrint test with commonly used clinical criteria, as calculated in a modified version of Adjuvant! Online, for decision-making about adjuvant chemotherapy.
2. True or False: The PAM50 was not predictive of the benefit of tamoxifen in premenopausal women in the NCIC MA12 trial.
3. True or False: Oncotype DX testing is incorporated into current NCCN and ASCO guidelines for breast cancer care.