Review Article

Utility of Genomic Platforms in Treatment Decisions in Axilla-Positive Breast Cancer

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ABSTRACT

Genomic platforms have proven to be more accurate as a prognostic tool than immunohistochemistry studies in patients with early, hormone receptor positive, HER 2 negative breast cancer and, in some cases, have also demonstrated predictive ability for chemotherapy benefit. They are now widely applied in node-negative disease, but their use in node-positive disease is more recent and more controversial, especially in premenopausal patients. In this article, we review the use of these tests in node-positive disease.

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INTRODUCTION

Human epidermal growth receptor 2 (HER2)-negative and estrogen receptor (ER) positive is the most common type of early breast cancer (BC) in postmenopausal women.

Although adjuvant chemotherapy (CT) decreases the risk of recurrence and improves survival, the absolute benefits in patients at low risk of recurrence are small. Therefore, in the decision to offer chemotherapy we must take into account several factors, related to the patient (age, menopausal status and comorbidity) and factors related to the disease (tumor size, grade, multifocality, histological subtype, lymphovascular infiltration (LVI), axillary involvement or ki67), although axillary involvement continues to be the most important clinical prognostic factor for indicating CT.

Adjuvant CT has demonstrated a relative risk reduction of BC mortality of approximately 20% to 30%, regardless of size, stage, grade, ER status, and whether patients received endocrine therapy, according to Early Breast Cancer Trialists' Collaborative Group (EBCTCG) metanalyses.[1]

This is an approximate absolute benefit of 7%, across all patients. However, the risk of recurrence in patients with axillary involvement (N+) is almost twice as high as in patients with negative lymph nodes (N0), in the absence of chemotherapy, so, benefit of chemotherapy is greater in this subgroup and its use is widely accepted. We now know that the therapeutic benefit may be different depending on the intrinsic subtypes and lymph node positivity may not confer the same degree of risk as before. Furthermore, in trials in early BC with negative node, it has been shown that chemotherapy offers no benefit in tumors with low recurrence score (RS), so the absolute benefit of chemotherapy may be even less than previously thought. On the other hand, data from the neoadjuvant setting suggest that chemotherapy may not provide benefit in many patients with ER-positive, HER2-negative disease.[2]

In this regard, it is useful to identify a subgroup of axilla-positive patients whose prognosis is so good that, even if chemotherapy reduces the relative risk by 20% to 30%, the absolute benefit would not exceed 1% to 3%, and this is approximately the risk of severe or life-threatening toxicity of chemotherapy, so it is reasonable to avoid chemotherapy.

DISCUSSION

Several genomic platforms are now available to estimate the risk of relapse and, based on this, to decide which patients are candidates for adjuvant chemotherapy.

The emergence of genomics (assessing DNA) and transcriptomics (assessing RNA) techniques and the ability to simultaneously measure the expression of thousands of genes has led to the identification of biology-based prognostic profiles, several of which have been validated and are in clinical use.

We will review in detail the main platforms and the studies that validate their prognostic role and, if applicable, predictive of treatment benefit.

Table 1 shows the main genomic platforms available and the differences between them. Oncotype DX 21-gene Recurrence Score (RS)

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Table 1 Main Genomic Platforms Available and the Differences Between Them.

Prognostic assay	ONCOTYPE	MAMMAPRINT	PAM50	Endopredict	BCI	GGI	IHC4
Genes	21 genes (16+5 control)	70 genes	50 genes	12 genes (8 +4 control)	7 genes	97 genes	IHQ markers
FFPA Underlying technology	Yes qRT-PCR	Yes DNA microarrays NGS	Yes DNA microarrays and qRT-PCR using nCounter technolog	Yes qRT-PCR	Yes qRT-PCR	Yes DNA microarrays	Yes IHQ
inclusion of Clinical Parameters	No	No	Yes	yes	No	No	No (IHQ markers)
Prognostic value	Yes, RR 10 years	Yes, RR 10 years	Yes, RR 10 years Late relapse	Yes, RR 10 years	Yes Late relapse	yes	yes
Predictive of chemotherapy benefict [62]	In N+ postmenopausal	no	no	no	Yes, benefit extended HT No prospective	no	no
Benefict extended HT predictive vaue	no	no	no	no	yes	no	no
score	RS scores (1-100) stratified low (<26) and high-risk.	low risk vs. high risk	ROR scores continuous (1-100) and stratified into low, intermediate and high risk. Intrinsic subtype.	Low or high risk on the basis of EP and EPClin scores.	Risk score and low risk vs. high risk category	low risk vs. high risk	Risk score and low risk vs. high risk category
Retrospective studies N+	transATAC[5], SWOG8814[6], PlanB[61], WSG-ADAPT[60], NSABPB-28[8], PACS01[9], E2197[11], SEER[11-15]	Netherlands Cancer Institute Cohort[19]	transATAC,[33] ABCSG 8[33,34], DBCG[37]	GEICAM 9906[44], ABCSG 6-ABCSG 8[42,45]	STOCKHOLM[48] TRANSATAC[49] ATTOM[50] IDEAL[51]	PACS01[54]	TransATAC[56] TEAM[55]
Prospective studies N+	RxPONDER/SWOG1007[4] (phase III)	MINDAC[20,21] (phase III) PROMIS[23]	OPTIMA[38]	UNIRAD RESCUE			
Category evidence [62]	1 2A (premenopausal)	1	2A	2A	2A	2B	2B

Abbreviations: IHQ = immunohistochemistry; NGS = next generation sequencing.

The Oncotype DX test is a genomic assay that generates a recurrence score (RS) that predicts 10 year recurrence risk. It comprises 16 cancer-related genes, selected on the basis of their statistical association with tumor proliferation, invasion and distant breast recurrence, and five 'housekeeping' reference control genes.[3] An algorithm generates a RS between 0 and 100, where higher scores indicate a higher likelihood of distant recurrence, although it is a continuous score, different cut-off points have been evaluated to classify patients at high or low risk of relapse and decide the role of chemotherapy administration based on this.

This platform has been validated as a prognostic and predictive tool in early axilla-negative BC,[4] but its role in N+ has been more debated. The first studies analyzed the role of RS retrospectively in patients included in phase III studies, In addition, 3 risk levels were established according to RS <18, 18-30 and ≥31, but we now have prospective data and a recently published phase III study.[4]

In the TransATAC study[5] involving N0 to N2 patients, the risk of distant relapse ratio recurrence (DRR) at 9 years was observed to be 17, 28 and 49% for patients with RS <18, 18-30 and ≥31, respectively, regardless of whether they were N0 or N1, although patients with ≥4 positive nodes had a higher risk of distant recurrence for any RS outcome.

Similarly, the SWOG S8814 study[6] (all N+), showed the RS prognostic capacity in 148 patients treated with tamoxifen (64% N1 and 36% with 4 or more positive nodes). Ten-years disease-free survival (DFS) adjusted by the number of positive nodes was 60% in RS<18, 49% in RS 18-30 and 43% if RS was 31 or more ($P = .017$). To assess RS predictive value, they compared the results between patients in the tamoxifen-only arm and those who received chemotherapy and tamoxifen, benefit of chemotherapy was shown only for patients with RS ≥ 31. The main limitation of the study is that it is a retrospective analysis, it had a small number

of patients and does not reflect current chemotherapy use and schedules.[7]

The prognostic ability of RS in N+ patients was too analyzed in other studies such NSABP-B28[8] or PACS 01 study[9] and the outcome of RS was again strongly associated with DRR, disease free survival (DFS) or overall survival (OS) in multivariate analysis. Furthermore, in the ECOG E2197[10] study RS outperformed a modified version of the Adjuvant! Online tool, which uses traditional clinicopathologic risk factors, in predicting 5 year recurrence risk.

All of these studies in addition to the population-based analysis of RS in N+ patients included in the Surveillance, Epidemiology and End Results (SEER) database[11–15]showed that RS is a useful prognostic tool and superior to classical clinicopathological factors.

The oncotype was validated prospectively in N1 patients by RxPONDER /SWOG1007,[4] a prospective, randomised, phase III study involving 5018 patients with early, HR-positive, HER2-negative, HER2-negative, 1-3 node involvement BC with an RS score of 0-25. Patients in this study were randomised to adjuvant hormonal treatment alone or CT followed by hormonal therapy, and stratified according to RS score, menopausal status and type of axillary surgery. The primary study objective was to determine the effect of CT on invasive disease free survival (IDFS) and whether the effect was influenced by RS.

When analyzing the results we should take into account the risk profile of the patients included in the study, since most of them coincided with a low-moderate risk group according to clinical criteria, since only 10% had grade 3 disease, 9% had 3 positive nodes and 66.8% were postmenopausal. No benefit for chemotherapy was found in the analysis of the whole population and no benefit for chemotherapy was found in the analysis of the postmenopausal group. However, in the pre-planned subgroup of premenopausal patients (n = 1665), the study found benefit in 5 year IDFS of 4.9% in favour of chemotherapy (HR 0.60, 95 % CI 0.43-0.83, $P = .002$) and this benefit was independent of RS score 0-13 or 14-25. In addition, although the number of events was small, an early OS benefit was found, as at 5 years, OS was 98.6% for those receiving CT and HT and 97.3% for those receiving HT alone (HR 0.47; 95% CI 0.24-0.94; $P = .032$), an absolute benefit of 1.3%

Regarding the use of ovarian ablation (OA) in premenopausal patients, it was more frequent among patients receiving HT alone (15.9 vs. 3.7%). One question is whether the benefit of CT in these patients may be partly attributed to the role of chemotherapy-induced menopause, as in an exploratory analysis of the TAYLORx study, some benefit of chemotherapy had already been observed in patients younger than 50 years and with a RS score between 16 and 25[16,17].

With these results we can assume that the recommendation in postmenopausal patients, N1, is the administration of CT when the RS is greater than 25 and hormonal treatment for the rest of the patients with RS ≤25, but in premenopausal patients these results oblige us to be more cautious and to take into account the profile of the patient and the tumour given that the benefit of CT cannot be excluded on the basis of this trial.[4]

MammaPrint (profile of 70 Amsterdam genes)

MammaPrint analyzes 70 genes related to breast cancer by microarray analysis on fresh tumor tissue, but currently the technique has already been implemented for paraffin-embedded tumor samples.[18] A subsequent algorithm determines the prognostic classification of patients at high or low risk of relapse and development of metastases at 10 years in the absence of adjuvant treatment.

The initial validation study in N+ was performed on tumor samples from 241 women with T1-T3 N1 breast cancer, they found that 10 year distant metastasis-free survival (DRFS) was 91% and 76% for low-risk group and high-risk respectively and BC-specific survival were 91% and 76% in the low and high-risk group . Moreover, in multivariate analysis, Mammaprint was significantly superior to traditional clinical prognostic factors (HR 7.17 CI 95% 1.81-28.43, $P = .005$).[19]

The MINDACT[20,21] is a randomized, phase III trial, what prospectively evaluates MammaPrint in patients with early BC, it included 6693 patients with stage I and II, the initial protocol only included N0 patients, but a later amendment allowed the inclusion of N1 patients.

Patients were classified as high and low genomic risk according to the Mammaprint result and high and low clinical risk according to clinical relapse risk defined by Adjuvant!Online. Patients classified as low risk according to both methods did not receive CT (n = 2745 p), and high-risk patients according to both methods received CT (n = 1806). Patients with discordant results (high clinical risk and/or low genomic risk, n = 1550) were randomized to treatment with HT alone versus CT plus HT. The primary endpoint was 5 years rate of survival without distant metastasis (DDFS) in patients with discordant results and no CT.

Among the randomized patients, 47.6% were N1, 93% were grade 2-3 and 4% were less than or equal to 50 years of age; in addition, a small number of patients with negative RH (11.6%) and her 2 positive (9.5%) were included which complicates the analysis of the study, but it provides for some interesting exploratory analysis in patients with tumors HR negative or her 2 positive. The 5 year DDFS results among patients with high clinical and/or low genomic risk are 94.7% without CT, and 95.9% if treated with CT (HR 0.78, $P = .267$). These results would imply no need for chemotherapy in these patients. With almost 9 years of follow-up, this difference was maintained.[21]

A secondary analysis performed among patients with high clinical/low genomic risk according to whether or not they receive CT treatment shows a small benefit of 1.5% in 5 year distant metastatic disease-free survival at 5 years if they receive chemotherapy.

An exploratory analysis in the HER2-negative, ER+ group of patients (1358 (90.7%)] of 1497 patients, of whom 676 received chemotherapy and 682 did not) shows different effects of chemotherapy on 8 year DDFS according to age: 93.6% with CT versus 88.6% without CT in women ≤50 years (n = 464) (absolute difference 5.0 percentage points) and 90,2% versus 90.0% in 894 women older than 50 years (absolute difference 0.2 points).[22]

Table 2 Main Differences Between PHASE III Studies That Have Included Positive Nodes.

design	RxPONDER [4] Phase III	MINDACT[20,21] Phase III
main criteria for inclusion	N1,RH+,her 2- RS 0-25	N0, N1, RH+/HER 2-, TN /HER 2+ RH- (2%), HER 2+ RH+ (7%) and Clinical-genomic discordant result
N Positive nodes	5018 patients 100%	6693 patients high clinical risk and low genomic risk (HCR/LGR):1550p (23,2%) 48% [a](658/1550)
Premenopausal Age <50	33%	NR n = 534p (43,5%)[a]
G 3	10%	29%[a]
Main objective	DFSi	DMFS in patients with high clinical risk/low genomic risk
Results	DFSi (5 years): Postmenopausal:91.6 % (CT+HT) vs. 91,9% (HT); NS Premenopausal: 93,9% (CT+HT) vs. 89,0% (HT); *P* = .0004.	ITT: DMFS 8 years: 92%(CT) vs. 89,4% (no CT) (hazard ratio 0·66; 95% CI 0·48–0·92) N1: DMFS 8 years: (91,2% (CT) vs. 89,9% (no CT); absolute difference 1.3 points 50ś or younger: 93,6 % (CT) vs. 88,6% (no CT), absolute difference 5%

Abbreviations: CT = chemotherapy; DFSi = invasive disease free survival; DMFS = distant metastasis-free survival; HT = hormonotherapy; HR = hormonal receptor; ITT = intention to treat population; NS = no significant; NR = no reported; TN = triple negative.
[a] patients with high clinical risk and low genomic risk.

In N+ patients the exploratory analysis showed that 5 year DDFS was 96.3% in chemotherapy group versus 95.6% in the non-chemotherapy group. At 8 years the difference for patients with CT versus without CT were similar (absolute difference 1.3 percentage points in favor of CT).[21,22]

Moreover 63% of patients with 1 N+ and 55.8% with 2-3 N+ and high clinical risk, were however of low genomic risk, suggesting that genomic risk is not influenced by tumor burden.[20]

In conclusion, it should be noted that with a follow-up of almost 9 years of the MINDACT study, Mammaprint is able to identify among those patients with high clinical risk, a subgroup of patients with low genomic risk, with excellent DDFS when treated with HT alone. For these women, the magnitude of the benefit of adding CT is small (2.6 percentage points) and does not increase with nodal positivity. The benefit of CT appears to be age-dependent, as it is mostly observed in women younger than 50 years where a clinically relevant threshold of 5 percentage points is reached. Similar results in terms of difficulty in validating these tests in N+ and premenopausal patients were found with oncotype, suggesting the possibility that the chemotherapy effect may be partially or potentially wholly due to its effect on cessation of menses rather than on a direct cytotoxic effect on the tumor.

PROMIS study[23] prospectively evaluated MammaPrint in 840 patients with an RS (oncotypeDx) of 18-30. The aim of the study was to assess the change in therapeutic decision after the MammaPrint test result. Initially only N0 were included but later an amendment was made to include N1. Patients were reclassified from intermediate risk to low risk in 374 cases (44.5%) and to high risk in 466 (55.5%), leading to a change in treatment decision in 33.6% of patients, regardless of nodal involvement.

Table 2 shows the main characteristics of the 2 randomized phase III studies that included the N+ population (RxPONDER and MINDACT).

BluePrint[24] is a genomic test that evaluates the expression of 80 RNA genes from breast cancer cells, identifies the biology driving tumor growth and provides more accurate molecular subtyping. It is composed of 3 gene signatures each of which measures the similarity of a tumor to a Luminal type (58 genes), basal type (28 genes) and HER2 type (4 genes). Used in combination with MammaPrint and Ki67 it subclassifies the luminal type into subtype A (Ki 67 <14w or MammaPrint low risk) and luminal B (ki 67 14 or MammaPrint high risk), the reproducibility and accuracy of BluePrint is 99% and 98.3%, below the discrepancies found in immunohistochemistry and FISH studies.[25]

Haan et al. show correlation of the 70 genes analyzed by MammaPrint with ten cancer footprints, finding the same correlation in all genes analyzed by BluePrint in the luminal and basal types, while for HER2 type they only found a relationship with 9 of 10 genes (perhaps limited by small number of genes).[26,27]

BluePrint showed the ability to predict sensitivity to neoadjuvant treatment, in an analysis of 426 patients from the Neoadjuvant Breast Registry Symphony Trial (NBRST), with 55% N1, BluePrint reassigned 94 patients to different molecular categories than those determined by immunohistochemistry (IHQ) or FISH. Those patients classified as luminal subtype had low pathologic complete response (pCR) rates (2% and 7%, respectively), while patients reclassified as HER2 positive had higher pCR rates than HER2 positive patients by IHC or FISH (53% vs. 38% *P* = .047).[28]

MammaPrint and BluePrint therefore allow a more precise identification of a group of patients with a better prognosis, in whom the use of chemotherapy can be avoided, even in the scenario of higher

tumor burden, and the BluePrint tool appears superior to IHC in categorizing BC biological subtypes.

The limitations of MammaPrint is that the phase III validation study (MINDACT) was less pure than RxPONDER, as it included N0-2 patients, with different biological profiles (her 2 positive, triple negative, luminal) which limits the interpretation of the results.

PAM50 (Predictor Analysis of Microarray 50)

PAM50 includes 58 genes whose design was oriented to the classification of BC into intrinsic subtypes: luminal A, luminal B, HER2-enriched and basal like.[17]

Data for the platform development was based on the use of microarrays of about 190 prototype samples, a continuous score composed of intrinsic subtype (defined by PAM50) and tumour size, called ROR, was obtained.[29] ROR stratifies patients (ER+) into high, medium, and low risk groups. It proved to be a prognostic predictor in both node-positive and node-negative populations, although the cut-off points were different for each group.[17,30,31] PAM50 has been incorporated in different guidelines for node positive tumours, although its level of evidence is 2A.[32] Its usefulness has been analysed in several studies.

In the ABCSG-8[33,34] study randomised more than 3700 postmenopausal women with early BC to receive either tamoxifen for 5 years or 2 years of tamoxifen followed by 3 years of anastrozole. 1246 patient samples included in the study were analyzed and determined that the ROR score and ROR-based risk groups provided additional prognostic information regarding late distance relapse compared to combined clinical factors score alone. Between 5 and 15 years, an absolute risk of distant recurrence (DR) of 2.4% was observed in the low-risk ROR-based group compared to 17.5% in the high-risk ROR-based group. Differences in distance relapse free survival (DRFS) by PAM50 ROR score were observed for both N+ and N0 patients,[35] and based on this analysis it is concluded that ROR-based risk groups can differentiate breast cancer patients with respect to their risk of late-distance recurrence.

Another analysis on 1478 patients in the same study evaluated intrinsic subtype (luminal A/B, HER2-enriched, basal-like) and ROR score, and in all subgroups, ROR score adds prognostic information to the clinical predictor ($P < .0001$). In addition, PAM50 assigns an intrinsic subtype to all cases, and the luminal A cohort had a significantly lower ROR at 10 years compared to luminal B ($P < .0001$). There was also a significant and clinically relevant discrimination between low and high risk groups within all subgroups analysed, with only a 3.5% risk of metastasis at 10 years in the low ROR group, which would allow us to avoid chemotherapy in this group.[36] Similar results were obtained after analysing samples from 2500 p (1480 were N+) includes in Danish group (DBCG), identifying 37% of N+ patients with a favourable prognosis and in whom chemotherapy could be avoided.[37] In addition, an analysis of PAM50 in patients included in this same study showed that ROR score was predictive of efficacy to cyclophosphamide, epirubicin and fluorouracil (FEC) versus cyclophosphamide, methotrexate and fluorouracil (CMF) (HR 1.01, 0.78, 0.54 for low, intermediate and high ROR).[38]

In a pooled analysis of ABCSG-8 - transATAC studies, the prognostic role of ROR added to standard clinical variables was evaluated in patients with 1 positive node (n = 331) and 2-3 positive nodes (n = 212), in the multivariate analysis, the ROR score provided additional prognostic information in both groups. The 10 year RRd was significantly increased in patients with 1 positive node classified as high risk by ROR and compared to the combined low and/or intermediate risk group and patients with 2-3 positive nodes. In addition, luminal intrinsic subtype A showed lower RRd compared to luminal subtype B in both patient groups.[33] in a retrospective analysis of more than 1000 patient samples included in the ATAC study using PAM50, IH4 and Oncotype Dx showed that the ROR score added prognostic information to the clinicopathological criteria in both N0 and N+, and was more effective in predicting late recurrence than the Oncotype RS.[39]

An analysis comparing different platforms has been published, 313 p cancer were randomized to standard (chemotherapy and endocrine therapy) or test-directed (chemotherapy if Oncotype DX recurrence score >25) treatment. Risk stratification was also determined with Prosigna (PAM50), MammaPrint, MammaTyper, NexCourse Breast (IHC4-AQUA), and conventional IHC4 (IHC4). Subtype classification was provided by Blueprint, MammaTyper, and Prosigna. Oncotype DX predicted a higher proportion of tumours as low risk (82.1%), followed by Prosigna (65.5%), IHC4 (72.0%), MammaPrint (61.4%), NexCourse Breast (61.6%), and low concordance between platforms was observed when dichotomising results between high and low-intermediate risk. Only 39.4% of cases were uniformly classified as low and/or intermediate or high risk, and 31.1% were concordant. Regarding subtype concordance, discordant subtyping between the PAM50, Blueprint and MammaTyper platforms was observed in 40% of cases.[40]

Prospective validation of PAM 50 is currently being studied in the OPTIMA study ("Optimal Personalized Treatment of early breast cancer using Multiparameter Analysis"),[38] 4500 patients with early BC, ER positive, HER2 negative, with axillary involvement or tumour size >3 cm, are randomised to receive CT and HT in patients with Prosigna Score >60 or only HT if the Prosigna Score ≤60.

EndoPredict (EP)

It is a genomic test that analyses by PCR on paraffin-embedded samples of the tumor the expression of 12 genes, 8 BC-related genes (3 cell proliferation cycle and 5 hormone signaling), 3 RNA control reference genes and a DNA control gene. Based on the expression levels of these genes, an EP index is established, then combined with tumor size and nodal stage to obtain another index, EPclin, increasing the prognostic capacity of EP.[41,42] EP offers a score from 0 to 15 with a cut-off point of 5 for low or high risk, while EPclin has a value range between 1 and 6.5 and classifies low and high risk of relapse with a cut-off point of 3.3.

EPclin has been evaluated in patients with positive nodes in 3 studies.

A retrospective analysis of 1700 samples of HT-treated patients included in the ABCSG-6/8 studies, 27% N1 and 5% with 4 or more positive nodes, showed that 10 years RFSd in the subgroup of N1 patients was 95.6% for patients with a low EPclin and 80.9% for high-risk EPclin, representing a significant reduction in the risk

of distant recurrence in patients with low EPclin (HR 3.65, 95% CI: 1,73-7,68, $P = .0003$).[42] These results are consistent with the low risk analysis of N1 patients included in the TransATAC study, where patients classified as EPclin low had an RFSd of 94.4%, which allows us to identify a group of low-risk N1 patients treated with HT alone.[43]

For patients who have received adjuvant CT treatment, EP has been retrospectively studied in patients included in the GEICAM 9906 study,[44] 25% classified as low risk for EP. Metastasis-free survival was 93% in the low-risk group and 70% in the high-risk group, with absolute risk reduction 23% (HR 4.8 CI 95: 2.5-9.5, $P < .0001$). Furthermore, according to the EP index, the risk of recurrence in the high-risk group was twice as high in premenopausal women (6.68) as in postmenopausal women (3.34). These results postulate EP as a prognostic factor in N+ BC patients treated with adjuvant CT and HT.[44]

To assess the predictive ability of EPclin to CT benefit, a comparative pooled analysis of EPclin in patients who received HT alone (ABCSG 6/8, TransATAC) versus patients who received TC+HT (GEICAM 2003-02/9906) was performed, 3746 p were included, those with high-risk EPclin had improved 10 year DRFI with chemotherapy addition versus HT alone (12% DRFI vs. 20% at 10 years) This indirect comparison suggested that a high-risk EPclin score may be predictive of benefit of chemotherapy in women with ER-positive and HER2-negative disease.[45]

An EBCTCG meta-analysis of 2185 samples analyzed by Myriad Genetics showed similar results with an absolute benefit of chemotherapy for women with high-risk EPclin between 5.3% and 7.3%.[46]

Since clinical validation studies of EP are retrospective, it is not possible to determine its usefulness as a tool for therapeutic decision making, prospective studies are needed and 2 prospective, randomized trials (UNIRAD and RESCUE) are currently underway to evaluate the prognostic and predictive efficacy of EP.

In addition, EP mostly analyses hormone signaling genes, so it has been suggested that it could be useful in predicting the benefit of extended hormonal therapy.[47]

BCI (BREAST CANCER INDEX H/I)

Breast Cancer Index (BCI) is a mixed platform that combines two tests: the expression ratio between HOXB13 and IL17BR and the so-called molecular grade index (genes related to cell cycle and proliferation). The score generated is used to determine the benefit of extending the duration of adjuvant hormonal therapy in postmenopausal node-negative patients. If we focus on the N+ population, we have some studies that have evaluated BCI in these patients as a prognostic and predictive factor for late relapse.[48] As a prognostic factor for late relapse, a study in 249 patients (1-3 node positive), 160 received up to 5 years of HT and 197 received CT. BCI classified 77% of patients as high risk (16.1% risk of recurrence). It therefore seems advisable based on this study to avoid extension of hormonal therapy in patients identified as low risk by BCI.[49] Patients included in the aTTom study were analyzed as a predictor of benefit from extended HT, and the role of extended HT in N+ patients was assessed. Patients classified as high risk by BCI appear to benefit from extending the duration of HT (10.2% absolute reduction in the risk of relapse, HR 0.35). Conversely, patients classified as low risk by BCI showed no benefit from adding another 5 years of hormone therapy.[50] BCI was also able to identify on samples of 908 patients participating in the IDEAL study the extended HT benefit group.[51]

Despite this, and given the lack of prospective studies, further studies in a node-positive population are needed to further evaluate the role of BCI in clinical practice

OTHER SIGNATURES

GENOMIC GRADE INDEX (GGI)

It is a signature that analyses 97 genes that can classify tumors according to grade better than immunohistochemistry.[52,53] GGI could reclassify grade 2 tumors into 2 groups of high and low risk of recurrence, thus improving the prognostic value of histological classification. Validation of GGI in patients with untreated or tamoxifen-treated HR+ tumors showed that GGI was a prognostic factor independent of classical markers. In a subgroup of 204 patients treated in the PACS01 trial, which included N+ patients, GGI outperformed histological grade and other proliferation markers (mRNA and Ki-67 protein, mitotic activity index) as a predictor of DFS, although this is a small study and a retrospective analysis, so further validation of GGI is needed.[54]

IHC4

IHC4 is based on a multivariate model using semi-quantitative information from immunohistochemical evaluation of ER, PR, HER2 and Ki67. The study is performed on FFPE samples, and a recurrence risk score is calculated using an algorithm. The use of the IHC4 algorithm was validated on the TEAM trial for predicting residual risk in patients with breast cancer.[55] Although some studies show that it can effectively separate luminal A from luminal B tumors and that it has a similar prognostic ability to RS,[56,57] Another study found that its prognostic ability was worse than that of PAM50,[58] and in the absence of prospective validation studies, it is not yet considered to have sufficient evidence to be implemented in clinical practice.[43]

CONCLUSIONS

When we decide to request a genomic test we must take into account that each of the available tests has differential characteristics and that the results are not concordant between the different platforms used, so we must interpret the results according to the evidence of the test and its context.

Genomic platforms provide prognostic information and, in some cases, are predictors of chemotherapy benefit in patients with early breast cancer. The number of publications in node-positive patients has increased in recent years, but only 2 platforms (OncotypeDx and Mammaprint) have reported phase III studies results (RXPONDER,[4] MINDACT[21]) supporting their use in postmenopausal patients with HR-positive, HER 2 negative early BC with limited nodal involvement (N1), thus allowing better selection of patients for chemotherapeutic treatment based on prognosis, thus avoiding overtreatment. However, it is worth noting the lack of long-term overall survival data, because as in the case of the oncotype (RxPONDER), 5 years is a short period of time, in view of the

risk of recurrence maintained over time, which is very relevant in patients with Her2-negative luminal breast cancer, a sustained risk directly proportional not only to the degree of lymph node involvement but also to the size of the tumor. However, this prognostic information is crucial since those patients with a low risk of recurrence have a low clinical impact of chemotherapy, regardless of the benefit of chemotherapy. In the case of Mammaprint the limitations were the heterogeneity of the cases and the number of patients includes. Both studies are positive and show with level I evidence that patients with low genomic risk have a good prognosis independent of chemotherapy administration. It should be noted that both platforms show that their prognostic role is limited in women <50 years, so we should be cautious in this population group, as shown by some recently published recommendation guidelines .Regarding the predictive value of the platforms, only OncotypeDx has been shown to predict the benefit of QT in patients with N1, although as we mentioned previously, in patients under 50 years of age, we did not find a cut-off point for SR in which patients did not benefit from QT.

In addition to Oncotype and mammaprint we have other molecular platforms available, PAM50 and BLUEPRINT have been shown to be superior to IHC in classifying breast tumors into molecular groups and thus we have another tool to improve prognostic and predictive profiles for HT or CT treatment.

Endopredict combines genomic and clinical factors in its risk algorithm, and has favorable results as a potential predictor of benefit to CT and as a short-term prognostic and late relapse prognostic tool. BCI, Endopredict and PAM50 also add predictors of late relapse in early breast cancer and, and, in the case of BCI, it has shown the ability to predict benefit at extended HT.[50]

Predictive information is very relevant in patients at high risk of relapse as it allows to establish the benefit of the addition of adjuvant chemotherapy, as well as in some cases the value of the addition of taxanes to anthracycline-based chemotherapy (Oncotype in the NSBP-28 trial population), treatments that carry side effects of great personal, occupational and social impact.

Regardless of the results of a given platform, often with data discordant with each other, the importance of interpreting these results in the context of clinical and pathological variables, taking into account each and every one of the clinical prognostic factors, must be emphasized. Thus, by way of example, although no cut-off point has been identified below which the benefit of chemotherapy can be ruled out in a premenopausal patient with N1 lymph node involvement, those with an otherwise low clinical risk could be spared chemotherapy. Thus, it seems clinically relevant whether the lymph node involvement is microscopic or not, whether the tumor size is less than 1 cm (pT1a-b), whether the tumor grade is low, whether there is no lymphovascular permeability or whether the histology is of lobular subtype or not. The latter represents approximately 10% of infiltrating breast cancers, has a more frequent lymph node involvement, despite having a generally luminal A molecular profile. In the latter case, molecular characterization with a genetic platform, could have additional importance for the final therapeutic decision in such a circumstance.

Regarding guidelines, it should be noted that not all of them have been updated. Recently the ASCO guidelines have been updated and adapted to the results of the published studies of each genomic signature,[59] and the most recent NCCN guideline[62] gives a category IA to the recommendation of the use of 2 platforms in the N1 breast cancer setting: MammaPrint and OncotypeDX, although OncotypeDx is the most recommended, with the recommendation level being 2A for premenopausal women. The level of recommendation for PAM50, PD and BCI in the NCCN guidelines is 2A. As for the ESMO guidelines, they recommend the use of platforms in conjunction with clinicopathologic factors rather than platforms alone, and establish the basis on which genomic testing is not recommended.

However, due to the low percentage of absolute benefit, the dichotomy of whether or not to administer chemotherapy to decrease the risk of recurrence in the era of precision medicine, even in cases at high risk of recurrence, deserves review, given that the benefit is small at best, with the identification of more effective treatments, scenarios where the predictive value of platforms would have greater clinical impact, being highly desirable.[59]

REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG)Increasing the dose intensity of chemotherapy by more frequent administration or sequential scheduling: a patient-level meta-analysis of 37 298 women with early breast cancer in 26 randomized trials. *Lancet Lond Engl*. 2019;393:1440–1452 Apr 6.
2. Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet Lond Engl*. 2014;384:164–172 Jul 12.
3. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004;351:2817–2826 Dec 30.
4. Kalinsky K, Barlow WE, Gralow JR, et al. 21-Gene assay to inform chemotherapy benefit in node-positive breast cancer. *N Engl J Med*. 2021;385:2336–2347 Dec 16.
5. Dowsett M, Cuzick J, Wale C, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. *J Clin Oncol Off J Am Soc Clin Oncol*. 2010;28:1829–1834 Apr 10.
6. Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomized trial. *Lancet Oncol*. 2010;11:55–65 Jan.
7. Natsuhara KH, Losk K, King TA, et al. Impact of genomic assay testing and clinical factors on chemotherapy use after implementation of standardized testing criteria. *The Oncologist*. 2019;24:595–602 May.
8. Mamounas EP, Tang G, Paik S, et al. 21-Gene Recurrence Score for prognosis and prediction of taxane benefit after adjuvant chemotherapy plus endocrine therapy: results from NSABP B-28/NRG Oncology. *Breast Cancer Res Treat*. 2018;168:69–77 Feb.
9. Penault-Llorca F, Filleron T, Asselain B, et al. The 21-gene Recurrence Score® assay predicts distant recurrence in lymph node-positive, hormone receptor-positive, breast cancer patients treated with adjuvant sequential epirubicin- and docetaxel-based or epirubicin-based chemotherapy (PACS-01 trial). *BMC Cancer*. 2018;18:526 May 4.
10. Goldstein LJ, Gray R, Badve S, et al. Prognostic utility of the 21-gene assay in hormone receptor-positive operable breast cancer compared with classical clinicopathologic features. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008;26:4063–4071 Sep 1.
11. Roberts MC, Miller DP, Shak S, Petkov VI. Breast cancer-specific survival in patients with lymph node-positive hormone receptor-positive invasive breast cancer and Oncotype DX Recurrence Score results in the SEER database. *Breast Cancer Res Treat*. 2017;163:303–310 Jun.
12. Stemmer SM, Steiner M, Rizel S, et al. Clinical outcomes in ER+ HER2 -node–positive breast cancer patients who were treated according to the Recurrence Score results: evidence from a large prospectively designed registry. *NPJ Breast Cancer*. 2017;3:32.
13. Hortobagyi. hortobagyi GN, Shak S, et al. Breast cancer specific mortality (BCSM) in patients (pts) with node-negative (N0) and node-positive breast cancer (BC) guided by the 21-gene assay: A SEER-genomic population-based study. In: [P3-11-02] San Antonio Breast Cancer Symposium 2018.
14. Petkov VI, Miller DP, Howlader N, et al. Breast-cancer-specific mortality in patients treated based on the 21-gene assay: a SEER population-based study. *NPJ Breast Cancer*. 2016;2:16017.

15. Wang M, Wu K, Zhang P, Zhang M, Ding A, Chen H. The Prognostic Significance of the Oncotype DX Recurrence Score in T1-2N1M0 Estrogen Receptor-Positive HER2-Negative Breast Cancer Based on the Prognostic Stage in the Updated AJCC 8th Edition. *Ann Surg Oncol*. 2019;26:1227–1235 May.
16. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. *N Engl J Med*. 2018;379:111–121 Jul 12.
17. Parker JS, Mullins M, Cheang MCU, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol Off J Am Soc Clin Oncol*. 2009;27:1160–1167 Mar 10.
18. Mittempergher L, de Ronde JJ, Nieuwland M, et al. Gene expression profiles from formalin fixed paraffin embedded breast cancer tissue are largely comparable to fresh frozen matched tissue. *PloS One*. 2011;6:e17163 Feb 11.
19. Mook S, Schmidt MK, Viale G, et al. The 70-gene prognosis-signature predicts disease outcome in breast cancer patients with 1-3 positive lymph nodes in an independent validation study. *Breast Cancer Res Treat*. 2009;116:295–302 Jul.
20. Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med*. 2016;375:717–729 Aug 25.
21. Cardoso F, Lvt Veer, et al.CARDOSO MINDACT: Longterm results of the large prospective trial testing the 70-gene signature MammaPrint as guidance for adjuvant chemotherapy in breast cancer patients. *J Clin Oncol*. 2020;38:506.
22. Piccart M, van 't Veer LJ, Poncet C, et al. 70-gene signature as an aid for treatment decisions in early breast cancer: updated results of the phase III randomized MINDACT trial with an exploratory analysis by age. *Lancet Oncol*. 2021;22:476–488 Apr 1.
23. Tsai M, Lo S, Audeh W, et al. Association of 70-Gene signature assay findings with physicians' treatment guidance for patients with early breast cancer classified as intermediate risk by the 21-gene assay. *JAMA Oncol*. 2018;4 Jan 11.
24. Krijgsman O, Roepman P, Zwart W, et al. A diagnostic gene profile for molecular subtyping of breast cancer associated with treatment response. *Breast Cancer Res Treat*. 2012;133:37–47.
25. Mittempergher L, Delahaye L, Witteveen A, et al. Mamma print and blueprint molecular diagnostics using targeted RNA next-generation sequencing technology. *J Mol Diagn JMD*. 2019;21(5):808–823. doi:10.1016/j.jmoldx.2019.04.007.
26. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144:646–674 Mar 4.
27. Haan JC, Bhaskaran R, Ellappalayam A, et al. Mamma print and blueprint comprehensively capture the cancer hallmarks in early-stage breast cancer patients. *Genes Chromosomes Cancer*. 2022. [Internet]637736544000000000 [citedJul 11]; Available at. https://www.sciencegate.app/document/10.1002/gcc.23014 .
28. Whitworth P, Stork-Sloots L, de Snoo FA, et al. Chemosensitivity predicted by BluePrint 80-gene functional subtype and MammaPrint in the Prospective Neoadjuvant Breast Registry Symphony Trial (NBRST). *Ann Surg Oncol*. 2014;21:3261–3267 Oct.
29. Ehsani S, Wisinski KB. Genomic Testing in the Management of Early-Stage Breast Cancer. *J Clin Outcomes Manag JCOM*. 2017;24:229–238 May.
30. Kwa M, Makris A, Esteva FJ. Clinical utility of gene-expression signatures in early stage breast cancer. *Nat Rev Clin Oncol*. 2017;14:595–610 Oct.
31. Markopoulos C, van de Velde C, Zarca D, Ozmen V, Masetti R. Clinical evidence supporting genomic tests in early breast cancer: Do all genomic tests provide the same information? *Eur J Surg Oncol J Eur Soc Surg Oncol Br Assoc Surg Oncol*. 2017;43:909–920 May.
32. Ovcaricek T, Takac I, Matos E. Multigene expression signatures in early hormone receptor positive HER 2 negative breast cancer. *Radiol Oncol*. 2019;53:285–292 Sep 24.
33. Gnant M, Sestak I, Filipits M, et al. Identifying clinically relevant prognostic subgroups of postmenopausal women with node-positive hormone receptor-positive early-stage breast cancer treated with endocrine therapy: a combined analysis of ABCSG-8 and ATAC using the PAM50 risk of recurrence score and intrinsic subtype. *Ann Oncol Off J Eur Soc Med Oncol*. 2015;26:1685–1691 Aug.
34. Makris A, Hughes-Davies L, MacPherson IR, et al. OPTIMA (Optimal Personalized Treatment of early breast cancer using Multi-parameter Analysis): A prospective trial to validate the predictive utility and cost-effectiveness of gene expression test-directed chemotherapy decisions. *J Clin Oncol*. 2016;34(15_suppl):TPS623 May 20–TPS623.
35. Filipits Martin, Nielsen Torsten O, et al.MARTIN FILIPITS The PAM50 risk-of-recurrence score predicts risk for late distant recurrence after endocrine therapy in postmenopausal women with endocrine-responsive early breast cancer clin cancer. *Res*. 2022;20:1298–1305.
36. Gnant M, Filipits M, Greil R, et al. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol Off J Eur Soc Med Oncol*. 2014;25:339–345 Feb.
37. Lænkholm AV, Jensen MB, Eriksen JO, et al. PAM50 Risk of Recurrence Score Predicts 10-Year Distant Recurrence in a Comprehensive Danish Cohort of Postmenopausal Women Allocated to 5 Years of Endocrine Therapy for Hormone Receptor-Positive Early Breast Cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 2018;36:735–740 Mar 10.
38. Jensen MB, Lænkholm AV, Balslev E, et al. The Prosigna 50-gene profile and responsiveness to adjuvant anthracycline-based chemotherapy in high-risk breast cancer patients. *NPJ Breast Cancer*. 2020;6:7 Feb 26.
39. Dowsett M, Sestak I, Lopez-Knowles E, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. *J Clin Oncol Off J Am Soc Clin Oncol*. 2013;31:2783–2790 Aug 1.
40. Bartlett JMS, Bayani J, Marshall A, et al. Comparing breast cancer multiparameter tests in the OPTIMA prelim trial: no test is more equal than the others. *J Natl Cancer Inst*. 2016;108(9). doi:10.1093/jnci/djw050.
41. Filipits M, Rudas M, Jakesz R, et al. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2011;17:6012–6020 Sep 15.
42. Filipits M, Dubsky P, Rudas M, et al. Prediction of Distant Recurrence Using EndoPredict Among Women with ER+, HER2- Node-Positive and Node-Negative Breast Cancer Treated with Endocrine Therapy Only. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2019;25:3865–3872 Jul 1.
43. Sestak I, Buus R, Cuzick J, et al. Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor–Positive Breast Cancer. *JAMA Oncol*. 2018;4:545–553 Apr.
44. Martin M, Brase JC, Calvo L, et al. Clinical validation of the EndoPredict test in node-positive, chemotherapy-treated ER+/HER2- breast cancer patients: results from the GEICAM 9906 trial. *Breast Cancer Res BCR*. 2014;16:R38 Apr 12.
45. Sestak I, Filipits M, Buus R, et al. Prognostic value of endopredict in women with hormone receptor–positive, HER2-negative invasive lobular breast cancer. *Clin Cancer Res*. 2020;26:4682–4687 Sep 1.
46. Soliman H, Flake DD, Magliocco A, et al. Predicting expected absolute chemotherapy treatment benefit in women with early-stage breast cancer using endopredict, an integrated 12-gene clinicomolecular assay. *JCO Precis Oncol*. 2019;3:1–10. doi:10.1200/po.18.00361.
47. Puppe J, Seifert T, Eichler C, Pilch H, Mallmann P, Malter W. Genomic signatures in luminal breast cancer. *Breast Care Basel Switz*. 2020;15:355–365 Aug.
48. Liefers GJ, Noordhoek I, Treuner K, et al. Breast cancer index (BCI) predicts benefit of two-and-a-half versus five years of extended endocrine therapy in HR+ breast cancer patients treated in the ideal trial. *J Clin Oncol*. 2020;38(15_suppl):512 May 20–512.
49. sgroi, Sgroi D, Zhang Y, Schnabel CA. Comparative performance of Breast Cancer Index (BCIN+) and CTS5 in hormone receptor-positive (HR+) lymph node-positive (N+) breast cancer patients with one to three positive nodes (N1). *J Clin Oncol*. 2019;37(15_suppl):555. doi:10.1200/JCO.2019.37.15_suppl.555.
50. Bartlett, Bartlett J, Sgroi D, et al. From extended endocrine therapy in breast cancer patients treated in the adjuvant tamoxifen–to offer more?(aTTom). *Trial*. 2019;30:1176–1183. doi:10.1093/annonc/mdz289.
51. Noordhoek I, Treuner K, Putter H, et al. Breast cancer index predicts extended endocrine benefit to individualize selection of patients with HR+ early-stage breast cancer for 10 Years of endocrine therapy. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2021;27:311–319 Jan 1.
52. Sotiriou C, Wirapati P, Loi S, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst*. 2006;98:262–272 Feb 15.
53. Filho OM, Ignatiadis M, Sotiriou C. Genomic Grade Index: An important tool for assessing breast cancer tumor grade and prognosis. *Crit Rev Oncol Hematol*. 2011;77:20–29 Jan 1.
54. Bertucci F, Finetti P, Roche H, et al. Comparison of the prognostic value of genomic grade index, Ki67 expression and mitotic activity index in early node-positive breast cancer patients. *Ann Oncol Off J Eur Soc Med Oncol*. 2013;24:625–632 Mar.
55. Bartlett JMS, Christiansen J, Gustavson M, et al. Validation of the IHC4 breast cancer prognostic algorithm using multiple approaches on the multinational TEAM clinical trial. *Arch Pathol Lab Med*. 2016;140:66–74 Jan 1.
56. Cheang MCU, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst*. 2009;101:736–750 May 20.
57. Cuzick J, Dowsett M, Pineda S, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 2011;29:4273–4278 Nov 10.
58. Nielsen TO, Parker JS, Leung S, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2010;16:5222–5232 Nov 1.
59. Andre F, Ismaila N, Allison KH, et al. Biomarkers for adjuvant endocrine and chemotherapy in early-stage breast cancer: ASCO guideline update. *J Clin Oncol*. 2022. [Internet]Apr 19 [cited 2022 Jul 3]; Available at. https://ascopubs.org/doi/pdf/10.1200/JCO.22.00069 .
60. Nitz U, Gluz O, Clemens M, et al. West German Study PlanB Trial: Adjuvant Four Cycles of Epirubicin and Cyclophosphamide Plus Docetaxel Versus Six Cycles of Docetaxel and Cyclophosphamide in HER2-Negative Early Breast Cancer. *J Clin Oncol Off J Am Soc Clin Oncol*. 2019;37:799–808 Apr 1.
61. Gluz O, Nitz UA, Christgen M, et al. West German Study Group Phase III PlanB Trial: First Prospective Outcome Data for the 21-Gene Recurrence Score Assay and Concordance of Prognostic Markers by Central and Local Pathology Assessment. *J Clin Oncol Off J Am Soc Clin Oncol*. 2016;34:2341–2349 Jul 10.
62. NCCN Guidelines Version 4; 2022. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf.