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The role of the 21-gene assay in the neoadjuvant setting for hormone receptor-positive HER2-negative breast cancer: Impact on systemic and surgical treatment decisions

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ABSTRACT

In breast cancer, one of the main objectives of neoadjuvant treatment, regardless of the specific breast cancer subtype, is to downstage the disease in both the breast and the lymph nodes, thereby allowing surgical de-escalation. In patients with inoperable disease, downstaging could lead to a feasible surgical resection; whereas, in patients presenting with operable disease, it could increase the eligibility for breast-conserving surgery (i.e., lumpectomy) instead of a mastectomy as well as spare some patients an axillary lymph node dissection. In patients with hormone receptor-positive (HR +) human epidermal growth factor receptor 2 (HER2)-negative early-stage breast cancer, neoadjuvant treatment modalities include chemotherapy (NCT) and endocrine therapy (NET). The 21-gene Oncotype DX Breast Recurrence Score® assay is a multigene assay that determines a Recurrence Score® (RS) result (range, 0–100) based on gene expression profile within the primary tumor. The RS is a validated prognosticator and predictor of benefit from adjuvant CT. Beyond its validation, it has shown clinical utility in 3 prospective randomized clinical trials in the adjuvant setting (TAILORx, RxPONDER, and WSG PlanB). In these studies, all assays were performed on surgical excision samples. This review focuses on the utility of the 21-gene assay in patients with HR + HER2-negative early-stage breast cancer in the neoadjuvant setting. It discusses the analytical validation of performing the assay on core biopsies, its clinical validation as a tool to guide neoadjuvant treatment decisions (primary surgery, NET, or NCT), the association of the RS result with clinical outcomes, the impact of the RS results on neoadjuvant treatment decisions in real-life clinical practice, the inclusion of the assay in the neoadjuvant setting in clinical-practice guidelines, access of patients worldwide to this approach, and potential directions for additional studies examining the role of the RS in other steps in the clinical pathway of HR + HER2-negative early-stage breast cancer.

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Introduction

The 21-gene Oncotype DX Breast Recurrence Score® assay is a quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR)-based assay performed on RNA extracted from formalin-fixed paraffin-embedded breast cancer (BC) tissue samples. [1,2] The assay measures the expression of 21 genes (16 breast cancer-related, 5 reference genes) and determines a Recurrence Score® (RS) result (range, 0–100), which corresponds to the risk of distant recurrence and the benefit (or lack thereof) from adjuvant chemotherapy (CT) in patients with hormone receptor positive (HR +) human epidermal growth factor receptor 2 (HER2)-negative early-stage breast cancer (EBC) treated with endocrine therapy (ET). [1,2] The RS result provides information independent of traditional parameters (e.g., age, tumor size, and grade). Furthermore, studies have consistently shown that a large proportion of grade 3 tumors have low RS results and may not benefit from adjuvant CT (up to 57% when low RS is defined as RS 0–25).[3–6] The assay was initially validated in a series of prospective-retrospective studies (i.e., studies evaluating archived specimens from patients in clinical trials) (level 1B evidence); [7–11] however, in the last decade, randomized prospective clinical utility studies were reported (TAILORx, RxPONDER, and WSG PlanB), reaching level 1A evidence. [3,12–16] In all of these studies, the assay was performed on surgical excision samples.

The 21-gene assay is widely used in clinical practice worldwide. Since its launch in 2004, over 2 million assays have been performed. For 1.5 million of them, the resultant RS result suggested it was reasonable to forego adjuvant CT (i.e., <1% absolute benefit of adjuvant CT).[17] The assay is included in major international guidelines as a decision-support tool in the adjuvant setting for HR + HER2-negative EBC, including those published by the European Society for Medical Oncology (ESMO), the St. Gallen International Breast Cancer Consensus, the American Society of Clinical Oncology (ASCO®), and the National Comprehensive Cancer Network® (NCCN®). [18–21] Notably, the ESMO 2024 guidelines specifically note that multigene assays can also be performed on diagnostic core biopsies, before any treatment. [18].

Among patients with HR + HER2-negative EBC, who constitute approximately 70% of female BC patients, [22] having definitive surgery first, followed by adjuvant systemic treatment guided by a multigene assay, is common. Neoadjuvant treatment is generally regarded similarly effective to adjuvant therapy, [23,24] and is often considered for patients with larger tumors or clinical nodal involvement.[18] The ESMO 2024 guidelines suggest that in all cases for this BC subtype, where systemic adjuvant CT is indicated, the same regimen can also be considered in the neoadjuvant setting, and that the considerations for selecting the appropriate CT regimen are similar in the neoadjuvant and adjuvant settings. [18].

The current review focuses on the utility of the 21-gene assay in HR + HER2-negative EBC in the neoadjuvant setting. It discusses the analytical validation of performing the assay on core biopsies, its clinical validation as a tool to guide neoadjuvant treatment decisions, the association of the RS result with clinical outcomes, its current inclusion in guidelines, its application in clinical practice, and future direction to further define its role in this setting.

Neoadjuvant treatment in HR + HER2-negative EBC

The main objectives of neoadjuvant treatment in BC, regardless of the specific BC subtype, are to downstage the disease both in the breast and the lymph nodes, thereby allowing surgical de-escalation (i.e., breast-conserving surgery [BCS] instead of mastectomy) and better surgical outcomes, [24–28] and to provide an opportunity to test tumor sensitivity to systemic therapy in vivo, thereby allowing early identification of resistance phenotypes and decisions of most appropriate adjuvant systemic therapy as determined by the pathological response. [20].

In patients with HR + HER2-negative BC, neoadjuvant treatment modalities include neoadjuvant CT (NCT) and neoadjuvant ET (NET). Among the HR + HER2-negative BC patient population NET studies have demonstrated clinical response rates that were comparable to those observed with NCT, contingent upon extended duration of treatment (at least 16 weeks).[29].

Several prospective randomized studies have directly compared NCT to NET in HR + BC.[30–32] Overall, the studies demonstrated no notable differences in clinical and radiological response rates between NCT and NET, with low pathological complete response (pCR) rates with either approach. [30–32] In the multicenter GEICAM/2006–3 phase 2 study, 95 patients with estrogen receptor-positive (ER +), progesterone receptor-positive (PgR +), HER2-negative BC, were randomized to NCT (epirubicin plus cyclophosphamide followed by docetaxel) or NET with the aromatase inhibitor (AI) exemestane for 24 weeks. For premenopausal patients, NET also included the gonadotropin-releasing hormone (GnRH) analog goserelin. Overall, the study showed that the clinical response rates, as assessed by MRI, were not statistically significantly different between NCT and NET (66% vs 48%, respectively, $P = 0.075$). [32] However, an exploratory subgroup analysis revealed a significant benefit for NCT over NET among the 51 premenopausal patients (clinical response rate, 75% vs 44%, $P = 0.027$), but not among the 44 postmenopausal patients (57% vs 52%, $P = 0.78$). Also, among the 53 patients with baseline Ki67 > 10%, a trend towards NCT benefit over NET was observed (clinical response rate, 67% vs 42%, $P = 0.075$), whereas no difference was reported for the 38 patients with baseline Ki67 ≤ 10% (63% vs 58%, $P = 0.74$). [32].

Validation of the 21-gene assay in the neoadjuvant setting

Extending the role of the 21-gene assay as a tool for personalizing treatment decisions from the adjuvant to the neoadjuvant setting required further validation, as its level 1A validation was established solely on surgical excision samples, not on core biopsies, and exclusively in the adjuvant setting. This validation was multifaceted. Analytical validation of the assay on core biopsies was required to demonstrate that the assay can be reliably performed on such samples and that the RS results from these samples are concordant with those from the corresponding resection specimens. Additionally, clinical validation was needed to demonstrate that the prognostic and predictive utility of the assay in the adjuvant setting extends to the neoadjuvant setting.

Analytical validation

The pivotal analytical validation study of the 21-gene assay included 119,715 core biopsies samples and 799,986 excision samples tested by Genomic Health Inc. (now Exact Sciences). [33] The initial assay technical success rate was similar on the core biopsies (94.5%) and excision samples (97.3%). The median RS results were similar between the specimen types (16 in both), as was the RS distribution. This similarity remained when assessed by age and nodal status. Quantitative gene expression and gene group score results were also similar between biopsies and excisions, and this similarity remained when assessed by age and nodal status. [33] Thus, this study demonstrated the reliability of the assay on core biopsies; however, it did not determine concordance between the specimen types, as the specimen types were not paired.

A recent concordance study examined RS results in paired samples from the same primary breast tumor of 134 patients who had core biopsies and subsequent surgical excision, with no intervening systemic therapy. [34] A high concordance was demonstrated, with a Lin's concordance correlation coefficient of 0.86 (95% CI, 0.80–0.90), and an overall agreement between the RS results of 91.8% (95% CI, 87.1–96.4%). The distribution of RS categories was similar between the paired samples, and this similarity remained when assessed by clinically relevant subgroups (tumor size, tumor grade, and nodal status). [34].

The studies demonstrating consistent RS results for core biopsies and surgical excision samples are aligned with those evaluating concordance

Table 1
Summary of studies investigating the role of the 21-gene assay in guiding NCT.

#	Author, reference	Study design, n, country	Neoadjuvant treatment[a]	Key eligibility criteria	Baseline patient characteristics[a]	Main endpoints	Main results
Prospective-retrospective, retrospective, and *meta*-analysis studies							
1	Gianni et al., 2005 [48]	Prospective-retrospective study[b] (patients enrolled in the NCT INT-MILAN study). n = 89 Italy	Paclitaxel plus doxorubicin x3 followed by weekly paclitaxel x12.	Patients enrolled in the NCT INT-MILAN study.	• Mean (range) age, 50 (29–65) yrs • N+, 84% • ER-negative, 35% • RS distribution: NR[c]	• pCR	• A probit regression analysis showed that the RS result (as a continuous variable) was significantly associated with the likelihood of pCR (P = 0.005).
2	Pivot et al., 2015 [49]	Prospective-retrospective study[b] (patients enrolled in 3 prospective NCT studies: Sequential, Satin and Erycost) n = 81 France	4 loretaxel-4EC: 7% 4 docetaxel-4EC: 85% 3 docetaxel-3EC: 5%	Patients with ER + HER2-negative BC, with ≤ 1 involved lymph node enrolled in Sequential, Satin or Erycost.	• Median (IQR) age, 50 (42–58) yrs • N+, 28% • RS distribution: o RS 0–17: 36% o RS 18–30: 35% o RS 31–100: 30%	• pCR • BCS	• pCR rate by RS group: o RS 0–17: 0% o RS 18–30: 18% o RS 31–100: 29% • BCS rate by RS group: o RS 0–17: 72% o RS 18–30: 64% o RS 31–100: 58% • In a univariate analysis, RS was significantly associated with pCR (P = 0.02). • In a univariate analysis, RS was not significantly associated with BCS (RS 31–100 vs RS 0–30) (P = 0.36).
3	Soran et al., 2016 [43]	Retrospective, single-center n = 60 USA	NCT, according to NCCN guidelines, was administered for 24 weeks. Treatment included doxorubicin, cyclophosphamide and a taxane.	Female, patients with ER + HER2-negative T1-3, N0-1, BC who subsequently received NCT	• Mean (range) age, 52 (28–86) yrs • N+, 17% • RS distribution: o RS 0–17: 45% o RS 18–30: 17% o RS 31–100: 38%	• pCR • Percent tumor reduction	• pCR rate by RS group: o RS 0–17: 0% o RS 18–30: 0% o RS 31–100: 0% • Mean (SD) percent tumor reduction by RS group (P = 0.21 for comparing the RS groups): o RS 0–17: 35% (26%) o RS 18–30: 54% (32%) o RS 31–100: 46% (37%)
4	Kantor et al., 2019 [44]	Retrospective analysis of NCDB data n = 1,377 (NCT-treated) USA	NR	Female patients with stage 1–3 HR + HER2-negative BC who had a RS result and received NCT	• Age ≤ 50 yrs, 39% • N+, 30% • RS distribution: o RS 0–10: 12% o RS 11–25: 44% o RS 26–100: 44%	• pCR	• pCR rate by RS group (P < 0.01 for comparing responses between the RS groups): o RS 0–10: 4.4% o RS 11–25: 1.3% o RS 26–100: 7.8%
5	Pease et al., 2019.[45]	Retrospective analysis of NCDB data n = 989 USA	NR	Patients with T1-3 ER + HER2-negative BC who had a RS result and received NCT	• Mean (range) age, 55 (23–83) yrs • N+, 23% • RS distribution: o RS 0–17: 23% o RS 18–30: 46% o RS 31–100: 32%	• pCR	• pCR rate by RS group (P < 0.001 for comparing responses between the RS groups): o RS 0–17: 2.2% o RS 18–30: 1.6% o RS 31–100: 9.6% • Significant association between pCR and RS 31–100 compared to RS 18–30 patients in MVA (OR, 4.9; 95%C CI, 2.0–11.8; P value, NR)
6	Ma et al., 2021 [46]	Retrospective analysis of NCDB data n = 2,075 (with RS results) USA	NR	Patients with stage 1–3 BC who had a RS result and received NCT[d]	• Age < 50 yrs, 38% • N+, 36% • RS distribution: o RS 0–30: 62% o RS 31–100: 38%	• pCR • BCS • OS	• Significant association between pCR and RS 31–100 in MVA (aOR, 1.99; 95% CI, 1.4–2.8; P < 0.001) • BCS rate by RS group (P, NR for comparing BCS rate between the RS groups): o RS 0–30: 39% o RS 31–100: 46% • In HR + HER2-negative patients, MVA revealed a significant association between high RS and worse OS outcomes (P =

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Table 1 (*continued*)

#	Author, reference	Study design, n, country	Neoadjuvant treatment[a]	Key eligibility criteria	Baseline patient characteristics[a]	Main endpoints	Main results
							0.006 for RS 26–100 vs RS 0–25). • In HR + HER2-negative patients, MVA found no significant association between, pCR and OS ($P = 0.07$), and no significant association between RS 31–100 plus pCR and OS outcomes ($P = 0.16$).
7	Pardo et al., 2021 [47]	Retrospective analysis of NCDB data n = 158 USA	NR	Patients with ER + HER2-negative T1-T2, T1/N2 BC who had a RS result and received NCT	• Age < 55 yrs, 48% • N1, 89%; ≥N2, 11% • RS distribution: o RS 0–17: 35% o RS 18–30: 39% o RS 31–100: 25%	• Axillary pCR	• Axillary pCR rate by RS group ($P = 0.027$ for comparing responses between the RS groups): o RS 0–17: 11% o RS 18–30: 10% o RS 31–100: 28%
8	Sella et al., 2021 [50]	Prospective-retrospective study[b] (patients enrolled in the Young Women's Breast Cancer Study) n = 76 USA	Anthracycline-taxane: 86% Taxane only: 13% Anthracycline only: 13% N/A: 1%	Patients with ER + HER2-negative BC, who were diagnosed ≤ 40 yrs and received NCT in the Young Women's Breast Cancer Study	• Median (range) age, 37 (24–40) yrs • N+, 74% • RS distribution: o RS 0–25: 50% o RS 26–100: 50%	• pCR	• pCR rate by RS group ($P = 0.09$): o RS 0–25: 5% (2 patients, both with RS between 20 and 25) o RS 26–100: 21% • In MVA, RS as a continuous variable was significantly associated with pCR (OR, 1.07; 95% CI. 1.01–1.12; $P = 0.01$).
9	Boland et al., 2021 [57]	Meta-analysis of 7 studies n = 1,744 USA, Europe	Differ by study	Female patients with ER + HER2-negative BC	• Weighted median (range) age, 50 (28–86) yrs • N+, 31% • RS distribution (as defined in each of the studies included in the *meta*-analysis): o Low: 13% o Intermediate: 42% o High: 45%	• pCR	• pCR rates by RS group: o Low-intermediate RS: 2.1% (weighted mean, 1.1%) o High RS: 9.5% (weighted mean, 10.9%) • Risk ratio, 4.5 (95% CI, 2.8–7.2, $P < 0.001$)
Prospective studies							
10	Yardley et al., 2015[51]	A prospective multicenter study n = 75 (ER +)[e] USA	Ixabepilone plus cyclophosphamide x 6.	Female patients with HER2-negative locally advanced BC	• Mean/median age for ER + patients: NR • RS distribution for ER + patients: o RS 0–17: 29% o RS 18–30: 29% • RS 31–100: 41%	• pCR	• pCR rate by RS group in evaluable ER + patients ($P = 0.03$ for comparing the RS groups): o RS 0–17: 0% o RS 18–30: 0% o RS 31–100: 17%
11	Bear et al., 2017[52]	A prospective, randomized multicenter study n = 55 (evaluable patients) USA, Canada	Patients received treatment according to the RS result: RS 26–100: NCT • RS 0–10: NET • RS 11–25: Randomized to NET or NCT	Female patients with HR + HER2-negative BC, tumor size ≥ 2 cm, which, in the opinion of the surgeon, were not suitable for BCS	Evaluable patients with final treatment received: • RS 0–10 (all NET): 12 • RS 11–25 (NET-treated): 18 • RS 11–25 (NCT-treated): 11 • RS 26–100 (all NCT): 14	• Proportion of patients refusing NCT assigned treatment (predefined threshold, 33%) • Rate of successful BCS (negative margins) • Clinical response rate (cCR + cPR), pCR in the breast	• None of the RS 11–25 NET-assigned patients refused their treatment assignment; 15% of the RS 11–25 NCT-assigned patients refused their assignment (significantly lower than the predefined threshold, $P = 0.029$) • Patients with successful BCS (NS difference between the groups): o RS 0–10 (NET): 75% o RS 11–25 (NET): 72% o RS 11–25 (NCT): 64% o RS 26–100 (NCT): 57% • Patients with cCR ($P = 0.042$ for comparing all groups): o RS 0–10 (NET): 8.3% o RS 11–25 (NET): 22% o RS 11–25 (NCT): 36% o RS 26–100 (NCT): 29% • Patients with clinical response (cCR + cPR) (P

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Table 1 (*continued*)

#	Author, reference	Study design, n, country	Neoadjuvant treatment[a]	Key eligibility criteria	Baseline patient characteristics[a]	Main endpoints	Main results
							= 0.049 for comparing all groups): o RS 0–10 (NET): 83% o RS 11–25 (NET): 50% o RS 11–25 (NCT): 73% o RS 26–100 (NCT): 93% • Patients with pCR in the breast (NS difference between the groups): o RS 0–10 (NET): 8.3% o RS 11–25 (NET): 6.0% o RS 11–25 (NCT): 0% o RS 26–100 (NCT): 21% • Patients with pCR in the breast and nodes (NS difference between the groups): o RS 0–10 (NET): 0% o RS 11–25 (NET): 0% o RS 11–25 (NCT): 0% o RS 26–100 (NCT): 14% • Comparison between RS 11–25 NET and RS 11–25 NCT for clinical response rate (cCT + cPR) showed that the difference (50% vs 73%) was statistically significant ($P = 0.037$) after adjusting for confounders.
12	Morales Murillo et al., 2021 [53][f]	A prospective single-center cohort study. n = 122 (consecutive patients, 60 of whom received NCT) Spain	Treatment was RS-based. RS 0–10 patients had initial surgery; postmenopausal RS 26–100 and premenopausal RS 21–100 patients received NCT; RS 11–25 patients were assigned mainly to initial surgery[g]	Female patients with HR + HER2-negative BC and tumor size ≥ 2 cm, who received NCT recommendation by a multidisciplinary team based on clinicopathological parameters.	• Mean (range) age, 57 (31–84) yrs[h] • Premenopausal, 37%[e] • N+: 48%[h] • RS distribution:[h] o RS 0–10: 17% o RS 11–25: 48% o RS 26–100: 35%	• pCR	• pCR rate by RS group: o In low-risk patients (RS 11–20 for premenopausal and RS 11–25 for postmenopausal, n = 20): 0% o In high-risk patients (RS 21–100 for premenopausal and RS 26–100 for postmenopausal, n = 40): 12% • Univariate analysis and MVA showed that the RS was the only variable associated with pCR to NCT (univariate: OR 0.94; 95% CI, 0.90–0.99; MVA: OR 0.95; 95% CI, 0.90–0.99)
13	Gluz et al., 2023 [54]	NCT sub-trial of the prospective randomized phase 3 WSG-ADAPT-HR + HER2-negative trial n = 864 of whom 582 had RS results. Germany	Solvent-based paclitaxel or nab-paclitaxel followed by epirubicin + cyclophosphamide	Female patients with HR + HER2-negative BC who were considered high risk: cN0-1, RS 12–25, who did not respond to NET [post-ET Ki67 > 10%]; cN0-1 RS 26–100. Patients with cN2-3 or (G3, baseline Ki67 ≥ 40%, and tumor size > 1 cm), could be included without RS and/or testing ET response.	Overall: • Median (range) age, 51 (20–79) yrs • Premenopausal, 49% • RS distribution among the dDFS evaluable patients (with imputation): o RS 0–25: 21% • RS 26–100: 79%	• pCR • dDFS	• pCR rate by RS group (significantly higher rates in RS 26–100 patients, $P = 0.021$): o RS 0–25: 8.4% o RS 26–100: 16.0% • Higher RS was associated with higher pCR rates (with imputation of RS values): OR, 3.11, 95% CI 1.71–5.63, $P < 0.001$ • In mediation analysis, the association between higher RS and dDFS adjusted for pCR was unfavorable (hazard ratio, 1.03; 95% CI, 1.01–1.05; $P = 0.01$).
14	Taylor et al., 2023 [55]	Pooled analysis of 2 small prospective trials, including a cohort from Bear et al (n = 59) [52] and a cohort from Zelnak et al[i] (n = 50) [56]	For the Bear et al cohort, see above. For the Zelnak et al cohort: NET, exemestane +/- goserelin for 6 to 12 months. NCT, docetaxel-	HR+, HER2-negative BC	For the Bear et al cohort, see above. For the Zelnak et al cohort: Evaluable patients with final treatment received for the combined cohort:[j]	• pCR in the breast	• pCR rates (in the breast). Comparing all 4 groups demonstrated statistical significance ($P = 0.0059$), the difference between pCR rates in patients with intermediate RS treated with NET or NCT was NS.

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Table 1 (*continued*)

#	Author, reference	Study design, n, country	Neoadjuvant treatment[a]	Key eligibility criteria	Baseline patient characteristics[a]	Main endpoints	Main results
		n = 109 (evaluable patients) US	cyclophosphamide for 6 cycles • RS 0–10: NET • RS 11–24: Randomized to NET or NCT • RS 25–100: NCT		• Median (range) age, 56 (35–75) yrs • Premenopausal, 30% • RS 0–10 (all NET): 21 • RS 11–24/25 (NET-treated): 23 • RS 11–24/25 (NCT-treated): 22 • RS 25/26–100 (all NCT): 37		o RS 0–10 (NET): 4.8% o RS 11–24/25 (NET): 0% o RS 11–24/25 (NCT): 0% o RS 25/26–100 (NCT): 21.6%

aOR, adjusted odds ratio; BC, breast cancer; BCS, breast conserving surgery; cCR, clinical complete response; CI, confidence interval; cPR, clinical partial response; dDFS, distant disease-free survival; EC, epirubicin cyclophosphamide regimen; ER, estrogen receptor; HER2, human Epidermal growth factor Receptor 2; HR, hormone receptor; IQR, interquartile range; MVA, multivariable analysis; N+, node-positive; N/A, not available; nab, nanoparticle albomin-bound; NCCN, National Comprehensive Cancer Network; NCDB, National Cancer Database; NCT, neoadjuvant chemotherapy; NET, neoadjuvant endocrine therapy; NR, not reported; NS, nonsignificant; OR, odds ratio; OS, overall survival; pCR, pathologic complete response; RS, RS, Recurrence Score result; SD, standard deviation; year, yr.

[a] Sum may be different than 100% due to rounding.
[b] Prospectively-designed study of archived tumor samples.
[c] The RS was determined internally (not by Genomic Health).
[d] The study also examined patients with 70-gene signature results.
[e] The table focuses on the ER + population out of the 138 patients for whom RS results were available.
[f] The decision impact component of this study is included in Table 3.
[g] Some patients with RS 11–20/25 did receive NCT due to nodal involvement.
[h] Patient characteristics for all 122 patients, only 60 of whom received NCT.
[I] The Zelnak et al study was presented as a poster at ASCO and was not published as a manuscript. Therefore, the data for this trial are not presented in the Table.
[j] The 2 studies differed in the RS range of the cohort that was randomized by 1 RS unit (11–25 in Bear et al and 11–24 in Zelnak et al).

for specific BC biomarkers (ER, PgR, HER2, and Ki67) using paired samples. [35–39] A *meta*-analysis of 21 studies that examined the concordance for ER and PgR, reported that the concordance rates in these studies were 84–100% for ER, and 69–97% for PgR, and that the overall agreement for ER (n = 2450) was 92.8%, and for PgR (n = 2448), 85.2%. [35] Studies conducted after this *meta*-analysis also reported high concordance for ER and PgR, as well as for HER2; whereas, the concordance for Ki67 varied between studies. For example, in a study with paired samples from 298 patients, the concordance rates for ER and PgR were 93.6% and 85.9%, respectively, and for HER2 and Ki67, 96.3%, and 79.5% respectively. [36] In another study with 1371 paired samples, these rates were 96.7% and 94.3% for ER and PgR, respectively, and 84.8% and 83.5% for HER2 and Ki67, respectively.[37] In a recent systematic review including 22 studies with a total of 5982 paired samples, the concordance rates for Ki67 were 70.3–92.7%, when measured as a percentage and 0.261–0.712, when measured as Cohen's kappa coefficient.[38] This wide range reflects reproducibility and interlaboratory variability issues associated with Ki67 assessment. [39–41] For example, in the REMAR study (n = 567), the correlation between local and central evaluation of Ki67 was weak (r = 0.35), with discrepancies in both directions. [39] Although the paired core/excisional concordance is moderate, the international Ki67 in Breast Cancer Working Group (IKWG) recommends using Ki67 thresholds of $\leq$ 5% and $\geq$ 30%, as the reproducibility was deemed acceptable in these ranges. [42].

Clinical validation: NCT

The utility of the RS result in the NCT setting has been investigated in a retrospective cohort study, [43] retrospective analyses of the National Cancer Database (NCDB), [44–47] prospective-retrospective studies, [48–50] and prospective trials. [51–56] These studies are summarized in Table 1. The retrospective and prospective-retrospective studies, which were conducted in the last 2 decades in Italy, Spain, and the United States, varied in sample sizes from 60 to 2075, varied in their RS cut-off values, and in the NCT regimens used. Clinical endpoints included mostly pCR rates, but also axillary pCR, BCS, percent reduction in tumor size, and survival. With the exception of a small study (Soran et al., [43] 60 patients), the results were overall consistent, showing that higher RS results were associated with higher pCR rates. An NCDB analyses found that higher RS results were significantly associated with higher axillary pCR rates. [47] Also, a small study evaluating the association between the RS and BCS rates reported that the association was nonsignificant. [49].

In 3 of the prospective studies (Yardley et al, Morales Murrilo et al, and the ADAPT chemotherapy sub-trial of the phase 3 WSG-ADAPT HR+/HER2-negative trial), patients across the RS range received NCT; however, the studies had distinct inclusion criteria (Table 1). [51,53,54] These 3 studies consistently demonstrated that higher RS results were associated with higher pCR rates. [51–54] The ADAPT chemotherapy sub-trial also examined distant disease-free survival (dDFS), and showed that high RS results were unfavorably associated with iDFS and dDFS [54] in the same prognostic range, as shown for adjuvant studies, like PlanB. Lastly, in the Bear et al prospective study, [52] RS 0–10 patients received NET and RS 26–100 patients received NCT, whereas the mid-range patients (RS 11–25) were randomized to NET vs NCT. Clinical complete response (cCR) and clinical response (cCR + clinical partial response [cPR]) rates varied significantly between the 4 treatment groups. Among NCT-treated patients, clinical response rates were 73% and 93% for the RS 11–25 and RS 26–100 groups, respectively. Among NET-treated patients, these rates were 83% and 50% for the RS 0–10 and RS 11–25 groups, respectively (P = 0.049 for comparing all 4 groups). Comparing NCT to NET in RS 11–25 patients while adjusting for age, race, menopausal status, and site, revealed that the clinical response rate (cCR + cPR) was significantly better among the NCT-treated patients (73% vs 50%, P = 0.037). Overall, higher pCR rates were noted with NCT vs NET, and higher rates of successful (i.e., negative pathological margins) BCS were noted with NET vs NCT; however, in both analyses, the differences were nonsignificant. In a pooled analysis, of 109 patients (59 from the Bear et al study [52] and 50 from another study by Zelnak et al [56]), among patients with intermediate RS, the pCR rates were similar between the NET- and NCT-treated patients [55] (Table 1).

A *meta*-analysis evaluating the role of the RS in predicting response to NCT in ER + HER2-negative BC patients, which included 7 studies,

Table 2
Summary of studies investigating the role of the 21-gene assay in guiding NET.

#	Author, reference	Study design, n, country	Treatment	Key eligibility criteria	Baseline patient characteristics	Main endpoints	Main results
Retrospective analysis of samples from prospective studies							
1	Akashi-Tanaka et al., 2009 [58]	Prospective-retrospective study[a] (patients enrolled in the neoTAM or neo ANZ study) n = 43 (with RS results) Japan	Neoadjuvant tamoxifen for 16 weeks (neoTAM study, n = 14), or neoadjuvant anastrozole for 16 weeks (neo ANZ study, n = 29)	Female postmenopausal BC patients with ER+, PgR+, HER2-negative, with tumors > 3 cm	• Mean (range) age, 61 (48–79) yrs • Node-positivity: 70% • RS distribution: o RS 0–17: 26% o RS 18–30: 37% o RS 31–100: 37%	• Clinical response (cCR + cPR) to NET in the 3 RS groups • RFS in the 3 RS groups	• Clinical response rate for all 43 patients did not differ significantly between the RS groups ($P = 0.11$): o RS 0–17: 64% o RS 18–30: 31% o RS 31–100: 31% • Analysis of patients treated with tamoxifen or anastrozole separately yielded similar results with no difference in response between RS groups. • Patients with RS 0–17 tended to have longer RFS, but the difference was nonsignificant ($P = 0.14$ for comparing the 3 groups).
2	Ueno et al., 2014[61] Ueno et al., 2019[59]	Prospective-retrospective study[a] (patients enrolled in a phase 2 prospective study of neoadjuvant exemestane treatment, JFMC34-0601) n = 64 (biopsy samples) for the response analysis [61] n = 59 (biopsy samples for the outcome analysis) n = 52 (matching resection samples) Japan	Neoadjuvant exemestane for 16 weeks, with a possible 8-week extension based on response. Patients underwent surgery at 24 weeks (unless they had PD), after which they received standard adjuvant treatment (ET, CT, radiation)	Female postmenopausal BC patients (using age ≥ 55 years as proxy) ER+, T2-3, N0-2, M0 disease	• Age distribution (yrs):[b] o 55–64: 53% o 65–74: 39% o 75–77: 8% • RS distribution:[b] o RS 0–17: 50% o RS 18–30: 27% o RS 31–100: 23% • RS distribution:[c] o RS 0–17: 53% o RS 18–30: 27% o RS 31–100: 20% • Received adjuvant CT:[c] 31%	• Clinical response (cCR + cPR) to NET in the 3 RS groups[b] • Evaluation of clinical response and rate of BCS by continuous RS[b] • DFS and OS in the 3 RS groups[c]	• Clinical response rate:[b,d] o RS 0–17: 59% o RS 18–30: 59% o RS 31–100: 20% ($P = 0.015$ compared to RS 0–17 group) • In a logistic regression analysis, continuous RS was significantly associated with clinical response ($P = 0.042$)[b] • BCS rate:[b] o RS 0–17: 91% o RS 18–30: 77% o RS 31–100: 47% (OR, 0.091, $P = 0.003$ compared to the RS 0–17 group) • In a univariable and multivariable analyses, continuous RS result was significantly associated with BCS[b] • Pre-treatment RS was significantly associated with DFS ($P = 0.005$), but not OS ($P = 0.064$). In a multivariate analysis, DFS was also no longer statistically significantly associated with DFS. The 5-yr DFS were: o RS 0–17: 90% o RS 18–30: 75% o RS 31–100: 50%
3	Iwata et al., 2019 (the TransNEOS study)[60]	Prospective-retrospective study[a] (translational study of the phase 3 New Primary Endocrine-therapy Origination Study [NEOS]). n = 295 Japan	Neoadjuvant letrozole for 24–28 weeks followed by surgery and subsequent adjuvant ET with or without adjuvant CT	Female post-menopausal BC patients with ER + HER2-negative, clinically N0 disease and tumor ≥ 2 cm.	• Median (range) age, 63 (49–75) yrs • Median (range) tumor size, 25 (20–65) mm • RS distribution: o RS 0–17: 53% o RS 18–30: 29% o RS 31–100: 18%	• Clinical response (CR + PR by MRI/CT scan) to NET in patients with RS 0–17 vs RS 31–100. • Evaluation of clinical response and rate of conversion from BCS noncandidacy to BCS recipient, by continuous RS	• Clinical response rate: o RS 0–17: 54% o RS 18–30: 42% o RS 31–100: 22% ($P <$ 0.001 compared to the RS 0–17 group) • In univariable and multivariable analyses, continuous RS result was significantly associated with clinical response • Nonsignificant difference in BCS candidacy between these RS groups ($P = 0.88$) o RS 0–17: 62% o RS 31–100: 63% • Significant difference in the proportion of patients having BCS ($P = 0.009$) o RS 0–17: 79% o RS 31–100: 60%

(continued on next page)

Table 2 (*continued*)

#	Author, reference	Study design, n, country	Treatment	Key eligibility criteria	Baseline patient characteristics	Main endpoints	Main results
							• Significant differences in pretreatment surgery recommendations from pre- to post-treatment in the RS 0–17 and not in the RS 31–100 group o RS 0–17: Of the 57 patients with pretreatment mastectomy recommendation, 58% converted to BCS and 42% underwent mastectomy; of the 92 patients with pretreatment BCS recommendation, 92% underwent BCS and 8% underwent mastectomy; $P < 0.001$. o RS 31–100: Of the 20 patients with pretreatment mastectomy recommendation, 35% converted to BCS and 65% underwent mastectomy; of the 28 patients with pretreatment BCS recommendation, 79% underwent BCS and 21% underwent mastectomy; $P = 0.075$.
Prospective studies[e]							
4	Al Saleh et al., 2021 [62] Al Saleh et al., 2023 [63]	Prospective, randomized phase 3 study (SAFIA) n = 273 with RS results Saudi Arabia, Egypt, Jordan, Lebanon, Algeria, and Tunisia.	Fulvestrant for 16 weeks. Pre/*peri*-menopausal women also received goserelin. Only patients who responded to the NET with CR/PR/MR were randomized to fulvestrant/goserelin + palbociclib or placebo for an additional 16 weeks, before surgery.	Patients with ER + HER2-negative stage II/IIIA BC. Patients with RS results (performed on a biopsy sample) of 31–100 were excluded.	• Median (range) age, **49 (25–84)** yrs • RS distribution: o RS 0–10: 13% o RS 11–25: 56% o RS 26–30: 10% o RS 31–100: 21%	• Radiological response after 16 weeks of NET by RS category • pCR rate after an additional 16 weeks by treatment	• Radiologic response after 16 weeks of NET (CR/PR) for 206 evaluable patients with RS 0–30. o RS 0–10: CR, 6%; PR, 65% o RS 11–25: CR, 3%; PR, 55% o RS 26–30: CR, 0%; PR, 72% • pCR after an additional 16 weeks of treatment in those who had a response to the first 16 weeks of ET (nonsignificant difference between the treatment arms): o Fulvestrant/goserelin + palbociclib: 2% o Fulvestrant/goserelin + placebo: 7%

BC, breast cancer; BCS, breast conserving surgery; cCR, clinical complete response; CR, complete response; cPR, clinical partial response; CT, chemotherapy; CT scan, computed tomography scan; DFS, disease-free survival; ER, estrogen receptor; ET, endocrine therapy; HER2, human Epidermal growth factor Receptor 2; MR, minimum response; MRI, magnetic resonance imaging; NCT, neoadjuvant chemotherapy; NET, neoadjuvant endocrine therapy; OR, odds ratio; OS, overall survival; pCR, pathologic complete response; PD, progressive disease; PgR, progesterone receptor; PR, partial response; RFS, relapse-free survival; RS, RS, Recurrence Score result; year, yr.

[a] A prospectively-designed study of archived tumor samples.
[b] For the biopsy cohort of 64 patients.
[c] For the biopsy cohort of 59 patients.
[d] No patient experienced a complete response.
[e] The Bear et al., 2017 study[52] is presented in Table 1.

also confirmed the association between higher RS results and higher pCR rates (risk ratio, 4.5; 95% CI, 2.8–7.2, $P < 0.001$) (Table 1).[57].

Combined, the data support the role of the RS as a predictor of response rate levels and pCR rates following NCT.

Clinical validation: NET

Five studies have published results on the association between the RS and response to NET in HR + HER2-negative BC, as assessed by clinical/radiologic response, and the feasibility of BCS. These studies are summarized in Table 2. Despite variability in study design, NET used, and endpoints, the studies have consistently shown that lower RS results were associated with better prognosis, higher response rates, and a higher proportion of patients eligible for BCS or having successful BCS (negative margins). Three studies had prospective-retrospective designs where RS results from pretreatment biopsy samples from post-menopausal patients enrolled in prospective NET trials were evaluated, and the association with the endpoints examined. [58–60] The largest of these trials was the translational study of the phase 3 NEOS trial (transNEOS), with 295 patients.[60] In transNEOS, patients received

Table 3
Summary of decision impact studies in the BC neoadjuvant setting.

#	Author, reference	Country	Study design, n	Key eligibility criteria	Baseline patient characteristics	Main endpoints	Main results
1	Nam et al., 2021[71]	US	A retrospective multicenter cohort study. n = 78 (evaluable patients with RS results)	Patients with ER + T1-T4 BC who underwent 21-gene testing on core-biopsy sample, and for whom clinicians were uncertain about neoadjuvant treatment.	• 98% female. • Mean (range) age, 58 (33–83) yrs • Node-positive: 22% • RS distribution: o RS 0–10: 18% o RS 11–25: 60% o RS 26–100: 22%	• Impact on systemic neoadjuvant treatment (NET vs NCT). • Impact on surgical decisions (BCS vs total mastectomy)	• 3 reasons were identified for performing test on biopsies: final excision sample not adequate for 21-gene testing (n = 6, group 1), decision support tool for primary surgery vs neoadjuvant treatment (n = 37, group 2), and a decision support tool for NET vs NCT (n = 35, group 3). • Group 2: proportion of patients receiving primary surgery with no neoadjuvant therapy. o RS 0–17: 93% o RS 18–30: 35% o RS 31–100: 75% (3 of 4 patients; all 3 had *BRCA1/2* mutations). • Group 3: proportion of patients receiving NET vs NCT: o RS 0–17: 76% vs 24% o RS 18–30: 50% vs 50% o RS 31–100: 33% vs 67%
2	Morales Murillo et al., 2021 [53],a	Spain	A prospective single-center cohort study. n = 122 (consecutive patients)	Female patients with HR + HER2-negative BC and tumor size ≥ 2 cm, who received NCT recommendation by a multidisciplinary team based on clinicopathological parameters.	• Mean (range) age, 56.5 (31–84) yrs • Premenopausal, 37% • Tumor size distribution: T1, 38%; T2, 47%; T3, 16%. • N+: 48% • RS distribution: o RS 0–10: 17% o RS 11–25: 48% o RS 26–100: 35%	• Changes in treatment recommendations. Treatments were assigned based on RS results by study protocol (RS 0–10, initial surgery; RS 26–100 in postmenopausal women or RS 21–100 in premenopausal women received NCT; mid-range patients were mainly assigned to initial surgery.	• Changes in treatment recommendation post testing: o NCT → no CT: 42% o NCT → adjuvant CT:9% • Changes were aligned with RS results. The proportions of patients who did not receive CT post testing were: o RS 0–10, 71% o RS 11–25, 57% o RS 26–100, 7%
3	Llombart-Cussac et al., 2023 (the KARMA Dx study)[72]	Spain	A prospective multicenter cohort study. n = 219 (consecutive patients) Of the 219 patients, 31 were in the neoadjuvant setting (Cohort C)	Cohort C: Female patients with ER + HER2-negative early-stage BC and high-risk clinicopathologic characteristics (cT2-3, cN0, and Ki67 ≤ 30%) for whom NCT was considered a standard recommendation by local guidelines.	Cohort C: n = 31 • Median (IQR) age, 49 (44–59) yrs • ≤50 yrs, 55% • Tumor size distribution: T1c, 19%; T2, 68%; T3, 13%. • Median (IQR) RS, 16 (13–22) • RS distribution: o RS 0–17: 68% o RS 18–30: 19% o RS 31–100: 13%	• Change in treatment recommendations after knowing the RS result. Treatment recommendations were made by the treating physician. • Changes in physicians' confidence in their treatment recommendations after knowing the RS result.	Cohort C: n = 29 (patients with pre-test recommendations that included CT). • NCT → NET: 76% • Changes were aligned with RS results. The proportion of patients with a NCT → NET change by RS category:[a] o RS 0–17, 80% o RS 18–30, 83% o RS 31–100, 33% • Physician's confidence in treatment recommendations increased post-testing in 45% of physicians..

BC, breast cancer; BCS, breast conserving surgery; CT, chemotherapy; ER, estrogen receptor; HER2, human Epidermal growth factor Receptor 2; HR, hormone receptor; IQR, interquartile range; N+, node-positive; NCT, neoadjuvant chemotherapy; NET, neoadjuvant endocrine therapy; pCR, pathologic complete response; RS, RS, Recurrence Score result; year, yr.

neoadjuvant letrozole for 24–28 weeks, after which response rates, determined by MRI/CT, and conversion from BCS noncandidacy to undergoing BCS were assessed. The clinical response rates (CR + PR) were 54%, 42%, and 22%, in the RS 0–17, RS 18–30, and RS 31–100 groups, respectively ($P < 0.001$ for the RS 0–17 vs the RS 31–100 group). The RS as a continuous variable was significantly associated with clinical response in univariable and multivariable analyses. The RS group was not associated with pretreatment eligibility for BCS (BCS candidacy of 62% vs 63% in the RS 0–17 and RS 31–100 groups, respectively; $P = 0.88$), but was significantly associated with having BCS (79% vs 60% in the RS 0–17 and RS 31–100 groups respectively; $P = 0.009$). Similarly, pretreatment surgery recommendations differed significantly from pre- to post-treatment in the RS 0–17 group (of those with pretreatment mastectomy recommendation, treatment changed to BCS in 58%; of those with pretreatment BCS recommendation, treatment changed to mastectomy in 8%; $P < 0.001$) but not in the RS 31–100 group (of those with pretreatment mastectomy recommendation, treatment changed to BCS in 35%; of those with pretreatment BCS recommendation, treatment changed to mastectomy in 21%; $P = 0.075$). Two prospective-retrospective studies also evaluated the association between the RS and survival outcomes; however, both were limited by a small sample size. In the Akashi-Tanaka et al study (n = 43), no significant association was identified with relapse-free survival; in the Ueno et al study (n = 59), the RS was not significantly associated with OS, only with DFS, and that significance was lost in a multivariable analysis. [58,59,61].

The 2 other studies were prospective. The Bear et al study [52] was

the only study directly comparing NET and NCT, as described above and is summarized in Table 1. In this study, the clinical response rate (cCR + cPR) in the RS 0–10 patients, all of whom received NET, was 83%, whereas that in the RS 11–25 patients who were randomized to NET was 50%. [52] The statistical significance of this pairwise comparison was not reported. The second prospective study was the phase 3 SAFIA study, [62,63] which investigated 16 weeks of NET with fulvestrant (plus goserelin for pre/perimenopausal patients). The study patients had stage II-IIIA ER + HER2-negative BC, and RS 0–30 as assessed on a core biopsy sample. Patients who responded to the NET (CR, PR, or minimal response) were included in the randomized phase of the study, where they received fulvestrant/goserelin plus either palbociclib or placebo for 16 additional weeks before surgery. After the first 16 weeks of the NET regimen, CR was reported for 6%, 3%, and 0% of the RS 0–10, 11–25, and 26–30 patients, respectively, and PR was reported for 65%, 55%, and 72%, respectively.[63] Although in both randomized trials the difference in response to NET as a function of the RS result was not evaluated, the findings are consistent with a hypothesis of higher odds of response to NET with lower RS results.

Recurrence Score and short-term preoperative NET

The first large phase 3 study investigating the utility of Ki67 testing after short-term preoperative NET as a means to identify HR + HER2-negative BC patients with good prognosis for whom CT can be potentially spared was the POETIC study. [64,65] This study represents an extension of the use of Ki67 as a prognostic biomarker for adjuvant treatment decisions.[66] In POETIC, 4,480 postmenopausal women with HR + BC (HER2-negative or positive) were randomized to 2 weeks of preoperative NET (AI) or no preoperative treatment. Adjuvant treatment was given based on standard-of-care local practice. The study examined whether short-term NET improved clinical outcomes and whether Ki67 levels in the tumor at baseline ($Ki67_B$) and/or after 2 weeks of NET ($Ki67_{2w}$) predicted outcomes, using a dichotomized approach for Ki67 (low $< 10\%$; high $\geq 10\%$). With a median follow-up for the entire study of 5.2 (IQR, 4.8–6.2) years, short-term NET was not associated with improved clinical outcomes (hazard ratio for time to recurrence, 0.92, 95% CI, 0.75–1.12, $P = 0.40$). Within the NET-treated HER2-negative subpopulation (n = 2,235), $Ki67_B$ and $Ki67_{2w}$ levels were found to predict clinical outcomes. Although only approximately 15% of patients with low $Ki67_B$ received adjuvant CT, the 5-year recurrence risk in those with low $Ki67_B$ and low $Ki67_{2W}$ was 4.3% (95% CI, 2.9–6.3%). In patients with high $Ki67_B$ and low $Ki67_{2W}$, it was 8.4% (95% CI, 6.8–10.5%); and in patients with high $Ki67_B$ and high $Ki67_{2W}$, 21.5% (95% CI, 17.1–27.0%). Thus, the POETIC trial demonstrated the utility of Ki67 as a dynamic biomarker by measuring levels at baseline and particularly after short-term NET treatment to guide adjuvant treatment decisions in the HR + HER2-negative population. [66].

The phase 3 WSG-ADAPT HR + HER2-negative endocrine therapy sub-trial took the POETIC concept a step further and combined the baseline RS result and a response to a 3-week course of preoperative NET to guide adjuvant treatment in HR + HER2-negative EBC with up to 3 involved lymph nodes.[67] In this study, adjuvant ET was given to patients with RS 0–11 (control arm, n = 868), or to those whose RS was 12–25 with response to NET ($Ki67_{post} \leq 10\%$) (experimental arm, n = 1422). The ADAPT data showed that 5-year invasive disease-free survival (iDFS) in the experimental arm was non-inferior to that of the control arm (92.6% [95% CI, 90.8–94.0%] vs 93.9% [95% CI, 91.8–95.4%], respectively). Age (≤50, >50 years), grade 3 status, and ER at baseline were not significantly associated with iDFS. Thus, this study demonstrated the feasibility of combining the RS result with Ki67 response to short-term preoperative NET in guiding treatment decisions. It also showed that not only patients with RS 0–11 can be potentially spared CT, but also those with up to 3 positive nodes, RS results of 12–25, and Ki67 response irrespective of menopausal status.[67].

Importantly, when NET or short-term preoperative ET has been administered, the 21-gene assay should be performed on core biopsies and not the surgical specimen. The RS was developed and validated on untreated tumor specimens, and is highly dependent on ER signaling, showing a strong inverse correlation with ER-related genes and limited association with proliferation. As therapies such as NET markedly suppress ER signaling, posttreatment RS result may increase independent of true tumor biology, making molecular downstaging an unreliable pharmacodynamic marker for ER-targeted therapies.[68].

Impact of using the 21-gene assay in real-life clinical practice

The clinical impact of using the 21-gene assay on adjuvant treatment decisions has been extensively studied, with numerous retrospective and prospective studies from different countries in Europe, North America, Asia, and Australia consistently showing that 21-gene testing leads to a change in treatment recommendation in both directions (i.e., from CT plus ET to ET alone and vice versa) with an overall net reduction in CT use (reviewed in [69,70]). The comprehensiveness and consistency of the evidence reflect the integration of the 21-gene assay into the decision-making process in the BC adjuvant setting. In contrast, only 3 studies investigating the impact of the assay on neoadjuvant treatment decisions were identified and are summarized in Table 3.[53,71,72] These studies included patients who were candidates for neoadjuvant treatment and focused on the use of the 21-gene assay as a tool to potentially omit CT from the treatment plan. These small studies from the United States and Spain showed that following the assay, CT use in the neoadjuvant setting decreased by up to 76%, and that the changes in treatment recommendations were aligned with the RS results (i.e., the lower the RS results, the more likely patients were to be spared NCT). One of the studies also assessed the confidence of the physicians in their treatment recommendations before and after the RS results were available, and found that 45% of the treating physicians reported increased confidence in their treatment decisions after knowledge of the RS result. [72].

An additional study evaluating the impact of 21-gene testing on core biopsies in clinical practice is the recently completed randomized controlled PRE-DX trial in the UK. This study evaluated the impact of testing core biopsies vs surgical excision samples on the management pathway and patient-reported outcomes in grade 2/3 ER + HER2-negative EBC. [73] Notably, the study was not intended to change the patient's treatment pathway, but rather to explore using the RS result obtained from core biopsies to inform adjuvant treatment decisions. The study (n = 341) found that either testing core biopsies or surgical excision samples were associated with a similar number of clinical touchpoints between the treating team and the patient in the timeframe between the diagnostic clinic appointment and the offer/prescription of the first adjuvant cancer treatment (mean, 2.9 vs 3.2, respectively, $P = 0.23$). Testing the biopsies was also associated with a nonsignificant reduction in the median time from surgery to adjuvant treatment (49.5 vs 57.5 days, $P = 0.24$), and a statistically significant reduction in patient-reported anxiety and depression scores, presumably stemming from expedited counseling regarding the treatment recommendation based on the earlier knowledge of the RS.[73,74].

The degree of change in treatment recommendations alongside the observed increase in physicians' confidence in the decision impact studies suggests considerable uncertainty among physicians when recommending neoadjuvant treatment in this BC subtype. These findings, combined with the reported decrease in patients' anxiety and depression upon testing on core biopsies, highlight the benefits of early 21-gene testing.

	Reimbursement of the assay on core biopsies	Reimbursement of the assay for guiding neoadjuvant treatment
Italy	Likely not	No (only adjuvant)
France	Yes	Likely not
Japan	Yes	Potentially
Canada	Yes (varies by province)	Likely not
Spain	Yes	Yes
UK	Yes	No (only adjuvant)
USA	Likely not	Likely not
Israel	Not universally	Not universally
Netherlands	Yes	Yes
Switzerland	Yes	Potentially
Germany	Yes	No (only adjuvant)

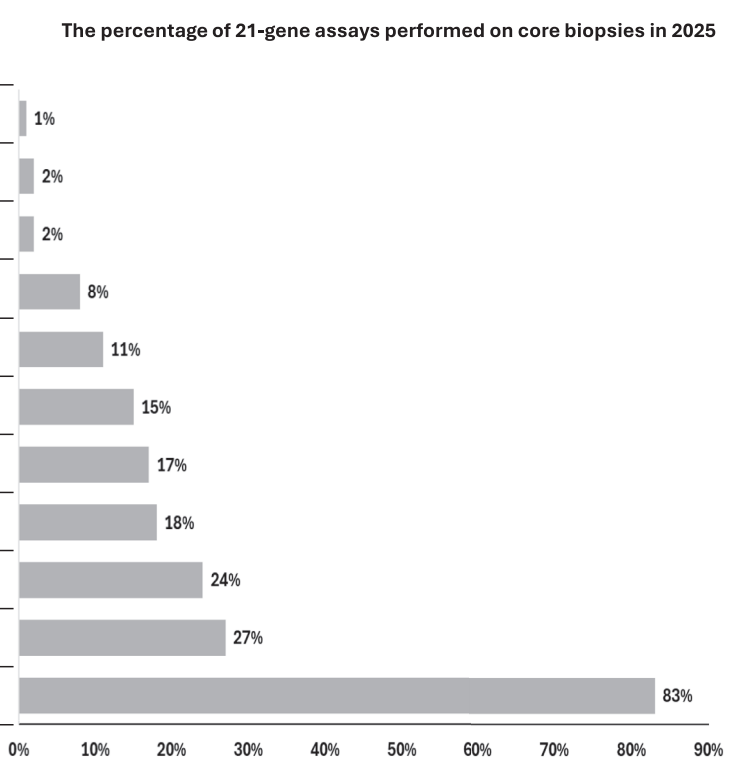

Fig. 1. The use of the 21-gene assay in the neoadjuvant setting: Summary of the reimbursement status and the extent of using the assay on core biopsies in select countries in 2025.

Use of the 21-gene assay on core biopsies in current clinical practice

Inclusion in treatment guidelines

The 21-gene assay is included as a tool to guide adjuvant treatment decisions in international guidelines published by the ESMO, St. Gallen, ASCO, and NCCN.[18–21] The recommendations of these guidelines regarding neoadjuvant treatment for HR + HER2- BC patients are summarized in **Supplementary Table S1**.[18,20,21,75,76] The guidelines are consistent in indicating that certain patients with HR + HER2-negative BC can be offered neoadjuvant treatment to improve surgical options. ASCO guidelines do not recommend the use of genomic assays to guide treatment decisions in the neoadjuvant setting, noting insufficient evidence.[76] In contrast, both ESMO and the St. Gallen guidelines indicate that the same considerations used to select adjuvant treatments also apply to the neoadjuvant setting, and suggest that gene expression profiling could provide useful information in the neoadjuvant setting as well. [18,75] The ESMO guidelines also specifically note that gene expression assays can be carried out on core biopsy samples.[18] The NCCN guidelines [20] point out that the 21-gene assay could guide treatment decisions in the neoadjuvant setting based on the studies by Pease et al [45] (for NCT) and TransNeos [60] (for NET).

Real-life use in recent years and current reimbursement status

The accumulation of evidence supporting the analytical validity of the assay on core biopsies and the clinical utility of the assay in the neoadjuvant setting coincided with changes in BC management due to the COVID-19 pandemic, such as the expanded use of NET in HR + HER2-negative patients in order to allow delays in surgery by 6–12 months. [77,78] Studies using the NCDB and the COVID-19-specific registry within the American Society of Breast Surgeons (ASBrS) Mastery demonstrated that NET rates in HR + HER2-negative BC increased by up to 5-fold during the pandemic. [79,80] The ASBrS study also reported that genomic testing using core biopsies approximately doubled during the pandemic (data on specific tests were not reported). [79] Accordingly, testing core biopsies has gained traction in real-life clinical practice in recent years, and this approach now represents a notable proportion of 21-gene testing based on testing volume from Exact Sciences.

The proportion of 21-gene assays being performed on core biopsies varies greatly by country. Fig. 1 presents data related to testing on core biopsies for 2025 in select countries, alongside the reimbursement status of performing the assay on core biopsies and using it to guide neoadjuvant treatment decisions in these countries. There is significant variability in testing on core biopsies from 83% in Germany to mere 1–2% in Italy, France, and Japan. [17] This variability is consistent with the reimbursement status of performing the assays on core biopsies and different conventional strategies of using the results to guide neoadjuvant treatment in each country (Fig. 1). Interestingly, in Italy, France, Japan, Canada, Spain, and the United States, the proportion of the assays being performed on core biopsies has been stable over time (2020–2025), whereas in the other countries, it has increased considerably in this timeframe (UK, from 10% to 15%; Israel, from 10% to 18%; Netherlands, from 0 to 24%; Switzerland, from 3% to 27%; and Germany from 25% to 83%).

Data gaps and future directions

Accumulating data support the use of the 21-gene assay to inform early treatment decisions in HR + HER2-negative breast cancer; however, randomized trials providing level 1A evidence in the neoadjuvant setting are lacking. In addition, certain clinical scenarios remain underexplored. One such setting is primary endocrine therapy (ET). In women ≥ 70 years with operable HR + HER2-negative disease, overall survival has been comparable between surgery plus ET and primary ET alone, although progression-free survival is inferior with ET alone. [81].

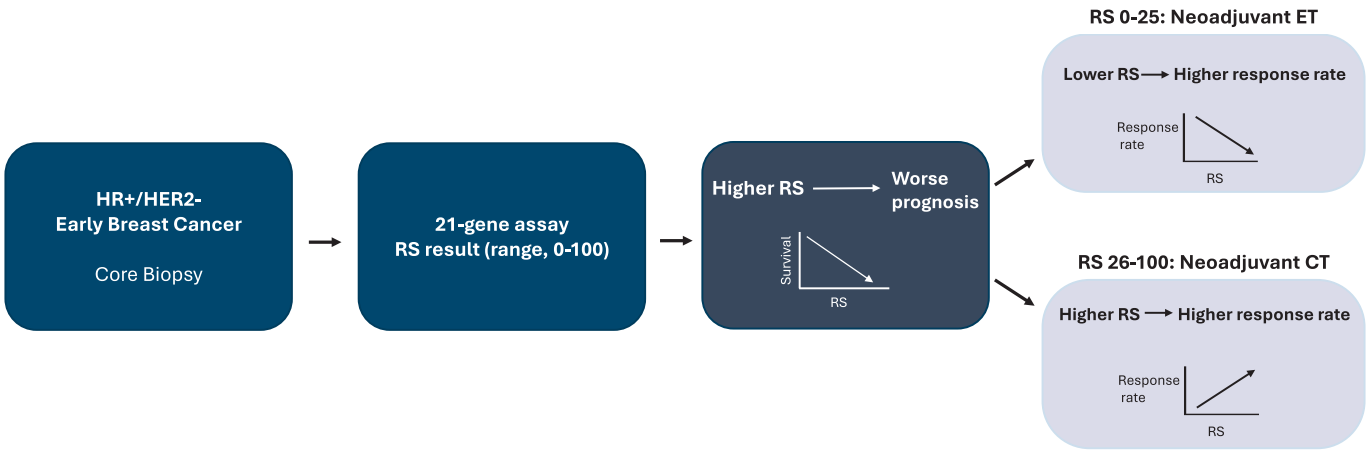

Fig. 2. Illustration of potential neoadjuvant treatment pathways informed by the 21-gene assay results on core-biopsy specimens.

In this context, a pretreatment core-biopsy-based RS could refine the selection of elderly or frail patients, those at high surgical risk, or those declining surgery. A low RS result may support de-escalation strategies, potentially including omission of surgery and/or radiotherapy. Trials such as CALGB 9343 and PRIME II have shown that radiotherapy omission in biologically low-risk older patients, in lieu of adjuvant ET alone, modestly increased local recurrence without affecting overall survival.[82,83] Integration of the RS into this framework could further improve biological risk stratification; however, prospective validation is needed.

Young women (≤40 years) account for approximately 10% of new breast cancer diagnoses, over half of whom have HR + disease, yet they remain underrepresented in clinical trials. [84] In TAILORx and RxPONDER, this age group comprised only 4.6% and 2.9% of participants, respectively. [12,14] Data in the neoadjuvant setting are similarly limited; only one small study specifically evaluated this subgroup and confirmed the association between high RS result and high pCR rates. [50] Given the distinct clinical behavior often observed in younger patients, the role of the 21-gene assay in guiding neoadjuvant decisions in this population remains insufficiently defined and warrants further investigation.

CDK4/6 inhibitors have been evaluated in the neoadjuvant HR + HER2-negative setting. In ADAPTcycle, biologically selected patients demonstrated similarly low pCR rates with ribociclib plus ET and with standard CT, highlighting limited chemosensitivity in this population. [85] In CARABELA, outcomes were comparable between endocrine-based CDK4/6 inhibitor therapy and CT in RS 0–25 tumors, whereas among RS 26–100 tumors, a trend in favor of CT was observed (P = 0.52). [86] Together, these findings suggest that baseline RS may help inform risk-adapted neoadjuvant strategies in the context of CDK4/6 inhibitors.

Building on these risk-adapted approaches, additional studies have explored whether CDK4/6 inhibitor–based regimens can also induce molecular downstaging. In CORALLEEN, patients with stage I-IIIA and tumor ≥ 2 cm received either neoadjuvant letrozole plus ribociclib or NCT. The proportion of patients with PAM50 low risk-of-relapse (ROR) at surgery (primary endpoint) was comparable between the groups, suggesting that select high-risk luminal tumors may achieve molecular risk reduction, as defined by ROR without cytotoxic therapy. [87] Similarly, in DxCARTES, patients with stage II–III HR + HER2-negative disease and baseline RS 18–100 received neoadjuvant endocrine therapy plus palbociclib. A substantial proportion achieved either pCR or reduction to RS ≤ 25 in the surgical specimen. [68] However, the biological interpretation of RS downstaging remains uncertain. As ET suppresses ER-driven transcriptional programs that contribute to the RS result, posttreatment changes may reflect pharmacodynamic effects rather than true shifts in intrinsic tumor biology. In contrast, proliferation-weighted signatures such as PAM50 may be less directly influenced by ER modulation. [88].

Another emerging area is the integration of the RS into neoadjuvant chemo-immunotherapy strategies. In KEYNOTE-756, the addition of pembrolizumab to CT improved pCR compared with CT alone in high-risk ER + HER2-negative disease. [89] In this context, tumors with low RS result, known to derive minimal benefit from CT, may also represent a population less likely to benefit from chemo-immunotherapy escalation, suggesting a potential role for genomic assays in treatment selection. Notably, I-SPY2 data indicate that other genomic signatures may help identify patients most likely to benefit from immunotherapy-based approaches. [90] The association between the RS result and immune-related biomarkers, including TILs and PD-L1, also remains poorly defined, with some evidence suggesting a weak positive correlation between these markers and the RS result. [91,92].

Conclusions

The 21-gene assay can be used to individualize treatment in the adjuvant and neoadjuvant settings, although the evidence supporting its use in the neoadjuvant setting is not as extensive and is evolving. Performing the assay as early as possible, namely, on a biopsy sample, provides relevant and clinically meaningful information at a critical crossroads in the decision-making process. The RS result could help inform decisions regarding whether to consider neoadjuvant therapy or proceed directly to surgery (with or without adjuvant CT), and if neoadjuvant treatment is considered, whether NCT or NET is more appropriate (Fig. 2). Furthermore, practically, using the assay early in the clinical pathway, even if no neoadjuvant treatment is given, can shorten the time to treatment decision, minimize the lag between diagnosis and treatment initiation, and help alleviate patients' anxiety regarding the key decision of adjuvant CT use.

Disclosures

AGZ: Advisory/consultancy role for AstraZeneca, Novartis, MSD, Pierre-Fabre, and Exact Science; participated in speaker bureau/expert testimony for Roche, AstraZeneca, Novartis, MSD, Pfizer, Lilly, and Pierre-Fabre; received research grant/funding (institution) from Pfizer and Lilly; and received travel/accommodation/expenses from Roche, Novartis, Pfizer, and Gilead. **TMA:** None declared. **RF:** employment, stock and other ownership interests, travel, accommodation, expenses from Exact Sciences (Abbott Cancer Diagnostics). **CP:** Advisory boards/honoraria for speaker events from AstraZeneca, Daiichi Sankyo, Eli Lilly, Eisai, Exact Sciences, Gilead, medac, MSD, Novartis, Pfizer, Roche, Seagen; support for attending meetings and/or travel from Gilead, Roche, Novartis. **OG:** Honoraria from Genomic Health/Exact Sciences, Roche, Pfizer, Novartis, Agendia, and AstraZeneca; served in consulting/advisory role for Genomic Health/Exact Sciences, Gilead, AstraZeneca, Lilly, Merck Sharpe and Dohme (MSD), Novartis, Pfizer, Daiichi Sankyo, and Roche; travel support from Pfizer and Daiichi Sankyo; and reports co-director position at West German Study Group. **SC:** Honorarium from Novartis, Hoffmann LaRoche, Pfizer, Eli Lilly, AstraZeneca, Amgen, Gilead, Merck, and Exact Sciences; research grants (to the BCCA) from Novartis, Hoffmann LaRoche, Pfizer, Genomic Health (Exact Sciences), AstraZeneca, Genentech, Celgene, Amgen, BMS, Merck, Sanofi, PUMA, and Gilead. **CS:** Honorarium from Astellas, Cepheid, Vertex, Seattle Genetics, PUMA, Amgen, Merck&Co., Inc, Novartis, Exact Sciences, Medquality; participated in a company-sponsored speaker's bureau for EISAI, Prime Oncology, Teva, Foundation Medicine, Genomic Health (Exact Sciences); other support from Genentech, Gilead, Pfizer, Roche. **GB:** Consulting fees from Roche, AstraZeneca, Novartis, MSD, Daiichi Sankyo, Pfizer, Menarini, Gilead and Exact Science; honoraria for speakers' bureaus from Roche, Pfizer, AstraZeneca, Eli Lilly, Novartis, MSD, Daiichi Sankyo, Gilead, Menarini and Exact Science; support for attending meetings from Roche, Pfizer, AstraZeneca, Takeda, Daiichi Sankyo, Gilead, MSD; and served on advisory boards for Roche, Pfizer, Daiichi Sankyo, Eli Lilly, MSD, Novartis, AstraZeneca, Exact Science, Gilead, Menarini and Helsinn.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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