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Oncotype DX Recurrence Score in premenopausal women

Shiliang Zhang, Kasey C. Fitzsimmons and Sara A. Hurvitz

***Abstract*:** In the past 20 years, clinicians have shifted away from relying solely on clinicopathologic indicators toward increasing use of multigene expression assays in guiding treatment decisions regarding adjuvant chemotherapy for early-stage hormone receptor (HR)-positive, HER2-negative breast cancer. Oncotype DX Recurrence Score (RS) is one of the most widely used multigene assays when considering indications for adjuvant chemotherapy, and guidelines have recently incorporated its use in women with early HR-positive HER2-negative breast cancer and up to three positive lymph nodes. While multiple retrospective and prospective clinical studies have demonstrated that most women with a low- to mid-range RS (0–25) can safely forgo chemotherapy, premenopausal women remain an important subgroup for which recommendations based on RS are ill-defined. The majority of patients included in clinical trials and retrospective analyses validating the use of RS have been postmenopausal women. In the subgroup of premenopausal women with HR-positive HER2-negative breast cancer, studies indicate that traditional clinicopathologic methods for assessing risk continue to be powerful tools when combined with RS to predict benefit from chemotherapy. This suggests that there is an element of uncaptured risk inherent to the premenopausal state that evades characterization by RS alone. This review describes the evidence that has supported the recommendation of RS in clinical guidelines, specifically focusing on data for its current use in premenopausal women. We review available data regarding the impact of the menstrual cycle on hormonally regulated gene expression, which may drive variations in the RS. Further research on the reliability and interpretation of the RS in the premenopausal subgroup is necessary and represents a gap in knowledge of how the RS should be applied in premenopausal women.

Keywords: breast cancer, genetic testing, hormone receptor–positive breast cancer, prognostic biomarkers, tumor biology

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Introduction

Annually, in the United States, nearly 200,000 women are diagnosed with hormone receptor (HR)-positive HER2-negative breast cancer, and of those, 32.1% are under the age of 50.[1,2] Among the different subtypes of breast cancer, HR-positive HER2-negative breast cancer is the most common – 68.2% of all breast cancers – and has the most favorable 5-year survival of 90.3%.[3,4] At the time of a new invasive breast cancer diagnosis, about 64% of patients with HR-positive HER2-negative breast cancer have local-stage, node-negative breast cancer with an estimated 5-year survival of over 99%.[4] However, age at diagnosis is a crucial prognostic indicator in HR-positive HER2-negative breast cancer. Women with HR-positive HER2-negative breast cancer diagnosed at a younger age (<40) have over double the rate of mortality compared with women diagnosed at an older age (51–60) when controlling for patient characteristics, disease, and treatment factors.[5] This significant disparity is not seen in other subtypes of breast cancer. Younger women with HER2-positive or triple-negative breast cancer do not have significantly worse outcomes compared to older women with

the same subtype of breast cancer.[5,6] In HER2-positive and triple-negative breast cancer, younger age at diagnosis is associated with either no increased mortality or borderline increased mortality, respectively, compared with older age at diagnosis.[5] While treatment guidelines for HER2-positive and triple-negative breast cancers do not differ based on age or menopausal status, treatment recommendations for HR-positive HER2-negative breast cancers are heavily reliant on these variables, which impact decisions regarding the addition of adjuvant chemotherapy. With the increasing adoption of multigene assays such as Oncotype DX Recurrence Score (RS), there is a trend toward personalizing treatment decisions based on an individual's tumor gene expression. However, the development of the RS has largely overlooked age and menopausal status as significant prognostic indicators. Subsequent prospective studies such as TAILORx and RxPONDER have distinguished different interpretations for the RS based on age and menopausal status as a result of subgroup analyses. At present, we review the development of the RS and the continued significance of age and menopausal status as prognostic indicators in the treatment of HR-positive HER2-negative breast cancer.

Development and uptake of Oncotype DX RS

Given the relatively promising outcomes of early HR-positive HER2-negative breast cancer, the ability to predict which subgroup of patients may benefit from aggressive therapy and which subgroup may forgo adjuvant chemotherapy is crucial to a clinician's treatment decisions. The likelihood of distant recurrence in node-negative patients treated with endocrine therapy alone at 10 years is about 15%.[7,8] Thus, while adjuvant endocrine therapy is recommended for nearly all patients with operable HR-positive breast cancer, only a subset of patients with a higher risk for recurrence benefit from adjuvant chemotherapy.[9] Traditionally, the decision to pursue aggressive treatment in HR-positive HER2-negative breast cancer has relied on a combination of clinical features such as age and menopausal status, as well as clinicopathologic factors such as tumor size, grade, lymph node involvement and intrinsic subtyping, which depends on the quantitative measurement of estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki67 by immunohistochemistry.[10,11] While intrinsic subtyping and histopathological evaluation can help determine prognosis, the reliance on these features as a risk stratification tool is limited by their lack of standardization across laboratories.[12] Multiple studies evaluating the reproducibility of Ki67 analysis demonstrate that even between experienced laboratories, there is significant interlaboratory variability.[13,14] Factors contributing to this discordance include differences in tissue region selection in a heterogeneous tumor, counting method, and subjective assessments of staining positivity. Furthermore, another shortcoming of the use of traditional clinicopathologic features and intrinsic subtyping is their lack of predictive ability in defining subsets of patients who may benefit most from adjuvant chemotherapy.[15] This has led to the development of commercially available, standardized multigene assays, including the 21-gene RS (Oncotype DX RS), the 70-gene signature (MammaPrint), the PAM50 risk of recurrence (ProSigna), and the 11-gene assay (Endopredict).[16] Arguably, the best-studied assay is the RS, which has been validated as both a prognostic and a predictive indicator in determining which patients may have reduced risk of distant recurrence with adjuvant chemotherapy.[17]

Oncotype Dx RS is a 21-gene quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay that evaluates the expression of 16 cancer-related genes and 5 reference genes. The cancer-related genes are further subdivided based on function and correlated expression to four group scores related to proliferation, estrogen, HER2, and invasion.[18] Expression of the 16 cancer-related genes is normalized against the reference genes and used to calculate the four group scores, which are then combined in a final composite RS on a scale from 0 to 100. Since the initial development of the RS in 2004, it has been validated retrospectively using data from NSABP B14, NSABP B20, ECOG 2197, SWOG 8814, and TransATAC and prospectively by the TAILORx and RxPONDER trials.[17–25] The test became commercially available in 2004, and subsequently there has been an abundance of supporting evidence for its use as a tool in guiding recommendations for adjuvant chemotherapy. This has resulted in its incorporation into major clinical guidelines by the American Society of Clinical Oncology (ASCO), National Comprehensive Cancer Network (NCCN), and European Society of Medical Oncology (ESMO), among others[26–28] (Figure 1).

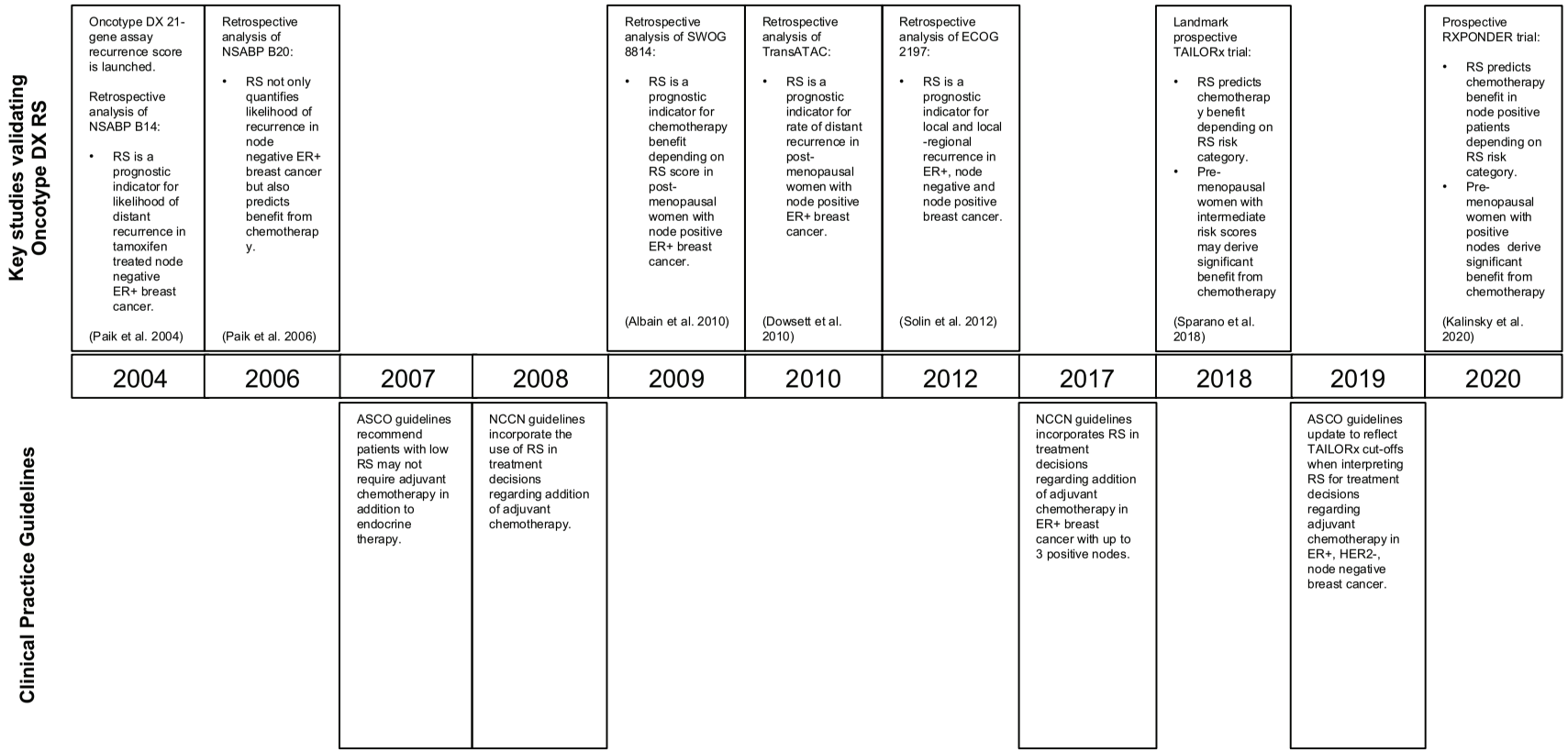

Figure 1. Timeline of key studies validating Oncotype DX RS and its incorporation into breast cancer treatment guidelines.

While several different multigene assays are commercially available, the RS remains one of the most well-researched, with validation from prospective clinical trials such as the recent RxPONDER.[24,25] Multiple studies have reported increased uptake of the RS among clinicians as a prognostic and predictive tool in guiding decisions regarding chemo-endocrine therapy and have demonstrated its contribution to reduced adjuvant chemotherapy prescription.[29–32] Data from Surveillance, Epidemiology, and End Results (SEER), SEER-Medicare, and SEER-Genomic Health indicate that among patients less than 50 years old, 57.2% received Oncotype DX RS testing from 2010 to 2015 in the period prior to the TAILORx trial. Among all patients with HR-positive HER2-negative early-stage breast cancers, 49.8% received RS testing, with an estimated cost of $115 million annually. This estimated cost of testing is only expected to rise in the post TAILORx trial era to approximately $231 million annually if 100% of eligible patients receive testing.[33]

Age-related interpretation of Oncotype DX RS and implications of menopausal status

Although Oncotype DX RS is often used to decide whether chemotherapy is necessary for early HR-positive HER2-negative breast cancer regardless of patient age, studies indicate that age and menopausal status significantly influence the interpretation of the RS, raising questions regarding the optimal use of this assay in younger women. The original development of the RS, based heavily on retrospective data from NSABP B14 and B20, resulted in the description of three categories for risk of recurrence: low risk (RS less than 18), intermediate risk (RS 18–30), and high risk (RS 31 or higher).[18,19] It is interesting to note that higher rates of distant recurrence at 10 years were observed in younger patients (<50 years of age) compared with older patients [21.1% (95% confidence interval: 15.1–26.8%) *versus* 12.3% (95% confidence interval: 9.1–15.3%)]. The decreased risk of recurrence in older patients was assumed to be secondary to higher expression of ER proteins in tumors of older patients, leading to a lower RS. Ultimately, the authors reported these findings as exploratory and did not offer age-dependent clinical recommendations for interpretation of the risk categories. In fact, the majority of retrospective analyses validating the RS overlooked the impact of age and menopausal status on risk classification. The TransATAC and SWOG8814 trials did not include any premenopausal patients, and retrospective studies evaluating the prognostic impact of RS using these trial data pertained only to postmenopausal patients.[22,23] Retrospective analyses of data from NSABP B14, B20, and ECOG 2197 did not include menopausal status as a demographic indicator. Instead, age was used as a surrogate.[20,21] From the original NSABP B14 and B20 trials, 69% and 52%, respectively, of participants were postmenopausal, and these trials used patient-reported menopausal status to guide their classification.[34] Patients who self-reported as perimenopausal were considered premenopausal by study definition. Both TAILORx and RxPONDER have since evaluated the effect of menopausal status as a subgroup of interest in their prospective investigations of Oncotype DX RS; however, postmenopausal participants continue to represent the majority of trial participants at about 66.0% and 66.8% respectively. With the goal of evaluating subgroups based on menopausal status, TAILORx and RxPONDER have since improved study definitions of menopause using a combination of age, date of last menstrual period, and serum FSH (follicle stimulating hormone) data. TAILORx defined menopausal status with criteria based on age-related cohorts: women 60 years and older were defined as postmenopausal and women less than 45 years old were defined as premenopausal. Among women with ages 45 to 59, menopause was defined as no menstrual period for at least 1 year or more prior to registration, or cessation of menstrual period for less than 1 year with an FSH level in the postmenopausal range, which the study defined as more than 34.4 IU/L.[35] RxPONDER similarly defined menopause as no menstrual period for at least 1 year. In addition, RxPONDER included premenopause criteria as having had a menstrual period within the past 6 months prior to registration. In patients where these definitions do not apply, the study used age-related cutoffs of less than 50 as premenopausal and 50 or older as postmenopausal.[25] Neither TAILORx nor RxPONDER reported the results of data collection based on prespecified definitions, and neither group reported how many participants' menopausal status defaulted to age-based categorization due to lack of data (Table 1).

Despite nonstandardized methods for defining menopausal status throughout landmark trials, the incorporation of menopausal status in evaluation of the RS has helped demonstrate its

Table 1. Percentage of premenopausal and postmenopausal patients enrolled in key studies validating oncotype DX RS.

Clinical trial	Total participants	Premenopausal	Postmenopausal	Summary findings
NSABP B-14[a] Paik *et al.* 2004	668 evaluated of 4028 in original trial	194 (29.0%)	474 (71.0%)	Patients less than 50 years old had higher rates of distant recurrence at 10 years compared with patients over 50 years old: 21.1% (95% CI: 15.1–26%) *versus* 12.3% (95% CI: 9.1–15.3%). Subsequent multivariate cox revealed that RS provides significant predictive power for distant recurrence independent of age.
NSABP B-20[a] Paik *et al.* 2006	651 evaluated of 2299 in original trial	289 (44.4%)	362 (55.6%)	Analysis of the entire NSABP B20 cohort revealed significant interaction between age and chemotherapy, with older age associated with less benefit from chemotherapy. In the subset of samples tested for RS in this study, there was no significant association between age and chemotherapy benefit ($p = 0.162$). However, there was a significant interaction between chemotherapy treatment and RS ($p = 0.038$), suggesting that RS is the stronger predictor of chemotherapy benefit.
ECOG 2197[a] Goldstein *et al.* 2008	465 evaluated of 2882 in original trial	193 (41.4%)	272 (58.6%)	This trial enrolled patients with 0–3 positive axillary lymph nodes and found significant correlation between age and recurrence when comparing patients <45 and >65 (HR: 2.39, 95% CI: 1.04–5.51, $p = 0.02$) with younger age correlating to higher chance of recurrence.
SWOG 8814 Albain *et al.* 2010	367 evaluated of 1477 in original trial	0 (0%)	367 (100%)	This trial included only postmenopausal women and demonstrated that RS predicts significant chemotherapy benefit in patients with positive nodes and a high RS (>31), while there was no significant benefit from chemotherapy in patients with positive nodes and low RS (<18).
TransATAC Dowsett *et al.* 2010	1231 evaluated of 5216 in original trial	0 (0%)	1231 (100%)	This trial included only postmenopausal women and demonstrated that RS is an independent predictor of distant recurrence in node-negative and node-positive patients.
TAILORx Sparano *et al.* 2018, 2019	9719	3300 (34.0%)	6419 (66.0%)	This trial demonstrated chemotherapy benefit in 5-year invasive disease-free survival (IDFS) in premenopausal women with RS of 11–25 (HR: 1.36, 95% CI: 1.06–1.75). There was no significant chemotherapy benefit in postmenopausal women with the same RS category 11–25 (HR: 0.99, 95% CI: 0.84–1.17). Benefit from chemo-endocrine therapy was especially pronounced in premenopausal women with RS 16–20 (HR: 1.76, 95% CI: 1.20–2.59, $p = 0.0034$).
RxPONDER Kalinsky *et al.* 2021	5015	1665 (33.2%)	3350 (66.8%)	Among premenopausal women with RS of 0–25, the 5-year IDFS was improved with chemo-endocrine therapy, 93.9%, over endocrine therapy alone, 89.0% (HR: 0.60; 95% CI: 0.43–0.83; $p = 0.002$). In contrast, in postmenopausal women with RS of 0–25, 5-year IDFS did not improve with chemo-endocrine therapy, 91.3% compared with endocrine therapy alone, 91.9% (HR: 1.02; 95% CI: 0.82–1.22, $p = 0.89$).

CI, confidence interval; HR, hormone receptor; RS, Recurrence Score.
[a]In studies where authors did not specify menopausal status, it was assumed participants less than 50 years old are premenopausal and participants 50 years or older are postmenopausal.

significant impact on the interpretation of the RS. TAILORx was the first prospective trial to validate the RS and to include age and menopausal status within its subgroup analysis of outcomes correlating with the RS. Using slightly lower RS thresholds for each risk category (low risk <11, intermediate risk RS 11–25, and high risk RS >25), the authors demonstrated that among women with HR-positive HER2-negative lymph node–negative breast cancer and a midrange RS of 11–25, endocrine therapy was noninferior to chemo-endocrine therapy.[36] However, the authors found significant interactions between age and RS, which when further analyzed were more clearly represented as interactions between menopausal status and RS. Subgroup analysis of women younger than 50 years old with an RS of 16–25 demonstrated lower rates of distant recurrence associated with chemo-endocrine therapy compared with endocrine therapy alone. In women with lower midrange RS of 16–20, the percentage point difference for distant recurrence was 1.6% at 9 years. In women with higher midrange RS of 21–25, the percentage point difference was increased to 6.5% at 9 years. Therefore, younger women with higher midrange RS had a significant clinical benefit of over 5% decrease in distant recurrence when adjuvant chemotherapy was added to endocrine therapy. These findings have led to increased caution when deciding to forgo chemotherapy in women younger than 50 years old, with guidelines recommending chemo-endocrine therapy in the subgroup of women younger than 50 with intermediate RS of 16–25.[27] In a more recently published iteration of the TAILORx data, these age-related chemotherapy benefits were further analyzed and found to be most evident in premenopausal women between 46 and 50 years old, women who are more likely to become menopausal with the use of chemotherapy than those in younger age groups. Of note, similar benefits with chemotherapy use in distant recurrence at 9 years were not seen in postmenopausal women of this same age group, suggesting that the chemotherapy benefit is likely dependent on menopausal status rather than age.[37]

Menopausal status-related chemotherapy benefits are further corroborated by data from the prospective clinical trial RxPONDER, in which patients with HR-positive HER2-negative breast cancer with up to three positive lymph nodes and an RS in the low and intermediate risk range (RS 0–25) were evaluated.[24] RxPONDER data demonstrate that benefit from chemotherapy for the outcome of invasive disease-free survival (IDFS) at 5 years is observed only within the premenopausal group. In this group, 5-year IDFS was 93.9% with chemo-endocrine therapy and 89.0% with endocrine therapy alone (HR: 0.60; 95% CI: 0.43–0.83; $p=0.002$). This represents a 40% risk reduction of an IDFS event for premenopausal women treated with adjuvant chemotherapy. Distant relapse-free survival (DRFS) showed a similar pattern, in which premenopausal women had improved DRFS at 5 years with adjuvant chemotherapy compared with those with only endocrine therapy – 96.1% *versus* 92.8% (HR: 0.58; 95% CI: 0.39–0.87; $p=0.009$). Overall survival data from the RxPONDER trial are not yet mature.[25] Postmenopausal patients with low and intermediate risk range RS derived no benefit from chemotherapy. These findings from the TAILORx and RxPONDER trials have led to guidelines incorporating menopause status as an important variable in the interpretation of the RS (Figure 2).

Function of ovarian suppression in premenopausal patients

Evidence of clinically significant chemotherapy benefits in premenopausal women has led to the primary hypothesis that chemotherapy acts as a form of ovarian suppression causing premature menopause in younger women.[17] This is an especially attractive theory given that the TAILORx subgroup found to benefit most from chemotherapy were premenopausal women from ages 46 to 50 – theoretically, the patients most likely to be on the precipice of menopause and for whom chemotherapy may have induced lasting menopause. Since the prospective trials TAILORx and RxPONDER began enrollment in 2006 and 2011, respectively, there has been increasing evidence for the benefits of ovarian suppression in premenopausal patients with early-stage HR-positive breast cancer.[38] The results of the TEXT and SOFT trials in 2014 and 2015 led to subsequent guideline modifications to include ovarian suppression with endocrine therapy in premenopausal patients.[39,40] It is possible that the increased chemotherapy benefit seen in TAILORx and RxPONDER was due to menopause induction during a time when most premenopausal trial participants were not prescribed ovarian suppression. In the TAILORx trial, only 13% of premenopausal women with intermediate RS were prescribed ovarian suppression, and outcomes

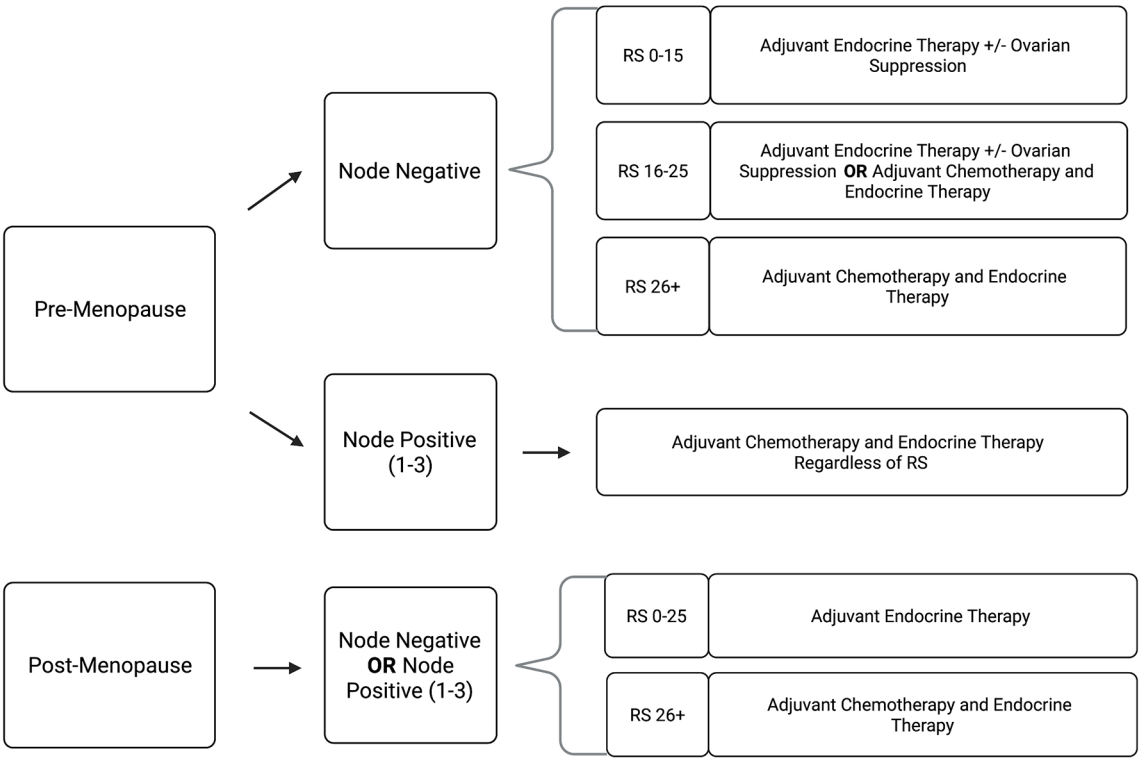

Figure 2. Oncotype Dx 21-gene assay recurrence score interpretation in premenopausal and postmenopausal women with early-stage ER-positive, HER2-negative breast cancer.[27]

based on this addition to endocrine therapy or chemo-endocrine therapy were not reported.[17] In the RxPONDER trial, for premenopausal patients, ovarian suppression was added to treatment plans of 19% of patients receiving endocrine therapy and 6.3% of patients receiving chemo-endocrine therapy. Exploratory analyses reported by the recent publication of the RxPONDER trial found no significant decrease in IDFS when comparing premenopausal women who received endocrine therapy and ovarian function suppression with those who did not receive ovarian function suppression (HR: 0.71; 95% CI: 0.38–1.30). However, the authors note that these findings are preliminary and exploratory in nature, do not adjust for multiplicity, and may lack power due to small sample sizes.[25]

Overall, the interpretation of intermediate RS of 16–25 in premenopausal women remains nuanced. Due to the clinically significant chemotherapy benefit in this subgroup, most guidelines recommend adjuvant chemotherapy, with some recommending the alternative option of ovarian function suppression combined with tamoxifen or aromatase inhibitor.[27] In younger breast cancer patients, whose cancers tend to be more aggressive and are subsequently associated with higher rates of distant recurrence, RS alone appears less reliable in guiding treatment decisions. In these cases, traditional methods of assessing clinical risk through tumor size and histologic grade continue to add important prognostic information. In a more recent iteration of the TAILORx trial, Sparano *et al.* evaluated RS with the additional variable of low or high clinical risk, as defined by a combination of tumor size and histologic grade, to evaluate whether the combination of clinical risk and RS may refine estimations on prognosis. In women <50 with intermediate RS (11–25) and high clinical risk, distant recurrence rates at 9 years were lower with adjuvant chemotherapy ($6.1 \pm 1.8\%$ in the treatment group compared with $12.3 \pm 2.4\%$ in the observation group), whereas in women with intermediate RS (11–25) and low clinical risk, the rates of distant recurrence were similar regardless of adjuvant chemotherapy ($3.9 \pm 1.0\%$ in the treatment group and $4.7 \pm 1.0\%$ in the observation group). Clinical risk appeared to estimate chemotherapy benefit best in the midrange intermediate risk group (RS of 16–20), where adjuvant chemotherapy in

patients with high clinical risk resulted in a $6.5 \pm 4.9\%$ difference in benefit from reduced distant recurrence compared with $-0.2 \pm 2.1\%$ difference in the low clinical risk group.[41] These findings have propelled further work investigating the integration of clinical risk factors into the RS to create novel tools such as RSClin.[42]

The role of the menstrual cycle on prognostic biomarkers and Oncotype DX

In addition to gaps in knowledge regarding how menopausal status and the use of ovarian suppression influence the interpretation and applicability of RS in younger women, questions also remain regarding whether tumor gene expression itself is directly influenced by circulating levels of estradiol and progesterone. If this is the case, then the RS could vary based on menstrual cycle phase at the time of tumor sampling and such variability could make the RS less consistent and reliable in a premenopausal woman. Most of the data relating to this question are circumstantial, highlighting the need for prospective studies to address this issue.

It has been established that morphological changes in breast tissue including proliferation, differentiation, and apoptosis correspond to changes in endogenous hormones throughout the menstrual cycle.[43] The association between changes in the hormonal milieu and treatment outcomes is less well established but has been evaluated in a variety of breast cancer studies. Some have hypothesized that the tumor microenvironment may be significantly influenced by menstrual cycling, such that tumors may have less metastatic potential when removed at certain points of the menstrual cycle.[44] This has been the focus of a number of clinical studies since the 1980s, though multiple systematic reviews have determined that there is insufficient evidence to recommend surgery during one phase of the menstrual cycle over another.[45–47] These inconclusive findings may be in part due to inherent challenges in studying the menstrual cycle, which include disagreement in the establishment of menstrual cycle windows, variability in hormone profiles between women, and the paucity of prospective randomized controlled trials.[44]

There are also data demonstrating that prognostic biomarkers may be influenced by the menstrual cycle. Horimoto *et al.* published an observational study of premenopausal patients with breast cancer showing that expression of Ki67, a marker of proliferation, fluctuates during different phases of the menstrual cycle, with higher mean expression seen in the luteal phase.[12] In addition to Ki67, studies have shown that ER concentration fluctuates in normal breast tissue as well as breast tumors, with higher ER expression in the follicular phase (when progesterone is decreased) compared with the luteal phase.[48–50] These hormonally influenced variations in biomarker expression in breast tumor tissue imply that gene expression profiles, including the RS, may vary based on the menstrual cycle. Much of the research investigating this question has been conducted by Haynes *et al.*, whose work has evaluated gene expression variation associated with the menstrual cycle in ER-positive breast cancer.[51] This group has sampled breast tumor tissue paired with serum hormone measurements to prospectively evaluate the effect of different menstrual cycle windows on RNA expression of 45 genes. The genes evaluated were subdivided into groups of estrogen-regulated genes, progesterone-regulated genes, and proliferation-associated genes. Composites of notable genes in each group were measured based on three menstrual period windows defined by varying levels of serum estrogen and progesterone. The authors found a significant increase in the average estrogen-regulated gene expression and average proliferation regulated-gene expression as the menstrual cycle progressed from early to mid and late cycle. The study included three genes evaluated within the RS panel – *PGR* (encoding PR), *MKI67* (encoding Ki67), and *AURKA* – and found significant changes in the expression of *PGR* and *MKI67* with increased expression in mid to late menstrual cycle (Table 2).[51]

Earlier retrospective data by the same group have also demonstrated significant changes in the expression of multiple genes evaluated by the RS including *PGR*, *MKI67*, *CCNB1*, *BIRC5*, and *MYBL2*.[52,54] This has propelled further interest in the evaluation of RS throughout the menstrual cycle. In a study by Bernhardt *et al.*, discordance in RS between time of breast biopsy and breast surgery was measured. This group found higher discordance in RS in women under 50 years old compared with those of women over 50 years old, and increasing discordance correlated inversely with age. In this study, differences in expression of the proliferation and HER2 group scores were largely responsible for the variation in RS.[55] Due to the retrospective nature of the study, menstrual

Table 2. Oncotype DX RS group score components and variation in gene expression associated with menstrual cycling.

Group score category	Gene expression	Changes in expression	References
Proliferation	Ki67	Higher expression on IHC in luteal phase. Higher gene expression in mid to late menstrual cycle.	(Horimoto *et al.*[12]) (Haynes *et al.*[51])
	STK15 (AURKA)	No significant change in gene expression when comparing early with mid and late phase of menstrual cycle.	(Haynes *et al.*[51])
	Survivin (BIRC5)	Higher gene expression in mid menstrual cycle.	(Haynes *et al.*[52])
	CCNB1 (Cyclin B1)	Higher gene expression in mid menstrual cycle.	(Haynes *et al.*[52])
	MYBL2	Higher gene expression in mid menstrual cycle.	(Haynes *et al.*[52])
Invasion	MMP11 (stromolysin 3)	No significant change in invasion group score throughout menstrual cycle.	(Haynes *et al.*[53])
	CTSL2 (cathepsin L2)		
HER2	GRB7	No significant change in HER2 group score throughout menstrual cycle.	(Haynes *et al.*[53])
	HER2		
Estrogen	ER	Higher gene expression in follicular phase.	(Pujol *et al.*[49]) (Kundaktepe *et al.*[50])
	PGR	Higher gene expression in mid to late menstrual cycle.	(Haynes *et al.*[54]) (Haynes *et al.*[53])
	BCL2	No significant change in gene expression when comparing early with mid and late phase of menstrual cycle.	(Haynes *et al.*[52])
	SCUBE2	No significant change in gene expression when comparing early with mid and late phase of menstrual cycle.	(Haynes *et al.*[52])
Other	GSTM1	Not evaluated or results not reported.	
	CD68		
	BAG1		

ER, estrogen receptor; IHC, immunohistochemistry; RS, Recurrence Score.

cycle data at the time of breast tumor sampling were not collected or assessed. However, interestingly, when the same group evaluated Oncotype DX RS in a HR-positive mammary tumor mouse model, Bernhardt *et al.* found increased expression in these same group genes (proliferation and HER2), which were responsible for increased RS in the mice diestrus phase, corresponding to the human luteal phase.[56]

Perhaps the best study evaluating the possible effect of the menstrual cycle on RS is the 2021 study by Haynes *et al.*[53] The authors evaluated paired tumor samples of ER-positive breast cancer taken at breast biopsy and again at 1 to 4 weeks later along with corresponding serum hormone concentrations and menstrual cycle history. The authors predefined two different menstrual cycle windows: W1, corresponding to low estrogen and low progesterone, occurring very early or late in the menstrual cycle at days 1–6 or 27–35; and W2, corresponding to high levels of estrogen and low or high levels of progesterone, occurring at mid to late cycle on days 7–26. The authors then calculated the RS for each of these tumor samples based on a previously validated gene expression

system used to approximate RS.[57] The authors found no significant change in the mean RS score when comparing between W1 and W2 (26.7 ± 3.5 *versus* 26.9 ± 3.9; Wilcoxon $p = 0.96$); however, they did find a clinically significant reassignment of risk category in six (27.3%) tumors, which would be classified differently in W2 compared with W1 ($\kappa = 0.54$, 95% CI: 0.27–0.80). It should be noted that the authors also evaluated variation in RS when sampling was done in the same window and found that four (50%) tumors were classified differently when measured in the same window. Evaluation of individual group scores of the RS showed significantly higher estrogen group score in W2 (+16.6%; $p = 0.046$), while trends of the other group scores did not reach statistical significance. Invasion group score trended toward being lower in W2, proliferation group score trended toward being higher in W2, and the HER2 group score showed no notable change.

Overall, the available evidence is inconclusive regarding the effects of the menstrual cycle on RS. While there was no evidence of statistical significance in RS variation in the Haynes-defined menstrual cycle windows, there was a clinically significant reassignment of risk in 27% of tumors sampled in different windows. However, the study also found that tumors tested within the same predefined windows had reassignment of RS risk category in 50% of cases. This suggests that the predefined menstrual cycle windows may not adequately capture gene expression variability. Another challenge in interpreting this study lies in the lack of clinical criteria in determining menstrual cycle regularity among study participants, who were included if they self-reported regular menstrual cycles. One-third of participants were 45 or older, and the presence of self-reported regular menstrual cycles may not accurately exclude those who have much longer cycles with infrequent periods and overall decreased hormonal variation. Further weaknesses of this study include the small sample size; the definition of the menstrual cycle based on serum estrogen levels and not on follicular or luteal phases, which prevents comparison with other previous studies; and the estimation of RS using their own gene expression system rather than the Oncotype DX RS from Exact Sciences. Given the limited data in this field, largely driven by one group, there is a need for larger prospective studies to better evaluate the impact of menopausal status on the interpretation of RS and the possible variability of RS risk categorization based on cyclical changes in endogenous hormones.

Conclusions and future directions

As strategies in the treatment of breast cancer continue moving toward reducing overtreatment and unnecessary toxicity, landmark studies such as TAILORx and RxPONDER have helped define prognostic and predictive criteria that breast oncologists use on a daily basis to risk stratify patients with early HR-positive HER2-negative breast cancer. While the Oncotype DX RS is indicated for use in these patients regardless of age, its interpretation is less clear in premenopausal patients, relying on subgroup analyses for treatment recommendations. The RS was created and validated using data primarily from postmenopausal women. Its predictive ability in premenopausal women failed in RxPONDER, which could not distinguish, as TAILORx had, any further subdivision of low and intermediate risk categories in which premenopausal women may forgo chemotherapy. There has yet to be a large prospective analysis of the predictive capacity of the RS in young women, nor has there been an in-depth biomarker study in a large group of premenopausal women.

While multiple studies cite ovarian suppression as the primary method by which adjuvant chemotherapy benefits premenopausal patients with HR-positive HER2-negative breast cancer, there have been no prospective studies with randomized treatment arms to evaluate this theory. TAILORx did not report outcomes based on addition of ovarian suppression to endocrine or chemo-endocrine therapy, and although RxPONDER did report these results, the authors concede that the data are exploratory in nature and not powered to evaluate the impact of ovarian suppression. While it would be optimal if studies evaluating therapies and outcomes in hormonally responsive breast cancer clearly defined menopausal status of participants, it is extremely challenging to do so. The TAILORx and RxPONDER trials both used as one of their criteria the lack of menstrual period for at least 1 year to define menopause. While this is adequate to define clinical menopause, it fails to recognize those women who may be peri-menopausal, with increasingly infrequent and irregular menstrual cycles. Inherently, research on the menstrual cycle is complicated – women can have varying lengths of cycles and irregular cycles. This is made even more complex in a patient with

breast cancer as medications used to treat this disease such as chemotherapy, tamoxifen, and luteinizing hormone–releasing hormone (LHRH) agonists may affect menstrual regularity as well as circulating hormone levels. Lack of menstruation for 12 months in the context of these factors may not be true menopause, nor is age a reliable marker of menopause. The TAILORx trial incorporated an elevated FSH as one criterion for menopause; however, it was not reported how often these data were readily available, nor whether these were affected by concomitant medication (e.g. tamoxifen) use. In large prospective studies on the scale of RxPONDER and TAILORx, with thousands of participants from multiple countries, standardization of hormone testing may also be difficult to establish. Moreover, patients may start on trial premenopausal and go into menopause during the course of the study, making it all the more complicated to analyze. It is therefore incredibly challenging to accurately distinguish pre- from postmenopausal women in trials as large as these, with follow-up as long as these studies have. There are data demonstrating that the absence of anti-mullerian hormone (AMH) is a reliable predictive indicator for loss of ovarian function in premenopausal women after chemotherapy.[58] Future studies can consider the use of AMH along with traditionally accepted criteria using FSH or menstrual history to diagnosis and predict premature ovarian insufficiency.

To isolate the benefits of chemotherapy from its ovarian suppressive effects, a prospective randomized trial is needed in which only premenopausal women with an intermediate RS are enrolled and receive either ovarian suppression plus endocrine therapy or chemotherapy plus ovarian suppression and endocrine therapy. Such a trial could enroll those with node-negative or 1–3 node-positive disease to address outstanding questions remaining from both TAILORx and RxPONDER.

In addition to establishing whether chemotherapy is exerting benefits outside of ovarian ablation, more research is needed to establish the impact of circulating hormones on RS. There is a clear biological rationale that fluctuations in endogenous hormones could impact tumor gene expression. While our review of current literature found inconclusive evidence for statistically significant variability of RS associated with menstrual cycling, Haynes *et al.* data suggest that variations in RS can be clinically significant, leading to reassignment of risk categories and possible subsequent changes in treatment recommendations.[53] Addressing this question would not require a large randomized trial. Instead, a relatively small study could be conducted in which serum hormone levels, tumor RS testing and menstrual history are obtained twice (at the time of breast biopsy and breast surgery) from premenopausal patients diagnosed with HR-positive HER2-negative breast. In this way, the effect of hormone levels and menstrual phases on RS can be measured.

In summary, further research is needed to understand the impact of hormone levels on RS results and to validate the use of RS in premenopausal women. If RS varies by menstrual cycle phase, this could have a major impact in treatment decision-making, possibly leading to over- or undertreatment of a large proportion of patients. Furthermore, the economic impact of inaccurate risk predictions in premenopausal women should not be overlooked. At a Medicare reimbursement rate of about $3400 a test, the RS is often cost prohibitive for patients lacking adequate insurance.[59] Estimated costs of testing are expected to increase as landmark trials such as TAILORx and more recently RxPONDER expand the indications for RS testing. Given the high cost of RS testing, one economic justification for its continued use is that it may reduce chemotherapy-related costs for patients who can forgo chemotherapy. Given that premenopausal women account for a large number of patients requiring chemotherapy based on the RS, imprecise risk stratification among this subgroup can have significant economic consequences. As we increasingly rely on tumor gene expression to tailor individualized treatment plans, we must consider whether our interpretation of these tests are broadly applicable to all patient subgroups and identify when further refinement is necessary. Among premenopausal women with HR-positive HER2-negative breast cancer, further research is essential to establish the validity and prognostic value of the RS.

Author contributions

Shiliang Zhang: Conceptualization; Data curation; Writing – original draft; Writing – review & editing.

Kasey C. Fitzsimmons: Data curation; Writing – review & editing.

Sara A. Hurvitz: Conceptualization; Data curation; Supervision; Writing – original draft; Writing – review & editing.

Conflict of interest statement

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