Ontario Health
Cancer Care Ontario

Guideline MOTAC-4 Version 2 IN REVIEW

A Quality Initiative of the Program in Evidence-Based Care (PEBC), Ontario Health (Cancer Care Ontario)

Clinical Utility of Multigene Profiling Assays in Early-Stage Invasive Breast Cancer

P. Blanchette, D. Sivajohanathan, J. Bartlett, A. Eisen, H. Feilotter, R. Pezo, G. Turashvili, P. Williams, and the Multigene Profiling Assays in Early-Stage Invasive Breast Cancer Expert Panel

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An assessment conducted in February 2025 placed Guideline MOTAC-4 Version 2 IN REVIEW. This means that it is undergoing a review for currency and relevance. It is still appropriate for this document to be available while this updating process unfolds. The PEBC has a formal and standardized process to ensure the currency of each document (PEBC Assessment & Review Protocol)

Guideline MOTAC-4 Version 2 is comprised of 5 sections. You can access the summary and full report here:
https://www.cancercareontario.ca/en/guidelines-advice/types-of-cancer/31766

Section 1:	**Recommendations Summary**
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PUBLICATIONS RELATED TO THIS REPORT

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Clinical Utility of Multigene Profiling Assays in Early-Stage Invasive Breast Cancer

Section 1: Recommendations

This section is a quick reference guide and provides the guideline recommendations only. For key evidence associated with each recommendation, see Section 2.

GUIDELINE OBJECTIVES
To update clinical guidance on the use of multigene profiling assays in individuals with early-stage invasive breast cancer.

TARGET POPULATION
Individuals diagnosed with early-stage invasive breast cancer for whom further information is needed for prognosis and treatment decision making. In this guideline, early-stage invasive breast cancer is defined as stage I to III breast cancers that are surgically operable and do not have evidence of inflammatory, locally recurrent or distant metastatic disease with pT1-T3, pN0-N1a based on surgical pathologic staging.

INTENDED USERS
This guideline is targeted for clinicians and policy makers involved in the diagnosis and treatment of breast cancer.

PREAMBLE
The purpose of this guideline is to determine the clinical utility of multigene profiling assays (i.e., Oncotype DX, MammaPrint, Prosigna, EndoPredict, and Breast Cancer Index), not to identify which assay is better. No prospective studies have compared these head-to-head. Given that the assays use different scoring systems and classification systems, please refer to Table 1-1 for a summary of each of the assays. Further, this guideline does not cover the utility of multigene profiling assays in helping to guide clinical treatment decisions regarding the use of either neoadjuvant chemotherapy or radiation.

RECOMMENDATIONS, KEY EVIDENCE, AND JUSTIFICATION

Recommendation 1
In patients with early-stage estrogen receptor (ER)-positive/human epidermal growth factor 2 (HER2)-negative breast cancer, clinicians should consider using multigene profiling assays (i.e., Oncotype DX, MammaPrint, Prosigna, EndoPredict, and Breast Cancer Index) to help guide the use of systemic therapy.
Qualifying Statements for Recommendation 1
• There is currently insufficient evidence to use multigene profiling assays among patients with either HER2-positive or triple negative breast cancers. • Multigene profiling assays are recommended for use in patients with lymph node-negative or lymph node-positive (1-3 lymph nodes) disease who are under consideration for adjuvant chemotherapy if the use is supported by other clinical, pathological, or patient-related factors. Clinical and pathological features include patient age, tumour grade, tumour size and nodal status. • One multigene profiling assay should be requested per patient to guide a specific treatment decision. Requesting multiple tests with different multigene profiling assays on an individual tumour specimen to guide a single treatment decision is discouraged.

Additional testing may be considered for patients with either repeat metachronous breast cancer diagnoses or synchronous breast cancer diagnoses where tumour specimens display varying morphologies, grade or hormone receptor status.

- Multigene profiling assays should be interpreted cautiously in premenopausal patients where a significant benefit from adjuvant chemotherapy may still exist despite a low-risk score.

Recommendation 2

In patients with early-stage node-negative ER-positive/HER2-negative disease, clinicians may use a low-risk result from Oncotype DX, MammaPrint, Prosigna, EndoPredict/EPclin, or Breast Cancer Index assays to support a decision not to use adjuvant chemotherapy.

Qualifying Statements for Recommendation 2

- Patients <50 years of age may still benefit from chemotherapy despite low-risk scores from multigene assay testing. Risk scores should be interpreted with caution and decisions should be made while considering other clinical, pathological, or patient-related factors.
- Treatment decisions should be based on all available clinical and pathological information for each patient, rather than depending entirely on multigene profiling test results.
- In patients with a low-grade tumour (i.e., grade 1) less than 1 cm in size, the Working Group members do not recommend a multigene assay profiling as this is unlikely to inform a treatment decision to use adjuvant chemotherapy.

Recommendation 3

In patients with node-negative ER-positive/HER2-negative disease, clinicians may use a high-risk result from Oncotype DX to support a decision to offer chemotherapy. A high Oncotype DX recurrence score is capable of predicting adjuvant chemotherapy benefit.

Qualifying Statements for Recommendation 3

- MammaPrint, Prosigna, EndoPredict or EPclin, and Breast Cancer Index do not have sufficient evidence to support a predictive benefit of adjuvant chemotherapy among clinically low-risk patients with breast cancer whose multigene profiling testing indicates a high-risk score.

Recommendation 4

In postmenopausal patients with ER-positive/HER2-negative tumours and one to three nodes involved (N1a disease), clinicians may withhold chemotherapy based on a low-risk Oncotype DX or MammaPrint score if the decision is supported by other clinical, pathological, or patient-related factors.

Qualifying Statements for Recommendation 4

- Premenopausal patients <50 years of age have a significant benefit from chemotherapy despite low-risk scores from multigene assay testing. Risk scores should be interpreted with caution and decisions should be made while considering other clinical, pathological, or patient-related factors.
- It is uncertain whether at least some of the benefit of chemotherapy among premenopausal patients may be due to chemotherapy induced amenorrhea versus the cytotoxic effects of treatment.
- The Prosigna, EndoPredict/EPclin, and Breast Cancer Index assays can identify low-risk node-positive patients whose prognostic outcomes are favourable; however, these assays have not demonstrated predictive evidence to support withholding adjuvant

chemotherapy among higher risk, node-positive, ER-positive, HER2-negative breast cancer patients.

Recommendation 5

The evidence to support the use of molecular profiling to select the duration of endocrine therapy is evolving. In patients with ER-positive disease, clinicians may consider using a Breast Cancer Index (BCI) (H/I) high assay result to support a decision to extend adjuvant endocrine therapy if the decision is supported by other clinical, pathological, or patient-related factors.

Qualifying Statements for Recommendation 5

- While a number of studies have demonstrated clinical utility of BCI for extending adjuvant endocrine therapy, the preliminary results of the NSABP B42 trial are negative leading to some uncertainty. Treatment decisions should be based on all available clinical and pathological information for each patient, rather than depending only on multigene profiling tests.
- MammaPrint, Oncotype DX, Prosigna, and EndoPredict currently have insufficient evidence to guide extension of adjuvant endocrine therapy; however, these molecular assays may prognosticate a very low rate of disease recurrence that might not justify an extension of endocrine therapy.

Figure 1-1. Multigene Profiling Assay Decision Tree for Adjuvant Chemotherapy in Node-Negative Patients

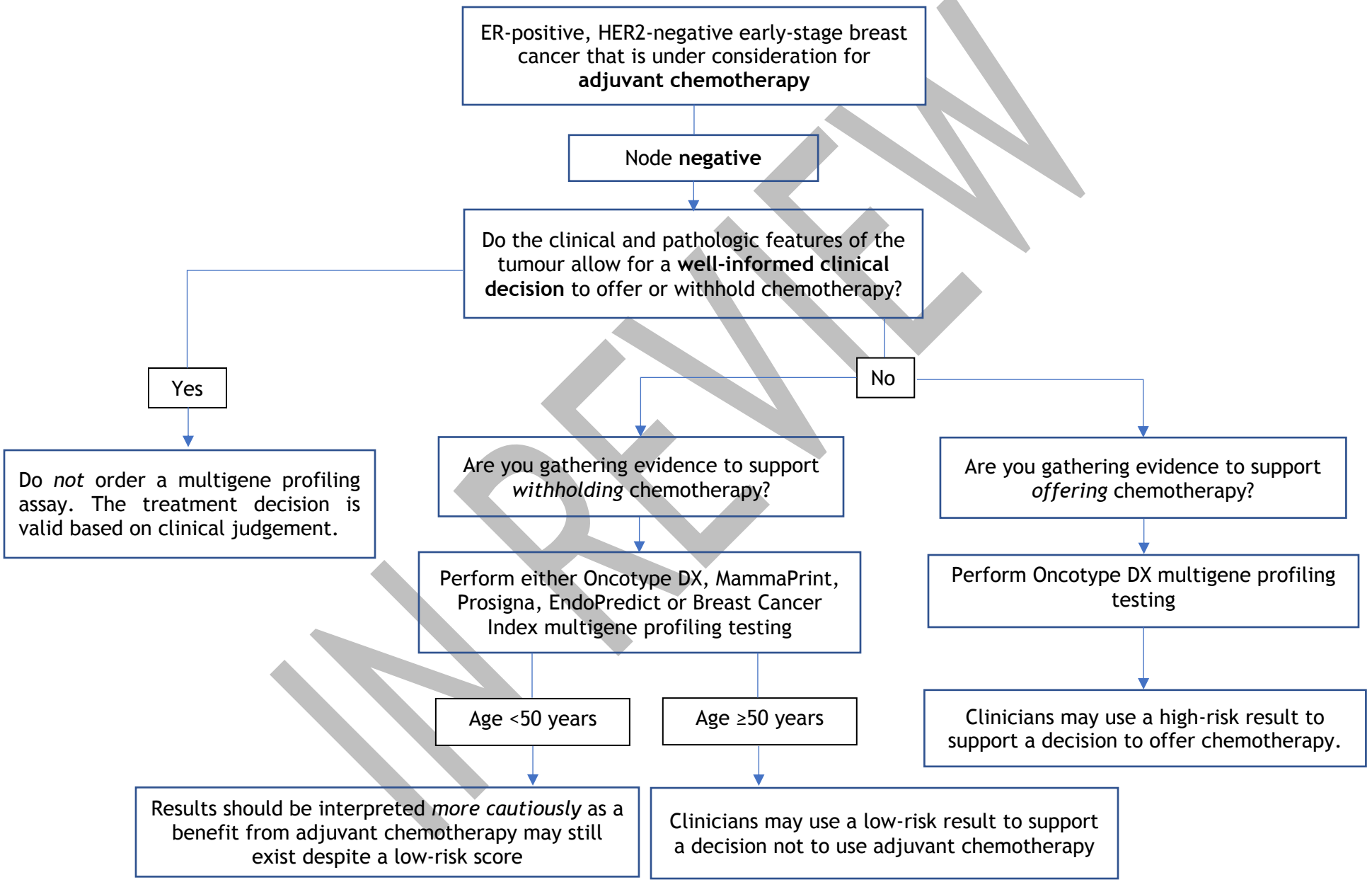

Figure 1-2. Multigene Profiling Assay Decision Tree for Adjuvant Chemotherapy in Node-Positive Patients

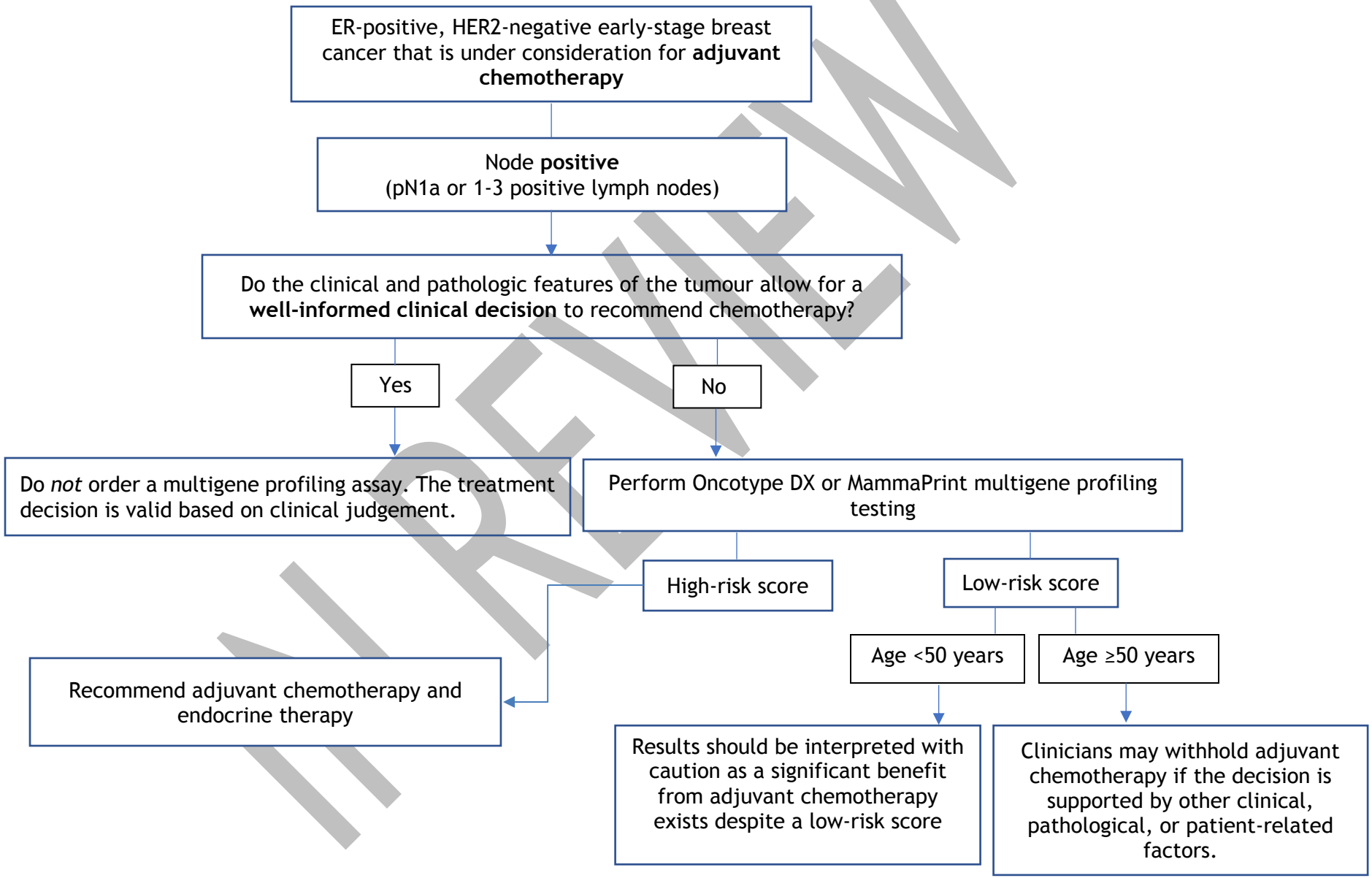

Figure 1-3. Multigene Profiling Assay Decision Tree for Extended Adjuvant Endocrine Therapy

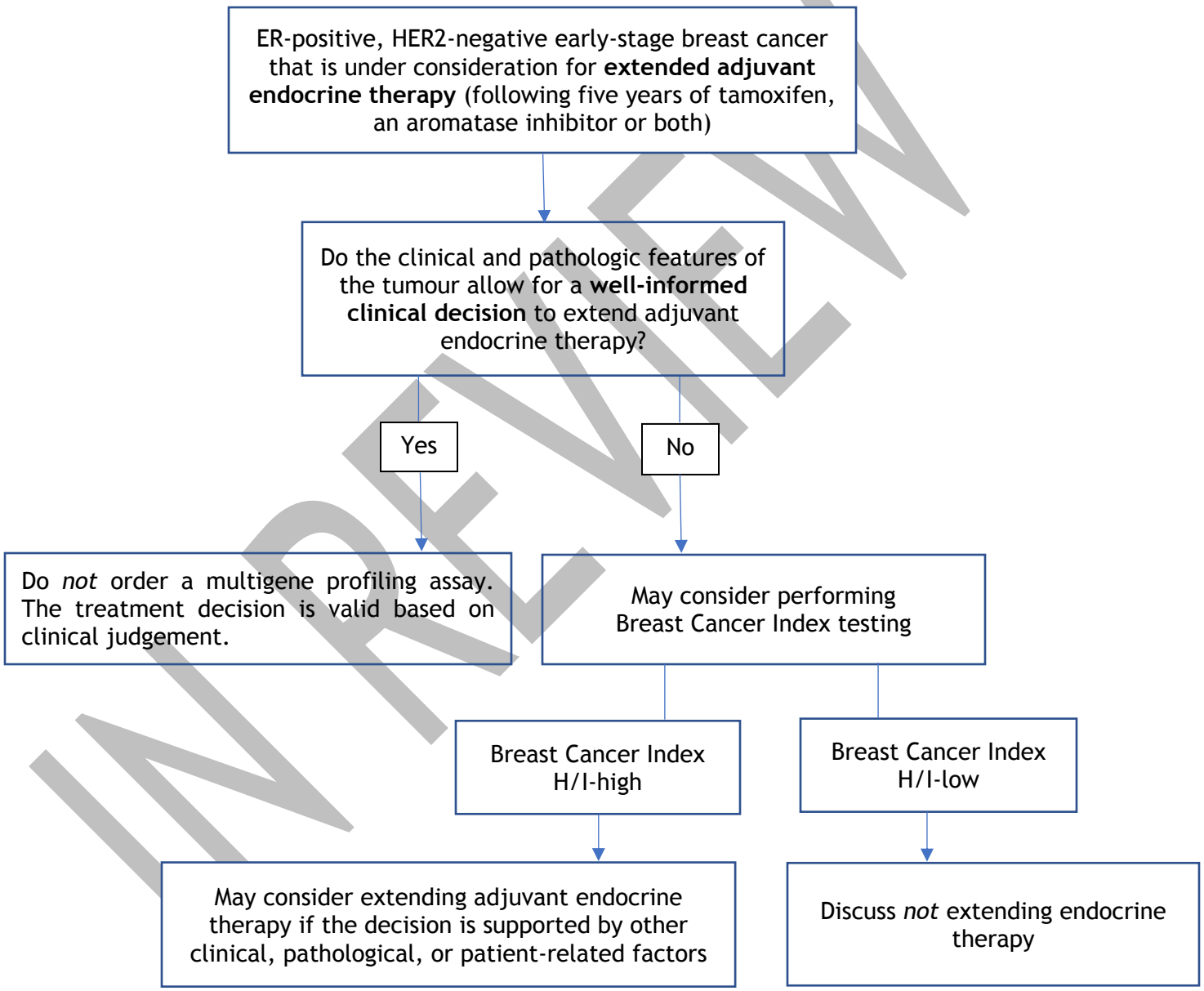

Table 1-1. Summary of assay characteristics.

	Oncotype DX	Prosigna	MammaPrint	EndoPredict	Breast Cancer Index
Tissue Required	FFPE	FFPE	FFPE or fresh tissue	FFPE	FFPE
Technique	qRT-PCR	qRT-PCR and nCounter DX Analysis System	Microarray	qRT-PCR	qRT-PCR
Assay Output	RS (0-100)	Intrinsic subtype and ROR score (0-100)	MammaPrint Index Risk of distant recurrence at 5 years	EPclin score (1-6) Molecular score (1-15)	BCI score (0-10) and BCI (H/I) low and BCI (H/I) high (ratio HoxB13 and interleukin-17B receptor)
Categories for Risk Measurement	*TAILORx categories* Low: ≤15 Intermediate: 16–25 High: 26–100 *Pre-TAILORx categories* Low: <18 Intermediate: 18–30 High: ≥31	*LN-negative* Low: 0–40 Intermediate: 41–60 High: 61–100 *LN-positive (1-3 nodes)* Low: 0–40 High: 41–100	Low: 0 to 1 High: −1 to 0	*EPclin score* Low: <3.3 High: ≥3.3 *Molecular score* Low: <5 High: ≥5	*BCI predictive H/I* Low: <0.06 High: ≥0.06 *BCI prognostic node-negative* Low: <5.0825 Intermediate: 5.0825-6.5025 High: ≥6.5025 *BCI prognostic node-positive* Low: <6.93 High: ≥6.93
Regulatory Approval or Endorsement	Assay conducted in centralized Exact Science's CLIA-certified lab	FDA cleared for decentralized testing (2014)	FDA cleared for Agendia centralized lab testing in FFPE (2015)	CE Mark for decentralized testing (2012)	Assay conducted in centralized CAP/CLIA-certified lab
Manufacturer	Exact Sciences Corp.	Veracyte	Agendia	Myriad Genetics, Inc.	Biotheranostics, Inc.
Testing Location	Central (1 laboratory in US)	Various labs across US, UK	Central (1 laboratory in the Netherlands, 1 in US)	Central laboratory in the US	Central (1 laboratory in US)
Genes, n	21-gene assay	50-gene assay	70-gene assay	12-gene assay EPclin score: 12-gene assay plus tumour size and nodal status	*HOXB13:IL17BR* expression ratio (H/I) and Molecular Grade Index

Abbreviations: BCI (H/I), Breast Cancer Index (HOXB13/IL17BR); CAP: College of American Pathologists; CLIA: Clinical Laboratory Improvement Amendments; EPclin, EndoPredict clinical score; ER, estrogen receptor; FDA, Food and Drug Administration; FDA: Food and Drug Administration; FFPE, formalin-fixed paraffin-embedded; LN, lymph node; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; ROR: risk of recurrence; RS, recurrence score; UK: United Kingdom US, United States

Clinical Utility of Multigene Profiling Assays in Early-Stage Invasive Breast Cancer

Section 2: Guideline – Recommendations and Key Evidence

GUIDELINE OBJECTIVES

To update clinical guidance on the use of multigene profiling assays in individuals with early-stage invasive breast cancer.

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- Multigene profiling assays should be interpreted cautiously in premenopausal patients where a significant benefit from adjuvant chemotherapy may still exist despite a low-risk score.

Key Evidence for Recommendation 1

Please see Key Evidence for Recommendations 2 through 4.

Justification for Recommendation 1

The main purpose of most multigene profiling assays is to determine whether a tumour has a high or low risk for recurrence. The five multigene profiling assays considered in this guidance evaluate the intrinsic molecular characteristics of a tumour to prognosticate behaviour with some being able to predict treatment benefit; however, the genes used to ascertain this predicted risk differ among assays. Although the results of different assays should be similar in terms of risk category, each individual assay uses a different scoring system and the results may not be directly comparable. The value in multigene profiling is more evident, and potentially limited to, providing support for decision-making regarding systemic therapy when such decisions remain difficult for the clinician and patient, even after considering all clinical, pathological, and patient-related factors. Although no males were included in any of the included studies, given the similarities in the management of male and female breast cancer, multigene profiling assays may be used in all individuals with early-stage ER-positive, HER2-negative invasive breast cancer.

Although multigene profiling assays may be used to guide treatment and ultimately improve patient outcomes, it is important to note the emotional impact such testing may have on patients, especially in those who receive a high score. Clinician and patient discussions should be conducted concerning the implications of results.

Recommendation 2

In patients with early-stage node-negative ER-positive/HER2-negative disease, clinicians may use a low-risk result from Oncotype DX, MammaPrint, Prosigna, EndoPredict/EPclin, or Breast Cancer Index assays to support a decision not to use adjuvant chemotherapy.

Qualifying Statements for Recommendation 2

- Patients <50 years of age may still benefit from chemotherapy despite low-risk scores from multigene assay testing. Risk scores should be interpreted with caution and decisions should be made while considering other clinical, pathological, or patient-related factors.
- Treatment decisions should be based on all available clinical and pathological information for each patient, rather than depending entirely on multigene profiling test results.
- In patients with a low-grade tumour (i.e., grade 1) less than 1 cm in size, the Working Group members do not recommend a multigene assay profiling as this is unlikely to inform a treatment decision to use adjuvant chemotherapy.

Key Evidence for Recommendation 2

For Oncotype DX, the evidence comes from one randomized controlled trial (RCT) [1,2] and two retrospective analyses of an RCT [3,4] with an overall low level of certainty as assessed using the GRADE approach.

- In the TAILORx trial [1], patients with a recurrence score (RS) ≤10 had an invasive disease-free survival (IDFS) rate of 94.0% and an overall survival (OS) rate of 98.0% with adjuvant endocrine therapy alone at five years and an IDFS rate of 84.0% and OS rate of 93.7% at nine years.
- No difference in freedom from distant recurrence (94.5% vs. 95.0%; p=0.48), IDFS (83.3% vs. 84.3%; p=0.26) or OS (93.9% vs 93.8%; p=0.89) was reported in patients with an RS of 11 to 25 between those who received endocrine therapy and chemoendocrine therapy at nine years follow-up in the intent-to-treat population [1].
- In a subgroup analysis from the TAILORx trial among women aged ≤50 years [1], there was a significant benefit in those that received chemoendocrine therapy for IDFS with an RS of 16 to 20 (hazard ratio [HR], 1.90, 95% confidence interval [CI], 1.27 to 2.84; p=0.0016) or 21 to 25 (HR, 1.70; 95% CI, 1.03 to 2.80; p=0.035). This corresponded to a 1.6% reduction in the rate of distant recurrence among patients with an RS of 16 to 20 and a 6.5% reduction in the rate of distant recurrence among patients with an RS of 21 to 25 at nine years' follow-up.
- In the initial retrospective analysis of NSABP B20 [3], in patients with low (RS <18) and intermediate scores (RS 18 to 20), there was no significant difference in 10-year freedom from distant recurrence between those that received chemotherapy and those that did not, (95.6% vs. 96.8%; p=0.61) and (89.1% vs 90.9%; p=0.39), respectively. There was a statistically significant interaction between chemotherapy treatment and RS score (p=0.038). In the analysis by Geyer et al [4] excluding patients with HER2-positive tumours, there was no benefit of chemotherapy in patients with low and intermediate scores. In a multivariable analysis, the test for interaction between chemotherapy and RS was statistically significant (p=0.023) when controlling for patient age, tumour size, ER and progesterone receptor (PR) status, and tumour grade. Similarly, when the patients were recategorized by RS using TAILORx cut-offs, a statistically significant benefit was shown with the addition of chemotherapy for patients with an RS >25, but there was no benefit in patients with RS <11 and RS 11 to 25.

For MammaPrint, the evidence comes from one RCT [5,6] with a low level of certainty as assessed using the GRADE approach.

- In a prespecified exploratory subgroup analysis of the MINDACT trial of node-negative, ER-positive, HER2-negative patients, there was no significant difference in distant metastasis-free survival between patients who received chemotherapy and no chemotherapy in the high clinical risk and low genomic risk group (p=NR) or in the low clinical risk and high genomic risk group (p=NR). However, after a median follow up of 8.7 years, there was a significant difference between the two treatment groups in the high clinical risk and low genomic risk group (HR, 0.60; 95% CI, 0.38 to 0.96; p=NR) but no significant difference in the low clinical risk and high genomic risk group (p=0.815).
- In a predefined exploratory analysis of hormone receptor (HR)-positive, HER2-negative women at high clinical risk and low genomic risk, a significant chemotherapy benefit was shown (HR, 0.54; 95% CI, 0.30 to 0.98; p=NR) with an absolute difference of 5.0% in the rate of survival without distant metastases between the treatment groups in women 50 years of age or younger. No significant benefit was shown in women older than 50 years (HR, 0.82; 95% CI 0.55 to 1.24; p= NR). However, it is important to note that premenopausal patients were not mandated to receive ovarian suppression prior to treatment.

For Prosigna, the evidence comes from two predictive studies of retrospective analyses of RCTs [7,8] and three prognostic studies assessing late recurrence [9-11]. The prognostic studies did not maintain randomization from the original trials and as a result are treated as observational studies with a very low certainty of the evidence as assessed using the GRADE approach.

- In both exploratory retrospective analyses of patients from the NCIC CTG MA.21 and DBCG 77B trials, categorical Risk of Relapse (ROR) score was not predictive of response to chemotherapy regimen (p=0.232) [7] for recurrence-free survival (RFS) or treatment (p=0.10) for disease-free survival (DFS) [8], respectively.
- In the retrospective analysis of the ATAC trial, Sestak et al [9] found that the risk of distant recurrence at five to 10 years was 1.4% (95% CI, 0.5 to 3.8) for low-risk patients.
- In the retrospective analysis of the ABSCG-8 trial, Filipits et al [10] found the probability for 15-year distant RFS (DRFS) was 97.6% (95% CI, 94.7 to 98.9) for low-risk patients with a significant difference in late DRFS between patients in the high- vs. low-risk group (HR, 4.74; 95% CI, 1.89 to 11.87; p<0.001).
- In the study combining both the ATAC trial and ABCSG-8 trial together [11], there was a significant difference in late distant recurrence (i.e., five to 10 years) between patients in the high- vs. low-risk group (HR, 5.49; 95% CI, 2.92 to 10.35; p=NR).

For EndoPredict, the evidence comes from two retrospective analyses of RCTs [9,12] assessing late recurrence. These prognostic studies did not maintain randomization from the original trials and as a result are treated as observational studies with a very low certainty of the evidence as assessed using the GRADE approach.

- In the retrospective analysis of the ATAC trial, Sestak et al [9] found the risk of distant recurrence for EPclin low-risk patients at five to 10 years was 4.3% (95% CI, 2.6 to 7.1).
- In an analysis of the both the ABCSG-8 and ABCSG-6 trial together [12], there was a significant difference in distant recurrence-free rate (DRFR) from five to 15 years in

women who were distant recurrence-free at five years between those with low and high EPclin scores (HR, 4.52; 95% CI, 2.65 to 7.72; p< 0.001).

For Breast Cancer Index, the evidence comes from three retrospective analyses of RCTs [9,13,14] assessing late recurrence. These prognostic studies did not maintain randomization from the original trials and as a result are treated as observational studies with a very low certainty of the evidence as assessed using the GRADE approach.

- In the retrospective analyses of the ATAC trial [14], there was a significant difference between high Breast Cancer Index scores (BCI-high) and BCI-low groups (13.3% vs. 3.5%; HR, 2.97; 95% CI, 1.23 to 7.13; p=NR). In a multivariate analysis for late recurrence, BCI molecular grade index MGI HOXB13/IL17BR (MGI H/I) was prognostic for risk of distant late recurrence in node-negative (HR, 1.95; 95% CI, 1.22 to 3.14) and node-negative HER2-negative populations (HR, 2.12; 95% CI, 1.30 to 3.47). Sestak et al [9] found the risk of distant recurrence at five to 10 years was 2.6% (95% CI, 1.3 to 5.0) for low-risk patients and 15.9% (95% CI, 8.9 to 27.6) for high-risk patients.
- Zhang et al [13] found there was a significant difference in late DRFS between the BCI-low, BCI-intermediate, and BCI-high-risk groups for patients in both the Stockholm cohort and the multi-institutional cohort (p=0.0152 and p=0.0002, respectively). In a multivariate Cox regression including clinicopathologic variables, BCI was significant for ER-positive, HER2-negative patients in both the Stockholm cohort (HR, 3.50; 95% CI, 1.09 to 11.21; p=0.035) and the multi-institutional cohort (HR, 9.24; 95% CI, 2.85 to 30.0; p=0.0002).

Justification for Recommendation 2

Patients from the Consultation Group rated both recurrence risk and survival as critical outcomes along with quality of life and adverse events. The benefits of withholding adjuvant chemotherapy would be large and acceptable to patients when there are no significant differences in survival benefit. Prognostic studies from Prosigna and EndoPredict demonstrate a low risk of late recurrence, which would make it acceptable to withhold chemotherapy given the potential side effects and toxicity associated with adjuvant chemotherapy. The Working Group notes that although the overall certainty of the evidence is low for both Oncotype DX and MammaPrint, the TAILORx and MINDACT trials provide the strongest available evidence and best trial design available for this population. Given the similarities in the management of male and female breast cancer, these data can be generalized to all individuals with early-stage ER-positive, HER2-negative invasive breast cancer.

Recommendation 3

In patients with node-negative ER-positive/HER2-negative disease, clinicians may use a high-risk result from Oncotype DX to support a decision to offer chemotherapy. A high Oncotype DX recurrence score is capable of predicting adjuvant chemotherapy benefit.

Qualifying Statements for Recommendation 3

- MammaPrint, Prosigna, EndoPredict or EPclin, and Breast Cancer Index do not have sufficient evidence to support a predictive benefit of adjuvant chemotherapy among clinically low-risk patients with breast cancer whose multigene profiling testing indicates a high-risk score.

Key Evidence for Recommendation 3

The evidence comes from one RCT [1,2,15] and two retrospective analyses of an RCT [3,4] with an overall low level of certainty as assessed using the GRADE approach.

- In the TAILORx trial [15], the rate of freedom from recurrence of breast cancer at a distant site for high-risk patients (RS 26-100) treated with endocrine therapy plus adjuvant chemotherapy was 93% at five years and 86.8% at nine years.
- In the retrospective analysis of the NSABP B20 trial [3], patients with high RS (RS ≥31) experienced a large chemotherapy benefit (60.5% vs. 88.1%; relative risk [RR], 0.26; 95% CI, 0.13 to 0.53) and a statistically significant interaction between chemotherapy treatment and RS score (p=0.038). In the second re-analysis by Geyer et al [4], a benefit of chemotherapy remained for patients with high RS (HR 0.18; 95% CI, 0.07 to 0.47; p<0.001); however, there was no benefit of chemotherapy in patients with RS <18 and RS 18 to 30. In a multivariable analysis, the test for interaction between chemotherapy and RS was statistically significant (p=0.023) when controlling for patient age, tumour size, ER and PR status, and tumour grade. Similarly, when the patients were recategorized by RS using TAILORx cut-offs, a statistically significant benefit was shown with the addition of chemotherapy for patients with an RS >25. In a multivariable analysis, the test for interaction between chemotherapy and RS was statistically significant (p=0.014) when controlling for patient age, tumour size, ER and PR status, and tumour grade. It is important to note the that the patients included in the tamoxifen-only arm were used in the initial development of the Oncotype DX assay and as a result, these results may be confounded.

Justification for Recommendation 3

Patients from the Consultation Group rated both recurrence rate and invasive DFS as critical outcomes along with quality of life and adverse events. The Working Group determined that the beneficial effects of lower recurrence rates and higher survival rates outweigh the adverse effects from adjuvant chemotherapy.

Given the similarities in the management of male and female breast cancer, these data can be generalized to all individuals with early-stage ER-positive, HER2-negative invasive breast cancer.

Recommendation 4

In postmenopausal patients with ER-positive/HER2-negative tumours and one to three nodes involved (N1a disease), clinicians may withhold chemotherapy based on a low-risk Oncotype DX or MammaPrint score if the decision is supported by other clinical, pathological, or patient-related factors.

Qualifying Statements for Recommendation 4

- Premenopausal patients <50 years of age have a significant benefit from chemotherapy despite low-risk scores from multigene assay testing. Risk scores should be interpreted with caution and decisions should be made while considering other clinical, pathological, or patient-related factors.
- It is uncertain whether at least some of the benefit of chemotherapy among premenopausal patients may be due to chemotherapy induced amenorrhea versus the cytotoxic effects of treatment.
- The Prosigna, EndoPredict/EPclin, and Breast Cancer Index assays can identify low-risk node-positive patients whose prognostic outcomes are favourable; however, these assays have not demonstrated predictive evidence to support withholding adjuvant chemotherapy among higher risk, node-positive, ER-positive, HER2-negative breast cancer patients.

Key Evidence for Recommendation 4

For Oncotype DX, the evidence comes from one RCT [16], the RxPONDER trial, and a retrospective study of the SWOG 8814 trial [17] with a low certainty of the evidence as assessed using the GRADE approach.

- The RxPONDER trial [16] reported there was no significant difference in IDFS at five years between patients (RS ≤25) who received chemoendocrine therapy or endocrine therapy (92.2% vs 91.0%; HR, 0.86; 95% CI, 0.72 to 1.03; p=0.10). The interaction between chemotherapy benefit and continuous recurrence score was not statistically significant for IDFS when controlling for continuous RS, menopausal status, and treatment group (p=0.35).
  In a prespecified analysis, a significant interaction was found between the addition of adjuvant chemotherapy and menopausal status (p=0.008) allowing for a subgroup analysis by menopausal status. In postmenopausal women, there was no significant difference in IDFS between those who received chemoendocrine therapy or endocrine therapy (91.3% vs. 91.9%; HR, 1.02; 95% CI, 0.82 to 1.26; p=0.89).
  In premenopausal women, a significant benefit was found in IDFS for women who received chemoendocrine therapy (93.9% vs. 89.0%; HR, 0.60; 95% CI, 0.43 to 0.83; p=0.002). In premenopausal women who were 50 years old or older, there was no significant chemotherapy benefit (HR, 0.98; 95% CI, 0.54 to 1.78); however, in premenopausal women younger than 50 years of age, a significant chemotherapy benefit was observed (HR, 0.48; 95% CI, 0.32 to 0.72; p=NR). The interaction between age and chemotherapy benefit in premenopausal women was not significant (p=0.06).
- In the retrospective analysis of the SWOG-8814 trial [17], there was no significant benefit for DFS or OS between patients who received either tamoxifen alone or cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) followed by tamoxifen at 10 years for those with RS <18 (p=0.97 and p=0.68, respectively) or RS between 18 and 30 (p=0.48 and p=065, respectively). For DFS, there was no significant interaction between RS and treatment (p=0.053); however, when assessing the first five years, a significant interaction was seen between RS and treatment for both DFS and OS (p=0.029 and p=0.016, respectively) but not after five years (p=0.58 and p=0.87, respectively).

For MammaPrint, the evidence comes from one RCT [5,6] with a low level of certainty as assessed using the GRADE approach.

- In node-positive patients in the MINDACT trial, there was no significant difference between patients who received chemotherapy and no chemotherapy in the high clinical risk and low genomic risk group for distant metastasis-free survival after a median follow-up of five years (absolute benefit of 0.7% in the chemotherapy arm) [5]; p=0.724) or eight years (absolute benefit of 1.3% in the chemotherapy arm; p=NS) [6]. The number of node-positive patients in the low clinical risk and high genomic risk were too small to be analyzed.

Justification for Recommendation 4

Patients from the Consultation Group rated both recurrence risk and survival as critical outcomes along with quality of life and adverse events. The benefits from withholding adjuvant chemotherapy would be large and acceptable to patients when there are no significant differences in survival benefit. Although favourable prognostic data exist for late recurrence with Prosigna, EndoPredict and Breast Cancer Index, given the increased clinical risk in lymph node-positive patients, strong predictive data regarding the use of these assays are needed. Given the similarities in the management of male and female breast cancer, these data can be generalized to all individuals with early-stage ER-positive, HER2-negative invasive breast cancer.

Recommendation 5

The evidence to support the use of molecular profiling to select the duration of endocrine therapy is evolving. In patients with ER-positive disease, clinicians may consider using a Breast Cancer Index (BCI) (H/I) high assay result to support a decision to extend adjuvant endocrine therapy if the decision is supported by other clinical, pathological, or patient-related factors.

Qualifying Statements for Recommendation 5

- While a number of studies have demonstrated clinical utility of BCI for extending adjuvant endocrine therapy, the preliminary results of the NSABP B42 trial are negative leading to some uncertainty. Treatment decisions should be based on all available clinical and pathological information for each patient, rather than depending only on multigene profiling tests.
- MammaPrint, Oncotype DX, Prosigna, and EndoPredict currently have insufficient evidence to guide extension of adjuvant endocrine therapy; however, these molecular assays may prognosticate a very low rate of disease recurrence that might not justify an extension of endocrine therapy.

Key Evidence for Recommendation 5

For Breast Cancer Index, the evidence comes from four retrospective analyses of RCTs [18-21], of which one [21] is currently available in abstract form, with a low certainty of evidence as assessed using the GRADE approach.

- In the retrospective review of the NSABP B42 trial [21], currently in abstract form, there was no significant difference between receiving an additional five years of letrozole or placebo for recurrence-free interval in those who were BCI (H/I)-low (HR, 0.69; 95% CI, 0.43 to 1.11; p=0.13) or BCI (H/I)-high (HR, 0.83; 95% CI, 0.55 to 1.26; p= 0.38). There was no significant interaction between BCI (H/I) level and treatment (p=0.55) for recurrence-free interval, breast cancer-free interval (p=0.07), DFS (p=0.62), or distant recurrence (p=0.14).
- In the translation IDEAL study [20], there was significant benefit in risk of recurrence for BCI (H/I)-high patients who received five years of extended letrozole (HR, 0.42; 95% CI, 0.21 to 0.84; p=0.011) with an absolute reduction of recurrence risk of 9.8%; however, this benefit was not observed in BCI (H/I)-low patients (HR, 0.95; 95% CI, 0.58 to 1.56; p=0.835). Similarly, in patients treated with primary adjuvant endocrine therapy with an aromatase inhibitor (AI), BCI (H/I)-high patients received a significant benefit from extended letrozole (HR, 0.34; 95% CI, 0.16 to 0.73; p=0.004), while no benefit was seen in BCI (H/I)-low patients (HR, 0.90; 95% CI, 0.53 to 1.55; p=0.712). There was a significant interaction between BCI (H/I) level and treatment in both the overall population (p=0.045) and in the subgroup of patients who received primary adjuvant endocrine therapy with an AI (p=0.025) after adjusting for age, tumour grade, pT stage, pN stage, prior endocrine therapy, and prior chemotherapy.
- In the Trans-aTTom study [19], consisting of node-positive patients only, those classified as BCI (H/I)-high showed a significant benefit from extended tamoxifen (HR, 0.35; 95% CI, 0.15 to 0.86; p=0.027) with an absolute recurrence risk difference of 10.2%. There was significant interaction between continuous BCI (H/I) and extended tamoxifen treatment (p=0.012) after adjusting for age, tumour size, tumour grade, and ER and PR status.
- In the retrospective review of the NCIC CTG MA, 17 trial [18], for patients with high H/I, there was a significant difference in the five-year RFS of 73% (95% CI, 56.6 to 84.1) and 89.5% (95% CI, 80.3 to 94.5) for patients receiving placebo and letrozole, respectively,

with an absolute risk of reduction of 16.5% (p=0.007). In an adjusted model, high H/I was significantly associated with patient benefit from letrozole (odds ratio [OR], 0.32; 95% CI, 0.14 to 0.72; p=0.006). The interaction between H/I and letrozole therapy was significant (p=0.03).
Justification for Recommendation 5
Patients from the Consultation Group rated both the recurrence risk and RFS as critical outcomes along with quality of life and adverse events. The Working Group determined the beneficial effects of lower recurrence and higher survival rates outweigh the adverse effects from extended adjuvant endocrine therapy. This recommendation can be generalized to all patients with node-negative and -positive, ER-positive breast cancer. The Working Group members acknowledge the emerging evidence for MammaPrint in this area [22] as well as the retrospective study of the NSABP B42 trial for BCI [21]; however, abstracts of studies are insufficient to make recommendations. Translational studies from the IDEAL, Trans-aTTom, and NCI CCTG MA 17 clinical trials all demonstrated a clinical benefit from extended adjuvant endocrine therapy among patients with a BCI (H/I) high assay result; however, the results from the recent analysis of the NSABP B42 trial were negative. While the NSABP B42 trial is only presented as an abstract, this preliminary result does raise some uncertainty regarding the predictive capacity of BCI and the Working Group has thus issued a weak recommendation for BCI (H/I) testing to guide extended adjuvant endocrine therapy.

IMPLEMENTATION CONSIDERATIONS

The recommendations are feasible to implement and would align with norms in the clinical community. The Working Group members believe that the availability of assays would help reduce inequities as assay scores may help inform care and treatment decisions. Historically, all assays covered in this guideline are conducted out of country. Clinicians would also need further education in interpreting scores from different assays and the communication of assay scores to patients. For a specific treatment decision, only one multigene profiling assay should be selected based on a discussion with the patient. Performing multiple tests could create uncertainty of results and anxiety in patients. Although multigene profiling assays may be able to guide treatment and ultimately improve patient outcomes, it is important to note the emotional impact it may have on patients, particularly in those who receive a high score. Most multigene profiling assay studies have been conducted in Caucasian populations and further study in racially and ethnically diverse populations is needed [23]. The Working Group members acknowledge the limited evidence regarding the use of multigene assays in male breast cancer and the potential for test results to underestimate risk. Further study on this special population is encouraged and warranted. Timeliness of care, including necessary test approvals, are important and as the use of assays increases particularly in lymph node-positive patients, it will be critical that the assays results are delivered quickly. Extended delays can cause anxiety in patients and impact quality of care.

FURTHER RESEARCH

The results of the prospective clinical trial OPTIMA, which studies the predictive value of Prosigna for adjuvant chemotherapy in high-risk patients, are awaited. The OPTIMA trial is enrolling breast cancer patients throughout the United Kingdom, Norway and Sweden who were pre- and postmenopausal with predominantly lymph node-positive disease with ovarian suppression being mandated for premenopausal patients.

Several clinical trials involving de-escalation of radiation therapy are underway both in node-positive and node-negative (DEBRA: NCT04852887, EXPERT: NCT02889874 and PRECISON:

NCT02653755), and node-positive (TAILOR RT: NCT03488693) ER-positive, HER2-negative breast cancer, which will help to determine whether regional radiotherapy may be safely omitted in patients with early-stage breast cancer with low-risk multigene profiling assay scores. The ELISA registry in Ontario will also evaluate the use of Oncotype DX in ductal carcinoma in situ to investigate whether a low-risk score may identify a group of women who can be treated safety with breast conserving surgery alone. A recent study defining an ultra-low-risk MammaPrint score also identified a group of very favourable risk patients with breast cancer who may not require adjuvant endocrine therapy [24]. This promising result also warrants further investigation.

Evidence around the use of multigene assays to guide the use of neoadjuvant chemotherapy, especially among patients with HR-positive breast cancer is needed. For example, the prospective registry NBRST (NCT01479101) is evaluating the MammaPrint assay in predicting response to patients treated with neoadjuvant preoperative systemic therapy.

Comparative studies investigating health system cost effectiveness are needed to evaluate the real-world value of these assays in clinical practice, especially in government-funded health care systems. In addition, specific studies investigating the clinical utility of these various assays in ethnically diverse backgrounds would also be of high benefit.

Clinical Utility of Multigene Profiling Assays in Early-Stage Invasive Breast Cancer

Section 3: Guideline Methods Overview

This section summarizes the methods used to create the guideline. For the systematic review, see Section 4.

THE PROGRAM IN EVIDENCE-BASED CARE

The Program in Evidence-Based Care (PEBC) is an initiative of the Ontario provincial cancer system, Ontario Health (Cancer Care Ontario). The PEBC mandate is to improve the lives of Ontarians affected by cancer through the development, dissemination, and evaluation of evidence-based products designed to facilitate clinical, planning, and policy decisions about cancer control.

The PEBC supports the work of Guideline Development Groups (GDGs) in the development of various PEBC products. The GDGs are composed of clinicians, other healthcare providers and decision makers, methodologists, and community representatives from across the province.

The PEBC is a provincial initiative of OH (CCO) supported by the Ontario Ministry of Health (OMH). All work produced by the PEBC is editorially independent from the OMH.

BACKGROUND FOR GUIDELINE

New evidence supporting the use of multigene profiling assays and its implication on patient treatment has emerged prompting an update of the original guideline from 2016.

GUIDELINE DEVELOPERS

This guideline was developed by the Multigene Profiling Assays in Early-Stage Invasive Breast Cancer GDG (Appendix 1), which was convened at the request of the Molecular Oncology and Testing Advisory Committee (MOTAC).

The project was led by a small Working Group of the Multigene Profiling Assays in Early-Stage Invasive Breast Cancer GDG, which was responsible for reviewing the evidence base, drafting the guideline recommendations, and responding to comments received during the document review process. The Working Group had expertise in medical oncology, pathology, molecular genetics, and health research methodology. Other members of the Multigene Profiling Assays in Early-Stage Invasive Breast Cancer GDG served as the Expert Panel and were responsible for the review and approval of the draft document produced by the Working Group. Conflict of interest declarations for all GDG members are summarized in Appendix 1, and were managed in accordance with the *PEBC Conflict of Interest Policy*.

GUIDELINE DEVELOPMENT METHODS

The PEBC produces evidence-based and evidence-informed guidance documents using the methods of the Practice Guidelines Development Cycle [25,26]. This process includes a systematic review, interpretation of the evidence by the Working Group and draft recommendations, internal review by content and methodology experts, and external review by Ontario clinicians and other stakeholders.

The PEBC uses the AGREE II framework [27] as a methodological strategy for guideline development. AGREE II is a 23-item validated tool that is designed to assess the methodological rigour and transparency of guideline development and to improve the completeness and transparency of reporting in practice guidelines.

The currency of each document is ensured through periodic review and evaluation of the scientific literature and, where appropriate, the addition of newer literature to the original evidence-base. This is described in the *PEBC Document Assessment and Review Protocol*. PEBC guideline recommendations are based on evidence of the magnitude of the desirable and undesirable effects of an intervention or accuracy of a test, and take into account the certainty of the evidence, the values of key stakeholders (e.g., patients, clinicians, policy makers, etc.), and the potential impact on equity, acceptability and feasibility of implementation. A list of any implementation considerations (e.g., costs, human resources, and unique requirements for special or disadvantaged populations, dissemination issues, etc.) is provided along with the recommendations for information purposes. PEBC guideline development methods are described in more detail in the *PEBC Handbook* and the *PEBC Methods Handbook*.

Search for Guidelines

As a first step in developing this guideline, a search for existing guidelines was undertaken to determine whether any guideline could be endorsed.

Evidence-based guidelines with systematic reviews that addressed at least one intervention in the research question were included. Guidelines published before November 2018 were excluded. Guidelines based on consensus or expert opinion were excluded.

The following sources were searched for guidelines on December 15, 2021 with the search terms "assay AND breast", "gene AND profiling AND breast", "biomarkers AND breast": ECRI Database, Canadian Partnership Against Cancer – Cancer Guidelines Database, National Institute for Health and Care Excellence Evidence Search, Canadian Medical Association Journal Infobase, Scottish Intercollegiate Guidelines Network, American Society of Clinical Oncology, National Health and Medical Research Council – Australia Clinical Practice Guidelines Portal, and Cancer Council Australia – Cancer Guidelines Wiki.

The MEDLINE and EMBASE databases were searched for guidelines on December 15, 2021. The full search strategy is available in Appendix 2. Two guidelines met the inclusion criteria [28,29]; however, both were excluded as they did not include the most recent published studies.

GUIDELINE REVIEW AND APPROVAL

Internal Review

For the guideline document to be approved, 75% of the content experts who comprise the GDG Expert Panel must cast a vote indicating whether or not they approve the document, or abstain from voting for a specified reason, and of those that vote, 75% must approve the document. In addition, the PEBC Report Approval Panel (RAP), a three-person panel with methodology expertise, must unanimously approve the document. The Expert Panel and RAP members may specify that approval is conditional, and that changes to the document are required. If substantial changes are subsequently made to the recommendations during external review, then the revised draft must be resubmitted for approval by RAP and the GDG Expert Panel.

Patient and Caregiver-Specific Consultation Group

Three patients/survivors/caregivers participated as Consultation Group members for the Multigene Profiling Assays in Early-Stage Invasive Breast Cancer GDG. They reviewed copies of the project plan and draft recommendations and provided feedback on their comprehensibility, appropriateness, and feasibility to the Working Group's Health Research Methodologist. The Health Research Methodologist relayed the feedback to the Working Group for consideration.

External Review

Feedback on the approved draft guideline is obtained from content experts and the target users through two processes. Through the Targeted Peer Review, several individuals with content expertise are identified by the GDG and asked to review and provide feedback on the guideline document. Through Professional Consultation, relevant care providers and other potential users of the guideline are contacted and asked to provide feedback on the guideline recommendations through a brief online survey.

DISSEMINATION AND IMPLEMENTATION

The guideline will be published on the OH (CCO) website and may be submitted for publication to a peer-reviewed journal. The Professional Consultation of the External Review is intended to facilitate the dissemination of the guideline to Ontario practitioners. Section 1 of this guideline is a summary document to support the implementation of the guideline in practice. OH (CCO)-PEBC guidelines are routinely included in several international guideline databases including the CPAC Cancer Guidelines Database, the CMA/Joule CPG Infobase database, NICE Evidence Search (UK), and the Guidelines International Network (GIN) Library.

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Clinical Utility of Multigene Profiling Assays in Early-Stage Invasive Breast Cancer

Section 4: Systematic Review

INTRODUCTION

Breast cancer is a common disease in Canada with approximately 25,000 new cases per year [30]. Survival outcomes with early-stage breast cancer have significantly improved over time with advances in systemic therapy, especially adjuvant chemotherapy and endocrine therapy [31].

Breast cancer is a heterogenous disease classified by the expression of the ER, PR and/or HER2 receptor. Clinical decision making regarding adjuvant systemic therapy may vary and is commonly influenced by patient, clinical and pathologic factors including tumour size, histologic grade, lymph node status, and ER, PR and HER2 expression, all of which have been shown to significantly influence risk of disease recurrence [32]. Given the potential side effects and toxicity of systemic therapy, several molecular gene expression profiling tests have been developed to assess the risk of recurrence. The use of these assays is meant to improve clinical decision making and optimize use of systemic therapy for breast cancer.

Clinical decision making regarding use of adjuvant chemotherapy has historically been based on a variety of factors including breast cancer stage, tumour biology or patient characteristics, all of which can be used to target patients at higher risk of disease recurrence. However, treatment decision remains challenging, especially among ER-positive, HER2-negative invasive breast cancers that are often less responsive to chemotherapy and may derive more clinical benefit from endocrine therapy alone. Previous treatment recommendations were generated from population-based or clinical trial data and were not necessarily indicative of clinical benefit at an individual patient level [33,34]. This imprecision has resulted in overuse of adjuvant chemotherapy in some patients with breast cancer, with unnecessary exposure to side effects and potential toxicity [35]. To mitigate this, several molecular profiling tests have been developed and validated that classify tumours into low-, intermediate-, or high-risk categories for risk of disease recurrence. These multigene profiling assays are generally prognostic of breast cancer outcome. Some may also predict the potential benefit from systemic therapy in terms of distant recurrence, IDFS, and OS [36]. Currently, several multigene profiling assays are approved by health regulatory agencies and supported for use by international breast cancer clinical guidelines. These assays are used in standard clinical practice to guide clinical decision making regarding the use of adjuvant chemotherapy for node negative ER-positive/HER2-negative invasive breast cancer.

In 2016, CCO's PEBC and the MOTAC published their first clinical practice guideline on *Clinical Utility of Multigene Profiling Assays in Invasive Early-Stage Breast Cancer*. That guideline reviewed several multigene expression assays including Oncotype DX (Exact Sciences Corporation, Madison, Wisconsin, USA), Mammaprint (Agendia, Irvine, California, USA), Prosigna (Veracyte, South San Francisco, California, USA) and EndoPredict (Myriad Genetics, Inc., Zurich, Switzerland). The assays were all commercially available but only Oncotype DX was provincially funded for clinical use at the time of guideline development.

Since 2016, the evidence supporting the use of multigene profiling assays in early-stage breast cancer has continued to evolve. Two significant prospective trials have reported further positive evidence for use of these assays among patients with lymph node-positive ER-positive/HER2-negative breast cancer [5,6,16]. Additional studies investigating the use in other clinical areas such as guiding clinical decision making regarding the use of neoadjuvant

chemotherapy, extended endocrine therapy, and radiation therapy have also been conducted or are ongoing. Given substantial development of new evidence in the field, this current updated version of the guideline was developed.

The current review was expanded to include evidence regarding the Breast Cancer Index (Biotheranostics, Inc., San Diego, California, USA) as data have also emerged regarding this assay's ability to predict benefit in extending adjuvant endocrine therapy [19,20]. Another assay, IHC4, was not included given potential concerns regarding the reproducibility of the Ki67 measurement across pathology laboratories. IHC4 is not a commercially available test; however, it can be calculated on the basis of ER, PR, and HER2 expression and Ki67 scoring [37]. The Working Group decided to not focus its investigation on the utility of multigene profiling assays with regard to supporting clinical decision making for neoadjuvant chemotherapy or radiation therapy given the number of ongoing trials.

The objectives of the current review were to assess the clinical utility of Oncotype DX, MammaPrint, Prosigna, EndoPredict, and Breast Cancer Index in terms of their ability to predict response to adjuvant chemotherapy and extended adjuvant endocrine therapy.

A specific aim was to investigate the evidence for the use of these molecular profiling assays in the setting of either node-negative or node-positive ER-positive/HER2-negative breast cancer patients in guiding clinical decisions to withhold or offer adjuvant chemotherapy. Additionally, important patient factors impacting the utilization of molecular profiling results including age at diagnosis and menopausal status were of special interest in this review. The systematic review focused on survival outcomes including distant recurrence, OS and IDFS and quality of life.

The Working Group of the Clinical Utility of Multigene Profiling Assays in Early-Stage Invasive Breast Cancer Guideline Development Group developed this evidentiary base to inform recommendations as part of a clinical practice guideline. Based on the objectives of this guideline (Section 2), the Working Group derived the research question outlined below.

RESEARCH QUESTION

What is the clinical utility (i.e., the ability of a test to provide information that is useful to direct treatment and ultimately improve patient outcomes) of multigene profiling assays (i.e., Oncotype DX, Prosigna, EndoPredict, MammaPrint, Breast Cancer Index) for patients with early-stage invasive breast cancer for:

a) chemotherapy in the adjuvant setting?

b) extended endocrine therapy?

METHODS

This systematic review is based on four different searches conducted over time: an original search for the first version of this guideline conducted in 2016, a search conducted as part of the PEBC Document Assessment and Review process in 2018, a search conducted by Ontario Health (Quality) for their Health Technology Assessment in 2018, and a new search to update the evidence for this new version of the guideline. Only the methods for this new search are described in detail here. The methods for the original guideline and for the Assessment and Review search are available on request from the PEBC (email: ccopgi@mcmaster.ca) and the methods for the Health Technology Assessment search were substantially similar [38].

The new search included an update of the previous searches to find new studies that examined the clinical utility of Oncoptye DX, Prosigna, EndoPredict and MammaPrint as well as the Breast Cancer Index.

Search for Systematic Reviews

A search was conducted for existing systematic reviews. This included original systematic reviews and systematic reviews published as a component of practice guidelines. The MEDLINE (November 1, 2018 to December 15, 2021) and EMBASE (November 1, 2018 to December 15, 2021) databases, as well as the Cochrane Database of Systematic Reviews (November 1, 2018 to December 15, 2021) were searched. The full search strategy is available in Appendix 2. Systematic reviews were included if they met the following criteria:

- The review addressed at least one research question with similar inclusion/exclusion criteria; and
- The review had a low risk of bias as assessed with the ROBIS tool or a moderate/high overall rating as assessed with the AMSTAR 2 tool; and
- The review had a literature search cut-off after November 2018.

Search for Primary Literature

A search for primary literature was conducted to locate literature where no existing systematic reviews were found or to update existing systematic reviews.

Literature Search Strategy

The MEDLINE (November 1, 2018 to December 15, 2021) and EMBASE (November 1, 2018 to December 15, 2021) databases were searched for studies meeting the inclusion criteria described below for Oncotype DX, MammaPrint, EndoPredict and Prosigna. The search start date was amended to 2000 for the Breast Cancer Index, which was not included in the previous version of this guideline.

An a priori list of population subgroups was developed by consensus. These subgroups of interest included nodal status, ER status, PR status, HER2 status, menopausal status, sex, tumour grade, and tumour size.

The full search strategy is available in Appendix 2. Reference lists of included primary literature were scanned for additional citations. The following conference proceedings were also searched from 2019 to 2021: San Antonio Breast Cancer Conference, American Society for Clinical Oncology, and European Society for Medical Oncology.

Study Selection Criteria and Process

Inclusion criteria

- RCTs designed with the assay as the intervention, and if none were available, then retrospective analyses of RCTs where archived tumour tissue was used retrospectively to evaluate the assay. If the previously described studies were unavailable, then

retrospective studies of prospective observational registries where archived tumour tissue is used to evaluate the assay were included; and
- Studies that only reported predictive data based on marker status (considering differential treatment effect). If there were no predictive studies available for either adjuvant chemotherapy or extended adjuvant endocrine therapy, then prognostic studies examining late recurrence (i.e., 5-10 years) were included.
- Studies using the Oncotype DX, MammaPrint, EndoPredict, Prosigna, and Breast Cancer Index assays; and
- Studies reporting on local and distant recurrence, OS, IDFS, adverse events, and quality of life; and
- Studies with patients with early-stage invasive breast cancer; and
- Studies using any of the following treatments: adjuvant chemotherapy and extended endocrine therapy.

Exclusion criteria
- Conference abstracts of non-randomized studies (single-arm clinical trials, case series, etc.); or
- Conference abstracts of interim analyses; or
- Papers or abstracts not available in English; or
- Letters and editorials that reported clinical trial outcomes; or
- Papers published before November 2018 for Oncotype DX, MammaPrint, EndoPredict, and Prosigna and before 2000 for Breast Cancer Index

A review of the titles and abstracts was conducted by one reviewer (DS), independently. For studies that warranted full-text review, one reviewer (DS) reviewed each study independently and confirmed the final included studies with the Working Group.

Data Extraction and Assessment of Study Quality and Risk of Bias

All included primary studies underwent data extraction by one reviewer (DS), independently, with all extracted data and information audited subsequently by an independent auditor. Ratios, including hazard ratios, were expressed with a ratio of <1.0 indicating benefit for the experimental group for a given outcome.

Risk of bias was assessed for each included RCT or retrospective analyses of RCTs, where the randomization was not broken using Cochrane's Risk of Bias tool, http://handbook.cochrane.org/ (Part 2, Section 8.5). Criteria from the QUIPS tool were used to assess the risk of bias for all prognostic studies.

Synthesizing the Evidence

Meta-analysis was not planned due to the anticipated heterogeneity in the included studies.

Assessment of the Certainty of the Evidence

The certainty of the evidence per outcome for each assay was assessed using criteria from the GRADE method: risk of bias, inconsistency, indirectness, imprecision, and publication bias.

RESULTS

Search for Systematic Reviews

A search for systematic reviews yielded 23 documents with five reviews undergoing full-text review. None of the reviews met the pre-specified inclusion criteria.

Search for Primary Literature

Literature Search Results

A search for primary literature yielded 1071 documents and 15 were included [2,6,9,13-16,18-22,39-43].

A PRISMA flow diagram of the complete search is available in Appendix 3. Table 4-1 provides a breakdown of the number of studies included from the current search as well as from the three previous searches. Where multiple reports and abstracts were published for a single trial, only the most recent full publication was included, unless other reports contained relevant data that were not available in the most recent publication. Study quality and study characteristics are provided in Appendices 4 and 5, respectively.

Table 4-1. Number of included trials and publications by multigene profiling assay

	Number of publications included*				Total number of publications†
	PEBC MOAC-4 Guideline, 2016	PEBC Document Assessment and Review, 2018	Ontario Health, Health Technology Assessment, 2019	Current guideline search	
Oncotype DX					
• Predictive studies for adjuvant chemotherapy	2 [3,17]	1 [1]	1 [4]	3 [2,15,16]	7 [1-4,15-17]
• Predictive studies for extended endocrine therapy	0	0	0	0	0
• Prognostic studies for late recurrence‡	0	1 [9]	0	0	1 [9]
MammaPrint					
• Predictive studies for adjuvant chemotherapy	0	1 [5]	0	1 [6,39,40]	2 [5,6] [39,40]
• Predictive studies for extended endocrine therapy	0	0	0	1 [22]	1 [22]
Prosigna					
• Predictive studies for adjuvant chemotherapy	1 [7]	0	1 [8]	0	2 [7,8]
• Predictive studies for extended endocrine therapy	0	0	0	0	0
• Prognostic studies for late recurrence‡	2 [10,11]	1 [9]	0	0	3 [9-11]
EndoPredict					
• Predictive studies for adjuvant chemotherapy	0	0	0	1 [41]	1 [41]
• Predictive studies for extended endocrine therapy	0	0	0	0	0
• Prognostic studies for late recurrence‡	1 [44]	1 [9]	1 [12]	0	3 [9,12,44]
Breast Cancer Index					
• Predictive studies for adjuvant chemotherapy	0	0	0	0	0
• Predictive studies for extended endocrine therapy	N/A	N/A	N/A	4 [18-21]	4 [18-21]
• Prognostic studies for late recurrence~	N/A	N/A	N/A	5 [9,13,14,42,43]	5 [9,13,14,42,43]

Abbreviations: N/A, not available; PEBC, Program in Evidence-Based Care

* A number of publications in the previous versions do not meet the current inclusion criteria and have not been included in the numbers in the table

† Multiple publications of trials were retained if they reported different aspects or outcomes

‡ No predictive studies for extended endocrine therapy were found for Oncotype DX, Prosigna and EndoPredict, and as a result, prognostic studies evaluating late recurrence were included

~ No predictive studies for adjuvant chemotherapy were found for Breast Cancer Index, and as a result, prognostic studies evaluating late recurrence were included

Certainty of the Evidence
Tables A4-1 and A4-2 in Appendix 4 provide the risk of bias assessments.

Oncotype DX

a. Adjuvant chemotherapy

Lymph node-negative

Risk of Bias

One RCT [1,2,15] and two retrospective studies of an RCT [3,4] were included and assessed using the Cochrane Risk of Bias tool. The TAILORx trial scored 'low' on most domains for risk of bias although the method and process of randomization was not described. Non-adherence was also higher than projected; therefore, the sample size of the group that underwent randomization was increased by 73%. The rate of non-adherence was significantly higher in the chemoendocrine group than the endocrine therapy-only group. Further, this trial also used a modified non-inferiority design due to concerns of non-adherence.

Certainty of the Evidence

The evidence for the predictive value of Oncotype DX for node-negative patients comes from one RCT [1,2,15] and two retrospective studies of one RCT [3,4]. The overall certainty for all outcomes from this evidence is low due to risk of bias and indirectness (i.e., the variation in treatment regimens used within and across trials and variation in the threshold for interpreting Oncotype DX's test results [i.e., the intermediate range for the TAILORx study was defined as an RS between 11 to 25, which is not consistent with previous definitions of intermediate RS]).

Lymph node-positive

Risk of Bias

One interim analysis of a RCT [16] and one retrospective study of a RCT [17] were included and assessed using the Cochrane Risk of Bias tool. The RxPONDER trial scored 'low' on all domains for risk of bias although the method and process of randomization was not clearly described. The retrospective analysis of the SWOG 8814 trial scored 'low' on most domains of the risk of bias tool apart from scoring 'moderate' for attrition bias as tumour data were not available for all patients from the original trial and there were some differences between the patients in this current subset and the parent trial.

Certainty of the Evidence

The overall certainty for all outcomes from this evidence is very low due to risk of bias, imprecision (i.e., low number of events in SWOG 8814), and indirectness (i.e., the variation in treatment regimens used within and across trials and 11.7% of the included patients were HER2-positive in SWOG 8814).

b. Prognostic studies of late recurrence

Risk of Bias

One retrospective study of an RCT [9] rated 'low' on most domains of the QUIPS risk of bias tool.

Certainty of the Evidence

The randomization of the RCT was not maintained in the reviews as a result, they are treated as observational studies. According to GRADE, observational studies without special strengths or important limitations provide evidence with a low level of certainty.

MammaPrint

a. Adjuvant chemotherapy

Risk of Bias

The MINDACT trial [5,6] scored 'low' on most domains of the Cochrane Risk of Bias tool except for blinding of participants and personnel. This trial was also revised to include women with up to three positive axilla nodes. Further, several patients were placed in the incorrect risk group due to a change in the RNA-extraction solution.

Certainty of the Evidence

The overall certainty for all outcomes from this evidence is low due to the risk of bias and indirectness (i.e., applicability of treatment regimens).

b. Extended endocrine therapy

Risk of Bias

The retrospective study of the NSABP B42 trial [22] is currently published in abstract form and as a result the risk of bias cannot be assessed.

Certainty of the Evidence

The overall certainty for all outcomes from this evidence is low due to imprecision (i.e., the effect estimate comes from one study with risk groups having wide confidence intervals).

Prosigna

a. Adjuvant chemotherapy

Risk of Bias

The retrospective analysis of two trials [7,8] were included and assessed using the Cochrane Risk of Bias tool. Both scored 'low' on most domains of the risk of bias tool apart from scoring 'moderate' for attrition bias as tumour data were not available for all patients from the original trial. There were significant differences in Jensen et al between the included population and the parent trial for histology and grade. Liu et al excluded patients who did not receive protocol-specified treatment from the original trial. As a result, both studies were assessed as having a high risk of bias.

Certainty of the Evidence

The evidence for the clinical utility of Prosigna for adjuvant chemotherapy comes from two retrospective studies of RCTs [7,8]. The overall certainty for all outcomes from this evidence is very low due to the risk of bias, imprecision (i.e., the effect estimate comes from two studies with risk groups having wide confidence intervals), and indirectness (i.e., the variation in treatment regimens used across trials, applicability of treatment regimens today).

b. Prognostic studies on late recurrence

Risk of Bias

All four prognostic studies [9-11] rated 'low' on most domains of the QUIPS risk of bias tool.

Certainty of the Evidence

The evidence for the prognostic value of Prosigna comes from three retrospective studies of RCTs [9-11]. The randomization of the RCTs was not maintained in the reviews and as a result, they are treated as observational studies. According to GRADE, observational studies without special strengths or important limitations provide evidence with a low level of certainty.

EndoPredict

a. Adjuvant chemotherapy

Risk of Bias

The risk of bias of one comparative study [41] comparing arms from two RCTs was assessed using the QUIPS tool. This study scored 'low' on most domains except for bias due to study participants and bias due to study attrition.

Certainty of the Evidence

As the randomization was not maintained for either of the original trials, this study is treated as an observational study. According to GRADE, observational studies without special strengths or important limitations provide evidence with a low level of certainty.

b. Prognostic studies of late recurrence

Risk of Bias

All three prognostic studies [9,12,44] rated 'low' on most domains of the QUIPS risk of bias tool.

Certainty of the Evidence

The evidence for the prognostic value of EndoPredict comes from three retrospective studies of RCTs [9,12,44]. The randomization of the RCTs was not maintained in the reviews and as a result, they are treated as observational studies. According to GRADE, observational studies without special strengths or important limitations provide evidence with a low level of certainty.

Breast Cancer Index

a. Extended endocrine therapy

Risk of Bias

Four retrospective studies of RCTs [18-21] were included and assessed using the Cochrane Risk of Bias tool. The retrospective study of the NSABP B42 [21] is currently published in abstract form and could not be assessed. All fully published studies scored 'low' on all domains of the risk of bias tool.

Certainty of the Evidence

The evidence for the predictive value of BCI comes from four retrospective studies of RCTs [18-21], including one abstract [21]. The overall certainty for all outcomes from this evidence is low due to indirectness (i.e., the inclusion of both node-positive and -negative populations, applicability of treatment regimens), imprecision (i.e., low patient numbers within risk groups) and inconsistency (i.e., the difference in the direction of effect).

b. Prognostic studies of late recurrence

Risk of Bias

All five prognostic studies [9,13,14,42,43] rated 'low' on all domains of the QUIPS risk of bias tool.

Certainty of the Evidence

The randomization of the RCTs was not maintained in the reviews and as a result, they are treated as observational studies. According to GRADE, observational studies without special strengths or important limitations provide evidence with a low level of certainty.

Outcomes

Question 1: What is the clinical utility of Oncotype DX for patients with early-stage invasive breast cancer?

A. PREDICTIVE STUDIES FOR ADJUVANT CHEMOTHERAPY

To date, there have been two RCTs [1,2,15,16] that have been designed with Oncotype DX as the intervention and two retrospective analyses of RCTs [3,4,17] using archived tumour tissue to evaluate the assay.

Tables 4-2 to 4-5 present a summary of the outcomes and subgroup analyses for node-negative and node-positive patients. Table A5-1 in Appendix 5 presents study details including treatment regimens.

i. Lymph node-negative patients

The TAILORx trial [1,2] and the retrospective analyses of the NSABP-20 trial [3,4] included only node-negative patients.

The TAILORx trial [1,2] is a prospective non-inferiority trial evaluating whether chemotherapy is beneficial for women with a mid-range RS of 11 to 25 utilizing Oncotype DX. Women with HER2-negative, node-negative early breast cancer were assigned to one of four treatment groups based on the 21-gene RS. Women with a score of 10 or lower were assigned to receive endocrine therapy only, and women with a score of 26 or higher were assigned to receive chemoendocrine therapy only. Women with a score of 11 to 25 were randomized to receive either endocrine therapy alone or chemoendocrine therapy. The most common chemotherapy regimens in patients randomly assigned to chemotherapy were the docetaxel-cyclophosphamide (56%) and anthracycline-containing regimens (36%), while the endocrine therapy regimens most commonly included an AI (78%); regimens were selected by the treating physician. Suppression of ovarian function was used in 13% of premenopausal women. Baseline characteristics were balanced in the intent-to-treat population.

Two retrospective analyses have been conducted of the NSABP B20 trial [3,4], an RCT that randomized node-negative, ER-positive patients to either tamoxifen or tamoxifen plus either cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) or methotrexate and 5-fluorouracil (MF). It is important to note that the patients included in the tamoxifen-only arm were used in the initial development of the Oncotype DX assay and as a result, these results may be confounded. The first retrospective analysis was published in 2006 by Paik et al [3] and the two chemotherapy arms were combined for the analysis. A total of 651 patients were included in the analysis and categorized into low-risk (RS <18), intermediate risk (RS ≥18 and <31), and high-risk (RS ≥31) groups. The second analysis by Geyer Jr et al [4] was an exploratory reanalysis of the original NSABP B20 trial excluding patients with HER2-positive gene expression while using the RS cut-offs from the TAILORx trial.

Recurrence

The TAILORx trial [1] reported no difference in freedom from distant recurrence in patients with an RS of 11 to 25 between those who received endocrine therapy and chemoendocrine therapy (HR, 1.10; 95% CI, 0.85 to 1.41; p=0.48) at nine years in the intent-to-treat population. The rate of freedom from recurrence of breast cancer at a distant site for patients with a score of ≥26 was 86.8% at nine years. Nine-year event rates should be interpreted with caution due to limited follow-up beyond five years.

In the initial retrospective analysis of NSABP B20 by Paik et al [3], patients with high RS (RS ≥31) experienced a large chemotherapy benefit (RR, 0.26; 95% CI, 0.13 to 0.53) with a mean absolute decrease in the rate of distant recurrence at 10 years of 27.6%. In patients with

intermediate (RS 18 to 30) and low (RS <18) RS, there was no significant difference between those that received chemotherapy and those that did not (RR, 0.61; 95% CI, 0.24 to 1.59) and (RR, 1.31; 95% CI, 0.46 to 3.78), respectively. There was a statistically significant interaction between chemotherapy treatment and RS score (p=0.038). Similar results were found when excluding patients with HER2-positive gene expression [4].

In the analysis by Geyer et al [4], when HER2-negative patients were recategorized by RS using cut-offs from the TAILORx study, a statistically significant benefit was shown with the addition of chemotherapy for high-risk patients (RS >25; p<0.001), but there was no benefit in low- (RS <11; p=0.46) or intermediate-risk (RS 11 to 25; p=0.43) patients. In a multivariable analysis, the test for interaction between chemotherapy and RS was statistically significant (p=0.014) when controlling for patient age, tumour size, ER and PR status, and tumour grade.

Survival

The TAILORx trial [1] reported no difference in IDFS (HR, 1.08; 95% CI, 0.94 to 1.24; p=0.26) or OS (p=0.89) in patients with an RS of 11 to 25 between those who received endocrine therapy and chemoendocrine therapy in the intent-to-treat population at nine years. Patients with an RS ≤10 had an IDFS rate of 84.0% and an OS rate of 93.7% at nine years.

Subgroup analyses

Tumour Size and Grade

In an exploratory subgroup analysis of the TAILORx trial [1], no significant interactions were found between chemotherapy treatment and tumour size or histologic grade in patients with an RS of 11 to 25; p-value not reported.

Menopausal status

In an exploratory subgroup analysis of the TAILORx trial [1], no significant interactions were found between chemotherapy treatment and menopausal status; p-value not reported.

Premenopausal women with an RS of 16 to 20 who received chemoendocrine therapy showed a significant benefit in IDFS (HR, 1.76; 95% CI, 1.20 to 2.59; p=0.0034). This benefit was not observed in premenopausal women with RS between 11 and 15 (HR, 0.85; 95% CI, 0.54 to 1.35; p=0.49) or 21 and 25 (HR, 1.50; 95% CI, 0.93 to 2.42; p=0.094).

Age

In the TAILORx trial [1], there was a significant interaction between chemotherapy treatment and age (≤50 vs. 51 to 65 vs. >65 years) for IDFS (p=0.03) and for freedom from recurrence of breast cancer at a distant or local–regional site (p=0.02) but not at a distant site (p=0.12).

In women aged ≤50 years, there was a significant benefit in those that received chemoendocrine therapy for IDFS with an RS of 16 to 20 (HR, 1.90, 95% CI, 1.27 to 2.84; p=0.0016) and 21 to 25 (HR, 1.70; 95% CI, 1.03 to 2.80; p=0.035). This difference was not observed in women with an RS between 11 and 15 (HR, 0.99; 95% CI, 0.62 to 1.58; p=0.97).

ii. Lymph node-positive patients

Both the RxPONDER trial [16] and the retrospective study of the SWOG-8814 trial [17] included only node-positive patients.

The RxPONDER trial [16] randomized 5083 women with node-positive, HR-positive, HER2-negative breast cancer with RS ≤25 to adjuvant endocrine therapy with or without chemotherapy. The preferred chemotherapy regimen for premenopausal women was an anthracycline and a taxane (54%) and for postmenopausal women was a taxane plus cyclophosphamide (57%); regimens were selected by the treating physician. Approximately

12.7% of premenopausal women had ovarian suppression. The results of this trial's third interim results were published with only 58% of the protocol-specified events being recorded after a median follow-up of 5.3 years upon recommendation from the Data and Safety Monitoring Committee.

Albain et al [17] conducted a retrospective analysis of the SWOG-8814 trial, a randomized trial where postmenopausal women with node-positive, HR- or ER-positive breast cancer were randomized to either tamoxifen alone, CAF followed by tamoxifen or CAF with concurrent tamoxifen. This analysis excluded patients who received CAF with concurrent tamoxifen due to inferior efficacy in the original trial. Of the original 927 patients included in the two trial arms, 367 were included in this analysis. These patients were representative of those in the parent trial.

Disease-free survival

The RxPONDER trial [16] reported the interaction between chemotherapy benefit and continuous recurrence score was not statistically significant for IDFS when controlling for continuous RS, menopausal status, and treatment group (p=0.35). There was no significant difference in IDFS at five years between patients who received chemoendocrine therapy or endocrine therapy (HR, 0.86; 95% CI, 0.72 to 1.03; p=0.10).

In the retrospective analysis of the SWOG-8814 trial [17], there was no significant benefit in DFS between patients who received either tamoxifen alone or CAF followed by tamoxifen at 10 years for low-risk patients (RS <18; HR, 1.02; 95% CI, 0.54 to 1.93; p=0.97) or intermediate-risk patients (RS 18-30; HR, 0.72; 95% CI, 0.39 to 1.31; p=0.48). However, there was a significant advantage in high-risk patients (RS >31) receiving CAF followed by adjuvant chemotherapy (HR, 0.59; 95% CI, 0.35 to 1.01; p=0.033). There was no significant interaction between RS and treatment (p=0.053); however, when assessing the first five years, a significant interaction was seen between RS and treatment (p=0.029) but not after five years (p=0.58).

Overall survival

In the retrospective analysis of the SWOG-8814 trial [17], there was no significant benefit between patients who received either tamoxifen alone or CAF followed by tamoxifen at 10 years for those with RS <18 (HR, 1.18; 95% CI, 0.55 to 2.54; p=0.68), between 18 and 30 (HR, 0.84; 95% CI, 0.40 to 1.78; p=0.65), and ≥31 (HR, 0.56; 95% CI, 0.31 to 1.02; p=0.057) after adjustment for the number of positive nodes. There was a significant interaction between RS and treatment over the entire period (p=0.026) and in the first five years (p=0.016); however, there was no significant interaction after five years (p=0.87).

Subgroup analysis

Menopausal status

In a prespecified analysis of the RxPONDER trial [16], a significant interaction was found between the addition of adjuvant chemotherapy and menopausal status (p=0.008) allowing for a subgroup analysis by menopausal status. In postmenopausal women, there was no significant difference in IDFS (HR, 1.02; 95% CI, 0.82 to 1.26; p=0.89) or distant RFS (HR, 1.05; 95% CI, 0.81 to 1.37; p=0.70) between those who received chemoendocrine therapy or endocrine therapy. No benefit of chemoendocrine therapy was observed in any of the subgroups for postmenopausal women (i.e., age, histologic grade of the tumour, tumour size, number of positive nodes or recurrence score). In premenopausal women, a significant benefit was found in IDFS (HR, 0.60; 95% CI, 0.43 to 0.83; p=0.002) and distant RFS (HR, 0.58; 95% CI, 0.39 to 0.87; p=0.0009) in women who received chemoendocrine therapy. In premenopausal women who were 50 years old or older, there was no significant chemotherapy benefit (HR, 0.98; 95% CI, 0.54 to 1.78); however, in women younger than 50 years of age a significant chemotherapy

benefit was observed (HR, 0.48; 95% CI, 0.32 to 0.72; p=NR). The interaction between age and chemotherapy benefit in premenopausal women was not significant (p=0.06).

B. PREDICTIVE STUDIES FOR EXTENDED ENDOCRINE THERAPY

There were no studies evaluating the predictive value of Oncotype DX for extended endocrine therapy.

C. PROGNOSTIC STUDIES FOR LATE RECURRENCE

One retrospective study [9] has been included that provided data on the prognostic utility of Oncotype DX for late recurrence (i.e., 5-10 years). Tables 4-6 and 4-7 present a summary of the outcomes for node-negative and node-positive patients, respectively. Table A5-1 in Appendix 5 presents study details including treatment regimens.

The ATAC trial evaluated the efficacy and safety of anastrozole versus tamoxifen given for five years in 9366 postmenopausal women with localized breast cancer. A subsequent substudy of the ATAC trial, the TransATAC, collected paraffin blocks from 2006 HR-positive women who were assigned to the monotherapy arms in the original trial. Sestak et al [9] assessed the risk of late distant recurrence in 774 women who had data available for all six signatures.

i. Lymph node-negative patients

In a retrospective analysis of the ATAC trial, Sestak et al [9] found the risk of distant recurrence at five to 10 years was 4.8% (95% CI, 2.9 to 7.9) for low-risk patients (RS <18), 9.6% (95% CI 5.6 to 16.3) for intermediate-risk patients (RS 18 to 30) and 16.1% (95% CI, 8.0 to 30.8) for high-risk patients (RS ≥31).

ii. Lymph node-positive patients

In a retrospective analysis of the ATAC trial, Sestak et al [9] found the risk of distant recurrence for five to 10 years in node-positive patients was 17.9% (95% CI, 11.5 to 27.3) for low-risk patients (RS <18), 19.5% (95% CI 10.9 to 33.5) for intermediate-risk patients (RS 18-30), and 27.5% (95% CI, 11.2 to 57.9) for high-risk patients (RS ≥31).

Table 4-2. Outcomes for the use of Oncotype DX for adjuvant chemotherapy in node-negative patients

Author, year, trial name	Intervention, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Freedom from distant recurrence (ET vs. CET)	Invasive disease-free survival (ET vs. CET)	Overall survival (ET vs. CET)
RCTs								
Sparano et al, 2018 2020 [1,15] TAILORx	RS ≤10, ET, 1629 RS 11-25 ET, 3399 CET, 3312 RS 26-100 CET, 1737	NR Median, 55 (24–75) Median, 56 (23-75)	Pre, 36% Post, 64%	ER+, 99.9% HER2−, 100%	9 yrs	RS ≤10 96.8% RS 11-25 94.5% vs 95.0% HR, 1.10; 95% CI (0.85-1.41); p=0.48 RS 26-100 86.8%	RS ≤10 93.7% RS 11-25 83.3% vs 84.3% HR, 1.08; 95% CI (0.94-1.24); p=0.26 RS 26-100 89%	RS ≤10 93.7% RS 11-25 93.9% vs 93.8% HR, 0.99; 95% CI (0.79-1.22); p=0.89 RS 26-100 89%
Retrospective analyses of RCTs								
Geyer Jr et al, 2018 [4] NSABP B20	RS <18 ET, 134 CET, 213 RS 18-30 ET, 42 CET, 83 RS ≥31 ET, 28 CET, 69 RS ≤10 ET, 66 CET, 110	Median, 51 (28-74)	NR	ER+, 100% HER2-, 100%	10 yr	RS <18 97% vs 96% HR, 1.19; 95% CI (0.40-3.49); p=0.73 RS 18-30 93% vs 88% HR, 0.64; 95% CI (0.23-1.75); p=0.62 RS ≥31 56.7% vs 89.6% **HR 0.18; 95% CI, (0.07-0.47); p<0.001** ***Interaction*** $p_{chemoXRS}$***=0.023***[a] RS ≤10 98% vs 95% HR, 1.19; 95% CI (0.41-3.51); p=0.46	NR	NR

Author, year, trial name	Intervention, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Freedom from distant recurrence (ET vs. CET)	Invasive disease-free survival (ET vs. CET)	Overall survival (ET vs. CET)
	RS 11-25 ET, 103 CET, 168 RS >25 ET, 35 CET, 87					RS 11-25 95% vs 94% HR, 0.61; 95% CI (0.26-1.35); p=0.43 RS >25 62% vs 88% **HR, 0.27; 95% CI, (0.12-0.62); p<0.001** ***Interaction*** $p_{chemoXRS}=0.014$[b]		
Paik et al, 2006 [3] NSABP B20	RS <18 ET, 135 CET, 218 RS 18-30 ET, 45 CET, 89 RS ≥31 ET, 47 CET, 117	NR	NR	ER+, 100% HER2-, 100%	10 yrs	RS <18 96.8% vs 95.6% 1.31; 95% CI (0.46-3.78); p=0.61 RS 18-30 90.9% vs 89.1% RR, 0.61; 95% CI (0.24 - 1.59); p=0.39 RS ≥31 60.5% vs 88.1% **RR, 0.26; 95% CI (0.13-0.53); p<0.001** ***Interaction*** $p_{chemoXRS}=0.038$	NR	RS <18 p=0.441 RS 18-30 p=0.826 RS ≥31 **p<0.001**

Abbreviations: CET: chemoendocrine therapy; CI: confidence interval; ER: estrogen receptor; ET: endocrine therapy; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; Mdn: median; mth: month; NR: not reported; OS: overall survival; PR: progesterone receptor; RCT: randomized controlled trial; RS: Recurrence score; yrs: years

[a, b] When controlling for patient age, tumour size, ER and PR status, and tumour grade

Table 4-3. Outcomes of subgroup analyses for the use of Oncotype DX for adjuvant chemotherapy in node-negative patients

Author, year, trial name	Subgroup, intervention, sample size	Freedom from distant recurrence (ET vs. CET)	Invasive disease-free survival (ET vs. CET)
RCTs			
Sparano et al, 2018 2019 [1,2] TAILORx	Tumour size		
	≤2cm RS 11-25, 5122	HR, 1.01; 95% CI, 0.73-1.39; p=NR	HR, 1.08; 95% CI, 0.92-1.28; p=NR
	>2cm RS 11-25, 1527	HR, 1.14; 95% CI, 0.75-1.74; p=NR	HR, 1.06; 95% CI, 0.82-1.37; p=NR
	Tumour grade		
	Low RS 11-25, 1893	HR, 1.82; 95% CI, 0.91-3.63; p=NR	HR, 1.09; 95% CI, 0.82-1.46; p=NR
	Intermediate RS 11-25, 3721	HR, 0.86; 95% CI, 0.62-1.20; p=NR	HR, 1.02; 95% CI, 0.85-1.23; p=NR
	High grade RS 11-25, 884	HR, 1.61; 95% CI, 0.88-2.94; p=NR	HR, 1.32; 95% CI, 0.92-1.90; p=NR
	Menopausal status		
	Premenopausal RS 11-25, 2415	HR, 1.42; 95% CI, 0.93-2.19; p=NR	**HR, 1.36; 95% CI, 1.06-1.75; p=NR**
	RS 11-15 ET, 472 CET, 415	HR, 0.88; 95% CI, 0.31-2.54; p=NR	HR, 0.85; 95% CI, 0.54-1.35; p=0.49
	RS 16-20 ET, 497 CET, 517	HR, 1.21; 95% CI, 0.64-2.31; p=NR	**HR, 1.76; 95% CI, 1.20-2.59; p=0.0034**
	RS 21-25 ET, 243 CET, 271	HR, 2.06; 95% CI, 1.03 to 4.14; p=NR	HR, 1.50; 95% CI, 0.93 to 2.42; p=0.094
	Postmenopausal RS 11-25, 4296	HR, 0.97; 95% CI, 0.71-1.34; p=NR	HR, 0.99; 95% CI, 0.84-1.17; p=NR
	RS 11-15, 1486	HR, 1.15; 95% CI, 0.62-2.13; p=NR	HR, 1.02; 95% CI, 0.76-1.37; p=NR

Author, year, trial name	Subgroup, intervention, sample size	Freedom from distant recurrence (ET vs. CET)	Invasive disease-free survival (ET vs. CET)
	RS 16-20, 1698	HR, 0.83; 95% CI, 0.49-1.42; p=NR	HR, 0.84; 95% CI, 0.64-1.09; p=NR
	RS 21-25, 1112	HR, 1.00; 95% CI, 0.60 to 1.68; p=NR	HR, 1.23; 95% CI, 0.90 to 1.70; p=NR
	Age		
	Age ≤50 RS 11-25, 1486	HR, 1.51; 95% CI, 0.97-2.33; p=NR	**HR, 1.51; 95% CI, 1.17-1.96; p=NR**
	RS 11-15 ET, 439 CET, 362	HR, 0.86; 95% CI, 0.31-2.39; p=NR	HR, 0.99; 95% CI, 0.62 to 1.58; p=0.97
	RS 16-20 ET, 454 CET, 469	HR, 1.36, 95% CI, 0.71-2.62; p=NR	HR, 1.90, 95% CI, 1.27-2.84; p=0.0016
	RS 21-25 ET, 246 CET, 246	HR, 2.19; 95% CI, 1.06-4.55; p=NR	HR, 1.70; 95% CI, 1.03-2.80; p=0.035
	Age 51-65 RS 11-25, 3545	HR, 0.93; 95% CI, 0.65-1.35; p=NR	HR, 0.89; 95% CI, 0.73-1.09; p=NR
	RS 11-15, 1250	HR, 1.10; 95% CI, 0.54-2.22; p=NR	HR, 0.74; 95% CI, 0.51-1.08; p=NR
	RS 16-20, 1425	HR, 0.72; 95% CI, 0.39-1.31; p=NR	HR, 0.76; 95% CI, 0.56-1.04; p=NR
	RS 21-25, 870	HR, 1.09; 95% CI, 0.59-1.99; p=NR	HR, 1.38; 95% CI, 0.94-2.03; p=NR
	Age >65 RS 11-25, 950	HR, 0.95; 95% CI, 0.48-1.86; p=NR	HR, 1.12; 95% CI, 0.81-1.53; p=NR
	RS 11-15, 322	HR, 0.73; 95% CI, 0.15-3.44; p=NR	HR, 1.36; 95% CI, 0.78-2.39; p=NR
	RS 16-20, 364	HR, 0.93; 95% CI, 0.29-2.94; p=NR	HR, 0.97; 95% CI, 0.58-1.62; p=NR
	RS 21-25, 264	HR, 1.07; 95% CI, 0.40-2.86; p=NR	HR, 1.07; 95% CI, 0.59-1.95; p=NR

Abbreviations: CET: chemoendocrine therapy; CI: confidence interval; ET: endocrine therapy; HR: hazard ratio; NR: not reported; RCT: randomized controlled trial; RS: recurrence score

Table 4-4. Outcomes for the use of Oncotype DX for adjuvant chemotherapy for node-positive patients

Author, year, trial name	Intervention, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Freedom from distant recurrence	Invasive disease-free survival (CET vs. ET)	Overall survival (CET vs. ET)
RCTs								
Kalinsky et al, 2021 [16] RxPONDER	RS ≤25 CET, 2487 ET, 2497	Median, 57.5 (18.3-87.6)	Pre, 33% Post, 67%	HR+, 100%, HER2-, 100%	Median, 5.3 yrs	NR	92.2% vs 91.0% HR, 0.86; 95% CI, 0.72-1.03; p=0.10	NR
Retrospective analyses of RCTs								
Albain et al, 2010 [17] SWOG 8814	RS <18 CET, 91 ET, 55 RS 18-30 CET, 57 ET, 46 RS ≥31 CET, 71 ET, 47	Mean, 60.4± 7.5	Post, 100%	ER+, 96.7% HER2+, 11.7%	10 yrs	NR	RS <18 64% vs 60% HR, 1.02; 95% CI, (0.54-1.93); p=0.97 RS 18-30 HR, 0.72; 95% CI, (0.39-1.31); p=0.48 RS ≥31 55% vs 43% **HR, 0.59; 95% CI, (0.35-1.01); p=0.033** *Interaction* $p_{treatmentXRS}$*=0.053*	RS <18 HR, 1.18; 95% CI, (0.55-2.54); p=0.68 RS 18-30 HR, 0.84; 95% CI, (0.40-1.78); p=0.65 RS ≥31 68% vs 51% HR, 0.56; 95% CI, (0.31-1.02); p=0.057

Abbreviations: CET: chemoendocrine therapy; CI: confidence interval; ER: estrogen receptor; ET: endocrine therapy; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; mth: month; NR: not reported; RCT: randomized controlled trial; RS: recurrence score

Table 4-5. Outcomes of subgroup analyses for the use of Oncotype DX for adjuvant chemotherapy in node-positive patients

Author, year, trial name	Subgroup, intervention, sample size	Distant relapse-free survival (CET vs. ET)	Invasive disease-free survival (CET vs. ET)
Randomized controlled trials			
Kalinsky et al, 2021 [16] RxPONDER	Menopausal status		
	Premenopausal RS ≤25 CET, 829 ET, 826	96.1% vs 92.8% **HR, 0.58; 95% CI, 0.39-0.87; p=0.009**	93.9% vs 89.0% **HR, 0.60; 95% CI, 0.43-0.83; p=0.002**
	Postmenopausal RS ≤25 CET, 1658 ET, 1671	94.4% vs 94.4% HR, 1.05; 95% CI, 0.81-1.37; p=0.70	91.3% vs 91.9% HR, 1.02; 95% CI, 0.82-1.26; p=0.89

Abbreviations: CET: chemoendocrine therapy; CI, confidence interval; ET: endocrine therapy; HR: hazard ratio; RS: recurrence score

Table 4-6. Prognostic ability of Oncotype DX for late recurrence in node-negative patients

Author, year, trial name	Recurrence score, sample size	Age, years (range)	Menopausal status	HER2 status	Follow-up	Risk for distant recurrence	Invasive disease-free survival	Overall survival
Retrospective analyses of RCTs								
Sestak et al, 2018 [9] ATAC	RS 0-10, 351 RS 11-25, 134 RS 26-100, 50	Mean, 63.4	Post, 100%	HR+, 100% HER2-, 100%	10 yrs (5-10 yrs)	RS 0-10 4.8% (95% CI, 2.9 to 7.9) RS 11-25 9.6% (95% CI 5.6 to 16.3) RS 26-100 16.1% (95% CI, 8.0 to 30.8)	NR	NR

Abbreviations: CI: confidence interval; ER: estrogen receptor; HER2: human epidermal growth factor 2; HR+; hormone receptor-positive; int: intermediate; NR: not reported; RCT: randomized controlled trial; RS: recurrence score; yrs: years

Table 4-7. Prognostic ability of Oncotype DX for late recurrence in node-positive patients

Author, year, trial name	Intervention, sample size	Age, years (range)	Menopausal status	HER2 status	Follow-up	Risk for distant Recurrence	Invasive disease-free survival	Overall survival
Retrospective analyses of RCTs								
Sestak et al, 2018 [9] ATAC	RS 0-10, 94 RS 11-25, 45 RS 26-100, 15	Mean, 63.4	Post, 100%	HR+, 100% HER2-, 100%	10 yrs (5-10 yrs)	RS 0-10 17.9% (95% CI, 11.5-27.3) RS 11-25 19.5% (95% CI 10.9-33.5) RS 26-100 27.5% (95% CI, 11.2-57.9)	NR	NR

Abbreviations: CI: confidence interval; HER2: human epidermal growth factor 2; HR+; hormone receptor-positive; NR: not reported; RCT: randomized controlled trial; RS: recurrence score; yrs: years

Question 2: What is the clinical utility (i.e., the ability of a test to provide information that is useful to direct treatment and ultimately improve patient outcomes) of MammaPrint for patients with early-stage invasive breast cancer?

A. PREDICTIVE STUDIES FOR ADJUVANT CHEMOTHERAPY

One RCT [5,6,39,40] was found that provides prospective evidence of the predictive value of MammaPrint for adjuvant chemotherapy. Tables 4-8 and 4-9 present a summary of the outcomes and subgroup analyses, respectively, while Table A5-2 in Appendix 5 presents study details including treatment regimens.

The MINDACT trial [5] is a non-inferiority trial that evaluated whether the addition of the 70-gene signature to standard clinical practice is beneficial in selecting patients for adjuvant chemotherapy. A follow-up exploratory analysis evaluated the survival of patients classified as ultra-low risk [40]. The initial study design enrolled patients with node-negative disease (79.0%) but was later revised to allow enrollment of women with up to three positive axillary nodes (20.9%). A total of 6693 patients were included in this study and divided into four main groups: low clinical risk and low genomic risk (41%); low clinical and high genomic risk (8.8%); high clinical and low genomic risk (23.2%); and high clinical risk and high genomic risk (27.0%). Patients with discordant results were randomly assigned to the adjuvant chemotherapy group or the no adjuvant chemotherapy group. Long-term follow-up data for a median of 8.7 years are available.

Distant metastasis-free survival

In patients with high clinical and low genomic risk, there was no significant difference between those who received adjuvant chemotherapy and those who did not after a median follow-up of five years (HR, 0.78; 95% CI, 0.50 to 1.21; p=0.27) with an absolute difference of 1.5% in the rate of survival without distant metastases between the groups [5]; however, a significant chemotherapy benefit was seen at 8.7 years (HR, 0.66; 95% CI, 0.48 to 0.92; p=NR) with an absolute difference of 2.5% [6]. Similarly, in patients with low clinical risk and high genomic risk, there was no significant difference between the two arms at five years (HR, 1.17; 95% CI 0.59 to 2.28; p=0.66) or 8.7 years (HR, 0.85; 95% CI 0.53 to 1.37; p=NR) with absolute differences of 0.8% and 1.5% between the two groups, respectively [5,6].

Disease-free survival

In patients with high clinical and low genomic risk, there was no significant difference between those who received adjuvant chemotherapy and those who did not with a median follow-up at five years (HR, 0.71; 95% CI, 0.50 to 1.01; p=0.06) [5] and at 8.7 years (HR, 0.79; 95% CI, 0.62 to 1.02; p=NR) [6]. Similarly, in patients with low clinical risk and high genomic risk, there was no significant difference between the two arms at five years (HR, 0.87; 95% CI 0.53 to 1.45; p=0.60) [5] or 8.7 years (HR, 0.79; 95% CI, 0.55 to 1.13; p=NR) [6].

Overall survival

In patients with high clinical and low genomic risk, there was no significant difference between those who received adjuvant chemotherapy and those who did not with a median follow-up at five years (HR, 0.69; 95% CI, 0.35 to 1.35; p=0.28) [5] and at 8.7 years (HR, 0.69; 95% CI (0.45 to 1.05; p=NR) [6]. Similarly, in patients with low clinical risk and high genomic risk, there was no significant difference between the two arms at five years (HR, 1.28; 95% CI, 0.54 to 3.02; p=0.58) [5] or 8.7 years (HR, 0.94; 95% CI, 0.54 to 1.67; p=NR) [6].

Subgroup analysis
Node negative

A prespecified exploratory subgroup analysis of patients according to nodal status was conducted [5]. In node-negative patients, there was no significant difference in distant metastasis-free survival between patients who received chemotherapy and no chemotherapy in the high clinical risk and low genomic risk group (p=0.193) or in the low clinical risk and high genomic risk group (p=0.815) after a median follow-up of five years. Similarly in node negative, ER-positive, HER2-negative patients, there was no difference between both treatment groups in the high clinical risk and low genomic risk group (p=NR) or in the low clinical risk and high genomic risk group (p=NR). However, after a median follow-up of 8.7 years, there was a significant difference between the two treatment groups in the high clinical risk and low genomic risk group (HR, 0.60; 95% CI, 0.38 to 0.96; p=NR). There was no significant difference in the low clinical risk and high genomic risk group (p=0.815)

Node positive

In node-positive patients, there was no significant difference in distant metastasis-free survival between patients who received chemotherapy and no chemotherapy in the high clinical risk and low genomic risk group (p=0.724) after a median follow-up of five years. The number of node-positive patients in the low clinical risk and high genomic risk was too small to be analyzed.

Age

In the follow-up publication by Piccart et al [6], a predefined exploratory analysis by age was conducted in HR-positive, HER2-negative women at high clinical risk and low genomic risk who were 50 years of age or younger and in women older than 50 years. In women 50 years or younger, a significant chemotherapy benefit was shown (HR, 0.54; 95% CI, 0.30 to 0.98; p=NR) with an absolute difference of 5.0% in the rate of survival without distant metastases between the treatment groups. No significant benefit was shown in women older than 50 years (HR, 0.82; 95% CI 0.55 to 1.24; p=NR).

Histology

In a follow-up abstract by Metzger et al [39], an exploratory subgroup analysis of patients with histologic data classified as invasive lobular carcinoma (ILC) or invasive ductal carcinoma (IDC) found that DMFS and DFS estimates were similar for both histological subtypes classified as either low or high genomic risk. No p-value was reported.

Ultra-low risk 70-gene signature

In a follow-up exploratory analysis currently available in abstract form [40], patients who had an ultra-low 70-gene signature had an eight-year distant metastasis-free interval of 97.6% (95% CI, 96.4 to 98.8) in the low clinical risk group and 95.0% (95% CI, 92.3 to 97.8) in the high clinical risk group. Similarly, the 8-year breast cancer specific survival was 99.7% (95% CI, 99.3 to 100) in the low clinical risk group and 99.2% (95% CI, 98.0 to 100) in the high clinical risk group.

B. PREDICTIVE STUDIES FOR EXTENDED ENDOCRINE THERAPY

One retrospective analysis of an RCT [22] was found in abstract form evaluating the predictive value of MammaPrint for extended endocrine therapy. Table 4-10 presents a summary of the outcomes, while Table A5-2 in Appendix 5 presents study details including treatment regimens.

Rastogi et al [22] conducted a retrospective analysis of the NSABP B42 trial, a randomized trial where postmenopausal, HR-positive women with breast cancer, who were disease-free after five years of endocrine therapy were randomized to letrozole or placebo

daily for five additional years. Of the original 3966 patients included in the original trial, 1866 were included in this analysis. There were no significant differences between the cohort aside from HER2 status. Exploratory analyses were conducted on patients primarily classified as low risk (MammaPrint score >0.000) by subcategorizing them into two new categories: ultra-low risk (MammaPrint score >0.355) and low but not ultra-low risk (MammaPrint score >0.000, ≤0.355). These subcategories were used to identify patients who would likely benefit from extended endocrine therapy.

Recurrence

In the low-risk group, there was a statistically significant benefit for extended letrozole (HR, 0.43; 95% CI, 0.25 to 0.74; p=0.002) for distant recurrence; however, this benefit was not observed in the high-risk group (HR, 0.65; 95% CI, 0.34 to 1.24; p=0.19) [22]. The treatment by risk group interaction was not statistically significant (p=0.38). There was a statistically significant benefit for extended letrozole in the low- but not ultra-low-risk group (HR, 0.42; 95% CI, 0.23 to 0.76; p=0.003); however, this benefit was not observed in the ultra-low-risk group (HR, 0.53; 95% CI, 0.13 to 2.15; p=0.37). No interaction term was reported.

Survival

For DFS, there was a statistically significant benefit with extended letrozole for patients in the low-risk group (HR, 0.67; 95% CI, 0.52 to 0.85; p<0.001); however, this benefit was not observed in the high-risk group (HR, 1.10; 95% CI, 0.82 to 1.47; p=0.55) [22]. The treatment by risk group interaction was statistically significant (p=0.015). There was a statistically significant benefit for extended letrozole in the low- but not ultra-low-risk group (HR, 0.64; 95% CI, 0.49 to 0.83; p<0.001); however, this benefit was not observed in the ultra-low-risk group (HR, 0.82; 95% CI, 0.45 to 1.48; p=0.50). No interaction term was reported.

Table 4-8. Outcomes for the use of MammaPrint for adjuvant chemotherapy

Author, year, trial name	Intervention, sample size	Age, years (range)	Menopausal status	Lymph Node	ER status, HER2 status	Follow-up	Distant metastasis-free survival	Disease-free survival	Overall survival
RCT									
Piccart et al, 2021 [6] Cardoso et al, 2016 [5] MINDACT	CT, 749 No CT, 748 CT, 344 No CT, 346	Median, 55 (23-71)	NR	LN-, 79.0% LN+, 21.0%	HR+, 88.4% HER2−, 90.3%	Median, 8.7 yrs	C-high/G-low CT vs. no CT 92.0% vs. 89.4% **HR, 0.66; 95% CI (0.48-0.92); p=NR** C-low/G-high CT vs. no CT, 92.3% vs. 90.8% HR, 0.85; 95% CI (0.53-1.37); p=NR	C-high/G-low CT vs. no CT 86.4% vs. 82.9% HR, 0.79; 95% CI (0.62-1.02); p=NR C-low/G-high CT vs. no CT, 86.2% vs. 81.9% HR, 0.79; 95% CI (0.55-1.13); p=NR	C-high/G-low CT vs. no CT 95.7% vs. 94.3% HR, 0.69; 95% CI (0.45-1.05); p=NR C-low/G-high CT vs. no CT, 93.8% vs. 93.0% HR, 0.94; 95% CI (0.54-1.67); p=NR
	CT, 749 No CT, 748 CT, n=344 No CT, 346					5 yrs	C-high/G-low CT vs. no CT 95.9% vs. 94.4% HR, 0.78; 95% CI (0.50-1.21); p=0.27 C-low/G-high CT vs. no CT, 95.8% vs. 95.0% HR, 1.17; 95% CI (0.59-2.28); p=0.66	C-high/G-low CT vs. no CT 92.9% vs. 90.1% HR, 0.71; 95% CI (0.50-1.01); p=0.06 C-low/G-high CT vs. no CT, 92.1% vs. 90.1% HR, 0.87; 95% CI (0.53-1.45); p=0.60	C-high/G-low CT vs. no CT 98.4% vs. 97.0% HR, 0.69; 95% CI (0.35-1.35); p=0.28 C-low/G-high CT vs. no CT, 97.1% vs. 97.8% HR, 1.28; 95% CI (0.54-3.02; p=0.58

Abbreviations: C-high/low: high/low clinical risk; CI: confidence interval; CT: chemotherapy; ER: estrogen receptor; G-high/low: high/low genomic risk; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; LN: lymph node; NR: not reported; yrs: years

Table 4-9. Outcomes of subgroup analyses for the use of MammaPrint for adjuvant chemotherapy

Author, year, trial name	Subgroup, intervention, sample size	Follow-up	Distant metastasis-free survival	Disease-free survival	Overall survival
Randomized controlled trials					
Piccart et al, 2021 [6] MINDACT	Lymph node status (HR+, HER2-)				
	Node negative C-high/G-low CT, 349 No CT, 350 C-low/G-high CT, 272 No CT, 262	8 yrs	C-high/G-low CT vs. no CT 91.7% vs. 89.2% **HR, 0.60; 95% CI, 0.38 to 0.96; p=NR** C-low/G-high CT vs. no CT, 91.1% vs. 91.8% p=0.815	C-high/G-low CT vs. no CT 87.5% vs. 83.4% p=NR C-low/G-high CT vs. no CT, 85.5% vs. 83.6% p=NR	C-high/G-low CT vs. no CT 95.5% vs. 93.9% p=NR C-low/G-high CT vs. no CT, 92.6% vs. 92.9% p=NR
		5 yrs	C-high/G-low CT vs. no CT 95.1% vs. 94.3% p=NR C-low/G-high CT vs. no CT, 94.1% vs. 95.6% p=NR	C-high/G-low CT vs. no CT 93.0% vs. 90.1% p=NR C-low/G-high CT vs. no CT, 91.3% vs. 91.6% p=NR	C-high/G-low CT vs. no CT 98.5% vs. 96.4% p=NR C-low/G-high CT vs. no CT, 96.1% vs. 98.4% p=NR
	Node positive C-high/G-low CT, 326 No CT, 332 C-low/G-high Too small to be analyzed	8 yrs	C-high/G-low CT vs. no CT 91.2% vs. 89.9% HR, 0.84; 95% CI, 0.51-1.37; p=NR	C-high/G-low CT vs. no CT 85.3% vs. 82.8% p=NR	C-high/G-low CT vs. no CT 95.5% vs. 94.9% p=NR
		5 yrs	C-high/G-low CT vs. no CT 96.0% vs. 95.9% p=NR	C-high/G-low CT vs. no CT 92.7% vs. 91.0% p=NR	C-high/G-low CT vs. no CT 98.4% vs. 98.8% p=NR
	Age (HR+, HER2-)				
	≤50 years C-high/G-low CT, 235	8 yrs	C-high/G-low CT vs. no CT 93.6% vs. 88.6%	NR	NR

Author, year, trial name	Subgroup, intervention, sample size	Follow-up	Distant metastasis-free survival	Disease-free survival	Overall survival
	No CT, 229		**HR, 0.54; 95% CI, 0.30-0.98; p=NR**		
		5 yrs	CT vs. no CT 96.2% vs. 93.6% p=NR		
	>50 years C-high/G-low CT, 441 No CT, 453	8 yrs	C-high/G-low CT vs. no CT 90.2% vs. 90.0% HR, 0.82; 95% CI, 0.55-1.24; p=NR		
		5 yrs	CT vs. no CT 95.0% vs. 95.8% p=NR		
Metger O et al, 2021 *Abstract* [39] MINDACT	ILC G-high, 79 G-low, 408	5 yrs	G-high, 89.4% (95% CI, 78.5-94.9) G-low, 96.6% (95% CI, 94.0-98.1)	G-high, 84.6% (95% CI, 73.5-91.3) G-low, 92.0% (95% CI, 88.6-94.4)	NR
	IDC, G-high, 1888 G-low, 2938		G-high, 92.3% (95% CI, 90.9-93.5) G-low, 96.5% (95% CI, 95.7-97.2)	G-high, 87.1% (95% CI, 85.3-88.6) G-low, 92.5% (95% CI, 91.4-93.4)	

Abbreviations: C-high/low: high/low clinical risk; CI: confidence interval; CT: chemotherapy; G-high/low: high/low genomic risk; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; ILC: invasive lobular carcinoma; IDC: invasive ductal carcinoma LN: lymph node; NR: not reported; yrs: years

Table 4-10. Outcomes for the use of MammaPrint for extended endocrine therapy

Author, year, trial	Intervention, sample size	Age, years (range)	Menopausal status	Lymph node	ER status, HER2 status	Follow-up	Distant recurrence (EET vs. PBO)	Disease-free survival (EET vs. PBO)	Overall survival
Retrospective analyses of RCTs									
Rastogi et al, 2021 *Abstract* [22] NSABP B42	1866	NR	Post, 100%	NR	HR+, 100%, HER2+, NR	NR	Low risk **HR, 0.43, 95% CI (0.25-0.74); p=0.002** High risk HR, 0.65, 95% CI (0.34-1.24); p=0.19 *Interaction* $p_{treatmentXriskgroup}=0.38$ Ultra low risk HR, 0.53, 95% CI (0.13-2.15); p=0.37 Low not ultra low risk **HR, 0.42, 95% CI (0.23-0.76); p=0.003**	Low risk **HR, 0.67, 95% CI (0.52-0.85); p<0.001** High risk HR, 1.10, 95% CI (0.82-1.47); p=0.55 *Interaction* $\boldsymbol{p_{treatmentXriskgroup}=0.015}$ Ultra low risk HR, 0.82; 95% CI (0.45-1.48); p=0.50 Low not ultra low risk **HR, 0.64, 95% CI (0.49-0.83); p<0.001**	NR

Abbreviations: CI: confidence interval; EET: extended endocrine therapy; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; NR: not reported; PBO: placebo; RCT: randomized controlled trial

Question 3: What is the clinical utility (i.e., the ability of a test to provide information that is useful to direct treatment and ultimately improve patient outcomes) of Prosigna for patients with early-stage invasive breast cancer?

A. PREDICTIVE STUDIES FOR ADJUVANT CHEMOTHERAPY

Two retrospective analyses of RCTs [7,8] have been included that examined the predictive effect of Prosigna for adjuvant chemotherapy. Table 4-11 provides study outcomes while Table A5-3 in Appendix 5 presents study details including treatment regimens.

Liu et al [7] retrospectively evaluated the role of Prosigna in patients from the NCIC CTG MA.21 trial, which randomized patients to either cyclophosphamide, epirubicin, and flurouracil (CEF), epirubicin, cyclophosphamide, and paclitaxel (EC/T), or doxorubicin, cyclophosphamide, and paclitaxel (AC/T). Of the 2104 women enrolled in the original trial, 1094 were included this analysis after excluding patients who did not receive protocol-specified treatment. There were no significant differences in baseline characteristics between the original population and this subset. The majority of the patients (70.3%) were node positive.

The study by Jensen et al retrospectively examined the Danish Breast Cancer Group (DBCG) 77B trial [8], a four-arm trial that randomized 1146 premenopausal women with high-risk early breast cancer to no systemic treatment, levamisole, oral cyclophosphamide or CMF. This retrospective review combined the no systemic treatment and levamisole arms (no systemic chemotherapy) and the two cyclophosphamide-based arms (cyclophosphamide-based chemotherapy) to evaluate the predictive ability of Prosigna. Of the 1146 patients included in the original trial, the Prosigna assay was conducted on samples obtained from 460 patients. There were significant differences between the included 460 and the remaining 612 including histologic type (p=0.03) and malignancy grade (p=0.02). The majority of the patients (87%) were node positive.

Recurrence-free survival

The NCIC CTG MA.21 trial [7] conducted exploratory multivariable analyses that determined categorical ROR score was not predictive of response to chemotherapy regimen (p=0.232) for RFS.

Disease-free survival and overall survival

In patients from the DBCG 77B trial [8], there was no significant difference between ROR score group and treatment for DFS (p=0.37) or OS (p=0.30). In a planned exploratory analysis of patients with ER-positive, HER2-negative breast cancer, a benefit from cyclophosphamide-based chemotherapy was shown in the high-risk group (ROR >40; HR, 0.48; 95% CI, 0.33 to 0.69), while no benefit was shown in the low-risk group (ROR ≤40; HR, 1.13; 95% CI, 0.42 to 3.07). There was no statistically significant interaction between ROR risk group and treatment (p=0.10).

Subgroup analysis

Prosigna intrinsic subtype

In the retrospective analysis of the NCIC CTG MA.21, intrinsic subtypes (non-luminal vs. luminal) were not predictive of treatment benefit (AC/T vs. EC/T + CEF; p=0.88) or of taxane benefit (EC/T vs. CEF; p=0.05).

In the retrospective analysis of the DBCG 77B trial [8], a statistically significant interaction was found between Prosigna subtypes and treatment in a multivariate analysis for both DFS (p=0.001) and OS (p=0.04) [8]. Patients with luminal B and basal-like subtypes showed significant benefit from cyclophosphamide-based chemotherapy, while the luminal A and HER2-enriched subtypes did not. P-values were not reported.

B. PREDICTIVE STUDIES FOR EXTENDED ENDOCRINE THERAPY

There were no studies evaluating the predictive value of Prosigna for extended endocrine therapy.

C. PROGNOSTIC STUDIES FOR LATE RECURRENCE

Three studies [9-11] have been included that examined the prognostic effect of Prosigna for late recurrence (i.e., 5-10 years). Tables 4-12 and 4-13 present a summary of the outcomes for studies with node-negative and node-positive disease, respectively. Table A5-3 in Appendix 5 presents study details including treatment regimens.

The ATAC trial evaluated the efficacy and safety of anastrozole versus tamoxifen given for five years in 9366 postmenopausal women with localized breast cancer. A subsequent sub study of the ATAC trial, the TransATAC, collected paraffin blocks from 2006 HR-positive women who were assigned to the monotherapy arms in the original trial. Sestak et al [9] assessed the risk of late distant recurrence in 774 women who had data available for all six signatures.

The ABCSG-8 trial, compared five years of adjuvant tamoxifen with sequential therapy consisting of tamoxifen for two years followed by anastrozole for three years in 3791 postmenopausal women with ER-positive breast cancer. Filipits et al [10] sought to determine whether the ROR score could prognosticate late distant recurrence in 1246 patients.

Sestak et al [11] examined both the ATAC trial and ABCSG-8 trial together to determine whether ROR score could prognosticate late distant recurrence in 2137 women.

i. Lymph node-negative patients

All three studies [9-11] reported prognostic utility for late recurrence in node-negative patients.

Recurrence

In the retrospective analysis of the ATAC trial, Sestak et al [9] found that the risk of late distant recurrence was 1.4% (95% CI, 0.5 to 3.8) for low-risk patients, 10.0% (95% CI, 6.0 to 16.5) for intermediate-risk patients and 23.2% (95% CI, 14.9 to 35.2) for high-risk patients as determined by the ROR score.

In the study combining both the ATAC trial and ABCSG-8 trial together, Sestak et al found there was a significant difference in late distant recurrence between patients in the high-risk versus low-risk group (HR, 5.49; 95% CI, 2.92 to 10.35; p=NR).

Distant recurrence-free survival

In the retrospective analysis of the ABSCG-8 trial, Filipits et al [10] found the probability for 15-year DRFS was 97.6% (95% CI, 94.7 to 98.9) for low-risk patients, 90.9% (95% CI, 85.9 to 94.2) for intermediate-risk patients and 82.5% (95% CI, 74.8 to 88.1) for high-risk patients as determined by the ROR score with a significant difference in late DRFS between patients in the high-risk versus low-risk group (HR, 4.74; 95% CI, 1.89 to 11.87; p<0.001). Patients with luminal A subtype had higher rates of late DRFS than patients with luminal B subtype (p=0.04).

ii. Lymph node-positive patients

All three studies [9-11] reported prognostic utility for late recurrence in node-positive patients.

Recurrence

In the retrospective analysis of the ATAC trial, Sestak et al [9] found that the risk of late distant recurrence was 13.0% (95% CI, 6.1 to 26.7) for intermediate-risk patients and 25.0% (95%

CI, 17.5 to 35.0) for high-risk patients. None of the node-positive low-risk women experienced distant recurrence at 10 years.

In the study combining both the ATAC trial and ABCSG-8 trial together, Sestak et al [11] found there was a significant difference in late distant recurrence between patients in the high-risk and low-risk group (HR, 7.94; 95% CI, 2.87 to 21.92; p=NR) with similar differences within the group in patients with one to three positive nodes (HR, 7.37; 95% CI, 2.63 to 20.65; p=NR).

Distant recurrence-free survival

In the retrospective analysis of the ABSCG-8 trial, Filipits et al [10] found the probability for 15-year DRFS was 100% for low-risk patients, 93.5% (95% CI, 84.0 to 97.5) for intermediate-risk patients and 79.9% (95% CI, 70.0 to 86.8) for high-risk patients as determined by the ROR score with a significant difference in late DRFS between patients in the high vs. intermediate group (HR, 3.15; 95% CI, 1.20 to 8.24; p=0.02). The hazard ratio between the high vs. low for node-positive patients could not be determined due to no late DRFS events in the low ROR score group. Patients with luminal A subtype had higher rates of DRFS than patients with luminal B subtype (p=0.03).

Table 4-11. Outcomes for the use of Prosigna for adjuvant chemotherapy

Author, year, trial name	Intervention, sample size	Age, years (range)	Menopausal status	Lymph Node	ER status, HER2 status	Follow-up	Recurrence-free survival	Disease-free survival	Overall survival
Retrospective analyses of RCTs									
Jensen et al, 2018 [8] DBCG 77B	ROR 0-51, 155 ROR 52-71, 148 ROR 72-100, 157	NR	Pre, 100%	LN-, 9% LN+, 87%	HR+, 72% HER2-, NR	10 yrs	NR	CT vs. NO CT HR, 0.55; 95% CI (0.38-0.79); p=NR ROR 8-51 HR, 0.74; 95% CI (0.38-1.43); p=NR ROR 52-71 HR, 0.42; 95% CI (0.24-0.73); p=NR ROR 72-100 HR, 0.58; 95% CI (0.35-0.95); p=NR	CT vs. NO CT HR, 0.85; 95% CI (0.65-1.12); p=NR ROR 8-51 HR, 1.15; 95% CI (0.72-1.84); p=NR ROR 52-71 HR, 0.76; 95% CI (0.49-1.17); p=NR ROR 72-100 HR, 0.74; 95% CI (0.49-1.11); p=NR
Liu et al, 2015 [7] NCIC CTG MA. 21	ROR ≤15, 37 ROR 16-40, 196 ROR >40, 861	Median, 47 yrs (23-61)	Pre, 69.2% Post, 30.8%	LN-, 29.7% LN+, 70.3%	ER+, 58.3% HER2-, 71.2%	10 yrs	Interaction $p_{chemotherapyregimenXRORscore}$=0.232	NR	NR

Abbreviations: CI: confidence interval; CT: chemotherapy; ER: estrogen receptor; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; LN: lymph node; NR: not reported; RCT: randomized controlled trial; ROR: risk of recurrence; yrs: years

Table 4-12. Prognostic ability of Prosigna for late recurrence in node-negative patients

Author, year, trial name	Prosigna category, n	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Risk for distant Recurrence	Distant recurrence-free survival	Overall survival
Retrospective analyses of RCTs								
Sestak et al, 2018 [9] ATAC	ROR low, 292 ROR intermediate, 165 ROR high, 78	Mean, 63.4	Post, 100%	HR+, 100% HER2-, 100%	10 yrs (5-10 yrs)	ROR low 1.4% (95% CI, 0.5-3.8) ROR intermediate 10.0% (95% CI, 6.0-16.5) ROR high 23.2% (95% CI, 14.9-35.2)	NR	NR
Filipits et al, 2014 [10] ABCSG-8	ROR low, 448 ROR intermediate, 292 ROR high, 179 Luminal A, 656 Luminal B, 240	NR	Post, 100%	HR+, 100% HER2-, NR	Median, 11 yrs (5-15 yrs)	NR	ROR low 97.5% (95% CI, 94.6-98.9) ROR intermediate 90.0% (95% CI, 83.6-94.0) ROR high 85.8% (95% CI, 72.5-93.0) ROR high vs. low **HR, 4.74; 95% CI (1.89-11.87); p<0.001** Luminal A 94.7% (95% CI, 91.5-96.7) Luminal B 88.7% (95% CI, 77.9-94.4) Luminal A vs B **p=0.04**	NR

Author, year, trial name	Prosigna category, n	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Risk for distant Recurrence	Distant recurrence-free survival	Overall survival
Sestak et al, 2015 [11] ATAC & ABCSG-8	ROR low, 1046 ROR intermediate, 378 ROR high, 156	NR	Post, 100%	HR+, 100% HER2-,	Median, 10 yrs (5-10 yrs)	ROR low 2.3% (95% CI, 1.5-3.5) ROR intermediate 8.5% (95% CI, 5.9-12.1) ROR high 9.3% (95% CI, 5.5-15.5) ROR high vs low **HR, 5.49; 95% CI (2.92-10.35); p=NR**	NR	NR

Abbreviations: CI: confidence interval; DR: distant recurrence; ER: estrogen receptor; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; NR: not reported; RCT: randomized controlled trial; ROR: risk of recurrence; yrs: years

Table 4-13. Prognostic ability of Prosigna for late recurrence in node-positive patients

Author, year, trial name	Prosigna category, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Risk for distant recurrence	Distant-recurrence free survival	Overall survival
Retrospective analyses of RCTs								
Sestak et al, 2018 [9] ATAC	ROR low, 15 ROR intermediate, 51 ROR high, 88	Mean, 63.4 yrs	Post, 100%	HR+, 100% HER2-, 100%	10 yrs (5-10 yrs)	ROR low 0 ROR intermediate 13.0% (95% CI, 6.1-26.7) ROR high 25.0% (95% CI, 17.5-35.0)	NR	NR
Filipits et al, 2014 [10] ABCSG-8	ROR low, 12 ROR intermediate, 124 ROR high, 191 Luminal A, 230 Luminal B, 91	NR	Post, 100%	HR+, 100% HER2-,	Median, 11 yrs (5-15 yrs)	NR	ROR low 100% ROR intermediate 93.5% (95% CI, 84.0-97.5) ROR high 79.9% (95% CI, 70.0-86.8) ROR high vs. intermediate **HR, 3.15; 95% CI (1.20-8.24); p=0.02** Luminal A 87.2% (95% CI, 76.9-93.1) Luminal B 79.9% (95% CI, 66.6-88.3) Luminal A vs B **p=0.03**	NR

Author, year, trial name	Prosigna category, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Risk for distant recurrence	Distant-recurrence free survival	Overall survival
Sestak et al, 2015 [11] ATAC & ABCSG-8	ROR low, 137 ROR intermediate, 160 ROR high, 260	NR	Post, 100%	HR+, 100% HER2-,	Median, 10 yrs (5-10 yrs)	ROR low 3.3% (95% CI, 1.2-8.6) ROR intermediate 7.8% (95% CI, 4.4-13.8) ROR high 20.9% (95% CI, 16.1-26.9) ROR high vs. low **HR, 7.94; 95% CI (2.87-21.92); p=NR**	NR	NR

Abbreviations: CI: confidence interval; DFS: disease-free survival; DR: distant recurrence; ER: estrogen receptor; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; int: intermediate; NR: not reported; RCT: randomized controlled trial; ROR: risk of recurrence; yr(s): years

[a] risk groups were tailored to the number of positive nodes

Question 4: What is the clinical utility (i.e., the ability of a test to provide information that is useful to direct treatment and ultimately improve patient outcomes) of EndoPredict for patients with early-stage invasive breast cancer?

A. PREDICTIVE STUDIES FOR ADJUVANT CHEMOTHERAPY

One retrospective, comparative study of five RCTs [41], which examined whether EPclin can predict chemotherapy benefit in patients with ER-positive, HER2-negative disease, was found. Table 4-14 presents a summary of the outcomes. Table A5-4 in Appendix 5 presents study details including treatment regimens.

This study by Sestak et al [41] compared patients from the GEICAM/9906 and GEICAM 2003/02 trials who received endocrine therapy plus chemotherapy with patients from the ABCSG-6, ABCSG-8, and TransATAC trials who received five years of endocrine therapy only. Chemotherapy and endocrine therapy agents varied across the trials. A total of 3746 patients were included in this review, with 1116 receiving endocrine therapy plus chemotherapy and 2630 receiving endocrine therapy only. There were significant differences between the baseline characteristics of patients. Patients in the endocrine therapy-only arm were all postmenopausal, significantly older, had significantly smaller tumours, significantly more node-negative disease, significantly fewer poorly differentiated disease and significantly lower median EPclin score compared with patients who received endocrine therapy plus chemotherapy (all $p<0.05$). Outcomes for this study were not analyzed by nodal status; 65.7% of patients were node-negative and 34.3% were node-positive.

Distant recurrence

Women with an EPclin score of 5 had a 10-year risk of distant recurrence of 46.1% (95% CI, 40.2 to 51.4) in the endocrine therapy arm and 25.8% (95% CI, 22.0 to 29.5) in the endocrine therapy plus chemotherapy arm [41]. A significant interaction was found between EPclin as a continuous variable and treatment ($p=0.022$). However, there was a non-significant interaction between treatment and EPclin score ($p=0.17$).

B. PREDICTIVE STUDIES FOR EXTENDED ENDOCRINE THERAPY

There were no studies evaluating the predictive value of EndoPredict for extended endocrine therapy. As a result, prognostic studies for late recurrence were included.

C. PROGNOSTIC STUDIES FOR LATE RECURRENCE

Three studies [9,12,44] have been included which examined the prognostic effect of EndoPredict for late recurrence (i.e., 5-10 years). Tables 4-15, 4-16, and 4-17 present a summary of the outcomes for studies with node-negative, node-positive, and mixed node populations, respectively. Table A5-4 in Appendix 5 presents study details including treatment regimens.

The ATAC trial evaluated the efficacy and safety of anastrozole versus tamoxifen given for five years in 9366 postmenopausal women with localized breast cancer. A subsequent substudy of the ATAC trial, the TransATAC, collected paraffin blocks from 2006 women who assigned to the monotherapy arms in the original trial. Sestak et al [9] assessed the risk of late distant recurrence in 774 women who had data available for all six signatures.

The ABCSG-6 compared patients who received five years of adjuvant tamoxifen, with or without the AI aminoglutethimide, for the first two years, while the ABCSG-8 trial compared five years of adjuvant tamoxifen with sequential therapy consisting of tamoxifen for two years followed by anastrozole for three years in 3791 postmenopausal women. Dubsky et al [44] and Filipits et al [12] examined both the ABCSG-8 and ABCSG-6 trial together. Dubsky et al included 1702 women who participated in the tamoxifen-only arm from both trials to determine whether

EndoPredict can identify patients with late recurrence. Similarly, Filipits et al reassessed the same cohort from Dubsky et al with a longer-term follow up of the DRFR.

i. Lymph node-negative patients

Two studies [9,12] reported the prognostic utility of EndoPredict for late recurrence in node-negative patients.

Recurrence

In examining the ABCSG-6 and ABCSG-8 trials together, Filipits et al [12] found that there was a significant difference in DRFR from five to 15 years between those with low and high EPclin scores (HR, 3.77; 95% CI, 1.84 to 7.72; p<0.0001).

In the retrospective analysis of the ATAC trial, Sestak et al [9] found that the risk of late distant was 4.3% (95% CI, 2.6 to 7.1) for EPclin low-risk patients and 14.6% (95% CI, 9.6 to 22.0) for EPclin high-risk patients.

ii. Lymph node-positive patients

Two studies [9,12] reported prognostic utility of EndoPredict for late recurrence for node-positive patients.

Recurrence

Filipits et al [12] found that in women who were distant recurrence-free at five years, there was a significant difference in DRFR from five to 15 years between those with low and high EPclin scores (HR, 3.59; 95% CI, 1.27 to 10.18; p=0.0100) with similar results being observed for the subset of women with one to three positive nodes (p=0.0337).

In the retrospective analysis of the ATAC trial, Sestak et al [9] found that the risk of late distant recurrence was 3.3% (95% CI, 0.5 to 21.4) for EPclin low-risk patients and 23.6% (95% CI, 17.0 to 32.1) for EPclin high-risk patients.

iii. Lymph node-positive and -negative patients

One retrospective study [44] provided outcomes for the overall population and did not conduct any node-specific analyses.

Recurrence

In retrospectively examining patients from tamoxifen-only arm of both the ABCSG-6 and ABCSG-8 trials together, Dubsky et al [44] found a significant difference in freedom from late distant recurrence between those who were EndoPredict low and EndoPredict high (HR, 3.28; 95% CI, 1.48 to 7.24; p=0.002) and between those who were EPclin low and EPclin high (HR, 6.25; 95% CI, 2.72 to 14.36; p<0.001). No p-value was reported.

Table 4-14. Outcomes for the use of EndoPredict for adjuvant chemotherapy

Author, year, trial name	Intervention, sample size	Age, years (range)	Menopausal status	Lymph Node	ER status, HER2 status	Follow-up	Risk for distant recurrence (ET vs. CET)	Disease-free survival	Overall survival
Retrospective study									
Sestak et al, 2019 [41]	ET, 2630 CET, 1116	NR	ET, 100% post CET, 51% pre, 49% post	LN-, 65.7% LN+, 34.3%	ER+, 100% HER2-, 100%	10 yrs	EPclin score 1 1.0% vs. 1.1% EPclin score 2 2.8% vs. 2.5% EPclin score 3 7.6% vs. 5.7% EPclin score 4 19.8% vs. 12.4% EPclin score 5 46.1% vs. 25.8% EPclin score 6 82.2% vs. 49.2% Interaction $p_{treatmentXEPclinscore}$=0.17 **$p_{treatmentxEPclincontinuous}$=0.022**	NR	NR

Abbreviations: CET: chemotherapy plus endocrine therapy; EPclin: EndoPredict score plus nodal status and tumour size; ER: estrogen receptor; ET: endocrine therapy; HER2: human epidermal growth factor 2; LN: lymph node; NR: not reported; RCT: randomized controlled trial; yrs: years

Table 4-15. Prognostic ability of EndoPredict for late recurrence in node-negative patients

Author, year, trial name	Assay classification, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Risk for distant recurrence	Disease-free survival	Overall survival
Retrospective analyses of RCTs								
Filipits et al, 2019 [12] ABCSG-6 & ABCSG-8	EPclin low, 764 EPclin high, 212	Median, 63	Post, 100%	ER+, 100% HER2-, 100%	Median, 9.6 yrs (5-15 yrs)	DRFR EPclin low 96.9% (95% CI, 95.2-98.5) EPclin high 84.9% (95% CI, 75.1-96.0) **HR, 3.77; 95% CI (1.84-7.72); p<0.0001**	NR	NR
Sestak et al, 2018 [9] ATAC	EPclin low, 393 EPclin high, 142	Mean, 63.4	Post, 100%	HR+, 100% HER2-, 100%	10 yrs (5-10 yrs)	EPclin low 4.3% (95% CI, 2.6 to 7.1) EPclin high 14.6% (95% CI, 9.6 to 22.0)	NR	NR

Abbreviations: CI: confidence interval; DRFR: distant recurrence-free rate; EPclin: EndoPredict score plus nodal status and tumour size; ER: estrogen receptor; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; Mdn: median; NR: not reported; RCT: randomized controlled trial; yrs: years

Table 4-16. Prognostic ability of EndoPredict for late recurrence in node-positive population

Author, year, trial name	Assay classification, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Risk for distant recurrence	Disease-free Survival	Overall survival
Retrospective analyses of RCTs								
Filipits et al, 2019 [12] ABCSG-6 & ABCSG-8	EPclin low, 133 EPclin high, 277	Median, 63	Post, 100%	ER+, 100% HER2-, 100%	Median, 9.6 yrs (5-15 yrs)	DRFR EPclin low 87.8% (95% CI, 74.0-100) EPclin high 83.0% (95% CI, 77.1-89.4) **HR, 3.59; 95% CI (1.27-10.18); p=0.0100**	NR	NR
Sestak et al, 2018 [9] ATAC	EPclin low, 40 EPclin high, 114	Mean, 66.4	Post, 100%	HR+, 100% HER2-, 100%	10 yrs (5-10 yrs)	EPclin low 3.3% (95% CI, 0.5 to 21.4) EPclin high 23.6% (95% CI, 17.0 to 32.1)	NR	NR

Abbreviations: CI: confidence interval; DRFR: distant recurrence-free rate; EPclin: EndoPredict score plus nodal status and tumour size; ER: estrogen receptor; HER2: human epidermal growth factor 2; HR: hormone receptor; Mdn: median; mths: months; NR: not reported; RCT: randomized controlled trial; yrs: years

Table 4-17. Prognostic ability of EndoPredict for late recurrence in studies with both node-negative and node-positive patients

Author, year, trial name	Assay classification, sample size	Age, years (range)	Menopausal status	LN status	ER status, HER2 status	Follow-up	Freedom from distant recurrence	Disease-free Survival	Overall survival
Retrospective analyses of RCTs									
Dubsky et al, 2013 [44] ABCSG-6 & ABCSG-8	EP low, 503 EP high, 495 EPclin low, 642 EPclin high, 356	Median, 63.8	Post, 100%	LN-, 68% LN+, 32%	ER+, 100% HER2-, 100%	NR (5-10 yrs)	EP low vs EP high **HR, 3.28; 95% CI (1.48-7.24); p=0.002** EPclin low vs EPclin high **HR, 6.25; 95% CI (2.72-14.36); p<0.001**	NR	NR

Abbreviations: CI: confidence interval; EP: EndoPredict; EPclin: EndoPredict score plus nodal status and tumour size; ER: estrogen receptor; HER2: human epidermal growth factor 2; HR: hazard ratio; LN: lymph node; LRFS: local recurrence-free survival; Mdn: median; mths: months NR: not reported; RCT: randomized controlled trial; yrs: years

Question 5: What is the clinical utility (i.e., the ability of a test to provide information that is useful to direct treatment and ultimately improve patient outcomes) of Breast Cancer Index for patients with early-stage invasive breast cancer?

A. PREDICTIVE STUIDES FOR ADJUVANT CHEMOTHERAPY

There were no studies evaluating the predictive value of Breast Cancer Index for adjuvant therapy. As a result, prognostic studies for late recurrence were included.

B. PREDICTIVE STUDIES FOR EXTENDED ENDOCRINE THERAPY

In total, four retrospective studies of RCTs [18-21], of which one is currently available in abstract form [21], have been published which examine the predictive value of the Breast Cancer Index on patient outcomes. Tables 4-18 and 4-19 present a summary of the outcomes and Table A5-5 in Appendix 5 presents study details including treatment regimens.

i. Lymph node-positive patients

The study conducted by Bartlett et al retrospectively examined the predictive performance of BCI (H/I) in the extended endocrine setting in the aTTom trial [19], a phase III RCT where breast cancer patients who were disease-free after having completed at least four years of adjuvant tamoxifen therapy were randomized to either continue or stop tamoxifen treatment for an additional five years. Of the 6956 patients included in the original trial, 583 node-positive, HR-positive patients with BCI (H/I) results were the focus of this study. There were no significant differences between the included patients and those in the original trial.

Recurrence-free interval

In the Trans-aTTom study [19], there was no significant benefit in risk of recurrence between the two arms (HR, 0.88; 95% CI, 0.65 to 1.18; p=0.388). In further prospectively planned subgroup analyses of patients classified as BCI (H/I)-high, a significant benefit from extended tamoxifen was shown (HR, 0.35; 95% CI, 0.15 to 0.86; p=0.027) with an absolute recurrence risk benefit of 10.2%; however, in patients classified as BCI (H/I)-low, there was no significant benefit from extended tamoxifen (HR, 1.07; 95% CI, 0.69 to 1.65; p=0.768). There was a significant interaction between continuous BCI (H/I) and extended tamoxifen treatment (p=0.012) after adjusting for age, tumour size, tumour grade, and ER and PR status.

Disease-free interval

In the Trans-aTTom study [19], a significant interaction between continuous BCI (H/I) and extended tamoxifen treatment (p=0.019) was shown after adjusting for age, tumour size, tumour grade, and ER and PR status.

ii. Lymph node-positive and -negative patients

Three retrospective studies of RCTs [18,20,21] included results of both node-negative and node-positive patients.

The most recent study, currently available in abstract form, retrospectively examined 2179 patients from the NSABP B42 trial [21], a phase III study which randomized HR-positive postmenopausal women who were disease-free after five years of treatment with an AI or tamoxifen followed by an AI to receive five years of letrozole or placebo.

The second study retrospectively examined the IDEAL trial [20], a phase III RCT that randomized HR-positive early-stage postmenopausal women to receive either 2.5 or five years of letrozole after five years of adjuvant therapy with either tamoxifen monotherapy (13%), tamoxifen followed by an AI (60%), or AI monotherapy (27%). Of the 1824 patients in the original

trial, 908 women were included in this retrospective study with no significant differences between the two populations.

The final study, published in 2013, retrospectively conducted a nested case-control study of the NCIC Clinical Trials Group MA.17 [18], a phase III study randomizing postmenopausal women with HR-positive tumours who remained disease-free after having completed approximately five years of standard adjuvant tamoxifen treatment to receive either letrozole or placebo as extended adjuvant therapy for five years. A total of 83 patients with disease recurrence were matched to 166 patients without disease recurrence, where the control patient had been recurrence-free for longer than the case patient, to evaluate the use of H/I for prediction of treatment benefit.

Recurrence-free survival

In the translational IDEAL study [20], there was significant reduction in the risk of recurrence for BCI (H/I)-high patients who received five years of extended letrozole (HR, 0.42; 95% CI, 0.21 to 0.84; p=0.011) with an absolute reduction of recurrence risk of 9.8%; however, this benefit was not observed in BCI (H/I)-low patients (HR, 0.95; 95% CI, 0.58 to 1.56; p=0.835). Similarly, in patients treated with primary adjuvant endocrine therapy with an AI, BCI (H/I)-high patients received a significant benefit from extended letrozole (HR, 0.34; 95% CI, 0.16 to 0.73; p=0.004), while no benefit was seen in BCI (H/I)-low patients (HR, 0.90; 95% CI, 0.53 to 1.55; p=0.712). There was a significant interaction between BCI (H/I) level and treatment in both the overall population (p=0.045) and in the subgroup of patients who received primary adjuvant endocrine therapy with an AI (p=0.025) after adjusting for age, tumour grade, pT stage, pN stage, prior endocrine therapy and prior chemotherapy. In a subgroup of node-positive patients, there was no significant interaction in a three-way test for interaction (p=0.624).

In the retrospective nested case-control study of NCIC CTG MA. 17 by Sgroi et al [18], the absolute four-year RFS was 86.6% (95% CI, 80.6 to 90.9) and 93.4% (95% CI, 90.2 to 95.6) for patients receiving placebo and letrozole, respectively. In patients with high H/I, there was a significant difference in the five-year RFS for patients receiving placebo (73%; 95% CI, 56.6 to 84.1) and letrozole (89.5%; 95% CI, 80.3 to 94.5; p=0.007). In patients with low H/I, there was no significant difference in RFS between the treatment arms (p=0.35). In an adjusted model, high H/I was significantly associated with patient benefit from letrozole (OR, 0.32; 95% CI, 0.14 to 0.72; p=0.006), whereas no benefit was observed in the low H/I group (p=0.23). The interaction between H/I and letrozole therapy was significant (p=0.03).

Recurrence-free interval

In the retrospective analysis of the NSABP B42 trial [21], currently only available in abstract form, there was no significant difference between receiving an additional five years of letrozole or placebo in those who were BCI (H/I)-low (HR, 0.69; 95% CI, 0.43 to 1.11; p=0.13) or BCI (H/I)-high (HR, 0.83; 95% CI, 0.55 to 1.26; p= 0.38). The interaction between BCI (H/I) level and treatment was not significant (p=0.55).

Distant recurrence

In the retrospective analysis of the NSABP B42 trial [21], there was no statistically significant difference in distant recurrence before four years between those who received extended letrozole therapy or placebo in the BCI (H/I)-low or -high group. However, after four years, BCI (H/I)-high patients had a significant benefit of extended letrozole therapy (HR, 0.29; 95% CI, 0.12 to 0.69; p=0.003), while there was no difference in BCI (H/I)-low patients (HR, 0.68; 95% CI, 0.33 to 1.39; p=0.28). There was no significant interaction between BCI (H/I) level and treatment (p=0.14).

C. PROGNOSTIC STUDIES FOR LATE RECURRENCE

Five retrospective studies [9,13,14,42,43] have been included that examined the prognostic utility of BCI-MGI H/I for late recurrence. Tables 4-20, 4-21 and 4-22 present a summary of the outcomes for node-negative patients, node-positive patients, and both node-negative and -positive patients, respectively. Table A5-5 in Appendix 5 presents study details including treatment regimens.

The ATAC trial evaluated the efficacy and safety of anastrozole versus tamoxifen given for five years in 9366 postmenopausal women with localized breast cancer. A subsequent substudy of the ATAC trial, the TransATAC, collected paraffin blocks from 2006 women who assigned to the monotherapy arms in the original trial. Sestak et al [9] assessed the risk of late distant recurrence in 774 women who had data available for all six signatures while Sgroi et al [14] assessed the prognostic value of BCI for early and late distant recurrence for women with localized lymph-node negative breast cancer.

The study by Zhang et al [13] retrospectively reviewed patients from two patient cohorts. The first cohort was from the Stockholm trial, a randomized trial comparing two or five years of adjuvant tamoxifen with untreated. Of the 2738 women included in the original Stockholm trial, 600 node-negative, postmenopausal patients were included in this retrospective analysis. The second cohort consisted of 358 ER-positive, LN-negative patients from two academic institutions.

Zhang et al [42] evaluated the prognostic risk of distant recurrence in ER-positive patients with one to three risk nodes using a distant recurrence risk model (BCIN+), which integrates BCI, tumour size and grade. This study included 402 patients from a single academic institution with HR-positive, invasive pN1 breast cancer who received adjuvant endocrine therapy with or without chemotherapy and remained distant recurrence free after five years.

The study by Nunes et al [43] retrospectively reviewed tumour specimens collected from 376 patients diagnosed with HR+, stage I to III ILC from four institutions to determine the prognostic utility of BCI for overall, early and late distant recurrence.

i. Lymph node-negative patients

Three retrospective studies [9,13,14] have been included that examined the prognostic utility of BCI for late recurrence in node-negative postmenopausal patients.

Recurrence

In the retrospective analysis of the ATAC trial [14], Sestak et al [9] found the risk of distant late recurrence was 2.6% (95% CI, 1.3 to 5.0) for BCI low-risk patients and 15.9% (95% CI, 8.9 to 27.6) for BCI high-risk patients. Similarly in a previous retrospective analysis of the ATAC trial, Sgroi et al found the risk found the risk of late distant recurrence was 3.5% (95% CI, 2.0 to 6.1) for BCI low-risk patient, 13.4% (95% CI, 8.5 to 20.8) for BCI intermediate-risk patients and 13.3% (95% CI, 7.4 to 23.4%) for BCI high-risk patients. In a multivariate analysis including clinical treatment score (CTS), BCI was prognostic for risk of distant late recurrence in all patients (HR, 1.95; 95% CI, 1.22 to 3.14) and in HER2-negative patients (HR, 2.12; 95% CI, 1.30 to 3.47).

Recurrence-free survival

Zhang et al [13] found there was a significant difference in late distant RFS between the BCI low-, intermediate-, and high-risk groups for patients in both the Stockholm cohort and the multi-institutional cohort (p=0.0152 and p=0.0002, respectively). In a multivariate Cox regression including clinicopathologic variables, BCI as a continuous variable was significant for

ER-positive, HER2-negative patients in both the Stockholm cohort (HR, 4.57; 95% CI, 1.28 to 16.37; p=0.020) and the multi-institutional cohort (HR, 9.33; 95% CI, 2.83 to 30.76; p=0.0002).

ii. Lymph node-positive patients

Two retrospective studies [9,42] have been included that examined the prognostic utility of Breast Cancer Index for late recurrence in node-positive patients.

Recurrence

In the retrospective analysis of the ATAC trial, Sestak et al [9] found the risk of late distant recurrence was 9.5% (95% CI, 8.3 to 23.9) for BCI low-risk patients and 36.5% (95% CI, 20.4 to 59.6) for BCI high-risk patients.

In the retrospective study by Zhang et al [42], there was a significant difference in late risk of distant recurrence at 15 years between the low-risk group (1.3%; 95% CI, 0.0% to 3.7%) and the high-risk group (16.1%; 95% CI, 10.6 to 21.3; p=0.0014). In a multivariate analysis adjusting for age, PR status, chemotherapy treatment, duration of endocrine treatment, type of endocrine treatments and number of positive lymph nodes, BCIN+ remained a significant prognostic factor for late distant recurrence (HR, 1.41; 95% CI, 1.06 to 1.89; p=0.02).

iii. Lymph node-positive and -negative patients

One retrospective study [43] has been included that examined the prognostic utility of BCI for late recurrence in both node-positive and -negative patients with ILC. Fifty-eight percent of the included patients were node-negative.

Recurrence

In this retrospective study, Nunes et al [43] found a significant difference in late distant recurrence between the low-intermediate risk group (6.5%; 95% CI, 2.0 to 10.9) and the high risk group (18.7%; 95% CI, 10.4 to 26.3; p=0.0224) in patients with ILC. In further subgroup analyses, there was a significant difference between the low-intermediate risk and the high-risk group for patients with well- and moderately differentiated tumours (7.5% vs 19.1%; p=0.0484) and for patients ≥50 years (6.9% vs. 24.9%; p=0.0087); however, no significant difference was observed for patients with stage II and III tumours (7.8% vs. 20.8%; p=0.1139), patients who received chemotherapy (8.9% vs 21.8%; 0.1876) and patients <50 years (5.5% vs. 3.7%; p=0.9235).

Table 4-18. Outcomes for the use of Breast Cancer Index for extended adjuvant endocrine therapy in node-positive patients

Author, year, trial name	BCI classification, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Recurrence-free interval (10 yrs vs. 5 yrs)	Disease-free interval (10 yrs vs. 5 yrs)	Overall survival
Retrospective analyses of RCTs								
Bartlett et al, 2019 [19] aTTom trial	Overall 10 yrs, 291 5yrs, 292 BCI (H/I)-high 10 yrs, 150 5 yrs, 137 BCI (H/I)-low 10 yrs, 141 5 yrs, 155	NR	Pre, 4% Post, 86% Peri, 4%	HR+, 100% HER2-, NR	8.9 yrs	Overall 28.4% vs. 33.1% HR, 0.88; 95% CI (0.65-1.18); p=0.388 BCI (H/I)-high 27.0% vs. 37.2% **HR, 0.35; 95% CI (0.15-0.86); p=0.027** BCI (H/I)-low 29.8% vs. 29.6% vs HR, 1.07; 95% CI (0.69-1.65); p=0.768 Interaction **$p_{BCI(H/I)continuousXEET}$=0.012[a]**	Overall 30.1% vs. 34.3% HR, 0.88; 95% CI (0.66-1.19); p=0.403 BCI (H/I)-high 29.6% vs. 38.8% **HR, 0.4; 95% CI (0.17-0.94); p=0.046** BCI (H/I)-low 30.4% vs. 30.6% HR, 1.05; 95% CI (0.69-1.61); p=0.82 Interaction **$p_{BCI(H/I)continuousXEET}$=0.019[b]**	NR

Abbreviations: BCI: Breast Cancer Index; CI: confidence interval; EET: extended endocrine therapy; ER: estrogen receptor; HER2: human epidermal growth factor 2; H/I: HOXB13/IL17BR; HR: hazard ratio; HR+; hormone receptor-positive; LN: lymph node; NR: not reported; PR: progesterone receptor; RCT: randomized controlled trial; yrs: years

[a,b] After adjusting for age, tumour size, tumour grade, and ER and PR status

Table 4-19. Outcomes for the use of Breast Cancer Index for extended adjuvant endocrine therapy in node-negative and node-positive patients

Author, year, trial name	BCI classification, sample size	Age, years (range)	Menopausal status	Lymph Node	ER status, HER2 status	Follow-up	Recurrence-free interval	Disease-free survival	Overall survival
Retrospective analyses of RCTs									
Mamounas et al (2021) *Abstract* [21] NSABP B42	BCI-high, 981 BCI-low, 1198	NR	100% post	LN-, 60%	HER2-, 80%	4 yrs	5 yrs vs. 10 yrs HR, 0.77; 95% CI (0.57-1.05); p=0.10 BCI-high HR, 0.83; 95% CI (0.55-1.26); p=0.38 BCI-low HR, 0.69; 95% CI (0.43-1.11); p=0.13	5 yrs vs. 10 yrs BCI-high HR, 0.81; 95% CI (0.64-1.04); p=0.09 BCI-low **HR, 0.75; 95% CI (0.58-0.95); p=0.017**	NR
Noordhoek et al, 2021 [20] IDEAL	Overall 10 yrs, 454 7.5 yrs, 454 BCI (H/I)-high 10 yrs, 221 7.5 yrs, 208 BCI (H/I)-low 10 yrs, 233 7.5 yrs, 246	NR	100% post	LN+, 73%	HR+, 100% HER2+, 9%	10 yrs	10 yrs vs. 7.5 yrs Overall 10.6% vs. 15.5% HR, 0.69; 95% CI (0.47-1.03); p=0.070 BCI (H/I)-high 5.9% vs. 15.7% **HR, 0.42; 95% CI (0.21-0.84); p=0.011** BCI (H/I)-low 14.9% vs. 15.4% HR, 0.95; 95% CI (0.58-1.56); p=0.835 Interaction $p_{BCI(H/I)Xtreatment}$**=0.045**[a]	NR	NR

Author, year, trial name	BCI classification, sample size	Age, years (range)	Menopausal status	Lymph Node	ER status, HER2 status	Follow-up	Recurrence-free interval	Disease-free survival	Overall survival
Sgroi et al, 2013 [18] NCIC CTG MA.17	Overall 10 yrs, 122 5 yrs, 127 H/I-high 10 yrs, 59 5 yrs, 62 H/I-low 10 yrs, 63 5 yrs, 65	NR	Post, 100%	LN-, 38% LN+, 59%	ER+, 97% PR+, 82% HER2-, 91%	NR	5 yr RFS, 10 yrs vs. 5 yrs Overall 90.1% vs. 80.4% H/I-high 89.5% vs. 73% **p=0.007** H/I-low 91% vs. 87% p=0.35 **Interaction** **$p_{H/I \times letrozole therapy}$=0.03**[b]	NR	NR

Abbreviations: BCI: Breast Cancer Index; CI: confidence interval; DFI: ER: estrogen receptor; HER2: human epidermal growth factor 2; H/I: HOXB13/IL17BR; HR: hazard ratio; HR+; hormone receptor-positive; LN: lymph node; NR: not reported; OR: odds ratio; PR: progesterone receptor; RFS: recurrence-free survival; yrs: years

[a] After adjusting for age, tumour grade, pT stage, pN stage, prior endocrine therapy and prior chemotherapy

[b] After adjusting for clinicopathological variables

Table 4-20. Prognostic ability of Breast Cancer Index for late recurrence in node-negative patients

Author, year, trial name	BCI category, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Risk for distant recurrence	Disease-free Survival	Overall survival
Retrospective analyses of RCTs								
Sestak et al, 2018 [9] ATAC	BCI low, 340 BCI intermediate, 126 BCI high, 69	Mean, 66.4	Post, 100%	HR+, 100% HER2-, 100%	10 yrs (5-10 yrs)	BCI low 2.6% (95% CI, 1.3-5.0) BCI intermediate 14.4% (95% CI, 9.0-22.6) BCI high 15.9% (95% CI, 8.9-27.6)	NR	NR
Sgroi et al, 2013 [18] ATAC	BCI low, 366 BCI intermediate, 146 BCI high, 84	Median, 62.7 (46.7-85.1)	Post, 100%	ER+, 100% HER2-, 100%	10 yrs (5-10 yrs)	BCI low 3.5% (95% CI, 2.0-6.1) BCI intermediate 13.4% (95% CI, 8.5-20.8) BCI intermediate vs. low **HR, 2.93; 95% CI, 1.37-6.29**[a] BCI high 13.3% (95% CI, 7.4-23.4) BCI high vs. intermediate **HR, 2.97; 95% CI, 1.23-7.13**[b]	NR	NR
Zhang et al (2013) [13] Stockholm & multi-institutional cohort	*Stockholm cohort* BCI low, 184 BCI intermediate, 58 BCI high, 43	NR	Post, 100%	ER+, 100% HER2-, 100%	10 yrs (5-10 yrs)	DRFS BCI low 97.2% (95% CI, 94.8-99.7) BCI intermediate 92.8% (95% CI, 86.2-99.9) BCI high 89.9% (95% CI, 80.9-99.8) **p=0.0152**	NR	NR

Author, year, trial name	BCI category, sample size	Age, years (range)	Menopausal status	ER status, HER2 status	Follow-up	Risk for distant recurrence	Disease-free Survival	Overall survival
Retrospective analyses of RCTs								
	Multi-institutional cohort BCI low, 181 BCI intermediate, 70 BCI high, 61					BCI low 97.5% (95% CI, 95.0-100.0) BCI intermediate 83.1% (95% CI, 73.8-93.5) BCI high 85.0% (95% CI, 76.4-94.5) **p=0.0002**		

Abbreviations: BCI: Breast Cancer Index; CI: confidence interval; DRFS: distant recurrence-free survival; ER: estrogen receptor; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; NR: not reported; yrs: years

[a,b] After adjustment for clinical treatment score

Table 4-21. Prognostic ability of Breast Cancer Index for late recurrence in node-positive patients

Author, year, trial name	BCI category, sample size	Age, years (range)	Menopausal status	HER2 status	Follow-up	Risk for distant recurrence	Disease-free survival	Overall survival
Sestak et al (2018) [9] ATAC	BCI low, 84 BCI intermediate, 50 BCI high, 20	Mean, 66.4	Post, 100%	HR+, 100% HER2-, 100%	10 yrs (5-10 yrs)	BCI low 9.5% (95% CI, 8.3-23.9) BCI intermediate 14.3% (95% CI, 8.3-23.9) BCI high 36.5% (95% CI, 20.4-59.6)	NR	NR
Zhang et al (2017) [42]	BCI low, 81 BCI high, 268	NR	NR	ER+, 99% HER2-, 64%	15 yrs (5-15 yrs)	BCI low 1.3% (95% CI, 0.0-3.7) BCI high 16.1% (95% CI, 10.6-21.3) **HR, 12.39; 95% CI, 1.7-90.35; p=0.0014**	NR	NR

Abbreviations: BCI: Breast Cancer Index; CI: confidence interval; ER: estrogen receptor; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; NR: not reported; yrs: years

Table 4-22. Prognostic ability of Breast Cancer Index for late recurrence in node-negative and node-positive patients

Author, year, trial name	BCI category, sample size	Age, years (range)	Menopausal status	HER2 status	Follow-up	Risk for distant recurrence	Disease-free survival	Overall survival
Nunes al (2021) [43]	BCI low-intermediate, 141 BCI high, 107	NR	NR	HR+, 100% HER2-, 94%	10 yrs (5-10 yrs)	BCI low-intermediate 6.5% (95% CI, 2.0-10.9) BCI high 18.7% (95% CI, 10.4-26.3) **HR, 3.04; 95% CI, 1.32-7.00; p=0.0224**	NR	NR

Abbreviations: BCI: Breast Cancer Index; CI: confidence interval; HER2: human epidermal growth factor 2; HR: hazard ratio; HR+; hormone receptor-positive; NR: not reported; yrs: years

Ongoing, Unpublished, or Incomplete Studies

A search for ongoing, unpublished, or incomplete phase II, III or IV trials was conducted on December 15, 2021 at clinicaltrials.gov using the terms "breast cancer" AND "Oncotype DX" OR "MammaPrint" OR "Prosigna" OR "EndoPredict" OR "Breast Cancer Index OR BCI".

Three trials were found for Oncotype DX, none for MammaPrint, three for Prosigna, three for EndoPredict and none for Breast Cancer Index. The trial details are provided in Appendix 6. The OPTIMA trial was added to the list of ongoing trials as it's a relevant trial that is not registered in clinicaltrials.gov.

DISCUSSION

This review highlights the current clinical utility of multigene expression testing in the care of patients with ER-positive, HER2-negative early-stage invasive breast cancer.

A number of multigene profiling assays including Oncotype DX, MammaPrint, Prosigna, EndoPredict/EPclin, and Breast Cancer Index can help to identify low-risk, node-negative, ER-positive, HER2-negative breast cancer patients who are unlikely to benefit from adjuvant chemotherapy. These assays may be used to aid or confirm a clinical decision to treat with endocrine therapy alone and safely withhold adjuvant chemotherapy. The evidence for this recommendation is supported from a variety of retrospective and prospective studies demonstrating favourable long-term outcomes among patients with low-risk molecular profiling test scores treated with endocrine therapy alone [1-14,45]. In addition, a high-risk Oncotype DX RS may also be used to predict adjuvant chemotherapy benefit [1-4,15], evidenced by the retrospective review of the NSABP B20 clinical trial demonstrating that patients with a high risk Oncotype DX recurrence score have a significant survival benefit when treated with chemoendocrine therapy as opposed to endocrine therapy alone [3]. The Working Group members acknowledge that not all node negative, ER-positive, HER2-negative breast cancer requires multigene assay testing and would discourage testing among patients with low grade (grade 1) invasive breast cancer less than 1 cm in size where testing has an extremely low likelihood of identifying patients with high-risk profiling scores benefitting from adjuvant chemotherapy [46]. Online clinical tools such as the NHS Predict (https://breast.predict.nhs.uk/) and CTS5 score (https://cts5-calculator.com/) also provide valuable modelling estimates of recurrence and survival to help guide clinical decisions regarding adjuvant systemic therapy [47,48].

This review also incorporates updated research supporting the use of molecular profiling assays in ER-positive, HER2-negative breast cancer among patients with limited lymph node-positive disease (pN1a or 1-3 lymph nodes positive). This hypothesis was previously generated through large observational studies and is now validated in two high-quality prospective RCTs providing strong evidence for the use of Oncotype DX and MammaPrint to guide the use of adjuvant chemotherapy in node-positive breast cancer [5,6,16]. The results of the MINDACT and RxPONDER clinical trials strongly support the ability to safely withhold adjuvant chemotherapy among patients ≥50 years of age with limited lymph node positivity based on either a low-risk Oncotype DX or MammaPrint score. These results will help oncologists to further de-escalate adjuvant breast cancer therapy, sparing patients the unnecessary side effects of chemotherapy and risk of potentially serious complications. Nonetheless, it is important to remember that molecular profiling assays must be carefully interpreted in conjunction with other clinical, pathological and patient-related factors. The Working Group would strongly caution against making treatment decision based on multigene profiling testing alone.

The use of either the MammaPrint or Oncotype DX assays in the node-positive (pN1 or 1-3 lymph nodes positive) disease setting is recommended. While other molecular assays have

demonstrated favourable prognostic data suggesting that withholding adjuvant chemotherapy may be warranted, they lack definitive prospective clinical trials conclusively testing this hypothesis. Therefore, stronger evidence from Prosigna, EndoPredict/EPclin, and Breast Cancer Index are needed before these assays are recommended for use among patients with node-positive breast cancer. Our recommendations highlight that younger breast cancer patients (≤50 years of age) experienced a clinically meaningful benefit from adjuvant chemotherapy irrespective of a low-risk Oncotype DX or MammaPrint score [6,16]. Therefore, clinical decision making, and interpretation of results should occur more conservatively in premenopausal breast cancer patients. One of the limitations of the MINDACT and RxPONDER clinical trials was the less-frequent use of ovarian suppression and AI therapy as primary adjuvant endocrine therapy as compared to tamoxifen. Some experts believe that the benefit of adjuvant chemotherapy among premenopausal breast cancer patients partially reflects chemotherapy-induced amenorrhea rather than direct cytotoxic effects of treatment. Therefore, investigators have speculated whether the greater use of ovarian suppression and AI therapy could have negated the small benefit of chemotherapy observed in the MINDACT and RxPONDER clinical trials. Nonetheless, this hypothesis remains unproven, and we recommend very careful consideration of any decision to withhold adjuvant chemotherapy in premenopausal patients with node-positive, ER-positive, HER2-negative breast cancer.

The evidence for using multigene profiling assays to guide clinical decision-making regarding duration of adjuvant endocrine therapy is less mature and more uncertain compared to the evidence for adjuvant chemotherapy. The strongest evidence to date supports the use of Breast Cancer Index to predict benefit of extended adjuvant endocrine therapy based on data from the IDEAL, MA17 and Trans-aTTOM and MA17 clinical trials [18-20]. Patients with BCI (H/I) high scores have been observed to have a significant DFS benefit with extended durations of adjuvant endocrine therapy. However, a recent abstract from the NSABP B42 clinical trial using the BCI (H/I) score was negative when investigating extended use of adjuvant aromatase inhibitor therapy and the Working Group has elected to issue a weak recommendation regarding use in clinical practice as further evidence evolves. The Breast Cancer Index Registry study (NCT04875351) will also help provide more confirmatory evidence in the future. We did not feel at present there were sufficient data to recommend MammaPrint, Oncotype DX, EndoPredict/EPclin or Prosigna to guide decisions to extend the duration of adjuvant endocrine therapy. An NSABP B42 trial abstract using Mammaprint is also promising; however, we await full publication of the study [22]. Many of these multigene profiling assays are prognostic for risk of late breast cancer recurrence and may identify patients at low risk of distant disease recurrence who most likely will not benefit from extended endocrine therapy. However, except for Breast Cancer Index, most assays have not established a true predictive benefit for extending endocrine therapy. In addition, the Working Group appreciates the relative perceived safety of endocrine therapy as compared to adjuvant chemotherapy and realize patients may be willing to extend endocrine therapy for a significantly small absolute survival benefit.

Our present guideline did not focus primarily on the utility of multigene profiling assays in helping to guide clinical treatment decisions regarding the use of either neoadjuvant chemotherapy or radiation. There are several trials ongoing evaluating the omission of adjuvant radiation therapy both in the node-negative and -positive setting [DEBRA: 04852887; EXPERT: 02889874; IDEAL: NCT 02400190; PRECISION: NCT2653755; TAILOR RT: NCT 03488693]. These trials will help determine whether adjuvant radiation may be safely omitted in selected breast cancer patients with low-risk molecular profiling scores. Further research in node-positive patients will be needed to determine whether both adjuvant chemotherapy and radiation treatment can be withheld together. Research has shown that molecular profiling assays may be accurately tested on paraffin embedded biopsy samples prior to surgery [49]. This observation may allow oncologists to guide treatment decisions regarding neoadjuvant

chemotherapy, especially among patients with ER-positive, HER2-negative breast cancer. This is of particular importance when deciding on optimal sequencing of surgery, chemotherapy, and endocrine therapy. Clinical trials are ongoing investigating the ability of various assays to predict neoadjuvant chemotherapy response rates and support the potential for successful surgical downstaging [NBRST: NCT011479101]. Larger coordinated trials will be needed to investigate survival endpoints to confirm the clinical utility of multigene assays in the neoadjuvant setting. Further study is also needed in male breast cancer patients and non-Caucasian populations underrepresented in clinical trials. Recent publications have suggested that Oncotype DX RS may require model calibration in populations with greater racial and ethnic diversity and among male breast cancer patients [23,50]. Additionally, studies directly evaluating the clinical utility and cost-effectiveness studies of these various multigene profiling assays are needed in public-funded health care systems to optimize value and accessibility. Given increased use of multigene profiling assay testing, ensuring timely testing and reporting will also be required to ensure that clinical treatment decisions are not delayed which may negatively impact patient quality of care.

CONCLUSIONS

The use of multigene profiling assays for early-stage, node-negative, ER-positive, HER2-negative breast cancer is well established. A variety of assays can be used to identify low-risk patients with a favourable disease prognosis who can be safely treated with endocrine therapy alone. We have now updated our clinical practice guideline demonstrating that both Oncotype DX and MammaPrint can also be used in patients with limited lymph node-positive disease (pN1a or 1-3 positive lymph nodes) to help identify patients at low risk who do not require treatment with adjuvant chemotherapy. Caution should be used in interpreting low-risk multigene assay scores in premenopausal women where a small adjuvant chemotherapy benefit may still exist even in those with low-risk scores. The role of multigene profiling assays to guide clinical decisions regarding the duration of adjuvant endocrine therapy is also emerging and Breast Cancer Index may be considered for use in aiding decisions regarding extending adjuvant endocrine therapy. Further research is required, and future studies will also help to clarify the potential use of multigene profiling assays to guide clinical decision making regarding neoadjuvant chemotherapy and adjuvant radiation therapy. Overall, multigene profiling assays are valuable clinical tools to be discussed with patients helping to guide and facilitate personalized clinical treatment decisions for adjuvant systemic therapy in patients with early-stage breast cancer.

Clinical Utility of Multigene Profiling Assays in Early-Stage Invasive Breast Cancer

Section 5: Internal and External Review

INTERNAL REVIEW

The guideline was evaluated by the GDG Expert Panel and the PEBC Report Approval Panel (RAP) (Appendix 1). The results of these evaluations and the Working Group's responses are described below.

Expert Panel Review and Approval

Of the 10 members of the GDG Expert Panel, eight members voted and zero abstained, for an 80% response in August 2021. Of those who voted, eight approved the document (80%). The main comments from the Expert Panel and the Working Group's responses are summarized in Table 5-1.

Table 5-1. Summary of the Working Group's responses to comments from the Expert Panel.

Comments	Responses
1. The last bullet of Recommendation 1 (Multigene profiling assays should be interpreted more cautiously in premenopausal patients) is not clear as to what it means. It is clearly stated in the Discussion later in the guideline.	We have modified this sentence to, "Multigene profiling assays should be interpreted more cautiously in premenopausal patients where a benefit from adjuvant chemotherapy may still exist despite a low-risk score."
2. For Recommendation 4, the first qualifying statement should say "despite **low-risk**" testing results	We have edited this statement.
3. I find that the recommendations could be better differentiated between pre- and post-menopausal patients or at least say in all patients, or in both pre- and post-menopausal patients.	We have included Qualifying Statements regarding premenopausal patients as needed.
4. Recommendation 1 should specify that premenopausal patient had tamoxifen and not ovarian function suppression + tamoxifen/AI. It has been stated by Sparano that ovarian function suppression + tamoxifen/AI could possibly used instead of chemotherapy plus endocrine therapy and it should be noted that is only a conjecture.	We have highlighted this issue in our discussion and have indicated the significant clinical benefit of adjuvant chemotherapy in premenopausal women. At present, the Working Group feels there is insufficient evidence that a particular choice of endocrine therapy may obviate the benefit of adjuvant chemotherapy in premenopausal patients.

RAP Review and Approval

Three RAP members reviewed this document in August 2021. The RAP approved the document on September 2021. The main comments from the RAP and the Working Group's responses are summarized in Table 5-2.

Table 5-2. Summary of the Working Group's responses to comments from RAP.

Comments	Responses

1. Define early-stage breast cancer. Which TNM edition is used? Are the references using the latest edition?	We have added a sentence to the target population to reflect the appropriate definition, "In this guideline, early-stage invasive breast cancer refers stage I to III breast cancers that are surgically operable and do not have evidence of locally recurrent or distant metastatic disease with with pT1-T3, pN0-N1a based on surgical pathologic staging."
2. It is apparent that the assays have not been directly compared and assessed under different conditions, so I wonder if that needs to be spelled out a bit more for context.	We have now added a Preamble to the Recommendations to address this. It states, "The purpose of this guideline is to determine the clinical utility of multigene profiling assays (i.e., Oncotype DX, MammaPrint, Prosigna, EndoPredict, and Breast Cancer Index), not to identify which assay is better. No prospective studies have compared these head-to-head. Given that the assays use different scoring systems and classification systems, please refer to Table 1-1 for a summary of each of the assays."
3. In the Justification for Recommendations section, it's mentioned, "Given the similarities between female and male breast cancer, these data can be generalized to all individuals with early invasive breast cancer." Which similarities? Similar biology? Prognosis for stage? Response to therapy?	We have revised this statement to read, "Given the similarities in biology and treatment recommendations between female and male breast cancer, these data can be generalized to all individuals with early invasive breast cancer."
4. It may be helpful to spell out which tests are available where in the Implementation Considerations section.	We have modified this section to read, "Historically, all assays covered in this guideline are conducted out of country."

Patient and Caregiver-Specific Consultation Group

Three patients/survivors/caregivers participated as Consultation Group members for the Working Group. They reviewed the draft recommendations and provided feedback on its comprehensibility, appropriateness, and feasibility to the Working Group's Health Research Methodologist. The main comments from the Consultation Group are summarized in Table 5-3.

Table 5-3. Summary of the Working Group's responses to comments from the Consultation Group.

Comments	Responses
1. Recommendations are clear and detailed with adequate evidence to support each recommendation and recognition when there is not enough evidence to support a recommendation.	-
2. Guideline addresses issues of concern to patients such as treatment versus survival benefits and takes into consideration the emotional impact of testing.	-
3. Guideline places importance on patient values by stating multiple times 'in consultation with patients' for treatment decisions.	-

4. How long do multigene assay test results take to come back? Waiting for results with no support plan is a major factor that needs to be considered.	We recognize how important this is to patient care and have edited the section on Implementation Considerations to include, 'Timeliness of care, including necessary test approvals, are important and as the use of assays increases particularly in lymph node positive patients, it will be critical that the assays results are delivered quickly. Extended delays can cause anxiety in patients and impact quality of care.'

EXTERNAL REVIEW

External Review by Ontario Clinicians and Other Experts

Targeted Peer Review

Four targeted peer reviewers from Ontario and British Columbia who are considered to be clinical and/or methodological experts on the topic were identified by the Working Group. Two agreed to be the reviewers; one response was received (Appendix 1). Results of the feedback survey are summarized in Table 5-4. The main comments from targeted peer reviewers and the Working Group's responses are summarized in Table 5-5.

Table 5-4. Responses to nine items on the targeted peer reviewer questionnaire.

	Reviewer Ratings (N=1)				
Question	Lowest Quality (1)	(2)	(3)	(4)	Highest Quality (5)
1. Rate the guideline development methods.	0	0	0	0	1
2. Rate the guideline presentation.	0	0	0	0	1
3. Rate the guideline recommendations.	0	0	0	1	0
4. Rate the completeness of reporting.	0	0	0	0	1
5. Does this document provide sufficient information to inform your decisions? If not, what areas are missing?	0	0	0	0	1
6. Rate the overall quality of the guideline report.	0	0	0	0	1
	Strongly Disagree (1)	(2)	Neutral (3)	(4)	Strongly Agree (5)
7. I would make use of this guideline in my professional decisions.	0	0	0	0	1
8. I would recommend this guideline for use in practice.	0	0	0	0	1
9. What are the barriers or enablers to the implementation of this guideline report?	None were noted				

Table 5-5. Summary of the Working Group's responses to comments from targeted peer reviewer.

Comments	Responses
1. A separate recommendation should be made about premenopausal women in node negative and node positive disease rather than within the	The Working Group wished to keep the current recommendation formatting. However, we have edited the node positive recommendation to clearly

qualifying statements of Recommendations 2 and 4. This would allow for discussion about the level of evidence for this patient population.	state "In **postmenopausal** patients with ER-positive/HER2-negative tumours and one to three nodes involved (N1a disease), clinicians may withhold chemotherapy based on a low-risk Oncotype DX or MammaPrint score if the decision is supported by other clinical, pathological, or patient-related factors." We have also more strongly stated the significant benefit of adjuvant chemotherapy among premenopausal patients.
2. Recommendation 5 for BCI may be too strong. Updated results at ASCO 2021 suggest that BCI may not be as strong.	We acknowledge this concern. Overall, the Working Group members felt a weak recommendation should be granted to consider the use of BCI as three published studies have demonstrated clinical benefit for BCI (H/I)-high in extended adjuvant endocrine therapy. The negative ASCO abstract from 2021 is now clearly referenced but has yet to be published and does not negate these other studies.

Professional Consultation

Feedback was obtained through a brief online survey of healthcare professionals and other stakeholders who are the intended users of the guideline. All clinicians with an interest in breast cancer or pathology and laboratory medicine in the PEBC database were contacted by email to inform them of the survey. A total of 292 clinicians who practice in Ontario were contacted. Fifty-five (18.8%) responses were received. Thirty stated that they did not have interest in this area or were unavailable to review this guideline at the time. The results of the feedback survey from 25 people are summarized in Table 5-6. The main comments from the consultation and the Working Group's responses are summarized in Table 5-7.

Table 5-6. Responses to four items on the professional consultation survey.

	N=25 (8.6%)				
General Questions: Overall Guideline Assessment	Lowest Quality (1)	(2)	(3)	(4)	Highest Quality (5)
1. Rate the overall quality of the guideline report.	0	0	1	14	10
	Strongly Disagree (1)	(2)	(3)	(4)	Strongly Agree (5)
2. I would make use of this guideline in my professional decisions.	0	0	4	9	12
3. I would recommend this guideline for use in practice.	0	0	2	9	14
4. What are the barriers or enablers to the implementation of this guideline report?	• Availability, accessibility, and funding of assays • Limitations in access to multidisciplinary care in remote areas • Education • Bureaucracy in filling out online forms for Ministry approval				

Table 5-7. Summary of the Working Group's responses to comments from professional consultants.

Comments	**Responses**

1. Guidance about which clinical and pathological features allow for a well-informed clinical decision to make treatment decision would be helpful.	We have added the following sentence to the Qualifying Statements for Recommendation 1, "Clinical and pathological features include patient age, tumour grade, tumour size and nodal status."
2. The recommendations should include the factors that define low-risk (favorable prognosis) vs. high-risk patients. Node-positive high-risk patients should not be offered multigene assays.	We have now included in a Qualifying Statement for Recommendation 2 that low-risk patients with small low-grade tumours < 1cm in size should not undergo multigene expression profiling based previous population-based research from Ontario. In regard to high-risk features, the MINDACT and RxPONDER clinical trials did not restrict entry except for N1a lymph nodal status (1-3 positive nodes) and inflammatory breast cancer. Therefore, the Working Group did not believe that other high risk clinical features could be used to identify patients who should not be tested.
3. The guideline comments, "Given the similarities in biology and treatment recommendations between female and male breast cancer, multigene profiling assays may be used in all individuals with early invasive breast cancer." This may not be true (DOI: 10.1158/1078-0432.CCR-19-2424) and perhaps should be acknowledged.	The Working Group strongly felt that male breast cancer patients should not be excluded from multigene testing due to limited data. We have revised our statement so it now states, "Although no males were included in any of the included studies, given the similarities in the management of male and female breast cancer, multigene profiling assays may be used in all individuals with early invasive breast cancer." The need for further investigation and better calibration of risk cut-offs in specialized populations (ethnically diverse and male breast cancer) is now noted in our discussion as well with the reference provided by the reviewer.
4. Reviewers commented on the lack of discussion about neoadjuvant chemotherapy and whether it was in the scope of this guideline.	The use of multigene profiling assays to guide the use of neoadjuvant chemotherapy is not covered in this guideline and is briefly discussed in the Discussion section. The Working Group members recognize this is an evolving field and we have included the following sentence to the preamble in Sections 1 and 2 to better clarify the guideline, "Further, this guideline does not cover the utility of multigene profiling assays in helping to guide clinical treatment decisions regarding the use of either neoadjuvant chemotherapy or radiation."
5. A reviewer commented on whether there should be a separate recommendation for premenopausal patients in both node-negative and -positive patients.	The Working Group wished to keep the current recommendation formatting. However, we have edited the node positive recommendation to clearly state "In postmenopausal patients with ER-positive/HER2-negative tumours and one to three nodes involved (N1a disease), clinicians may withhold chemotherapy based on a low-risk Oncotype DX or MammaPrint score if the decision is supported by other clinical, pathological, or patient-related factors." We have also more strongly stated the significant benefit of adjuvant chemotherapy among premenopausal patients.
6. A few reviewers commented on the wording regarding premenopausal women and the use of the assays. In women with	The Working Group has reworded the Qualifying Statement for node-positive patients to, "Premenopausal patients <50 years of age have a

node-positive breast cancer, there is the same caution using the assay in premenopausal patients as there is in node-negative. Further, the guideline states a caveat in several places that low scores do not rule out a chemotherapy benefit for premenopausal woman, which is not entirely true. There is no evidence that node-negative premenopausal women with low or intermediate recurrence scores from Oncotype DX derive any benefit from chemotherapy.	significant benefit from chemotherapy despite low-risk scores from multigene assay testing. Risk scores should be interpreted with caution and decisions should be made while considering other clinical, pathological, or patient-related factors." In node-negative premenopausal women, the TAILORx trials showed a significant benefit in IDFS for chemoendocrine therapy for those with an RS of 16 to 20 (HR, 1.76; 95% CI, 1.20 to 2.59; p=0.0034). This benefit was not observed in premenopausal women with RS between 11 and 15 (p=0.49) or 21 and 25 (p=0.094). In women aged ≤50 years, there was a significant benefit in those that received chemoendocrine therapy for IDFS with an RS of 16 to 20 (HR, 1.90, 95% CI, 1.27 to 2.84; p=0.0016) and 21 to 25 (HR, 1.70; 95% CI, 1.03 to 2.80; p=0.035). This difference was not observed in women with an RS between 11 and 15 (HR, 0.99; 95% CI, 0.62 to 1.58; p=0.97).
7. Many reviewers commented on the use of multiple assays and whether patients can take different assays and what to do if scores differ.	The Working Group has added a Qualifying Statement to Recommendation 1 to help clarify that only one assay should be ordered per treatment decision, "One multigene profiling assay should be requested per patient to guide a specific treatment decision. Requesting multiple tests to guide a single treatment decision is discouraged." This is further expanded in the Implementation Considerations, "For a specific treatment decision, only one multigene profiling assay should be selected based on a discussion with the patient. Performing multiple tests could create uncertainty of results and anxiety in patients."
8. The recommendations and flowchart (Figures 1-1 and 1-2) ask the question whether the clinical and pathologic features allow a well-informed clinical decision to offer or withhold chemotherapy. It would be helpful to provide more specific guidance about these criteria.	For Recommendation 2 (node-negative patients), the Working Group has added in a Qualifying Statement stating, "In patients with a low-grade tumour (i.e., grade 1) less than 1cm in size, the Working Group does not recommend a multigene assay profiling as this is unlikely to inform a treatment decision to use adjuvant chemotherapy." For Recommendation 4 (node-positive patients), no high-risk exclusion criteria other than inflammatory breast cancer was used in the MINDACT or RxPONDER clinical trials. Therefore, we are not able to provide further factors, although we would recommend that clinicians use their best clinical judgement when ordering testing using the philosophy of choosing wisely.

CONCLUSION

The final guideline recommendations contained in Section 2 and summarized in Section 1 reflect the integration of feedback obtained through the external review processes with the document as drafted by the GDG Working Group and approved by the GDG Expert Panel and the PEBC RAP.

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Appendix 1: Affiliations and Conflict of Interest Declarations

Table A1-1: Members of the Working Group

Name	Affiliation	Declarations of interest
Phillip Blanchette Medical oncologist	London, ON	No conflict of interest declared
John Bartlett Molecular pathologist	Toronto, ON	Has received $500 or more in a single year to act in a consulting capacity for Insight Genetics, Inc., BioNTech AG, Biotheranostics Inc., Pfizer, RNA Diagnostics Inc., oncoXchange/MedcomXchange Communications Inc., Herbert Smith French Solicitors, OncoCyte Corporation, NanoString Technologies, Inc., Oncology Education, Due North; has received other financial or material support from Biotheranostics Inc., NanoString Technologies, Inc., Breast Cancer Society of Canada; has received grants/research support as a principal or co-investigator from Thermo Fisher Scientific, Genoptix, Agendia, NanoString Technologies, Inc., Stratifyer GmbH, Biotheranostics, Inc., MammaPrint; has been a principal investigator of the OPTIMA clinical trial; and has published several papers[1] related to the guideline.
Andrea Eisen Medical oncologist	Toronto, ON	No conflict of interest declared
Harriet Feilotter Molecular pathologist	Kingston, ON	No conflict of interest declared
Rossanna Pezo Medical oncologist	Toronto, ON	Has received $500 or more in a single year as an advisory board member for Bristol-Myers Squibb, EMD Serono, Novartis, Pfizer, AstraZeneca, Lilly, Exact Sciences, Myriad, and Daiichi Sankyo; has received $500 or more in research funding from Merck; and has received research funding as a principal or co-investigator from Novartis, Pfizer, AstraZeneca, and Daiichi Sankyo
Gulisa Turashvili Anatomic pathologist	Toronto, ON	No conflict of interest declared
Phillip Williams Anatomic pathologist	Toronto, ON	No conflict of interest declared
Duvaraga Sivajohanathan Health research methodologist	Program in Evidence-Based Care McMaster University Hamilton, ON	No conflict of interest declared

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Table A1-2: Report Approval Panel

Name	Affiliation	Declarations of interest
William (Bill) Evans Medical oncologist	Hamilton, ON	No conflict of interest declared
Donna Maziak Surgical oncologist	Ottawa, ON	No conflict of interest declared
Jonathan Sussman Radiation oncologist	Hamilton, ON	No conflict of interest declared

Table A1-3: Expert Panel Members

Name	Affiliation	Declarations of interest
Anita Bane Pathologist	London, ON	No conflict of interest declared
Christine Brezden-Masley Medical oncologist	Toronto, ON	Has received $500 or more in a single year to act in a consulting capacity for Myriad, Agendia, Genomic Health (Exact Sciences)
Janet Dancey Medical oncologist	Kingston, ON	No conflict of interest declared
Sukhbinder Dhesy-Thind Medical oncologist	Hamilton, ON	Serves as CCTG co-chair for the RxPONDER trial (MAC.15) and is an author of the upcoming manuscript of trial results; serves as a sponsorship committee member for the Juravinski Cancer Centre BRIGHT Run (non-profit charitable run) which has received sponsorship funds from Genomic Health (Exact Sciences)
Leta Forbes Medical oncologist	Oshawa, ON	No conflict of interest declared
John Hilton Medical oncologist	Ottawa, ON	Has received $500 or more in a single year to act in a consulting capacity for BMS, AstraZeneca, Novartis, Pfizer, Merck, GSK, Puma Biotechnology, Genomic Health and Agendia
Bryan Lo Molecular geneticist	Ottawa, ON	Has served on advisory boards for AstraZeneca, Pfizer, Novartis, Bayer in the last 5 years; owns stocks, bonds or stock options valued at $500 or more at Merck and Johnson & Johnson; has received grants for quality assurance and improvement projects from AstraZeneca, Bayer, EMD Serono; serves as co-investigator on multi-center projects (ex. EXACTIS/PMT, CanTRK ring project) that has received financial support from pharmaceutical companies.
Brian Pinchuk Surgical oncologist	Toronto, ON	No conflict of interest declared
Trevor Pugh Molecular geneticist	Toronto, ON	T.J.P. provides consultation for Merck, Chrysalis Biomedical Advisors and the Canadian Pension Plan Investment Board, and receives research support from Roche; received research support from Roche/Genetech imCORE program to support correlative genomic analysis from an interventional circulating tumour DNA study in ovarian cancer.
Maureen Trudeau Medical oncologist	Toronto, ON	Currently owns stocks, bonds or stock options valued at $500 or more at RNA Diagnostics.

Table A1-4: Members of the Patient Consultation Group

Name	Declarations of interest
Lise Craig	No conflict of interest declared
Patricia Sevean	No conflict of interest declared
Robert Tuck	No conflict of interest declared

Table A1-5: Targeted Peer Reviewer

Name	Affiliation	Declarations of interest
Karen Gelmon Medical oncologist	Vancouver, BC	No conflict of interest declared

Appendix 2: Literature Search Strategy

MEDLINE search for guidelines and systematic reviews

1 exp breast cancer/
2 breast cancer.mp.
3 (breast adj2 (cancer$ or neoplasm$ or carcinoma$ or malignan$ or tumo?r)).mp.
4 or/1-3
5 (oncotype$ or 21 gene or recurrence score).mp.
6 (prosigna or PAM50).mp.
7 (mammaprint or 70 gene).mp.
8 endopredict.mp.
9 (breast cancer index or BCI).mp.
10 or/5-9
11 tailorx.mp.
12 rxponder.mp.
13 (swog adj (S1007 or "8814")).mp.
14 (nsabp adj (b20 or b-20 or b 20)).mp.
15 (nsabp adj (b14 or b-14 or b 14)).mp.
16 transatac.mp.
17 ((ma17 or ma 17 or ma-17 or ma12 or ma 12 or ma-12) adj (trial or study)).mp.
18 (ABCSG-6 or ABCSG 6 or ABCSG-8 or ABCSG 8).mp.
19 mindact.mp.
20 (raster adj2 study).mp.
21 (geicam 9906 or geicam-9906 or geicam9906).mp.
22 (OPTIMA adj2 study).mp.
23 or/11-22
24 (systematic adj (review: or overview:)).mp.
25 (meta-analy: or metaanaly:).mp.
26 (pooled analy: or statistical pooling or mathematical pooling or statistical summar: or mathematical summar: or quantitative synthes?s or quantitative overview:).mp.
27 (exp review literature as topic/ or review.pt. or exp review/) and systematic.tw.
28 (cochrane or embase or psychlit or psyclit or psychinfo or psycinfo or cinhal or cinahl or science citation index or scisearch or bids or sigle or cancerlit or pubmed or pub-med or medline or med-line).ab.
29 (reference list: or bibliograph: or hand-search: or handsearch: or relevant journal: or manual search:).ab.
30 or/24-29
31 (selection criteria or data extract: or quality assess: or jadad score or jadad scale or methodologic:quality).ab.
32 (stud: adj1 select:).ab.
33 (31 or 32) and review.pt.
34 30 or 33
35 (guideline or practice guideline).pt.
36 exp consensus development conference/
37 consensus/
38 (guideline: or recommend: or consensus or standards).ti.
39 or/35-38
40 34 or 39
41 4 and 10
42 23 or 41

43 40 and 42
44 limit 63 to yr="2018 -Current"

MEDLINE search for primary studies

1 exp breast cancer/
2 breast cancer.mp.
3 (breast adj2 (cancer$ or neoplasm$ or carcinoma$ or malignan$ or tumo?r)).mp. (395514)
4 or/1-3
5 (oncotype$ or 21 gene or recurrence score).mp.
6 (prosigna or PAM50).mp.
7 (MammaPrint or 70 gene).mp.
8 EndoPredict.mp.
9 (breast cancer index or BCI).mp.
10 or/5-9
11 tailorx.mp.
12 rxponder.mp.
13 (swog adj (S1007 or "8814")).mp.
14 (nsabp adj (b20 or b-20 or b 20)).mp.
15 (nsabp adj (b14 or b-14 or b 14)).mp.
16 transatac.mp.
17 ((ma17 or ma 17 or ma-17 or ma12 or ma 12 or ma-12) adj (trial or study)).mp
18 (ABCSG-6 or ABCSG 6 or ABCSG-8 or ABCSG 8).mp.
19 mindact.mp.
20 (raster adj2 study).mp.
21 (geicam 9906 or geicam-9906 or geicam9906).mp.
22 (OPTIMA adj2 study).mp.
23 or/11-22
24 exp randomized controlled trials as topic/ or exp clinical trials, phase III as topic/ or exp clinical trials, phase IV as topic/
25 (randomized controlled trial or clinical trial, phase III or clinical trial, phase IV).pt.
26 random allocation/ or double blind method/ or single blind method/
27 (randomi$ control$ trial? or rct or phase III or phase IV or phase 3 or phase 4).tw.
28 or/24-27
29 (phase II or phase 2).tw. or exp clinical trial/ or exp clinical trial as topic/
30 (clinical trial or clinical trial, phase II or controlled clinical trial).pt.
31 (29 or 30) and random$.tw.
32 (clinic$ adj trial$1).tw.
33 ((singl$ or doubl$ or treb$ or tripl$) adj (blind$3 or mask$3 or dummy)).tw.
34 placebos/
35 (placebo? or random allocation or randomly allocated or allocated randomly).tw.
36 (allocated adj2 random).tw.
37 Prospective study/
38 Retrospective study/
39 Cohort study/
40 or/31-39
41 28 or 40
42 (4 and 10 and 41) or 23
43 (comment or letter or editorial or note or erratum or short survey or news or newspaper article or patient education handout or case report or historical article).pt.
44 42 not 43

45 exp animal/ not human/
46 44 not 45
47 limit 46 to english
48 limit 47 to yr="2018 -Current"

EMBASE search for guidelines and systematic reviews

1 breast cancer/
2 breast cancer.mp.
3 (breast adj2 (cancer$ or neoplasm$ or carcinoma$ or malignan$ or tumo?r)).mp.
4 or/1-3
5 (oncotype$ or 21 gene or recurrence score).mp.
6 (prosigna or PAM50).mp.
7 (mammaprint or 70 gene).mp.
8 endopredict.mp.
9 (breast cancer index or BCI).mp.
10 or/5-9
11 tailorx.mp.
12 rxponder.mp.
13 (swog adj (S1007 or "8814")).mp.
14 (nsabp adj (b20 or b-20 or b 20)).mp.
15 (nsabp adj (b14 or b-14 or b 14)).mp.
16 transatac.mp.
17 ((ma17 or ma 17 or ma-17 or ma12 or ma 12 or ma-12) adj (trial or study)).mp.
18 (ABCSG-6 or ABCSG 6 or ABCSG-8 or ABCSG 8).mp.
19 mindact.mp.
20 (raster adj2 study).mp.
21 (geicam 9906 or geicam-9906 or geicam9906).mp.
22 (OPTIMA adj2 study).mp.
23 or/11-22
24 (systematic adj (review: or overview:)).mp.
25 (meta-analy: or metaanaly:).mp.
26 (pooled analy: or statistical pooling or mathematical pooling or statistical summar: or mathematical summar: or quantitative synthes?s or quantitative overview:).mp.
27 (exp review literature as topic/ or review.pt. or exp review/) and systematic.tw.
28 (cochrane or embase or psychlit or psyclit or psychinfo or psycinfo or cinhal or cinahl or science citation index or scisearch or bids or sigle or cancerlit or pubmed or pub-med or medline or med-line).ab.
29 (reference list: or bibliograph: or hand-search: or handsearch: or relevant journal: or manual search:).ab.
30 or/24-29
31 (selection criteria or data extract: or quality assess: or jadad score or jadad scale or methodologic:quality).ab.
32 (stud: adj1 select:).ab.
33 (31 or 32) and review.pt.
34 30 or 33
35 consensus development conference/
36 practice guideline/
37 *consensus development/ or *consensus/
38 *standard/

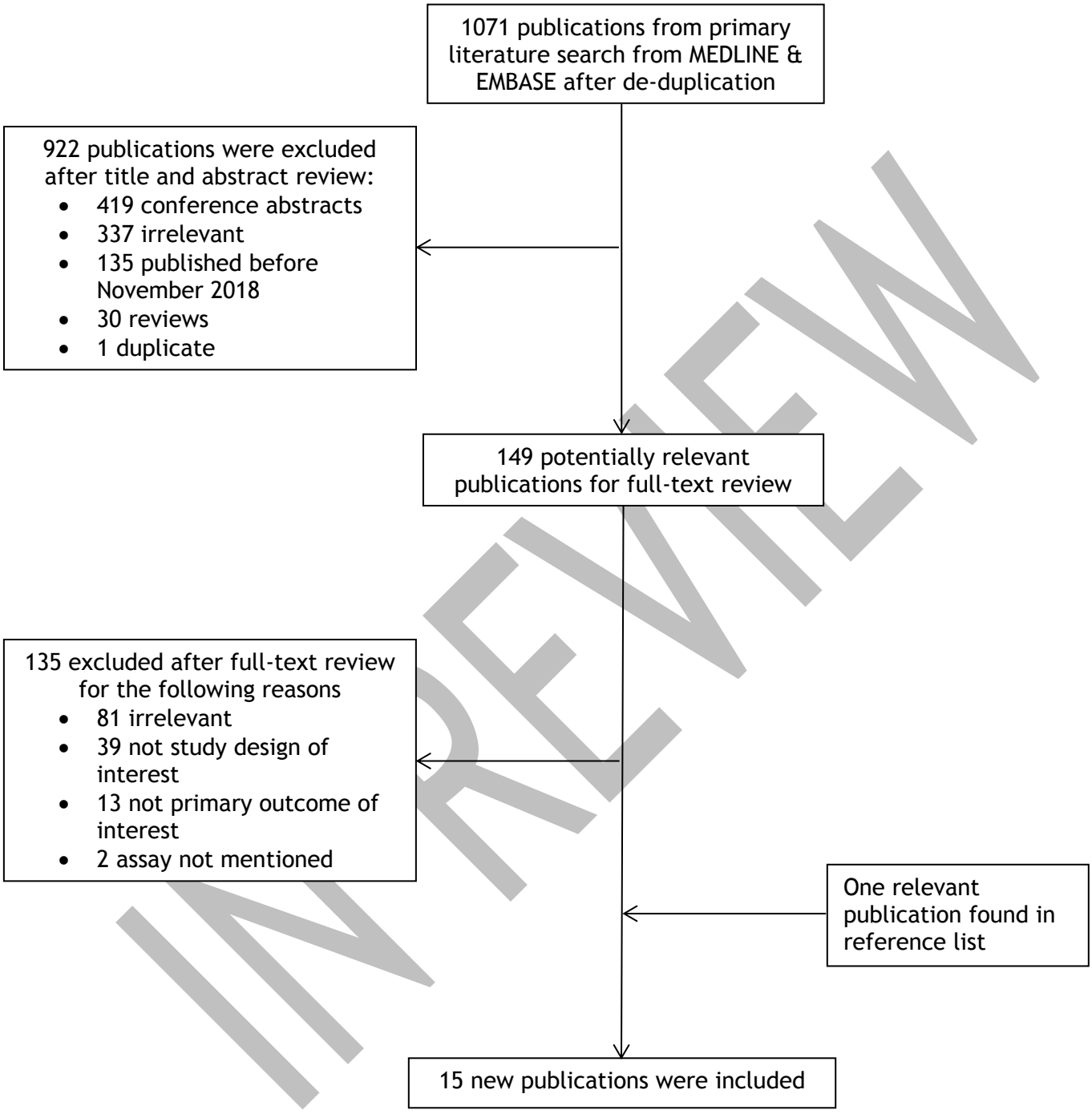
39 (guideline: or recommend: or consensus or standards).kw.
40 (guideline: or recommend: or consensus or standards).ti.
41 or/35-40
42 34 or 41
43 4 and 10
44 23 or 43
45 42 and 44
46 limit 45 to yr="2018 -Current"

EMBASE search for primary studies

1 exp breast cancer/
2 breast cancer.mp.
3 (breast adj2 (cancer$ or neoplasm$ or carcinoma$ or malignan$ or tumo?r)).mp. (395514)
4 or/1-3
5 (oncotype$ or 21 gene or recurrence score).mp.
6 (prosigna or PAM50).mp.
7 (MammaPrint or 70 gene).mp.
8 EndoPredict.mp.
9 (breast cancer index or BCI).mp.
10 or/5-9
11 tailorx.mp.
12 rxponder.mp.
13 (swog adj (S1007 or "8814")).mp.
14 (nsabp adj (b20 or b-20 or b 20)).mp.
15 (nsabp adj (b14 or b-14 or b 14)).mp.
16 transatac.mp.
17 ((ma17 or ma 17 or ma-17 or ma12 or ma 12 or ma-12) adj (trial or study)).mp
18 (ABCSG-6 or ABCSG 6 or ABCSG-8 or ABCSG 8).mp.
19 mindact.mp.
20 (raster adj2 study).mp.
21 (geicam 9906 or geicam-9906 or geicam9906).mp.
22 (OPTIMA adj2 study).mp.
23 or/11-22
24 exp randomized controlled trial/ or exp phase 3 clinical trial/ or exp phase 4 clinical trial/
25 randomization/ or single blind procedure/ or double blind procedure/
26 (randomi$ control$ trial? or rct or phase III or phase IV or phase 3 or phase 4).tw.
27 or/ 24-26
28 (phase II or phase 2).tw. or exp clinical trial/ or exp clinical trial as topic/
29 27 and random$.tw.
30 (clinic$ adj trial$1).tw.
31 ((singl$ or doubl$ or treb$ or tripl$) adj (blind$3 or mask$3 or dummy)).tw.
32 placebo/
33 (placebo? or random allocation or randomly allocated or allocated randomly).tw.
34 (allocated adj2 random).tw.
35 Prospective study/
36 Retrospective study/
37 Cohort study/
38 or/28-37
39 27 or 29 or 38
40 (4 and 10 and 39) or 23

41 (editorial or note or letter erratum or short survey).pt. or abstract report/ or letter/ or case study/
42 40 not 41
43 animal/ not human/
44 42 not 43
45 limit 44 to english
46 limit 45 to yr="2018 -Current"

Appendix 3: PRISMA Flow Diagram



Appendix 4: Quality Assessment

Table A4-1: Risk of Bias for Included Trials* Assessed Using Cochrane's Risk of Bias Tool
*RCTs or retrospective studies of RCTs where randomization has not been broken

Trial	SELECTION BIAS		PERFORMANCE BIAS	DETECTION BIAS	ATTRITION BIAS	REPORTING BIAS	OTHER BIAS
	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Oncotype DX							
Kalinsky et al (2021) RxPONDER	Low	Low	Low	Low	Low	Low	-
Sparano et al (2018) TAILORx	Unknown	Low	Low	Low	Low	Low	-
Albain et al (2010) SWOG 8814	Low	Low	Low	Low	High	Low	-
Geyer et al (2018) NSABP B20	Low	Low	Low	Unknown	High	Low	-
Paik et al (2006) NSABP B20	Low	Low	Low	Unknown	Low	Low	-
MammaPrint							
Cardoso et al (2016) Piccart et al (2021) MINDACT	Low	Low	Low	Unknown	Low	Low	-
Rastogi et al (2021) *Abstract* NSABP B42	Low	Low	Low	Unknown	Unknown	Unknown	-
Prosigna							
Jensen et al (2018) DBCG 77B	Low	Low	Low	Unknown	High	Low	-

Trial	SELECTION BIAS		PERFORMANCE BIAS	DETECTION BIAS	ATTRITION BIAS	REPORTING BIAS	OTHER BIAS
	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Liu et al (2015) NCIC CTG MA.21	Low	Low	Low	Unknown	Unknown	Low	-
Breast Cancer Index							
Mamounas et al (2021) NSABP B42 *Abstract*	Low	Low	Low	Unknown	Unknown	Unknown	
Noordhoek et al (2021) IDEAL trial	Low	Low	Low	Low	Unknown	Low	-
Bartlett et al (2019) aTTom trial	Low	Low	Low	Low	Low	Low	-
Sgroi et al (2013) NCIC MA.17	Low	Low	Low	Low	Unknown	Low	-

Abbreviation: RCT: randomized controlled trial

Table A4-2: Risk of Bias of Prognostic Studies using the QUIPS Tool

Study	Bias Domains					
	Study Participants	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Study Confounding	Statistical Analysis and Reporting
Oncotype DX						
Sestak et al (2018) ATAC	Low	Low	Low	Low	Low	Low
Prosigna						
Sestak et al (2018) ATAC	Low	Low	Low	Low	Low	Low
Sestak et al (2015) ATAC & ABCSG-8	Low	High	Low	Low	Low	Low
Filipits et al (2014) ABCSG-8	Low	Moderate	Low	Low	Low	Low
EndoPredict						
Filipits et al (2019) ABCSG-6 & ABCSG-8	Low	Low	Low	Low	Low	Low
Sestak et al (2018) ATAC	Low	Low	Low	Low	Low	Low
Dubsky et al (2013) ABCSG-6 & ABCSG-8	Low	Low	Low	Low	Low	Low
Breast Cancer Index						
Nunes et al (2021)	Low	Low	Low	Low	Low	Low
Sestak et al (2018) ATAC	Low	Low	Low	Low	Low	Low

Study	Bias Domains					
	Study Participants	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Study Confounding	Statistical Analysis and Reporting
Zhang et al (2017)	Low	Low	Low	Low	Low	Low
Sgroi et al (2013) ATAC	Low	Low	Low	Low	Low	Low
Zhang et al (2013)	Low	Low	Low	Low	Low	Low

Appendix 5: Study Characteristics

Table A5-1. Study Characteristics of Included Studies for Oncotype DX

Oncotype DX	Inclusion Criteria	Exclusion Criteria	Outcomes	Sample Size Calculation	Risk Category & Treatment	Treatment Regimen
ADJUVANT CHEMOTHERAPY (PREDICTIVE STUDIES)						
RCTs						
RxPONDER Kalinsky et al (2021) Abstract *Funded by NCI grants, Cure Research Program, Hope Foundation for Cancer Research, Breast Cancer Research Foundation and Genomic Health, Inc.*	Women >18 years of age with HR+, HER2- breast cancer and one to three positive lymph nodes	Patients with contraindications to taxane and/or anthracycline-based chemotherapy	Primary: invasive disease-free survival, overall survival	NR	RS ≤ 25: ET vs. CET stratified by RS (0-13 vs.14-25), menopausal status and axillary nodal dissection vs. sentinel node biopsy	NR
TAILORx Sparano et al (2020) (2019) (2018)	Women who were 18-75 years of age, HR-positive, HER2-negative, node-negative, met NCCN guidelines for the recommendation or consideration of adjuvant chemotherapy	Patients with a previous ipsilateral or contralateral invasive breast cancer or ductal carcinoma in situ, or with bilateral synchronous cancers. Patients who developed breast cancer after 8 or more months of receiving a selective estrogen-receptor modulator or an aromatase inhibitor for breast cancer prevention, or a SERM for other indicators.	Primary: Invasive disease-free survival Secondary: freedom from recurrence of breast cancer at a distant site, freedom from recurrence of breast cancer at a distant site or local-regional site, overall survival	Required a 73% increase in the number of patients randomized relative to a design with 100% adherence to ensure adequate power. Based on assuming an accrual of 6860 patients over 3.81 years, of whom up to 5% would be ineligible, it was projected that 6517 eligible patients would be required	RS ≤ 10: ET only RS ≥ 26: chemotherapy plus endocrine (chemoendocrine) therapy RS 11-25: ET vs CET	ET among postmenopausal women: most commonly included an aromatase inhibitor (91%) ET among premenopausal women: most commonly included either tamoxifen alone or tamoxifen followed by an aromatase inhibitor (78%) CT: Docetaxel, cyclophosphamide (56%) and anthracycline-

						containing regimens (36%) Suppression of ovarian function was used in 13% of premenopausal women
Retrospective Analysis of RCTs						
NSABP B20 Paik et al (2006) *Funded by Public Health Service Grants from NCI and NIH, and Genomic Health* Geyer et al (2018) *Funded by Public Health Service Grants from NCI and NIH*	Women with primary operable, histologically node-negative, ER-positive breast cancer from the NSABP B20 trial with tumor blocks available in the NSABP Tumor Bank were included in this retrospective analysis	Insufficient tumor (<5% of the overall tissue) as assessed by histopathology, insufficient RNA (<0.5 µg), or weak RT-PCR signal (average cycle threshold for the reference genes >35) *Geyer et al excluded patients with HER2-gene expression ≥11.5*	Primary: freedom from distant recurrence	NR	ET vs CET Low risk, RS <18 Intermediate risk, RS ≥18 and <31 High risk, RS ≥31	ET: Tamoxifen CET: Tammoxifen plus sequential methotrexate and fluorouracil, or tamoxifen plus cyclophosphamide, methotrexate, and fluorouracil *Both chemotherapy arms were combined in the re-analyses.*
SWOG 8814 Albain et al (2010) *Funded by NCI and Genomic Health, Inc*	Postmenopausal women with axillary node-positive breast cancer were eligible for inclusion if they had ER-positive or PR-positive tumours, or both, classified by local institutional standards from the SWOG 8814 trial with available tumour blocks	Evidence of cancer by liver enzymes, chest radiograph, contralateral mammogram, and bone scan	Primary: disease-free survival Secondary: overall survival	NR	Low risk, RS <18 Intermediate risk, RS 18-30 High risk, RS ≥ 31	ET: Tamoxifen CET: Cyclophosphamide, doxorubicin and fluorouracil followed by tamoxifen *Original SWOG 8814 trial included a third arm (CAF with concurrent tamoxifen) which was excluded in the re-analysis due to inferior efficacy.*
LATE RECURRENCE (PROGNOSTIC STUDIES)						
Retrospective studies of RCTs						
TransATAC Sestak et al (2018)	Postmenopausal women with histologically proven, operable, HR-positive,	Received chemotherapy or received the combination treatment	Primary: time to distant recurrence	NR	Low risk, RS <18 Intermediate risk, RS 18-30 High risk, RS ≥31	Arm 1: 5 years of anastrozole

Funded by Cancer Research UK, the Royal Marsden, NIH Biomedical Research Centre and Breast Cancer Now	ER-positive, ERBB2-negative, early-stage breast cancer from the ATAC trial for whom all assay signatures were available.	(i.e., anastrozole plus tamoxifen); patients who had 4 or more positive lymph node.				Arm 2: 5 years of tamoxifen

Abbreviations: CET: chemoendocrine therapy; CT: chemotherapy; ER: estrogen receptor; ERBB2: erythroblastic oncogene B 2; ET: endocrine therapy; HER2: human epidermal growth factor 2; HR: hazard ratio; HR; hormone receptor; LN: lymph node; NCCN: National Comprehensive Cancer Network; NCI: National Cancer Institute; NIH: National Institutes of Health; NSABP: National Surgical Adjuvant Breast and Bowel Project; PR: progesterone receptor; NR: not reported; RCT: randomized controlled trial; RNA: ribonucleic acid; RS: recurrence score; RT-PCR: reverse transcription polymerase chain reaction; SERM: selective estrogen receptor modulator; UK: United Kingdom

Table A5-2. Study Characteristics of Included Studies for MammaPrint

MammaPrint	Inclusion Criteria	Exclusion Criteria	Study Outcomes	Statistical Power and Required Sample Size	Risk Category and Treatment	Treatment Regimen
ADJUVANT CHEMOTHERAPY (PREDICTIVE STUDY)						
RCTs						
MINDACT Cardoso et al (2016) Piccart et al (2021) *Funded by grants from several non-industry organizations but also Sanofi-Aventis, Novartis, F. Hoffmann-La Roche, Eli Lily, and Veridex*	Women (18-70 years) with histologically confirmed primary invasive breast cancer. Initial study design only included patients who were LN-negative but in 2009 the protocol was revised to include LN-positive (up to 3 lymph nodes) patients.	Serious cardiac illness or medical condition, serous uncontrolled intercurrent infection or concomitant disease, previous or concurrent cancer or have received previous chemotherapy, hormonal therapy or radiotherapy.	Primary: Survival without distant metastasis Secondary: proportion of patients who received chemotherapy according to the clinical risk as compared with the genomic risk, overall survival, disease-free survival	Total sample size modified from 6000 to 6600. Low clinical and high genomic risk: 80% power to reject a one-sided test at α=0.025 of 5-year DMFS of 92%	Low clinical and genomic risk: ET High clinical and low genomic risk OR low clinical and high genomic risk: Randomized to ET or CET High clinical and genomic risk: CET	Refer to original trial publication for details Supp. Appendix pg 24
EXTENDED ENDOCRINE THERAPY (PREDICTIVE STUDY)						
Retrospective studies of RCTs						
NSABP B42 Rastogi et al (2021) Abstract *Funded by US NIH and pharmaceutical/biotech company*	Postmenopausal women status with stage I-IIIA invasive carcinoma of the breast, ER-positive, and/or PR-positive who were disease free after 5 years of endocrine therapy from the NSABP B42 trial with available tumour blocks	History of nontraumatic osteoporotic fracture of wrist, hip, or spine; diagnosis of contralateral breast cancer including ductal carcinoma in situ; other malignancies unless the patient is disease free for 5 years before randomization; use of hormone-	Primary: distant recurrence Secondary: Disease-free survival and breast cancer-free interval	NR	MP-high: ≤0.000 MP-low: >0.000 MP-ultra-low: >0.355 MP-low but not ultra-low: >0.000 but ≤0.355	ET: 5 years of endocrine therapy EET: additional 5 years of letrozole

		replacement therapy, oral contraceptives, or hormonal therapy for osteoporosis (raloxifene).				

Abbreviations: CET: chemoendocrine therapy; DMFS: distant metastasis-free survival; EET: extended endocrine therapy; ER: estrogen receptor; ET: endocrine therapy; LN: lymph node; MP: MammaPrint; NIH: National Institutes of Health; NSABP: National Surgical Adjuvant Breast and Bowel Project; PR: progesterone receptor; RCT: randomized controlled trial; US: United States

Table A5-3. Study Characteristics of Included Studies for Prosigna

Prosigna	Inclusion Criteria	Exclusion Criteria	Study Outcomes	Statistical Power and Required Sample Size	Risk Category and Treatment	Treatment Regimen
ADJUVANT CHEMOTHERAPY (PREDICTIVE STUDIES)						
Retrospective studies of RCTs						
DBCG 77B Jensen et al (2018) *Funded by the Danish Breast Cancer Cooperative Group*	All patients previously randomized in the DBCG 77B trial (i.e., premenopausal women without distant metastasis, and had either positive lymph nodes, tumours >5 cm, and/or invasion of the deep fascia) with available FFPE primary resection tumor blocks	NR	Primary: disease-free survival Secondary: overall survival	NR	ROR score Low risk, 0-51 Intermediate risk, 52-71 High risk, 72-100	Arm 1: Radiotherapy alone OR radiotherapy + levamisole Arm 2: Radiotherapy + cyclophosphamide OR radiotherapy + CMF *Original DBCG 77B trial had 4 arms; however, this retrospective analysis combined the chemotherapy arms into one arm and the no chemotherapy arms into another*
NCIC CTG MA.14 Liu et al (2014) *Funded by a grant from the Susan G Komen Foundation*	All patients previously randomized in the NCIC CTG MA.14 trial (i.e., postmenopausal women who had surgical removal of histologically proven adenocarcinoma of the breast by segmental or total mastectomy with axillary dissection, ECOG PS 0-2) with available FFPE primary resection tumor blocks	NR	NR	NR	ROR score Low risk, ≤15 Intermediate risk, 16-40 High risk, >40	Arm 1: Cyclophosphamide, epirubicin, and fluorouracil Arm 2: Epirubicin, cyclophosphamide, and paclitaxel Arm 3: Doxorubicin, cyclophosphamide, and paclitaxel
LATE RECURRENCE (PROGNOSTIC STUDIES)						
Retrospective studies of RCTs						
TransATAC Sestak et al (2018) *Funded by Cancer Research UK,*	Postmenopausal women with histologically proven, operable, HR-positive, ER-positive, ERBB2-negative, early-stage breast cancer from the ATAC trial for whom	Received chemotherapy or received the combination treatment (i.e., anastrozole plus	Primary: time to distant recurrence		ROR score Low risk, 0-26 Intermediate risk, 26-68 High risk, >68	Arm 1: 5 years of anastrozole Arm 2: 5 years of tamoxifen

the Royal Marsden, NIH Biomedical Research Centre and Breast Cancer Now	all assay signatures were available.	tamoxifen); patients who had 4 or more positive lymph node.				
ABCSG-8 Filipits et al (2014) *Both studies funded by AstraZeneca (original trial) and NanoString Technologies*	All patients previously randomized in the ABCSG-8 trial (i.e., postmenopausal women with primary, operable, histologically verified, ER-positive and/or PR-positive, grade 1 or 2 ductal and Gx lobular invasive breast cancer) with available FFPE primary resection tumor blocks	Any type of preoperative chemotherapy or hormone or radiation therapy; other previous or current malignoma; G3 patients; contraindication against tamoxifen or anastrozole; random assignment fails to occur within <6 weeks of surgery; ductal carcinoma in situ (without invasive cancer); T4; uncertain or unknown hormone receptor status; any comorbidity	Primary: distant recurrence-free survival	NR	ROR score Node negative: Low risk, ≤40 Intermediate risk, 41-60 High risk, >60 1-3 positive nodes: Low risk, ≤15 Intermediate risk, 16-40 High risk, >40 ≥4 positive nodes Low risk, NR Intermediate risk, NR High risk, all	Arm 1: 5 years of tamoxifen Arm 2: 2 years tamoxifen + 3 years anastrozole
ABCSG-8/TransATAC Sestak et al (2015) *Funded by Breakthrough Breast Cancer, NIH Biomedical Research Centre, Cancer Research UK*	Postmenopausal women with HR-positive breast cancer who received five years of endocrine treatments and who did not have a recurrence in the first 5 years.	Women who had a recurrence in the first five years	Primary: time to first distant recurrence	NR	ROR score Low-risk, 0-26 Intermediate risk, 26-68 ROR high-risk, >68	ATAC: 5 years of anastrozole VS. 5 years of tamoxifen ABCSG-8: 5 years of tamoxifen VS. 2 years tamoxifen + 3 years anastrozole

Abbreviations: ABCSG: Austrian Breast & Colorectal Cancer Study Group; DBCG: Danish Breast Cancer Cooperative Group; ECOG PS: Eastern Cooperative Oncology Group Performance Status; ER: estrogen receptor; ERBB2: erythroblastic oncogene B 2; FFPE: formalin-fixed, paraffin-embedded; HER2: human epidermal growth factor 2; HR; hormone receptor; NCIC CTG: National Cancer Institute of Canada Clinical Trials Group; NIH: National Institutes of Health; NR: not reported; PR: progesterone receptor; RCT: randomized controlled trial; ROR: risk of recurrence; UK: United Kingdom; US: United States

Table A5-4. Study Characteristics of Included Studies for EndoPredict

EndoPredict	Inclusion Criteria	Exclusion Criteria	Study Outcomes	Statistical Power and Required Sample Size	Risk Category and Treatment	Treatment Regimen
ADJUVANT CHEMOTHERAPY (PREDICTIVE STUDIES)						
Comparative study						
Sestak et al (2019) *Funded by Cancer Research UK and Myriad Genetics*	All pre- and postmenopausal women with ER-positive, HER2-negative breast cancer from the GEICAM/9906 and GEICAM 2003/02 trials (treated with endocrine therapy + chemotherapy) and from the ABCSG-6, ABCSG-8 and TransATAC trials (treated with endocrine therapy alone)	NR	Primary: distant recurrence-free survival Secondary: breast cancer-free interval	NR	EPclin low risk, <3.3 EPclin high risk, ≥3.3	GEICAM/9906: Fluorouracil, epirubicin and cyclophosphamide (FEC) or FEC + paclitaxel GEICAM 2003/02: Fluorouracil, doxorubicin and cyclophosphamide (FAC x 6) or FAC x 4 + paclitaxel ABCSG-8: Either tamoxifen only for 5 years or tamoxifen for 2 years followed by anastrozole for 3 years. ABCSG-6: Either tamoxifen only for 5 years alone or 5 years of tamoxifen plus aminoglutethimide for the first 2 years TransATAC: 5 years of tamoxifen or anastrozole alone
LATE RECURRENCE (PROGNOSTIC STUDIES)						
Retrospective studies of RCTs						
TransATAC/ATAC Sestak et al (2018) *Funded by Cancer Research UK, the Royal Marsden, NIH Biomedical Research Centre and Breast Cancer Now*	Postmenopausal women with histologically proven, operable, HR-positive, ER-positive, ERBB2-negative, early-stage breast cancer from the ATAC trial for whom all assay signatures were available.	Received chemotherapy or received the combination treatment (i.e., anastrozole plus tamoxifen); patients who had 4 or more positive lymph node.	Primary: time to distant recurrence	NR	EP low risk, < 5 EP high risk ≥ 5 EPclin low risk, <3.3 EPclin high risk, ≥3.3	Arm 1: 5 years of anastrozole Arm 2: 5 years of tamoxifen

ABCSG-6/ABCSG-8 Dubsky et al (2013) *Funding source not reported* Filipits et al (2019) *Funded by Myriad Genetics, Inc.*	All patients previously included in ABCSG-6 (tamoxifen-only arm) or ABCSG-8 (tamoxifen-only and tamoxifen plus anastrozole arms).	Breast cancer patients with ER-negative and/or HER2-positive tumours.	Filipits et al, Primary: distant recurrence-free rate Secondary: risk of late recurrence Dubsky et al, Primary: distant metastasis	NR	EP low risk, <5 EP high risk ≥5 EPclin low risk, <3.3 EPclin high risk, ≥3.3	Either tamoxifen only for 5 years or tamoxifen for 2 years followed by anastrozole for 3 years.

Abbreviation: ABCSG: Austrian Breast & Colorectal Cancer Study Group; EP: EndoPredict; EPclin: EndoPredict clinical score; ER: estrogen receptor; ERBB2: erythroblastic oncogene B 2; GEICAM: Spanish Breast Cancer Research Group; HER2: human epidermal growth factor 2; HR; hormone receptor; NIH: National Institutes of Health UK: United Kingdom

Table A5-5: Study Characteristics of Included Studies for Breast Cancer Index

Breast Cancer Index	Inclusion Criteria	Exclusion Criteria	Study Outcome	Statistical power and required sample size	Risk Category	Treatment Regimen
EXTENDED ENDOCRINE THERAPY (PREDICTIVE STUDIES)						
Retrospective studies of RCTs						
NSABP B42 Mamounas et al (2021) Abstract *Funded by US NIH & pharmaceutical/biotech company*	All patients previously randomized in the NSABP B42 trial (i.e., postmenopausal women with stage I to III, HR+ breast cancer who were disease-free after 5 years of endocrine) with available primary tumour tissue	NR	Primary: Recurrence-free interval Secondary: Distant recurrence, breast cancer-free interval, and DFS	NR	NR	ET: 5 years of endocrine therapy EET: additional 5 years of letrozole
IDEAL trial Noordhoek et al (2021) *Funded by Biotheranostics Inc.*	All patients previously randomized in the IDEAL trial (i.e., women with HR+ early-stage postmenopausal breast cancer) with available FFPE primary resection tumour blocks	Not recurrence-free for at least 2.5 years post randomization, lack of invasive tumour, incorrect tumour specimen, insufficient or poor RNA signal, patients with a follow-up time or had recurred <2.5 years after randomization	Primary: recurrence-free interval Secondary: disease-free interval, DFS	At 80% power, it was estimated that a total of 768 patients would be required to detect the benefit in BCI (H/I)-high patients at the 5% significance level	BCI (H/I)-high, >0.06 BCI (H/I)-low, ≤0.06	After completing 5 years of adjuvant therapy with either tamoxifen monotherapy, tamoxifen followed by an AI, or AI monotherapy ET: additional 2.5 years of letrozole (total 7.5 years of ET) EET: additional 5 years of letrozole (total 10 years of ET)
aTTom Trial Bartlett et al (2019) *Funded by Biotheranostics Inc., Breast Cancer Research Foundation, and Ontario Institute for Cancer Research*	All patients previously randomized in the aTTom study (women, > 50 to ≥ 70 years, with ER+ or ER untested invasive breast cancer, who remained disease free after having completed at least 4 years of adjuvant tamoxifen therapy), with available FFPE primary resection tumour blocks	Lack of invasive tumour as assessed by histopathology review, insufficient tissue on tissue microarray analysis, and insufficient RNA signal	Primary: recurrence-free interval Secondary: DFS	At 80% power, approximately 1800 HR-positive patients would be required to detect a 9.4% absolute benefit in DFI within the BCI (H/I)-high subset at a 5% significance level	BCI (H/I)-high, >0.06 BCI (H/I)-low, ≤0.06	ET: 5 years of adjuvant tamoxifen therapy EET: 5 years of adjuvant tamoxifen therapy + an additional 5 years

NCIC CTG MA.17 Sgroi et al (2013) *Funded by Avon Foundation, NIH, Department of Defense Breast Cancer Research Program and the NCI SPORE in breast cancer*	All patients previously randomized in the NCIC CTG MA. 17 trial (i.e., postmenopausal breast cancer patients, hormone receptor-positive [ER-positive and/or PR-positive] tumours) with disease recurrence and available FFPE primary resection tumour blocks	-	Primary: breast cancer recurrence	A post hoc power analysis indicated that with the sample size used, the power to detect an odds ratio of 3 for comparing risk of late recurrence without extended letrozole vs that with letrozole in the H/I-high group was 82% at a 5% significance level	BCI (H/I)-high, >0.06 BCI (H/I)-low, ≤0.06	After having completed approximately 5 years of standard adjuvant tamoxifen treatment, ET: Placebo EET: Letrozole for 5 years After a demonstrated statistically significant DFS benefit and trend toward survival advantage in patients who received letrozole, patients on the placebo arm were offered letrozole for a planned period of 5 years.
LATE RECURRENCE (PROGNOSTIC STUDIES)						
Retrospective studies of RCTs						
Nunes et al (2021) *Funded by Biotheranostics, Inc., and in part by the Breast Cancer Research Foundation*	Patients diagnosed with HR-positive, stage I to III ILC between 1992 and 2011 from four institutions with any nodal status, and pure lobular or mixed lobular/ductal histology and had available FFPE tumour blocks	Patients treated with neoadjuvant therapy, missing clinical information (i.e., tumor size or nodal status), or inadequate survival follow-up	Time to distant recurrence	NR	NR	NR
TransATAC/ATAC Sestak et al (2018) *Funded by Cancer Research UK, the Royal Marsden, NIH Biomedical Research Centre and Breast Cancer Now* Sgroi et al (2013) *Funded by several non-industry organizations*	Postmenopausal women with histologically proven, operable, hormone receptor-positive, ER-positive, ERBB2-negative, early-stage breast cancer *Sestak et al only included patients for whom all assay signatures were available.*	Received chemotherapy, did not have ER-positive disease, received the combination treatment (i.e., anastrozole plus tamoxifen) *Sestak et al excluded patients*	Sestak et al, Primary: time to distant recurrence Sgroi et al, Primary: distant recurrence Secondary: all recurrences, breast cancer	NR	Low-risk, BCI <5.0825 Intermediate risk, 5.0825 ≤ BCI <6.5025 High risk, BCI ≥6.5025	Arm 1: 5 years of anastrozole Arm 2: 5 years of tamoxifen

but also industry including, AstraZeneca		*who had 4 or more positive lymph node*.	deaths, overall survival			
Zhang et al (2017) *Funded by The US Department of Defense Breast Cancer Award*	Women with HR-positive, invasive pN1 breast cancer diagnosed between 1993 and 2007 who received adjuvant endocrine therapy with or without chemotherapy, had at least 5 years of follow-up, and had available FFPE tumour blocks	Women with four or more positive lymph nodes	Primary: Time to distant recurrence Secondary: Time to any recurrences (locoregional or distant, whichever occurred first	NR	NR	Adjuvant ET: Tamoxifen only, AI only, or a sequence of tamoxifen and AI Adjuvant ET + CT: Tamoxifen only, AI only, or a sequence of tamoxifen and AI + Adjuvant doxorubicin and cyclophosphamide or cyclophosphamide followed by paclitaxel-based chemotherapy.
Stockholm study Zhang et al (2013) *Funded by Avon Foundatio, NCI, The US Department of Defense Breast Cancer Award, and the Susan G. Komen for the Cure*	All patients randomized in the Stockholm study (i.e., postmenopausal women with histologically verified invasive early-stage, unilateral breast cancer, low-risk LN-negative patients with tumor size 30 mm or less in diameter) with available FFPE tumour blocks AND Patients with ER-positive, LN-negative breast cancer identified from University of Pittsburgh Medical Center and Massachusetts General Hospital between 1990 and 2000 with available FFPE tumour blocks	Inoperable local disease, distant metastases at the time of primary diagnosis, other concurrent cancers, medical contraindications to the treatment, surgery which deviated from that stipulated in the protocol	Primary: Distant recurrence-free survival	NR	Low-risk, BCI <5.0825 Intermediate risk, 5.0825 ≤ BCI <6.5025 High risk, BCI ≥6.5025	Stockholm trial: Arm 1: 2 years of adjuvant tamoxifen given post-operatively. Arm 2: Disease free at 2 years randomized to tamoxifen for 3 years or no further therapy

Abbreviation: AI: aromatase inhibitors; BCI: Breast Cancer Index; DFI: disease-free interval DFS: disease-free survival; ER: estrogen receptor; ERBB2: erythroblastic oncogene B 2; ET: endocrine therapy; EET: extended endocrine therapy; FFPE: formalin-fixed, paraffin-embedded; H/I: HOXB13/IL17BR; HR; hormone receptor; LN: lymph node; NCIC CTG: National Cancer Institute of Canada Clinical Trials Group; NIH: National Institutes of Health; NSABP: National Surgical Adjuvant Breast and Bowel Project; PR: progesterone receptor; UK: United Kingdom; US: United States

Appendix 6: Ongoing, Unpublished or Incomplete Studies

Table A6-1: Oncotype DX

Program for the Assessment of Clinical Cancer Tests (PACCT-1): Trial Assigning Individualized Options for Treatment: The TAILORx Trial	
Protocol ID:	NCT00310180
Type of trial:	Interventional, Phase III
Primary endpoint:	5-year DFS
Accrual:	10273
Sponsorship:	National Cancer Institute (NCI)
Status:	Active, not recruiting
Date last updated:	May 3, 2021
Estimated study completion date:	September 30, 2030
A Phase III, Randomized Clinical Trial of Standard Adjuvant Endocrine Therapy +/- Chemotherapy in Patients With 1-3 Positive Nodes, Hormone Receptor-Positive and HER2-Negative Breast Cancer With Recurrence Score (RS) of 25 or Less. RxPONDER: A Clinical Trial Rx for Positive Node, Endocrine Responsive Breast Cancer	
Protocol ID:	NCT01272037
Type of trial:	Interventional, Phase III
Primary endpoint:	Recurrence scores
Accrual:	10000
Sponsorship:	National Cancer Institute (NCI)
Status:	Active, not recruiting
Date last updated:	May 3, 2021
Estimated study completion date:	Not provided
The IDEA Study (Individualized Decisions for Endocrine Therapy Alone)	
Protocol ID:	NCT02400190
Type of trial:	Interventional
Primary endpoint:	Loco-regional recurrence
Accrual:	202
Sponsorship:	University of Michigan Rogel Cancer Center
Status:	Active, not recruiting
Date last updated:	November 3, 2020
Estimated study completion date:	March 2026

Table A6-2: Prosigna

A Randomised Phase III Trial of Adjuvant Radiation Therapy Versus Observation Following Breast Conserving Surgery and Endocrine Therapy in Patients with Molecularly Characterised Luminal A Early Breast Cancer	
Protocol ID:	NCT02889874
Type of trial:	Interventional
Primary endpoint:	Local recurrence rate after breast conserving surgery
Accrual:	1167
Sponsorship:	Breast Cancer Trials, Australia and New Zealand
Status:	Recruiting
Date last updated:	April 13, 2021
Estimated study completion date:	December 2023
LA LEAST- Luminal A, Limited Endocrine Adjuvant Systemic Therapy. A Trial of Abbreviated Hormone Therapy for Low-Risk Hormone Receptor Positive, HER2 Negative Early Breast Cancer	
Protocol ID:	NCT03917082
Type of trial:	Interventional, Phase II
Primary endpoint:	Distant relapse free interval at five years
Accrual:	290
Sponsorship:	British Columbia Cancer Agency
Status:	Recruiting
Date last updated:	December 9, 2020
Estimated study completion date:	May 1, 2029
OPTIMA: Optimal Personalised Treatment of early breast cancer using Multiparameter Analysis: a randomised study	
Protocol ID:	ISRCTN42400492
Type of trial:	Interventional
Primary endpoint:	Invasive disease-free survival
Accrual:	4500
Sponsorship:	NIHR Health Technology Assessment Programme
Status:	Recruiting
Date last updated:	July 11, 2019
Estimated study completion date:	December 31, 2031

Table A6-3: EndoPredict

EndoPredict® Extended Endocrine Trial (EXET): A Prospective Registry to Evaluate the Impact of EndoPredict® Test on Extended Endocrine Treatment Decisions and Patient Outcomes.	
Protocol ID:	NCT04016935
Type of trial:	Observational
Primary endpoint:	DRFS
Accrual:	2800
Sponsorship:	Myriad Genetic Laboratories, Inc.
Status:	Recruiting
Date last updated:	March 19, 2021
Estimated study completion date:	June 1, 2029

A Phase III Randomized, Double-Blind, Neoadjuvant Study of Hormonal Therapy Plus Palbociclib Versus Hormonal Therapy Plus Placebo in Women With Operable, Hormone Sensitive and HER2-Negative Primary Breast Cancer	
Protocol ID:	NCT03969121
Type of trial:	Interventional, Phase III
Primary endpoint:	PEPI score, EndoPredict EPclin score
Accrual:	200
Sponsorship:	Kyoto Breast Cancer Research Network
Status:	Recruiting
Date last updated:	April 6, 2021
Estimated study completion date:	June 1, 2022

Randomized, Double Blind, Multicentric Phase III Trial Evaluating the Safety and Benefit of Adding Everolimus to Adjuvant Hormone Therapy in Women With High Risk of Relapse, ER+ and HER2- Primary Breast Cancer Who Remain Free of Disease After Receiving at Least 1 Year of Adjuvant Hormone Therapy	
Protocol ID:	NCT01805271
Type of trial:	Interventional, Phase III
Primary endpoint:	DFS
Accrual:	1279
Sponsorship:	UNICANCER
Status:	Active, not recruiting
Date last updated:	February 21, 2021
Estimated study completion date:	June 2030

Appendix 7: Guideline Document History

GUIDELINE VERSION	SYSTEMATIC REVIEW		PUBLICATIONS	NOTES and KEY CHANGES
	Search Dates	Data		
Original MOAC-4 2016	2002 to Feb 2016	Full Report	Web publication.	NA
Reviewed version 2018	2016 to Apr 2018	New data found in Document and Assessment Review		2016 recommendations require an UPDATE