Clinical utility of multigene profiling assays in early-stage breast cancer

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ABSTRACT

Background This clinical practice guideline was developed to determine the level of evidence supporting the clinical utility of commercially available multigene profiling assays and to provide guidance about whether certain breast cancer patient populations in Ontario would benefit from alternative tests in addition to Oncotype dx (Genomic Health, Redwood City, CA, U.S.A.).

Methods A systematic electronic Ovid search of the MEDLINE and EMBASE databases sought out systematic reviews and primary literature. A systematic review and practice guideline was written by a working group and was then reviewed and approved by Cancer Care Ontario’s Molecular Oncology Advisory Committee.

Results Twenty-four studies assessing the clinical utility of Oncotype dx, Prosigna (NanoString Technologies, Seattle, WA, U.S.A.), EndoPredict (Myriad Genetics, Salt Lake City, U.S.A.), and MammaPrint (Agendia, Irvine, CA, U.S.A.) were included in the evidence base.

Conclusions The clinical utility of multigene profiling assays is currently established for an appropriate subset of patients with estrogen receptor–positive, her2-negative, node-negative breast cancer for whom a decision to give chemotherapy is difficult to make. For patients with estrogen receptor–positive tumours who receive tamoxifen alone, Oncotype dx, Prosigna, and EndoPredict validly identify a low-risk population with favourable outcomes, indicating that a low-risk assay result is actionable and the decision to withhold chemotherapy is supported. Clinical evidence indicates that a high Oncotype dx recurrence score can predict for chemotherapy benefit, but a high Prosigna or EndoPredict score, although prognostic, is not, based on clinical trial evidence, directly actionable. Prosigna and EndoPredict are statistically more likely to identify a population at risk for recurrence beyond 5 years, but that information is currently not actionable because of a lack of interventional studies.

Key Words Practice guidelines, breast cancer, multigene profiling assays, Oncotype dx, Prosigna, EndoPredict, MammaPrint, recurrence

INTRODUCTION

Breast cancer is a heterogeneous disease, and risk of recurrence depends on several factors, including tumour size, histologic grade, regional lymph node involvement, lymphovascular invasion, and expression of both the estrogen (ER) and progesterone hormone receptors, and on HER2 (human epidermal growth factor receptor 2) protein overexpression or gene amplification (or both). For women with early-stage breast cancer, adjuvant systemic therapy is based on risk of recurrence. In most cases, patients with ER-positive or progesterone receptor–positive early-stage breast cancer are offered endocrine therapy, and patients with HER2-positive tumours are offered both chemotherapy and HER2-targeted therapies. However, determining which ER-positive, HER2-negative patients should be offered chemotherapy is a more complex decision, because biomarkers for chemotherapy benefit are less well established than those that predict for recurrence.

Since the early 2000s, multigene profiling assays, which use modern molecular quantitation technologies, have been developed to aid in the risk-stratification of early
breast cancers. Several assays are commercially available and have received regulatory clearance, either through the U.S. Food and Drug Administration or the European Economic Area (Conformité Européenne designation), or as a laboratory-developed test in a Clinical Laboratory Improvement Amendments–approved laboratory. The Molecular Oncology Advisory Committee (MOAC) at Cancer Care Ontario (CCO) sought to systematically review the clinical utility of the regulatory-cleared assays that are commercially available in Canada.

At the request of the MOAC, development of a recommendation report was organized by CCO’s Program in Evidence-Based Care. A systematic review by a Working Group informed recommendations that would be submitted to the MOAC. The main objective of the review was to determine the level of evidence supporting the clinical validity and utility of the commercially available assays. The secondary objective was to determine whether certain breast cancer patient populations in Ontario would benefit from alternative tests in addition to Oncotype DX, because, at time of development, Oncotype DX was the only assay publicly funded in Ontario. The review focuses on the Oncotype DX (Genomic Health, Redwood City, CA, U.S.A.), Prosigna (NanoString Technologies, Seattle, WA, U.S.A.), MammaPrint (Agendia, Irvine, CA, U.S.A.), and EndoPredict (Myriad Genetics, Salt Lake City, U.S.A.) assays, all of which have evidence to support their ability to identify intrinsically low-risk and high-risk molecular profiles in breast cancer. A description of each assay can be found in Appendix A. All work produced by the Program in Evidence-Based Care is editorially independent from CCO. The entire recommendation report is freely available on the CCO Web site.

METHODS

Systematic Review

A systematic electronic Ovid search of the Medline and Embase databases for systematic reviews and primary literature from 2002 through February 2016 included keywords to identify the multigene assays of interest and important studies that were known a priori (Tables I and II). In addition to the Medline and Embase searches, systematic reviews and primary literature identified in the main search were scanned for citations of potentially useful studies. Web sites and databases of guideline developers and systematic review producers were also searched.

Studies that used prospectively enrolled patients and that prospectively collected tumour specimens were identified for inclusion in the evidentiary base of the systematic review. Retrospective cohort studies, case–control studies, case series, letters, editorials, and studies not published in English were excluded. The flow diagram of the literature search can be found in Figure 1. Reference management software was used to remove duplicate citations.

A review of titles and abstracts was performed by one reviewer (LHS) and verified by a second (MCC). For items that warranted a full-text review, one reviewer (LHS) determined whether the inclusion and exclusion criteria were met. The resulting list of studies was verified by the Working Group. Data were extracted from studies on the verified list.

Unles otherwise indicated, ratios (including hazard ratios) are expressed such that a ratio of less than 1.0 indicates a reduced risk for recurrence or death. All extracted data and information were audited by an independent auditor.

A framework to evaluate the clinical utility of tumour markers was proposed by Hayes et al.3 in 1996. That tumour marker utility grading system was further refined in 20095 and was used to grade the levels of evidence of the prognostic and predictive studies included in the present review. The framework assigns a study type category (A–D) based on 5 elements: clinical trial design; patients and patient data; specimen collection, processing, and archive; statistical design and analysis; and study validation. A level of evidence (I–IV) is then determined based on the aggregate quality of the identified studies (Table III). In addition to quality assessment based on the tumour marker grading system, sources of bias, country in which the study was conducted, and sources of funding were extracted and considered to determine the overall quality of the studies.

Development of Recommendations and Report Review

Draft recommendations were developed based on the considered judgment of the Working Group after review of the aggregate evidence quality and the likely benefits and harms of ordering each assay. The recommendation report was internally reviewed by the director of the Program in Evidence-Based Care and then presented to the MOAC. The MOAC reviewed and formally approved the document in May 2016.

RESULTS

Details of the characteristics and quality of the systematic reviews and primary studies included and excluded in the present guideline document can be found in the full guideline report.6 All 24 studies chosen for inclusion in the evidence base are fully detailed in Table IV. Key evidence supporting each recommendation is summarized in the text.

In the context of the report, recurrence at between 1 and 5 years after resection is considered early recurrence, and recurrence at more than 5 years after resection is defined as late recurrence. The primary outcomes were risk of recurrence (local and distant) at 5 and 10 years, and overall survival. For studies that did not report overall survival, data for disease-free or relapse-free survival were extracted as surrogate outcomes.

Multigene Profiling Assays

The main purpose of multigene profiling assays is to determine, based on intrinsic tumour biology, the risk that a tumour will recur. In common practice, that determination is most important in the ER-positive, HER2-negative population, because a subset of those patients will have a low risk of recurrence without the addition of adjuvant chemotherapy to standard endocrine therapy. All multigene profiling assays therefore evaluate the intrinsic molecular characteristics of a tumour; however, the molecular markers used to ascertain risk differ between the available assays (Appendix A). The value of multigene profiling is
| Search term | Hits | Description |
|-------------|------|-------------|
| 1. exp breast cancer/ | 235,614 | |  
| 2. breast cancer.mp. | 194,555 | Breast cancer terms  
| 3. (breast adj2 (cancer$ or neoplasm$ or carcinoma$ or malignan$ or tumo?r$)).mp. | 290,158 |  
| 4. or/1–3 | 290,182 |  
| 5. (oncotype or 21 gene or recurrence score).mp. | 812 | Terms for the multigene profiling assays  
| 6. (prosigna or PAM50).mp. | 129 |  
| 7. (mammaprint or 70 gene).mp. | 550 |  
| 8. endopredict.mp. | 25 |  
| 9. or/5–8 | 1,388 |  
| 10. tailorx.mp. | 15 | Terms for important studies known a priori  
| 11. rxponder.mp. | 7 |  
| 12. (swog adj (S1007 or “8814”)).mp. | 10 |  
| 13. (nsabp adj (b20 or b-20 or b 20)).mp. | 8 |  
| 14. (nsabp adj (b14 or b-14 or b 14)).mp. | 15 |  
| 15. transatc.mp. | 13 |  
| 16. ((ma17 or ma 17 or ma-17 or ma12 or ma 12 or ma-12) adj (trial or study)).mp. | 61 |  
| 17. (ABCSG-6 or ABCSG 6 or ABCSG-8 or ABCSG 8).mp. | 17 |  
| 18. mindact.mp. | 22 |  
| 19. (raster adj2 study).mp. | 10 |  
| 20. (geicam 9906 or geicam-9906 or geicam9906).mp. | 8 |  
| 21. (OPTIMA adj2 study).mp. | 20 |  
| 22. or/10–21 | 179 |  
| 23. exp randomized controlled trials as topic/ or exp clinical trials, phase III as topic/ or exp clinical trials, phase IV as topic/ | 107,740 | Methodology terms  
| 24. (randomized controlled trial or clinical trial, phase III or clinical trial, phase IV).pt. | 410,812 |  
| 25. random allocation/ or double blind method/ or single blind method/ | 229,854 |  
| 26. (random$ control$ trial? or rct or phase III or phase IV or phase 3 or phase 4).tw. | 138,935 |  
| 27. or/23–26 | 664,107 |  
| 28. (phase II or phase 2).tw. or exp clinical trial/ or exp clinical trial as topic/ | 1,077,720 |  
| 29. (clinical trial or clinical trial, phase II or controlled clinical trial).pt. | 537,237 |  
| 30. (28 or 29) and random$.tw. | 379,415 |  
| 31. (clinical$ adj trial$1).tw. | 245,790 | Combining of terms  
| 32. (((sing$ or doubl$ or treb$ or tripl$) adj (blind$3 or mask$3 or dummy$)).tw. | 140,226 |  
| 33. placebos/ | 33,849 |  
| 34. (placebo$ or random allocation or randomly allocated or allocated randomly).tw. | 191,968 |  
| 35. (allocated adj2 random).tw. | 747 |  
| 36. Prospective study/ | 401,247 |  
| 37. Retrospective study/ | 550,579 |  
| 38. Cohort study/ | 186,361 |  
| 39. or/30–38 | 1,638,866 |  
| 40. 27 or 39 | 1,852,392 |  
| 41. (4 and 9 and 40) or 22 | 323 |  
| 42. (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. | 1,987,212 | Exclusions and limits  
| 43. 41 not 42 | 318 |  
| 44. exp animal$ not human/ | 4,096,239 |  
| 45. 43 not 44 | 317 |  
| 46. limit 45 to English | 314 |  
| 47. limit 46 to yr=“2002–2016” | 309 |  

**Table I** Literature search strategy: Ovid MEDLINE in-process and other non-indexed citations and Ovid MEDLINE 1946 to present.
### TABLE II  Literature search strategy: EMBASE 1996 to week 7, 2016

| Search term | Hits | Description |
|-------------|------|-------------|
| 1. breast cancer/ | 221,856 | |
| 2. breast cancer.mp. | 291,233 | Breast cancer terms |
| 3. (breast adj2 (cancer$ or neoplasm$ or carcinoma$ or malignan$ or tumo?r)).mp. | 334,758 | |
| 4. or/1–3 | 334,758 | |
| 5. (oncotype or 21 gene or recurrence score).mp. | 1,747 | Terms for the multigene profiling assays |
| 6. (prosigna or PAM50).mp. | 317 | |
| 7. (mammaprint or 70 gene).mp. | 994 | |
| 8. endopredict.mp. | 56 | |
| 9. or/5–8 | 2,756 | |
| 10. TAILORx.mp. | 48 | Terms for important studies known a priori |
| 11. rxponder.mp. | 16 | |
| 12. (SWOG adj (S1007 or “8814”)).mp. | 16 | |
| 13. (nsabp adj (b20 or b-20)).mp. | 16 | |
| 14. (nsabp adj (b14 or b-14)).mp. | 16 | |
| 15. transatac.mp. | 27 | |
| 16. ((ma17 or ma17 or ma-17 or ma12 or ma12 or ma-12) adj (trial or study)).mp. | 76 | |
| 17. (ABC123 or ABC124 or ABC125 or ABC126).mp. | 27 | |
| 18. mindact.mp. | 64 | |
| 19. (raster adj2 study).mp. | 17 | |
| 20. (geicam 9906 or geicam-9906 or geicam9906).mp. | 21 | |
| 21. (OPTIMA adj2 study).mp. | 69 | |
| 22. or/10–21 | 354 | |
| 23. exp randomized controlled trial/ or exp phase 3 clinical trial/ or exp phase 4 clinical trial/ | 347,436 | Methodology terms |
| 24. randomization/ or single blind procedure/ or double blind procedure/ | 174,384 | |
| 25. (randomi$ control$ trial? or rct or phase III or phase IV or phase 3 or phase 4).tw. | 182,769 | |
| 26. or/23–25 | 517,584 | |
| 27. (phase II or phase 2).tw. or exp clinical trial/ or exp prospective study/ or exp controlled clinical trial/ | 1,127,414 | |
| 28. 27 and random$.tw. | 347,289 | |
| 29. (clinic$. adj trial$.).tw. | 286,181 | |
| 30. ((sing$ or doubl$ or treb$ or tripl$) adj (blind$3 or mask$3 or dummy)).tw. | 127,920 | |
| 31. placebo/ | 215,324 | |
| 32. (placebo$ or random allocation or randomly allocated or allocated randomly).tw. | 192,316 | |
| 33. (allocated adj2 random).tw. | 303 | |
| 34. Prospective study/ | 283,378 | |
| 35. Retrospective study/ | 391,863 | |
| 36. Cohort study/ | 180,186 | |
| 37. or/29–36 | 1,322,563 | |
| 38. 26 or 28 or 37 | 1,622,632 | |
| 39. (4 and 9 and 38) or 22 | 709 | Combining of terms |
| 40. (editorial or note or letter erratum or short survey).pt. or abstract report/ or letter/ or case study/ | 1,787,525 | |
| 41. 39 not 40 | 689 | Exclusions and limits |
| 42. animal/ not human/ | 506,080 | |
| 43. 41 not 42 | 688 | |
| 44. limit 43 to english | 671 | |
| 45. limit 44 to exclude medline journals | 24 | |
| 46. limit 45 to yr="2002–2016” | 24 | |
more evident in clinical settings in which systemic therapy recommendations are difficult for the clinician to make. Figure 2 graphically summarizes the recommendations presented here in a decision-tree format for clinical use.

In this practice guideline, a distinction is made between utility for prognosis (recurrence) and for prediction (treatment response). The latter does not follow from the former; it requires an evidence base that includes prospective interventional trials. Although all of the assays reviewed have evidence supporting prognostic utility, the evidence supporting predictive utility remains limited. Another limitation of some assays is the inclusion of an “intermediate risk” category. That category includes patients with an elevated risk of recurrence, but at a magnitude that does not make a clinical decision clear because of lack of evidence. Both limitations are currently being addressed by ongoing trials.

**Oncotype DX**

Five identified studies assessed the prognostic ability of Oncotype dx (Tables vi and v). Based on the tumour-marker utility grading system, all five studies were assessed as category B and were found to support the overall prognostic role of Oncotype dx for tumour recurrence at an evidence level of IB (Table v). When comparing distant with local recurrence, four of five studies were determined to provide level IB evidence supporting the ability of Oncotype dx to prognosticate distant recurrence. The fifth study was category B, but evaluated only the ability of the Oncotype dx assay to prognosticate locoregional recurrence. That evaluation constitutes level II evidence supporting the ability of Oncotype dx to prognosticate for local recurrence.

Two studies assessed as category B, reported that patients with high recurrence scores received a significant benefit from chemotherapy, and patients with low recurrence scores received minimal or no benefit from chemotherapy (Tables vi and v). Even though both studies were category B and reported consistent results, one study enrolled node-positive patients, and one enrolled node-negative patients, with their evidence therefore being considered level II supporting the predictive ability of Oncotype dx in node-positive and node-negative

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**TABLE III** Study categories and levels of evidence based on the tumour marker utility grading system

| Descriptor                  | Meaning                                                                 |
|-----------------------------|-------------------------------------------------------------------------|
| **Study categories**        |                                                                         |
| A                           | Randomized controlled trial designed with tumour biomarker or biomarker assay as the intervention |
| B                           | Randomized controlled trial designed to address a treatment intervention that is not the tumour biomarker or biomarker assay; study prospectively enrolls and follows patients and collects tumour samples, and then uses archived tumour tissue retrospectively to evaluate the tumour biomarker or biomarker assay |
| C                           | Prospective observational registry study that prospectively enrolls patients in a registry and collects, processes, and archives tumour specimens, but that uses standard-of-care treatment and follow-up; archived tumour tissue is used retrospectively to evaluate the tumour biomarker or biomarker assay |
| D                           | Retrospective study                                                     |
| **Levels of evidence**      |                                                                         |
| IA                          | 1 Category A study                                                     |
| IB                          | At least 2 category B studies with consistent results                  |
| II                          | 1 category B study,                                                     |
|                             | or multiple category B studies with inconsistent results,               |
|                             | or at least 2 category C studies with consistent results               |
| III                         | 1 Category C study                                                     |
|                             | or multiple category C studies with inconsistent results               |
| IV                          | Any number of category D studies:                                      |
|                             | (level IV evidence is not sufficient for determining clinical utility) |

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* The tumour marker utility grading system provides a framework to evaluate the clinical utility of tumour markers and was used to grade the levels of evidence of the prognostic and predictive studies that form the evidentiary base of this systematic review.
| Study | LoE a | Population characteristics | Outcomes |
|-------|-------|----------------------------|----------|
|       | Receptor status | Nodal status | Risk of recurrence: |
|       |      |                         | Rate of distant recurrence at 10 years |
|       |      |                         | Rate of distant recurrence at 9 years for N0 patients |
|       |      |                         | Rate of distant recurrence at 9 years for N+ patients |
|       |      |                         | Overall survival at 9 years for N0 |
|       |      |                         | Overall survival at 9 years for N+ |

**Oncotype DXb – prognosis ability**

| Study | LoE a | Population characteristics | Outcomes |
|-------|-------|----------------------------|----------|
| Paik et al., 2004² | B | 668 | ER+ | N– |
| (NSABP B-14) | Tamoxifen-treated | | |

**Risk of recurrence:**
- Rate of distance recurrence at 10 years
- Risk of distant recurrence at 9 years for N0 patients
- Risk of distant recurrence at 9 years for N+ patients
- Overall survival at 9 years for N0
- Overall survival at 9 years for N+

| Study | LoE a | Population characteristics | Outcomes |
|-------|-------|----------------------------|----------|
| Dowsett et al., 2010⁹ | B | 1231 | ER+ | 872 (70.8%) N0 |
| (TransATAC) | 243 (19.7%) N1–3 | 63 (5.1%) N≥4 | 53 (4.3%) Unknown | |

**Risk of recurrence:**
- Distant recurrence: 50-point change in RS (5 vs. 55)
- Multivariate (age, tumour size, type of initial treatment, tumour grade) risk of locoregional recurrence (50-point change in RS)

| Study | LoE a | Population characteristics | Outcomes |
|-------|-------|----------------------------|----------|
| Mamounas et al., 2010⁹ | B | 895 | ER+ | N– |
| (NSABP B-14 and B-20 [tamoxifen-only arms]) | Tamoxifen-treated | | |

**Risk of recurrence:**
- RS significantly associated with risk of locoregional recurrence (log-rank p<0.001)
- 10-Year Kaplan–Meier risk of locoregional recurrence (p<0.001)
- Multivariate (age, tumour size, type of initial treatment, tumour grade) risk of locoregional recurrence (50-point change in RS)

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### TABLE IV  Continued

| Study                        | LoEa | Population characteristics | Outcomes |  |
|------------------------------|------|----------------------------|----------|---|
|                              | (n)  | Receptor status | Nodal status |  |
| Oncotype DXb – prognostic ability continued | | | | |
| Dowsett et al., 201311 (TransATAC) | B 1007 | ER+ | 739 N0 | Risk of recurrence: |
|                              |      |      |      | - Prognostic information beyond prognostic CTS based on risk of recurrence at 10 years |
|                              |      |      |      | - Used a change in likelihood ratio (LR-$\Delta \chi^2$) to quantitatively measure the relative amount of information provided by one score compared with another |
|                              |      |      |      | - N- or HER2- patients |
|                              |      |      |      | LR-$\Delta \chi^2$ for RS + CTS vs. CTS: 10.2; p=0.001; LR-$\Delta \chi^2$ for ROR + CTS vs. CTS: 23.4; p<0.001 |
|                              |      |      |      | N- patients |
|                              |      |      |      | LR-$\Delta \chi^2$ for RS + CTS vs. CTS: 15.0; p<0.001; LR-$\Delta \chi^2$ for ROR + CTS vs. CTS: 24.6; p<0.001 |
|                              | PAM50 ROR | compared with Oncotype DX RS |  |
| Sestak et al., 201312 (TransATAC) | B 940 | ER+ | 683 N0 | Risk of recurrence: |
|                              |      |      |      | - Prognostic information beyond CTS based on univariate and multivariate proportional hazards models |
|                              |      |      |      | - Used a change in likelihood ratio (LR-$\Delta \chi^2$) to quantitatively measure the relative amount of information provided by one score compared with another |
|                              |      |      |      | - Distant recurrence in years 0–5 |
|                              |      |      |      | All patients |
|                              |      |      |      | Univariate LR-$\Delta \chi^2$ for RS: 24.2; p<0.001; Multivariate LR-$\Delta \chi^2$ for RS: 13.22; p<0.001 |
|                              |      |      |      | Univariate LR-$\Delta \chi^2$ for ROR: 37.32; p<0.001; Multivariate LR-$\Delta \chi^2$ for ROR: 11.41; p<0.001 |
|                              |      |      |      | N- patients |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 14.52; p<0.001; Multivariate LR-$\Delta \chi^2$ for ROR: 10.41; p<0.001 |
|                              |      |      |      | N+ patients |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 0.81; p=0.40; Multivariate LR-$\Delta \chi^2$ for ROR: 1.33; p=0.20 |
|                              |      |      |      | HER2- patients |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 10.35; p=0.001; Multivariate LR-$\Delta \chi^2$ for ROR: 8.69; p=0.003 |
|                              |      |      |      | HER2- or N- patients (n=615) |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 6.84; p=0.008; Multivariate LR-$\Delta \chi^2$ for ROR: 8.61; p=0.008 |
|                              |      |      |      | HER2- or N+ patients (n=230) |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 4.01; p=0.05; Multivariate LR-$\Delta \chi^2$ for ROR: 1.96; p=0.20 |
|                              | 95 HER2+ | 845 HER2- | 257 N+ | Distance recurrence in years 5–10 |
|                              |      |      |      | All patients |
|                              |      |      |      | Univariate LR-$\Delta \chi^2$ for RS: 12.17; p<0.001; Multivariate LR-$\Delta \chi^2$ for RS: 5.55; p=0.02 |
|                              |      |      |      | Univariate LR-$\Delta \chi^2$ for ROR: 40.64; p<0.001; Multivariate LR-$\Delta \chi^2$ for ROR: 16.29; p<0.001 |
|                              |      |      |      | N- patients |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 1.01; p=0.30; Multivariate LR-$\Delta \chi^2$ for ROR: 8.93; p=0.003 |
|                              |      |      |      | N+ patients |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 5.17; p=0.02; Multivariate LR-$\Delta \chi^2$ for ROR: 8.37; p=0.004 |
|                              |      |      |      | HER2- patients |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 2.81; p=0.09; Multivariate LR-$\Delta \chi^2$ for ROR: 18.18; p<0.001 |
|                              |      |      |      | HER2- or N- patients (n=615) |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 2.23; p=0.10; Multivariate LR-$\Delta \chi^2$ for ROR: 13.85; p<0.001 |
|                              |      |      |      | HER2- or N+ patients (n=230) |
|                              |      |      |      | Multivariate LR-$\Delta \chi^2$ for RS: 0.38; p=0.50; Multivariate LR-$\Delta \chi^2$ for ROR: 4.78; p=0.03 |
| Study                  | LoE | Population characteristics | Outcomes                                                                 |
|-----------------------|-----|-----------------------------|--------------------------------------------------------------------------|
|                       |     | (n)  | Receptor status | Nodal status | Risk of recurrence: | |
| **OncoType DX**b – predictive ability |     |      |                |              |                        | |
| Paik et al., 2006<sup>13</sup> (NSABP B-20 reanalysis) | B   | 651  | ER+            | N–           | RS stratification      | |
|                       |     |      |                |              | 353 RS <18 (54.2%) | |
|                       |     |      |                |              | 134 RS 18–30 (20.6%) | |
|                       |     |      |                |              | 164 RS ≥31 (25.2%) | |
|                       |     |      |                |              | Risk of distant recurrence with chemotherapy at 10 years | |
|                       |     |      |                |              | RS <18: RR: 1.31; 95% CI: 0.46 to 3.78 | |
|                       |     |      |                |              | RS 18–30: RR: 0.61; 95% CI: 0.24 to 1.59 | |
|                       |     |      |                |              | RS ≥31: RR: 0.26; 95% CI: 0.13 to 0.53 | |
|                       |     |      |                |              | Absolute change (mean ± standard error) in 10-year distant recurrence rate | |
|                       |     |      |                |              | RS <18: 1.1%±2.2% increase | |
|                       |     |      |                |              | RS 18–30: 1.8% increase (standard error not provided) | |
|                       |     |      |                |              | RS ≥31: mean 27.6%±8.0% decrease | |
| Albain et al., 2010<sup>14</sup> (SWOG 8814) | B   | 367  | ER+            | N+           | Risk of distant recurrence (50-point change in RS) in years 1–10 | |
|                       |     |      |                |              | HR: 2.64; 95% CI: 1.33 to 5.27; p=0.006 | |
|                       |     |      |                |              | Risk of distant recurrence in first 5 years | |
|                       |     |      |                |              | HR: 5.55; 95% CI: 2.32 to 3.28; p=0.0002 | |
|                       |     |      |                |              | Risk of distant in years 5–10 | |
|                       |     |      |                |              | HR: 0.86; 95% CI: 0.27 to 2.74; p=0.80 | |
|                       |     |      |                |              | Survival: | |
|                       |     |      |                |              | Improvement in disease-free survival for patients treated with chemotherapy (cyclophosphamide, doxorubicin, and fluorouracil), who had a high RS (≥31) | |
|                       |     |      |                |              | HR: 0.59; 95% CI: 0.35 to 1.01; log-rank p=0.033 | |
|                       |     |      |                |              | No improvement in disease-free survival for patients with a low or intermediate RS | |
|                       |     |      |                |              | RS <18: HR: 1.02; 95% CI: 0.54 to 1.93; log-rank p=0.97 | |
|                       |     |      |                |              | RS 18–30: HR: 0.72; 95% CI: 0.39 to 1.31; log-rank p=0.48 | |
| Prosignac<sup>a</sup> |     |      |                |              | Risk of recurrence: | |
| Dowsett et al., 2013<sup>11</sup> (TransATAC) | B   | 1007 | ER+            | N–           | Prognostic information beyond CTS based on risk of recurrence at 10 years | |
|                       |     |      |                |              | Used a change in likelihood ratio (LR-Δχ²) to quantitatively measure the relative amount of information provided by one score compared with another | |
|                       |     |      |                |              | N– or HER2– patients | |
|                       |     |      |                |              | LR-Δχ² for ROR: 23.4; p<0.001 | |
|                       |     |      |                |              | LR-Δχ² for RS: 10.2; p<0.001 | |
|                       |     |      |                |              | N– patients | |
|                       |     |      |                |              | LR-Δχ² for ROR: 24.6; p<0.001 | |
|                       |     |      |                |              | LR-Δχ² for RS: 15.0; p<0.001 | |

<sup>a</sup> Prosignac on CHEK-2 study

<sup>b</sup> Esoteric Inc.; RS, recurrence score

<sup>c</sup> PAM50 ROR compared with Oncotype DX RS
| Study                        | LoE | Population characteristics | Outcomes | Risk of recurrence:                                                                                                                                                                                                 |
|-----------------------------|-----|-----------------------------|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                             | (n) | Receptor status             | Nodal status |                                                                                           |                                                                                                                                                                                                 |
|                             |     |                             | (1N or 2–3N) | Risk of distance recurrence at 10 years for 1N N+ patients was significantly different between the ROR risk groups (p=0.0002) and between the intrinsic subgroups (p<0.0001) |
|                             |     | ER+                         | N+        | Low-risk ROR: 6.6%; 95% CI: 3.3% to 12.8%                                                                                                             |
|                             |     |                             |           | Intermediate-risk ROR: 15.5%; 95% CI: 9.5% to 25.0%                                                                                                 |
|                             |     |                             |           | High-risk ROR: 25.5%; 95% CI: 17.5% to 36.1%                                                                                                          |
|                             |     |                             |           | Luminal A: 8.4%; 95% CI: 5.3% to 13.3%                                                                                                                 |
|                             |     |                             |           | Luminal B: 25.3%; 95% CI: 17.4% to 36.0%                                                                                                                |
|                             |     |                             |           | Risk of distant recurrence at 10 years for 2–3N N+ patients was significantly different between the ROR risk groups and between the intrinsic subgroups (p values not provided) |
|                             |     |                             |           | Low- and intermediate-risk ROR: 12.5%; 95% CI: 6.6% to 22.8%                                                                                          |
|                             |     |                             |           | High-risk ROR: 33.7%; 95% CI: 25.5% to 43.8%                                                                                                           |
|                             |     |                             |           | Luminal A: 16.5%; 95% CI: 10.7% to 24.8%                                                                                                               |
|                             |     |                             |           | Luminal B: 38.8%; 95% CI: 27.2% to 53.2%                                                                                                               |
| Prosigna – continued        |     |                             |           |                                                                                                                                                                                                                              |
| Gnant et al., 2013[15]      | B 543| ER+                         | N+        | Risk of recurrence:                                                                                                                                                                                                 |
| (TransATAC and ABCSG-8)     |     |                             |           | Risk of distant recurrence at 10 years for 1N N+ patients was significantly different between the ROR risk groups (p=0.0002) and between the intrinsic subgroups (p<0.0001) |
|                             |     |                             |           | Low-risk ROR: 6.6%; 95% CI: 3.3% to 12.8%                                                                                                             |
|                             |     |                             |           | Intermediate-risk ROR: 15.5%; 95% CI: 9.5% to 25.0%                                                                                                 |
|                             |     |                             |           | High-risk ROR: 25.5%; 95% CI: 17.5% to 36.1%                                                                                                          |
|                             |     |                             |           | Luminal A: 8.4%; 95% CI: 5.3% to 13.3%                                                                                                                 |
|                             |     |                             |           | Luminal B: 25.3%; 95% CI: 17.4% to 36.0%                                                                                                                |
|                             |     |                             |           | Risk of distant recurrence at 10 years for 2–3N N+ patients was significantly different between the ROR risk groups and between the intrinsic subgroups (p values not provided) |
|                             |     |                             |           | Low- and intermediate-risk ROR: 12.5%; 95% CI: 6.6% to 22.8%                                                                                          |
|                             |     |                             |           | High-risk ROR: 33.7%; 95% CI: 25.5% to 43.8%                                                                                                           |
|                             |     |                             |           | Luminal A: 16.5%; 95% CI: 10.7% to 24.8%                                                                                                               |
|                             |     |                             |           | Luminal B: 38.8%; 95% CI: 27.2% to 53.2%                                                                                                               |
|                             |     |                             |           |                                                                                                                                                                                                                              |
| Sestak et al., 2013[12]     | B 940| ER+                         | N0        | Risk of recurrence beyond CTS based on univariate and multivariate proportional hazards models                                                                                                                      |
| (TransATAC)                 |     |                             |           | Used a change in likelihood ratio (LR-Δχ²) to quantitatively measure the relative amount of information provided by one score compared with another                                                                   |
|                             |     |                             |           | Distant recurrence in years 0–5                                                                                                                        |
|                             |     |                             |           | All patients                                                                                                                                                                                                      |
|                             |     |                             |           | Univariate LR-Δχ² for ROR: 37.32; p<0.001; Multivariate LR-Δχ² for ROR: 11.41; p<0.001                                                              |
|                             |     |                             |           | Multivariate LR-Δχ² for RS: 13.22; p<0.001                                                                                                           |
|                             |     |                             |           | N– patients                                                                                                                                                                                                       |
|                             |     |                             |           | Multivariate LR-Δχ² for ROR: 10.41; p=0.001; Multivariate LR-Δχ² for RS: 14.52; p<0.001                                                              |
|                             |     |                             |           | HER2– patients                                                                                                                                                                                                   |
|                             |     |                             |           | Multivariate LR-Δχ² for ROR: 1.33; p=0.20; Multivariate LR-Δχ² for RS: 0.81; p=0.40                                                                  |
|                             |     |                             |           | HER2– or N– patients (n=615)                                                                                                                         |
|                             |     |                             |           | Multivariate LR-Δχ² for ROR: 8.69; p=0.003; Multivariate LR-Δχ² for RS: 10.35; p=0.001                                                             |
|                             |     |                             |           | HER2– or N+ (n=230)                                                                                                                                                                                          |
|                             |     |                             |           | Multivariate LR-Δχ² for ROR: 8.61; p=0.008; Multivariate LR-Δχ² for RS: 6.84; p=0.008                                                              |
|                             |     |                             |           | HER2– or N+ (n=230)                                                                                                                                                                                          |
|                             |     |                             |           | Multivariate LR-Δχ² for ROR: 1.96; p=0.20; Multivariate LR-Δχ² for RS: 4.01; p=0.05                                                             |

TABLE IV  Continued
| Study | LoE² | Population characteristics | Outcomes |
|-------|------|-----------------------------|----------|
|       | $(n)$ | Receptor status | Nodal status | Risk of recurrence: continued |
|       |       |              |            | Distant recurrence in years 5–10 |
|       |       |            |     | All patients |
|       |       |            |     | Univariate LR-$\Delta\chi^2$ for ROR: 40.64; $p<0.001$; Multivariate LR-$\Delta\chi^2$ for ROR: 16.29; $p<0.001$ |
|       |       |            |     | N– patients |
|       |       |            |     | Multivariate LR-$\Delta\chi^2$ for ROR: 8.93; $p=0.003$; Multivariate LR-$\Delta\chi^2$ for RS: 1.01; $p=0.30$ |
|       |       |            |     | N+ patients |
|       |       |            |     | Multivariate LR-$\Delta\chi^2$ for ROR: 8.37; $p=0.004$; Multivariate LR-$\Delta\chi^2$ for RS: 5.17; $p=0.02$ |
|       |       |            |     | HER2– patients |
|       |       |            |     | Multivariate LR-$\Delta\chi^2$ for ROR: 18.18; $p=0.001$; Multivariate LR-$\Delta\chi^2$ for RS: 2.81; $p=0.09$ |
|       |       |            |     | HER2– or N– patients (n=615) |
|       |       |            |     | Multivariate LR-$\Delta\chi^2$ for ROR: 13.85; $p=0.001$; Multivariate LR-$\Delta\chi^2$ for RS: 2.23; $p=0.10$ |
|       |       |            |     | HER2– or N+ patients (n=230) |
|       |       |            |     | Multivariate LR-$\Delta\chi^2$ for ROR: 4.78; $p=0.03$; Multivariate LR-$\Delta\chi^2$ for RS: 0.38; $p=0.50$ |
| Prosigna® – continued | | | | | |
| Sestak et al., 2013²² continued (TransATAC) | B | 940 | ER+ | 683 N0 | 257 N+ |
| | | | | | Risk of recurrence: continued |
| | | | | | Distant recurrence in years 5–10 |
| | | | | | All patients |
| | | | | | Univariate LR-$\Delta\chi^2$ for ROR: 40.64; $p<0.001$; Multivariate LR-$\Delta\chi^2$ for ROR: 16.29; $p<0.001$ |
| | | | | | N– patients |
| | | | | | Multivariate LR-$\Delta\chi^2$ for ROR: 8.93; $p=0.003$; Multivariate LR-$\Delta\chi^2$ for RS: 1.01; $p=0.30$ |
| | | | | | N+ patients |
| | | | | | Multivariate LR-$\Delta\chi^2$ for ROR: 8.37; $p=0.004$; Multivariate LR-$\Delta\chi^2$ for RS: 5.17; $p=0.02$ |
| | | | | | HER2– patients |
| | | | | | Multivariate LR-$\Delta\chi^2$ for ROR: 18.18; $p=0.001$; Multivariate LR-$\Delta\chi^2$ for RS: 2.81; $p=0.09$ |
| | | | | | HER2– or N– patients (n=615) |
| | | | | | Multivariate LR-$\Delta\chi^2$ for ROR: 13.85; $p=0.001$; Multivariate LR-$\Delta\chi^2$ for RS: 2.23; $p=0.10$ |
| | | | | | HER2– or N+ patients (n=230) |
| | | | | | Multivariate LR-$\Delta\chi^2$ for ROR: 4.78; $p=0.03$; Multivariate LR-$\Delta\chi^2$ for RS: 0.38; $p=0.50$ |
| Filipits et al., 2014¹⁶ | B | 1246 | ER+ | 919 N0 | 327 N+ |
| (ABCSDG-8) | | | | | Risk of recurrence: |
| | | | | | Low-risk patients |
| | | | | | 2.4% Absolute risk for distance recurrence between 5 and 15 years |
| | | | | | High-risk patients |
| | | | | | 17.5% Absolute risk for distance recurrence between 5 and 15 years |
| | | | | | Survival: |
| | | | | | All patients |
| | | | | | Distinct recurrence-free survival significantly different for high-risk and low-risk patients |
| | | | | | HR: 6.90; 95% CI: 3.08 to 15.45; $p<0.001$ |
| | | | | | N– patients |
| | | | | | Distinct recurrence-free survival significantly different for high-risk and low-risk patients |
| | | | | | HR: 4.74; 95% CI: 1.89 to 11.87; $p<0.001$ |
| | | | | | N+ patients |
| | | | | | HR could not be calculated; no late distinct recurrence-free survival events occurred in the low-risk group |
| Liu et al., 2015¹⁷ | B | 1094 | 58.3% ER+ | 29.7% N0 | 41.7% ER– |
| (NCIC CTG MA.21) | | | | | 42.5% N1–3 |
| | | | | | 22.5% Luminal B |
| | | | | | 22.0% N4–10 |
| | | | | | 17.0% HER2- enriched |
| | | | | | 32.9% Basal-like |
| | | | | | 5.8% N>10 |
| | | | | | Risk of recurrence: |
| | | | | | ROR classification: 3.4% low risk, 17.9% intermediate risk, 78.7% high risk |
| | | | | | Survival: |
| | | | | | No significant univariate effect on recurrence-free survival was observed when a high ROR was compared with low or intermediate ROR (HR: 1.27; 95% CI: 0.83 to 1.95; $p=0.28$) |
| | | | | | On multivariate analysis (adjusted for patient and tumour characteristics), higher continuous ROR was associated with a worse recurrence-free survival ($p=0.03$); risk group assignment was not prognostic ($p=0.31$) |
| | | | | | Intrinsic subtype had significant prognostic effect on recurrence-free survival ($p=0.002$) |
TABLE IV  Continued

| Study                  | LoE | Population characteristics | Outcomes                                      |
|-----------------------|-----|----------------------------|-----------------------------------------------|
|                       |     |                            | Risk of recurrence:                           |
|                       |     | n | Receptor status | Nodal status |                                      |
|                       |     |   |               |              | ■ Risk of distance recurrence in years 5–10 |
|                       |     |   |               |              | ■ Low-risk group                        |
|                       |     |   |               |              |  2.4%; 95% CI: 1.6% to 3.5%              |
|                       |     |   |               |              | ■ Intermediate-risk group                 |
|                       |     |   |               |              |  8.3%; 95% CI: 6.1% to 11.2%              |
|                       |     |   |               |              | ■ High-risk group                        |
|                       |     |   |               |              |  16.6%; 95% CI: 13.1% to 20.9%           |
|                       |     |   |               |              | ■ N– women                                |
|                       |     |   |               |              | More prognostic info was added by the ROR than by the CTS |
|                       |     |   |               |              | LR-χ²: 30.95 vs. 21.48 (univariable)      |
|                       |     |   |               |              | LR-χ²: 17.25 vs. 7.79 (bivariable)        |
|                       |     |   |               |              | ■ N+ women                                |
|                       |     |   |               |              | More prognostic info was added by the ROR than by the CTS |
|                       |     |   |               |              | LR-χ²: 35.60 vs. 25.67 (univariable)      |
|                       |     |   |               |              | ■ HER2–, N– women                         |
|                       |     |   |               |              | More prognostic info was added by the ROR than by the CTS (data not provided) |

| Study                  |     |                            | Risk of recurrence:                           |
|                       |     | n | Receptor status | Nodal status |                                      |
|                       |     |   |               |              | ■ MammaPrint score: 180 poor prognosis, 115 good prognosis |
|                       |     |   |               |              | ■ Distant metastases within 5 years for subsets of patients (poor score vs. good score) |
|                       |     |   |               |              | ■ N0 patients                             |
|                       |     |   |               |              | OR: 15.3; 95% CI: 1.8 to 127; p=0.003    |
|                       |     |   |               |              | ■ N+ patients                             |
|                       |     |   |               |              | OR: 13.7; 95% CI: 3.1 to 61; p<0.001     |
|                       |     |   |               |              | Survival:                                 |
|                       |     |   |               |              | ■ 5-Year overall survival, 10-year overall survival |
|                       |     |   |               |              | ■ Overall good-prognosis: 97.4%±1.5%, 94.5%±2.6% |
|                       |     |   |               |              | ■ Overall poor-prognosis: 74.1%±3.3%, 54.6%±4.4% |
|                       |     |   |               |              | ■ Good-prognosis, N0: 96.7%±2.3%, 96.7%±2.3% |
|                       |     |   |               |              | ■ Good-prognosis, N+: 98.2%±1.8%, 92.0%±4.8% |
|                       |     |   |               |              | ■ Poor-prognosis, N0: 71.5%±4.8%, 49.6%±6.1% |
|                       |     |   |               |              | ■ Poor-prognosis, N+: 76.5%±4.6%, 59.5%±6.3% |

Prosigna® – continued
Sestak et al., 2015¹⁸
(TransATAC and ABCSG-8)

Prosigna® continued
Sestak et al., 2015¹⁸
(TransATAC and ABCSG-8)
| Study                                      | LoE² | Population characteristics | Outcomes                                                                 |
|-------------------------------------------|------|-----------------------------|--------------------------------------------------------------------------|
|                                           |      | Receptor status              | | Risk of recurrence:                                                                 |
|                                           |      | Nodal status                | || Risk of 5-year distant recurrence-free interval                          |
|                                           |      |                             | | - Low MammaPrint score and low Adjuvant! Online score                     |
|                                           |      |                             | |   95.3%; 95% CI: 90.9% to 100%                                            |
|                                           |      |                             | | - High MammaPrint score and high Adjuvant! Online score                   |
|                                           |      | 358 HER2–                   | |   89.8%; 95% CI: 85.1 to 94.8%                                            |
|                                           |      |                             | | - Low MammaPrint score and Adjuvant! Online high score                    |
|                                           |      |                             | |   98.4%; 95% CI: 96.1 to 100%                                            |
|                                           |      |                             | | - High MammaPrint score and low Adjuvant! Online score                    |
|                                           |      | 85 ER–                      | |   98.4%; 95% CI: 100 to 100%                                             |
|                                           |      | 293 PR+                     | | **Survival**:                                                             |
|                                           |      |                             | | - Risk of 5-year distant disease-free survival                          |
|                                           |      |                             | | - Low MammaPrint score and low Adjuvant! Online score                    |
|                                           |      |                             | |   94.3%; 95% CI: 89.5 to 99.3%                                            |
|                                           |      |                             | | - High MammaPrint score and high Adjuvant! Online score                   |
|                                           |      |                             | |   88.7%; 95% CI: 83.8 to 93.8%                                            |
|                                           |      |                             | | - Low MammaPrint score and high Adjuvant! Online high score               |
|                                           |      | 48 HER2+                    | |   97.6%; 95% CI: 94.9 to 100%                                            |
|                                           |      |                             | | - High MammaPrint score and low Adjuvant! Online score                    |
|                                           |      | 342 ER+                     | |   94.6%; 95% CI: 87.6 to 100%                                            |
|                                           |      |                             | | **Risk of recurrence**:                                                  |
|                                           |      |                             | || Risk of 5-year distant recurrence-free interval (p=0.03)               |
|                                           |      |                             | | - Low risk (n=219)                                                       |
|                                           |      |                             | |   97.0%; 95% CI: 94.7% to 99.4%                                           |
|                                           |      |                             | | - High risk (n=208)                                                      |
|                                           |      |                             | |   91.7%; 95% CI: 87.9% to 95.7%                                           |
|                                           |      | 21 HER2–                    | | **Significantly more prognostic information was added with EndoPredict** |
|                                           |      |                             | | compared with clinical parameters alone (p<0.001)                        |
|                                           |      |                             | | - At 10 years, absolute freedom from distant recurrence (by EPclin score) |

**TABLE IV** Continued

*LoE² = Level of evidence; RASTER = Randomized, Assessment of Strategies in Triaging Early breast cancer (RASTER) study; EPclin = EndoPredict clinical score.*
populations (Table v). Further category B validation studies in both populations would be required to attain level IB evidence in support of predictive ability.

Two category A studies to further evaluate the predictive utility of Oncotype dx are currently ongoing. If results from those randomized controlled trials are favourable, they will provide level I evidence supporting a predictive benefit for Oncotype dx in node-negative and node-positive women.

The TAILORX study randomized women with known intermediate recurrence scores to endocrine therapy alone or to endocrine therapy plus chemotherapy. Accrual for TAILORX is complete, but final results have not been published. A recent preliminary report provided data concerning survival and the rate of freedom from distant recurrence for the 1626 low-risk patients who received endocrine monotherapy. Given that the preliminary report provided data only for the low-risk cohort, the reviewers did not believe that those data yet provide level IA evidence for the prognostic ability of Oncotype dx.

To further evaluate the clinical utility of Oncotype dx for node-positive patients, the rPOND trial (SWOG S1007) enrolled ER-positive, HER2-negative patients with 1–3 involved regional lymph nodes and low-to-intermediate recurrence scores (≤25). Patients are being randomly allocated to endocrine therapy alone or to endocrine therapy plus chemotherapy.

**Prosigna**

Six studies evaluated the prognostic ability of the Prosigna (formerly PAM50) assay (Tables vi and v). Based on the tumour marker utility grading system, all six studies were assessed as category B and as supporting the overall prognostic role of the Prosigna assay; that evidence is considered to be level IB (Table v). All six studies assessed prognostication for distant recurrence, constituting level II evidence.

The rPOND trial has been designed to evaluate the clinical utility of Oncotype dx for patients with 1–3 positive nodes; however, the Prosigna assay will be used on tumour samples as a secondary risk assessment tool and might provide level I evidence for Prosigna.

**MammaPrint**

Three studies assessed the prognostic ability of MammaPrint (Tables vi and v). Based on the tumour marker utility grading system, all three studies were assessed as category C studies. They reported consistent support for the prognostic ability of MammaPrint; however, because the studies were assessed as category C, the evidence supporting the overall prognostic ability of MammaPrint is considered to be level II (Table v). Given that all studies assessed the role of MammaPrint in prognostication of distant recurrence, there is level II evidence supporting that ability.

Accrual has now completed for MINDACT, which is evaluating the ability of MammaPrint to predict benefit from chemotherapy. In that study, patients who are node-negative and who have 1–3 positive nodes are being evaluated by clinicopathologic risk factor assessment and by MammaPrint. It should be noted that MINDACT includes...
both estrogen receptor-positive and estrogen receptor-negative patients, and that only estrogen receptor-positive patients are receiving endocrine therapy. Patients with discordance between the risk predicted by clinicopathologic features and by MammaPrint were randomized to receive chemotherapy or no chemotherapy. Preliminary results for patients whose tumor was high-risk according to clinicopathologic features, but low-risk according to MammaPrint, were presented at the 2016 American Association for Cancer Research annual meeting. Compared with the studies involving the other assays highlighted here, the Mindact study is designed to address a somewhat different patient population and clinical utility. Further analysis of the final study results is needed to determine whether a role for the routine clinical use of MammaPrint is supported.

EndoPredict

Two studies supported the prognostic ability of EndoPredict (Tables vi and v). Based on the tumor marker utility grading system, both studies were assessed as category B, and thus the evidence supporting the overall prognostic ability of EndoPredict is considered to be level IB (Table v). Given that the Austrian Breast and Colorectal Cancer Study Group 6 and 8 trials support the ability to prognosticate for distant recurrence, there is level II evidence supporting a role for EndoPredict in prognosticating for local recurrence and level IB evidence for distant recurrence (Table v).

Recommendation 1

Clinicians may offer multigene profile assay testing to potential chemotherapy candidates with invasive breast carcinoma that is estrogen receptor-positive, HER2-negative (Recommendation type: evidence-based; Evidence quality: level IB; Recommendation strength: moderate).

Qualifying Statement: If the patient management plan has been decided based on any or all of clinical, pathologic, or patient-related factors and is unlikely to change, a multigene profiling assay should not be requested.

Predictive Ability of Multigene Profiling Assays with Respect to Withholding Chemotherapy

There is level IB evidence that even in the absence of chemotherapy, patients stratified as low-risk by Oncotype dx, Prosigna, and EndoPredict all validated and have utility for this indication (level IB). Given that the Austrian Breast and Colorectal Cancer Study Group 6 and 8 trials support the ability to prognosticate for local recurrence, there is level II evidence supporting a role for EndoPredict in prognosticating for distant recurrence at 5 and 10 years after treatment. The ability of Oncotype dx to identify a very low-risk population (rate...
of freedom from distant recurrence at 5 years: 99.3%; 95% confidence interval: 98.7% to 99.6%) has been confirmed by an interim analysis from a prospective trial 25.

Although the Oncotype dx, Prosigna, and EndoPredict assays have all shown that, in the absence of chemotherapy treatment, patients stratified as low risk have a low risk of recurrence, prospective validation data are limited. Nevertheless, a low-risk result from a multigene profiling assay can support the decision to withhold chemotherapy in this subset of patients. Because such a decision is also informed by available clinical and pathology information, not all patients with low-risk tumour features require a multigene profiling assay.

**Recommendation 2**

In patients with node-negative, ER-positive, HER2-negative disease, clinicians may use a low-risk result from the Oncotype dx, Prosigna, or EndoPredict assay to support a decision to withhold chemotherapy (Recommendation type: evidence-based; Evidence quality: level IB; Recommendation strength: moderate).

**Qualifying Statement:** A treatment decision should be based on all available clinical and pathology information, and not depend solely on multigene profiling results.

**Predictive Ability of Multigene Profiling Assays with Respect to Offering Chemotherapy**

Level IB evidence 7,8 indicates that withholding chemotherapy is associated with a high-risk of recurrence in the high-risk subgroups identified through Oncotype dx2,9–12, Prosigna11,12,15–18, and EndoPredict22,23. Although Oncotype dx, Prosigna, and EndoPredict are supported by level IB evidence for prognosticating high risk of recurrence in a subgroup of patients, only Oncotype dx has been evaluated to determine its ability to predict a benefit from chemotherapy. Two studies evaluating the clinical validity of Oncotype dx in predicting chemotherapy benefit in high-risk groups13,14 were not perfectly designed to validate that use, but reported consistent results, resulting in level II evidence to support it. Although the tamoxifen-only arm of the National Surgical Adjuvant Breast and Bowel Project B-20 trial13 included samples that were used in the initial

### TABLE V Summary of assay characteristics

| Characteristic | Oncotype DXa | Prosignab | MammaPrintc | EndoPredictd |
|---------------|-------------|-----------|-------------|--------------|
| ER status     | Positive    | Positive  | Positive    | Positive     |
| HER2 status   | Negative    | Negative  | Negative    | Negative     |
| Nodal status  | Negative    | Negative  | Negative    | Negative     |
| Tissue required | Formalin-fixed paraffin-embedded (FFPE) | qRT-PCR | Microarray | qRT-PCR |
| Technique     | qRT-PCR     | qRT-PCR   | Microarray  | qRT-PCR      |
| Assay output  | Recurrence score | Intrinsic subtype and risk of relapse score | Risk of distant recurrence at 5 years | EPclin score |
| Regulatory approval or endorsement | Assay conducted in centralized Genomic Health’s CLIA-certified lab | FDA-cleared for decentralized testing (2014) | FDA-cleared for Agendia centralized lab testing in FFPE (2015) | CE Marking for decentralized testing (2012) |

**Level of evidence h**

| Prognostic ability | Overall | IB | IB | II | IB |
|--------------------|---------|----|----|----|----|
| Risk of recurrence | Local   | II | NA | NA | II |
|                    | Distant | II | IB | II | IB |
|                    | Predictive utility | II | NA | NA | NA |
| Ongoing studies    | TAILORx (I) | RxPONDER (I) | RxPONDER (I) | MINDACT (I) |

a Genomic Health, Redwood, CA, U.S.A.
b NanoString Technologies, Seattle, WA, U.S.A.
c Agendia, Irvine, CA, U.S.A.
d Myriad Genetics, Salt Lake City, U.S.A.
e The intrinsic subtype determination of the Prosigna assay can be performed in ER-negative patients and in HER2-positive patients; however, clinical utility has been established only in ER-positive, HER2-negative patients.
f Although node-positive patients have been enrolled in at least one study for each assay, the clinical utility reported in the table reflects the utility in the more frequently studied node-negative population.
g MammaPrint was FDA-cleared for use with frozen fresh tissue in 2007.
h Based on the Tumor Marker Utility Grading System 7,8.
i Evaluation of Prosigna scores is included as a secondary objective in the RxPONDER randomized controlled trial.

ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; qRT-PCR = quantitative reverse-transcriptase polymerase chain reaction; CLIA = clinical laboratory improvement amendments (U.S. Food and Drug Administration); FDA = U.S. Food and Drug Administration; CE = Conformité Européenne; NA = not applicable.
development of the Oncotype dx test, and some critics believe that this design flaw is sufficient to invalidate the evidence, the Working Group and others29 consider the consistent result of the later study34 to be a mitigating factor.

Although prognostication for recurrence in the absence of treatment and prediction of benefit with adjuvant chemotherapy are distinct aspects of clinical validity, they are often addressed together. Oncotype dx has sufficient evidence to simultaneously address prognostic and predictive validity for high-risk tumours. However, the two studies that were identified to evaluate the predictive ability of Oncotype dx used chemotherapy regimens that are no longer widely used in Ontario. An ongoing trial (TAILORx) that will provide additional data about the predictive ability of Oncotype dx in the context of more modern adjuvant chemotherapy treatment has not yet reported primary outcome data. Thus, current evidence for the predictive validity of Oncotype dx is low-level (level II) and supports only a weak recommendation. Based on prognostic ability, other multigene profiling assays likely also have similar predictive utility; however, further validation is needed to support a recommendation for their use.

**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-risk Oncotype dx result in this subpopulation has been associated with both poor prognosis without chemotherapy and a predicted benefit from chemotherapy (Recommendation type: evidence-based; Evidence quality: level IB–II; Recommendation strength: weak).

**Qualifying Statements:** High-risk stratification by Oncotype dx may support a decision to offer chemotherapy, but the treatment decision or decisions should be based on all available clinical and pathology information and should not solely depend on Oncotype dx.

**Nodal Status**
There is level IB evidence2,8 indicating that node-positive low-risk subgroups identified using Oncotype dx9,14 and Prosigna15,18 experience lower recurrence rates, even in the absence of adjuvant chemotherapy. However, the recurrence risks associated with a low-risk Oncotype dx recurrence score for a node-positive patient and with an intermediate-to-high recurrence score for a node-negative patient are similar in magnitude9,11. Analysis from the swog 8814 trial14 shows that a high-risk Oncotype dx recurrence score is predictive for chemotherapy benefit, albeit with a wide confidence interval (level II evidence).

Although there is evidence for the prognostic ability of Oncotype dx and Prosigna in node-positive patients, the clinical utility of multigene profiling assays depends on potential benefit to patients with node-positive disease. Currently, the routine use of multigene profiling assays for ER-positive, HER2-negative, node-positive tumours is not supported by evidence. Clinical judgment is therefore needed in considering a role for multigene profiling assays in the subset of patients with a low nodal disease burden (for example, micrometastases only). In current practice, that subset represents a small proportion of node-positive patients.

**Recommendation 4**
In some patients with ER-positive, HER2-negative tumours and with 1–3 involved nodes (N1a disease), clinicians may withhold chemotherapy based on a low-risk Oncotype dx or Prosigna score if the decision is supported by other clinical, pathology, or patient-related factors (Recommendation type: consensus-based; Evidence quality: level II; Recommendation strength: weak).

**Qualifying Statements:** Node-positive disease is associated with a relatively high risk of recurrence, and chemotherapy is frequently recommended. Currently, multigene profiling assays are not approved and funded in Ontario for node-positive disease, unless the largest metastatic deposit measures 2 mm or less (micrometastatic disease, pN1mi). The clinical outcome in patients with pN1mi disease is considered to be more similar to that for patients with node-negative disease; thus, clinicians may offer multigene profile assay testing for those patients. The presence of isolated tumour cells (largest deposit less than 0.2 mm or 200 cells) is considered node-negative disease in this setting.

**Late Disease Recurrence**
Intervention studies that assess the predictive ability of multigene profiling assays for late recurrence are lacking. The prognostic value of Prosigna11,15 and EndoPredict22,23 for late recurrence is based on a multivariate statistical analysis performed retrospectively on completed prospective trials (level II evidence7,8). Although a high Oncotype dx recurrence score is also associated with late recurrence, the statistical difference between early and late recurrence loses significance on multivariate analysis12,14.

**Recommendation 5**
In patients with ER-positive disease, the evidence is insufficient to recommend the use of multigene profiling assays to inform clinical decision-making for late risk of recurrence. A high-risk score using Prosigna or EndoPredict prognosticates for late recurrence; however, evidence that those tests predict a benefit for the use of extended adjuvant endocrine treatment beyond 5 years is lacking. (Recommendation type: consensus-based; Evidence quality: lack of evidence; Recommendation strength: weak).

**DISCUSSION**
Clinical staging and histopathologic assessment remain the principal means of prognosis and basis for treatment decisions in breast cancer. It is now well established that breast tumours also have intrinsic molecular patterns that can be informative concerning their biologic potential29. As an example, the discovery of a low-risk ER-positive molecular profile (“luminal A”) in contrast to a high-risk ER-positive profile (“luminal B”) with implications for chemo-responsiveness20 was a major impetus for developing the multigene profiling assays.
No single gene expression profile is considered to be a “gold standard” for molecular classification. Even among the multigene profiling assays that have been clinically validated, the actual panels of genes evaluated have little to no overlap. However, in all of the assays, the genes involved in proliferation, survival, stromal invasiveness, and inflammatory responses are heavily represented.

It must be emphasized that the key determinant of whether to order a multigene profiling assay remains clinical judgment. Before such a test is performed, a clinician would already have access to several decision-making tools, including standard pathology (tumour grade, subtype) and risk-assessment nomograms [for example, Adjuvant! Online (https://www.adjuvantonline.com/)]. Based on those factors, a reasonable decision to withhold chemotherapy might already be made, and further testing would therefore not be of use.

**Evaluation for Clinical Utility**

**Major Considerations**

Even when an assay is fully validated for its ability to separate a patient population into two distinct groups, it might not serve a useful purpose. Evaluation for clinical utility must take into account the designs of all the relevant trials with respect to the clinical scenarios that arise in patient care. For example, the MammaPrint assay has prognostic validity, as shown by multiple retrospective analyses; however, those trials were category C or retrospective studies in which treatment was not a consideration in the study design. The diagnosis of a “low-risk” tumour in the absence of a validated treatment recommendation is not clearly actionable. Further consideration of the MammaPrint assay awaits the final publication of MINDACT, which has a prospective design to address chemotherapy treatment and has been presented only in abstract form to date.

By contrast, the trials for Oncotype dx, Prosigna, and EndoPredict all examined prognosis in prospective clinical trials in which at least one arm received a standard treatment. Although the blocks were tested retrospectively, the trials themselves were prospective in design and constitute level IB evidence for prognostic validity. Given the designs of those trials, the same clinical utility is established for all three assays. For patients with ER-positive tumours who receive tamoxifen alone, all three assays validly identify a low-risk population (low recurrence score, risk of recurrence, or EPClin score respectively) with a favourable outcome. A low-risk assay result is therefore actionable, and the decision to withhold chemotherapy is supported by evidence. It should be emphasized that the information obtained from such a test must still be interpreted in the context of the overall clinical and pathologic features of the tumour. In a recent review of key biomarkers in breast cancer, an American Society of Clinical Oncology working group reached similar conclusions, although they chose to emphasize the prognostic utility of the tests over the predictive.

Oncotype dx, Prosigna, and EndoPredict are also able to validly identify a high-risk population of ER-positive tumours with poorer outcome when treated with tamoxifen alone. However, to demonstrate the clinical utility of a high risk score, a clinical trial designed with both tamoxifen alone and tamoxifen plus chemotherapy arms is required. Oncotype dx is associated with two studies having such a design; however, one is flawed, and the other was limited to a node-positive population. Nevertheless, the prognostic validity was consistent in the two studies, and the evidence that a high Oncotype dx recurrence scores can predict for chemotherapy benefit is therefore considered to be level II. A high Prosigna risk of recurrence score and a high EndoPredict EPClin score, although prognostic, are not directly actionable based on clinical trial evidence.

Some authors argue that tests that have been validated for prognostication, such as Prosigna, could also be used to predict chemotherapy benefit based on their similarity to Oncotype dx in head-to-head comparison studies. The MDC has previously considered that question, concluding that the information provided by the Prosigna and EndoPredict tests are similar but not fully replace, the information provided by Oncotype dx. Therefore, although it is plausible that Prosigna and EndoPredict—and potentially other tests (covered later in the Discussion)—can perform the same function as Oncotype dx, it is the Working Group’s consensus that, at this time, the evidence base is more extensive for Oncotype dx with respect to both prognostic and predictive value.

**Minor Considerations**

Some of the studies assessed in the current practice guideline include analyses of early compared with late recurrence. That question is of potential interest, because overall clinical presentation and natural history are known to differ significantly between tumours that recur early (within 5 years) and those that recur late (after 5 years). Those findings point to a molecular or biologic basis for the difference and could have an effect on patient surveillance. However, at present time, an assay that prognosticates for late recurrence in an ER-positive tumour is not clearly actionable, and therefore clinical utility cannot be established. Further interventional studies designed to determine the benefit of extended hormone therapy in patients at risk for late recurrence are needed.

**Other Tests for Future Consideration**

In addition to the multigene profiling assays that have been reviewed here, other multigene profiling assays that have been associated with prognostication for early-stage breast cancer are on the market. The most notable is the Breast Cancer Index (bioTheranostics, San Diego, CA, U.S.A.), which was also assessed by the American Society of Clinical Oncology panel as having level IB evidence in support of its prognostic ability. The Breast Cancer Index is based on a gene-expression ratio of HOXB13 to IL17BR, combined with a 5-gene molecular grade index. The evidence cited by the American Society of Clinical Oncology panel supported both the analytic and clinical validity of the test, leading toward clinical utility (prognostic only) similar to that of EndoPredict and Prosigna. At the time of writing, the Breast Cancer Index is not available for samples originating in Canada. As for EndoPredict and Prosigna, data from prospective interventional trials are not currently available for the Breast Cancer Index.
Other assays that were not included in the present guideline lacked high-quality studies assessing their clinical utility. The Rotterdam 76-gene signature was developed using microarray data from frozen archival samples from both ER-negative and ER-positive patients. The assay has been retroactively validated in three datasets, but its analytical validity and clinical utility have not been addressed. The Genomic Grade Index was developed to grade tumours more accurately. It consists of a 97-gene assay. Several small prospective–retrospective studies have suggested that the Genomic Grade Index might have clinical utility, but no study has directly assessed the analytic validity of the assay.

CONCLUSIONS

The clinical utility of multigene profiling assays is currently established for an appropriate subset of patients with ER-positive, HER2-negative, node-negative breast cancer for whom a decision to give chemotherapy is difficult to make. The clinical utility of multigene testing lies in its ability to identify low-risk and high-risk populations based on a tumour’s molecular profile.

Oncotype DX is actionable whether the score is low-risk (supporting the withholding of chemotherapy) or high-risk (indicative of likely chemotherapy benefit). Additional evidence concerning intermediate-risk scores is currently being gathered (TAILORx trial). Although Oncotype DX can also be prognostic and predictive for node-positive patients, it is unclear whether its results are sufficient to guide treatment. The NXTponder trial is expected to help resolve that question.

Prosigna, EndoPredict, and MammaPrint—other commercially available multigene profiling assays—use the same biologic principles as Oncotype DX, but different gene panels. The evidence supports the concept that those tests are at least as informative as Oncotype DX with respect to finding clinically relevant intrinsic molecular profiles, but prospective clinical trials both for assessing the clinical validity of those assays and for providing relevant clinical (interventional) utility are lacking. Prosigna and EndoPredict are statistically more likely to identify a population at risk for recurrence beyond 5 years, but that information is currently not actionable because of a lack of interventional studies.

ACKNOWLEDGMENTS

The authors thank the following individuals for their assistance in developing this report: Melissa Brouwers, Sheila McNair, Hans Messersmith, and Caroline Zwaal for providing feedback on draft versions; Terence Tang for conducting a data audit; and Sara Miller for copyediting.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood Current Oncology’s policy on disclosing conflicts of interest, and we declare the following interests: AE, MT, and PB received institutional funding from Genomic Health during development of the Ontario Clinical Oncology Group Oncotype DX field study. MC, LS, SKR, MR, and JH all have no conflicts to disclose.

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APPENDIX A: ASSAY DETAILS

The Oncotype dx assay (Genomic Health, Redwood, California, U.S.A.) provides clinicians with a recurrence score (rs) based on a multigene expression profile. This assay was first developed in patients with ER-positive, HER2-negative, node-negative breast cancer who had been randomized to the tamoxifen-only arm of the National Surgical Adjuvant Breast and Bowel Project B-20 trial1. The expression levels of messenger RNA for 250 candidate genes previously implicated in breast cancer pathogenesis were tested in three independent studies involving those patients. The final gene panel used to calculate the rs contains the 16 cancer-related genes that were found, in the three studies, to be the most highly correlated with recurrence, plus five reference genes. An algorithm generates the rs, which is an estimate of the 10-year risk of distant recurrence. The rs is reported on a scale of 0–100, with scores of 17 or less indicating low risk of recurrence, scores of 18–30 indicating intermediate risk, and scores greater than 30 indicating a high risk of recurrence2.

The Prosigna assay (NanoString Technologies, Seattle, Washington, U.S.A.) was developed to make the categorization of breast tumours into their intrinsic subtypes clinically applicable. The PAM50 breast cancer intrinsic classifier algorithm uses microarray and quantitative reverse-transcriptase polymerase chain reaction to classify all patients—regardless of hormone receptor status, HER2 amplification, or nodal status—into the subtypes based on the expression patterns of 50 genes3. The Prosigna risk of recurrence score classifies patients into a high-, intermediate-, or low-risk group based on an algorithm that incorporates the 50 gene signature, intrinsic subtype, and tumour size3. To gain clearance from the U.S. Food and Drug Administration (FDA) for decentralized testing using the Prosigna assay, the original microarray assay had to be transferred to a FDA-cleared medical instrument, the nCounter system.

In 2007, the MammaPrint assay (Agendia, Irvine, California, U.S.A.) became the first multigene profiling assay to obtain FDA clearance. The assay was developed to determine prognosis in patients with early breast cancer regardless of hormone receptor status or HER2 amplification status. Using DNA microarray analysis of frozen fresh tissue from selected untreated primary breast tumours, a 70-gene signature that was predictive of the early development of distant metastasis was developed4. Based on the expression signature of the 70 genes, patients are classified as either good or poor prognosis4. To compete with the other assays, which use formalin-fixed paraffin-embedded (FFPE) tissue and not frozen tissue, the MammaPrint assay has recently been updated to use FFPE tissue. The assay received FDA clearance for centralized laboratory testing with FFPE tissue in 2015.

EndoPredict (Myriad Genetics, Salt Lake City, U.S.A.) is an RNA-based assay that was developed to prognosticate for distant recurrence in patients with ER-positive, HER2-negative breast cancer who are receiving adjuvant endocrine therapy5. EndoPredict uses reverse-transcriptase polymerase chain reaction to measure the mRNA expression levels of 11 genes5. The EndoPredict risk score ranges from 0 to 15, with higher values indicating a higher risk of recurrence5. Additionally, an EPclin score, which combines the EndoPredict risk score with tumour size and nodal status, was also developed, and it categorizes patients into either low- or high-risk groups5. The EndoPredict assay has received the Conformité Européene mark and is in routine use in parts of Europe.