Health Policy Advisory Committee on Technology

Technology Brief Update

Gene expression profiling of breast cancer

April 2016
2016 Summary of findings

Numerous gene expression profiling (GEP) tools, using various methodologies, are available, typically through clinician request, or are being trialled in validation studies. Since the original brief on GEPs for early breast cancer, new evidence is available for three tests, two have entered into the MSAC process for public funding, two had no new evidence and six new tests emerged, including one that is in the MSAC process and another that is approved for use in Australia. Most studies provided a low-level of prognostic evidence, and no studies that considered direct evidence or the impact of the prognostic tool on change in management and health outcomes were identified. In general, this update does not provide any further evidence on the clinical utility of these tests; the results of two trials (using OncotypeDX and Mammaprint) are awaited to inform this.

2016 HealthPACT Advice

There are numerous gene expression profiling tests approved for marketing in Australia for the identification of women who may respond to treatment for breast cancer. Clinical utility of these tests has not, as yet, been proven, as the current evidence base and a lack of long-term follow-up data does not demonstrate that testing results in changed clinical outcomes for these patients. Of concern is that, on the basis of these test results, women may be denied treatment that may, in fact, be of benefit to them. It is also of concern that only a small subset of women would be good candidates for gene expression profiling testing, however, consumer demand for these tests is likely to increase.

HealthPACT does not support public investment in this technology in clinical practice at this time and not until after consideration of published results of studies demonstrating clinical utility. Therefore, HealthPACT recommends that the evidence for gene expression profiling tests for breast cancer prognosis be reviewed in 36 months.
This update will consider whether pertinent evidence has emerged since the Brief on Gene expression profiling of breast cancer was considered by HealthPACT at the May 2012 meeting. At that time, a systematic review (SR) conducted by the National Institute for Health and Care Excellence (NICE) determined that this body of evidence could not substantiate the clinical utility of tests intended to determine gene expression signatures for identifying the subpopulation of lymph node negative breast cancer patients most likely to respond to chemotherapy. Worldwide validation of these testing methods is ongoing.

The Target Group for the May 2012 Brief was the population of ‘lymph node negative breast cancer patients’, however the report focused on gene expression profiling tests (GEPs) for human epidermal growth factor receptor 2 negative (HER2-ve), hormone receptor positive (ER+ve and/or PR+ve), lymph node negative or positive (N+ve/-ve) breast cancer, as was included in the NICE SR. While trial populations vary slightly, the vast majority of GEPs identified are designed for this latter group of patients for whom, according to Breast Cancer Guidelines, treatment decisions can be the most difficult (see Table 3). For these reasons, and because of time restraints related to the scope of this update, the patient indication for this update is HER2-ve, ER+ve or PR +ve and N+/-ve breast cancer.

In Australia, the GEP test, Oncotype DX®, was considered and rejected for public funding by the Medical Services Advisory Committee (MSAC), and has recently resubmitted an application. The gene profiling test MammaPrint® has similarly been considered by MSAC but was not approved for funding. For this reason, evidence regarding OncoType Dx and MammaPrint will not be considered in this Brief update. Rather, this document considers new information pertaining to the other testing methods identified in the original Brief, or any other relevant tests that have emerged.

A more recent GEP test called EndoPredict® has an application currently under consideration by the Protocol Advisory Sub-Committee (PASC) of MSAC. EndoPredict will be briefly described but evidence for this test will not be discussed at length in this report.

**Description of the technology**

One of the most significant problems facing clinicians treating women with breast cancer is tumour heterogeneity; different tumour and patient characteristics need to be taken into account before treatment can commence. Characteristics including patient age, tumour size, histological grade, lymph node status and oestrogen (ER), progesterone (PR) or HER2
(also known as ERBB2) status are all used to guide the need for adjuvant chemotherapy and to assess the risk of distant recurrence. Algorithms that incorporate these factors include the National Institute of Health (NIH) and the St Gallen’s consensus statements, the Nottingham prognostic index and the online decision tool Adjuvant!Online.\textsuperscript{4,5}

One current focus of breast cancer research is the identification of genes or biomarkers that may improve the quality of life of patients already diagnosed with breast cancer. In addition to determining which treatments may be the most effective for individual patients or which treatments should be avoided due to potential toxic side effects, the identification of biomarkers may be able to predict tumour behaviour and the prognosis or response of patients to treatment.\textsuperscript{6}

The purpose of gene expression analysis is to determine which genes are actively transcribed into messenger RNA (mRNA) and therefore translated into proteins in tissue samples of interest, in this case breast cancer. Gene expression profiling assays may assist in the classification of breast cancers into prognostic categories depending on the expression of a panel of genes or biomarkers, predicting those patients who \textit{would}, as well as those who \textit{would not}, benefit from adjuvant chemotherapy.\textsuperscript{6,7} In addition to patient benefits, stratification of patients may result in cost savings with many patients being spared unnecessary and expensive chemotherapy.\textsuperscript{8}

There are several platforms used for gene profiling and each has advantages and disadvantages (Table 1). Fluorescent in situ hybridisation (FISH) measures gene copy number, but it does not provide direct information about gene or protein expression. Reverse transcriptase – polymerase chain reaction (RT-PCR) can quantitatively measure ribonucleic acid (RNA) expression levels of a small number of genes with high-levels of precision and sensitivity but does not provide direct information about protein expression or distribution, or about gene amplification. Although RNA is degraded in formalin-fixed paraffin-embedded (FFPE) tissues, RT-PCR is well-suited to amplify from these short RNA fragments. Microarrays also measure RNA expression levels, not gene copy number or protein expression and distribution. Unlike RT-PCR, microarrays are high throughput and can assess gene expression of many genes at once. Results from microarrays tend to be regarded as semi-quantitative and may reflect differences in tumour biology by characterising differences in patterns of gene expression. The primers used in microarrays are more efficient with length; therefore fresh frozen tissue is preferred over FFPE tissue.\textsuperscript{9} Immunohistochemistry (IHC) measures protein expression and distribution in a cell.
Table 1  A comparison of the advantages and disadvantages of methods used to measure gene expression (printed with permission Genomic Health).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FISH</th>
<th>RT-PCR</th>
<th>Microarray</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured molecule</td>
<td>DNA</td>
<td>RNA</td>
<td>RNA</td>
<td>Protein</td>
</tr>
<tr>
<td></td>
<td>Does not provide direct information about gene or protein expression</td>
<td>Does not provide direct information about protein expression or distribution</td>
<td>Does not provide direct information about protein expression or distribution</td>
<td>Provides information about protein expression and distribution</td>
</tr>
<tr>
<td>Quantitative nature</td>
<td>Quantifies gene copy number of relatively small number of genes</td>
<td>Quantifies gene expression levels of relatively small number of genes</td>
<td>Qualitative assessment of gene expression of many genes at once Modest precision for expression levels of individual genes</td>
<td>Assesses protein expression and distribution of a relatively small number of proteins Dichotomous</td>
</tr>
<tr>
<td>Tissue fixation</td>
<td>DNA is preserved</td>
<td>RNA is degraded FFPE tissue, but RT-PCR is well suited to amplify from short RNA fragments</td>
<td>RNA is degraded with FFPE tissue; fresh frozen tissue is preferred</td>
<td>May denature proteins May affect antibody epitope binding</td>
</tr>
<tr>
<td>Amount of tissue sample needed</td>
<td>2 × 5-µm sections</td>
<td>3-6 × 10-µm sections</td>
<td>30 × 30-µm sections</td>
<td>2-4 core samples per patient</td>
</tr>
<tr>
<td>Interpretation of results</td>
<td>Subject to inter-observer variability.</td>
<td>Normalised against expression levels of reference genes.</td>
<td>Normalised against reference genes or global expression.</td>
<td>Subject to inter-observer variability.</td>
</tr>
</tbody>
</table>

FISH, fluorescent in situ hybridisation; RT-PCR, reverse transcriptase polymerase chain reaction; IHC, immunohistochemistry; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; FFPE, formalin-fixed paraffin-embedded

There are several GEPs commercially available or in development, all of which use either use IHC, quantitative RT-PCR (qRT-PCR) or a microarray platform to measure the level of gene transcription into mRNA. These tests classify patients who have a poor prognosis by identifying those who express high levels of proliferation genes, usually the same genes that are the target of conventional chemotherapy.

For detailed information regarding different molecular methods used in gene profiling and generic descriptions of the technology used (e.g. microarrays), the reader should refer to the full Brief considered by HealthPACT at the May 2012 meeting.

2016 Stage of development in Australia

- [ ] Yet to emerge
- [ ] Experimental
- [ ] Investigational
- [x] Nearly established
- [ ] Established
- [ ] Established but changed indication or modification of technique
- [ ] Should be taken out of use
2016 Licensing, reimbursement and other approval

Prosigna has been approved by FDA and CE marked and is available for sale in markets that recognise the CE mark, and in the US. MammaPrint has been FDA approved and in 2015 received FDA marketing clearance for use in FFPE breast cancer tissue. EndoPredict is trademarked in Europe, Australia and the US and is commercially available in Europe and the US.

The following GEPs are also commercially available in the US: Oncotype DX, MammaPrint, BluePrint, TargetPrint, Breast Cancer Index, Mammastrat, BreastOncPx, NexCourse Breast IHC4 and BreastPRS.

2016 Australian Therapeutic Goods Administration approval

☐ Yes
☐ No
☐ Not applicable

MammaPrint and Oncotype DX tests are performed in the USA, therefore Australian Therapeutic Goods Administration (TGA) approval is not required (as reported in the protocols submitted to PASC).

Myriad Genetics has applied for registration of EndoPredict® (IVD Class 3) with the TGA. TGA approval has been granted for the kPCR Amplification/Detection (A/D) Module manufactured by Siemens Healthcare Diagnostics (IVD Class 1) and used specifically for EndoPredict®.

According to a press release in August 2014 by nanoString Technologies Inc. the Prosigna Assay has been approved for use in Australian qualified clinical laboratories. The test is run on the nCounter® Dx Analysis System. Although the manufacturer says it is listed on the ARTG it was not found in a search of the ARTG.

No other GEP technologies were identified on the ARTG, however it is possible that they could have been missed due to different descriptions or naming conventions.

2016 Diffusion of technology in Australia

It is expected that this technology is diffusing in Australia, given that three GEP tests are currently within the MSAC process for public funding, and others are available.
2016 International utilisation

<table>
<thead>
<tr>
<th>Country</th>
<th>Level of Use</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Trials underway or completed</td>
</tr>
<tr>
<td>European Union</td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td></td>
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<tr>
<td>United States</td>
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</tbody>
</table>

2016 Cost infrastructure and economic consequences

Any information publicly available about the costs of these tests is limited, and the costs also vary depending on the hardware and software required to run them. A European Health Technology Assessment (HTA) report noted that Mammaprint and Oncotype DX are both expensive tests; Mammaprint cost €2,675 (AU$4,106) and Oncotype DX US$3,400 (AU$4,789), and most RT-PCR and microarray analyses usually cost in the range of US$3,500 (AU$4,930). The MSAC submission for Oncotype DX, considered in 2014, suggested a fee of $4,200, however it should be noted that this test is performed in the US rather than locally. The expense of the test was used as an argument for public funding, as it was claimed it would increase access to women who could not afford it without the rebate; however MSAC argued that the out-of-pocket expense would be high for patients, even if only the MBS fee was charged. The European HTA (which considered Oncotype DX, MammaPrint and FEMTELLE\(^a\)) reported that there is the possibility of savings to be made with a reduced use of chemotherapy and its side effects in women who will derive little or no benefit from it, and possibly reduced mortality in women who appropriately receive chemotherapy; however they noted that the key evidence to draw conclusions about cost-effectiveness is missing.\(^b\)

Since TGA approval of the Prosigna Assay, Sonic Genetics has begun offering the test to Australian women at a cost of $2,900. According to their website, Sonic Genetics require pre-payment and there is a 10 day turn around for test results. The assay is performed on the nanoString nCounter Dx Analysis System which makes it possible to perform in local laboratories that have that equipment, rather than overseas.

\(^a\) FEMTELLE\(^a\) is an American Diagnostica Inc. enzyme-linked immunoassay (ELISA) test for the protein markers uPA (urokinase-type plasminogen activator) and PAI-1 (plasminogen activator inhibitor type-1). Large validation studies have assessed its prognostic value for disease recurrence in breast cancer patients. It is registered as a CE marked in vitro product, is now used routinely in Europe, and for research use only in the USA. Other in-house ELISA kits for uPA and PAI-1 are also available and can be used across a number of different cancer types. Source: [http://meka.thl.fi/htacore/113.aspx](http://meka.thl.fi/htacore/113.aspx)
2016 Evidence and Policy

Safety and effectiveness

Research is currently very active in the field of gene profiling for cancer. Several new tests were identified in this update that were not included in the original Brief. A number of systematic and descriptive reviews were identified which have been published since the 2012 NICE HTA. A large proportion of the evidence identified was related to Oncotype DX and MammaPrint and has not been considered here as these tests have already been the subject of submissions to MSAC for public funding. Importantly, no evidence that shows the impact of any of these tests in prospective studies is yet available; trials to assess this important step are currently underway for Oncotype DX and MammaPrint. New evidence was identified for MapQuant, Mammastrat and the Rotterdam signature; no new evidence was found for Theros BCI or BreastOnc PX. The new tests identified and discussed here are EndoPredict (considered only briefly in this update as submitted to MSAC for public funding), Prosigna, eXagenBC, BreastPRS, TargetPrint and BluePrint.

Three reviews (including one systematic review) assessing gene expression profiling for risk prediction in breast cancer, published between 2013 and 2015 inform the major part of this update. Limited descriptive reviews which discuss recently developed gene profiling tools, and recent publications related to individual GEPs, are also considered. Articles describing evidence for specific relevant GEPs were pearled from the reviews.

The majority of studies on individual GEPs identified were validation studies, based on retrospective cohorts from breast cancer databases or trials, and using either FFPE or frozen tumour samples collected from patients. The majority of validity studies on individual tests were of low level prognostic evidence (level III-3), and others were analyses of prognostic factors in a single arm of a randomised trial (level III-2). One prospective study assessing the impact on patient treatment of the Genomic Grade Index (GGI) was considered to provide moderate level interventional evidence (level III-2). No direct evidence was identified, or prospective studies for linked evidence of clinical utility or therapeutic effectiveness. The prospective TAILORx trial is underway to determine the need for adjuvant chemotherapy in the Oncotype DX stratified intermediate risk group with ER+ve, N-ve breast cancer. Trial completion is expected in 2017. In addition the prospective MINDACT trial, currently ongoing, will assess the clinical utility of MammaPrint, which includes both N-ve and N+ve patients.

An increasing number of research groups have been applying gene profiling technologies to genetic cancer libraries or databases to determine the underlying molecular biology of disease. As a result, many profiles are being created and tested, some of which are designed

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b Trial Assigning Individualized Options for treatment (Rx) trial is sponsored by the National Cancer Institute
c Microarray in Node-negative Disease May Avoid Chemotherapy trial is sponsored by the European Organisation for Research and Cancer Treatment
to be applied broadly to many cancer types, and others which apply to narrow populations, specified by other markers. The IHC4 test, discussed in the original Brief, is one of several IHC kits and algorithms now available which measure ER, PR, HER2 and Ki67 status. It is also used in conjunction with other markers to make treatment decisions in early breast cancer.\textsuperscript{20, 22} It is not discussed further here. The plasminogen activator system markers uPA and PAI-1, which are assessed by enzyme-linked immunosorbent assay (ELISA, not discussed in this update) are now used routinely in Europe to assist with treatment decisions in breast cancer, although they can also be used to assess other cancers. The markers are correlated with tumour aggressiveness and poor clinical outcome and there is high level evidence of the clinical utility of these markers.\textsuperscript{23}

Combinations of GEPs are also being trialled for their prognostic ability. For example IHC4 has been tested in combination with the mRNA based Oncotype DX for its ability to add prognostic information.\textsuperscript{19} Agendia have designed two additional microarrays, TargetPrint and BluePrint to be performed in conjunction with MammaPrint also with the aim of adding prognostic information.\textsuperscript{24}

This update will refer only to gene expression tests specific to early human HER2-ve breast tumours, that are also ER+ve or PR+ve.

To summarise the current prognostic tools available within the early breast cancer setting, the narrative review by Berse and Lynch published in 2015, provides a comprehensive synopsis, including the markers used, relevant populations and intended clinical use; they are summarised in Table 2.\textsuperscript{20} Note that HER2+ve populations and the relevant profiling tests have been not included, nor have technologies which are used to classify HER2 status. Some genetic profiles are used in HER2-ve populations with lymph node negative or positive (N-ve or N+ve) tumours and they are included here, although the majority of tools for HER2-ve populations apply only to N-ve tumours. More information about each individual test is provided below the table.

Other than Oncotype Dx and MammaPrint, Prosigna had the most literature identified in this update. Prosigna has disseminated further than other GEPs in Australia probably due to its ability to be performed using technology available in this country. It also has performed well when compared to Oncotype DX.\textsuperscript{19} Most of the other GEPs have progressed little beyond validation studies. One exception is MapQuant GGI, which has been assessed for its impact on treatment decisions in a level III-1 study.\textsuperscript{21}
<table>
<thead>
<tr>
<th>Relevant population</th>
<th>Intended clinical utility</th>
<th>Test name (Vendor)</th>
<th>Genes</th>
<th>Biological material Technology</th>
<th>Prognostic or predictive results available? From SRs?</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ve, N-ve</td>
<td>Estimates the recurrence risk (risk index score) following surgery and initial treatment</td>
<td>Insight Dx Mammostrat* (Clarien Diagnostic Services)</td>
<td>SLC7A5; HTF9C/TRMT2A; TP53; NDRG1; CEACAM5/CD66e</td>
<td>Tumour tissue (FFPE) IHC</td>
<td>Analytic validity: NA Clinical validity: significant predictor of distant PFS and OS; validated in patients samples from the NSABP B-14 and B-20 trials; Clinical utility: NA</td>
</tr>
<tr>
<td>ER+ve, N-ve</td>
<td>Improves histologic grade classification (high-grade vs. low-grade tumours).</td>
<td>MapQuant Dx Genomic grade index (GGI)* (Ipsogen)</td>
<td>Multiple 97 genes</td>
<td>Tumour tissue (fresh or flash-frozen) Microarray</td>
<td>Analytic validity: NA Clinical validity: prognosis validated in public databases, and in 650 patients either untreated or treated with tamoxifen. High GGI reported to be associated with increased sensitivity to specific chemotherapies. Predictor of worse survival in ER+ve patients; Clinical utility: NA</td>
</tr>
<tr>
<td>ER+ve, N-ve</td>
<td>Prognostic information associated with risk of distant metastasis (metastasis score). Helps identify higher-risk patients who might benefit from additional therapy</td>
<td>BreastOncPx* (US Labs)</td>
<td>Multiple 14 genes (not including ER or HER2)</td>
<td>Tumour tissue (FFPE) RT-PCR</td>
<td>Analytic validity: NA Clinical validity: found to be associated with DMFS and OS in 279 untreated patients after adjustment for age, tumour size and grade; Clinical utility: NA</td>
</tr>
<tr>
<td>ER+ve, N-ve</td>
<td>Predicts distant recurrence</td>
<td>EndoPredict* (Svidon Diagnostics)</td>
<td>Multiple 11 genes (8 cancer-related, 3 controls)</td>
<td>Tumour tissue (FFPE) RT-PCR</td>
<td>Analytic validity: intra-laboratory testing showed high reproducibility in one study; Clinical validity: A validation study of 1,702 samples from the ABCSG-6 and 8 trials were reported to have significantly different DRFS rates between the high and low risk groups. The EPclin score was prognostic for excellent survival after 5 years of ET in a subgroup of patients; Clinical utility: NA</td>
</tr>
<tr>
<td>Relevant population</td>
<td>Intended clinical utility</td>
<td>Test name (Vendor)</td>
<td>Genes</td>
<td>Biological material Technology</td>
<td>Prognostic or predictive results available? From SRs?</td>
</tr>
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</tbody>
</table>
| ER+ve, N-ve         | Evaluates likelihood of distant early and late recurrence  
Risk of overall recurrence (0-10 years)  
Benefit from extended (>5 years) endocrine therapy | THEROS BCI*  
(H/I and MGI)  
(Aviara Diagnostics/ Biotheranostics) | HOXB13; IL17BR | Tumour tissue (FFPE) RT- PCR | Analytic validity: NA  
Clinical validity: Prognosis in 852 untreated or tamoxifen treated patients for early and late recurrence risk; association in 264 randomised postmenopausal and 93 untreated premenopausal patients of high H/I ratio with aggressive tumour characteristics; in 1252 tumour samples H/I was significantly associated with PFS and OS but not in a cohort of 58 patients with resectable tumours following tamoxifen treatment.  
Clinical utility: NA |
| ER+ve, N+/ve        | Stratifies patients with breast cancer into low or high risk of recurrence  
Eliminates intermediate grade (reclassifies grade 2 tumours into grade 1-like and grade 3-like tumors) | MGI*  
(also used in conjunction with THEROS H/I ratio – see THEROS BCI)  
(Biotheranostics) | BUB1B; CNEPA; RACGAP1; RRM2; NEK2 | Tumour tissue (FFPE) RT-PCR | Analytic validity: NA  
Clinical validity: NA  
Clinical utility: NA |
| ER+ve, N+/ve        | Evaluates distant recurrence-free survival at 10 years when used in conjunction with other clinicopathologic factors  
Provides a risk category (low, intermediate, high)  
Aimed at post-menopausal women to be treated with adjuvant endocrine therapy alone | Prosigna breast cancer prognostic gene signature assay*  
(NanoString Technologies) | Multiple 50 genes (based on the PAM50 microarray) | Tumour tissue (FFPE)  
Gene expression profiling by proprietary nCounter Dx analysis system | Analytic validity: NA  
Clinical validity: samples form a prospective randomised trial of tamoxifen versus placebo in premenopausal patients showed the microarray to be prognostic for DFS and OS for classified subtypes and luminal subtype was predictive of tamoxifen benefit.  
Clinical utility: NA |
| ER+ve, N+/ve        | Proprietary Coperna algorithm calculates the prognostic index | eXagenBC*  
(eXagen Diagnostics) | CYP24; PDCD61P; BIRC5 | Tumour tissue (FFPE) FISH | Analytic validity: NA  
Clinical validity: NA  
Clinical utility: NA |
**Relevant population** | **Intended clinical utility** | **Test name** (Vendor) | **Genes** | **Biological material Technology** | **Prognostic or predictive results available? From SRs?**
---|---|---|---|---|---
**ER+/-, N-ve** | Assist in assessing a patient’s risk of systemic recurrence of cancer following successful initial treatment with surgery and tamoxifen alone to identify patients at risk of distant metastases within 5 and 10 years of first diagnosis | Rotterdam signature* (Veridex) | Multiple 76 genes (no genes in common with Oncotype DX or MammaPrint) | Tumour tissue (fresh or frozen) Microarray | Analytic validity: NA
Clinical validity: validated for low risk and high risk DFS in N-patients regardless of age, tumour size, grade or ER status19
Clinical utility: NA

**ER+/-, N-ve** | Prognostic test | BreastPRS* (ChipDX/Signal Genetics) | Multiple (validation done on a 200-gene data set) | Tumour tissue (FFPE or fresh) Microarray | Analytic validity: NA
Clinical validity: validated on a 200-gene data set; a significant difference in 10 year RFS was reported when 59 patients of intermediate risk according to Oncotype DX were reclassified into high and low-risk groups27
Clinical utility: NA

ABCSG, Austrian Breast and Colorectal Study Group; DFS, disease free survival; DMFS, distant metastasis free survival; DRFS, disease recurrence-free survival; EPclin, EndoPredict score combined with tumour size and nodal status in a linear model; ER, estrogen receptor; ET, endocrine therapy; FFPE, formalin-fixed paraffin embedded; FISH, fluorescent in-situ hybridisation; GGI, genomic grade index; IHC, immunohistochemistry; NA, not available; NSABP, National Surgical Adjuvant Breast and Bowel Project; OS, overall survival; PFS, progression-free survival; PR, progesterone receptor; RT-PCR, reverse transcription-polymerase chain reaction; IHC, immunohistochemistry; FISH, fluorescence in situ hybridisation; BCI, breast cancer index; NSCLC, non–small cell lung cancer; MGI, molecular grade index; DCIS, ductal carcinoma in situ.

*Insight Dx Mammostrat (Clarient Diagnostic Services, Aliso Viejo, CA); Insight Dx Mammostrat Plus (Clarient Diagnostic Services, Aliso Viejo, CA); MapQuant Dx Genomic Grade Index (Ipsogen, Marseilles, France and New Haven, CT); BreastOncPx (Integrated Oncology, Phoenix, AZ); EndoPredict (Sividon Diagnostics, Köln, Germany); THEROS Breast Cancer Index (BCI) (Biotheranostics, San Diego, CA); VeriStrat (Biodexis, Boulder, CO); Molecular Grade Index (MGI) (Biotheranostics, San Diego, CA); Prosiga Breast Cancer Prognostic Gene Signature Assay (NanoString Technologies, Seattle, WA); eXagenBC (eXagen Diagnostics, Albuquerque, NM); MammaPrint/Amsterdam Signature (Agendia, Amsterdam, The Netherlands and Irvine, CA); TargetPrint (Agendia, Amsterdam, The Netherlands and Irvine, CA); BluePrint (Agendia, Amsterdam, The Netherlands and Irvine, CA); Rotterdam signature (Veridex, Raritan, NJ); BreastPRS (Signal Genetics, Carlsbad, CA);
Tests considered in the original Brief

MapQuant Dx genomic grade index

The MapQuant GGI was mentioned in a number of reviews identified and published after 2012. According to one source it is the first clinically validated molecular test to measure tumour grade as an indicator of tumour proliferation, risk of metastasis and chemotherapy response, and as such has clinical potential.\(^\text{19}\) The GGI is applicable to all cancer types. The test is based on a 97 gene microarray which was constructed from genes identified when the expression levels of grade 3 and grade 1 tumours were compared. The GGI has also been adapted as a RT-PCR test of 6-genes which can be conducted on FFPE tissue. The test is used to reclassify histologically intermediate grade (HG2) tumours into high or low molecular grade.\(^\text{25, 26, 28}\)

In a prospective study by Metzger-Filho et al (2013) (level III-2 interventional evidence) assessment of the impact on treatment decisions based on GGI was performed.\(^\text{21}\) Classification of tumours using GGI was compared to classification based on clinicopathologic characteristics, following which its subsequent impact on treatment decisions was assessed. GGI was found to reclassify 69 per cent of 54 HG2 tumours as GG1 (54%) or GG3 (46%). Amongst patients reclassified as GG1, the proportion receiving hormone therapy alone increased and those receiving hormone and chemotherapy decreased, whilst among those classified as GG3, the changes were reversed. Clinicopathologic characteristics were judged independently of the GGI and treating oncologists were given the opportunity to change their treatment decision once the GGI classification was known. The authors provided further insight into the impact of reclassification by GGI, finding that in 22.5 per cent of 89 early breast cancer cases, treating oncologists changed their treatment recommendations from hormone plus chemotherapy to hormone therapy alone, but when patients’ preferences were considered this proportion was reduced to in 10.1 per cent.\(^\text{21}\)

THEROS BCI

BCI is a prognostic tool which uses incorporates two independent biomolecular markers. The first of these consists of a qRT-PCR profile of 5 genes (the Molecular Grade Index or MGI) and the second reports a ratio of the expression rate of the two genes HOXB13 and IL7BR (H/I) to classify patients into recurrence risk groups. The BCI was validated in a level III-2 (prognostic evidence) study which included two cohorts of ER+ve, N-ve patients treated with adjuvant tamoxifen.\(^\text{29}\) The prognostic performance of an optimised model of the BCI algorithm was assessed retrospectively in tamoxifen treated patients from the Stockholm study\(^\text{d}\), and a multi-institutional cohort.\(^\text{30}\) In the Stockholm cohort the 5-year distant recurrence-free survival in the low-risk, intermediate-risk and high-risk groups were 98% (95% CI 96% - 100%), 95.2% (95% CI 90.1% - 100%), and 87.8% (95% CI 79.0% - 97.4%),

\(^\text{d}\) The Stockholm Breast Cancer Study Group conducted a randomised trial from 1976 to 1990 in which 2738 postmenopausal patients with invasive early stage breast cancer were randomised to tamoxifen for 3 or 5 years or no adjuvant endocrine therapy\(^\text{10}\)
respectively. In a Kaplan-Meier univariate analysis there was a significantly lower risk of distant recurrence in the low risk group compared with other groups: the hazard ratio (HR) was 2.31 (95% CI 0.52-10.3) for intermediate versus low-risk and 6.19 (95% CI 1.75 - 21.92) for high versus low-risk. However little difference was found between the intermediate and high risk groups. In a multivariate Cox regression BCI was found to outperform clinicopathological factors for 0 to 5 years and more than 5 years in both cohorts. In ER+ve, HER2-ve patients in the Stockholm cohort (n=295) BCI was the only significant predictor with a HR of 16.25 (95% CI 2.59-102.0; p= 0.003), and in the multi-institutional cohort (n=281) the HR was 13.71 (95% CI 4.54-41.36). The authors concluded that BCI has prognostic sustainability for early and late-distant recurrence risk and potential clinical use for decisions of chemotherapy at diagnosis for ER+ve, N-ve patients.29

*InsightDx Mammostrat and InsightDx Mammostrat Plus*

The InsightDx Mammostrat® (Clarient Inc, California, USA) test uses five independent immunohistochemical markers (SLC7A5, HTF9C, P53, NDRG1, and CEACAM5) that do not measure proliferation or hormone receptor status. However, initial validation studies with large sample numbers found Mammostrat to be an independent prognostic tool for women with ER+ve, tamoxifen treated breast cancer.8 The studies were all retrospective (level III-3), based on two large cohorts in the UK (n=1,540) (2010) and the US (n=1,109) (2006) and a third cohort taken from the NSABP B14 and B20 trials which was not explicit on how the data was combined (n=1267) (2008). The more recent Mammostrat Plus test includes an assessment of ER, PR, MKI67 and HER2 genes, however studies assessing this extended test were not identified. In a meta-analysis by Issa, Chaudhari and Marchant (2015) an average adjusted multivariate Cox hazard ratio (HR) of 1.605 (95% CI 1.300, 2.015) for 10-year relapse free survival was reported for Mammostrat. This outcome was averaged from two of the validation studies published in 2008 and 2012.32

*BreastOncPx*

This 14-gene signature assay using RT-PCR provides prognostic information for N-ve, ER+ve breast cancer patients and is associated with risk of distant metastasis. It can be used to identify higher-risk patients who might benefit from additional therapy.33 No results published after the NICE 2012 review were identified.

*Rotterdam signature (veridex-76)*

The Rotterdam Signature assay uses 60 genes to evaluate ER+ve samples and 16 genes to evaluate ER-ve samples. The assay is intended to predict the risk of 5-year breast cancer recurrence in lymph node-negative patients. Testing for RNA expression is performed on a fresh frozen tumour sample and results are expressed as a hazard ratio for distant recurrence.31

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6 The National Surgical Adjuvant Breast and Bowel Project (NSABP), based in Pittsburgh, Pennsylvania has conducted several large, randomised clinical trials evaluating surgical and adjuvant therapies in patients with operable breast cancer (including B14 and B20) 31. NSABP NSABP. Available from: http://www.nsabp.pitt.edu/ [Accessed 8/02/2016].
recurrence. In a meta-analysis of published Cox hazard ratios, Issa, Chaudhari and Marchant (2015) compared the likelihood of 10-year relapse free survival amongst GEPs. They reported an adjusted multivariate Cox HR for disease free survival of 5.407 (95% CI 1.771, 18.502) for the Rotterdam Signature. This result was averaged from six studies (level III-3) published between 2005 and 2012, with average patient enrolment of 424. The authors also reported the Rotterdam Index HR to have the widest confidence intervals, and therefore the least stability when compared to HRs for Oncotype DX, Mammostrat and Mamprint.

**New tests identified in the Update**

**Prosigna breast cancer prognostic gene signature assay**

Apart from OncoType DX and MammaPrint, Prosigna was the most frequently discussed BC profiling tool in the literature identified. Prosigna uses nanoString technology to produce the 50-gene profile which is based on the original in the PAM50 algorithm. The test produces a Risk of Recurrence score (ROR) which is based on the identification and profiling of the four intrinsic subtypes of breast cancer, Luminal A, Luminal B, HER2-enriched, and Basal-like. It is performed using a diagnostic kit which quantifies mRNA expression and can be performed in local laboratories provided they have the nanoString nCounter Dx technology.

Prosigna was approved by the FDA in 2013 as a prognostic test (but not as a tool for selecting therapy or predicting response to therapy), and has also received clearance in the European Union. This test has advanced further than most in its diffusion as it was approved by the FDA in 2013 for use in the US, and is now being offered in Australia through Sonic Genetics.

Prosigna was validated retrospectively (level III-3) using samples of 1,478 N+ve and N-ve patients from the ABCSG-8 trial where the test was found to provide significant prognostic information over and above clinicopathological features, for 10-year distant recurrence. Following this validation Prosigna was assessed in a further 2,485 patients from the ABCSG-8 trial and the translational arm of the ATAC (arimidex, tamoxifen alone or in combination) trial (level III-2). According to one review author, there were inconsistencies for higher risk recurrence associated with high and intermediate Prosigna scores between the two cohorts. It should be noted that the ATAC study used the remaining RNA from an Oncotype DX assay, which involved microdissection and RNA extraction, for testing with Prosigna. As a result, the integrity of the samples has been questioned.

A retrospective comparison (level III-2) of PAM50 ROR and Oncotype DX RS was performed on 1,017 FFPE samples from the ATAC trial (post-menopausal hormone receptor +ve primary breast cancer patients from the tamoxifen or anastrozole-only arms). A trial-

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1 The Austrian breast and Colorectal Cancer Study Group-8 (ABCSG-8) trial is a Phase III clinical trial comparing anastrozole with tamoxifen treatment in low-intermediate risk postmenopausal patients (source: [http://jco.ascopubs.org/content/30/7/722.full](http://jco.ascopubs.org/content/30/7/722.full))
defined endpoint was time from randomisation to first disease recurrence (DR). Using a likelihood ratio (LR) analysis, the ROR score added prognostic information for DR beyond clinical treatment score in all patients ($\Delta LR-\chi^2 = 33.9; p < 0.001$). More prognostic information was added by ROR than the Oncotype DX RS score and more patients were scored as high risk and fewer as intermediate risk by ROR than Oncotype DX. ROR provided similar information to IHC4 when the two were compared in all patients, but the ROR provided more in the HER2-ve, N-ve patient group.22

**EndoPredict**

Endopredict is an 8-gene expression profile which uses qRT-PCR on FFPE samples. It identifies HER2-ve, ER+ve patients who will benefit from adjuvant endocrine therapy alone rather than combined endocrine and chemotherapy. In addition to the 8-gene profile, the test algorithm includes three control genes and clinical factors of tumour size and the number of lymph nodes affected. EndoPredict uses the EP score (EP score assesses distant recurrence risk using the 8-gene profile only) or the EPclin score (the EPclin score algorithm incorporates clinical factors and eight-gene profile) to classify early breast cancer patients as high or low-risk for distant recurrence, using a continuous scale.

The EP score and EPclin score were validated in a retrospective study (level III-3) of 1,702 samples from the ABCSG-6 and ABCSG-8 trials.6,36 At 10 years the distant recurrence rates for patients with EP low and EP high rating were 8% (3%-13%) and 22% (15%-29%) in ABCSG-6 and 6% (2%-9%) and 15% (11%-20%) in ABCSG-8 respectively. Distant recurrence rates for patients with EPclin low and EPclin high were 4% (1%-8%) and 28% (20%-36%) in ABCSG-6 and 4% (2%-5%) and 22% (15%-29%) in ABCSG-8. A Kaplan-Meier analysis was used.

In an additional publication a comparison of EPclin score risk stratification was made against risk assessment using clinical guidelines (German s3, National Comprehensive Cancer Network (NCCN) and St Gallen guidelines), to test if classification by EndoPredict was corresponding to actual distant recurrence-free survival of patients.37 The same ABCSG-6 and ABCSG-8 trial samples were used. EPclinic reassigned 58-61 per cent of women classified as high or intermediate-risk according to clinical guidelines, to low-risk. Low-risk patients reclassified by EndoPredict showed a five per cent rate of distant metastasis at 10 years. The authors claim that if EndoPredict is performed on patients assigned a risk of intermediate or high risk by clinical guidelines, it may be able to reduce indications for chemotherapy in ER+ve postmenopausal women with limited clinical risk factors. The analysis was restricted in power by the small number of samples included from clinically high-risk patients, particularly those with Grade 3 tumours.37

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6 FFPE samples from two randomised trials were used to validate EndoPredict. Samples included from the Austrian Breast and Colorectal Study Group–6 (ABCSG-6) trial were from breast cancer patients randomised to the tamoxifen-only arm. Samples included from the ABCSG-8 trial were from patients who received tamoxifen for either 5 years or 2 years followed by anastrozole for 3 years.
The IMPAKT (Improving Care and Knowledge through Translational Research) 2012 Working Group, a European collaboration, published a consensus statement commenting that EndoPredict was the only GEP to report analytic validity results for intra-laboratory testing. EndoPredict was found to have high reproducibility with a success rate of >95 per cent. The IMPAKT Statement claimed to present an independent evaluation of six genomic tests based on EGAPP criteria, however there was a disclosure from one author who had received honoraria from Sividon Diagnostics GmgH, the manufacturer of EndoPredict.  

**MGI**

The Molecular Grade Index (MGI) consists of a 5 gene qRT-PCR profile which measures the expression level of five cell-cycle related genes. The gene expression can indicate the level of tumour cell proliferation and tumour grade. The GEP provides a risk stratification of low or high and the intermediate risk category is removed, as MGI reclassifies grade 2 tumours into grade 1-like or grade 3-like tumours. The MGI can be used independently and is also used in conjunction with the H/I ratio to give the Breast Cancer Index (see THEROS BCI). No studies relating to the independent evaluation of MGI were identified.

**BreastPRS**

BreastPRS is a 200 gene expression signature which was originally generated using fresh frozen tissue, and validated in publically available data from breast cancer patients where it was found to be associated with recurrence-free survival. More recently it has been translated to be used with FFPE tissue. The assay uses the Affymetrix U133 GeneChip hybridisation system, requiring RNA isolated from FFPE samples. BreastPRS classifies patients as low- or high-risk. The test was compared with an Oncotype DX approximation algorithm trained to predict Oncotype DX risk groups in untreated ER+ve, N-ve patients and showed statistically significant correlation between recurrence score and risk group (p<0.0001) (level III-2). In further work Breast PRS has been used to reclassify patients classified as intermediate risk by OncoType DX into high and low-risk groups. At 10 years after diagnosis the high and low-risk groups showed recurrence-free survival rates of 60 per cent and 90 per cent, respectively.

**eXagenBC**

This test assesses three genes using the FISH technique on FFPE tissue. No further information was identified.

**Tests available which can be performed with MammaPrint**

**TargetPrint and BluePrint**

TargetPrint and BluePrint are two recent microarray GEPs developed by Agendia which can be performed alone or in conjunction with MammaPrint. TargetPrint performs a quantitative assessment of ER, PR and HER2 status and BluePrint classifies the tumour into molecular subtypes (basal-type, luminal-type and ERBB2-type/HER2) using an 80-gene
microarray profile. The tests can be performed on FFPE or fresh tumour tissue and are suitable for N-ve, ER+ve or -ve tumours. In a recent concordance study (level III-3) MammaPrint was found to have only 68 per cent concordance with Ki67 for stratification of luminal A and B subgroups. TargetPrint was found to have high concordance with IHC/FISH for stratification of ER, PR and HER2 subgroups (97%, 80% and 95% respectively) and was thought to have the potential to add prognostic information in settings where pathology standards are insufficient.

**Current guidelines**

Recently, four international breast cancer guidelines published updates. Current recommendations from the listed publications for early HER2-ve breast cancer are summarised in Table 3. The guidelines agree that the determination from core biopsy of HER, ER and PR status in addition to tumour grade and lymph node involvement are critical in the diagnoses of early breast cancer.

The National Comprehensive Cancer Network (NCCN) guideline recommends treatments based on the RS using the 21 gene RT-PCR assay (Oncotype DX) for invasive N-ve breast cancer tumours >0.5 cm in size. The European Society for Medical Oncology (ESMO) guideline lists four GEPs which may be used at the clinicians’ discretion, in cases of uncertainty for indications of adjuvant treatment with chemotherapy, after consideration of other tests. Both guidelines make the recommendations on the basis of lower level evidence, but with strong consensus for its benefit.

The St Gallen guideline is a consensus based document informed by expert panel input at the 14th St Gallen International Breast Cancer conference. The guideline acknowledges current available evidence for GEPs Oncotype DX, PAM50 and EndoPredict in specific patients groups, and their value in assistance with making treatment decisions. Subgrouping and treatment according to prognosis is recommended for low, intermediate and high risk hormone receptor +ve & HER2 –ve luminal disease as assessed by a multi-parameter molecular marker and clinical markers.

The American Society for Clinical Oncology guidelines are based on evidence review and considered many breast tumour biomarker assays, and like the other guidelines, found sufficient evidence of clinical utility for Oncotype DX, EndoPredict, PAM50, and Breast Cancer Index certain subgroups, but did not recommend Mammaprint for any group.

In 2014, the NHMRC Clinical Practice Guidelines for the Management of Early Breast Cancer (2011) were rescinded, and at the time of writing there was no published update.
Table 3  Summary of current relevant guideline recommendations for risk assessment using gene expression profiles and treatment for early breast cancer

<table>
<thead>
<tr>
<th>Guideline</th>
<th>Population</th>
<th>Recommendation/Statement</th>
<th>Level of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015</td>
<td>Early BC</td>
<td>Prognostic value of multi-parameter molecular markers: Oncotype DX was predictive of late distant recurrence in NSABP B-14 but not predictive of late distant recurrence after ET in the ATAC study. PAM-50 ROR score and the IHC4 test each remained prognostically significant beyond 5 years of endocrine treatment.</td>
<td>Recent research findings presented at the 14th International Conference on Primary Therapy of Early Breast Cancer and their implications for patient care</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EndoPredict was prognostically significant beyond 5 years in ABCSG trials 6 and 8, particularly when combined with clinical factors. Breast Cancer Index was prognostic for early and late distant recurrence in two series.</td>
<td>As above</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sub-grouping and treatment according to prognosis:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multi-parameter molecular marker ‘favourable prognosis’ if available. High ER/PR and clearly low Ki-67. Low or absent nodal involvement (n0-3), smaller T size (T1 T2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multi-parameter molecular marker ‘intermediate’ if available. Uncertainty persists about degree of risk and responsiveness to endocrine and cytotoxic therapies.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multi-parameter molecular marker ‘unfavourable prognosis’ if available. Lower ER/PR with clearly high Ki-67. More extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, larger T size (T3).</td>
<td></td>
</tr>
<tr>
<td>NCCN 2015 Clinical Practice Guidelines in Oncology National Comprehensive Cancer Network. Breast Cancer</td>
<td>Invasive BC; pT1, pT2, pT3; and pNO or pN1mi (≤2mm axillary mode metastasis) tumour &gt;0.5cm</td>
<td>consider 21-gene RT-PCR assay not done: adjuvant ET ± CT low RS: adjuvant ET intermediate RS: adjuvant ET ± CT high RS: adjuvant ET ± CT</td>
<td>Category 1 Category 2A Category 2A Category 2A</td>
</tr>
<tr>
<td>ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, 2015</td>
<td>Early BC (general)</td>
<td>In cases of uncertainty regarding indications for adjuvant CT (after consideration of other tests), gene expression assays, such as MammaPrint, Oncotype DX, Prosigna and EndoPredict, may be used, where available. These assays can determine the individual’s recurrence risk as well as potentially predict the benefit of CT.</td>
<td>IV, A</td>
</tr>
<tr>
<td>Guideline</td>
<td>Population</td>
<td>Recommendation/Statement</td>
<td>Level of evidence</td>
</tr>
<tr>
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</tr>
<tr>
<td>ASCO Clinical Practice Guideline on Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women with Early-Stage Invasive Breast Cancer, 2016</td>
<td>Early invasive BC with known ER/PgR and HER2 status under consideration for adjuvant systemic therapy</td>
<td>ER/PgR+, HER2-, node-negative BC: Recommended: Oncotype DX, EndoPredict, PAM50, BCI</td>
<td>Evidence quality; strength of recommendation: high, strong intermediate, moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not recommended: Mammaprint</td>
<td>mostly insufficient evidence</td>
</tr>
<tr>
<td></td>
<td>ER/PgR+, HER2-, node-positive BC</td>
<td>No tests recommended</td>
<td></td>
</tr>
</tbody>
</table>

ABCSG, Austrian breast and colorectal study group; ATAC, arimidex, tamoxifen alone or in combination trial; BC, breast cancer; CT, chemotherapy; ER, oestrogen receptor; ESMO, European Society for Medical Oncology; ET, endocrine therapy; Ki67, cellular marker for proliferation; NR, not reported; PR, progesterone receptor; RS, recurrence score; RT-PCR, reverse transcriptase polymerase chain reaction; NCCN, National Comprehensive Cancer Network; NSABP B-14, National Surgical Adjuvant Breast and Bowel Project trial B-14; ASCO, American Society for Clinical Oncology

⁎NCCN categories of evidence and consensus: Category 1 – based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate; Category 2A - based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate; Category 2B - based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate; Category 3 - based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate

⁎ESMO levels of evidence: I – evidence from at least one large, randomised controlled trial of good methodological, quality (low for potential bias) or meta-analysis of well-conducted, randomised trials without heterogeneity; II – small, randomised trials of large, randomised trials with a suspicion of bias (lower methodological quality) or meta-analysis or such trials or trials with demonstrated heterogeneity; III – prospective cohort studies; IV – retrospective cohort studies or case-control studies; V – studies without the control group, case reports, experts opinions.

ESMO Grades of recommendation: A – Strong evidence for efficacy with a substantial clinical benefit, strongly recommended; B – Strong or moderate evidence for efficacy but with a limited clinical benefit, generally recommended; C – insufficient evidence for efficacy or benefit does not outweigh the risks or disadvantages (adverse events, costs), optional; D – moderate evidence against the efficacy or for adverse outcomes, generally not recommended; E – Strong evidence against the efficacy or for adverse outcomes, never recommended.

⁎⁎ESMO Levels of evidence and Grades of recommendations are adapted from the Infectious Diseases Society of America-United States Public Health Service Grading System

ASCO levels of strength of evidence: high – high confidence that available evidence reflects true magnitude and direct of net effect; intermediate – moderate confidence that available evidence reflects true magnitude and direct of net effect; insufficient – evidence is insufficient to discern true magnitude and direction of net effect.

ASCO levels of strength of recommendation: strong – high confidence that recommendation reflects best practice; moderate – moderate confidence that recommendation reflects best practice.
2016 Economic evaluation

Systematic reviews of cost-effectiveness analyses of GEPs published after the study by Ward et al (2013) were not identified. The majority of cost-effectiveness studies identified for individual GEPs for early breast cancer reported on the Oncotype DX and MammaPrint assays, however studies were also identified for EndoPredict and BCI. Results for EndoPredict will not be discussed here as the technology is the subject of a submission to MSAC.

In the US based study regarding BCI, Gustaven et al reported on two economic models which were developed to project cost and effectiveness in a hypothetical population of ER+ve, N-ve breast cancer patients. The first model evaluated BCI conducted at diagnosis and included its impact on the decision for adjuvant chemotherapy and for extended endocrine therapy. In the second model BCI was conducted at five years post-diagnosis for patients who were recurrence free at that time point, and included the impact on decision making for duration of endocrine therapy. A comparator of standard management was used, which was based on interviews with disease-state experts. Key assumptions for the models included implementation of extended endocrine therapy for patients with high BCI (H/I), and patients with BCI (H/I) would stop endocrine therapy after 5 years (based on published outcomes). Modelling for disease-free survival in years 5 to 10 and utilisation of extended endocrine therapy was similarly based on published data for BCI.

For the first model, in the base case scenario without the use of BCI, 10 years of follow-up treatment had an average cost of $45,437 (US) per patient, compared to a cost of $41,634 (US) per patient when BCI was used at diagnosis, resulting in a net saving of $3,803 (US) per patient tested. Cost benefits were found to be driven by targeted use of adjuvant chemotherapy, targeted and extended use of endocrine therapy, and increased patient compliance. In the second model, the base case scenario costs per patient were $22,708 (US), and with the use of BCI at 5 years post diagnosis were $20,904 (US) per patient. The savings in model two amounted to $1,803 (US) per patient and was driven mainly by the targeted use of extended endocrine therapy.

A cost-effectiveness analysis in an Australian setting is likely to be provided should one of the GEPs which currently have submitted applications to MSAC, be approved for full assessment.

2016 Ongoing research

The websites ClinicalTrials.gov and Australian New Zealand Clinical Trials Registry (ANZCTR) were searched for registered trials related to genetic profiling tools for breast cancer. No relevant trials were identified through the ANZCTR. A number of trials were registered with ClinicalTrials.gov that relate to GEPs under current investigation and listed in the previous sections of this report. The trials, including those that are currently
recruiting participants, ongoing or recently completed are briefly described in the following table. Note that registered trials for BreastOncPx, eXagenBC, BreastPRS and Rotterdam Signature were not identified, and trials related to EndoPredict, Oncotype DX and MammaPrint were not included here. It should be noted that a prospective study using Oncotype DX (the Trial Assigning Individualised Options for Treatment, or TAILORx study) has begun reporting, and published results on the group of women in the study with the lowest risk based on the Oncotype DX recurrence score. These early results from this subgroup of women (n=1,626) showed that 5-year recurrence rates were very low without chemotherapy treatment. Results on rest of the women with higher recurrence scores (n=8,627) are not yet available, but will be very important in terms of the adoption into practice and possible reimbursement of the test.

Table 4: summary of ongoing clinical trials of selected GEPs identified on ClinicalTrials.gov

<table>
<thead>
<tr>
<th>Trial Status</th>
<th>Clinicaltrials.gov identifier and name</th>
<th>Outcome/expected primary or relevant outcome</th>
<th>Completion date (estimated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed</td>
<td>NCT01899079: A Prospective Observational Study of Clinical Outcomes for the NanoString® Technologies Prosigna Gene Signature Assay</td>
<td>The proportion of patients whose choice of treatment is changed as a result of receiving the Prosigna test result</td>
<td>January 2014</td>
</tr>
<tr>
<td>Completed</td>
<td>NCT01974856: A Prospective Observational Study of Clinical Outcomes for the NanoString® Technologies Prosigna™ Gene Signature Assay</td>
<td>The proportion of patients whose choice of treatment is changed as a result of receiving the Prosigna test result</td>
<td>October 2014</td>
</tr>
<tr>
<td>Active, not recruiting</td>
<td>NCT02625935: Prospective Observational Study Evaluating Treatment Decision Impact of Prosigna® in Early Stage Breast Cancer Patients</td>
<td>The proportion of patients for whom the choice of treatment was changed as a result of receiving the Prosigna test results</td>
<td>August 2016</td>
</tr>
<tr>
<td>Active, not recruiting</td>
<td>NCT00991263: Study of Tissue Samples From Women Treated With Paclitaxel for Breast Cancer on Clinical Trial CALGB-9344 or CALGB-9741 (Prosigna)</td>
<td>Disease-free survival (up to 10 years) To determine the relationship between PAM50-defined ROR score and DFS in CALGB-9344 and CALGB-9741 To evaluate the relationship between PAM50-defined ROR score and DFS in the HER2-negative subsets in CALGB-9344 and CALGB-9741 To examine the relationship between PAM50-defined proliferation score and DFS in CALGB-9344 and CALGB-9741 in multivariate Cox-proportional hazards models</td>
<td>May 2010</td>
</tr>
<tr>
<td>Recruiting</td>
<td>NCT02213042: Evaluation of Biomarkers Associated With Response to Subsequent Therapies in Subjects With HER2-Positive Metastatic Breast Cancer (open-label, Phase II study) Prosigna</td>
<td>Evaluate changes in biomarkers between pre-treatment biopsy and disease progression biopsy</td>
<td>August 2018</td>
</tr>
<tr>
<td>Recruiting</td>
<td>NCT02395575: A Study of Clinical Outcomes for the NanoString® Technologies Prosigna™ Gene Signature Assay (prospective)</td>
<td>The proportion of patients whose choice of treatment is changed as a result of receiving the Prosigna test results</td>
<td>December 2015</td>
</tr>
<tr>
<td>Recruiting</td>
<td>NCT01373660: PAM50 HER2-enriched</td>
<td>Pathological complete response to dual HER2 blockade</td>
<td>June 2016</td>
</tr>
<tr>
<td>Study ID</td>
<td>Title</td>
<td>Description</td>
<td>Status</td>
</tr>
<tr>
<td>---------</td>
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<td>--------</td>
</tr>
<tr>
<td>NCT02400567</td>
<td>Efficacy of Letrozole + Palbociclib Combination as Neoadjuvant Treatment of Stage II-IIIA HER2+ Early Breast Cancer</td>
<td>Evaluation of the number of patients with a RCB 0-I index as a measure of efficacy</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02445391</td>
<td>Platinum Based Chemotherapy or Observation in Treating Patients With Residual Triple-Negative Basal-Like Breast Cancer Following Neoadjuvant Chemotherapy (randomized phase III trial)</td>
<td>Rate of basal-like gene expression using PAM50 analysis by digital mRNA quantification</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01916837</td>
<td>Genomic Grade Index (GGI): Feasibility in Routine Practice and Impact on Treatment Decisions in Early Breast Cancer (MapQuant/GGI)</td>
<td>The success rate in obtaining the Genomic Grade Index in clinical practice</td>
<td>Completed</td>
</tr>
<tr>
<td>NCT02600442</td>
<td>Assessment of Breast Cancer Response to Neoadjuvant Anthracycline-based Chemotherapy by FDG-PET and Molecular Markers</td>
<td>To determine 5 year event-free survival rates in breast cancer patients according to PET response, biological markers and biomarkers identified with molecular high throughput analysis (GGI).</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT01564056</td>
<td>Adjuvant Systemic Treatment for (ER)+HER2– Breast Carcinoma in Women Over 70 According to Genomic Grade (GG): Chemotherapy + Endocrine Treatment Versus Endocrine Treatment (MapQuant/GGI)</td>
<td>Overall survival (median follow-up 4 years)</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02057029</td>
<td>Assessment of the Decision-making Impact of the Breast Cancer Index in Recommending Extended Adjuvant Endocrine Therapy for Patients With Early Stage ER+ Breast Cancer (HEROS BCI)</td>
<td>Patient Endocrine Therapy Questionnaires - patient's perceived risk of recurrence, preference for continuing endocrine therapy after 5 years, concerns surrounding taking additional medication including concerns of cost, side effects, safety and benefit, as well as the level of comfort with the choice made both before and after the BCI test results are known</td>
<td>Active, not recruiting</td>
</tr>
</tbody>
</table>

CALGB 9344/CALGB C9741, Cancer and Leukaemia trials Group B trials; DFS, disease free survival; HER2, human epidermal growth factor receptor 2; GGI, genomic grade index; ROR, risk of recurrence; RCB, residual cancer burden; ER, oestrogen; BCI, breast cancer index; FDG-PET, fluorodeoxyglucose positron emission tomography
ClinicalTrials.gov reported the termination of trial NCT02067416, due to withdrawal of funds by the sponsor (last update on ClinicalTrials.gov was September 2015). According to the website the non-randomised prospective study was designed to determine whether Mammostrat can be used to predict those who will best benefit from neo adjuvant chemotherapy. No other trials are currently listed.

2016 Other issues

The majority of trials are funded by companies that own the GEP technology under investigation. As a result it is not always possible to rule out publication bias from these types of studies. Authorship declarations of conflict of interests help in the assessment of this type of bias. Authors of a number of the articles referenced in this report disclosed interests in companies funding the study. 21, 25-28, 37, 41

2016 Number of studies included

Evidence included for assessment in this Technology Brief has been assessed according to the revised NHMRC levels of evidence for prognostic tests or interventions where possible. Some evidence was included which is not typically permitted in the NHMRC hierarchy, and could not be assessed according to their guidelines. A document summarising these levels may be accessed via the HealthPACT web site.

Total number of studies providing evidence in this update: 20

Total number of Level I studies: 3

Total number of Level III-2 interventional evidence studies: 1

Total number of level III-2 prognostic evidence studies: 3

Total number of Level III-3 prognostic evidence studies: 5

Total number of narrative reviews without specific search/inclusion criteria: 4

Guidelines: 3

AHRQ report: 1

Search Criteria to be used (MeSH Terms)

Breast Neoplasms
Gene Expression Profiling
Gene Expression
Prognosis
Individualized Medicine
Microarray Analysis
Reverse Transcriptase Polymerase Chain Reaction
Neoplasm Recurrence, Local
Biological Assay
Female
Humans

References (2012 and 2015)


43. FDA (2008). 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY. [Internet]. Food and Drug Administration. Available from:
Gene expression profiling of breast cancer: Update April 2016

44. ECRI (2011). *Gene expression profiling to guide management of early-stage breast cancer*, ECRI Institute, Plymouth Meeting, Pennsylvania


Gene expression profiling of breast cancer: May 2012

2012 TECHNOLOGY BRIEF

REGISTER ID
WP039

NAME OF TECHNOLOGY
GENE EXPRESSION PROFILING OF BREAST CANCER

PURPOSE AND TARGET GROUP
FOR THE CLASSIFICATION, PROGNOSTICATION AND PREDICTION OF TREATMENT OUTCOME IN BREAST CANCER PATIENTS

STAGE OF DEVELOPMENT IN AUSTRALIA

☐ Yet to emerge
☐ Experimental
☐ Investigational
☒ Nearly established

☐ Established
☐ Established but changed indication or modification of technique
☐ Should be taken out of use

AUSTRALIAN THERAPEUTIC GOODS ADMINISTRATION APPROVAL

☐ Yes
☐ No
☒ Not applicable

INTERNATIONAL UTILISATION

<table>
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<tr>
<th>COUNTRY</th>
<th>LEVEL OF USE</th>
<th>Trials under way or completed</th>
<th>Limited use</th>
<th>Widely diffused</th>
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<td>Israel</td>
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2012 IMPACT SUMMARY

There are several products currently undergoing world-wide validation that may be used to determine a prognostic gene expression signature for breast cancer to enable identification of those lymph node-negative patients who would best respond to, and benefit from, chemotherapy:

- MammaPrint® microarray, produced by Agendia (Amsterdam, Netherlands);
- MapQuant™ Dx microarray with qRT-PCR in development, produced by Ipsogen (Marseille, France) and CE marked;

qRT-PCR = quantitative reverse transcription polymerase chain reaction
• Oncotype DX® qRT-PCR, produced by Genomic Health (California, USA);
• Breast Cancer Index\textsuperscript{SM}, also known as THEROS Breast Cancer Index\textsuperscript{SM}, qRT-PCR, produced by bio Theranostics (California, USA);
• BreastOncPx™, RT-PCR offered by US LABS (California, USA); and
• the Veridex 76-gene microarray, also known as the Rotterdam Signature 76-Gene Panel, which is not currently commercially available.

\textit{In vitro} diagnostic medical devices (IVDs), such as these tests, are currently not required to be registered on the Australian Register of Therapeutic Goods, however, under the TGA regulatory framework introduced in July 2010, IVDs will be required to be registered as of July 2014.

The only gene expression signature assay to gain FDA approval is the MammaPrint assay, however the Oncotype DX\textsuperscript{®}, the Breast Cancer Index and the BreastOncPx assays are all analysed in accredited reference laboratories that are certified by the Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP). The assays are classified by the FDA as \textit{in vitro} diagnostic multivariate index assays (IVDMIAs) and as such, are not required to be FDA approved, however the CLIA certification is overseen by FDA. The classification of these IVDMIAs has been under review by the FDA since 2008, with no date set for a final ruling. It should be noted that MammaPrint has FDA clearance as a prognostic assay and a Class II device and as such “is not intended for diagnosis, or to predict or detect response to therapy or help select the optimal therapy for patients”.\textsuperscript{43}

Gene expression profiling for breast cancer is an area of intense interest for clinicians, manufacturers and especially, patients. A general search of PubMed reveals over 6,000 papers published since 1997. In recent years, many overviews of current research have been published including the 2007 AHRQ\textsuperscript{9} technology assessment\textsuperscript{9}, the 2011 emerging technology evidence report by the ECRI Institute\textsuperscript{44}, the 2010 Tec report by BlueCross BlueShield (for oestrogen receptor positive (ER+) and lymph node-positive women (LN+))\textsuperscript{45} and the 2008 Tec report.\textsuperscript{46} In addition, several peer reviewed systematic reviews have been published.\textsuperscript{4, 5, 47-49}

The most recent overview of the evidence is the 2012 HTA conducted on behalf of the United Kingdom’s National Institute for Health and Clinical Excellence (NICE) “\textit{Gene expression profiling and expanded immunohistochemistry tests to guide the use of adjuvant chemotherapy in breast cancer management}”.\textsuperscript{14} This systematic review updated the evidence for Oncotype DX and MammaPrint by Marchionni et al (2008) in addition to identifying other potential products for gene profiling. It is

\textsuperscript{9} AHRQ = Agency for HealthCare, Research and Quality (USA)
anticipated that the NICE guidance, based on this report, will be finalised by August 2012.

Due to the extensive body of evidence and the number of gene profiling products currently on the market, this Brief will attempt to paraphrase the NICE systematic review to give an overview of the current evidence.

2012 BACKGROUND

A number of treatment options for breast cancer may be offered to patients including surgery, radiotherapy, hormonal therapy, chemotherapy, directed antibody therapy and targeted small molecule therapy. Current NHMRC clinical practice guidelines for the treatment of early breast cancer\(^\text{10}\) state that:

- up to the age of 70 years, multi-agent chemotherapy reduces the risk of recurrence and death for women with breast cancer;
- moderately prolonged (several months) combined chemotherapy is recommended as it is more effective than single agent therapy and treatment lasting less than one month;
- Anthracycline-containing regimes are superior to cyclophosphamide, methotrexate and 5-fluorouracil (CMF) for both recurrence-free survival and overall survival at the increased risk of alopecia, cardiac toxicity and febrile neutropenia;
- Dose intensity is important to outcome in adjuvant cytotoxic therapy, at least in dose ranges achievable without colony stimulating factor (CSF) support;
- Treatment with high-dose chemotherapy outside of clinical trials is not recommended;
- Women should be fully informed of the short- and long-term effects of cytotoxic chemotherapy on general functioning and on body image, sexuality and fertility;
- Tamoxifen is recommended for most women with ER+ tumours, as it significantly improves recurrence-free and overall survival in women of all age groups\(^\text{11}\);
- Tamoxifen reduces the incidence of contralateral breast cancer;
- Women should be informed of the potential side effects of tamoxifen, including endometrial cancer, stroke, pulmonary embolism, deep vein thrombosis, hot flushes and vaginal dryness and discharge, but not excess weight gain. For most women, the protective effect of tamoxifen against the recurrence of breast cancer will vastly outweigh the increased risk of side effects;

\(^{10}\) Early breast cancer is defined by the NHMRC as tumours ≤ 5cm, either impalpable or palpable but not fixed lymph nodes and with no evidence of distant metastases

\(^{11}\) Aromatase inhibitors may be used in post menopausal women, as Tamoxifen has been shown to be inferior in overall survival and disease free survival in comparison (clinical opinion QH).
• Chemotherapy in combination with tamoxifen yields an increase in disease-free survival compared with tamoxifen alone; and
• Tamoxifen in combination with chemotherapy yields an increase in disease-free survival compared with chemotherapy alone.\(^5\)

One of the most significant problems facing clinicians treating women with breast cancer is the heterogeneity of the tumour itself, with different tumour and patient characteristics needed to be taken into account before treatment can commence. Characteristics including patient age, tumour size, histological grade, lymph node status (positive or negative) and oestrogen (ER), progesterone (PR) or human epidermal growth factor receptor 2 (HER2 also known as ERBB2) status are all used to guide the need for adjuvant chemotherapy and to assess the risk of distant recurrence. Algorithms that incorporate these factors include the National Institute of Health (NIH) and the St Gallen’s consensus statements, the Nottingham prognostic index and the online decision tool Adjuvant!Online.\(^4,5\)

In the past, research has focussed on the prevention and detection of breast cancer, however, current research is concentrating on the identification of genes or biomarkers that may improve the quality of life of patients already diagnosed with breast cancer. In addition to determining which treatments may be the most effective for individual patients or which treatments should be avoided due to potential toxic side effects, the identification of biomarkers may be able to predict tumour behaviour and the prognosis or response of patients to treatment.\(^6\)

Lymph node status remains the best prognostic marker for survival, with approximately 50 per cent of node positive breast cancer patients not developing recurrence (with or without adjuvant chemotherapy), whereas recurrence may occur in 25 per cent of node negative.\(^5\) Adjuvant poly-chemotherapy may significantly improve the period of time patients are disease free and their overall survival from breast cancer for both lymph node-negative and positive patients. However, for patients who are node-negative, who have a better prognosis when compared to those who are node-positive, treatment with adjuvant chemotherapy results in only a small improvement in survival rates. In randomised controlled trials of women younger than 50 years of age, when poly-chemotherapy was compared to no chemotherapy, 10-year disease-free survival only increased from 58 to 68 per cent. Improvements in disease-free and overall survival decreased with increasing age. In addition, other studies have indicated that node-negative women treated with only tamoxifen after surgery have an average 10-year recurrence rate of approximately 15 per cent indicating that 85 per cent of these women may have been subjected to toxic chemotherapy unnecessarily.\(^5\) Despite the use of all of these clinical tools, it has been estimated that 60 per cent of all patients with early stage breast cancer receive adjuvant chemotherapy and only 2-15 per cent of these patients derive a benefit.\(^5\)
The purpose of gene expression analysis is to determine which genes are actively transcribed into messenger RNA (mRNA) and therefore translated into proteins in tissue samples of interest, in this case breast cancer. Gene expression profiling assays may assist in the classification of breast cancers into prognostic categories depending on the expression of a panel of genes or biomarkers, predicting which patients who would, as well as patients who would not, benefit from adjuvant chemotherapy. In addition to patient benefits, stratification of patients may result in cost savings with many patients being spared unnecessary and expensive chemotherapy.

The central dogma of molecular biology dictates that DNA (genes) can either undergo replication (DNA→DNA) or transcription (DNA→mRNA) and then translation (mRNA→protein) (Figure 1). The term ‘gene expression’ describes the transcription of information encoded within DNA sequences into messenger RNA (mRNA), and the subsequent translation of the mRNA information into proteins that regulate and control cell function. Analysis of gene expression was previously conducted on a single gene-by-gene basis, by low-throughput techniques such as those which use a nucleic acid probe including in situ hybridisation, northern blotting, Southern blotting, RNase protection assays or real-time PCR, or those which use a protein probe, including immunocytochemistry or western blotting. It should be noted that the expression of many genes is regulated after transcription by enzymes, therefore the measurement of mRNA concentrations may not reflect the true level of gene expression.
Figure 1  The central dogma of molecular biology illustrating methods available for measuring gene expression. At the DNA level, fluorescent in situ hybridisation (FISH) measure the number of copies of a gene in each cell. At the translation/RNA level both RT-PCR and microarrays measure the level of gene transcription into messenger RNA. At the translation level, immunohistochemistry (IHC) detects the presence and distribution of a protein within a cell (printed with permission Genomic Health).

Each diagnostic platform has advantages and disadvantages (Table 1). FISH measures gene copy number, but it does not provide direct information about gene or protein expression. RT-PCR quantitatively measures RNA expression levels of a small number of genes with high-levels of precision and sensitivity but does not provide direct information about protein expression or distribution, or about gene amplification. Although RNA is degraded in formalin-fixed paraffin-embedded (FFPE) tissues, RT-PCR is well-suited to amplify from these short RNA fragments. Microarrays also measure RNA expression levels, not gene copy number or protein expression and distribution but unlike RT-PCR, microarrays are high throughput and can assess gene expression of many genes at once. Results from microarrays tend to be regarded as semi-quantitative and may reflect differences in tumour biology by characterising differences in patterns of gene expression. The primers used in microarrays are more efficient with length; therefore fresh frozen tissue is preferred over FFPE tissue. Immunohistochemistry measures protein expression and distribution in a cell.
Table 5  
A comparison of the advantages and disadvantages of methods used to measure gene expression (printed with permission Genomic Health).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FISH</th>
<th>RT-PCR</th>
<th>Microarray</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured molecule</td>
<td>DNA</td>
<td>RNA</td>
<td>RNA</td>
<td>Protein</td>
</tr>
<tr>
<td></td>
<td>Does not provide direct information about gene or protein expression</td>
<td>Does not provide direct information about protein expression or distribution</td>
<td>Does not provide direct information about protein expression or distribution</td>
<td>Provides information about protein expression and distribution</td>
</tr>
<tr>
<td>Quantitative nature</td>
<td>Quantifies gene copy number of relatively small number of genes.</td>
<td>Quantifies gene expression levels of relatively small number of genes.</td>
<td>Qualitative assessment of gene expression of many genes at once.</td>
<td>Assesses protein expression and distribution of a relatively small number of proteins.</td>
</tr>
<tr>
<td></td>
<td>High precision</td>
<td>High analytical sensitivity.</td>
<td>Modest precision for expression levels of individual genes.</td>
<td>Dichotomous</td>
</tr>
<tr>
<td>Tissue fixation</td>
<td>DNA is preserved</td>
<td>RNA is degraded FFPE tissue, but RT-PCR is well suited to amplify from short RNA fragments.</td>
<td>RNA is degraded with FFPE tissue; fresh frozen tissue is preferred.</td>
<td>May denature proteins.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>May affect antibody epitope binding.</td>
</tr>
<tr>
<td>Amount of tissue sample needed</td>
<td>2 × 5-µm sections</td>
<td>3-6 × 10-µm sections</td>
<td>30 × 30-µm sections</td>
<td>2-4 core samples per patient.</td>
</tr>
<tr>
<td>Interpretation of results</td>
<td>Subject to inter-observer variability.</td>
<td>Normalised against expression levels of reference genes.</td>
<td>Normalised against reference genes or global expression.</td>
<td>Subject to inter-observer variability.</td>
</tr>
</tbody>
</table>

FISH = fluorescent in situ hybridisation, RT-PCR = reverse transcriptase PCR, IHC = immunohistochemistry

As mentioned above, there are several gene profiling tests commercially available or in development, all of which use either use reverse transcription PCR or a microarray platform to measure the level of gene transcription into mRNA. These tests classify patients who have a poor prognosis by identifying those patients who express high levels of proliferation genes, which are usually the same genes that are the target of conventional chemotherapy. The most recent St Gallen consensus statement recommends that a validated gene expression assay be considered in order to stratify patients when other clinical factors return an equivocal result in respect to proceeding to treatment with chemotherapy.  

**Microarray technology**

For an in-depth overview of the production of DNA microarrays, how they work and issues associated with their use (noise, accuracy, sensitivity, reproducibility, sample preparation and analysis of data) please see the 2007 horizon scanning Emerging Technology Bulletin. Briefly, high throughput DNA microarrays use small fragments of DNA, or oligonucleotides, which are attached to a glass substrate by a variety of manufacturing processes. Each microarray may hold 100,000s of these elements. Complementary DNA from test and reference samples are labelled with a visualisation tag such as a fluorophore and hybridised to the microarray. The relative
intensity of the signal of the bound DNA is an indication of a given gene’s activity, with an active gene giving a more intense, brighter signal than less active genes.\textsuperscript{48} See Figure 2.

Figure 2 Schematic of a DNA microarray hybridisation\textsuperscript{54}

DNA microarray analysis for the prognosis of breast cancer was first described by van ‘t Veer et al in 2002. Primary breast tumours from 98 women were examined. The study group included ‘sporadic’ cases who were lymph node-negative and <55 years when diagnosed (n=78) of whom 34 developed distant metastases within five years and 44 were disease-free after five years. RNA was isolated and used to derive complementary RNA (cRNA). Hybridisations for each tumour were carried out on microarrays containing approximately 25,000 human genes and fluorescent intensities were scanned, quantified, normalised and corrected to yield the transcript abundance of a gene as an intensity ratio with respect to that of a signal of a reference pool. Using clustering algorithms, a ‘poor prognosis’ gene expression signature, predictive of a short interval to distant metastases in lymph node-negative women was identified. This signature consisted of a set of 70 genes with functions ranging from regulation of cell cycle, metastasis, invasion and angiogenesis. When the gene expression profile was used to classify lymph node-negative patients for eligibility for adjuvant chemotherapy, it performed as well the St Gallen and NIH
Using the St Gallen and NIH criteria, between 82 and 92 per cent of lymph node-negative (LN-) women would have been candidates for adjuvant therapy. However, in the absence of adjuvant chemotherapy, 70-80 per cent of these women would not go on to develop distant metastases, and would therefore not benefit from the treatment. Using the ‘poor prognosis’ gene expression profile, only 43/78 (55%) of women would be candidates for adjuvant therapy, significantly reducing the number of patients who would otherwise receive unnecessary treatment (Table 6). This study formed the basis for the MammaPrint® assay.

Table 6  Breast cancer patients eligible for adjuvant therapy using 70-gene panel

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient group</th>
<th>Total (n=78)</th>
<th>Metastatic disease at 5-years (n=34)</th>
<th>Disease free at 5-years (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St Gallen</td>
<td></td>
<td>64/78 (82%)</td>
<td>33/34 (97%)</td>
<td>31/44 (70%)</td>
</tr>
<tr>
<td>NIH</td>
<td></td>
<td>72/78 (92%)</td>
<td>32/34 (94%)</td>
<td>40/44 (91%)</td>
</tr>
<tr>
<td>Poor prognosis profile</td>
<td></td>
<td>43/78 (55%)</td>
<td>31/34 (91%)</td>
<td>12/44 (27%)</td>
</tr>
</tbody>
</table>

Microarray assays such as MammaPrint® may be used in women who are ER+ or oestrogen receptor negative (ER-) but are lymph node-negative (Figure 3). It should be noted that the requirement for fresh or frozen tumour samples for microarray testing is a major limitation of these assays.

Figure 3  Patient criteria for the use of the 70- or 76-gene microarray: ER- = Oestrogen negative, ER+ = oestrogen positive, N+ = lymph node-positive, N- = lymph node-negative

MammaPrint®

MammaPrint® is a 70-gene expression profile (microarray) test that measures 70 cancer-related genes in triplicate and measures reference genes (normalisation genes and negative controls). It is intended for use in women under the age of 61.
years with newly diagnosed Stage I or Stage II lymph node-negative breast cancer with a tumour size ≤5.0cm. It aims to predict the likelihood of breast cancer recurrence within five to 10 years. Testing is performed on a fresh frozen tumour sample that has a minimum of 30 per cent malignant cells. The test is a DNA microarray. The results are expressed as a “MammaPrint Index”, which assigns a prognosis of low- or high-risk. Worldwide, all collected samples are sent to the one laboratory for processing in the Netherlands. It has recently been reported that MammaPrint is suitable for use in women with up to three positive lymph nodes. In addition, Blueprint, an 80 gene microarray (Agendia) may be used in addition to MammaPrint for molecular sub-typing in patients with early-stage (stage I or II), LN- or LN+ (up to 3), ER+ or ER- breast cancer. The MammaPrint® assay was approved by the United States’ FDA in 2007 as a prognostic, not diagnostic, assay and is CE marked. An application for public reimbursement was received by the Medical Services Advisory Committee for the MammaPrint® test in 2007 but was deemed ineligible. MammaPrint® was previously processed in Australia by MedVet, however, this is no longer the case and the test is no longer offered in Australia. The parent company did not respond to email enquiries.

*MapQuant Dx (may also be marketed under Genomic Grade test)*

Is a 97-gene signature microarray test that is currently marketed in Europe. An eight gene RT-PCR gene expression assay is also under development. No information regarding this assay could be ascertained. This test is not currently offered in Australia.

*The Veridex-76 or Rotterdam Signature 76-Gene Panel*

The Rotterdam Signature test is not currently commercially available. The assay uses 60 genes to evaluate ER+ samples and 16 genes to evaluate ER- samples. Of the 76-genes, this test has only three in common with the MammaPrint® assay. The assay is intended to predict the risk of 5-year breast cancer recurrence in lymph node-negative patients. Testing for RNA expression is performed on a fresh frozen tumour sample and results are expressed as a hazard ratio for distant recurrence.

*Quantitative reverse transcription PCR*

Reverse transcription PCR (RT-PCR) is used to detect and quantify mRNA, thereby demonstrating whether or not a specific gene is being expressed in a given sample. The first step in RT-PCR uses reverse transcriptase and a primer to anneal and extend a target mRNA sequence. If the target mRNA is present, the reverse transcriptase and primer anneals to the mRNA sequence and transcribes a complimentary strand of DNA. This strand is then replicated with primers and a polymerase, and amplified using a standard PCR protocol. Once amplified, the PCR product is separated on an agarose gel, with bands corresponding to mRNAs of interest, indicating gene
RT-PCR was first investigated as a breast cancer prognostic tool by Paik et al (2004) in a retrospective study conducted on a cohort of 668 lymph node-negative women (level III-3 prognostic evidence). A putative 21-gene panel consisted of 16 cancer-related and five reference genes, and their levels of gene expression were used to define an algorithm to calculate a recurrence score and to determine a risk group (low, intermediate, or high) for each patient. The RT-PCR assay categorised 51, 22 and 27 per cent of woman as having a low, intermediate, or high-risk, respectively, of breast cancer recurrence. The 10-year distant metastases recurrence rates in the low, intermediate, and high-risk groups were 6.8, 14.3 and 30.5 per cent, respectively. A multivariate analysis indicated that women in the high-risk category were more likely to experience recurrence than those in the low risk group (HR =2.81, 95%CI [1.70, 4.64], \( p < 0.001 \)).

This study formed the basis of the \textit{Onco\textsuperscript{type} DX\textsuperscript{®}} assay.

\textit{Onco\textsuperscript{type} DX\textsuperscript{®}}

\textit{Onco\textsuperscript{type} DX\textsuperscript{®}} is a 21-gene expression profile that measures 16 cancer-related genes in triplicate and has 5 reference genes. There is only one gene overlap between the \textit{Onco\textsuperscript{type} DX\textsuperscript{®}} and the MammaPrint assay. It is intended for use in women with newly diagnosed Stage I or II breast cancer who are ER+, lymph node-negative and who will be treated with tamoxifen. It aims to evaluate the likelihood of breast cancer recurrence and assess the potential benefit of adjuvant chemotherapy.

Testing is performed on formalin-fixed, paraffin-embedded tumour tissue. The test is a quantitative reverse transcriptase-polymerase chain reaction (q RT-PCR). The results are expressed as a “Recurrence Score” between zero and 100. Patients with a score less than 18 are defined as low-risk of distant recurrence within 10-years, greater than 31 as high-risk, and between 18-30 as an intermediate risk. Patients with a high-risk score are more likely to benefit from adjuvant chemotherapy. It is important to note that although the \textit{Onco\textsuperscript{type} DX\textsuperscript{®}} assay was originally developed for use in ER+ and lymph node-negative women, recent evidence has suggested that the recurrence score is prognostic in patients who are ER+ with up to three positive lymph nodes. The \textit{Onco\textsuperscript{type} DX\textsuperscript{®}} test received CE marking in 2007. The \textit{Onco\textsuperscript{type} DX\textsuperscript{®}} test is offered to women in Australia under licence to the parent US company, Genomic Health. Formalin-fixed, paraffin embedded biopsy samples are sent to Healthscope (Dandenong, Victoria) for processing. A similar arrangement occurs in New Zealand, with samples sent via the pathology group, Labtests, a subsidiary of Healthscope Pathology. The tumour sample is sectioned and directly

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\( ^{12} \) Corresponding 95%CI values: 6.8% [4.0, 9.6], 14.3% [8.3, 20.3], and 30.5% [23.6, 37.4]
shipped to Genomic Health in the USA for analysis. Onco
type DX® is not currently covered by private health insurance in Australia or New Zealand.61

**Breast Cancer Index, 2-gene ratio, H/I or HOXB13/IL17BR**

The Breast Cancer Gene Expression Ratio is a 2-gene expression test that evaluates the expression ratio of the homeobox gene (HOXB13) to the interleukin-17B receptor gene (IL-17BR). It is intended for use in women with newly diagnosed ER+ and LN-breast cancer, with the aim of predicting 5-year disease recurrence. Testing is performed on formalin-fixed, paraffin-embedded tumour tissue. The test is a quantitative reverse transcriptase-polymerase chain reaction (q RT-PCR). Results are reported as a ratio (H:I) of the expression of the two genes, with an increase in the ratio indicating an increase in the risk of disease recurrence. The American Society of Clinical Oncology (ASCO) has recommended that more clinical data is collected regarding the use of this assay.55 This test is not currently offered in Australia.

**BreastOncPx**

BreastOncPx™ is a 14-gene signature assay. It is intended for use in women with ER+ and LN-breast cancer to assess their risk of distant metastasis and to identify high-risk patients who may benefit from additional therapy. Testing is conducted on sections of primary breast tumour obtained from paraffin block in sections.62 This test is not currently offered in Australia.

The systematic review by Ward et al (2012) described tests other than those outlined above. The Randox Assay (BCA) (Randox Laboratories) is a cDNA-based expression biochip assay that aims to accurately define the clinical sub-types of breast cancer tumours prior to initiating treatment; however no studies describing the use of this assay could be identified. The PAM50 gene expression assay (ARUP Laboratories Inc.) classifies subtypes of breast cancer using quantitative values of ER, PR, HER2, proliferation, and Luminal score. The current version of the test does not provide a risk of recurrence score. The majority of the evidence for this test identified in the review was either in abstract form or unpublished and therefore not included for assessment. Two expanded immunohistochemistry tests for protein expression were, however, included for assessment. The IHC4 assesses levels of four key proteins in a breast cancer sample: ER, PgR, Her-2 and Ki-67. The final algorithm for IHC4 calculates a risk score for distant recurrence based on these four proteins in addition to clinical and pathological variables, resulting in five tumour categories which determine treatment and prognosis. The IHC4 test is under development by an academic research team. The Mammostrat® (Clarient Inc, California, USA) test uses five independent immunohistochemical markers (SLC7A5, HTF9C, P53, NDRG1, and CEACAM5) that do not measure proliferation or hormone receptor status. Results are used inform treatment decisions, stratifying women into risk groups.14, 63
Although gene expression signature tests may be regarded as a replacement for clinical parameters, stratification by clinical features such as tumour size and lymph node status provide prognostic information that is independent of that offered by gene expression signature assays. A good prognosis is associated with small tumour size, lymph node-negative status and ER+ and progesterone (PR+) receptor positive status. A poor prognosis is associated with HER2 over-expression.

The features of some of the current available gene expression signature assays are summarised in Table 7.

Currently none of the tests described are conducted in Australia. Although the MammaPrint assay has been offered in the past, and the OncoType DX assay is currently offered to Australian patients, this is done on a user pays basis, with the patient bearing the full cost of the test. Diagnostic tests such as these cannot be listed on the Medicare Benefits Schedule unless they are conducted in Australia.
<table>
<thead>
<tr>
<th>Table 7</th>
<th>Summary of the features of current prognostic gene expression signatures for breast cancer$^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MammaPrint</td>
</tr>
<tr>
<td>Analysis</td>
<td>Microarray</td>
</tr>
<tr>
<td>Assay</td>
<td>70-gene signature</td>
</tr>
<tr>
<td>Tissue type</td>
<td>Frozen or stabilised mRNA</td>
</tr>
<tr>
<td>Discovery set</td>
<td>78 ER$^\pm$, N0, &lt;5 cm diameter cancers, age &lt;55 years</td>
</tr>
<tr>
<td>Initial validation set</td>
<td>295 ER$^\pm$, N$^\pm$, &lt;5 cm diameter cancer, age &lt;52 years</td>
</tr>
<tr>
<td>Outcome</td>
<td>Distant metastasis at 5 years</td>
</tr>
<tr>
<td>Clinical application</td>
<td>Prognosis of N0, &lt;5 cm diameter, stage I/II disease, age &lt;61 years</td>
</tr>
<tr>
<td>Results presentation</td>
<td>Dichotomous; good or poor prognosis</td>
</tr>
<tr>
<td>Prognostic value in other populations</td>
<td>Up to 3 positive nodes, and HER2$^+$ disease</td>
</tr>
<tr>
<td>Predictive value</td>
<td>Chemotherapy response (poor prognosis group)</td>
</tr>
<tr>
<td>Level of evidence</td>
<td>II</td>
</tr>
</tbody>
</table>

MGI = molecular grade index, FFPE = formalin fixed, paraffin embedded, ER = oestrogen receptor (+ve or –ve), N+ = lymph node-positive, GGI = genomic grade index, PR = progesterone receptor.
2012 CLINICAL NEED AND BURDEN OF DISEASE

In Australia during 2007, the most commonly diagnosed cancer in females was breast cancer, with 12,567 cases at an age-standardised incidence rate (ASR) of new breast cancer cases 109.2 per 100,000. In addition, the leading cause of burden of disease from cancer in females during 2010 was breast cancer, representing a total of 24 per cent of the total cancer burden and four per cent of the total burden of disease in Australia, accounting for 61,100 DALYs. In 2007, breast cancer was the second most common cancer causing death in Australian females, accounting for 2,680 deaths with an ASR of 22.1 per 100,000. Survival rates are of importance when considering prognostic assays. The 5-year relative survival for breast cancer in females has increased from 72 per cent in 1982–1986 to 88 per cent in 1998–2004. Based on 1997 data, women aged 50-59 years fared better than all age groups with a 5-year relative survival rate of 90 per cent compared to 81, 86, 89 and 80 per cent survival in women ages 0-39, 40-49, 60-69 and >70 years, respectively. In addition, the 5-year relative survival rate was significantly higher in women who were LN- (97%) compared to a rate of 80 per cent in LN+ women or 71 per cent when the lymph node status was unknown. Five year survival rates were also significantly higher for women with small tumours: 98 percent for tumours ≤ 10mm reducing to 73 per cent for those with tumours ≥ 30mm.

In New Zealand in 2007, the number of new breast cancer cases registered was 90.3 per 100,000 females, representing 2,575 women diagnosed with the disease. The number of deaths from breast cancer in the same year was 643 at an ASR of 20.8 per 100,000.

It is worth noting the number of women who may benefit from gene profiling assays. Oestrogen and progesterone receptors are the only immunohistochemistry (IHC)-based breast markers to have received the imprimatur of a consensus committee of the College of American Pathologists. They are weak prognostic markers of outcome and strong predictive markers of response to endocrine therapy (e.g. tamoxifen). Oestrogen receptors (ER+) are present in approximately 70 per cent of breast cancers in unselected Australian women. ER status is strongly influenced by tumour grade and histology; with almost all grade I tumours and lobular carcinomas being ER positive. The HER2 oncogene protein, a transmembrane glycoprotein, is over-expressed in 10–20 per cent of primary breast cancers.

13 DALYs = disability adjusted life year
14 Clinical opinion suggests that ER negative women have a high early death rate (prior to 5-years), which flattens out after 5-years. The 10-year relative survival rate may give a more accurate understanding of the overall survival of women. The corresponding 10-year survival data for these time periods could not be obtained, however, 10-year relative survival rates have increased from 61% in women diagnosed with breast cancer between 1982 to 1987, to 78% in women diagnosed between 1994 and 1999. AHW & NBOCC (2009). Breast cancer in Australia: an overview, 2009. Australian Institute of Health and Welfare & National Breast and Ovarian Cancer Centre, Canberra http://www.aihw.gov.au/publication-detail/?id=6442468297.
HER2 status in breast cancer has been demonstrated to be a prognostic as well as a predictive marker.

**2012 Diffusion of Technology in Australia**

Currently only the Oncotype DX® (ODX) assay can be accessed by women in Australia and New Zealand. As described above, all samples are collected locally, sectioned and sent to the parent US company for analysis. In Australia in 2009-10, 61 patients privately paid for an ODX test. This number increased slightly in 2010-11 with 85 women paying for ODX testing. During this same period, 151 women took part in the Australian Decision Impact Study. As of March 2012, 71 women had privately paid for ODX testing (personal communication Healthscope).

**2012 Comparators**

The comparator for gene profiling assays is standard clinical practice. This may include the use of tools such as those described in the background section. Algorithms such as the Nottingham prognostic index and the online decision tool Adjuvant!Online may be used to guide treatment decision making. These tools provide information about prognosis which is largely based on pathological features of the tumour including tumour size, grade, lymph node and oestrogen receptor status.

**2012 Safety and Effectiveness**

The NHMRC developed a paper to inform health professionals on the clinical utility of personalised medicine and its potential to improve health outcomes for individuals. Predictive tests may be used to diagnose a disease, assess an individual’s risk of disease, identify whether or not an individual will benefit from a particular intervention and/or tailor dosing regimens to individual variations in metabolism. To assess the efficacy of these tests, the paper suggests that, in conjunction with ethical considerations, that three elements should be assessed:

- **Analytical validity:** the reproducibility and repeatability of the assay, that is, the ability of the test to accurately and reliably measure gene expression, in this case levels of mRNA or protein.

- **Clinical validity:** measures the test’s ability to predict the presence or absence of disease, that is, the sensitivity, specificity and positive and negative predictive values, in this case, to accurately predict the risk of distant recurrence.

- **Clinical utility:** a measure of the health care value provided by the test – in this case, the test’s ability to discriminate between those who will have more, or less, benefit from chemotherapy. The clinical utility for a breast cancer prognostic assay should
include outcome measures including overall survival, disease-free survival, adverse
effects of chemotherapy and quality of life.\textsuperscript{14, 50}

The NICE systematic review identified 32 full text papers (30 studies) that described the use
of nine prognostic assays used for guiding treatment in early breast cancer patients. The
majority reported on the use of either the MammaPrint or ODX assays. Most were small,
retrospective cohort studies (level III-3 prognostic evidence) with a relatively short follow-
up. Only seven studies reporting on a sample size in excess of 1,000 participants (3 for ODX
and Mammostrat, 1 for IHC4). Four studies reported analytical validity, 20 studies reported
clinical validity and 18 studies reported clinical utility. For a full break down of the results of
each individual study included in the review, please see the full version of the review. Only a
brief summary of the results is presented below.

\textit{Oncotype DX}

The 2008 Blue Cross Tec report summarised the results from studies conducted with
OncoType DX as follows: “\textit{Oncotype DX ...............identifies a subset of patients who would
otherwise be recommended for chemotherapy, but are actually at lower risk of recurrence
(average 7–9\% risk at 10 years; 95\% CI [11, 14]). Oncotype DX testing also identifies a subset
of conventionally classified low-risk patients who are reclassified at higher risk of recurrence.
However, due to wide confidence intervals, it is not clear that all reclassified higher-risk
individuals would realise a net benefit from chemotherapy}”.\textsuperscript{46}

The NICE systematic review identified 12 additional studies, not already assessed by
previous systematic reviews, which reported on the use of ODX in ER+, lymph node-negative
women.

\textbf{Analytical validity:} No new evidence since previously published systematic reviews.

\textbf{Clinical validity (prognostic ability):} Early systematic reviews indicated that the ODX
recurrence score (RS) correlated significantly with disease-free survival and overall
survival, with the RS being a better predictor of distant recurrence at 10-years than
traditional clinical tools. This finding was supported by the larger studies included in
the NICE review.

\textbf{Clinical utility:} Previous evidence included in past systematic reviews describing the
clinical utility of ODX was regarded as flawed due to differences in chemotherapy
regimes. The current review did not identify any prospective studies that reported
the effect of ODX testing on long-term outcomes such as overall survival. Four
studies were included for assessment that described changes in treatment decision
making, with one small study (n=106) describing results from a UK trial. The use of
ODX resulted in a change in decision making in between 31.5-38 per cent of women.
Although three of the studies had long follow up times, were moderate to large in
size and were considered to be of medium to high quality, there was a lack of
standardisation in patient selection and the use of decision-making tools. The authors concluded that further direct evidence of the clinical utility of ODX was required.

As mentioned in the diffusion section, the Australian Decision Impact Study recruited 151 (101 lymph node-negative and 50 N+) women from three Melbourne hospitals for testing with Oncotype DX. This study is possibly being conducted at the Peter MacCallum Cancer Centre in Victoria and initial results were presented at the San Antonio Breast Cancer Symposium in December 2011. The goal of the study was to characterise how the results from testing with ODX would impact on the decision making process of treating clinicians. The primary endpoint was the overall change in eth treatment recommendation for node negative and node positive women. Although results from this study have not yet been published in the peer-reviewed literature, the results presented at the conference were as follows: knowledge of the risk score resulted in a change in treatment recommendations in 36/151 (24%) of women with the largest shift observed in node-positive women. Recommendations changed from chemotherapy (CHT) to hormone therapy (HT) in 15.9 per cent of women and from hormonal to chemotherapy in 7.9 per cent. In node-negative women the overall change was 22.8 per cent (11.9% from CHT to HT and 10.9% from HT to CHT). In node-positive women the overall change was 26 per cent (24% from CHT to HT and 2% from HT to CHT). 

**MammaPrint**

The 2008 Blue Cross Tec report summarised the results from studies conducted with MammaPrint as follows: “Adjusted hazard ratios for distant metastases suggest that the test provides recurrence risk information in addition to conventional classification criteria; the strongest associations appear in the first 5 years of follow-up. Average 10-year disease-free recurrence in low-risk patients by MammaPrint® was approximately 85–88% in two studies, with a lower confidence limit of 74–79%. However, ROC analysis in an independent multicenter validation study suggests only slightly improved predictive accuracy for time to distant metastases with MammaPrint® compared to other conventional criteria. In one study, after Adjuvant! Online risk classification, patients reclassified as low risk by the 70-gene signature in either Adjuvant! Online risk group had 10-year disease-free survival rates of 88–89%, with lower confidence limits of 74–77%. Patients reclassified as high risk had 10-year disease-free survival rates of 69%, with lower confidence limits of 45–61% and upper confidence limits of 76–84%”. 

The NICE systematic review concluded:

- **Analytical validity**: No new evidence since previously published systematic reviews.
- **Clinical validity (prognostic ability)**: Early systematic reviews reported variable results on the prognostic ability of the MammaPrint (MP) assay. The NICE review identified
four additional small studies (ranging from n=102-272) which reported on the clinical validity of MP. The MP score was found to be a strong, independent prognostic factor which may be reliable at predicting outcome at 5-years, rather than the stated 10-years.

**Clinical utility:** Previous systematic reviews identified only one study (n=427) that demonstrated that MP impacted on clinical decision making, however, follow-up was short. The NICE review identified a further six studies of high to moderate quality, that reported on clinical utility. There was a high level of discordance between MP and current clinical practice, however how this would impact on decision making was not made clear. One study reported that the use of MP would alter treatment advice for 40 per cent of patients, however it was assumed that all patients classified as high-risk would receive chemotherapy and that all patients classified as low-risk would not receive chemotherapy. No evidence of a change in actual clinical practice was provided. The authors concluded that further evidence is required.

**Breast Cancer Index**

The 2008 Blue Cross Tec report summarised the results from studies conducted with the Breast Cancer Index test as follows: "The Breast Cancer Gene Expression ratio was significantly and independently associated with poorer disease-free survival in two studies of lymph node-negative, ER+, tamoxifen-treated patients with breast cancer. Patients who were low risk by the 2-gene expression ratio had average 10-year recurrence rates of about 17–25%. Two additional studies in heterogeneous populations of patients also support a statistical association between the 2-gene expression ratio and recurrence-free survival".\(^{46}\)

The NICE systematic review concluded:

**Analytical validity:** No available evidence.

**Clinical validity (prognostic ability):** Only one retrospective study was identified for inclusion in the NICE review. This study used a subset of 588 samples from a larger cohort, with some samples dating back as far as 1976, which may bring into question the criteria used for patient selection. The training set of samples and the test/validation set of samples were obtained from the same pool of samples, introducing the potential of incorporation bias, which may make the test appear more powerful in differentiating a low-risk case from a high-risk case than it really is. From the results the BCI appears to be a strong prognostic factor for distant recurrence and breast cancer specific death independent of tumour size, grade, HER2 status and PR status. When BCI was compared to Adjuvant! Online both were significant predictors of breast cancer specific death; and distant recurrence. The authors of the NICE review concluded that more evidence of clinical validity would be required.
**Clinical utility:** No available evidence.

**Mammostrat**

No previous assessment results were published describing the use of this assay. The NICE systematic review concluded:

**Analytical validity:** No available evidence.

**Clinical validity (prognostic ability):** Three moderate to high quality, retrospective studies were identified for inclusion in the NICE review. Sample sizes were large, ranging from 1,109 to 1,540. The one UK study, which may be relevant to the Australian health system, had a long follow-up (9 years). The risk score was found to be a significant independent predictor of distant recurrence free survival and overall survival. In addition, the Mammostrat test was an independent prognostic tool for ER+, tamoxifen-treated breast cancer possibly regardless of node status and ER-negative tumours. Samples in this study were again quite old (1981) and patient selection criteria may have varied. The NICE systematic review concluded that although results look promising more evidence is required.

**Clinical utility:** No available evidence.

**IHC4 test**

No previous assessment results were published describing the use of this test. The NICE systematic review concluded:

**Analytical validity:** No available evidence.

**Clinical validity (prognostic ability):** Only one retrospective cohort, high quality with a large sample size (n=1,911) was available for inclusion in the NICE review. Although this study found the IHC4 test to be a highly significant predictor of distant recurrence ($\chi^2 = 39.1, p<0.0001$) further evidence is required.

**Clinical utility:** No available evidence.

**MapQuant Dx**

No information regarding this assay could be ascertained.

**The Veridex-76 or Rotterdam Signature 76-Gene Panel**

The Rotterdam Signature test is not currently commercially available.

**BreastOncPx**

No information regarding this assay could be ascertained.
2012 COST IMPACT

Australian women wishing to access gene expression profile assays for breast cancer must currently do so on a user-pays basis. None of the current assays are covered by private health insurance. In 2007, when the MammaPrint® assay was offered to Australian women by MedVet, the cost of the test was $3,600. The current listed price of the Oncotype DX® assay is $4,000, however as this is based on a US price the cost will fluctuate due to exchange rates (personal communication Healthscope). The cost of the Breast Cancer Index is estimated to be US$3,200 ($3,104). Costs of the IHC4 and Mammostrat immunohistochemistry tests are estimated to be £100-200 ($154-300) and £1,120-1,620 ($1,726-2,497) (NICE 2012).

Two recent cost-analyses reported on costs associated with gene expression profiling. The study by Yang et al (2012) developed a 10-year Markov model that compared costs and QALY’s of US patients treated with therapy guided by either the MammaPrint or Oncotype DX assay. The model assumed a hypothetical cohort of 1,000 lymph node-negative, ER+ women with breast cancer who received either one of the two gene profiling tests. Baseline risk was obtained using the Adjuvant! Online assessment tool. Assumptions included that patients could only have one recurrence and that they would not progress to a better health state, so that during each cycle of the model patients would either be disease-free or develop recurrence. It was also assumed that 90 per cent of patients deemed to be at high-risk by both Oncotype DX and Adjuvant! Online would receive chemotherapy, whereas as 90 per cent of those at low-risk with both tests did not receive chemotherapy. For those patients with an equivocal result between the two tests, it was assumed that 50 per cent would receive chemotherapy. Costs, in 2009 US dollars, included:

- the gene signature assay: MammaPrint = $4,200 and Oncotype DX = $3,975;
- adjuvant chemotherapy: $19,618 (an assumed willingness to pay of $50,000);
- the cost of treating recurrence: $10,837;
- other costs associated with chemotherapy per cycle: $727;
- treatment of adverse events: minor= $2,709, major = $18,061, fatal = $45,153; and
- end-of-life care costs: $34,778.

For strategy 1, of the 1,000 patients, 354 were classified as low-risk by Adjuvant! Online and of these, 138 were reclassified by Oncotype DX as high-risk. Of the 314 patients classified as high-risk by Adjuvant! Online, 192 remained high-risk with Oncotype DX but 122 were reclassified as low-risk. For strategy 2, 80 patients were classified as low-risk with Adjuvant! Online, 192 remained high-risk with Oncotype DX but 122 were reclassified as low-risk. For strategy 2, 80 patients were classified as low-risk with Adjuvant! Online, 192 remained high-risk with Oncotype DX but 122 were reclassified as low-risk. For strategy 2, 80 patients were classified as low-risk with Adjuvant! Online, 192 remained high-risk with Oncotype DX but 122 were reclassified as low-risk.

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15 QALY = quality adjusted life year
risk with MammaPrint. When Oncotype DX was used to guide treatment, costs were $27,882 and patients gained 7.364 QALYs. MammaPrint was found to be more cost-effective when used to guide treatment, with patients spending $21,598 with a gain of 7.461 QALYs. These results were found to be robust when a sensitivity analysis was conducted, with Oncotype DX found to be the dominant strategy with an ICER of $6,284. It should be noted that this study is conducted in the US health system and may not be applicable to Australia or New Zealand’s health system.

Klang et al (2010) modelled costs based on the results on the first 368 Israeli women (median age 57 years, range 29-81 years) tested with the Oncotype DX assay and their real life treatment decisions. A Markov model was used to simulate the costs associated with and without the use of Oncotype DX, with the analysis from the payer’s perspective (public health insurer) not taking into account patient associated costs such as lost productivity. Although it wasn’t explicitly stated in the paper, costs were in US dollars (year not stated) and were as follows:

- retail price of assay: $3,460;
- chemotherapy: $3,540;
- supportive care: $243;
- management of chemotherapy adverse events: $2,249; and
- the cost of treating recurrence: $10,000.

Of the 368 women tested with Oncotype DX (ODX), the first 55 women did not have a reported treatment recommendation prior to testing. Based on traditional clinical parameters, 174 (56%) women were offered chemotherapy prior to testing with ODX. Of the 174 women classified as high-risk prior to testing, 63 were found to be low-risk (risk score 0-17), 72 were an intermediate risk (RS 18-30) and 39 (RS >30) were high-risk after testing with ODX. Chemotherapy was recommended for 89 women after testing with ODX, with 125 (40%) women having their treatment plan altered in light of their results. Hormone therapy alone was administered to 105 (34%) women instead of the initial recommendation of adjuvant chemotherapy plus hormone therapy. Of importance were the 63 low-risk women at baseline who were recommended for chemotherapy, however after testing with ODX, none of these women received chemotherapy.

Taking into account the cost of the ODX test plus savings resulting from the reduced use of adjuvant chemotherapy, the net average cost of ODX testing was $1,828 per patient. The average QALY gained per patient due to reduced chemotherapy was 0.136 years. For the small number of women (8%) who were originally assigned to hormonal therapy alone but switched to chemotherapy after testing with ODX, the average QALY associated with reduction in recurrence was 0.034 years. The net QALY gained was therefore 0.170 years, with a cost per QALY gained of $10,770, well below the generally acceptable cost-
effectiveness value of $50,000 per QALY. ODX testing increased actual costs for the payer (insurance fund) by $1.5 million in the first year after diagnosis. The NICE systematic review conducted a thorough cost-effectiveness analysis and identified four studies, two of which compared MammaPrint to Adjuvant! Online and two that compared ODX to Adjuvant! Online. The above study by Klang et al (2010) was excluded from the assessment as the exact nature of the comparator defined as clinical practice in Israel was unclear. Of the two MammaPrint studies, one modelled treatment decision making in Dutch patients and costs were expressed in 2005 Euros (Retel et al 2010). The other was modelled from a US payer perspective and costs were expressed in 2007 US dollars (Chen et al 2010). The two ODX economic analyses were Canadian and conducted from a health care perspective. The costs of the first study by Tsoi et al (2010) were expressed in 2008 Canadian dollars and was conducted as part of an evaluation in the Ontario Health Technology Assessment series. This study was later updated, with patients classified slightly differently into low, intermediate and high-risk groups, with costs expressed in 2010 Canadian dollars (Paulden et al 2010). Generally, all studies were considered to be well conducted but their generalisability to the UK health system was limited due to a number of reasons. This reasoning may apply to the Australian health system.

The results from the four economic analyses are tabulated in Table 8. Comparison of the ICER values is difficult in the current economic climate; however, it would appear that the MammaPrint assay is the more cost-effective at A$6-9,000 per QALY gained. These values were obtained with reasonably old data (2005 and 2007) and a more reasonable estimate may be the cost-effectiveness data from the two later Canadian studies. The cost-effectiveness varied greatly between these two studies, with the later study likely to be the more accurate, with an ICER of A$23,226. Although this is considerably higher than ICERs obtained with the MammaPrint assay, an ICER of this size is still well below the acceptable $50,000 threshold.
The manufacturers of the ODX and the Mammostrat (immunohistochemistry) assays submitted economic evaluations considering the cost-effectiveness of their products if used in current clinical practice in the UK health care system, from the perspective of the National Health Service. The details of the Mammostrat analysis were not publicly available. A summary of the ODX analysis is at the bottom of Table 8. The ICER of A$9,581 per QALY gained is close to those obtained with the MammaPrint assay. Although this analysis was deemed to be more relevant for decision making in the UK, the authors of the NICE review identified several concerns regarding the assumptions used in the model including baseline levels of chemotherapy, the risk of distant recurrence, the cost of chemotherapy in the UK, the number of women who would be offered the gene profiling test and the number that would be offered chemotherapy after reclassification.

The authors of the NICE review constructed an economic model using the ODX assay addressing the limitations outlined above. An ICER of £26,940 (A$41,404) per QALY gained was obtained when the test was offered to all women with early breast cancer who were ER+ and lymph node-negative. An ICER of £9,007 was obtained if ODX testing was only offered to those women with a Nottingham Prognostic Index score of ≥ 3.4. In addition, economic modelling was conducted for the Mammostrat and IHC4 tests; however the models cannot be compared due to differences in methodology and patient characteristics. Despite this, it would appear that guiding treatment with IHC4 is the strategy most likely to be cost-effective if offered to all ER+, lymph node-negative, HER2 women or to women with a Nottingham Prognostic Index score ≥ 3.4. In the model, when the IHC4 test was directly compared to Oncotype DX, the IHC4 test was dominant, in that it cost less and was as effective as ODX (Table 9). Due to the limited effectiveness evidence base, these results should be viewed with caution (NICE 2012).

### Table 8  Summary from the main results from the four cost-effectiveness studies included in the NICE systematic review (Ward et al 2012)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Test evaluated</th>
<th>Costs</th>
<th>QALYs</th>
<th>ICER (compared to Adjuvant! Online)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic Health</td>
<td>Oncotype DX Usual care</td>
<td>£12,735 £11,847</td>
<td>11.54 11.39</td>
<td>£6,232 (A$9,581)</td>
</tr>
</tbody>
</table>
Clearly, a cost-effectiveness analysis from the Australian health care perspective is required.

### Table 9 Comparison of the cost-effectiveness of the Oncotype DX and IHC4 tests (NICE 2012)

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean cost</th>
<th>Mean QALY</th>
<th>ICER</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>When offered to all women with early breast cancer who are ER+, lymph node-negative, HER2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncotype DX</td>
<td>£9,094</td>
<td>13.54</td>
<td>£26,940 Dominant</td>
</tr>
<tr>
<td>IHC4</td>
<td>£6,340</td>
<td>13.49</td>
<td>13.44</td>
</tr>
<tr>
<td>Normal clinical practice</td>
<td>£6,519</td>
<td>13.44</td>
<td></td>
</tr>
<tr>
<td><strong>When offered to all women with early breast cancer with a NPI score ≥ 3.4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncotype DX</td>
<td>£10,911</td>
<td>13.90</td>
<td>£9,007 Dominant</td>
</tr>
<tr>
<td>IHC4</td>
<td>£8,318</td>
<td>12.97</td>
<td>12.83</td>
</tr>
<tr>
<td>Normal clinical practice</td>
<td>£8,816</td>
<td>12.83</td>
<td></td>
</tr>
</tbody>
</table>

NPI = Nottingham Prognostic Index, ICER = incremental cost-effectiveness ratio

### 2012 Ethical, Cultural or Religious Considerations

Gene profiling assays may offer patients the opportunity to access personalised medicine, giving them an increased choice in treatment pathways. Patients considered to be at low-risk may choose to forego treatment with potentially toxic chemotherapy agents whilst those found at high-risk may opt for chemotherapy. As with all diagnostic tests, the consequences for those patients who return either a false positive or false negative result may be serious. Patients who base their treatment options solely on a result that indicates they are at low-risk of metastases, when they are in fact at high-risk, may forego life-saving chemotherapy. Conversely, those patients wrongly diagnosed as high-risk will expose themselves to highly toxic chemotherapy. Of concern is that with current clinical tools a great number of women expose themselves unnecessarily to harmful and ineffective chemotherapy, with little effect on health outcomes but a detrimental effect on their quality of life. In addition, it should be remembered that a subset of patients with a good prognosis may still develop recurrence after curative surgery and adjuvant therapy.

Gene profiling assays are currently only accessible on a user-pays basis, which raises issues of equity in that only those women who can afford to pay $4,000 can access this additional information to guide their treatment. Consideration should also be given to the fact that all gene signature assays available to Australasian women must be sent overseas to the parent company for processing, necessitating a 2-3 week delay in the decision of whether or not to offer chemotherapy, which in some cases may be crucial.

### 2012 Other Issues

Since 28th November 2010, a total of 18 voluntary reports have been made to the FDA’s MAUDE (Manufacturer and User Facility Device Experience) adverse events database by treating physicians. Several reports were made by the same physician, describing an adverse event in different patients. Of these, three were false negative results for the oestrogen receptor, 11 were false negative results for the HER2 gene and three patients were false

Gene expression profiling of breast cancer: May 2012
positives in that they were found to be unequivocal for the HER2 gene by the FDA approved vysis probe fish kit but equivocal by the Oncotype DX® Assay. The outcome of these results may be the inappropriate treatment of patients which may have life threatening consequences. One remaining reported adverse event described a Oncotype DX® quality assurance exercise conducted by a chief of hospital pathology:

“The Oncotype DX® test has grossly inaccurate results for the HER2 gene by qrt-pcr. The sensitivity for a positive result is only 31%........... We have found that the HER2 gene has a false negative rate of 69%, which means that pts who are supposed to receive the special targeted therapy may not receive it, and the recurrence score will be erroneous because of the erroneous HER2 result. Another serious concern is that, if this test is missing 69% of positive results for HER2, what else is it missing? is the test reliable and robust? this test appears to have systemic problems and needs to being thoroughly examined by an independent entity. The test is bound to cause serious pt harm by using inappropriate therapies for breast cancer pts.”.73

In a right-of-reply, Genomic Health was contacted for a response to this Brief and commented on the MAUDE database entries as follows:

“Although the Oncotype DX assay reports a the quantitative gene expression of HER2 based on triplicate measurements of gene expression, Genomic Health, Inc. currently does not advocate use of its assay as a means of assessing HER2 status specifically, as no clinical outcomes data have yet been produced linking the HER2 by RT-PCR measurements with response to anti-HER2 therapies. Further, the company does not advocate ordering of Oncotype DX in HER2 positive patients, and it is unclear why this single center has submitted a sizable number of assays in patients known to be HER2 positive by IHC or FISH. Genomic Health has offered to collaborate with this investigator to better understand the findings, but to date, he has not responded.”

No adverse events were reported on the MAUDE database for the other assays described in this Brief.

Clinical trials

Several studies currently underway have been identified that specify the use of gene profiling in their protocols. No current clinical trials using the MapQuant, Veridex-76 or the Breast Cancer Index tests were identified on either ClinicalTrials.gov or the Australian New Zealand Clinical Trials Registry.

One of the largest on-going studies is the TAILORx (Trial Assigning Individualized Options for Treatment (Rx) randomised controlled trial which aims to enrol 10,000 women who will undergo testing with ODX. Those women with an ODX risk score of less than 11 will receive hormone therapy, those with a score >25 will receive hormone therapy and chemotherapy and those with a score between 11 and 25 will be randomised to receive either hormone
therapy or chemotherapy. This study will not provide direct evidence for the value of Oncotype DX, as all patients in the trial will receive the test. It is expected that the first results from this trial will be published in 2013.\(^9\)

I-SPY 2 TRIAL, a multi-centre phase II trial of 800 adult women with histologically confirmed invasive breast cancer, randomised to treatment with novel drugs in combination with standard chemotherapy compared to standard therapy alone (Clinical Trials Identifier NCT01042379). The tumour profile of enrolled women will be determined by the MammaPrint\textsuperscript{TM} assay in addition to ER and HER2 status. The goal is to identify improved treatment regimens for subsets of patients based on the molecular characteristics (biomarker signatures) of their disease. The estimated primary completion date for this study is February 2014.

A large, long-term multi-centred European trial, MINDACT, has currently enrolled 3,142 breast cancer patients from 93 institutions in nine countries, with the aim of enrolling over 6,600 patients (Clinical Trials Identifier NCT00433589). Primary data completion is not expected until 2019, with the primary outcome measure being distant metastasis-free survival at 5-years. This study aims to compare the MammaPrint test to a clinical-pathological prognostic tool (Adjuvant! Online) for the selection of patients with negative or 1-3 positive nodes for adjuvant chemotherapy in breast cancer. Women assessed as “high-risk” by both assessment tools are advised to undergo chemotherapy and those assessed as “low-risk” are advised to undergo hormonal therapy. Cases that are discordant for the two tools are randomised to receive either chemotherapy or hormonal therapy. This study aims to confirm that breast cancer patients with a “low risk” molecular prognosis by MammaPrint and “high risk” clinical prognosis can be spared chemotherapy without affecting distant metastases free survival.

The SWITCH trial, a multi-centre French study, run in conjunction with Genomic Health\textsuperscript{TM} Inc, began recruiting 100 women with HR+, N- breast cancer in 2011 and should be nearing completion in March 2012 (Clinical Trials Identifier NCT01446185). The primary aim of this study was to determine the impact of the Oncotype DX\textsuperscript{TM} recurrence score on the treatment recommendation made (administration of chemotherapy or not, in addition to hormone therapy).

Two studies were identified with patient inclusion criteria of patients with breast cancer and an Oncotype DX\textsuperscript{TM} recurrence score>25:

- A randomised phase III trial comparing high-dose radiation therapy to standard radiation therapy in treating patients with early-stage breast cancer that was removed by surgery (Clinical Trials Identifier NCT01349322);

- A Canadian randomised controlled trial comparing Metformin, an agent that is commonly used to treat diabetes, to placebo (Clinical Trials Identifier NCT01101438).
Metformin may decrease or affect the ability of breast cancer cells to grow and may work with other therapies to keep cancer from recurring. Health Canada has not approved the sale or use of Metformin to treat breast cancer. Although Metformin is approved by the FDA for the treatment of diabetes, its use in breast cancer is considered investigational;

A US study that began the enrolment of 70 women in 2009 is estimated to be finalised in mid-2012 (Clinical Trials Identifier NCT00941330). This study randomised participants to two active comparator arms: chemotherapy using docetaxel and cytoxan or hormonal therapy using exemestane, with the aim of ascertaining whether or not therapy would shrink the size of the breast tumour, preserving the breast or resulting in less extensive surgery. All participants underwent testing with the Oncotype DX® assay prior to entering the study to determine the likelihood of benefit from chemotherapy or hormonal therapy. Only those women with an Oncotype DX® recurrence score less than or equal to 24 were eligible to participate. Patients with hormone receptor-positive breast cancers with recurrence scores less than or equal to 24 have been previously demonstrated to obtain a larger benefit from hormonal therapy, compared to chemotherapy, when these agents are given after surgery.

A similar multi-centre study began enrolment of 60 women with breast cancer in 2011 and aims to finalise data collection in early 2013 (Clinical Trials Identifier NCT01293032). The primary aim of this study was to determine the effectiveness of hormone therapy or chemotherapy, based on gene expression analysis prior to surgery, in the treatment of patients with breast cancer. Participants were stratified according to their risk of recurrence as determined by the Oncotype DX® assay. Two patient groups (group I: risk score of <11, group II: risk score 11-25) received neoadjuvant hormonal therapy comprising tamoxifen (pre-menopausal women) or an aromatase inhibitor (post-menopausal women) for 4-6 months in the absence of disease progression or unacceptable toxicity. The outcomes in these women were compared to two patient groups (group III: risk score 11-25, group IV: risk score >25) who received 6-8 courses of neoadjuvant chemotherapy comprising an anthracycline/taxane based regimen over 4-6 months in the absence of disease progression or unacceptable toxicity.

SUMMARY OF FINDINGS

No randomised controlled trials were identified. The majority of literature identified described the use of either the Oncotype DX or MammaPrint assays; however there are weaknesses in the evidence base for these two assays. Most studies of the included studies were retrospective with a relatively small sample size. Data suggests that both the Oncotype DX and MammaPrint tests are highly predictive of distant recurrence within 5-10 years. Test such as the Breast Cancer Index and IHC4 appear promising but are still in the early phases of development and validation and require more research. Although both of the Oncotype DX and MammaPrint assays returned acceptable incremental cost-effectiveness ratios, the
IHC4 test may be the more cost-effective option. A number of randomised controlled trials are currently underway and it would be prudent to await the results of these studies and of those on the newer tests such as the IHC4. No current or planned studies could be identified that compare all profiling tests on the same patients. In addition, a cost-effectiveness analysis conducted from the Australian/New Zealand perspective would be informative with consideration given to the additional cost of tests that may require the use of fresh frozen tissue instead of paraffin embedded, formalin fixed tissue samples.

2012 HealthPACT Advisory:

There is a large amount of low-level, though moderate to high quality, evidence currently available describing many of these technologies. The NICE systematic review is an update of the current evidence and it states that the clinical utility of these technologies has not as yet been demonstrated. It is unlikely that additional research at this time would add further to this evidence base, therefore HealthPACT has recommended that these technologies be monitored for further information in 24 months.

2012 Number of Studies Included

All evidence included for assessment in this Technology Brief has been assessed according to the revised NHMRC levels of evidence. A document summarising these levels may be accessed via the following link on the HealthPACT web site.

This brief is based on the results of the NICE systematic review, and although usually considered a high level of evidence, the strength of a systematic review is only as good as the strength of the studies included for assessment. The majority of the studies included in the NICE systematic review were retrospective cohorts and therefore the overall level of evidence of this review is level III-3 prognostic evidence.

Search Criteria to be used (MeSH Terms)

Breast Neoplasms
Gene Expression Profiling
Gene Expression
Prognosis
Individualized Medicine
Microarray Analysis
Reverse Transcriptase Polymerase Chain Reaction
Neoplasm Recurrence, Local
Biological Assay
Female
Humans