The Oncotype DX® Assay
A Genomic Approach to Breast Cancer
Pathology: 20\textsuperscript{th} and 21\textsuperscript{st} Century

<table>
<thead>
<tr>
<th>Size</th>
<th>Age</th>
<th>Phenotype</th>
<th>Nodal status</th>
<th>Protein/Gene “Genomic Profiling”</th>
</tr>
</thead>
</table>
Prognostic & Predictive Markers Used in Breast Cancer Management

<table>
<thead>
<tr>
<th>Prognostic (recurrence risk)</th>
<th>Predictive (treatment benefit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Axillary node status</td>
<td>• ER/PR status</td>
</tr>
<tr>
<td>• Histologic type/grade</td>
<td>• HER2 \textit{neu} status</td>
</tr>
<tr>
<td>• Tumour size</td>
<td>• \textit{Oncotype DX} \textsuperscript{®} test</td>
</tr>
<tr>
<td>• Patient age</td>
<td></td>
</tr>
<tr>
<td>• Lymphatic/Vascular invasion</td>
<td></td>
</tr>
<tr>
<td>• ER/PR status</td>
<td></td>
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<tr>
<td>• HER2 \textit{neu} status</td>
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<tr>
<td>• Endopredict</td>
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<td>• Prosigna</td>
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<tr>
<td>• uPA PAI1</td>
<td></td>
</tr>
<tr>
<td>• \textit{Oncotype DX} \textsuperscript{®} test</td>
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</tr>
</tbody>
</table>

These markers can be used to estimate the risk of disease recurrence

These markers can be used to predict treatment benefit

Role of traditional markers in ER+ EBC

• Markers are used to determine diagnosis, estimate prognosis and to inform treatment decisions
• Some markers (tumour grade, nodal status and genomic tests) are prognostic (PR); some are predictive of treatment benefit. Some are both predictive and prognostic (ER, HER2 and the Oncotype DX® test)
• Standardisation and / or reproducibility of a test may present a challenge
• Variability in interpretation of results
• Despite the use of many markers, a large proportion of patients are classified as intermediate risk and there is a population in whom treatment decisions are not clear

The **OncoType DX**® Breast Cancer Test

- It is a 21 gene genomic test (16 tumour genes and 5 reference genes)\(^1\)
- Uses RT-PCR technology on formalin fixed tissue\(^1\)
- Quantitatively predicts the likelihood of breast cancer recurrence in women with newly diagnosed, invasive EBC\(^2,3\)
- Is the only assay that has been demonstrated to be predictive of likelihood of benefit from chemotherapy\(^2,3\)
- Is included clinical practice guidelines (St Gallen, ESMO, ASCO\(^\circledR\), NCCN\(^\circledR\)) and NICE\(^4-7\)

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The **Oncotype DX®** Assay Uses a Genomic Approach to Predict Recurrence Risk and Response to Adjuvant Therapy

16 Cancer and 5 Reference Genes From 3 Studies

**PROLIFERATION**
- Ki-67
- STK15
- Survivin
- Cyclin B1
- MYBL2

**OESTROGEN**
- ER
- PR
- Bcl2
- SCUBE2

**INVASION**
- Stromelysin 3
- Cathepsin L2

**HER2**
- GRB7
- HER2

**CD68**
- Beta-actin
- GAPDH
- RPLPO
- GUS
- TFRC

**GSTM1**
- BAG1

**RS** = + 0.47 x HER2 Group Score - 0.34 x ER Group Score + 1.04 x Proliferation Group Score + 0.10 x Invasion Group Score + 0.05 x CD68 - 0.08 x GSTM1 - 0.07 x BAG1

<table>
<thead>
<tr>
<th>Category</th>
<th>RS (0 -100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>RS &lt;18</td>
</tr>
<tr>
<td>Int risk</td>
<td>RS 18 - 30</td>
</tr>
<tr>
<td>High risk</td>
<td>RS ≥ 31</td>
</tr>
</tbody>
</table>


RS, Recurrence Score® result
The Oncotype DX® Assay Process

GHI has processed >450,000 tests from >70 countries; > 2,800 tests from the UK

ORDER ENTRY
- Online or Fax
- Fax Request FedEx

SHIPPING
- Specimen Retrieval
- Specimen Accessioning

PATHOLOGY
- Pathology Review
- Histopath

ANALYTICAL LABORATORY
- Extraction
- Quantitation
- gDNA Detection
- Reverse Transcription
- QPCR

REPORT FULFILLMENT
- Results Generation
- Report Delivery
- Materials Return

MATERIAL RETURN
- FedEx

Secure Online Portal

10-14 working days

1. GHI Data on file August 2014
Suitable Tumour Material for the Oncotype DX® Invasive Breast Cancer Assay

FFPE tumour blocks (or 15 unstained slides) are used for the analysis, they should contain

• The largest area of highest grade invasive carcinoma (a minimum of 2mm)
• Microinvasive carcinomas (foci <0.1 cm are not unsuitable)
• The preferred fixative is 10% neutral buffered formalin
• The Oncotype DX Breast Cancer Assay was clinically validated using tumour tissue from primary breast tumours and not from a lymph node
• All unused material is returned to the pathology laboratory

Validated as a prognosticator and a predictor of chemotherapy benefit in ER+ EBC

Quantifies expression of a panel of genes which is expressed as a Recurrence Score® result

The Recurrence Score result is a continuous value ranging from 0 to 100; and classifies patients into 3 risk categories for guidance:

- Low (<18) minimal if any benefit from adjuvant chemotherapy\(^1,2\)
- Intermediate (18-30)\(^1,2\)
- High (≥31) significant benefit from adjuvant chemotherapy\(^1,2\)

The OncoType DX® Recurrence Score® result is a Continuous Predictor of Distant Recurrence Risk

What is the 10-year probability of distant recurrence for a patient with a Recurrence Score result of 30?

Dotted lines represent 95% CI

Recurrence Score® Result 30 = 20% risk of distant recurrence at 10 years

The Recurrence Score® Result Assesses Individual Tumour Biology for ER+ Breast Cancer

The graph shows the distant recurrence at 10 years against the Recurrence Score value. The continuous biology is indicated by two curves, one for LOW RECURRENCE SCORE DISEASE and another for HIGH RECURRENCE SCORE DISEASE.

LOW RECURRENCE SCORE DISEASE
- Indolent
- Hormone therapy-sensitive
- Minimal, if any, chemotherapy benefit

HIGH RECURRENCE SCORE DISEASE
- Aggressive
- Less sensitive to hormone therapy
- Large chemotherapy benefit

The Recurrence Score® Result Quantifies the Risk of Distant Recurrence (Prognosis)

Likelihood of recurrence according to Recurrence Score categories in the NSABP B14 study\(^1\)

28 recurrences in low risk group
25 in intermediate group
56 in high risk group

UK decision impact data\(^2\)
(n=142), N0,1 ER+, EBC patients

\(p<0.001\)


RS, Recurrence Score® result
The Oncotype DX® Report Provides Valuable Information Along a Continuum of ER+ Breast Cancer

- The Oncotype DX® report currently provides information on node positive and negative disease:
  - Prognosis
  - Predicted chemotherapy benefit
  - Quantitative data on ER, PR and HER2

- There are three separate Recurrence Score® reports:
  - Node negative
  - 1-3 positive nodes
  - ≥4 positive nodes

- Node-positive report contains an additional page with prognosis and predicted chemo benefit information specific to node-positive patients
Node Negative Report: Risk of Recurrence and Prediction of Chemotherapy benefit

Prognosis: 10-Year Risk of Distant Recurrence after 5 Years of Tam, Based on the Recurrence Score Result (from NSABP B-14)

- Tam Alone: 5% (95% CI: 3%-7%)
- Tam + Chemo

10-Year Risk of Distant Recurrence

Recurrence Score Result:

- Oncotype DX Breast Cancer Assay uses RT-PCR to determine the expression of a panel of 21 genes in tumor tissue. The Recurrence Score result is calculated from the gene expression results and ranges from 0-100.

Clinical Experience: The following results are from a clinical validation study that included 2751 patients from the NSABP B-14 study. The study included female patients with stage I or II node-negative (N0) breast cancer treated with 5 years of tamoxifen (tam). It is unknown whether the findings apply to other patients outside these criteria.

The findings are applicable to women who had stage I or II node-negative (N0) estrogen receptor positive (ER+) breast cancer who were treated with 5 years of tamoxifen (tam). It is unknown whether the findings apply to other patients outside these criteria.

Prediction of Chemotherapy Benefit after 5 Years of Tam, Based on the Recurrence Score Result (from NSABP B-20)

- Low Risk: 10-Year Risk of Distant Recurrence
- Intermediate Risk: 10-Year Risk of Distant Recurrence
- High Risk: 10-Year Risk of Distant Recurrence

Absolute Benefit of Chemotherapy at 15 Years by Recurrence Score Risk Group

- Low Risk
- Intermediate Risk: 15-35
- High Risk: >35

The findings are applicable to women who had stage I or II node-negative (N0) estrogen receptor positive (ER+) breast cancer who were treated with 5 years of tamoxifen (tam). It is unknown whether the findings apply to other patients outside these criteria.
Node positive report 1-3 N+ (SWOG 8814 study, 5 yr Risk of Recurrence)
Report Guidance

Both the node negative and node positive invasive breast cancer reports are provided because the patient’s nodal status was marked Micromets (pN1mi, 0.2-2.0 mm) on the test requisition form.

Advances in breast cancer diagnostics and in histopathological and molecular analysis techniques have resulted in an increase in the number of women diagnosed with micrometastatic (pN1mi) breast cancer (≤2 mm axillary node metastasis). Studies report conflicting results regarding the clinical significance and implications of these micrometastases, with some data suggesting they do not confirm increased risk for distant recurrence and other data suggesting that they do.

For women with disease that is ER+ and who have micrometastasis, clinical practice varies, with some women receiving adjuvant chemotherapy and others prescribed endocrine therapy alone (NCCN 2013).

Should you have any questions, please contact Genomic Health Customer Service.
Quantitative Single Gene Expressions within The Onco\textit{type} DX\textsuperscript{®} Breast Cancer Assay
Single-Gene Testing in the Oncotype DX® Assay Addresses Limitations with Current Methodologies

- Both IHC and FISH are associated with variability that can affect the accuracy of test results
- The impact of preanalytical variability can be minimised by “normalisation” strategies used in quantitative gene expression assessment as performed by quantitative RT-PCR in the Oncotype DX assay
Continuous Measurement of ER/PR is Reflective of Tumour Biology

Reproducibility of the assay has a standard deviation of less than 0.4 units

# High Degree of Concordance between RT-PCR and FISH for HER2

## HER2 concordance 2 x 2

<table>
<thead>
<tr>
<th>ECOG 2197*</th>
<th>Central IHC+</th>
<th>Central IHC–</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncotype® DX+</td>
<td>94 (78%)</td>
<td>4 (1%)</td>
<td>98</td>
</tr>
<tr>
<td>Oncotype DX–</td>
<td>27 (22%)</td>
<td>439 (99%)</td>
<td>466</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>121</td>
<td>443</td>
<td>564</td>
</tr>
</tbody>
</table>

**CONCORDANCE 95%* [95% CI (92%, 96%) Kappa 83%, 95% CI (77%, 88%)]**

Concordance calculated as (94 + 439)/564

<table>
<thead>
<tr>
<th>Kaiser Study*</th>
<th>Central FISH+</th>
<th>Central FISH–</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncotype DX+</td>
<td>55 (98%)</td>
<td>11 (3%)</td>
<td>66</td>
</tr>
<tr>
<td>Oncotype DX–</td>
<td>1 (2%)</td>
<td>408 (97%)</td>
<td>409</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>56</td>
<td>419</td>
<td>475</td>
</tr>
</tbody>
</table>

**CONCORDANCE 97%* [95% CI (96%, 99%), Kappa 89%, 95% CI (82%, 95%)]**

Concordance calculated as (55 + 408)/475

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*See Appendix (slides 34-39) for in-depth information on these studies*
The Oncotype DX® Assay also provides Quantitative Data for ER, PR, HER2

- Provides additional insight into the biology of individual tumours
- A useful independent "control" for pathologists
- Not validated for prediction of treatment benefit
Weak correlation between Recurrence Score® Result and Ki67

There is only moderate correlation between the Recurrence Score result and Ki-67 even when Ki67 is assessed centrally by a single pathologist.

Adapted from Glutz et al. SABCS 2011 Abstract S4-3
Onco
type DX® Clinical Validation: NSABP B-14 Trial

- Objective: Prospectively validate the Recurrence Score® result as a predictor of distant recurrence in node-negative, ER+ patients

- Multicentre study with prespecified 21-gene assay, algorithm, endpoints, analysis plan

Onco
type DX® Clinical Validation:
NSABP B-14 Trial, Distant Recurrence

Distant recurrence over time

Proportion without distant recurrence

- All Patients, n = 668
- RS < 18, n = 338
- RS 18-30, n = 149
- RS ≥ 31, n = 181

10-Year rate of recurrence = 6.8%*
95% CI: 4.0%, 9.6%

10-Year rate of recurrence = 14.3%
95% CI: 8.3%, 20.3%

10-Year rate of recurrence = 30.5%*
95% CI: 23.6%, 37.4%

*10-Year distant recurrence comparison between low- and high-risk groups: \( P < 0.001 \)


RS, Recurrence Score® result
Objective: Prospectively determine the relationship between Recurrence Score® result and chemotherapy benefit in node-negative, ER+ patients

Multicenter study with prespecified 21-gene assay, algorithm, endpoints, analysis plan

High Recurrence Score® Result Correlates with Greater Benefit from Chemotherapy (NSABP B-20 trial)

Poor Concordance between Central and Local Grade Assessment

Comparison from the Phase III WSG-Plan B Trial

Concordance ~68%

<table>
<thead>
<tr>
<th>Local grade</th>
<th>Central grade</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1 N (%)</td>
<td>G2 N (%)</td>
</tr>
<tr>
<td>G1 N (%)</td>
<td>57 (1.9)</td>
<td>116 (4.0)</td>
</tr>
<tr>
<td>G2 N (%)</td>
<td>76 (2.6)</td>
<td>1305 (44.6)</td>
</tr>
<tr>
<td>G3 N (%)</td>
<td>6 (0.2)</td>
<td>222 (7.6)</td>
</tr>
<tr>
<td>Overall</td>
<td>139 (4.7)</td>
<td>1643 (56.1)</td>
</tr>
</tbody>
</table>

Classical Clinicopathological Criteria can't Predict the Recurrence Score® Result

- Small tumours have proportionately fewer high Recurrence Score values
- However, there is a range of Recurrence Score values across both categories of tumour size

The Oncotype DX® Assay
The Only Multi-gene Assay Incorporated into all Major Guidelines to Predict Adjuvant Chemotherapy Benefit in ER+, HER2- EBC

<table>
<thead>
<tr>
<th><strong>NCCN Guidelines</strong>&lt;sup&gt;®&lt;/sup&gt;</th>
<th>&gt; 0.5 cm, node negative, N1mi</th>
<th>Quantifies risk of recurrence as a continuous variable and predicts responsiveness to both tamoxifen and chemotherapy&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASCO® Guidelines</strong></td>
<td>Node negative</td>
<td>Predicts the risk of recurrence and may be used to identify patients likely to benefit from tamoxifen or chemotherapy&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>ESMO</strong></td>
<td>Node negative</td>
<td>Provides additional prognostic and/or predictive information to complement pathology assessment and to predict response to adjuvant chemotherapy&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>St Gallen Consensus</strong></td>
<td>Node negative, node positive</td>
<td>Provides not only prognostic but also predictive information regarding the utility of cytotoxic therapy in addition to endocrine therapy&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>NICE</strong></td>
<td>Node negative</td>
<td>Recommended as an option for guidance of chemotherapy decisions in patients at intermediate risk&lt;sup&gt;*&lt;/sup&gt; of distant recurrence&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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*Intermediate risk of distant recurrence is defined as NPI score ≥ 3.4 or at intermediate risk by other decision making tools or protocols.
NICE and UK Funding
NICE Recommendations for the Oncotype DX® Breast Cancer Assay

• The Oncotype DX assay is recommended as an option for guiding adjuvant chemotherapy decisions for people with oestrogen receptor positive (ER+), lymph node negative (LN−) and human epidermal growth factor receptor 2 negative (HER2−) early stage invasive breast cancer if:

  – the person is assessed as being at intermediate risk and

  – information on the biological features of the cancer provided by the Oncotype DX assay is likely to help in predicting the course of the disease and would therefore help when making the decision about prescribing chemotherapy and

  – the manufacturer provides the Oncotype DX assay to NHS organisations according to the confidential arrangement agreed with NICE

• Genomic Health is making every effort to achieve NHS funding for this test
NICE Recommends the Oncotype DX® Breast Cancer Assay for Intermediate Risk Patients

- NPI>3.4 used as a proxy for the cost-effectiveness analysis
- Does not restrict the use of the Oncotype DX test to patients whose risk is assessed by NPI
- Access to the Oncotype DX test where treatment is not clear based on conventional clinicopathologic measures

The Nottingham Prognostic Index is calculated using the following formula:

- tumour size in cm x 0.2
- + lymph-node stage (1, 2 or 3)*
- + histologic grade (1, 2 or 3)

NPI Score

Therefore a patient with:
- Tumour size= 2.1
- Grade II
- Node Negative

NPI = 3.42
(Eligible for the Oncotype DX® test)

*When only node negative patients are analysed the lymph-node stage is 1 for all cases.

Which Patients may benefit from the Oncotype DX® Assay?

Clinical indication

Newly Diagnosed Early Stage Invasive Breast Cancer

- Node-negative or with *1 to 3 positive nodes
- Metastatic or locally advanced breast cancer with 4+ positive nodes
- ER-pos, HER2-neg
- HER2-pos
- Triple-neg

Use of the Oncotype DX® breast cancer assay in the N+ setting validated for post-menopausal patients

NICE guidance

Newly Diagnosed Early Stage Invasive Breast Cancer

- Node-negative, ER-positive, HER2-negative

The patient is assessed as being at intermediate risk; the decision to prescribe chemotherapy remains unclear, so that information on the biological features of the cancer provided by the Oncotype DX assay is likely to help in predicting the course of the disease


ER: Oestrogen receptor
HER2: Human Epidermal Growth Factor Receptor 2
How are Eligible Patients Defined according to NICE Guidance?

According to NICE guidance DG10:

- Intermediate risk of distant recurrence being defined as an NPI score > 3.4, which can be calculated from information that is routinely collected about people with breast cancer

- Intermediate risk is not defined by Adjuvant!

- Other decision-making tools or protocols are also currently used in the NHS may also be used to identify people at intermediate risk

- In reality, in the ER+, N0 group, this may be
  - Grade 1 tumours: >7cm
  - Grade 2 tumours: Between 2cm and 12 cm
  - Grade 3 tumours: <7cm

Conclusions

- Recurrence Score® results reflects individual tumour biology
- The Oncotype DX® test quantitatively predicts the Risk of distant Recurrence and assesses the likely benefit of chemoendocrine therapy (Level IB Evidence)
- The risk of distant recurrence or chemotherapy benefit can't be accurately predicted by relying on clinical and pathological variables alone
- The Oncotype DX test is supported by the most substantial body of evidence as a prognosticator and is the only assay that has proved predictive of chemotherapy benefit
- Only assay incorporated into ASCO®, NCCN®, ESMO, St Gallen and NICE guidelines
- More than 450,000 patients tested to date worldwide, with more than 2,800 patients tested in the UK by June 2014
Thank you