MammaPrint
The current situation and future developments

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株式会社DNAチップ研究所 -
MammaPrint（マンマプリント）-
Breast Cancer
Invasive Ductal Carcinoma

- invasive ductal carcinoma
- lymphangiogenesis
- blood vessel
Breast Cancer - Survival
Kaplan-Meier Survival Curves

~30% die of breast cancer
-> adjuvant therapy can be beneficial

~70% survive breast cancer
-> adjuvant therapy is not beneficial

Which Breast Cancers Return?
Breast Cancer Treatment Options

After surgery and Radiotherapy:
1) Who to treat, 2) How to treat - what drugs

Surgery  Local Radiotherapy  (Neo-)Adjuvant systemic therapy
1. Chemotherapy
2. Endocrine therapy
3. Targeted therapy
Need and benefit of adjuvant treatment

- Risk of recurrence and death → Prognosis
- Likelihood of benefit from therapy based on overall biology and/or expression of target → Prediction
Of 100 women with breast cancer (stage 1/2)
…………~25% will develop a recurrence
75% of all patients is treated with chemotherapy
So, overall 50% of patients receive toxic chemotherapy of which they do not benefit, but may suffer the toxic side-effects.

Can we do better?
The Microscope, 350 years

Van Leeuwenhoek microscope, 17th century
(Hospital of Netherlands Cancer Institute is named Antoni van Leeuwenhoek Hospital)

Digital microscope, 21st century
30 years of progress in cancer research

1979

2009
New diagnostics of cancer: from micro-scope to micro-array to micro-xxx
Comprehensive set shows the picture

70 gene MammaPrint signature; Recurrence Score H/ITM (HOXB13/IL17BR); Genomic Grade; 76 gene Rotterdam signature
DNA microarray technology:

- Allows us to determine the activity of thousands of genes in a single experiment

*gene expression signature*

*expression profiling*
DNA microarray technology

- Provides patterns that allow you to recognize different etiological origin, different classes of outcome of disease (prognosis, treatment response)
Multi Gene Expression Profiles in Clinical Practice

Unfixed sample of tumor tissue

Surgical removal of tumor tissue

Tumor RNA

Labeled control cDNA or cRNA

Labeled tumor cDNA or cRNA

Comparative analysis of gene expression

Molecular signature

Poor prognosis

Good prognosis
Breast Cancer - Survival
Kaplan-Meier Survival Curves

~30% die of breast cancer
-> adjuvant therapy can be beneficial

~70% survive breast cancer
-> adjuvant therapy is not beneficial

Which Breast Cancers Return?
Current Clinical Management

*lymph node negative breast cancer*

*adjuvant treatment selection criteria*

- (US or EU) consensus criteria: > 80%

As only 25-30% of these patients develop distant metastases, some 40-60% of patients are over-treated with adjuvant (chemo)therapy, some may be undertreated
Clinicopathological Risk Assessment
Adjuvant! Online

Patient Information
Age: 50
Comorbidity: Average for Age
ER Status: Positive
Tumor Grade: Grade 3
Tumor Size: 2.1 - 3.0 cm
Positive Nodes: 0
Calculate For: Mortality
10 Year Risk: 24 Prognostic

Adjuvant Therapy Effectiveness
Horm: Overview 98 (Tamoxifen)
Chemo: Overview 98 (CMF-Like)
Hormonal Therapy: 28
Chemotherapy: 11
Combined Therapy: 36

No additional therapy:
- 72.2 alive in 10 years.
- 23.5 die of cancer.
- 4.3 die of other causes.

With hormonal therapy: Benefit = 5.9 alive.

With chemotherapy: Benefit = 2.3 alive.

With combined therapy: Benefit = 7.7 alive.

www.adjuvantonline.com/
The Problem For Using Chemotherapy
(Most Common Presentation Of Breast Cancer Today: T1 N0 ER+ Grade 2)

Need To Treat 100 Women

And Only One Benefits!

Courtesy Peter Ravdin
Scanned image of 25K human oligonucleotide microarray

Hybridized with mixture of ‘red’-labeled cRNA of a tumor sample and ‘green’-labeled reference cRNA

Determine:
- fluorescence intensities
- recognize patterns related to clinical parameter over a series of tumors

Breast Cancer retrospective series
n=78 with known outcome

Nature, 2002
Tumor samples of known clinical outcome

Development of 70 gene MammaPrint

Unbiased full genome gene expression analysis

Prognosis reporter genes

Distant metastases group

No distant metastases group

Nature, 2002
70 Gene MammaPrint Signature

Supervised analysis on n=78 tumors, >96% adjuvantly untreated

van’t Veer et al., Nature 415, p. 530-536, 2002

threshold set with 10% false negatives
91% sensitivity, 73% specificity
Proliferation, angiogenesis, adhesion to extracellular matrix, local invasion, intravasation, survival, extravasation.

**Proliferation, Angiogenesis, Adhesion to Extracellular Matrix, Local Invasion, Intravasation, Survival, Extravasation**

**COL4A2, FLT1, FGF18, MMP9**

**MMP9, COL4A2**

**COL4A2, FLT1, MMP9, TGFB3, MTDH, DIAPH3, PALM2, DCLK2, NMU, NMUR1, NMUR2**

**MMP9, COL4A2**

**COL4A2, FLT1, FGF18, MMP9**

Nature, 2002
Breast Cancer – MammaPrint signature
Confirmation on Retrospective Consecutive series

n= 151; Distinguishes in 40% good profile, 60% poor profile

151 patients, <53, LN0
~95% adjuvantly untreated
10 year survival curve

NEJM, 2002
Improved Clinical Management
MammaPrint and tumor diameter (LN0, <53)

Small tumors, < 15mm

Profiling:
40% in good profile
60% in poor profile

Small Tumors generally considered low risk, more than half may be at (MammaPrint) high risk.

UNDERTREATMENT!

NEJM, 2002
International Validation 70-gene signature

TransBIG - 5 European Hospitals, 302 pts, adjuvantly untreated

Overall survival by gene signature risk

Buyse, JNCI, 2006
ADJUVANT! ONLINE FOR BREAST CANCER

“Clinical low risk” defined as predicted 10-year survival probability

≥ 88% for ER+ patients
≥ 92% for ER- patients

Buyse, JNCI, 2006
Risk assessment 302 patients
TransBIG - 5 European Hospitals

MammaPrint identifies
High risk 63%
Low risk 37%

Adjuvant!online identifies
High risk 74%
Low risk 26%
more high risk!

but also:
Concordant cases 65%
Discordant cases 35%

Adjuvant! “Clinical low risk” defined as 10-year survival probability
≥ 88% for ER+ patients
≥ 92% for ER- patients

Buyse, JNCI, 2006
Metastasis-free survival
70 genes vs Adjuvant!
TransBIG - 5 European Hospitals

70 gene signature

Adjuvant!

Low risk signature
Low risk Adjuvant!

High risk signature
High risk Adjuvant!

Discordant cases better predicted by 70 gene prognosis signature

Buyse, JNCI, 2006
75% of patients receive toxic chemotherapy
Current clinicopathological risk assessment

50% of patients receive toxic chemotherapy of which they do not benefit, but may suffer the toxic side-effects. Some patients who need chemotherapy may not be selected.
MammaPrint risk assessment

Improving assignment: less over- and under-treatment
MammaPrint from Research to Diagnostics

Current Achievements:

- Retrospective validation - Completed
- Prospective Technology assessment - Cost-effectiveness
- Diagnostic test - International CE marked
- Laboratory - CLIA registered
- Diagnostic test - ISO17025 certified
- Diagnostic test - CAP accredited
- Diagnostic test and clinical use - FDA approved, IVD MIA feb07
- Treatment Recommendations - Dutch Guidelines 08
- Treatment Recommendations - StGallen 09 International Guidelines

Reproducibility
Test Result >98%
Success rate >95%
Glas et al,
BMC Genomics 2006
Clinical Utility MammaPrint

Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER)

Jolien M Bueno-de-Mesquita, Wim H van Harten, Valesca P Retel, Laura J van ’t Veer, Frits S A M van Dam, Kim Karsenberg, Kirsten F L Douma, Harm van Tinteren, Johannes L Peters et al., Jelle Wesseling, Tim S Wu, Douwe Atsma, Emiel J T Rutgers, Guido Brink, Arno N Floore, Annuiska M Glas, Rudi M H Roumen, Frank E Bellot, Cees van Krimpen, Sjoerd Rodenhuis, Marc J van de Vijver, Sabine C Linn

Prospective trial implementing MammaPrint, 2003-2006
PIs Sabine Linn, Marc van de Vijver
Sponsor: Dutch Health Insurance Council

Discordant cases MammaPrint signature versus Guidelines The Netherlands and Adjuvant-on-line

<table>
<thead>
<tr>
<th>Clinical risk (Dutch CBO guidelines)</th>
<th>70-gene prognosis signature, n (%) (n=427)</th>
<th>Discordant findings, n (%), 95% CI, kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good (n=219)</td>
<td>Poor (n=208)</td>
</tr>
<tr>
<td>Low (n=243)</td>
<td>167 (39)</td>
<td>76 (18)*</td>
</tr>
<tr>
<td>High (n=184)</td>
<td>52 (12)*</td>
<td>132 (31)</td>
</tr>
<tr>
<td>Clinical risk (Adjuvant! Online)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (n=133)</td>
<td>96 (22)</td>
<td>37 (9)*</td>
</tr>
<tr>
<td>High (n=294)</td>
<td>123 (29)*</td>
<td>171 (40)</td>
</tr>
</tbody>
</table>

~30 % discordant cases led in ~20% to adapted treatment advise

Clinical Utility of MammaPrint

1. Risk Assessment

- Assign patients to risk categories with high specificity (low risk vs high risk for recurrence)
- Low risk sufficiently low to forego chemotherapy
Adjuvant treatment decided by risk
MammaPrint stratification in low and high risk of relapse

Low risk: no chemotherapy, ERpos endocrine th
High risk: chemotherapy and endocrine therapy

Do high risk patients benefit from chemo?
Clinical Utility and Clinical Benefit
MammaPrint

1. Risk Assessment
   - Assign patients to risk categories with high specificity (low risk vs high risk for recurrence)
   - Low risk sufficiently low to forego chemotherapy

2. Chemo Benefit “the chemotherapy choice”
   - High risk should identify patients with early relapse (relevant for chemotherapy benefit)
   - High risk should show clinical benefit for chemotherapy
Response to neo-adjuvant Chemotherapy and MammaPrint

- Netherlands Cancer Institute
- 2 clinical trials
- T-stage >3 cm
  - and/or LNplus (SNB/FNA)
- ultrasound guided
  - 14 gauge biopsies
- MRI imaging
- Pathology

Eligible patients
N=167

Good prognosis-signature
N=23 (14%)

pCR(axilla+breast)
n=0

Poor prognosis-signature
N=144 (86%)

pCR(axilla+breast)
n=29 (20%)
P=0.015

pCR: pathological complete remission

MammaPrint low risk signature -> no benefit of chemotherapy
MammaPrint high risk signature -> benefit of chemotherapy

Straver et al, BCRT 2009, presented at AACR2009

- Antracycline-like
- Antracyclin-Taxane
- Taxane
Neo-adjuvant Standard Chemotherapy and MammaPrint Clinical Benefit

• MammaPrint High Risk Signature patients show significantly higher chemosensitivity

• All pCR are found in the High Risk Signature group

High Risk Signature Patients show Clinical Benefit of Chemotherapy

Low Risk Signature Patients do not show Clinical Benefit of Chemotherapy
Adjuvant Standard Chemotherapy and MammaPrint Clinical Benefit

Meta-analysis 70 gene signature in Lymph node negative and 1-3 positive node patients

• adjuvant endocrine therapy (tam)

OR

• endocrine (tam) plus chemotherapy

Knauer et al, abstracts StGallen, ASCO and submitted, Albain et al 2009
Breast Cancer Specific Survival (5 yrs) 
Endocrine vs Endocrine-Chemo 
within MammaPrint low and high risk (n=772)

MammaPrint Low risk (n=268)

- 99% Endocrine & Chemo (n=78)
- Benefit ns
- 97% Endocrine (n=174)

MammaPrint High risk (n=307)

- 94% Endocrine & Chemo (n=148)
- Benefit 13%
- 81% Endocrine (n=141)

Interaction term for differential effect p=0.45

Knauer et al, abstracts StGallen, ASCO and submitted, Albain et al 2009
MammaPrint Low risk - Cox multivariate analysis:
BCSS at 5 years for ET vs. ET + CT

<table>
<thead>
<tr>
<th>MammaPrint low risk</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Age at diagnosis (by year)</td>
<td>1.00 (0.88-1.15)</td>
<td>0.95</td>
</tr>
<tr>
<td>Tumorsize (by cm)</td>
<td>0.98 (0.89-1.10)</td>
<td>0.77</td>
</tr>
<tr>
<td>No. of positive nodes (0-3)</td>
<td>1.09 (0.37-3.16)</td>
<td>0.88</td>
</tr>
<tr>
<td>Grade</td>
<td>0.57 (0.12-2.82)</td>
<td>0.49</td>
</tr>
<tr>
<td>ER-positive status</td>
<td>∞ (0-∞)</td>
<td>0.99</td>
</tr>
<tr>
<td>PR-positive status</td>
<td>0.09 (0.01-0.90)</td>
<td>0.04</td>
</tr>
<tr>
<td>HER2-positive status</td>
<td>∞ (0-∞)</td>
<td>0.99</td>
</tr>
<tr>
<td>Adjuvant therapy: ET vs. ET+CT</td>
<td>∞ (0-∞)</td>
<td>0.98</td>
</tr>
</tbody>
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Knauer et al, abstracts StGallen, ASCO and submitted 2009
MammaPrint High risk - Cox multivariate analysis: BCSS at 5 years for ET vs. ET + CT

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<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>Age at diagnosis (by year)</td>
<td>0.96 (0.91-1.02)</td>
<td>0.17</td>
</tr>
<tr>
<td>Tumorsize (by cm)</td>
<td>1.05 (1.01-1.09)</td>
<td>0.02</td>
</tr>
<tr>
<td>No. of positive nodes (0-3)</td>
<td>1.39 (0.95-2.03)</td>
<td>0.09</td>
</tr>
<tr>
<td>Grade</td>
<td>1.03 (0.48-2.19)</td>
<td>0.94</td>
</tr>
<tr>
<td>ER-positive status</td>
<td>0.48 (0.18-1.34)</td>
<td>0.16</td>
</tr>
<tr>
<td>PR-positive status</td>
<td>0.31 (0.09-1.03)</td>
<td>0.06</td>
</tr>
<tr>
<td>HER2-positive status</td>
<td>0.72 (0.25-2.10)</td>
<td>0.55</td>
</tr>
<tr>
<td>Adjuvant therapy: ET vs. ET+CT</td>
<td>0.21 (0.06-0.80)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Knauer et al, abstracts StGallen, ASCO and submitted 2009
Adjuvant Standard Chemotherapy and MammaPrint Clinical Benefit

- MammaPrint High Risk signature patients show significant chemo-sensitivity
  (number needed to treat 30)
- MammaPrint Low Risk Signature group does not show significant chemo benefit
  (number needed to treat 333)

MammaPrint High Risk Signature Patients show substantial Clinical Benefit of Adjuvant Chemotherapy

(Cave: not a randomized trial!)

Knauer et al, abstracts StGallen, ASCO and submitted, Albain et al 2009
MammaPrint
current clinical implementation

• FDA approved (only prognostic IVDMIA for breast cancer)
• Dutch CBO guidelines for treatment of breast cancer (2008)
• StGallen International guidelines for treatment of breast cancer (published July 2009)
## St Gallen International Expert Consensus 2009

**Table 3. Chemoendocrine therapy in patients with ER-positive, HER2-negative disease**

<table>
<thead>
<tr>
<th>Clinicopathological Features</th>
<th>Relative Indications for Chemoendocrine therapy</th>
<th>Factors Not Useful for Decision</th>
<th>Relative Indications for Endocrine Therapy Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER, PgR</td>
<td>Lower ER and PgR level</td>
<td></td>
<td>Higher ER and PgR level</td>
</tr>
<tr>
<td>Histological Grade</td>
<td>Grade 3</td>
<td>Grade 2</td>
<td>Grade 1</td>
</tr>
<tr>
<td>Proliferation</td>
<td>High&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Intermediate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Low&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nodes</td>
<td>Node positive (4 or more involved nodes)</td>
<td>Node positive (1-3 involved nodes)</td>
<td>Node negative</td>
</tr>
<tr>
<td>Peritumoral Vascular Invasion (PVI)</td>
<td>Presence of extensive PVI</td>
<td></td>
<td>Absence of extensive PVI</td>
</tr>
<tr>
<td>pT-size</td>
<td>&gt; 5cm</td>
<td>2.1 – 5 cm</td>
<td>≤ 2cm</td>
</tr>
<tr>
<td>Patient Preference</td>
<td>Use all available treatments</td>
<td></td>
<td>Avoid side effects</td>
</tr>
</tbody>
</table>

### Multi-gene Assays

<table>
<thead>
<tr>
<th>Gene Signature&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Relative Indications</th>
<th>Factors Not Useful</th>
<th>Relative Indications for Endocrine Therapy Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 recurrence score</td>
<td>High score</td>
<td>Intermediate score</td>
<td>Low score</td>
</tr>
<tr>
<td>70 gene signature</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
NCCN 2008
‘consider multi-gene assay’

• Consider 21-gene recurrence score for
  - hormone receptor pos, her2 neg
  - pT1, pT2, pT3 and pN0 or pN1min, that are 0.6-1 cm and moderately/poorly differentiated or unfavorable characteristics
  - or > 1cm
MammaPrint prediction in ‘NCCN considers multi-gene assay’

BCSS according to MammaPrint (NCCN considers CT)

Breast cancer specific survival

- low risk
- high risk

p < 0.001

HR: 3.8 (1.9 - 7.7)
MammaPrint additions: 2009 and future developments 2010

- MammaPrint all ages (FDA expected Oct 09)
- Mammmaprint validated for 1-3 positive lymph nodes
- MammaPrint tested in Japanese patients (Prof Kato, Osaka)
- estrogen receptor, progesterone receptor, her2 (TargetPrint) (2009)
- molecular subtypes (luminal, her2, basal)
- drug targets (62 gene research panel)
MammaPrint in Japanese Patients

Osaka Medical Center for Cancer and Cardiovascular Diseases
Pof Kikuya Kato
N=102, treated, 1998-2001

Makoto Ishitobi, Teodora Goranova, Yoshifumi Komoiike, Kazuyoshi Motomura,
Hiroki Koyama, Annuska Glas, Ellen van Lienen, Hideo Inaji Laura van’t Veer
and Kikuya Kato
MammaPrint additions: 2009 and future developments 2010

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MammaPrint for Guiding Therapeutic Decisions

RNA “active genes” → Tumor Sample → Gene activity profile → Breast Cancer: Assess the risk of distant metastases
The Netherlands Cancer Institute
Amsterdam, NL (Marc van de Vijver, Hans Peterse, Jelle Wesseling, Emiel Rutgers, Rene Bernards, Marieke Straver, Sjoerd Rodenhuis, Sabine Linn, Stella Mook, Michael Knauer)

Dutch Cancer Society
Amsterdam, NL

Netherlands Health Insurance Board
Amsterdam, NL

Rosetta Inpharmatics, Merck
Seattle WA, USA (Hongyue Dai, Yudong He, Stephen Friend)

University of North Carolina
Chapel Hill and Stanford, US (Chuck Perou, Zhiyuan Hu, Cheng Fan)

Massachusetts General Hospital
Boston, MA (Shridhar Ramaswamy, Dennis Sgroi, Ben Wittner, Paula Ryan, Daniel Haber)

Agenda
Amsterdam, NL (Richard Bender, Femke de Snoo, Annuska Glas, Arno Floore, Guido Brink)

EORTC breast group
Brussels, BE (Herve Bonnefoi, Jan Bogaerts, Emiel Rutgers)

TransBig EU 6th framework program
Brussels, Paris, Amsterdam, BIG groups, FECS, Europa Donna, EU/Canada/other (Martine Piccart, Fatima Cardoso, Philippe Bedard, Christos Sotiriou, Giancarlo Pruneri, Beppe Viale, Sherene Loi, Mahasti Saghatelian, Marc Buyse)

UCSF Cancer Center
San Francisco, US (Laura Esserman, Joe Gray, I-SPY investigators)
Thank You