Prediction of distant relapses on tamoxifen in early-stage breast cancer using gene expression profiling:

A potential tool for AI tailoring

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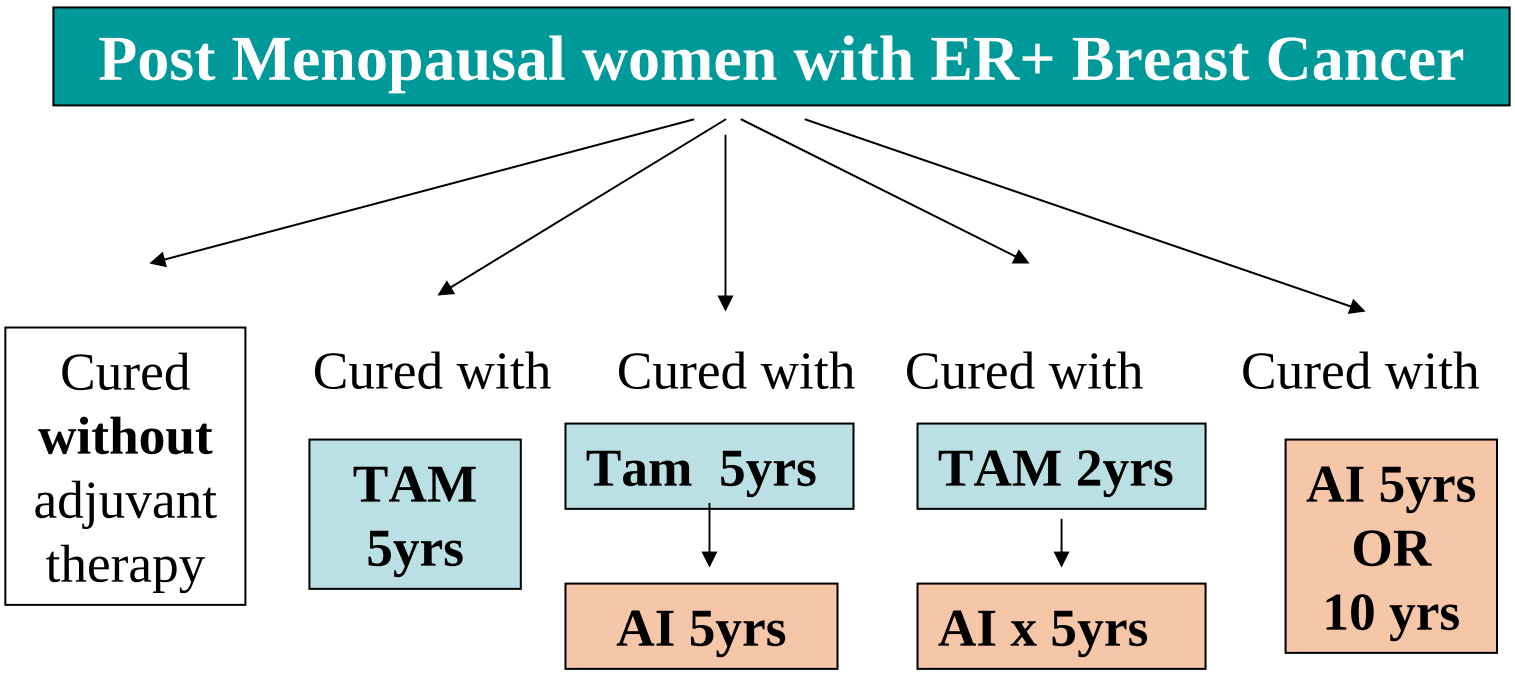
Background

- The majority of early stage BC express estrogen receptors, yet up to 40% will relapse on tamoxifen in the adjuvant setting.

- Recent evidence from large randomized controlled trials show benefit of aromatase inhibitors (AIs) over tamoxifen alone

Introduction of AIs in the adjuvant setting- for WHOM & at what COST?



Identification of a gene predictor

Aim:

To discover a set of genes that can predict for early distant relapse in ER+ early-stage BC patients on adjuvant Tamoxifen

⇨ the high risk group may benefit from an alternative endocrine approach

(ie: upfront AI)

METHODS

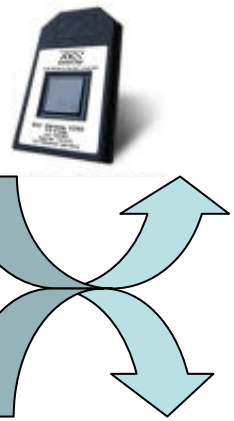
Discovery-based approach

ALL 255 samples were ER+ & RECEIVED ADJUVANT TAM ONLY

Three different institutions

Training set (TS)
John Radcliffe, Oxford

99 samples



42,700 probe sets
Affymetrix U133set

Validation set A
Karolinska Institute, Sweden

69 samples

Hybridized in another laboratory!

Validation set B
Guys Hospital, UK

87 samples

Methods: Predictor development

1. Cox proportional hazards model to identify genes associated with distant relapse ($p<0.001$)
2. 1000 random permutations
3. Clustering of top variant genes to identify highly correlated groups
4. Average expression (cluster centroid) was calculated per group
5. The classification model was fitted using a multivariate Cox regression with the cluster centroids
6. Risk score calculated from linear combination of fitted coefficients and cluster centroids

Methods: Predictor development

This method was chosen because:

1. **You can include many genes without overfitting**
2. **Provides redundancy against noise and laboratory failures**
3. **Facilitates mapping across platforms**

Tamoxifen Relapse Score=

$$\sum_{i \in G} w_i \sum_{j \in P_i} \frac{x_{ij}}{n_i}$$

Determination of BEST CUT-OFF on TS

LOW risk group to have an excellent <u>3yr %DMFS</u> (>90%)

ie: safe for "switch" after 2 yrs as opposed to upfront aromatase inhibition

RESULTS

Results: demographics of TS

N=99

Median age: 64yrs

Median tumor size: 2.2cm

Grade 1/2/3: 20/46/16%

Node negative: 58%

Median f/u: 6.1yrs

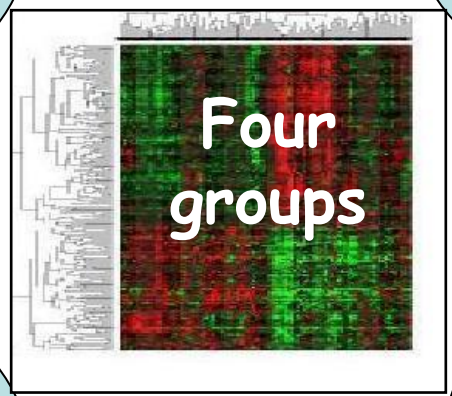
10yr% DMFS: 75%

769 probe sets identified significantly correlated with distant relapse after 1000 random permutations ($p<10^{-5}$)

Result: Risk Score from 62 probe sets

Group 1 : 8 genes
"immune-related"
C1R,CXCL12,SERPING1

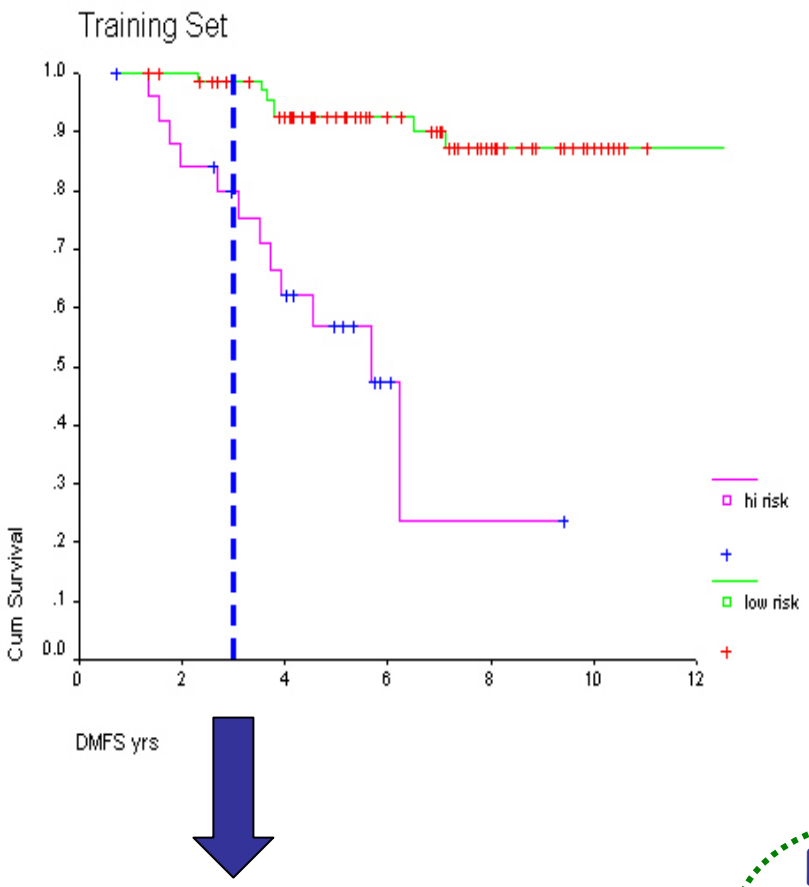
Group 2 : 9 genes
Chromosome 8q11-24



Group 3 : 4 genes
Ie: TOP2A, MYBL1,
DCC1

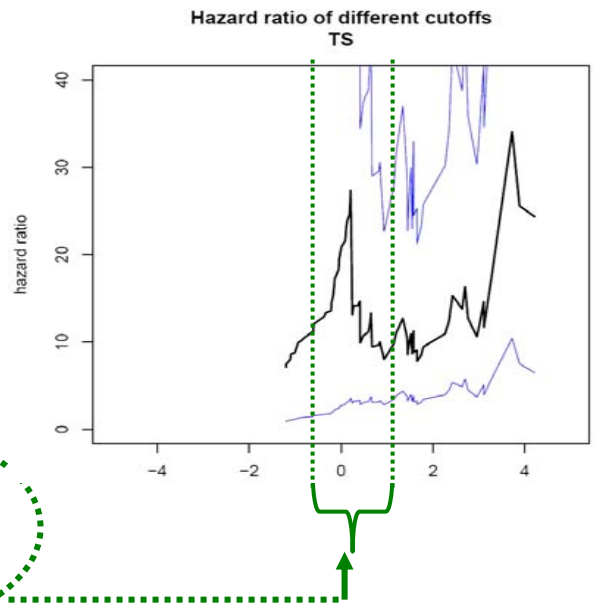
Group 4 : 33 genes
"cell cycle"
BUB1, CCNB, KI67, STK6

Predictor application on TS



HR: 9.1 (3.4-24)
p=<0.00001

Low risk group n=74 (73%)



Range of suitable cutoffs

3yr %DMFS=98%

Independent Validation Set

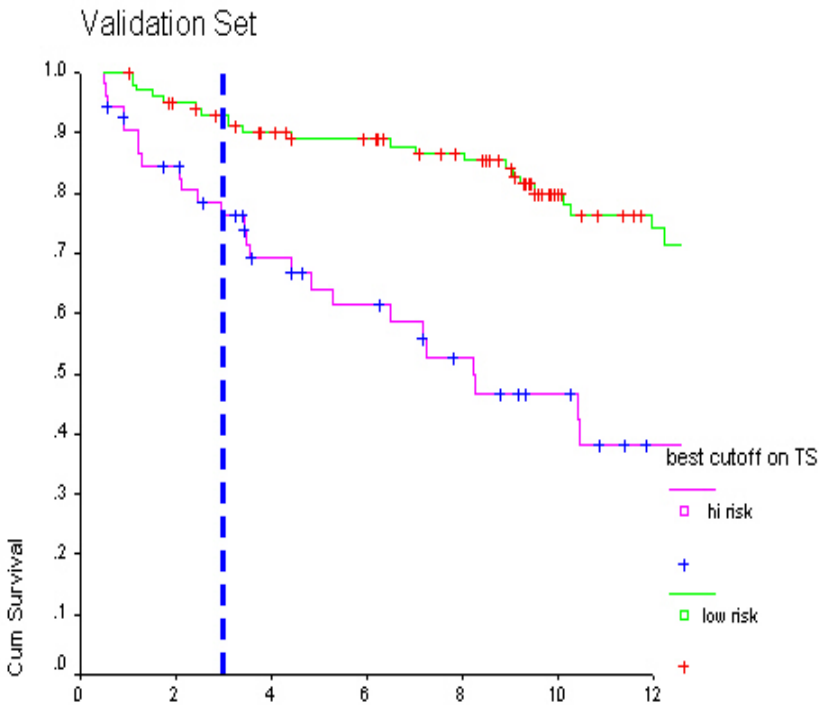
Two validation sets combined: n=156

- Median age: 62.5yrs
- Median tumor size: 2.4cm
- Grade 1/2/3: 19/50/19%
- Node negative: 33%

Median f/u: 10.1yrs

10yr% DMFS: 64%

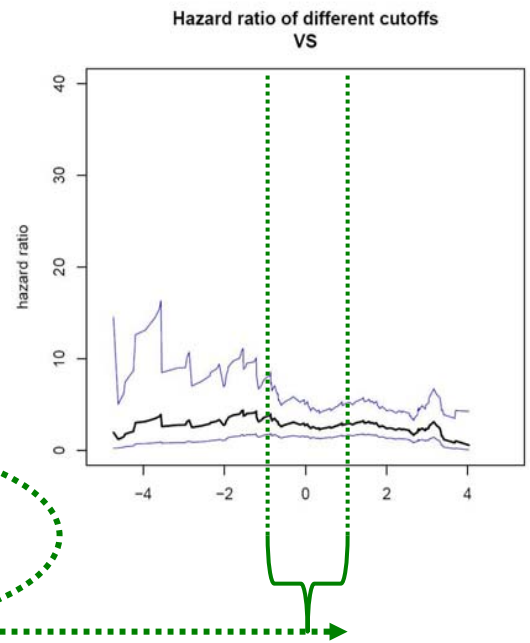
Results of Independent Validation



3yr %DMFS=91%

HR 3.3 (1.8-5.8) p<0.001

Low risk group n= 103 (66%)



Low risk group n= 50 to 103, HR 3.7 to 3.3

Multivariate Cox Proportional Analysis

Variable	P value	Hazard Ratio (95%CI)
Clinical Tumor size (T1vsT2)	0.357	1.4 (0.7-2.8)
Grade (I vs 2,3)	0.52*	0.6 (0.14-2.7)
Nodal status (pos vs neg)	0.79	1.1 (0.5-2.2)
ER[1]	0.92	1.0 (0.5-2.0)
PgR[1]	0.97*	1.0. (0.8-1.2)
HER2[1]	0.75	1.1 (0.8-1.5)
62 probe set predictor	0.003	3.0 (1.5-6.0)

*significant at the univariate level $p<0.05$, without the predictor in the model

[1] log expression values from microarray measurements

Current published literature

Comparison with published predictors using a microarray approach:

1. 2 gene ratio (Agilent platform)
   - No common genes (*Ma et al, Cancer Cell June 2004)*
2. 44 gene predictor (cDNAs)
   - No common genes (Jansen *et al. JCO Feb 2005)*

ON THE SAME VALIDATION SET!

A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen

Xiao-Jun Ma,[1] Zuncai Wang,[2] Paula D. Ryan,[3] Steven J. Isakoff,[4,5] Anne Barmettler,[2] Andrew Fuller,[2] Beth Muir,[2] Gayatry Mohapatra,[2] Ranelle Salunga,[1] J. Todd Tuggle,[1] Yen Tran,[1] Diem Tran,[1] Ana Tassin,[1] Paul Amon,[1] Wilson Wang,[1] Wei Wang,[1] Edward Enright,[1] Kimberly Stecker,[1] Eden Estepa-Sabal,[1] Barbara Smith,[3] Jerry Younger,[3] Ulysses Balis,[2] James Michaelson,[2] Atul Bhan,[2] Karleen Habin,[3] Thomas M. Baer,[1] Joan Brugge,[4] Daniel A. Haber,[3] Mark G. Erlander,[1] and Dennis C. Sgroi[2]

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80 patients (60 in TS)
Early stage breast cancer
Median age= 65yrs
Node neg 30%
Adjuvant Tamoxifen-treated
FU= 10yrs
Agilent platform

Neither ratio or individual genes (HOXB13, IL27BR) were significant at the p=0.05 level

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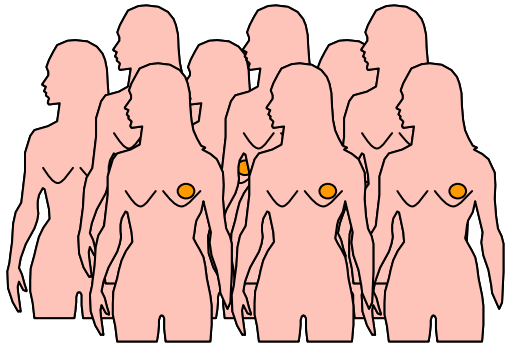
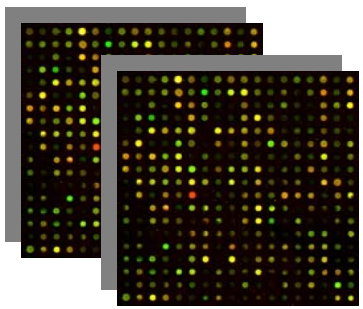
Molecular Classification of Tamoxifen-Resistant Breast Carcinomas by Gene Expression Profiling

Maurice P.H.M. Jansen, John A. Foekens, Iris L. van Staveren, Maaike M. Dirkzwager-Kiel, Kirsten Ritstier, Maxime P. Look, Marion E. Meijer-van Gelder, Anieta M. Sieuwerts, Henk Portengen, Lambert C.J. Dorssers, Jan G.M. Klijn, and Els M.J.J. Berns



112 patients (46 in TS)
recurrent breast cancer
Tamoxifen (40mg/day)
as first line treatment



Hybridized in duplicates
cDNA glass array
19,200 spots

Unfortunately detailed methods not published so predictor could not be reproduced on our dataset.

Advantages of our study

- Over 33,000 genes used
  - Whole genome based approach
  - New candidate genes & biological pathways
- Largest training & independent validation set to date using microarray approach
  - Homogeneous treatment
  - Different populations from different countries
  - Population-based, consecutive archival samples.

Conclusions (1)

- We have identified using microarray technology a set of genes that can independently predict for early distant relapse on adjuvant tamoxifen
  - The high risk group may potentially be targeted for other adjuvant endocrine therapies apart from tamoxifen (such as upfront AIs)
  - The low risk group may potentially be suitable for the tamoxifen (2yrs) then sequential AI approach

Conclusions (2)

- This list of genes may offer us new insights into the biology of endocrine therapy response & resistance in breast cancer

- Whilst our results on a relatively large and independent validation set are promising, prospective & a larger validation series is crucial

THE END

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