Molecular Predictors of response to aromatase inhibitor treatment in breast cancer

Anita Dunbier, Helen Anderson, Jody Hazlett, Briar Hunter, and Mitch Dowsett

Breast Cancer Research Group
Department of Biochemistry
University of Otago
Background

- In modern practice ~80% breast cancer oestrogen receptor positive (ER+)
- Oestrogen antagonism or withdrawal reduces tumour cell proliferation of ER+ breast cancers
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- In modern practice ~80% breast cancer oestrogen receptor positive (ER+)
- Oestrogen antagonism or withdrawal reduces tumour cell proliferation of ER+ breast cancers
- Adjuvant therapy with tamoxifen almost halves the rate of disease recurrence amongst ER+ve, no effect ER-ve
- Reduces the annual breast cancer death rate by one-third
Androstenedione → Aromatase → Oestrone → ER → EREs → ER target genes → ↑ Proliferation

Androstenedione ↔ Testosterone

Testosterone → Aromatase → Oestradiol
Background

• ~80% breast cancer oestrogen receptor positive (ER+ve)
• Aromatase inhibitors superior to tamoxifen for treatment of hormone responsive postmenopausal BrCa
• most patients diagnosed at early stage and have a good prognosis
• BUT: 10-year recurrence rate remains above 20%
• ER+ve BrCa kills more women than any other breast cancer subset
Challenges of ER+ve Breast Cancer Management
Aims

• To identify in ER+ve breast cancers:
  - the molecular features of response
  - phenotypic and genotypic determinants of benefit and prognosis in patients treated with aromatase inhibitors
Outline

- Effectiveness of prognostic biomarkers
- Novel biomarkers for resistance to aromatase inhibitor treatment in post-menopausal ER+ve Breast Cancer
Outline

➢ Effectiveness of prognostic biomarkers

➢ Novel biomarkers for resistance to aromatase inhibitor treatment in post-menopausal ER+ve Breast Cancer
Existing prognostic biomarkers

1. 21-gene recurrence score/OncotypeDx
   - Paik et al. (2004), *NEJM*, 351, 2817.
   - validated estimate of prognosis for patients with node-negative (N0), estrogen receptor (ER)–positive disease if treated with tamoxifen alone
Existing prognostic biomarkers

1. 21-gene recurrence score/OncotypeDx
   - Paik et al. (2004), NEJM, 351, 2817.
   - Validated estimate of prognosis for patients with node-negative (N0), estrogen receptor (ER)–positive disease if treated with tamoxifen alone
   - Current list price USD$4,175
   - Used on over 230,000 patients

2. PAM50 Intrinsic Subtyping/ROR Score
   - Based on intrinsic subtypes defined by Perou et al. (2000); Parker et al. (2009)
   - Risk of recurrence score
   - ROR Score = \( R_{\text{LumA}} + R_{\text{LumB}} + R_{\text{Basal}} + R_{\text{Her2}} + eP \)
   - Released as “Prosigna™” on Nanostring platform in Europe and Israel
3. Immunohistochemistry Score (IHC4)

- Oestrogen receptor, Progesterone Receptor, Her2, and Ki67 all previously shown to be prognostic and/or predictive
- IHC4 score = \(100 \times \{-0.105 \text{ER}_{10} - 0.0760 \text{PgR}_{10} + 0.528 \text{HER2} + 0.214 \ln (1 + 10 \times \text{Ki67})\}\)

4. Clinical Treatment Score

- Uses nodal status, tumour size, grade and age
- Clinical score = \(100 \times \{0.473 \text{N}_{1-3} + 1.728 \text{N}_{4+} + 0.707 \text{T}_{2-5} + 1.190 \text{T}_{>5} + 0.598 \text{Gr}_{2} + 0.990 \text{Gr}_{3} + 0.220 \text{Age}(\geq65y) - 0.153 \text{Ana}\}\)

Scores developed by Jack Cuzick, QMUL
The ATAC trial

Anastrozole vs Tamoxifen vs Combination

Surgery

9366 patients

RANDOMIZE

Tamoxifen 20 mg (n=3116)

Anastrozole 1 mg (n=3125)

Tamoxifen 20 mg
Anastrozole 1 mg (n=3125)

5 years

TransATAC

Anastrozole vs Tamoxifen vs Combination

9366 patients

Surgery

Randomize

Tamoxifen 20 mg (n=3116)

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5 years

TransATAC

Anastrozole vs Tamoxifen vs Combination

Surgery

9366 patients

R A N D O M I Z E

Tamoxifen 20 mg (n=3116)

Anastrozole 1 mg (n=3125)

TransATAC: 1782, centrally confirmed HR+

RNA extracted from 1372 blocks

1125 Eligible with reportable RNA results and IHC
Results – RS in node negative vs positive patients

**Node negative**

- **Dowsett et al., JCO, 2010**

**Node positive**

- **Dowsett et al., JCO, 2010**
Results – RS in Anastrozole vs Tamoxifen

All (N = 1,231)

Node negative, both arms (n = 872)
  - Tamoxifen (n = 432)
  - Anastrozole (n = 440)

Node positive, both arms (n = 306)
  - Tamoxifen (n = 152)
  - Anastrozole (n = 154)

Dowsett et al., JCO, 2010
Comparison of CTS, RS, IHC4 and ROR

All patients

CTS

C-index (predictive accuracy)

Dowsett et al., JCO, 2013
Comparison of ROR vs RS

Dowsett et al., JCO, 2013
Conclusions, Part I

• Recurrence Score/OncotypeDx, PAM50 ROR, and IHC4 all add significant information to clinicopathological parameters
• None of these scores predict benefit of anastrozole over tamoxifen
• Combination of CTS, ROR and IHC4 provides most accurate prediction
• Potential for use of ROR or IHC4 in New Zealand?
Effectiveness of prognostic biomarkers

Novel biomarkers for resistance to aromatase inhibitor treatment in post-menopausal ER+ve Breast Cancer
Challenges of ER+ve Breast Cancer Management

ER+ve Breast Cancer Patients

Genomics-Biomarkers

Good Signature

Endocrine Therapy

Chemotherapy (?) and Targeted Therapy

Poor Signature

FGFR4 mutation

ALK mutation

ERBB2 mutation
RESEARCH ARTICLE

ERα-Dependent E2F Transcription Can Mediate Resistance to Estrogen Deprivation in Human Breast Cancer

Todd W. Miller1,4, Justin M. Balko2, Emily M. Fox2, Zara Ghazoui3, Anita Dunbier1, Helen Anderson5, Mitch Dowsett6,7, Aixiang Jiang1, R. Adam Smith1,2, Sauveur-Michel Maia10, H. Charles Manning13, Ana M. Gonzalez-Angulo11,12, Gordon B. Mills1,2, Catherine Higham1, Siprochan Chanthaphaychith2, Maria G. Kuba3, William R. Miller4, Yu Shyr5,13, and Carlos L. Arteaga1,2,4

Preclinical and clinical studies of estrogen deprivation support the PDGF/Abl pathway as a novel therapeutic target for overcoming endocrine resistance in breast cancer

Marion T Weigel1, Zara Ghazoui3, Anita Dunbier1, Sunil Pancholi1, Mitch Dowsett6,7, and Lesley-Ann Martin1

Research article

Hyperactivation of phosphatidylilin kinase promotes escape from hormone dependence in estrogen receptor-negative breast cancer

Todd W. Miller,1 Bryan T. Hennessy,2,3 Ana M. Gonzalez-Angulo,2,4, Emily M. I Heidi Chen,5 Catherine Higham,6 Carlos Garcia-Echeverria,7 Yu Shyr3,13, and Carlos L. Arteaga1,2,4

A Gene Expression Signature from Human Breast Cancer Cells with Acquired Hormone Independence Identifies MYC as a Mediator of Antiestrogen Resistance

Todd W. Miller1,4, Justin M. Balko2, Zara Ghazoui3, Anita Dunbier1, Helen Anderson5, Mitch Dowsett6,7, Ana M. Gonzalez-Angulo11,12, Gordon B. Mills1,2, William R. Miller4, Huiyun Wu1, Yu Shyr5,13, and Carlos L. Arteaga1,2,4

Abstract

Purpose: Although most patients with estrogen receptor α (ER)-positive breast cancer initially respond to endocrine therapy, many ultimately develop resistance to antiestrogens. However, mechanisms of antiestrogen resistance and biomarkers predictive of such resistance are underdeveloped.

Experimental Design: We adapted four ER+ human breast cancer cell lines to grow in an estrogen-depleted medium. A gene signature of estrogen independence was developed by comparing expression profiles of long-term estrogen-deprived (LTED) cells to their parental counterparts. We evaluated the ability of the LTED signature to predict tumor response to neoadjuvant therapy with an aromatase inhibitor and disease outcome following adjuvant tamoxifen. We utilized Gene Set Analysis (GSA) of LTED cell gene expression profiles and a loss-of-function approach to identify pathways causally associated with resistance
16 week Neo-adjuvant Aromatase Inhibitor Study

Presentation

Anastrozole

2 weeks

14 weeks

Surgery

112 ER+ve post-menopausal Stage I-IIIB, early breast cancers
16 week Neo-adjuvant Aromatase Inhibitor Study

Presentation

- Anastrozole
- 2 weeks
- 112 biopsies

Surgery

- 14 weeks
- 97 biopsies
- 29 biopsies
Anastrozole

112 biopsies  97 biopsies  29 biopsies

2 weeks  14 weeks
Anastrozole

- 2 weeks
- FFPE
- RNAlater

Expression profiling
Illumina Human-6 v2 48K probes
Histopathology
% malignant epithelial etc.
Array CGH
32K BAC array

IHC
Ki67, ER, PR, HER2, EGFR, pAKT, pERK1/2
Histopathology
Grade, tumour type etc.

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Histopathology
Grade, tumour type etc.
Anastrozole

2 weeks

Expression profiling Illumina Human-6 v2 48K probes

Proportional 2 wk change in Ki67

= \frac{\text{2 wk Ki67}}{\text{Baseline Ki67}} \times 100\%
Relapse Free Survival by 2 week LnKi67

Relapse Free Survival %

Years since Randomisation

<=0.8  N = 45  O = 3  E = 7.9
0.81-1.99  N = 60  O = 9  E = 10.6
2+  N = 54  O = 14  E = 7.4

χ² = 8.65  df = 1  p = 0.003

2.7 fold ↑ Ki67  ➤ 2-fold ↑ Hazard ratio

2wk Ki67 relates to outcome on anastrozole more closely than baseline Ki67.
Anastrozole

Expression profiling
Illumina Human-6
v2 48K probes

Predict

Proportional 2 wk change in Ki67 = \frac{2 \ wk \ Ki67}{Baseline \ Ki67} x 100%
Response predictors

- Pre-treatment expression of 471 genes correlated with % decrease in Ki67 (p<0.005)

Dunbier et al., Clin Can Res, 2013
### Genes correlated with poor 2 week change in Ki67

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Description</th>
<th>Correlation coefficient</th>
<th>Parametric p-value</th>
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<tbody>
<tr>
<td>1</td>
<td>SLAMF8 SLAM family member 8 (CD2 family member)</td>
<td>0.52</td>
<td>0.000003</td>
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<td>2</td>
<td>P2RY6 Pyrimidinergic receptor P2Y, G-protein coupled, 6</td>
<td>0.507</td>
<td>5.6E-06</td>
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<td>4</td>
<td>ZBED2 Zinc finger, BED-type containing 2</td>
<td>0.492</td>
<td>1.11E-05</td>
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<td>5</td>
<td>PITPNM1 Phosphatidylinositol transfer protein, membrane-associated 1</td>
<td>0.487</td>
<td>0.000014</td>
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<tr>
<td>6</td>
<td>IL21R Interleukin 21 receptor</td>
<td>0.481</td>
<td>1.81E-05</td>
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<tr>
<td>7</td>
<td>LAIR2 Leukocyte-associated immunoglobulin-like receptor 2</td>
<td>0.476</td>
<td>2.29E-05</td>
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<tr>
<td>8</td>
<td>RGS19 Regulator of G-protein signaling 19</td>
<td>0.474</td>
<td>2.54E-05</td>
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<tr>
<td>9</td>
<td>HAVCR2 Hepatitis A virus cellular receptor 2</td>
<td>0.465</td>
<td>3.75E-05</td>
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<tr>
<td>10</td>
<td>IL32 Interleukin 32</td>
<td>0.464</td>
<td>3.92E-05</td>
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<tr>
<td>11</td>
<td>ADAM8 ADAM metallopeptidase domain 8 (CD156)</td>
<td>0.464</td>
<td>3.84E-05</td>
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<tr>
<td>12</td>
<td>PLCL3 Phospholipase C, eta 1</td>
<td>0.464</td>
<td>3.77E-05</td>
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<tr>
<td>13</td>
<td>FPRL2 Formyl peptide receptor-like 2</td>
<td>0.462</td>
<td>4.25E-05</td>
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<td>14</td>
<td>LAG3 Lymphocyte-activation gene 3</td>
<td>0.461</td>
<td>4.31E-05</td>
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<td>15</td>
<td>SGK Serum/glucocorticoid regulated kinase</td>
<td>0.46</td>
<td>4.66E-05</td>
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<td>16</td>
<td>TNF Tumor necrosis factor alpha</td>
<td>0.458</td>
<td>4.93E-05</td>
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<td>17</td>
<td>CARD9 Caspase recruitment domain family, member 9</td>
<td>0.457</td>
<td>5.24E-05</td>
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<td>18</td>
<td>TRAF3 TNF receptor-associated factor 3</td>
<td>0.452</td>
<td>6.47E-05</td>
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<tr>
<td>19</td>
<td>AKR1B1 Aldo-keto reductase family 1, member B1 (aldose reductase)</td>
<td>0.452</td>
<td>6.38E-05</td>
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<tr>
<td>20</td>
<td>MSL3L1 Male-specific lethal 3-like 1 (Drosophila)</td>
<td>0.447</td>
<td>0.0000789</td>
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<tr>
<td>24</td>
<td>TNFAIP3 Tumor necrosis factor, alpha-induced protein 3</td>
<td>0.438</td>
<td>0.000113</td>
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<tr>
<td>25</td>
<td>CD53 CD53 molecule</td>
<td>0.438</td>
<td>0.000112</td>
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<tr>
<td>33</td>
<td>IRF8 Interferon regulatory factor 8</td>
<td>0.427</td>
<td>0.000173</td>
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<tr>
<td>35</td>
<td>CD86 CD86 molecule</td>
<td>0.426</td>
<td>0.000177</td>
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<tr>
<td>36</td>
<td>IL10RA Interleukin 10 receptor, alpha</td>
<td>0.426</td>
<td>0.000177</td>
</tr>
<tr>
<td>37</td>
<td>CD84 CD84 molecule</td>
<td>0.425</td>
<td>0.000184</td>
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<tr>
<td>40</td>
<td>ITGAL Integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)</td>
<td>0.423</td>
<td>0.000199</td>
</tr>
</tbody>
</table>

**Top 20 genes**

**Selected genes 21-40**

**Immune-related genes**

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Dunbier et al., 2013
Relationship between SLAMF8 and change in Ki67

Dunbier et al., Clin Can Res, 2013
## Genes correlated with *good* 2 week change in Ki67

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>Parametric p-value</th>
<th>FDR</th>
<th>Gene symbol</th>
<th>Gene name</th>
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<td>0.394</td>
<td>0.000975</td>
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<td>HIST1H2BD</td>
<td>Histone cluster 1, H2bd</td>
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<td>0.395</td>
<td>0.000949</td>
<td>0.127925</td>
<td>CRIM1</td>
<td>Cysteine rich transmembrane BMP regulator 1 (chordin-like)</td>
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<tr>
<td>0.398</td>
<td>0.000843</td>
<td>0.121047</td>
<td>HTR1F</td>
<td>5-hydroxytryptamine (serotonin) receptor 1F</td>
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<tr>
<td>0.399</td>
<td>0.000829</td>
<td>0.120653</td>
<td>MYH14</td>
<td>Myosin, heavy chain 14</td>
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<td>0.402</td>
<td>0.000758</td>
<td>0.115071</td>
<td>NDUFA7</td>
<td>NADH dehydrogenase (ubiquinone) 1 alpha subcomplex)</td>
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<tr>
<td>0.403</td>
<td>0.000717</td>
<td>0.111167</td>
<td>TMC4</td>
<td>Transmembrane channel-like 4</td>
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<tr>
<td>0.403</td>
<td>0.000717</td>
<td>0.111167</td>
<td>RLN2</td>
<td>Relaxin 2</td>
</tr>
<tr>
<td>0.406</td>
<td>0.000648</td>
<td>0.109505</td>
<td>MAP2K3</td>
<td>Mitogen-activated protein kinase kinase 3</td>
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<td>0.41</td>
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<td>0.102707</td>
<td>TRAM1L1</td>
<td>Translocation associated membrane protein 1-like 1</td>
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<td>0.416</td>
<td>0.000474</td>
<td>0.092842</td>
<td>RAI2</td>
<td>In multiple clusters</td>
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<tr>
<td>0.417</td>
<td>0.000452</td>
<td>0.092195</td>
<td>IFRG15</td>
<td>Interferon responsive gene 15</td>
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<tr>
<td>0.421</td>
<td>0.000392</td>
<td>0.088171</td>
<td>RAB17</td>
<td>RAB17, member RAS oncogene family</td>
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<tr>
<td>0.421</td>
<td>0.00004</td>
<td>0.088318</td>
<td>PAK6</td>
<td>P21(CDKN1A)-activated kinase 6</td>
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<td>0.422</td>
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<td>0.087623</td>
<td>GATA3</td>
<td>GATA binding protein 3</td>
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<td>0.422</td>
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<td>NEK7</td>
<td>NIMA (never in mitosis gene a)-related kinase 7</td>
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<tr>
<td>0.424</td>
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<td>AHNAK</td>
<td>AHNAK nucleoprotein</td>
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<td>0.424</td>
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<td>0.075186</td>
<td>CELSR2</td>
<td>Cadherin, EGF LAG seven-pass G-type receptor 2</td>
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<td>0.434</td>
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<tr>
<td>0.294</td>
<td>0.012783</td>
<td>0.483010</td>
<td>ESR1</td>
<td>Estrogen receptor 1</td>
</tr>
</tbody>
</table>
### Pathway analysis of genes correlated p<0.005

**Ingenuity Pathway Analysis:**

<table>
<thead>
<tr>
<th>Category</th>
<th>Function Annotation</th>
<th>p-value</th>
<th># Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammatory Response</strong></td>
<td>immune response</td>
<td>6.83E-23</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>activation of leukocytes</td>
<td>7.20E-12</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>activation of mononuclear leukocytes</td>
<td>5.93E-11</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>activation of lymphocytes</td>
<td>1.02E-10</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>activation of T lymphocytes</td>
<td>2.84E-10</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>inflammatory response</td>
<td>1.21E-05</td>
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<tr>
<td></td>
<td>chemotaxis of leukocytes</td>
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<td></td>
<td>chemotaxis of granulocytes</td>
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<td></td>
<td>chemotaxis of eosinophils</td>
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<td>binding of phagocytes</td>
<td>1.13E-04</td>
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<td>chemotaxis of T lymphocytes</td>
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<td></td>
<td>antibody response</td>
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<tr>
<td></td>
<td>activation of granulocytes</td>
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<td>4</td>
</tr>
</tbody>
</table>
Association between inflammatory signature and change in Ki67

Dunbier et al., Clin Can Res, 2013
Validation set

- Validated inflammatory signature as predictor of change in Ki67 in an independent set of 58 letrozole-treated tumours*.  

* Miller et al., 2009, JCO, 27, 1382
Lymphocytic Infiltration

- Investigated association between lymphocytic infiltration and change in Ki67 after AI
- Tumours with lymphocytic infiltration showed poorer anti-proliferative response to AI treatment

* Smith et al., 2005, JCO, 23, 5108
Lymphocytic Infiltration

- Investigated lymphocytic infiltration over 16 weeks
Association between inflammatory signature and change in Ki67

Proportional change in Ki67 (% 2-week/Baseline)

Pre-treatment expression of Inflammatory Response Metagene

Rs = 0.44
p < 0.0001

No change
90% fall
What type of immune cell is associated with poor Ki67 response on AI?

- Used profiles from Reference Database of Immune Cells (Hijikata et al., 2007)
- Matched relative expression of 471 genes correlated to change in Ki67

Response correlated profile

<table>
<thead>
<tr>
<th></th>
<th>B cell</th>
<th>dendritic cell</th>
<th>HSCs</th>
<th>T cell</th>
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→ 100% accuracy in leave-one out cross validation

Prediction of unknown → Dendritic cell
Immune infiltration and breast cancer

• Associated with good prognosis in patients treated with chemotherapy (DeNardo et al., 2011; Mahmoud et al., 2011; Denkert et al., 2010)

• Could outcomes be different in patients treated only with endocrine therapy?

• Hormonal influences on the immune system?
Could oestrogen deprivation prompt recruitment of inflammatory cells?

Culture in steroid-depleted media

- 1 nM Oestradiol (equivalent to AI-treated)

+ 1 nM Oestradiol

Extract RNA, QPCR for CCL5, CCL22, CXCL16, CXCL14 over 3 day timecourse
CCL5
7-fold up-regulated at Day 5 (p=0.02)

CCL22
8-fold up-regulated at Day 5 (p=0.05)

CXCL16
2-fold up-regulated at Day 5 (p=0.003)

CXCL14 - not detectable
Change in chemokine expression in tumours treatment with aromatase inhibitors

<table>
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<tr>
<th>Chemokine</th>
<th>Dunbier <em>et al.</em></th>
<th></th>
<th>Miller <em>et al.</em></th>
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</table>
Model of effect of oestrogen deprivation on immune cell infiltration
Could oestrogen deprivation prompt recruitment of inflammatory cells?

Culture in steroid-depleted media

MCF7 cells → - 1 nM E2 → PBMCs (Immune cells)

MCF7 cells → + 1 nM E2 →
Could oestrogen deprivation prompt recruitment of inflammatory cells?

![Box plot showing the number of migrated cells with and without oestradiol. The plot indicates a statistically significant difference between the two conditions (p=0.004).]
Model of effect of oestrogen deprivation on immune cell infiltration

Pharmacologic invention? NSAIDs?
Summary Part II

- Pre-treatment expression an inflammatory signature associated with poor anti-proliferative response to AI treatment in two independent datasets
- Lymphocytic infiltration associated with poor response
- Preliminary evidence that dendritic cells may be involved
- Lymphocytic infiltration increases during treatment
- Oestrogen deprivation appears to induce chemokine expression in vitro and in vivo
- Don’t forget the stroma!
- Don’t forget the host immune response!
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Patients
IHC tests

- $\text{ER}_{10}$ – 3 x 600u cores, 6F11 antibody, H-score/300
- $\text{PgR}_{10}$ – full sections, clone 16, % positive cells/100
- HER2 – full sections, *HercepTest* with FISH+(ratio >2) on 2+ cases, +/-
- Ki-67 – 3 x 600u cores, SP6 antibody (Abcam),
  \[ \log (1 + 10 \times \% \text{positive cells}) \]