TUMOUR INFILTRATING LYMPHOCYTES IN YOUNG WOMEN WITH TRIPLE NEGATIVE BREAST CANCER

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DR. GUY MARTLAND
DISCLOSURES

• None
BACKGROUND

• TNBC 15-20%

• Poor outcomes, no targeted therapies

• Overlap with basal-like/BRCAness phenotype

• Genomic/transcriptonomic data highlights immune-active subtype

• Could immunotherapy have a role?
THE POSH STUDY

• UK, 2000-2008, prospective observational cohort
• 2956 women aged 40
• First invasive BC
• Baseline clinicopathological data at diagnosis, annual follow-up
• Complete germline BRCA testing
PROGNOSTIC FACTORS

• T-stage, N-stage
• Subtype
• BMI
• Ethnicity
• BRCA status ×

Family history and outcome of young patients with breast cancer in the UK (POSH study)

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Prospective Observational Study of Breast Cancer Treatment Outcomes for UK Women Aged 18–40 Years at Diagnosis: The POSH Study

Ellen Copson, Bryony Eccles, Tom Maishman, Sue Gerty, Louise Stanton, Ramsey I. Cutress, Douglas G. Altman, Lorraine Durcan, Peter Simmonds, Gill Lawrence, Louise Jones, Judith Bliss, Diana Eccles; POSH Study Steering Group

Correspondence to: Diana Eccles, University of Southampton Clinical Trials Unit, Faculty of Medicine, University of Southampton and University Hospital Southampton Foundation Trust, Trenance Road, Southampton SO16 6YD, UK (e-mail: D.M.Eccles@soton.ac.uk).

Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study

Ellen R Copson*, Tom C Maishman*, Wilf Tapper, Ramsey I Cutress, Stephanie Greer-Hegate, Douglas G Altman, Bryony Eccles, Sue Ger Lorraine T Durcan, Louise Jones, D Gareth Evans, Alastair M Thompson, Paul Pharma, Douglas F Easton, Alisk M Dunneing, Andrew Harb Sundi Lahiani, Ros Eles, Fiona J Gilbert, Muslim Hamid, Shirley Hodgson, Peter Simmonds, Louise Stanton, Diana M Eccles

Correspondence to:
### ADJUVANT TIL STUDIES TO DATE

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>PR status</th>
<th>No. cases</th>
<th>HR LPBC</th>
<th>HR per 10% TIL↑</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loi¹</td>
<td>2013</td>
<td>Unknown</td>
<td>256</td>
<td>0.86 (0.08-9.45)</td>
<td>-</td>
<td>M=49*</td>
</tr>
<tr>
<td>Adams²</td>
<td>2014</td>
<td>Known</td>
<td>481</td>
<td>0.81 (0.69-0.95)</td>
<td>21.6% &lt;40</td>
<td></td>
</tr>
<tr>
<td>Loi³</td>
<td>2014</td>
<td>Known</td>
<td>134</td>
<td>0.80 (0.62-1.03)</td>
<td>M=59*</td>
<td></td>
</tr>
<tr>
<td>Dieci⁴</td>
<td>2015</td>
<td>Unknown</td>
<td>199</td>
<td>0.85, (0.74–0.99)</td>
<td>M=56*</td>
<td></td>
</tr>
<tr>
<td>Pruneri⁵</td>
<td>2016</td>
<td>Known</td>
<td>647</td>
<td>0.48 (0.25–0.90)</td>
<td>M=52</td>
<td></td>
</tr>
</tbody>
</table>

*=whole cohort

LPBC vs TIL-low¹

2 S. Adams, J Clin Oncol. 2014.
3 S. Loi, Ann Oncol. 2014.
CD8 T CELL MODULE (LIGHTPINK4) ADJ.P = 3.2E-72

ECM (MAGENTA2 FN1) ADJ.P = 2.6E-45
OBJECTIVES

• Do TILs \(\uparrow\downarrow\) outcome in young patients?
• By what mechanisms?
• How can we increase infiltration of lymphocytes in immune-cold TNBC?
METHODS

• ER-ve, PR-ve, HER2-ve

• Stage I-III, neoadjuvant excluded

• H&E full face – stromal TILs

• 10 hpf’s at x40, in 5% increments (International TILs Group guidelines¹)

• TMAs - x3 cores for antibody staining – 1,2,3

¹R. Salgado, Ann Oncol. 2015.
TILS AND SURVIVAL

• N = 350

• High (n=25) >55%

• Moderate (n=122), 20-55%

• Low (n=203), <20%

Hazard ratios

High: 0.104 (0.014-0.751) p=0.018

Mod: 0.568 (0.355-0.908) p=0.026

Log-rank Mantel Cox p=0.002.
• Median TIL count = 15%
## TMA Survival/Correlates

<table>
<thead>
<tr>
<th>Marker (high)</th>
<th>Multivariable HR</th>
<th>P-value</th>
<th>R correlation (TILs)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8</td>
<td>0.460</td>
<td>0.005</td>
<td>+0.599</td>
<td>4.50(\times) 10^{-33}</td>
</tr>
<tr>
<td>FOXP3</td>
<td>0.280</td>
<td>0.000371</td>
<td>+0.398</td>
<td>5.77(\times) 10^{-14}</td>
</tr>
<tr>
<td>SMA</td>
<td>1.151</td>
<td>0.533</td>
<td>-0.211</td>
<td>0.000126</td>
</tr>
<tr>
<td>MHC I</td>
<td>0.709</td>
<td>0.142</td>
<td>+0.436</td>
<td>1.27(\times) 10^{-16}</td>
</tr>
<tr>
<td>PD-L1 tumour</td>
<td>0.413</td>
<td>0.004</td>
<td>+0.400</td>
<td>3.68(\times) 10^{-16}</td>
</tr>
<tr>
<td>PD-L1 lymphs</td>
<td>0.478</td>
<td>0.006</td>
<td>+0.598</td>
<td>1.79(\times) 10^{-32}</td>
</tr>
</tbody>
</table>

**Image:**
- **Survival Functions:**
  - Survival curves for different groups (e.g., MHCCat, FOXP3, CD8).
  - Time on the x-axis, event-free survival on the y-axis.
  - Legend indicating high and low expression levels.
DIGITAL PATHOLOGY

• Definiens software

• Automatic identification of positive & negative nuclei through dynamic thresholding and morphology based separation.

• QC1 = removal of invalid cores

• QC2 = removal of cores with very small area of invasive tumour/tumour islands

• Positive nuclei/total no. nuclei = positivity index
DIGITAL PATHOLOGY (Q1 Q2)
CD8
R = +0.809
(p=6.1987^-79; n=335)
## ROC Analysis

<table>
<thead>
<tr>
<th>Marker</th>
<th>PPV</th>
<th>Marker</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki67</td>
<td>0.4959</td>
<td>Tumour size</td>
<td>0.6118</td>
</tr>
<tr>
<td>ALDH1</td>
<td>0.5732</td>
<td>Age</td>
<td>0.5579</td>
</tr>
<tr>
<td>CK56</td>
<td>0.5377</td>
<td>BMI</td>
<td>0.5304</td>
</tr>
<tr>
<td>EGFR</td>
<td>0.4965</td>
<td>Clinical T Stage</td>
<td>0.6234</td>
</tr>
<tr>
<td>MHC</td>
<td>0.6297</td>
<td>Path T Stage</td>
<td>0.6403</td>
</tr>
<tr>
<td>P53</td>
<td>0.5117</td>
<td>Invasive size</td>
<td>0.6353</td>
</tr>
<tr>
<td>TIL %</td>
<td>0.6868</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-L1 (lymphs)</td>
<td>0.6609</td>
<td>Combined (n=24)</td>
<td>0.8324</td>
</tr>
</tbody>
</table>

Survival at 3 yrs (50 alive)

<table>
<thead>
<tr>
<th>Marker</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIL %</td>
<td>0.6823</td>
</tr>
<tr>
<td>No. nodes involved</td>
<td>0.7049</td>
</tr>
<tr>
<td>Combined</td>
<td>0.7603</td>
</tr>
</tbody>
</table>
ROC ANALYSIS

**Clinical factors**
- No. lymph nodes - 0.7049
- Max invasive tumour size – 0.6353

**TILs**
- TIL score - 0.6868
- TIL category - 0.6335

**TMAs**
- PD-L1 (lymphocytes) - 0.6609
- CD8 - 0.6562
- MHC - 0.6297
- FOXP3 - 0.6213

**Final score:** TIL % + no. nodes = PPV +0.7603
CONCLUSIONS + ONGOING WORK

• TILs more predictive than traditional risk factors (grade, tumour size)

• Automated scoring a useful alternative?

• PD-L1 positive prognostic factor in this cohort (cf. melanoma)

• SMA ↑↓ TILs → exploring CAF inhibition in TNBC mouse models
ACKNOWLEDGEMENTS

• Thank you to all patients who participated in POSH
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• Ellen Copson, Gareth Thomas
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• Scott Harris