Importance of obtaining tissue for research – A case study in NSABP B-27

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NSABP
Current prognostic tools in adjuvant setting

- Can identify high risk patients
- High risk patients derive greater benefit from chemotherapy
- However, the tools are probabilistic
- The tools cannot tell who actually benefited from chemotherapy and who need more than chemotherapy after chemotherapy is administered
Oncotype DX 21 Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

\[ RS = +0.47 \times \text{HER2 Group Score} \]
\[ -0.34 \times \text{ER Group Score} \]
\[ +1.04 \times \text{Proliferation Group Score} \]
\[ +0.10 \times \text{Invasion Group Score} \]
\[ +0.05 \times \text{CD68} \]
\[ -0.08 \times \text{GSTM1} \]
\[ -0.07 \times \text{BAG1} \]

**Category** | **RS (0 – 100)**
--- | ---
Low risk | RS < 18
Int risk | RS ≥ 18 and < 31
High risk | RS ≥ 31
Recurrence Score and prognosis (NSABP B-14 tamoxifen arm)
Higher risk = Greater benefit (NSABP B-20)

![Graph showing benefit from chemotherapy based on recurrence score.](Image)
pCR provides patient specific in-vivo assessment of tumor response

- However, not a perfect surrogate for survival endpoint
- Even doubling of pCR rate did not result in improvement in survival endpoint (NSABP B-27)
- Does not provide base-line risk assessment
NSABP B-27

Operable Breast Cancer

Randomization

AC x 4

Taxotere x 4

Surgery

Surgery

Surgery

Taxotere x 4
NSABP B-27: pCR as a surrogate for clinical end-points regardless of treatment

P < 0.0001
NSABP B-27
Doubling of pCR in AC-T vs AC

*p<0.001 for test of heterogeneity across groups
NSABP B-27

Doubling of pCR did not translate to clinical outcome differences

<table>
<thead>
<tr>
<th>TRT</th>
<th>N</th>
<th>Deaths</th>
<th>HR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>801</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-T</td>
<td>803</td>
<td>143</td>
<td>0.94</td>
<td>0.57</td>
</tr>
<tr>
<td>AC-pT</td>
<td>799</td>
<td>163</td>
<td>1.07</td>
<td>0.53</td>
</tr>
</tbody>
</table>
No perfect tools

• **Current prognostic tools in adjuvant setting**
  – Can identify high risk patients
  – High risk patients derive greater benefit from chemotherapy
  – However, the tools are probabilistic
  – The tools cannot tell who actually benefited from chemotherapy and who need more than chemotherapy

• **pCR is a patient specific in-vivo assessment of tumor response**
  – Not a perfect surrogate for survival endpoint
  – Even doubling of pCR rate did not result in improvement in survival endpoint (NSABP B-27)
  – Does not provide base-line risk assessment
Is pCR a valid surrogate endpoint?
Extrapolation of B-18 data to B-27

AC arm

15% 85%

pCR No-PCR

90% 5YS 75% 5YS

5YS of all patients in AC arm =

\[
\frac{90 \times 15 + 75 \times 85}{100} = 77.25\%
\]
Extrapolation of B-18 data predicted that B-27 clinical outcome data could not be robust.

**AC arm**

- 15% pCR
- 85% 5YS

5YS of all patients in AC arm = \[
\frac{90 \times 15 + 75 \times 85}{100} = 77.25\%
\]

**AC-\rightarrow T arm**

- 30% pCR
- 70% 5YS

5YS of all patients in AT arm = \[
\frac{90 \times 30 + 75 \times 70}{100} = 79.50\%
\]
B-27 could not be robust for survival endpoint due to relatively good outcome of no-pCR patients

<table>
<thead>
<tr>
<th>% pCR</th>
<th>expected 5YS</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>77.25%</td>
</tr>
<tr>
<td>30</td>
<td>79.5</td>
</tr>
<tr>
<td>50</td>
<td>82.5</td>
</tr>
<tr>
<td>60</td>
<td>84</td>
</tr>
<tr>
<td>80</td>
<td>87</td>
</tr>
</tbody>
</table>
NSABP B-27
pCR as a surrogate for clinical end-points

Why is the curve for no-pCR not steeper?
NSABP B-27
Problem of patient selection?

Mixed with good prognosis patients who did not benefit from chemotherapy??
NSABP B-27 Pathology

- Pretreatment core biopsy paraffin block procurement protocol (B-27.2) started one year after initiation of the main trial (B-27)
- Initial planned markers – p53, Ki67, ER, PR, HER2 – but technology evolved
- Had to develop a new method for microarray gene expression profiling of paraffin embedded tumor tissue
- Affymetrix U133 2.0 plus GeneChip data available from 326 cases
Gene expression profiling of B-27 pre-treatment core biopsy specimens

- RNA extraction using ROCHE kit
- 100 ng total RNA as starting material
- Hybridization to Affymetrix GeneChip U133 2.0 plus
- PAM and SUPERPC used for prediction of ER, pCR, and outcome
NSABP B-27
Gene expression and survival outcome

Low-risk (n=163)
High-risk (n=163)
No-pCR group included both low and high risk patients

No-pCR & low-risk (n=147)
No-pCR & high-risk (n=125)
NSABP B-27
Problem of patient selection

Mixed with good prognosis patients who did not benefit from chemotherapy
B-27 could have been more robust if only high-risk patients were enrolled (no-pCR in high-risk has 65% rather than 75% 5YS)

<table>
<thead>
<tr>
<th>% pCR</th>
<th>expected 5YS with no selection</th>
<th>expected 5YS with high-risk only</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>77.25%</td>
<td>68.8%</td>
</tr>
<tr>
<td>30</td>
<td>79.5</td>
<td>72.5</td>
</tr>
<tr>
<td>50</td>
<td>82.5</td>
<td>77.5</td>
</tr>
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</table>
Low-risk patients had good outcome regardless of pCR.
Combination of prognostic genes and pCR defines residual risk after chemotherapy

Candidates for post-neoadjuvant trials for targeted therapy
Can we predict pCR with gene expression profiling?
Prognostic Profile and pCR

<table>
<thead>
<tr>
<th></th>
<th>No-pCR</th>
<th>pCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk</td>
<td>147 (90%)</td>
<td>16 (10%)</td>
</tr>
<tr>
<td>High-risk</td>
<td>125 (79%)</td>
<td>34 (21%)</td>
</tr>
</tbody>
</table>

The proportion of No-pCR in low-risk group is higher than expected (p-value=0.0067).
Microarray analysis of formalin fixed paraffin embedded B-27 core biopsy specimens

While prediction of ER status is very good……

<table>
<thead>
<tr>
<th>Predicted by microarray</th>
<th>IHC (central lab)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ER-</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>ER+</td>
<td>4</td>
</tr>
<tr>
<td>ER-</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>ER+</td>
<td>206</td>
<td></td>
</tr>
</tbody>
</table>

Error rate = 3.8%
Microarray analysis of formalin fixed paraffin embedded B-27 core biopsy specimens

Prediction of pCR is poor

<table>
<thead>
<tr>
<th>Predicted by microarray</th>
<th>Pathology</th>
<th>pCR</th>
<th>No pCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCR</td>
<td>59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No pCR</td>
<td>213</td>
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</tr>
</tbody>
</table>

Error rate = 25.1%
Microarray analysis of formalin fixed paraffin embedded B-27 core biopsy specimens

Prediction of pCR in ER negative subset is better

<table>
<thead>
<tr>
<th>Predicted by microarray</th>
<th>Pathology</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No pCR</td>
<td>67</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>pCR</td>
<td>6</td>
<td>14</td>
</tr>
</tbody>
</table>

Error rate = 15.6%
• Pre-treatment core biopsy mandatory
  – RNAlater for gene expression profiling
  – Formalin for validation and clinical adaptation of discovered expression profiles
  – Hank’s buffer for In-vitro chemosensitivity assay
Conclusion

• Gene expression analysis of pre-treatment core biopsy provided biological explanation of NSABP B-27 data

• Combination of gene expression and pCR may identify patients who need more than chemotherapy
  – Validation study with ECTO and NSABP B-40