Personalized Medicine for Triple Negative Breast Cancer - New Dimensions in Therapeutic Individualization

Bryan P. Schneider & Milan Radovich
Medicine & Medical Molecular Genetics
Indiana University School of Medicine
AT LEAST THREE types of breast cancer

Schneider, et al CCR, 2008
The inability to define is a bad start

TRIPLE NEGATIVE BREAST CANCER (ER-, PR-, HER2-)
TNBC Clinical Characteristics

• Risk Factors:
  – Young
  – African American
  – BRCA carrier
  – >3 children
  – First birth <26

• Relapse Pattern:
  – Higher risk
  – Early timing
  – More visceral & CNS (46%)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>bone</th>
<th>-viscera</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNBC</td>
<td>79</td>
<td>13%</td>
<td>74%</td>
</tr>
<tr>
<td>ER+</td>
<td>123</td>
<td>39%</td>
<td>54%</td>
</tr>
<tr>
<td>HER2+</td>
<td>78</td>
<td>7%</td>
<td>81%</td>
</tr>
</tbody>
</table>

Dent et al; CCR 2007
Liedtke et al; JCO 2008
A link between TNBC and tumors in those who carry a BRCA mutation

• Most BRCA-1 carriers develop TNBC
• Sporadic TNBC:
  – Phenotypic similarity to BRCA-1
    • Patient & tumor characteristics
  – Gene expression similarities/aberrant DNA repair
  – BRCA dysfunction: “BRCA-ness”
• Similarities may be important!
  – Mechanism of development
  – Therapeutic strategies
Basal-like breast cancer and BRCA1

• Most BRCA1 carriers get basal-like breast cancers
• Shared characteristics with sporadic basal-like: “BRCAness”

Sorlie, PNAS 2003
Basal-Like Breast Cancer and BRCA1

Basal-like carcinomas

- BRCA1 downregulation (ID4 overexpression?)
- BRCA1 gene promoter methylation

Salivary gland-like tumors
IDC basal-like
Metaplastic
Medullary

Rakha, EA et al. J Clin Oncol 26:2568-2581 Copyright © 2010, Research To Practice, All rights reserved.
Standard cytotoxic therapeutics for TNBC

• Traditional “chemotherapy” remains the standard of care
• Traditional chemotherapy is “relatively” more important
• Some respond **VERY** well to chemotherapy others do not
• Is TNBC really one disease?
# TNBC “targeted” therapies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Target</th>
<th>Rationale/evidence</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinating agents</td>
<td>DNA: inter-strand breaks</td>
<td>*defective DNA repair</td>
<td>*neoadjuvant DFCI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*in vitro chemo-sensitivity in BRCA-1</td>
<td>*CALGB 40603</td>
</tr>
<tr>
<td>Anti-VEGF</td>
<td>VEGF</td>
<td>over-expression of VEGF</td>
<td>*positive phase III trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(unplanned subgroup)</td>
</tr>
<tr>
<td>PARP-1 inhibitors</td>
<td>PARP-1</td>
<td>*over-expression</td>
<td>*positive randomized phase II trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*in vitro sensitivity</td>
<td>*accrued phase III</td>
</tr>
</tbody>
</table>
Randomized phase II trial of PARPi BSI-201:

Metastatic TNBC (n=120)

Gemcitabine Carboplatin

Gemcitabine Carboplatin BSI-201

21 day cycle

<table>
<thead>
<tr>
<th></th>
<th>Gem/carbo (n=44)</th>
<th>Gem/carbo/BSI (n=42)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>7 (16%)</td>
<td>20 (48%)</td>
<td>0.002</td>
</tr>
<tr>
<td>CBR</td>
<td>9 (21%)</td>
<td>26 (62%)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

$\text{CBR} = \text{CR} + \text{PR} + \text{SD} \geq 6\text{mo}$
ASCO-Plenary; 2009
PARP inhibitor: Overall Survival

BSI-201 + Gem/CARBO (n = 57)
Median OS = 9.2 months

Gem/CARBO (n = 59)
Median OS = 5.7 months

P = 0.0005
HR = 0.348 (95% CI, 0.189-0.649)

O’Shaughnessy et al.
Finding new ways to attack triple negative breast cancer (next generation sequencing)
The Transcriptome

DNA

RNA

Protein
Why look at a Transcriptome

• Catalog of all mRNAs in the cell
• **Ability to determine therapeutic targets**
• Search space is limited to only actively transcribed areas... compared to studying whole genomes
• Allows for the study of coding and non-coding RNAs
So if the answer may lie in RNA.... How do we find it??

Next generation sequencing

The answer is here
How do we study it?... Next-Generation Sequencing

• A new method of high-throughput sequencing
• What once took 10 years and millions of $$ ... now takes 2 weeks and $30K
• Can analyze all RNA in a tissue
RNA is attached to beads and placed on glass slides
Data Capture

Measuring all the RNA that genes are producing
• 10 Triple Negative Breast Tumors from premenopausal women

Ribosomal depleted RNA

• 10 Normal Breast Tissues
• Microdissected ductal epithelium
• Samples split by menstrual phase

Sequence reads mapped to human genome using ABI BioScope 1.2

Statistical & biological analyses using custom & commercially available software

*Wikipedia
LASER CAPTURE MICRODISSECTION

For each sample: 70 frozen sections were microdissected. Four 11-hour days per sample at the University of Illinois.
Normals are quite different...
Menstrual Cycle can explain some of the difference
Tumor vs. Normal

7140 Genes that are significantly different between tumor and normal!
Heterogeneity in chemo-sensitivity & outcome may be explained by fundamental differences at the gene expression level.
Gene expression & SNPs as potential markers for outcome in ongoing HOG trial

*TNBC (local) or ER+/HER2- with BRCA mutation
*s/p neoadjuvant therapy with residual disease (>2cm or LN+)

Primary endpoint: 2 yr DFS

Gene expression as a marker of outcome
PARP SNPs as a predictor of outcome for PARPi
Impact of having tumor vs normal comparison for therapeutic discovery

• Tumor vs. tumor comparisons
  – Increased likelihood of “passenger” findings
  – Must sort out whether expression differences are due to:
    • overexpression of gene in a particular tumor type OR
    • Underexpression of “tumor control”

• Tumor vs. normal
  – By definition ALL differences have potential to be important

Schneider et al, CCR 14, 2008
The tale of 3 therapies in TNBC...

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Target</th>
<th>Rationale (prior data)</th>
<th>Next-Gen Transcriptome</th>
<th>Next-Gen Fold Change/P-value</th>
<th>Clinical Trial Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab &amp; Gefitinib</td>
<td>EGFR</td>
<td>Overexpression of EGFR</td>
<td>Not Overexpressed</td>
<td>-1.61 (p = 0.09)</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>Imatinib</td>
<td>c-KIT</td>
<td>Overexpression of c-KIT</td>
<td>Not Overexpressed</td>
<td>-6.82 (p = 1.8E-06)</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>BSI-201</td>
<td>PARP</td>
<td>Overexpression of PARP/Synthetic lethality in DNA repair</td>
<td>Overexpressed</td>
<td><strong>3.97 (p = 2.0E-05)</strong></td>
<td>POSITIVE</td>
</tr>
</tbody>
</table>
The $Million Question:

How do we find drugs that work?
Both COBRA1 & CSNK1G2 are known inhibitors of ER-alpha (ESR1) activity.
Using computers to find new therapies

Energy = -52.98 kcal/mol  RMSD = 0.89 Angstrom
Conclusions

• Breast cancer is more than one disease
• TNBC has variable chemo-sensitivity
• TNBC is more than one disease
• Targets & improved therapies are needed for TNBC
Acknowledgements

• Lab:
  – Milan Radovich
  – Bradley Hancock
  – Nawal Kassem
  – Abdulateef Aregbe

• IU Collaborators:
  – George Sledge
  – Susan Clare
  – Anna Maria Storniolo
  – Connie Rufenbarger
  – David Flockhart
  – Sunil Badve
  – Lang Li
  – Yunlong Liu
  – Samy Meroueh