Advances in Genomics and Proteomics in Breast Cancer Care

Hawaii Society of Clinical Oncology
November 15, 2014

Vered Stearns, M.D.
Breast and Ovarian Cancer Program
Sidney Kimmel Comprehensive Cancer Center
Johns Hopkins School of Medicine
Off-Label Use Disclosure(s)

I do not intend to discuss an off-label use of a product during this activity.
Financial Disclosure(s)

I currently have or have had the following relevant financial relations to disclose:

- *Commercial Interest -* Abbvie, Pfizer, Celgene, Merck, Novartis, Medimmune
- *Relationship with Commercial Interest -* Grant/Research Support
Clinical Utility of Tumor Markers in Breast Cancer

- Determine risk
- Screening
- Determine diagnosis
  - Benign vs. malignant states
  - Different types of malignancy
- Determine prognosis
  - Predict relapse (primary)
  - Predict progression (metastatic)
  - Predict survival
- Predict response to therapy (primary and metastatic)
  - Hormone therapy
  - Chemotherapy
  - Novel therapies
- Monitor course
  - Predict relapse (primary)
  - Follow detectable disease (metastatic)

Stearns et al. BCRT 1998;52:239-259
Prognostic and Predictive Factors in Breast Cancer

• Accepted
  – Age
  – Stage
  – Grade
  – ER Content, PR Content
  – HER2
  – Multiparameter gene expression, e.g., Onco\textit{type} DX

• Investigational
  – Multiparameter gene expression
  – Genomics
  – Proteomics
  – Circulating
  – Functional imaging
Future Clinical Trial Design: Must Consider Patient Subsets

Genetics
Epigenetics
Gene Expression
Proteomics
Metabolomics
Genetics
Genetics of Cancer

• Genes we are born with = Germline
  – A germline mutation can be found in every type of cell
  – e.g., BRCA1 and BRCA2

• Genes within the cancer = Somatic
  – Most genes within the cancer are the genes we are born with, but the genes within the cancer collect a series of mutations that are not seen in non-cancerous cells
Hereditary Breast Cancer
How Much Breast and Ovarian Cancer Is Hereditary?

Breast Cancer
- Sporadic
- Family clusters
- Hereditary (50% due to BRCA1/2)

5%–10%
15%–20%

Ovarian Cancer
- Sporadic
- Family clusters
- Hereditary (50% due to BRCA1/2)

5%–10%
Clinical Utility of BRCA Testing

• Determine risk
• Interventions to reduce risk
• Implications for treatment
  – Metastatic disease
  – Adjuvant trials
    • e.g., Olympia
Neoadjuvant chemotherapy

- gBRCA, TNBC patients

Surgery

- Axillary node positive (any tumour size)
  - Axillary node negative (T > 2cm)

Adjuvant Chemotherapy

- gBRCA, TNBC patients

Radiotherapy/additional surgery as required

Informed consent for participation in the study and confirmation of BRCA status

Randomisation

- (within 8 weeks after last treatment (surgery, chemotherapy or radiotherapy))

Olaparib 300 mg orally twice daily, continuous for 12 months OR

Placebo orally twice daily, continuous for 12 months

Mammogram/breast MRI 6 months from randomisation

Follow up for local and distant recurrence and survival status

Patients will continue to be followed clinically on a 3 monthly basis during the first 2 years, followed by 6 monthly assessments for the 3rd, 4th and 5th year, and annually thereafter.

Yearly breast imaging (mammogram/MRI) for 10 years
Tumor Genomics
Somatic Mutations

- Cancer is characterized by frequent somatic mutations
- Mutation profile may differ between a primary and metastatic lesion or among different metastatic sites
- Mutation status may also change with cancer progression
- Serial biopsies of metastatic sites is not often feasible
  - Invasive, not always accessible, costly, may delay treatment
Cancer Genome Landscape

PIK3CA (chromosome 3)

TP53 (chromosome 17)

Wood L, Science 2007
TCGA: Integrated data set for comparing and contrasting multiple tumor types
TCGA: Comprehensive Molecular Portraits of Human Breast Tumors

Nature 2012
<table>
<thead>
<tr>
<th>Subtype</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>Basal-like</th>
<th>HER2E</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+/HER2− (%)</td>
<td>87</td>
<td>82</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>HER2+ (%)</td>
<td>7</td>
<td>15</td>
<td>2</td>
<td>68</td>
</tr>
<tr>
<td>TNBCs (%)</td>
<td>2</td>
<td>1</td>
<td>80</td>
<td>9</td>
</tr>
<tr>
<td>TP53 pathway</td>
<td>TP53 mut (12%); gain of MDM2 (14%)</td>
<td>TP53 mut (32%); gain of MDM2 (31%)</td>
<td>TP53 mut (84%); gain of MDM2 (14%)</td>
<td>TP53 mut (75%); gain of MDM2 (30%)</td>
</tr>
<tr>
<td>PIK3CA/PTEN pathway</td>
<td>PIK3CA mut (49%); PTEN mut/loss (13%); INPP4B loss (9%)</td>
<td>PIK3CA mut (32%); PTEN mut/loss (24%); INPP4B loss (16%)</td>
<td>PIK3CA mut (7%); PTEN mut/loss (35%); INPP4B loss (30%)</td>
<td>PIK3CA mut (42%); PTEN mut/loss (19%); INPP4B loss (30%)</td>
</tr>
<tr>
<td>RB1 pathway</td>
<td>Cyclin D1 amp (29%); CDK4 gain (14%); low expression of CDKN2C; high expression of RB1</td>
<td>Cyclin D1 amp (58%); CDK4 gain (25%)</td>
<td>RB1 mut/loss (20%); cyclin E1 amp (9%); high expression of CDKN2A; low expression of RB1</td>
<td>Cyclin D1 amp (38%); CDK4 gain (24%)</td>
</tr>
<tr>
<td>mRNA expression</td>
<td>High ER cluster; low proliferation</td>
<td>Lower ER cluster; high proliferation</td>
<td>Basal signature; high proliferation</td>
<td>HER2 amplicon signature; high proliferation</td>
</tr>
<tr>
<td>Copy number</td>
<td>Most diploid; many with quiet genomes; 1q, 8q, 8p11 gain; 8p, 16q loss; 11q13.3 amp (24%)</td>
<td>Most aneuploid; many with focal amp; 1q, 8q, 8p11 gain; 8p, 16q loss; 11q13.3 amp (51%); 8p11.23 amp (28%)</td>
<td>Most aneuploid; high genomic instability; 1q, 10p gain; 8p, 5q loss; MYC focal gain (40%)</td>
<td>Most aneuploid; high genomic instability; 1q, 8q gain; 8p loss; 17q12 focal ERRB2 amp (71%)</td>
</tr>
<tr>
<td>DNA mutations</td>
<td>PIK3CA (49%); TP53 (12%); GATA3 (14%); MAP3K1 (14%)</td>
<td>TP53 (32%); PIK3CA (32%); MAP3K1 (5%)</td>
<td>TP53 (84%); PIK3CA (7%)</td>
<td>TP53 (75%); PIK3CA (42%); PIK3R1 (8%)</td>
</tr>
<tr>
<td>DNA methylation</td>
<td>–</td>
<td>Hypermethylated phenotype for subset</td>
<td>Hypomethylated</td>
<td>–</td>
</tr>
<tr>
<td>Protein expression</td>
<td>High oestrogen signalling; high MYB; RPPA reactive subtypes</td>
<td>Less oestrogen signalling; high FOXM1 and MYC; RPPA reactive subtypes</td>
<td>High expression of DNA repair proteins, PTEN and INPP4B loss signature (pAKT)</td>
<td>High protein and phospho-protein expression of EGFR and HER2</td>
</tr>
</tbody>
</table>

Percentages are based on 466 tumour overlap list. Amp, amplification; mut, mutation.
MDACC Phase I Experience

Reviewed records of 106 consecutive patients with metastatic TNBC treated in phase I clinic at MD Anderson between August 2005 and May 2012:

Table 2: Therapies received in the phase I clinical trials program.

<table>
<thead>
<tr>
<th>Phase I therapy</th>
<th>No. of patients</th>
<th>Evaluable (n)</th>
<th>CR (n)</th>
<th>PR (n)</th>
<th>SD≥6mo (n)</th>
<th>SD≥6mo/PR/CR (n)</th>
<th>Median PFS mo (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy alone(^a)</td>
<td>8 (8)</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/7 (0)</td>
<td>2.1 (0.9-3.3)(^c)</td>
</tr>
<tr>
<td>Chemotherapy and targeted agent(^b)</td>
<td>63 (59)</td>
<td>57</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>11/57 (19)</td>
<td>3.0 (1.9-4.1)(^c)</td>
</tr>
<tr>
<td>Single agent targeted drug</td>
<td>15 (14)</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/15 (0)</td>
<td>1.1 (0.7-1.4)(^c)</td>
</tr>
<tr>
<td>≥2 targeted agents</td>
<td>20 (19)</td>
<td>19</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1/19 (5)</td>
<td>1.9 (1.4-2.4)(^c)</td>
</tr>
<tr>
<td>Total</td>
<td>106 (100)</td>
<td>98</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>12/98 (12)</td>
<td>2.1 (1.5-2.6)</td>
</tr>
</tbody>
</table>

\(^a\) one or more agents
\(^b\) one or more of each
\(^c\) p value <0.0001 for comparison of PFS by log rank test across all 4 groups

Abbreviations: CR, complete response; CI, confidence interval; mo, months; PR, partial response; PFS, progression-free survival; SD, stable disease

Mol cancer Ther 2014
Table 4: Summary of multivariate analysis for response, progression-free survival, and, overall survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimated Effect</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response (SD ≥6 months/PR/CR)</td>
<td>OR(^{a})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic sites ≤2 (vs &gt;2)</td>
<td>10.62</td>
<td>1.52-74.09</td>
<td>0.017</td>
</tr>
<tr>
<td>Chemotherapeutic and targeted agents (vs chemotherapeutic or targeted agent only)</td>
<td>27.02</td>
<td>1.43-511.4</td>
<td>0.028</td>
</tr>
<tr>
<td><strong>Progression-free survival</strong></td>
<td>HR(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic sites ≤2 (vs &gt;2)</td>
<td>0.44</td>
<td>0.26-0.75</td>
<td>0.003</td>
</tr>
<tr>
<td>Chemotherapeutic and targeted agents (vs chemotherapeutic or targeted agent only)</td>
<td>0.38</td>
<td>0.22-0.633</td>
<td>0.0002</td>
</tr>
<tr>
<td>PI3K pathway inhibitors, yes (vs no)</td>
<td>0.49</td>
<td>0.27-0.88</td>
<td>0.018</td>
</tr>
<tr>
<td>Anti-angiogenic agents, yes (vs no)</td>
<td>0.52</td>
<td>0.29-0.91</td>
<td>0.023</td>
</tr>
<tr>
<td><strong>Overall survival</strong></td>
<td>HR(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDACC score ≤2 (vs &gt;2)</td>
<td>0.25</td>
<td>0.15-0.41</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^{a}\)OR>1 is associated with higher response.

\(^{b}\)HR<1 is associated with longer progression-free survival or overall survival.

Abbreviations: CR, complete response; CI, confidence interval; HR, hazards ratio; OR, odds ratio; PR, partial response; SD, stable disease; MDACC, MD Anderson Cancer Center
A) PFS of 16 patients treated on matched therapy (6.4 mo) or other therapies (1.9 mo), p < 0.001
B) PFS of 63 patients treated on CT+targeted agents (3.0 mo) or 43 on other therapies (1.6 mo), p < 0.0001
Johns Hopkins Effort: IMAGE: Individualized Molecular Analyses Guide Efforts in Breast Cancer

• Primary Objective:
  – To demonstrate the feasibility of real-time molecular profiling of metastatic breast cancer patients in less than 28 days from consent to analysis and suggestions.

• Secondary Objective:
  – To prospectively follow plasma tumor DNA in all patients who take part.
Fig. 5. J12129 Schema. A schema for collecting tissues and blood samples to perform NGS on a limited number of cancer genes.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>n=23</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>54</td>
<td>25-67</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>n=11 (48%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>n=10 (43%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>n=2 (9%)</td>
<td></td>
</tr>
<tr>
<td>ECOG PS</td>
<td>1.0</td>
<td>0-2</td>
</tr>
<tr>
<td>Lines of Therapy</td>
<td>3.0</td>
<td>1-6</td>
</tr>
</tbody>
</table>
Tumor Profiling Summary: 19/23 Evaluable Specimens

Genetic Aberrations by Frequency

<table>
<thead>
<tr>
<th>Locus Affected</th>
<th>Percentage harboring aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>100.0%</td>
</tr>
<tr>
<td>MYC</td>
<td>31.6%</td>
</tr>
<tr>
<td>CCNE1</td>
<td>26.3%</td>
</tr>
<tr>
<td>MCL1</td>
<td>26.3%</td>
</tr>
<tr>
<td>NOTCH1</td>
<td>21.1%</td>
</tr>
<tr>
<td>FGFR1</td>
<td>15.8%</td>
</tr>
<tr>
<td>MYST3</td>
<td>15.8%</td>
</tr>
</tbody>
</table>

Heather Parsons, Vered Stearns, Ben Park
Ongoing and Planned Studies

- NCI Match
- My Pathway
- Signature
- Many others
Genetic Sequencing (3000) → Actionable mutation detected → Study agent

- Stable disease\(^1\), Complete or partial response (CR+PR)\(^2\) → Continue on study agent until progression
- Progressive disease (PD)\(^2\) → Check for additional actionable mutations \(^3\)

\(^1\) Stable disease is assessed relative to tumor status at re-initiation of study agent
\(^2\) CR, PR, SD, and PD as defined by RECIST
\(^3\) Rebiopsy; if additional mutations, offer new targeted therapy

No additional actionable mutations, or withdraw consent → Off Study
NCI-MATCH

- Umbrella protocol for multiple, single-arm phase II trials
  - Each molecular subgroup matched to a targeted agent
- CTEP-IND for protocol template
  - Arms could be added or deleted without affecting other arms
  - Device discussions with CDRH
- Initially focused on single-agents (commercial or experimental)
  - Combinations will be considered for targets that have validated combination targeted therapy
  - Need minimum dose/safety established in phase 1 trials
- Study will be reviewed by the CIRB
NCI-MATCH In Progress

- Currently 20 “arms”

- EGFR, HER2, MET, BRAF, NF1, GNAQ, GNA11, TSC1/2, PTEN, Patch, NF2, ALK, ROS, FGFR
ML28897/PRO 02

MY PATHWAY: AN OPEN-LABEL PHASE IIA STUDY EVALUATING TRASTUZUMAB/PERTUZUMAB, ERLOTINIB, VEMURAFENIB, AND VISMODEGIB IN PATIENTS WHO HAVE ADVANCED SOLID TUMORS WITH MUTATIONS OR GENE EXPRESSION ABNORMALITIES PREDICTIVE OF RESPONSE TO ONE OF THESE AGENTS

Sponsor: Genentech
NCT02091141

http://clinicaltrials.gov/ct2/show/NCT02091141?term=my+pathway&rank=1
<table>
<thead>
<tr>
<th>Molecular Abnormality</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 overexpression, amplification, or mutation</td>
<td>Trastuzumab/Pertuzumab</td>
</tr>
<tr>
<td>EGFR activating mutation</td>
<td>Erlotinib</td>
</tr>
<tr>
<td>BRAF mutation (V600E or others)</td>
<td>Vemurafenib</td>
</tr>
<tr>
<td>Hedgehog pathway mutation (SMO, PTCH-1, others)</td>
<td>Vismodegib</td>
</tr>
</tbody>
</table>
PRO 02 My Pathway Study: Schema

N=500
125 per pathway
Novartis: Signature

- A modular Phase II trial to link targeted therapy to patients with pathway-activated tumors
- Rapidly matches patients to therapies that target their tumors' genetic alterations
- Features a rapid trial deployment model and patient-sparing adaptive statistical design
Compounds included in Signature

• BKM120 (Pan PI3Ki)
• TKI258 (FGFRi)
• MEK162 (MEKi)
• LGX818 (RAFi)
• LDE225 (SMOi)
• BGJ398 (FGFRi)
• LEE011 (CDK4/6i)
• LDK378 (ALKi)
• Combinations TBD
Detecting DNA Somatic Mutations in the Circulation

- DNA somatic mutations are highly tumor specific
- Tumor DNA fragments can be detected in the circulation
- As tumors enlarge and invade, they outgrow their blood supply, necrosis increases and the amount of circulating mutant DNA rises
- Cell-free tumor DNA is present in plasma of cancer patients and can reflect the mutations present in the tumors, however, the frequency compared to germline DNA is low
- Novel and highly sensitive techniques are now available to detect these mutations quantitatively and qualitatively
- Can be used as a biomarker or as a therapeutic target
Measuring Circulating DNA

• Methods
  – Digital PCR
    • BEAMing
    • Droplet digital PCR
  – Quantitative PCR using primers that only amplify unique somatic rearrangements in cancer cells

• Clinical utility
  – Treatment decisions in the metastatic setting
  – Adjuvant and neoadjuvant decisions
Digital PCR

Normal + Mutant

Courtesy of Ben Park
Detection of Tumor \textit{PIK3CA} Status in Peripheral Blood

- Accurate identification of tumors with sensitizing or desensitizing mutations to specific drugs are critical
- Enrolled 49 women with metastatic breast cancer
- Obtained a prior breast cancer tissue sample
- Purified and amplified DNA, and PIK3CA exons 9 and 20 mutations were identified
- 10 mL peripheral venous blood sample to detect plasma-derived DNA fragments (ptDNA) by BEAMing
- Compared mutational status in tissue and plasma

<table>
<thead>
<tr>
<th>Description of Tissue Tumor DNA and Plasma-Derived ptDNA for Mutation Analysis</th>
<th>Number of Samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate archival tissue available for sequencing</td>
<td>51/60 (85)</td>
</tr>
<tr>
<td>Adequate plasma available for ptDNA extraction and BEAMing</td>
<td>60/60 (100)</td>
</tr>
<tr>
<td>PIK3CA mutations identified by sequencing archival tissue</td>
<td>14/51 (27.4)</td>
</tr>
<tr>
<td>PIK3CA mutations identified by BEAMing of plasma-derived ctDNA</td>
<td>17/60 (28.3)</td>
</tr>
<tr>
<td>PIK3CA wild-type by sequencing of archival tissue and</td>
<td>37/51 (72.5)</td>
</tr>
<tr>
<td>PIK3CA wild-type by BEAMing of plasma-derived ctDNA</td>
<td></td>
</tr>
<tr>
<td>PIK3CA mutant by sequencing of archival tissue and</td>
<td>8/51 (15.6)</td>
</tr>
<tr>
<td>PIK3CA mutant by BEAMing of plasma-derived ctDNA</td>
<td></td>
</tr>
<tr>
<td>PIK3CA wild-type on sequencing of archival tissue and</td>
<td>8/51 (15.6)</td>
</tr>
<tr>
<td>PIK3CA mutant by BEAMing of plasma-derived ctDNA</td>
<td></td>
</tr>
<tr>
<td>PIK3CA mutant on sequencing of archival tissue and</td>
<td>6/51 (11.7)</td>
</tr>
<tr>
<td>PIK3CA wild-type by BEAMing of plasma-derived ctDNA</td>
<td></td>
</tr>
</tbody>
</table>
ptDNA in Early Breast Cancer

- **PIK3CA** mutation status changes in 20% of patients with metastatic breast cancer compared to primary tumors.
- May be used for assessment at trial entry: “Liquid biopsy.”
- Mutations can also be detected in the circulation of women with early (Stage I) breast cancer.

Courtesy of Ben Park
Decisions to treat are based on results from clinical trials

- Large randomized multi-institutional trials comparing adjuvant therapy vs. controls
- Takes years to prove effectiveness
- A consequence of this type of study is that it leads to “overtreatment”
Limitations of Current Approach

• How can we determine on an individual level who truly needs additional therapies, i.e. has microscopic metastatic disease?
• How can we determine in patients who need adjuvant therapies whether systemic treatment is working?
• How can we accelerate the pace of clinical trials?
Genetic alterations (mutations) in tumor DNA are specific for cancer cells.

Cell-free, plasma-derived tumor DNA fragments (ptDNA) can be readily found in the blood.

ptDNA can therefore be a marker of whether micrometastatic disease is present.
ptDNA & pCR in Neoadjuvant Setting: Methods

NGS tumor: TSM discovery

Choose primary TSM, Confirm ptDNA TSM via ddPCR

Evaluate post-treatment plasma for TSM

Correlate ptDNA results with pCR vs. residual disease, clinical outcomes

TSM - Tumor-specific mutation

von Minckwitz G et al. JCO 2012;30:1796-1804
Case 1

• 63 years old woman
• 2009: Left-sided DCIS, ER+
  – Lumpectomy and radiation therapy
• 2012: Inflammatory changes in the left breast
  – Biopsy showed TNBC
  – Neoadjuvant TAC x 5 cycles → Initial response then progression
  – Left mastectomy with a rotating flap for a surgical closure
  – Path: extensive disease in positive margins
  – Capecitabine x 6 → evidence of disease progression in lymph nodes and both axilla, skin, and a growing left supraclavicular lymph node
  – Eribulin 2 weeks on and 2 weeks off → initial PR then PD
• No significant family history
Case 1 (cont.)

• Enrolled in IMAGE
• FoundationOne testing revealed the following alterations:
  – MAP2K1 (MEK1) amplification
  – CCNE1 amplification
  – MCL1 amplification
  – MYC amplification
  – TP53 N247K, R175H
  – CREBBP TRAP1-CREBBP fusion
  – SETD2 T2354A
Case 1: What Would You Recommend Next?

- Cisplatin or carboplatin
- CDK2 inhibitors
- Trials with MEK inhibitors
- Trials with immune checkpoints
- Off label trametinib
Case 1: Tumor Board Recommendation

- MAP2K1 (MEK1) amplification may be actionable
  - Encodes the signaling protein mitogen-activated protein kinase kinase 1 (MKK1 or MEK1) Unknown whether amplification of this gene would lead to a functional consequence, but based on role in the MAPK pathway it can be presumed
  - Consider pursuing a clinical trial which targets MEK
  - Trametinib is an FDA approved MEK inhibitor for V600-mutant melanoma in combination with Dabrafenib, and is in clinical trials in solid tumors, if a trial is not possible, consider off label use

- CCNE1 amplification
  - Encodes for Cyclin E2
  - Preclinical data in ovarian cancer showing responsiveness of cell lines with CCNE1 amplification to CDK2 inhibitors, compared to cell lines without amplification
Case 1: Conclusion

• The primary oncologist was able to obtain off label Trametinib (Mekinist)
• Initial response was noted within 2 weeks
Gene Expression Profiles
Breast Cancer Subtypes

Sorlie 2001; Proc. Natl. Acad. Sci. USA 98, 10869-10874
### Surrogate Definitions of Intrinsic Subtypes: St Gallen

<table>
<thead>
<tr>
<th>Intrinsic subtype</th>
<th>Clinico-pathologic surrogate definition</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>‘Luminal A-like’&lt;br&gt;all of:&lt;br&gt;ER and PgR positive&lt;br&gt;HER2 negative&lt;br&gt;Ki-67 'low'&lt;br&gt;Recurrence risk 'low' based on multi-gene-expression assay (if available)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>The cut-point between ‘high’ and ‘low’ values for Ki-67 varies between laboratories.&lt;sup&gt;a&lt;/sup&gt; A level of &lt;14% best correlated with the gene-expression definition of Luminal A based on the results in a single reference laboratory [23]. Similarly, the added value of PgR in distinguishing between ‘Luminal A-like’ and ‘Luminal B-like’ subtypes derives from the work of Prat et al. which used a PgR cut-point of ≥20% to best correspond to Luminal A subtype [24]. Quality assurance programmes are essential for laboratories reporting these results.</td>
</tr>
<tr>
<td>Luminal B</td>
<td>‘Luminal B-like (HER2 negative)’&lt;br&gt;ER positive&lt;br&gt;HER2 negative&lt;br&gt;and at least one of:&lt;br&gt;Ki-67 'high'&lt;br&gt;PgR ‘negative or low’&lt;br&gt;Recurrence risk ‘high’ based on multi-gene-expression assay (if available)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>‘Luminal B-like’ disease comprises those luminal cases which lack the characteristics noted above for ‘Luminal A-like’ disease. Thus, either a high Ki-67&lt;sup&gt;a&lt;/sup&gt; value or a low PgR value (see above) may be used to distinguish between ‘Luminal A-like’ and ‘Luminal B-like (HER2 negative)’.</td>
</tr>
<tr>
<td>Luminal B</td>
<td>‘Luminal B-like (HER2 positive)’&lt;br&gt;ER positive&lt;br&gt;HER2 over-expressed or amplified&lt;br&gt;Any Ki-67&lt;br&gt;Any PgR</td>
<td></td>
</tr>
<tr>
<td>Erb-B2 overexpression</td>
<td>‘HER2 positive (non-luminal)’&lt;br&gt;HER2 over-expressed or amplified&lt;br&gt;ER and PgR absent</td>
<td></td>
</tr>
<tr>
<td>‘Basal-like’</td>
<td>‘Triple negative (ductal)’&lt;br&gt;ER and PgR absent&lt;br&gt;HER2 negative</td>
<td>There is an 80% overlap between ‘triple-negative’ and intrinsic ‘basal-like’ subtype. Some cases with low-positive ER staining may cluster with non-luminal subtypes on gene-expression analysis. ‘Triple negative’ also includes some special histological types such as adenoid cystic carcinoma.</td>
</tr>
</tbody>
</table>
Hormone Receptor-Positive Tumors

- Assess likelihood of response to endocrine manipulations
- Benefit from CT or novel targeted therapies
- Mechanisms of resistance: HER2, AKT/mTOR/PI3K pathway, angiogenesis, epigenetics
  - De novo or acquired
  - Early vs. late recurrence

Program for the Assessment of Clinical Cancer Tests (PACCT-1): Trial Assigning Individualized Options for Treatment: The TAILORx Trial
Node-positive HR-positive and HER2-negative breast cancer

Number of positive nodes?

1-3 positive

Patients consent to study-sponsored RS testing if not already done

RECURRENCE SCORE evaluated

RS > 25

Completion of chemotherapy

RS = 25

Eligible for S1007 (RxPONDER) if other criteria are met

4+ positive

Eligible for S1207 (everolimus trial) if other criteria are met
HER2-Positive Tumors

• Assess likelihood of response to anti-HER2 therapies
• HER2 testing
• Dual targeting
• ER status matters
• Benefit from CT or novel targeted therapies
• Mechanisms of resistance: PI3K pathway, PTEN loss
Triple-Negative Breast Cancer

- Approximately 20% of newly diagnosed breast cancers
- Mostly basal: high proliferation index, CK5/6+, EGFR, Ckit, p53 mutation, genomic instability
- Common in BRCA1 mutation carriers and in Blacks
- Most women diagnosed with stage I-III disease will receive third-generation chemotherapy
  - Reduce risk of relapse by ~33-50%
- Many women will suffer a recurrence or will be diagnosed with metastatic disease
- Metastatic disease is often visceral and associated with poor survival
- Further molecular stratification is needed
A

Training Set
14 Human Breast Cancer Expression Datasets
(n = 2353)

Validation Set
7 Human Breast Cancer Gene Expression Datasets
(n = 894)

Bimodal Filter on
ER, PR, and HER2 Expression

386 Breast Tumors with TN Phenotype

201 Breast Tumors with TN Phenotype

k-means clustering

7 TNBC Subtypes

Best-fit TNBC Subtype

Correlation to Training Set GE Signature

GSEA

B

ER

PR

HER2

Expression (log2)

Frequency

C

Controls

386 Tumor Samples

Expression (log2)

ER

PR

HER2

Lehmann et al. JCI 2011;121:2750-67
Therapeutic Targets

- DNA damaging agents
- Tyrosine kinase inhibitors
- Anti-angiogenic agents
- Androgen receptor antagonists
- Epigenetic modulators
- Mesenchymal stem like
- Immune modulation
Case 2

- 67 years old and was first diagnosed with bilateral breast cancer in 2008
  - Left sided TNBC, clinical stage II
  - Right sided ER+ cancer
  - Received neoadjuvant chemotherapy with AC→T
  - Bilateral mastectomy and SNB, right axillary dissection
  - Path:
    - Left : T2 (2.7 cm) N0, grade 2 TNBC
    - Right : T1 (1.9 cm) N1 (1 pos node), grade 1, ER>95%, PR>95%, Her-2 negative invasive ductal carcinoma
  - Right-sided radiation
  - Aromatase inhibitor from 2009 until June 2012
Case 2 (cont.)

Recurred with metastatic TNBC in 4/2012: left axilla and pulmonary mets

Treatment:

- 7-9/2012 Olaparib plus carboplatin no response
- 9-10/2012 Capecitabine 9-10/2012) no response
- 11/12-5/13 Docetaxel PR (axillary, skin mets)
- 7-9/13 anti-PDL1 antibody PD, new skin mets

Family history is notable for a cousin with breast cancer and “some uterine cancer,” and she is BRCA negative per report
Case 2: What Would You Recommend Next?

A. Third line chemotherapy agents such as eribulin
B. Genetic testing
C. A tumor profiling test
D. B and C
Case 2: What Would You Recommend Next?

A. Third line chemotherapy agents such as eribulin
B. Genetic testing
C. A tumor profiling test
D. B and C
Case 2 (cont.)

Genetic testing
• BRCA negative

Foundation medicine test obtained
• Genetic alterations
  – PTEN (T319fs*6 and Y188fs*2)
  – NF 1 duplication
  – TP53 mutation
Case 2 (cont.)

Genetic testing
- BRCA negative

Foundation medicine test obtained
- Genetic alterations
  - PTEN (T319fs*6 and Y188fs*2)
  - NF 1 duplication
  - TP53 mutation

Tumor tested for Androgen (AR) receptor and was AR+
Case 2: What Would You Recommend Next?

A. Standard chemotherapy agent, eg eribulin
B. Targeting PI3K and MAPK pathways
C. Targeting AR
Case 2: Conclusion

- She was placed by her local oncologist on bicalutamide (Casodex) with a relatively rapid progression
- About 10% of TNBC are AR+
- Small clinical benefit observed with bicalutamide

<table>
<thead>
<tr>
<th>Pts with clinical benefit on bicalutamide</th>
<th>AR%</th>
<th>ER%</th>
<th>PgR%</th>
<th>HER2</th>
<th>Site of testing</th>
<th>Site of metastases</th>
<th>Prior therapy LABC/MBC</th>
<th>DOR on prior therapy, wks</th>
<th>DOR on bicalutamide, wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>10-20</td>
<td>1</td>
<td>0</td>
<td>Neg</td>
<td>P</td>
<td>LN</td>
<td>0</td>
<td>NA</td>
<td>231+</td>
</tr>
<tr>
<td>#2</td>
<td>&gt;80</td>
<td>3</td>
<td>0</td>
<td>Neg</td>
<td>Met</td>
<td>GI</td>
<td>0</td>
<td>NA</td>
<td>54</td>
</tr>
<tr>
<td>#3</td>
<td>&gt;80</td>
<td>0</td>
<td>0</td>
<td>+/-</td>
<td>P</td>
<td>Breast LN</td>
<td>1</td>
<td>NR</td>
<td>25</td>
</tr>
<tr>
<td>#4</td>
<td>&gt;90</td>
<td>0</td>
<td>0</td>
<td>Neg</td>
<td>P</td>
<td>LN Bone</td>
<td>1</td>
<td>158</td>
<td>35</td>
</tr>
<tr>
<td>#5</td>
<td>&gt;50</td>
<td>0</td>
<td>0</td>
<td>Neg</td>
<td>P</td>
<td>LN Bone</td>
<td>1</td>
<td>15</td>
<td>43+</td>
</tr>
</tbody>
</table>

Abbreviations: DOR, duration of response; GI, gastrointestinal; LABC, locally advanced breast cancer; LN, lymph node; Met, metastasis; NA, not applicable; NR, no response; P, primary tumor.

- New studies will target AR and PI3K

Proteomics
Proteomics to Identify Biomarkers

Akhilesh Pandey, M.D., Ph.D.
Proteomics to Identify Novel Tumor Suppressors in Breast Cancer

Uncovering a Tumor Suppressor for Triple-Negative Breast Cancers

John G. Albeck and Joan S. Brugge

Activation of Multiple Proto-oncogenic Tyrosine Kinases in Breast Cancer via Loss of the PTPN12 Phosphatase

Tingting Sun, Nicola Aceto,1,8,9 Kristen L. Meerbey,1,8,9 Jessica D. Kessler,1,8 Chuanghui Zhou,1,8 Ilene Migliaccio,1,8 Don X. Nguyen,1,8 Natalia N. Pavlova,1,8 Maria Botero,1,8 Jian Huang,1,8 Ronald J. Bernard,1,8 Barrie Schmitt,1,8 Guang Hu,1,8 Marrie Z. Li,1,8 Noah Daphourl,1,8 Steven P. Gygi,1,8 Mitchell Faso,1,8 Chad J. Creighton,1,8 Susan G. Hiseanbeck,1,8 Chad A. Shaw,1,8 Donna Mazaryk,1,8 Richard A. Gibbs,1,8 David A. Wheeler,1,8 C. Kent Osborne,1,8,9 Rachel Schmitt,1,8,9,10 Mohamed Bertins-Menji,1,8,10 Stephen J. Eledge,1,8,10 and Thomas F. Westbrook1,8,9

Akhilesh Pandey, M.D., Ph.D.

Sun et al., Cell, 2012
Proteomics to study Mechanisms of Drug Resistance in Breast Cancer

Akhilesh Pandey, M.D., Ph.D.

Duncan et al. Cell, 2012
Proteomics to Dissect Pathway Alterations Due to Mutations in Breast Cancer

Wu et al. Nature Communications, 2014

Akhilesh Pandey, M.D., Ph.D.
Proteomics to Identify Drug Targets in Breast Cancer

LETTER

doi:10.1038/nature10167

Selective killing of cancer cells by a small molecule targeting the stress response to ROS

Lakshmi Raj¹, Takao Ide¹, Aditi U. Gurkar¹, Michael Foley², Monica Schenone², Xiaoyu Li², Nicola J. Tolliday², Todd R. Golub², Steven A. Carr², Alykhan F. Shamji², Andrew M. Stern², Anna Mandinova¹,², Stuart L. Schreiber² & Sam W. Lee¹,²

Akhilesh Pandey, M.D., Ph.D.

Raj et al., Nature, 2011
Clinical Utility of Tumor Markers in Breast Cancer

- Determine risk
- Screening
- Determine diagnosis
  - Benign vs. malignant states
  - Different types of malignancy
- Determine prognosis
  - Predict relapse (primary)
  - Predict progression (metastatic)
  - Predict survival
- Predict response to therapy (primary and metastatic)
  - Hormone therapy
  - Chemotherapy
  - Novel therapies
- Monitor course
  - Predict relapse (primary)
  - Follow detectable disease (metastatic)

Stearns et al. *BCRT* 1998;52:239-259
Tumor Marker Development Flow Chart

LOE

Types of studies

None

Cell lines

V

Expression in unlinked tissue
Some outcomes

IV

Apparent incidence of expression
Some outcomes, not linked to clinical trial

III

Outcomes, hypothesis generation, MVA
Linked to small trial or to non-trial

II

Validation of hypothesis from archived specimens from prospective trials

I

Prospective clinical trial specifically of tumor marker

Research

Clinical utility

Broad Idea

General Hypothesis

More focused hypothesis

Develop hypothesis re: specific use

Test specific hypothesis

New ideas/new hypothesis

Relative expertise

Biologic

+++ +++ ++ ++ ++ +

Technical

+ ++ +++ +++ + + +

Clinical/statistical

+/- + + + ++ +++ +++

Henry NL and Hayes. Oncologist 2006;11:541-552
As part of the Cancer Center’s Personalized Medicine effort, there is a need to have a “molecular profiling tumor board” to interpret genetic alterations found in a patient’s tumor sample.

The GAITWAY tumor board was formed in response to this need.
GAITWAY Tumor Board: Mission

- To review a patient’s molecular tumor profile including but not limited to mutations, copy number changes, immunohistochemical staining of potentially “actionable” genes/proteins from commercial and in house assays
- To review the relevant literature on the evidence that such genetic alterations are of functional consequence and therefore could be actionable
- To review the current state of FDA approved therapies and clinical trials evaluating therapies for the individual genetic alterations.
- To evaluate the weight of evidence for current standard of care therapies versus pursuing “actionable” alterations and the likelihood of best responses for each patient
- To discuss and prioritize recommendations based upon the genetic assays and convey the GAITWAY tumor board’s suggestions back to the referring physician and patient

Ben Ho Park, M.D., Ph.D.
Definition of “actionable”

• A genetic alteration that has an FDA approved therapy for the given tumor type (highest priority), e.g., Vemurafenib for V600E mutant metastatic melanoma
• A genetic alteration that has an FDA approved therapy for a different tumor type, e.g., vemurafenib for V600E mutation found in thyroid cancer
• A genetic alteration that may provide rationale for participation in a clinical trial of a targeted therapy
• A genetic alteration that may lead to recommendations for genetic counseling and germline mutation testing

Ben Ho Park, M.D., Ph.D.
Additional Considerations

- Ethical considerations for pursuing “nth” line standard of care therapies versus targeted therapies with unproven value
- Discovering and interpreting potential germline variants and need for genetic counseling/testing
- Legal implications for reporting or not reporting incidental findings, e.g., finding proviral HIV DNA in cancers using whole genome sequencing
Thank You