Next Generation Sequencing Assays in Primary and Metastatic Breast Cancers:

Implications for Clinical Practice

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NGS = targeted sequencing of selected panel of genes or whole exome sequencing

**Routine Clinical Use (in Breast Cancer)**
- To facilitate accrual to clinical trials

**Research Use**
- Identify novel and currently drugable therapeutic targets
- Study tumor heterogeneity and disease evolution
NGS in Routine Clinical Practice

• There is no FDA cleared NGS assay for cancer therapeutic target profiling
  – 2013 FDA cleared the Illumina MiSeqDx instrument and the Universal Kit reagents, MiSeqDx Cystic Fibrosis 139-Variant Assay, and MiSeqDx Cystic Fibrosis Clinical Sequencing Assay for clinical use.

• There are several commercial CLIA labs that provide targeted NGS for cancer profiling
  – Foundation Medicine (NGS of 315 genes + 28 genes often rearranged in cancer)
  – Caris Life Sciences (multiple panels, multiplatform assays: IHC, FISH, PCR, NGS)
  – Paradigm PCDx (NGS of 114 cancer genes)

• Many academic institutions perform targeted NGS-based cancer target profiling in their CLIA laboratories
  – Platforms vary (Illumina, IonTorrent, etc…)
  – With or without reflex validation with pCR or Sanger (ABI)
  – Assay performance or analytic validity of tests run in CLIA labs are not public
Melanoma and Lung and Colorectal cancers have validated DNA sequence-based predictive markers but Breast Cancer does not.

The Hypothesis: Cancers that harbor a “driver” mutation, regardless of histologic type, will respond to a targeted drug while cancers without the mutation will not.

**Support**

- Selected preclinical data, “enticing hypotheses”
- Several clinical success stories
  - HER2 / trastuzumab: Breast + Gastric
  - Bcr-Abl / imatinib: CML
  - cKIT/PDGFR / imatinib: GIST
  - EML4/ALK / crizotinib: NSCLC
  - BRAF / vemurafenib: Melanoma + Thyroid + NSCLC
- Clinical anecdotes (N=1 cases)

**Caveats**

- Most cancers harbors >30 mutations
- Several negative trial results
  - BRAF / vemurafenib: Colon
  - PI3K / everolimus: Breast
  - CDK4/6 / palbociclib: Breast
  - HER2 / trastuzumab: Lung, Ovarian
  - ER / tamoxifen: Ovarian
- Clinical anecdotes lack the denominator
The most frequent “potentially targetable” NGS findings in breast cancer (Foundation One assay)

Samples with actionable alterations, 84%

% of samples

Genes with potentially actionable alterations
Genes with alterations, not currently actionable

N Vassan et al. The Oncologist, 19:1-6, 2014
The clinical utility of molecular target profiling of cancer and treatment selection, outside of a few FDA indications, is not yet proven.

Testing this concept in clinical trials is a high priority.

- **Institutional clinical trials**
- **Novartis – Signature** (open)
  - N=1 local trials
- **Genentech – My Pathway** (open)
  - N=1 local trials
- **NCI-MATCH** (yet to be activated)
  - ECOG-ACRIN-NCTN
  - CTSU-CCOP
Institutional Trials

Yale Cancer Center Molecular Analysis Prior to Investigational Therapy (MAP-IT)

http://clinicaltrials.gov: NCT01855503

Metastatic Breast Cancer

Yale Pathology
IHC for, AR, ER, HER2, (PDL1)

Yale Research Lab:
Whole exome sequencing

Phase II clinical trial
(PIK3C, FGFR, AR, NOTCH, BRCA)

Phase I program

Signature;
My Pathway;
(MATCH)

http://medicine.yale.edu/lab/pusztai/clinicaltrials/index.aspx#page1
Novartis – Signature Trial
www.signaturetrial.com, or Tel: 1-855-SIGN-P2P (1-855-744-6727)

Step 1
Patient
• Tissue sample collected by treating physician.
• Micro array based genomic test ordered.

Step 2
Selection
• Treating physician evaluates gene expression results
• Patient evaluated for drug trial designed for intervention of impacted molecular pathway

Step 3
Novartis Protocol
• Eligible patient can participate on open Novartis study with a trial drug expected to block impacted molecular pathway.

Tier 1 Compounds OPEN in 2013
1. BKM120 (Pan PI3Ki)
2. TKI258 (FGFRi)*
3. MEK162 (MEKi)
4. LGX818 (RAFi)
5. LDE225 (SMOi)

Tier 2 Compounds OPEN in 2014
6. LDK378 (ALKi)
7. LEE011 (CDK4/6i)
8. BGJ398 (FGFRi)
9. Combo 1 TBD
10. Combo 2 TBD

Tier 3 Compounds Planned
11. PKC412 (FLT3i)
12. BYL719 (PI3Kαi)
13. INC280 (cMETi)
14. AEB071 (PKCi)
15. Other Combo TBD

• Patient-triggered, target-specific and tissue-agnostic clinical trial program
• Local test results accepted
• 70-100 patients per treatment arm
• Drug specific inclusion/exclusion criteria
• Non-competing design with ongoing Novartis trials
My Pathway – Genentech
http://clinicaltrials.gov/show/NCT02091141

- Patient-triggered, target-specific and tissue-agnostic clinical trial program
- 5 FDA approved drugs
  - Vismodegib
  - Erlotinib
  - Trastuzumab/Pertuzumab
  - Vemurafenib
- Local test results accepted
- New biopsy not required
- 125 patients per treatment arm
### Currently available drugs for breast cancer through the Signature and My Pathway trials

<table>
<thead>
<tr>
<th>Molecular abnormality</th>
<th>Drug</th>
<th>Currently Open Basket Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>activating mutations in: <strong>RAS, RAF, NF1, MEK</strong></td>
<td>MEK162</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td><strong>BRAF V600</strong> mutation</td>
<td>LGX878</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td>Activating mutations in <strong>SMO</strong>, loss of function in <strong>PATCH1</strong></td>
<td>LDE225</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td>CDK4/6 amplification or activating mutation, <strong>CCND1/CCND3 amplification, p16 (CDKN2A)</strong></td>
<td><strong>LEE011</strong>*</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td>ALK activating mutation or rearrangement</td>
<td>LDK378</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td><strong>PIK3CA</strong> activating mutation, <strong>PIK3R1</strong> activationg mutation, <strong>PTEN</strong> loss of function</td>
<td>BKM120***</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td>FGFR1/2/3 amplification, activation mutations in <strong>VEGFR2, FLT3, cKITRET, NTRK1, CSFR1</strong></td>
<td><strong>TKI258</strong>*</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td>Activating mutations in <strong>SMO</strong>, loss of function in <strong>PATCH1</strong></td>
<td>vismodegib</td>
<td>Genentech My Pathway</td>
</tr>
<tr>
<td>activating mutations in <strong>EGFR</strong></td>
<td>Erlotinib</td>
<td>Genentech My Pathway</td>
</tr>
<tr>
<td><strong>HER2 amplification</strong></td>
<td><strong>Trastuzumab/Pertuzumab</strong>*</td>
<td>Genentech My Pathway</td>
</tr>
<tr>
<td>activating mutations in <strong>BRAF</strong></td>
<td>vemurafenib</td>
<td>Genentech My Pathway</td>
</tr>
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</table>

* breast cancer is excluded
NCI-MATCH (not yet open)

- Basket trial
- Parallel Phase II trials (n=30)
  - 40 drugs pledged
  - 20 arms currently
    - EGFR, HER2, MET, BRAF, NF1, GNAQ, GNA11, TSC1/2, PTEN, Patch, NF2, ALK, ROS, FGFR
- Fresh Bx required
- Central testing

1 CR, PR, SD, and PD as defined by RECIST
2 Stable disease is assessed relative to tumor status at re-initiation of study agent
3 Rebiopsy; if additional mutations, offer new targeted therapy
Most patients will not have access to these trials – but they have access to the tests and using them is tempting

- Thinking outside of the box to fully harness community interest in molecularly targeted therapies and assess their clinical validity:
  - On-line registry of off-label use of molecularly targeted therapies
    - Drug is covered only if patient is entered into the registry
    - Basic info on test, disease and treatment history
    - Outcome measure: Length of stay on treatment
    - Cause of discontinuation
    - Brief Informed consent for EMR audit
    - Penalty for non-compliance with data: provider exclusion
Research Use

**NGS to study** tumor heterogeneity and disease evolution

Clinical NGS assays = targeted sequencing of panels of genes

Research tools = whole exome and whole genome sequencing

- Within tumor spatial heterogeneity
- Between site heterogeneity in a single patient
- Heterogeneity at disease subtype level
- Can a metric of “heterogeneity” be used as a biomarker
- Biological implications of genomic and cellular heterogeneity
The challenge of heterogeneity research

**Causes of variability**

- **Technical variation:**
  - Noise
  - Bias
  - Tissue composition
  - Methodology (variable sensitivity & specificity)

- **Biological variation:**
  - Variable cell states
  - Spatial multi-clonality

**Interpretation**

- "The assay has limited reproducibility"
- "The tumor is spatially heterogeneous"

In experimental results both causes of variability *always* coexist, correct interpretation requires understanding each component.
The “biological signal” to ‘technical noise” ratio in whole exome sequencing experiments is low

- 1 erroneous base call out of 100,000 calls yields 30,000 false variants over the entire human genome
  - random PCR amplification errors
  - variable coverage level
  - alignment errors (low mapability regions, missing annotations, paralogs)
  - algorithmic variant calling errors

- Multiple filters are applied to reduce error rates, but false base calls cannot be completely eliminated
  - PCR duplications, base call quality, minimum and maximum coverage filters, allele balance, strand filter, low complexity filter, end-base trimming, variant call p-value
  - Restrict analysis to well known mutations (Safe, but discards most of the data!)

H Li. Towards better understanding of artifacts in variant calling from high coverage samples. Cornell University; http://arxiv.org/abs/1404.0929.
Biological versus technical variability in whole exome sequencing of different biopsies of the same cancer

N=11 surgically resected tumors, Each sampled at 3 distinct locations

33 specimens total
8 DNA samples split and sequenced twice

What is the extent of variant discordance in technical replicates?
Contribution of alignment?
Contribution of variant calling?
Impact of filters?

What is the extent of between biopsy discordance?
Difference by ER status or grade?

Is discordance differ by variant type?
Lower for SNP?
Higher for COSMIC?
What does this suggest?

• In common invasive ductal carcinoma of the breast, within-tumor spatial heterogeneity is small.

• Variation from **unavoidable** technical noise is of the same magnitude as variation from spatial heterogeneity
  – Chance of false discovery is high; orthogonal validation is critical.

• The within-tumor spatial heterogeneity of grade 1 or 2 ER+ and TNBC appears similar.

• The significance of “private mutations” in different regions of the same tumor is uncertain
  – We found that most COSMIC mutations are shared across all biopsies from the same primary cancer.
Metrics of Heterogeneity or Complexity May Represent Biomarkers on Their Own
How can we quantify breast cancer genomic heterogeneity?

• Group level comparisons; e.g. is one clinical outcome cohort more heterogeneous than the other?
  – Pearson distance
  – Dispersion distance
  – Cosine distance

• How to measure “complexity” or “heterogeneity” within a single microarray or sequencing experiment?
  – Some metric of Entropy
  – Some metric of Fractal dimensions
  – Gini coefficient
Which of these gene expression data sets is the most heterogeneous?

Mean dispersion distance tracks heterogeneity closely

T Jiang et al. BMC Genomics 2014, 15:876
Different breast cancer subtypes have distinct heterogeneity

Among the basal-like cancers, the residual disease subgroup is more heterogeneous than the pCR group suggesting a multiplicity of paths to resistance.

Basal-like cancers have the greatest transcriptional, DNA copy number and mutational heterogeneity and the Luminal A the least.
Basal like cancers with RD are more heterogeneous than cancers with pCR across most KEGG pathways.

The majority of pathways exhibit higher transcriptional heterogeneity in basal chemo-resistant phenotype compared to chemo-sensitive.
Entropy of MAF as metric of clonal heterogeneity

1. Normal filtered variant call
2. 435 potential driver genes
3. Variant in cancer related gene
4. Find normal SNPs in neighborhood
5. MAF distribution of 10-20 nearest SNPs (>50X)

\[ P = \frac{MAF(\text{mutation})}{\text{mean}(MAF(\text{neighbor SNPs}))} \]

6. Derive clonal proportion of minor allele for each variant

\[ E = \frac{p \times \log(p)}{\text{mutation number}} \]

Entropy for clonal heterogeneity
Mutational load and clonal entropy; two different aspects of heterogeneity

RD cases have higher clonal entropy but lower mutational load.
Conclusions

• The clinical utility of targeted NGS assays for tumor target profiling has not yet been established for metastatic breast cancer

• Several trials started to examine clinical utility
  – Randomized SAFIR-2 (breast cancer; not yet open)
  – Randomized NCI-Lung MATCH, ALCHIMIST (Lung cancer; open)
  – Conduct your N=1 clinical experiment in the context of the “My Pathway”, “Signature”, “Institutional” or NCI-MATCH trials
  – Logistically even simpler registry studies would also be welcome

• Whole exome and whole genome sequencing results contain considerable noise but some fascinating results are emerging:
  – In breast cancer within tumor spatial heterogeneity is modest (unlike renal cancer).
  – Biological interpretation of the totality of genomic anomalies in a cancer and the genomic differences between regions of the same tumor remains a major challenge.
  – “Tumor heterogeneity” “genome entropy” may predict outcome