Practical Implications of Next Generation Sequencing (NGS) in Breast Cancer: Promises and Challenges

Naoto T. Ueno, MD, PhD, FACP
Professor of Medicine
Executive Director, Morgan Welch Inflammatory Breast Cancer Research Program and Clinic
Chief, Section of Translational Breast Cancer Research
Enhanced Drug-Development Guide & Evaluation (EDGE) Preclinical Solutions
Department of Breast Medical Oncology
TeamOncology
The Field of Molecular Diagnostics in 2017

- Stage I-III breast cancer
  - ER+
    - Refine prognostic prediction & assist select patients for chemotherapy: OncoType Dx, Prosigna, MammaPrint, Breast Cancer Index, etc
    - Predict late recurrence > 5 years, assist selecting patients for extended adjuvant endocrine therapy: Breast Cancer Index, (Prosigna, Endopredict)
  - TNBC
    - Select patients with pCR: MD Anderson Signature, ARTEMIS study
    - Predict prognosis: Immune signatures are weekly prognostic but not enough to withheld adjuvant chemotherapy.
    - Select patients for inclusion of platinum therapy: Maybe germ line BRCA status (DNA repair deficiency tests do not seem to be drug specific)
  - HER2+
    - Select one HER2-tareted drug over the other: NONE
    - Predict HER2-targeted therapy resistance: NONE, PIK3CA mutation is associated with slightly lower RR but not enough to be clinically useful
- Stage IV disease
  - Molecular target profiling: Foundation Medicine, Caris Life Science, etc…
NGS-based Tumor Target Profiling

• 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)

• Academic institutions perform their own targeted NGS or exome sequencing in their molecular pathology laboratories
  – Platforms vary (Illumina, IonTorrent, etc. with and without reflex validation)
  – Assay performance or analytic validity of tests are often not public
Promises?

- Prognostic estimation
- Personalized/Precision Treatment
  - High tumor response rates
  - Less treatment toxicities
  - Exceptional Responder Identification
- Understanding the treatment resistance mechanisms
What do we find with NGS-based tumor profiling?

N=100 metastatic breast cancers assayed with Foundation One

Samples with actionable alterations, 84%
Greater PFS Benefit With EVE in Patients With Minimal Alterations in PIK3CA/PTEN/CCND1 or FGFR1/2

Presented By Gabriel Hortobagyi at 2013 ASCO Annual Meeting

HR (95% CI): 0.27 (0.18 - 0.41)
What is a “targetable/actionable” mutation?

### Type I targets
- Proven clinical target function in a specific disease type coupled with a FDA approved drug
  - Melanoma BRAF V600E
  - NSCLC EGFR L858R or exon19del
  - Mastocytosis KIT D816V
  - CML BCR-ABL translocation
  - NSCLC ALK-AML4 fusion gene
  - Breast /Gastric Ca HER2 amp
  - GIST KIT/PDGFRA Y288C

### Type II targets
- Clinically valid mutation targets in a non-approved cancer type
  - BRAF V600E in Colon Ca
- Activating mutations in known targets validated in vitro:
  - HER2 D769H, V777L
  - ER Y537S
  - PDGFRA Y288C
  - FGFR2 K292
  - FGFR1 amp
  - PIK3CA H1047R
  - AKT1 E17K
- Mutations with predicted deleterious function but not yet tested in the lab

### Type III targets
- “Might-be-targets” based on current (rudimentary) understanding of cancer biology
  - Mutations in a targetable pathway up-stream or down-stream to type I-II targets

Depending on how “actionable” is defined, the number of actionable mutations can vary substantially.
**Proposed 3-tier NGS-based drug recommendations**

**Level 1 for breast cancer:** HER2 amplification

**Level 2A for breast cancer:** none (maybe Topo II amp, PIK3CA mut)

**Level 2B for breast cancer:** <10% of all reported gene level variants (BRAF, ALK, EGFRmut, KIT)

**Level 3A/B for breast cancer:** >90% of all reported findings (ER and HER2 mutations, FGFR1 amp, etc…)

Meric-Bernstam et al, JNCI, 107(7): djv098, 2015
Challenges

• Robustness of the Assays
• The impact of tumor heterogeneity (i.e. sampling “error”) on profiling results
• The impact of timing of the collection of the samples
• Not enough clinical studies
NGS in Breast Cancer

- **Analytical Validity:** Are we really measuring what we think that we are measuring?

- **Clinical Validity:** Do the measurements reflect a particular clinical state or associated with outcome (e.g. prognosis, response)?

- **Clinical Utility:** Is patient outcome better because of using the test?
Table 2. Mutations status and previous treatment exposure

<table>
<thead>
<tr>
<th></th>
<th>Previous treatment exposure (n=186), n (%)</th>
<th>Treatment naïve (n=736), n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>115 (61.8)</td>
<td>557 (75.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mutated</td>
<td>71 (38.2)</td>
<td>179 (24.3)</td>
<td></td>
</tr>
<tr>
<td>PIK3CA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>123 (66.1)</td>
<td>607 (82.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mutated</td>
<td>63 (33.9)</td>
<td>129 (17.5)</td>
<td></td>
</tr>
<tr>
<td>AKT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>180 (96.8)</td>
<td>716 (97.3)</td>
<td>0.63</td>
</tr>
<tr>
<td>Mutated</td>
<td>6 (3.2)</td>
<td>20 (2.7)</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>180 (96.8)</td>
<td>728 (98.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Mutated</td>
<td>6 (3.2)</td>
<td>8 (1.1)</td>
<td></td>
</tr>
</tbody>
</table>
Concordance of Genomic Alterations in Primary vs Metastatic Tumors

- 33 matched primary and recurrent tumors
- Somatic mutations: 97 of 112 (86.6%) somatic mutations were concordant
- Copy number alterations: 136 of 159 (85.5%) were concordant, 37 (23.3%) were concordant, but below the reporting threshold in one of the matched samples, 23 (14.5%) discordant

Alterations potentially targetable with established or investigational therapeutics were considered “actionable”

40 of 43 (93%) patients had actionable alterations that could inform targeted treatment options.

There were both losses and gains of actionable alterations

Meric-Bernstam, Mol Can Ther 2014
The challenge of heterogeneity research

Causes of variability

Technical variation:
- Noise pre-analytical
- Bias analytical
- 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.
Targeted sequencing of 111 genes (0.00001% of the total genome) in 58 cancers

Whole exome (3% of the total genome) sequencing of 100 cancers

Web-based tools to assist treatment recommendations based on tumor profiling data
Comparison of drug recommendations from four on-line tools and the FM report for the same genomic results (N=75 NGS data)

<table>
<thead>
<tr>
<th>Table 1: Comparison of 5 Mutation Based Treatment Recommendation Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affiliation</strong></td>
</tr>
<tr>
<td><strong>Access</strong></td>
</tr>
<tr>
<td><strong>Intended Audience</strong></td>
</tr>
<tr>
<td><strong>Genes Covered</strong></td>
</tr>
<tr>
<td><strong>Data Entry</strong></td>
</tr>
<tr>
<td><strong>Recommendations</strong></td>
</tr>
<tr>
<td><strong>Output</strong></td>
</tr>
<tr>
<td><strong>Algorithm</strong></td>
</tr>
<tr>
<td><strong>Background Source</strong></td>
</tr>
<tr>
<td><strong>Last update noted</strong></td>
</tr>
</tbody>
</table>

* Total number of genes listed under any 1 of 21 malignancies, excluding repeats. Note: Gene list & access to information is restricted by required selection of malignancy.
Occurrence of FDA Approved Drug Recommendations: Mutated genes from highest to lowest number of sources are listed along x-axis and number of drug recommendations grouped by number of contributing sources are stacked along y-axis.
Enrollment on Genotype-Matched Trials

Underwent Genomic Testing  
N = 2000

Mutation in Potentially Actionable Gene

Yes (789)  No (1211)

Genotype-matched trial after genomic testing?  

Yes (83)  No (706)

Genotype-Selected Trial N = 54  Genotype-Relevant Trial N = 29

11% of pts with mutations in actionable genes went on genotype-matched trials

Meric-Bernstam et al, JCO, 2015
Prospective testing of the clinical validity and utility of molecular target profiling for treatment selection

- Institutional / Pharma clinical trials (*several open*)

- Novartis – Signature Basket Study (*open*)
  - N=1 local trials

- Genentech – My Pathway Basket Study (*open*)
  - N=1 local trials

- NCI-MATCH Basket Study (*open*)
  - NCTN – NCORP

- Targeted Agent and Profiling Utilization Registry (TAPUR)
  - TAPUR@asco.org. (*yet to be activated*)
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>activiating mutations in: RAS, RAF, NF1, MEK</td>
<td>MEK162</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td><strong>BRAF V600 mutation</strong></td>
<td>LGX878</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td>Activating mutations in SMO, loss of function in PATCH1</td>
<td>LDE225</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td><strong>CDK4/6 amplification</strong> or activating mutation, CCND1/CCND3 amplification, p16 (CDKN2A) loss or inactivating mutation</td>
<td>LEE011*</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td><strong>ALK activating mutation</strong> or rearrangement</td>
<td>LDK378</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td>PIK3CA activating mutation, PIK3R1 activating mutation, PTEN loss of function</td>
<td>BKM120*</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td>FGFR1/2/3 amplification, activation mutations in VEGFR2, FLT3, cKIT RET, NTRK1, CSFR1</td>
<td>TKI258*</td>
<td>Novartis Signature</td>
</tr>
<tr>
<td>Activating mutations in SMO, loss of function in PATCH1</td>
<td>vismodegib</td>
<td>Genentech My Pathway</td>
</tr>
<tr>
<td>activating mutations in EGFR</td>
<td>Erlotinib</td>
<td>Genentech My Pathway</td>
</tr>
<tr>
<td>HER2 amplification</td>
<td>Trastuzumab/Pertuzumab*</td>
<td>Genentech My Pathway</td>
</tr>
<tr>
<td>activating mutations in BRAF</td>
<td>vemurafenib</td>
<td>Genentech My Pathway</td>
</tr>
</tbody>
</table>

* breast cancer is excluded
### Parallel Phase II trials (n=30 each)
- 40 drugs pledged
- 20 arms planned

### Fresh Bx required

### Central testing required:

<table>
<thead>
<tr>
<th>Current Drugs</th>
<th>Molecular Targets</th>
<th>Estimated Mutation Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crizotinib</td>
<td>ALK rearrangement</td>
<td>4%</td>
</tr>
<tr>
<td>Crizotinib</td>
<td>ROS1 translocations</td>
<td>5%</td>
</tr>
<tr>
<td>Dabrafenib and Trametinib</td>
<td>BRAF V600E or V600K mutations</td>
<td>7%</td>
</tr>
<tr>
<td>Trametinib</td>
<td>BRAF Fusions/ Non-V600E/Non-V600K BRAF mutations</td>
<td>2.8%</td>
</tr>
<tr>
<td>Afatinib</td>
<td>EGFR activating mutations</td>
<td>1–4%</td>
</tr>
<tr>
<td>Afatinib</td>
<td>HER2 activating mutations</td>
<td>2–5%</td>
</tr>
<tr>
<td>AZD9291</td>
<td>EGFR T790M mutations and rare EGFR activating mutations</td>
<td>1–2%</td>
</tr>
</tbody>
</table>
| Ado–trastuzumab–
emtansine | HER2 amplification                                    | 5%                            |
| VS6063                 | NF2 loss                                               | 2%                            |
| Sunitinib              | cKIT mutations                                         | 4%                            |

**Current Mutations:**
- EGFR, HER2, MET, BRAF, NF1, GNAQ, GNA11, TSC1/2, PTEN, Patch, NF2, ALK, ROS, FGFR
What do patients think of tumor target profiling?

N=400 out patients at Yale Cancer Center

Figure 1: Patient Preferences for Tumor Profiling

- I would want tumor profiling...
  - even if there is only a 50% chance the the information learned might help to improve my cancer treatment.
    - Agree: 72
    - Neutral: 13
    - Disagree: 5
  - even if there is only a 10% chance the the information learned might help to improve my cancer treatment.
    - Agree: 66
    - Neutral: 17
    - Disagree: 8
  - even if there is only a 1% chance the the information learned might help to improve my cancer treatment.
    - Agree: 61
    - Neutral: 20
    - Disagree: 9
  - if the information learned would be used for research to potentially help someone in the future.
    - Agree: 72
    - Neutral: 14
    - Disagree: 4
  - but only if there was no way of accidentally or unintentionally finding out information about the genes that I am born with and might pass down to my children.
    - Agree: 46
    - Neutral: 23
    - Disagree: 19
  - even if I might find out about a gene that could impact my health and might cause a disease other than cancer.
    - Agree: 65
    - Neutral: 18
    - Disagree: 9

M Yushak et al, AACR 2015. Abs 3879, Cancer Res 75 (Aug 1, 2015);
doi: 10.1158/1538-7445.AM2015-3879
What do patients think of incidental germ line findings discovered during cancer target profiling?

Figure 2: Desire for Information Disclosure

I would...

- want to know if I have a gene variant even if modern science does not yet understand if this particular variant will have any impact on my health.
  - Agree: 49
  - Neutral: 29
  - Disagree: 12

- want to know if I have a gene variant that ALWAYS causes a serious disease or condition other than cancer, EVEN IF THERE IS NOTHING I CAN DO to prevent the disease.
  - Agree: 56
  - Neutral: 22
  - Disagree: 11

- want to know if I have a gene variant that increases my risk of having a serious disease other than cancer, EVEN IF THERE IS NOTHING I CAN DO to lower my chances of developing the disease.
  - Agree: 58
  - Neutral: 21
  - Disagree: 11

- want to know if I have a gene variant that increases my risk of having a serious but PREVENTABLE illness other than cancer.
  - Agree: 77
  - Neutral: 12
  - Disagree: 2

- only want to know the information that impacted my treatment for cancer.
  - Agree: 52
  - Neutral: 19
  - Disagree: 20

- want to know all of the information that is learned including unintentional or accidental results for genes that may not be related to my cancer but could affect my health.
  - Agree: 72
  - Neutral: 13
  - Disagree: 6

Precision Oncology Decision Support
Getting to the Right Patient, with the Right Drug at the Right Time

Selected publications
Johnson A et al., Drug Discov Today. 2015
Meric-Bernstam F, J Natl Cancer Inst. 2015
Meric-Bernstam F et al, J Clin Oncol. 2015

Chen K et al, Clin Chem. 2015
Zhou W et al., Nat Methods. 2015
Boland GM et al. Oncotarget. 2015
Johnson A et al, ASCO, 2016

Shared Resources
Functional Genomics Core
Clinical and Translational Research Center

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Conclusions

- NGS-based “mutation” calling is more variable than we would hope for
  - Analytical validity PCR > IonTorrent > Targeted exome sequencing > WES
  - Differences in mutation calling algorithms can lead to different results
  - Assignment of “deleterious” versus “tolerated” effect to a given mutation is an art
  - Which gene is targetable is “in the eye of the beholder”
  - The level of evidence to support the use of a drug for a given anomaly is usually weak

- The proper clinical validity and utility of target profiling results is yet to be established. Please try to use these tests in the context of structured trials or institutional registries.

- Genomic heterogeneity due to spatial effect, timing of the biopsy

- Must retain a critical mind even if we are enthusiastic about the potential of cancer target profiling and targeted therapies
  - A lot more types of “driver events” than we previously thought and currently check for
  - Each cancer harbors a large number of genomic abnormalities

- Patients are willing to undergo testing even if the likelihood of “success” is small
MD Anderson Cancer Center: Funda Meric-Bernstam, M.D.

Yale University: Lajos Pusztai

NCI: Takebe Naoko

- nueno@mdanderson.org
- Twitter: @teamoncology, @InflammatoryBCa
- Facebook: http://www.mdanderson.org/IBCProgram
- https://www.facebook.com/ntueno