Paired tumor and cfDNA in patients with HER2-mutant solid tumors treated with neratinib reveals convergence of multiple on-target resistance mechanisms: Results from the SUMMIT “Basket” Trial

Helen H. Won¹, S. Duygu Selcuklu¹, Sarina A. Piha-Paul², Cristina Saura³, Jordi Rodon², Ingrid A. Mayer⁴, Sherene Loi⁵, Geoffrey I. Shapiro⁶, Janice Lu⁷, Adam Brufsky⁸, Catherine Zimel¹, Myra Melcer¹, Maurizio Scaltriti¹, Lisa D. Eli⁹, Richard E. Cutler Jr.⁹, Alshad S. Lalani⁹, Richard P. Bryce⁹, Carlos Arteaga¹⁰, Funda Meric-Bernstam¹¹, Michael F. Berger¹, David B. Solit¹, Alison Schram¹, David M. Hyman¹.

¹Memorial Sloan Kettering Cancer Center, New York, NY; ²MD Anderson Cancer Center, Houston, TX; ³Vall d’Hebron University Hospital, Barcelona, Spain; ⁴Vanderbilt-Ingram Cancer Center, Nashville, TN; ⁵Peter MacCallum Cancer Centre, Melbourne, Australia; ⁶Dana Farber Cancer Institute, Boston, MA; ⁷Keck School of Medicine of USC, Los Angeles, CA; ⁸University of Pittsburg, Pittsburgh, PA; ⁹Puma Biotechnology, Los Angeles, CA; ¹⁰UTSW Harold C. Simmons Comprehensive Cancer Center, Dallas, TX; ¹¹MD Anderson, Department of Investigational Cancer Therapeutics, Houston, TX
HER2 (ERBB2) mutations

- Somatic HER2 mutations are seen at relatively low frequencies across multiple tumor types
- Unlike many oncogenic drivers, mutations in HER2 occur across multiple domains of the protein, specifically in the extracellular, transmembrane, and kinase domain, resulting in constitutive kinase signaling, oncogenic transformation and enhanced tumor growth in preclinical models
Neratinib (HKI-272; PB272; NERLYNX®)

- Neratinib is an oral, irreversible pan-HER tyrosine kinase inhibitor and suppresses intracellular signaling, cell proliferation and colony formation in HER2-mutant tumor cell lines *in vitro*\(^1,2\)
- In preclinical models, HER2-mutant alleles activate to different degrees and have differential sensitivity both within and across HER tyrosine kinase inhibitors

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2. Carmona et al. Cancer Res 2016:76(14 Suppl); abst 298
SUMMIT neratinib ‘basket’ study

- 125 patients enrolled in the HER2 cohort
- 31 unique mutations, 87% hotspot mutations


<table>
<thead>
<tr>
<th>HER2-mutation positive</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer</td>
<td>26 (18.4)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>25 (17.7)</td>
</tr>
<tr>
<td>Bladder/urinary tract cancer</td>
<td>16 (11.3)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>12 (8.5)</td>
</tr>
<tr>
<td>Biliary tract cancer</td>
<td>9 (6.4)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>7 (5.0)</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>5 (3.5)</td>
</tr>
<tr>
<td>Gastroesophageal cancer</td>
<td>5 (3.5)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>4 (2.8)</td>
</tr>
<tr>
<td>Other solid tumors (NOS)</td>
<td>15 (10.6)</td>
</tr>
</tbody>
</table>
Efficacy in HER2-mutant patients by tumor type/allele

- Neratinib activity was influenced by both tumor lineage as well as mutation type
- Greatest degree of response was observed in breast cancer

Breast Combination cohort: Neratinib with fulvestrant in ER+ MBC patients

ORR: 32%

Response criteria
- RECIST 1.1
- PET response criteria
- Not evaluated

*No target lesion measurement

MSK-IMPACT for tissue sequencing

Deep coverage, targeted sequencing of 468 genes to guide treatment

1. Patient Consent
2. Sample Accessioning
3. Sample Preparation
4. Sequencing
5. Bioinformatics Analysis
6. Case Review and Sign Out

Cancer Gene Exons (468 genes):
- actionable mutations
- targets of investigational agents
- frequently mutated in cancer
- cancer susceptibility genes

Cancer Gene Introns (20 genes):
- recurrent rearrangements

Noncoding Regions
- TERT promoter
- microsatellites
- >1000 common SNPs

Target Territory = 1.52 Mb
Average Coverage = 720x

Zehir, Benayed et al., Nature Medicine, 2017
MSK-ACCESS for cfDNA sequencing

Ultra-deep coverage, targeted sequencing of 129 genes

Leveraged experience from sequencing 25,000 tumors with MSK-IMPACT

Selected exons of 129 genes for mutation detection
- OncoKB Level 1-4
- Hotspot sites
- High rates of mutations
- Protein kinase domains
- Tumor suppressor genes

Microsatellite regions
SNPs for zygosity and copy number of 13 genes
Common SNPs for genome-wide copy number & QC
Introns for structural variants of 10 genes
Clonal hematopoiesis genes

Submitted to NYS Department of Health for clinical use approval
Acquired Resistance Patient Cohort Description

- **Pts with Clinical Benefit (n=11)**
  - CR, PR (n=9)
  - SD ≥ 24 weeks (n=2)

- **Tumor tissue**
  - Paired pre- & post-tx (n=11)

- **Plasma cfDNA**
  - Baseline & post-tx (n=11)

- **MSK-IMPACT sequencing**
  - ~715X coverage, LOD 2%

- **MSK-ACCESS sequencing**
  - ~20,770X coverage, LOD 0.1%

- **Identification of resistance mechanism**
  - On-target (82%, n=9)
  - Other, none (18%, n=2)

**LOD**: Limit of Detection
Clinical Response by Tumor Type and Allele

- 11 patients with significant clinical response (RECIST 1.1 or PET)
- All enrolling HER2 mutations were clonal

CUP: Cancer of unknown primary
Acquired HER2 resistance in tissue

- Pretreatment HER2 mutation retained in 10/11 tissues at progression
- 73% (8/11) acquired at least one alteration at progression
  - 38% (3/8) acquired a secondary HER2 alteration
Acquired HER2 resistance in plasma cfDNA

- 73% (8/11) acquired a secondary HER2 alteration post-neratinib in cfDNA
Acquired HER2 mutations in cfDNA seem subclonal

- Subclonal HER2 mutations acquired near, at, or post progression (range 0.1% - 9.1%)
Acquisition of Gatekeeper Mutations in an ER+ Invasive Lobular Breast Cancer Patient

83 year-old female ER+/PR-/HER2- CR, 80 weeks
Overall ERBB2 evolution

- On-target mutations identified in 82% (9/11)
  - 78% activating (7/9)
  - 22% gatekeeper (2/9)
  - 11% non-hotspot (1/9) with polyclonal resistance
Conclusions

• In patients with clinical benefit on neratinib, a potential on-target resistance mechanism was identified in 82% (9 of 11 patients)
  – Gatekeeper mutations were acquired in two patients treated on combination fulvestrant therapy with prior HER2-targeted therapies

• HER2 mutations lead to oncogene addiction in solid tumors and HER2 signaling may select for acquisition of additional activating events

• Tumor sequencing in combination with plasma cfDNA sequencing can be utilized to provide insight into intra-tumor and subclonal heterogeneity
  – Additional tissue and longitudinal plasma sampling is essential in providing a more comprehensive overview of molecular response and resistance mechanisms
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