Improvement of clinical outcome in refractory cancer patients by individualized genome-guided therapy

Woong-Yang Park
Samsung Genome Institute
Role of biomarkers for targeted therapy


Stewart, Transl Lung Cancer Res, 2015
Biomarker testing for targeted therapy

- Mutations associated with drug sensitivity
  - EGFR Gly719X, exon 19 deletion, Leu858Arg, Leu858Gln
- Mutations associated with primary drug resistance
  - EGFR exon 20 insertions
- Mutations associated with acquired drug resistance
  - EGFR Thr790Met, Asp761Tyr, Leu747Ser, Thr854Ala

Pao, Lancet, 2010

Kohno, Transl Lung Cancer Res, 2015
Screening biomarkers for targeted therapy

Patient → Drug selection → Personalized targeted therapy
Precision medicine for refractory cancer

Patient → Actionable Genome Information → High-throughput Drug screening → Clinical trials for targeted therapy → Personalized targeted therapy
Akt inhibitor for refractory gastric cancer

First visit (2014 Sep) → 6 months of conventional chemotherapy → 5 months of Akt inhibitor trial

- Pre-treatment
- At 2 months: PR
- At 5 months: PD
- At 8 months: PR

NGS with biopsy tissue from EGD: Detection of PIK3CA E542K (4%)
**Actionable genome analysis workflow**

**Patient**
- Refractory/advanced cancer
- Informed consent

**Oncology clinic**
- Patient selection
- Clinical trials
- Biopsy

**Pathology/Lab Medicine**
- Pathologic diagnosis
- DNA/RNA extraction
- Validation

**Clinical sequencing**
- Clinical sequencing
- Automation with LIMS

**Bioinformatics**
- Software package
- User interface (report)
- Integrated database

**Tumor board**
- Review of patient information
- Harmonizing clinical decision

**Clinical utility of personal genome analysis**
- How many patients with actionable variants?
- How much improvement expected by matched therapy?

**Sample acquisition**
- What amount of DNA required for sequencing?
- Can I use FFPE samples? Biopsy?

**Clinical sequencing**
- Which gene should I include for sequencing?
- What type of capture? What scale of sequencing needed?

**Bioinformatics solution**
- Which caller should I choose for the best performances?
- How can I control the data quality?

**Clinical report**
- Which variants should be reported?
CancerSCAN™ is the best-in-class genomic tests for cancer patients. The assay have undergone robust analytic validation and have been proven to be among the most accurate, sensitive, and comprehensive tests available today to provide more treatment options for patient care.

**Sample acquisition**
to include minimal amount of samples (FFPE, FNA, liquid biopsy etc)

**Clinical sequencing**
with QC/QA and reasonable turnaround time for clinic

**Bioinformatics solution**
optimized to the clinical actions

**Clinical report**
providing actionable information for personalized therapy

**Tumor board**
review the list of actionable targets with QC information

- FFPE-compatible pipeline
- Optimized to use small input DNA (>50ng)

- Deep sequencing (X1,000) for 1% VAF with HiSeq2500
- Rapid turnaround time (<2weeks) with automation

- Optimized callers for SNV/Indel, CNV, and gene fusion, MSI
- QC on bioinformatic analysis process

- Classification of actionable mutation with annotation
- Quality control report on individual actionable variants

- Bi-weekly tumor board for lung, stomach, breast and colon cancer
- Data mining for clinico-genomic database
CanerSCAN™ workflow

1. Sample reception/Report (documents, barcode)
2. Pre-PCR (DNA preparation, quantitation, QC)
3. Post-PCR (library preparation, QC)
4. Sequencing (high output mode, QC)
5. Analysis server (analysis pipeline, storage)
6. Data analysis (reports, DB, QC)
## Annotation and classification of somatic mutations

<table>
<thead>
<tr>
<th>Tier 1: Known as drug targets</th>
<th>Tier 2: Reported in human cancer (COSMIC, TCGA)</th>
<th>Tier 3: Novel variants</th>
<th>Tier 4: Polymorphic variants (dbSNP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR L858R in NSCLC</td>
<td>EGFR G719S in NSCLC</td>
<td>TP53 R273H (COSM10660)</td>
<td>FGFR2 G872F</td>
</tr>
<tr>
<td>BRAF V600E in melanoma</td>
<td>BRAF V600E in non-melanoma</td>
<td>BRCA1 K1183R (COSM148277)</td>
<td>NTRK1 P690L (COSM4516579)</td>
</tr>
<tr>
<td>EGFR A289V in GBM</td>
<td></td>
<td>TP53 C185T</td>
<td></td>
</tr>
<tr>
<td>BRAF V600G in melanoma</td>
<td></td>
<td>FGFR2 G872F</td>
<td></td>
</tr>
</tbody>
</table>

### Categories

**Category 1:** Candidates for KFDA-approved therapy

- EGFR L858R in NSCLC
- BRAF V600E in melanoma

**Category 2:** Candidates for clinical trials in the hospital

- EGFR G719S in NSCLC
- BRAF V600E in non-melanoma

**Category 3:** Candidates for investigator-initiated trials

- EGFR A289V in GBM
- BRAF V600G in melanoma

**Category 4:** Providing information on for patient ID

- TP53 R273H (COSM10660)
- BRCA1 K1183R (COSM148277)
- NTRK1 P690L (COSM4516579)
- TP53 C185T
- FGFR2 G872F

EGFR L858R in NSCLC, BRAF V600E in melanoma, TP53 R273H (COSM10660), BRCA1 K1183R (COSM148277), EGFR A289V in GBM, BRAF V600G in melanoma, TP53 C185T, NTRK1 P690L (COSM4516579), FGFR2 G872F.
Clinical utility of actionable genome sequencing

**Investigator initiated clinical trial**
Functional analysis with PDC screening
e.g. BRAF fusion in thyroid cancer brain metastasis

**Further experimental study**
Candidate pathway analysis with RNA-seq

- **Category 1**
  - 18.9%
- **Category 2**
  - 15.5%
- **Category 3**
  - 36.2%
- **Category 4**
  - 29.4%

**Clinical trial**
Off-label drugs in stomach, colon, and lung cancer
e.g. RET fusion in colon cancer

**MFDS-approved target therapy**
e.g. EGFR L858R in NSCLC

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**Clinical sequencing platform**
- total 6,563 cases of CancerSCAN (2016, 3)

**Clinical implementation**
- weekly tumor board meeting

**Genome-based clinical trials**
- Hanmi, AstraZeneca, TGEn, and Pfizer

**Clinico-genomic database**
- data mining and gene-drug interaction map
Clinical benefit of genome-based clinical trial

NEXT (Next generation pErsonalized tx with mulTi-omics and preclinical model) trial: master protocol to route participants to different candidate drugs in trials based on clinical sequencing reports.

- 541 Patients consented to the NEXT1 trial as a master protocol
- Fresh tumor biopsy or FFPE, pathology confirmation/tumor > 60%
- Comprehensive molecular profiling using NGS and IHC

513 Were successfully matched-profiled

418 Patients with molecular profiling receive molecular matched or non-matched therapy

Genome sequencing report provided to clinicians at the time of SOC † failure

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Type</th>
<th>Gastric cancer</th>
<th>Colorectal cancer</th>
<th>Pancreas, Biliary tract cancer</th>
<th>Sarcoma &amp; others</th>
<th>Genitourinary cancer</th>
<th>Total patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matched</td>
<td></td>
<td>33</td>
<td>10</td>
<td>1</td>
<td>16</td>
<td>0</td>
<td>60</td>
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<tr>
<td>Convertional</td>
<td></td>
<td>67</td>
<td>22</td>
<td>22</td>
<td>48</td>
<td>40</td>
<td>199</td>
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<tr>
<td>Not Eligible</td>
<td></td>
<td>27</td>
<td>90</td>
<td>39</td>
<td>3</td>
<td>0</td>
<td>159</td>
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<tr>
<td>Total</td>
<td></td>
<td>127</td>
<td>122</td>
<td>62</td>
<td>67</td>
<td>40</td>
<td>418</td>
</tr>
</tbody>
</table>
Profile of actionable mutations

- **Treatment type**
  - Matched (N = 60, 14.4%)
  - Conventional (N = 199, 47.6%)
  - Not eligible (N = 159, 38.0%)

- **Detected variant type**
  - Potentially treatable (N = 184)
  - Any (N = 211)
  - No (N = 23)

- **Cancer type**
  - GC (N = 127)
  - CRC (N = 122)
  - PC/BTC (N = 62)
  - sarcoma/others (N = 67)
  - GUC (N = 40)
Improved clinical response by matched therapy

The overall response rate between matched and conventional treatment groups were significant for the gastric cancer cohort (9/29 vs. 4/62, p-value = 0.0018, based on the chi-square test) and the sarcoma/other cancer cohort (9/16 vs. 6/48, p-value = 0.0003), while they were not significant for the other two groups.

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<tr>
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<th>Gastric Cancer</th>
<th>Colorectal Cancer</th>
<th>Pancreas/Biliary Tract Cancer</th>
<th>Sarcoma &amp; Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>33</td>
<td>68</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>CR (%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>PR (%)</td>
<td>9 (27.3%)</td>
<td>4 (5.9%)</td>
<td>1 (10.0%)</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>SD (%)</td>
<td>16 (48.5%)</td>
<td>31 (45.6%)</td>
<td>5 (50.0%)</td>
<td>12 (54.5%)</td>
</tr>
<tr>
<td>PD (%)</td>
<td>4 (12.1%)</td>
<td>27 (39.7%)</td>
<td>4 (40.0%)</td>
<td>9 (41.0%)</td>
</tr>
<tr>
<td>N.E (%)</td>
<td>4 (12.1%)</td>
<td>6 (8.8%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>ORR</td>
<td>31.0%</td>
<td>6.5%</td>
<td>10.0%</td>
<td>4.5%</td>
</tr>
</tbody>
</table>

Kim ST manuscript in review
Variable tumor purity in TCGA cancer types

Low tumor purity in clinical samples
Deep sequencing for low allele friction variants

Shin HT, manuscript in review
Single-cell genome analysis

Average capture: 72 ± 5 single cells per chip

<table>
<thead>
<tr>
<th>Cell capture</th>
<th>Cell lysis</th>
<th>Reverse transcription</th>
<th>cDNA amplification</th>
<th>cDNA yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 nl</td>
<td>9 nl</td>
<td>9 nl</td>
<td>9 nl</td>
<td>135 nl</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>135 nl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.2 ± 2 ng per cell</td>
</tr>
</tbody>
</table>

Nature Biotech, 2014
Combinatorial therapy based on single cell profile

Kim et al. Genome Biology 2016
Validation of combinatorial therapy in PDC and PDX

A. 2D Cell viability (%)

B. 3D Cell viability (%)

C. Western blot analysis of p-EGFR, p-Src, p-AKT, p-ERK, and GAPDH.

D. Tumor volume over time.

E. Body weight over time.

F. Western blot analysis of p-EGFR, p-Src, p-AKT, and GAPDH.

G. Immunohistochemistry staining for p-ERK and Ki-67.

H. Proliferative index (%)

I. Apoptotic index (%)

Kim et al. Genome Biology 2016
Separation of cancer and normal cell population
Characterization of tumor infiltrating lymphocytes

Chung WS, manuscript in review
Precision medicine for refractory cancer

- Liquid biopsy
- Actionable genome analysis
- Gene-drug interaction
- Prediction model
- Clinical trials for targeted therapy
- Lifestyle data
- Clinical information
- Data mining
- Clinical data warehouse
- Reference database (Korea Central Cancer Registry)
- Public database (genetic variations, clinical trials)

Personalized therapy
Prediction model using clinic-genomic data

Kim JH, manuscript in review
Genomic analysis on somatic alterations in patient tumor samples is essential in the precision cancer medicine cycle. From sample acquisition to precise analysis on clinical samples, efficient and secure process will be guaranteed.

Precision medicine requires high quality sequencing data, which is optimized for various kinds of clinical setting such as sample acquisition, therapeutic protocol and clinical trials.

Finding the right indication is still a challenge in the clinical utility of genome-based targeted therapy. To this end, the clinical implementation of laboratory-developed test for NGS may be applied nationwide.

Integration of genomic and clinical information in cancer patient will help us to find novel biomarkers and drug targets. To ensure the value of cancer genome cohort, the quality and the scale of genome data should be assured by multi-institutional consortium.
Korean Actionable Genome Consortium

Proposed Goal

- Establish recommendations for the use of NGS panels in Korean patients with cancer
- Define clinical utility for the assessment of somatic mutations

KAGC
(Keunchil Park & Dong-Young Noh)

Korea Cancer Association

Steering Committee
(AMC, SNUH, YUHS, SMC, CMC, KUMC)

CONTENT
(Keunchil Park)
- Actionability principles
- Actionable content

SAMPLE & REPORT
(Jihoon Kim)
- Sample handling & preservation
- NA extraction & QC / QA
- Standards for reports that will survive evolution of knowledge & content

SEQUENCING & DATA ANALYSIS
(Woong-Yang Park)
- NGS technical performance standards
- Standards for validating analytical tools
- Germline vs. tumor recommendations

SAMSUNG GENOME INSTITUTE
Workflow for KAGC

- Clinical study design
- Selection and recruitments of refractory cancer patients
- Sample acquisition with matched blood
- Targeted sequencing on actionable genes
- Bioinformatic analysis pipeline
- KAGC tumor board
- Clinical reports
- Data sharing and analysis including clinical information
- Database for actionable cancer genome cohort