**Introduction**

• Much evidence has shown that genomic profiling of tumors provides patients with treatment options specific to their tumor’s biology in the US. Within the Kyoto University Hospital Cancer Center, we offer patients access to these tests through the OncoPrime™ for those patients of cancer with unknown primary (CUP), rare tumor types or for which standard treatment options have failed.

• Our institution sees over 6,000 cancer patients each year with over 1,500 receiving chemotherapy.

• EA Genomics performs all DNA extraction, isolation, library preparation, sequencing and bioinformatics analysis of the samples.

• Clinical test results are available within 3 weeks, demonstrating efficient of genomic profiling along with successful execution of international specimen logistics.

**Patient and Sample Data**

• QCCP was officially launched in May of 2015. Since then EA Genomics has received 75 patient samples from Kyoto University Hospital Cancer Center.

• Patients going through the testing were generally successful (89.3%) with the specimen failures likely indicative with FFPE artifacts or severe tumor heterogeneity.

• 60% of samples were from female patients, and the mean patient age at time of collection was 57.6 years, which and max age at collection of 8 years and 82 years, respectively (see Figure 1a).

• The majority (88%) of sample received were from stage IV tumors, with an equal distribution of sample coming from metastatic and primary sites (see Figure 1b).

• The 2 most frequent known indications received were cancers of the pancreas (25%) and of the liver/bile duct (18%). 21% of samples were metastatic samples from cancers of unknown origin (see Figure 1c).

• Overall, 88% of sequenced patients were matched with at least 1 actionable result (see Figure 1d), defined here as either an approved therapy in the given indication or another cancer indication, or a relevant clinical trial.

**Methods**

• The Q2 Comprehensive Cancer Panel is built around Agilent SureSelect™ chemistry with several modifications to enable library preparation from low input DNA derived from formalin-fixed, paraffin-embedded (FFPE) tissue or fresh frozen tissue.

• In this assay, genomic DNA is fragmented and captured using a library that targets a specific set of regions that have been implicated in human cancer, many of which have specific targets to available FDA-approved therapies.

• After PCR amplification, the captured DNA libraries are sequenced and the resulting sequenced DNA is aligned to the reference genome to identify single nucleotide variant (SNV) alleles, indels and rearrangements.

• Sequencing is performed on Illumina HiSeq 2500 machine using paired-end, 150 bp configuration with either V4 or RapidRun Chemistry, depending on batch volume.

• QCCP is a targeted sequencing assay for analysis of genomic variation predictive of response to therapies in clinical development. The 233 gene cancer panel includes the genes most commonly associated with cancers and can be used to assess mutations, deletions, insertions and rearrangements.

• Variant calling is done using our in-house variant calling software, VarPROWLC. For this assay, the limit of detection (LOD) for SNVs is 4% and 10% for indels. LOD estimates assume a minimum depth support of 250 reads or more in both directions.

• Fusion detection is done using the STAR-SEQR algorithm. All reported fusions involve at least one of the genes specifically baited to detect fusion events. In addition, fusions must have at least 10x read depth support in both directions, with the number of spanning reads being greater than or equal to the number of junction reads supporting the fusion event.

**Variant Prioritization and Clinical Reporting**

(Tables and figures are not transcribed as they are not relevant to the natural text representation.)

**Panel and Software Details**

• While the median number of variants submitted for clinical reporting was 7 (mean = 7.3), only 24% of these variants were found to be actionable after clinical annotation (see Figure 2).

• 10 patient reports had only variants of unknown significance (VUS) reported, and so were not matched with a clinically actionable result.

• Presence of at least 1 clinically actionable result was not found to be associated with the sample being collected from a metastatic vs. primary tumor site (see Table 1).

**Observations and Conclusions**

• 75% of all actionable biomarkers were found in 12 well known oncogenes (see Figure 3).

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**Table 1: Contingency table of actionable results**

<table>
<thead>
<tr>
<th>Metastatic Site</th>
<th>Primary Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>At least 1 actionable variant found</th>
<th>Only VUS's returned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastatic Site</td>
<td>29</td>
</tr>
<tr>
<td>Primary Site</td>
<td>28</td>
</tr>
</tbody>
</table>

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**Figure 2: Figure 2. Actionability of variants selected for clinical reporting**

- **Approved therapy for given indication (1)**
- **Approved therapy in other indication (86)**
- **VUS (376)**
- **Relevant Clinical Trial (31)**

**Figure 3: Figure 3. Most frequently observed actionable biomarkers**

- **Approved therapy**
- **Relevant clinical trial**