Aims of the project

• Learn methods and processes involved in Whole Exome Sequencing
• Help develop bioinformatics pipeline for easy and feasible variant detection for implementation
  • Knowledge exchange for accurate somatic variant detection
  • Implement on data derived from sequencing
• Develop custom panel based on Whole Exome Sequencing
• Build relationship for future collaborations
Endometrial Cancer

- Tends to have good prognosis
- Incidence is increasing
- 20% recurrence rate
- Recurrent EC is much more aggressive
  - Median survival < 1 year
- Treatment options are limited
Circulating tumour DNA

<table>
<thead>
<tr>
<th>Event</th>
<th>Cancer screening</th>
<th>Localized cancer</th>
<th>Metastatic cancer</th>
<th>Refractory cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment strategy</td>
<td>Early intervention</td>
<td>Risk of dissemination and detection of recurrence</td>
<td>Treatment selection and monitoring response</td>
<td>Mechanism of resistance and new treatment</td>
</tr>
<tr>
<td></td>
<td>Multiple DNA abnormalities</td>
<td>RNA expression and fusion transcripts</td>
<td>Protein expression and phosphorylation</td>
<td>In vitro/in vivo culture</td>
</tr>
<tr>
<td></td>
<td>Amplification and deletion</td>
<td>Translocation</td>
<td>Point mutations</td>
<td>Chromosomal abnormalities</td>
</tr>
</tbody>
</table>
Patients & Samples

Four patients with EC; CT46, CT58, CT37 & CT14

Tumours were predominantly all high grade (3) except CT14 (grade 1)

- Matched tumour-normal samples were analysed
  - Tumour samples were formalin-fixed paraffin embedded
  - Normal samples derived from buffy coat containing lymphocytes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histology</th>
<th>Stage</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT14</td>
<td>Endometrioid</td>
<td>1B</td>
<td>1</td>
</tr>
<tr>
<td>CT37</td>
<td>Endometrioid</td>
<td>1B</td>
<td>3</td>
</tr>
<tr>
<td>CT46</td>
<td>Serous</td>
<td>1A</td>
<td>3</td>
</tr>
<tr>
<td>CT58</td>
<td>Carcinosarcoma/Serous</td>
<td>4B</td>
<td>3</td>
</tr>
</tbody>
</table>
Whole Exome Sequencing

- Library preparation and capture hybridisation protocol used as developed by Nonacus
- Initially trialled DNANexus – cloud platform with pipelines for variant calling
- Platypus, VarScan & ANNOVAR used
- Variant allele frequency calculated using:
  \[
  \frac{\text{Total number of variant reads}}{\text{Total number of reads}} \times 100
  \]

Mutation Tracking

- Custom ctDNA panel developed based on results from Whole Exome Sequencing
Whole Exome Sequencing

- High number of exonic nonsynonymous single nucleotide variants (SNVs) for CT14 & CT37 identified
- Germline variant analysis was conducted
  - Identify if patients had mutations in mismatch repair genes (Lynch Syndrome)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Number of exonic SNVs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT46</td>
<td>59</td>
</tr>
<tr>
<td>CT58</td>
<td>52</td>
</tr>
<tr>
<td>CT14</td>
<td>301</td>
</tr>
<tr>
<td>CT37</td>
<td>131</td>
</tr>
</tbody>
</table>

21 MUTATIONS IDENTIFIED WITH KNOWN IMPACT IN CANCER AND VAF > ~15%

Custom ctDNA panel

- PMS2 p.G225C
- PMS2 p.K435E
- MLH1 p.I219V

Lynch Syndrome

- PMS2 p.G751A
- PMS2 p.K435E
- PMS2 p.G59A
- MLH1 p.I219V
- EPCAM p.M115T

Lynch Syndrome
Academia perspective

- Different working environment vs academia
- Skills needed to transfer to academia gained
- Better understanding of the science behind each process
- Highlighted benefit of working in collaboration with industry

Nonacus perspective

- Academic/clinical guidance on the benefits and use of our technology in this case endometrial cancer
- Access to clinical samples and validation of our technology across another cancer type
- We hope that further projects and development work and grant applications going forwards.

“This IAX grant has enabled clinical validation of our technology both in terms of determining the clinical need and validity of a test but also allows validation of our technology on real patient samples and for a given cancer type.”
THANK YOU!

Diviya Gorsia & Nonacus

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