Tumour testing versus germline testing

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# Disclosure conflicts of interest speaker

| relationships with companies | AstraZeneca  
|                             | Bayer       
|                             | Bristol-Myers Squibb  
|                             | Illumina    
|                             | Janssen Pharmaceuticals  
|                             | Merck       
|                             | Nimagen     
|                             | Roche       |
Hereditary cancer

age and number of mutations

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no genetic predisposition

normal cell somatic mutations

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with genetic predisposition

normal cell with germline mutation somatic mutations
What is evaluated in tumor testing

- Somatic alterations
- Somatic alterations and germline variants
What is evaluated in tumor testing

- Somatic alterations

- Somatic alterations and germline variants

- Depends on:
  - the use of normal DNA as a reference to define germline variants
  - In tumour-only setting: bio-informatic tools to define germline variants in a mixture of somatic and germline variants
Genomic variation

Normal/constitutive/germline DNA

- Copy number variants (CNV)
- Single nucleotide variants (SNV) \(\sim 30,000\) in exons of individual
  - Single nucleotide polymorphisms (SNP) population frequency \(>1\%\)
- Variants with unknown significance (VUS)
- ( Likely) pathogenic variants leading to a hereditary condition / genetic susceptibility

Tumour DNA

- All variants present in normal DNA
- Driver mutations: a dozen
- Passenger mutations number depends on etiology / repair mechanisms

Often only potentially clinically relevant variants are reported
Advantages of tumour-only testing

• Detection of both somatic an germline variants in a single sample (no reference sequence needed)
  • Easier sample logistics
  • Lower costs
Commonly used with smaller-intermediate sized NGS panels
  • E.g. cancer hotspot panels / FoundationOne / ......

• Detection of relevant germline findings without making a definitive hereditary diagnosis allowing for less strict informed consent procedures

• Tumour test can function as a prescreen for a genetic test
  • Intention to identify persons at risk (e.g. BRCA in ovarian carcinoma)
  • Create additional opportunities to trace rare germline - tumour combinations (e.g. BRCA in prostate cancer)
Tumor test as prescreen for germline test

- Most tumour tests are not designed and validated for this purpose and thus may not be able to detect all relevant germline mutations

Special attention is needed for:
- Coverage of all relevant regions: for most tumour suppressor genes
  - Complete coding region and flanking intronic regions
  - Detection of (multiple) exon deletions
- Bio-informatic pipeline should not filter-out germline variants based on variant allele frequency of variant in comparison to neighbouring SNPs
Suspicion of genetic susceptibility based on a tumour test?

- Frequency of somatic mutations – genetic syndrome
  - E.g. TP53, PTEN, CDKN2A often have somatic mutations, while they are rarely affected in the germline
- Tumour type – gene combination
  - STK11 mutations common in lung cancer; Peutz Jegher syndrome is rare
  - POLE mutations common in endometrial; PPAP is rare
- Tumour type and clinical context
  - Commonly mutated genes in CRC:
    - APC associated with Familial Adenomatous Polyposis
    - SMAD4 associated with Juvenile Polyposis
    - Only consider genetic susceptibility in case of polyposis
- Mutational burden of a tumour
  - High mutational burden > high a priori risk of mutation being somatic
Tumour sequencing (1)

Barbara Gaynor (dob 10/12/1975) has non-small cell lung cancer and a biopsy from the adenocarcinoma has been tested using a lung cancer panel.

- The estimated neoplastic cell content was 70% within the area macrodissected and used for DNA extraction.

### Samples tested and results obtained

<table>
<thead>
<tr>
<th>Name and date of birth</th>
<th>Sex</th>
<th>Sample details</th>
<th>Testing performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbara GAYNOR 10/12/1975</td>
<td>Female</td>
<td>Sample taken – 04/01/2015</td>
<td>Your laboratory’s lung cancer NGS panel</td>
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<tr>
<td></td>
<td></td>
<td>Sample received – 09/01/2015</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block number – 2015-001156</td>
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</table>

**Results obtained**

c.817C>T p.(Arg273Cys) detected in the TP53 gene. The mutation level is approximately 50%.

All other variants considered to be non-pathogenic are not reported.

TP53 reference sequence: NC_000017.9

consider genetic susceptibility

NOT consider genetic susceptibility

Source: UKNEQAS 2015
Tumour sequencing (2)

Carol Mathews has colorectal cancer. The estimated neoplastic cell content was 85% within the area macrodissected and used for DNA extraction.

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</table>
| Carol MATHEWS 28/02/1972 | Female | Sample taken – 12/01/2015  
Sample received – 14/01/2015  
Block number – 2015-002061 | Your laboratory’s NGS cancer panel |

**Results obtained**

c.35G>A p.(Gly12Asp) detected in the KRAS gene. The mutation level is approximately 20%.

All other variants considered to be non-pathogenic are not reported.

BRCA1 reference sequence: NM_007294.3

KRAS reference sequence: NM_004985.3

consider genetic susceptibility  

NOT consider genetic susceptibility

Source: UKNEQAS 2015

Radboudumc
Tumour sequencing (3)

- A patient with malignant melanoma and has been referred for cancer gene analysis.
- Neoplastic cell content was 90% within the area macrodissected and used for DNA extraction.

### Samples tested and results obtained

<table>
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<th>Sex</th>
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<th>Testing performed</th>
</tr>
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</table>
| David MAYBERRY 03/10/1969 | Male | Sample taken – 14/01/2015  
Sample received – 19/01/2015  
Block number – 2015-003357 | Your laboratory’s NGS cancer panel                      |

### Results obtained

c.1564_1565delAT detected in the APC gene. The mutation level is approximately 50%.
All other variants considered to be non-pathogenic are not reported.
APC reference sequence: NM_000038.5

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consider genetic susceptibility  NOT consider genetic susceptibility

Source: UKNEQAS 2015
How to deal with possible germline variants in cancer susceptibility genes?

• Discuss variants in a Molecular Tumor Board with participation of a clinical (molecular) geneticist
• Define criteria for germline genetic testing/referral for genetic counseling
  • Only (likely) pathogenic variants (ACMG/IARC class 4 or 5)
  • A minimum *a priori* risk of a germline origin
• List of genes to consider clinically actionable
  • E.g. define what to do with frequently occurring moderate risk variants like c.1100del in *CHEK2*
Take home messages

• Check whether a given gene panel is suitable for the clinical need: genes of interest need to be sufficiently covered

• Tumor-based testing may unravel both somatic and germline mutations, however germline variants may be filtered out depending on the test specifications

• Not all class 4 and 5 variants in cancer predisposition genes that are detected in tumor-based testing need to be considered as an indication of putative tumor predisposition: context is important.