Cancer Validation in the 100,000 genomes project

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Cancer is a lesion of the Genome

**Lifetime risk**

50%

1 in 2 people born after 1960 in the UK will be diagnosed with some form of cancer during their lifetime.

**Cancer-Associated Mutations**

- Oncogenes
- Tumor suppressor genes
- DNA repair genes
- Carcinogen
  - activating genes
  - deactivating genes
- Cell cycle genes
- Cell cycle checkpoint genes
- Cell death genes
- Cell signaling genes
- Cellular differentiation genes
- Cellular senescence genes
- Metastasis/invasion genes
Diagnosis → Cancer Molecular Lesions → Prognosis

Personalized Cancer Therapy

Molecular Profiling
- Prognostic Markers
- Markers predictive of drug sensitivity/resistance
- Markers predictive of adverse events

Disease Monitoring
Why is Cancer Challenging?

• **Sample pathway issues**
  - Hard to get the DNA – why leukaemia has led the way!
  - Complex sample pathway, well established and difficult to change
  - Often not much tissue available for DNA extraction after other diagnostic tests have been done
  - Sample quality is poor due to tissue processing required for other tests – i.e. formalin. Not DNA friendly

• **Sample issues**
  - Samples will be contaminated with normal tissue
  - Difficulties in assessing neoplastic content – training required
  - Tumours are heterogeneous – different mutations spatially and in frequency.
  - Mutation Frequencies can vary from 0-100%
Current clinical pathway for DNA diagnostics from biopsy/resection material

1. Surgical Biopsy/resection
2. Cut up
3. Formalin fixation
4. Processing & Paraffin embedding
5. DNA extraction
6. Macro dissection of neoplastic areas from mounted sections
7. Microscopic examination to identify neoplastic areas
Evolution of Existing Molecular Pathology Services (or lessons learn ’t the hard way......)

- Careful tumour assessment is required to ensure tumour present - work closely with pathologists
- Macro-dissection to improve neoplastic content
- Use robust methods that will work on low level and poor quality fragmented DNA obtained from FFPE samples
- Select sensitive methods that will detect variants at low frequency (due to tumour heterogeneity or normal tissue contamination) e.g Pyrosequencing & Real Time
- Use of NGS panels increasing – Good sensitivity due to high coverage, often targeted and amplicon based as they work well on low level/poor quality DNA
- Test must be cost effective – Cancer is common and diagnostic tests are not centrally commissioned – it is often unclear who should pay for the test
- Turnaround times must be short.
Cancer 100,000 genomes Project

**WGS:** Potential to detect all Cancer mutations (CNS, indels, SV, CNVS) – exome and intron

**CRUK & BRC WGS Pilots:** WGS quality from FFPE tissue is highly variable between centres and often of poor.

**GEL experimental phase 1:**

- Tissue handling variables (fixative, length of time in formalin, block size) have a critical effect on the quality of the DNA extracted from FFPE and the downstream WGS

- Optimal tissue handling can certainly improve the quality of WGS from FFPE but currently not to the quality seen from fresh frozen samples.
Current clinical pathway for DNA diagnostics from biopsy/resection material:

1. Surgical Biopsy/resection
2. Cut up
3. Formalin fixation
4. Processing & Paraffin embedding
5. DNA diagnostic test
6. Macro dissection of neoplastic areas from mounted sections
7. DNA extraction
8. Fresh Frozen
9. Microscopic examination to identify neoplastic areas
Validation

• GEL WGS pathway not accredited

• Fresh frozen preferred but there will always be occasions when this is not possible. FFPE will be the only option for some patients/tumours

• Confirmatory test used will need to have been validated for FF and FFPE (UKAS requirement)
GMCs will need to validate any results that are going to be clinically acted upon:-

- Drugable
- Diagnostic
- Prognostic

- Absence of a mutation may be just as clinically actionable as the presence of a mutation. Therefore likely to be necessary to validate negative results on some occasions.

- Potentially actionable variants will be flagged on the GEL WGS result but the decision as to whether a variant should be acted upon will depend entirely on the Clinical context.
Genomic Block FF or FFPE

Routine Diagnostic Block FFPE

Standard of care testing
- Histology /Morphology
- Immunocytochemistry
- DNA tests
- FISH

Genomic Block FF or FFPE

DNA Retained by GMC for validation

DNA sent to GEL Biorepository/Illumina

DNA sent to GEL Biorepository/Illumina

Genome analysis results:
- Virtual cancer panel & tiering
- Complementary analysis

GMC
Laboratory assessment then MDT review:
Validation of clinically actionable variants
- ? In house
- ? Validation hubs
- ? Validation net work

Excess DNA retained by biorepository

?Technical validation of a representative selection of variant types

Clinical Report

Updated Integrated Clinical Report
How will GMCs validate clinically actionable results?

• Standard of care testing will have already been done
  ➢ wide variation throughout the UK in what people do – some do individual tests others gene panels.

• In-house
  ➢ Generally too time consuming/expensive to set up a whole new assay – Sanger often too insensitive. If panels are already up and running as part of standard of care, the additional genes on them may be useful for validation in some cases.

• Validation Network or Hubs
  ➢ Labs who can test for the various mutations.

• How will the validation tests be funded (in-house or sending away)?
Impact of read depth on somatic variant detection
(Somatic Variant discovery in Cancer - Illumina Application Note: Cancer genomics)

- 70T-30N
  - alleles with VF of >30% detected with sensitivity of 99%
  - Alleles with VF of 20% detected with sensitivity of 90%
  - alleles with VF of 15% detected with sensitivity of 80%
  - alleles with VF of 10% detected with sensitivity of 55%
  - alleles with VF of 5% detected with sensitivity of 18%
Issue of WGS sensitivity and negative results

• Negative results can be just as important as positive results
• The Sensitivity of WGS is not as good as most of the methods currently used in molecular pathology.
• This means standard of care testing will likely flag up some lower level mutations missed by WGS
• How will we ensure Clinicians appreciate the significance of differences in sensitivity between WGS and routine standard of care tests?
Cancer Validation Questions

• How will the validation networks/hubs be organised?

• How will we validate the different mutation types?
  ➢ SNPs, indels, CNVs, SVs

• Sensitivity of Cancer validation tests
  ➢ How sensitive do the tests we use to validate negative results have to be?
  ➢ Should we all be using methods of similar sensitivity for validation?
  ➢ How will we ensure clinical teams understand the differences in sensitivity between WGS and routine services?

• How can we ensure there is enough DNA for validation?
  ➢ How much DNA is likely to be left over at the GMC?
  ➢ Is there potential for using the routine diagnostic DNA sample?

• How can we fund the validation test?
## Acknowledgements

### NHSE Genomic Medicine Centres

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