Cancer Validation in the 100,000 genomes project

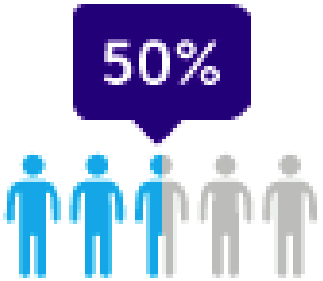
Dr Shirley Henderson

ACGS spring meeting

06/07/16

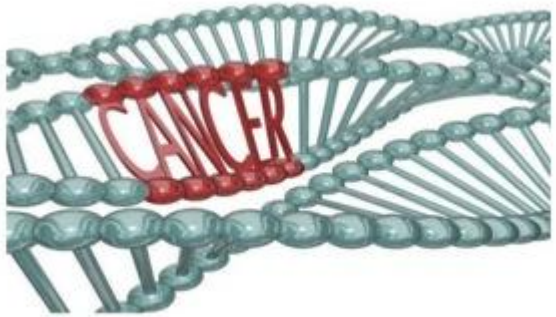
CANCER RESEARCH UK

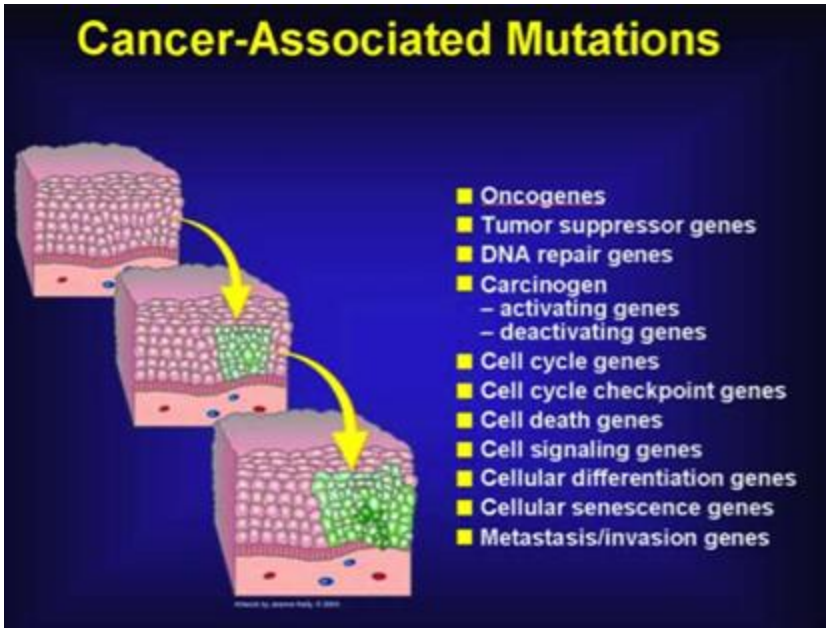
Lifetime risk



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

Cancer is a lesion of the Genome

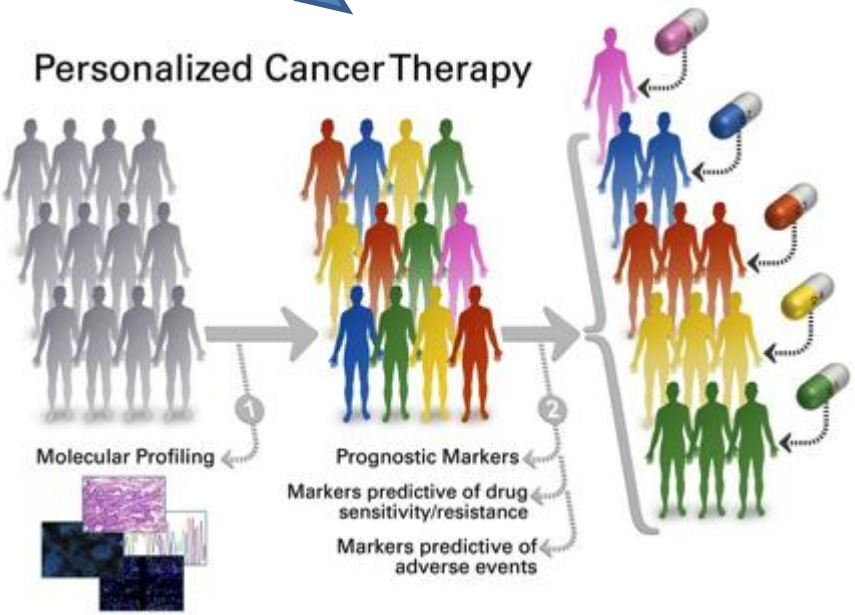


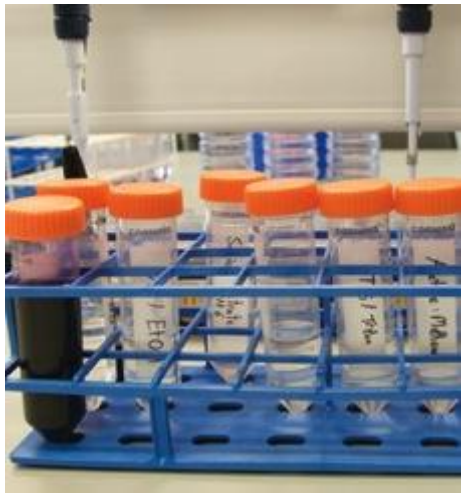


Diagnosis

Prognosis

Cancer Molecular Lesions





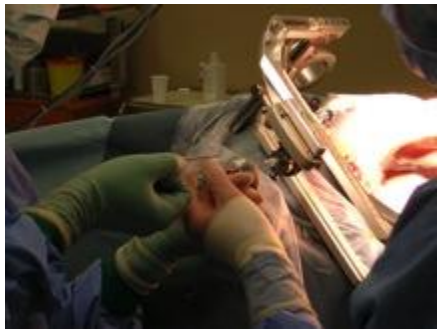
Disease Monitoring

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

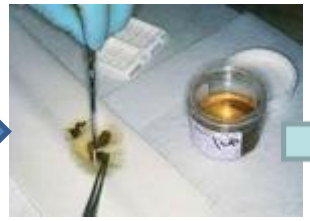
- **Sample issues**
  - Samples will be contaminated with normal tissue
  - Difficulties in assessing neoplastic content – training required
  - Tumours are heterogeneous – different mutations spatially and in frequency.
  - Mutation Frequencies can vary from 0-100%

Current clinical pathway for DNA diagnostics from biopsy/resection material



Surgical Biopsy/resection

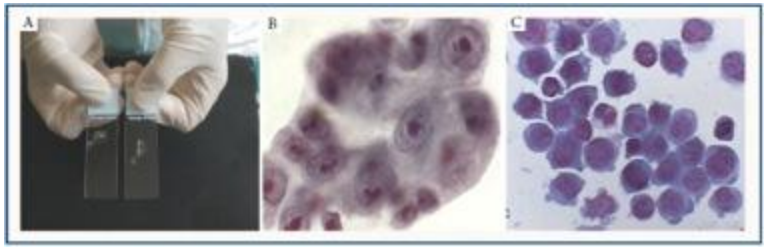
Cut up



Formalin fixation

Processing & Paraffin embedding

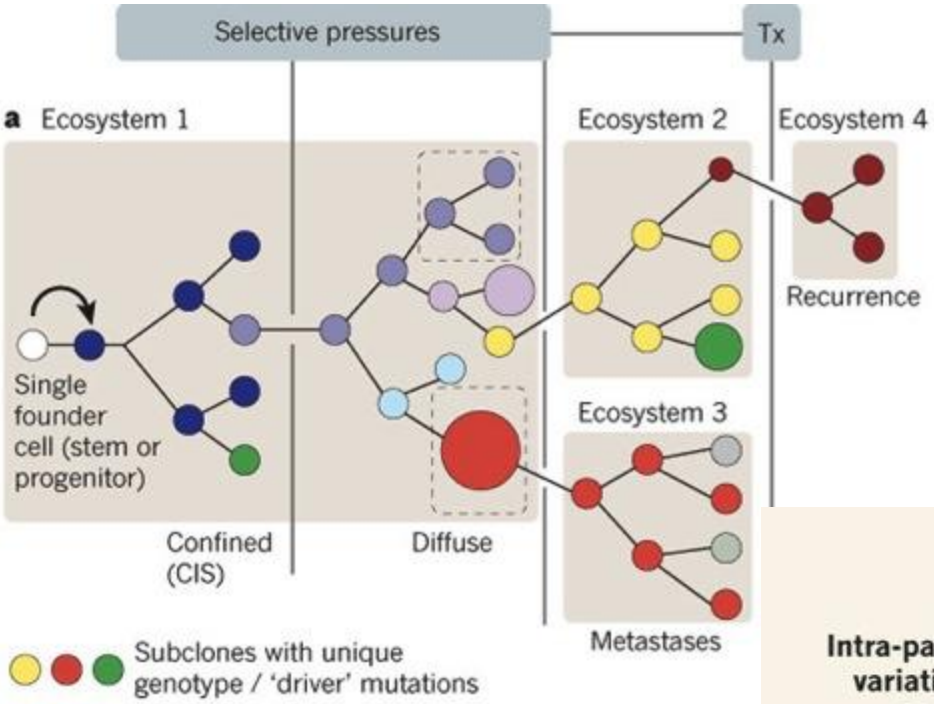
Microscopic examination to identify neoplastic areas

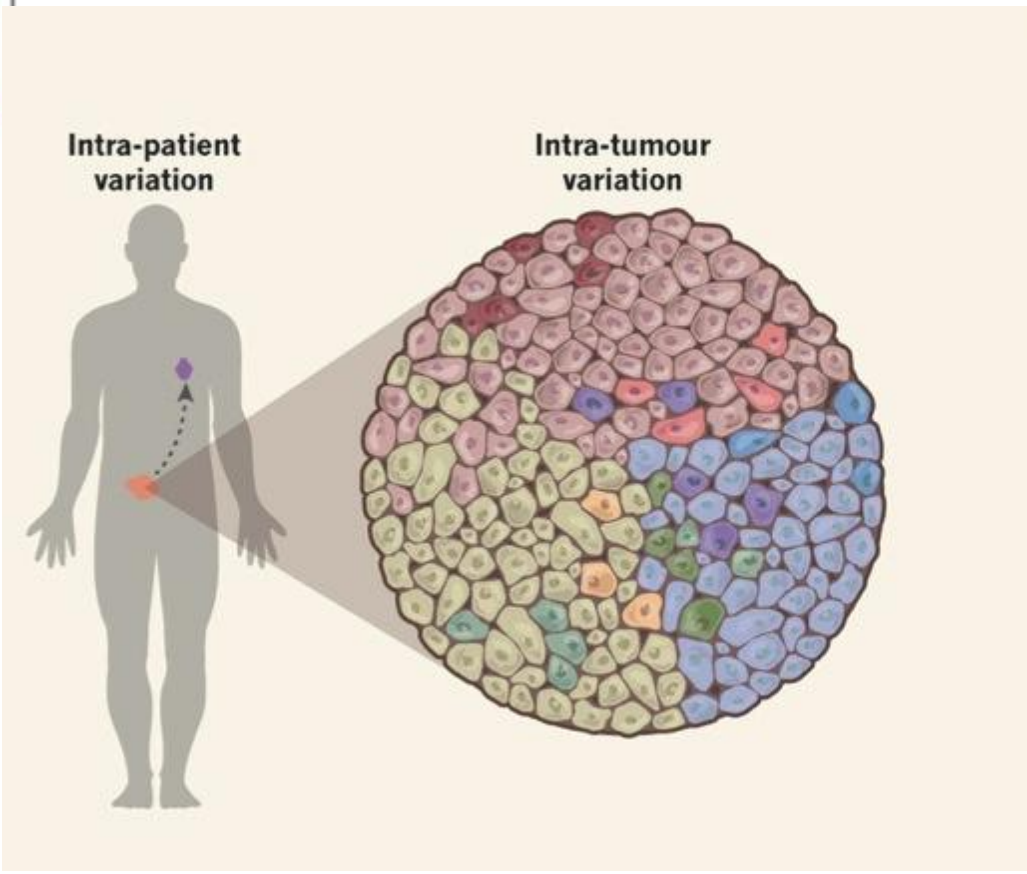


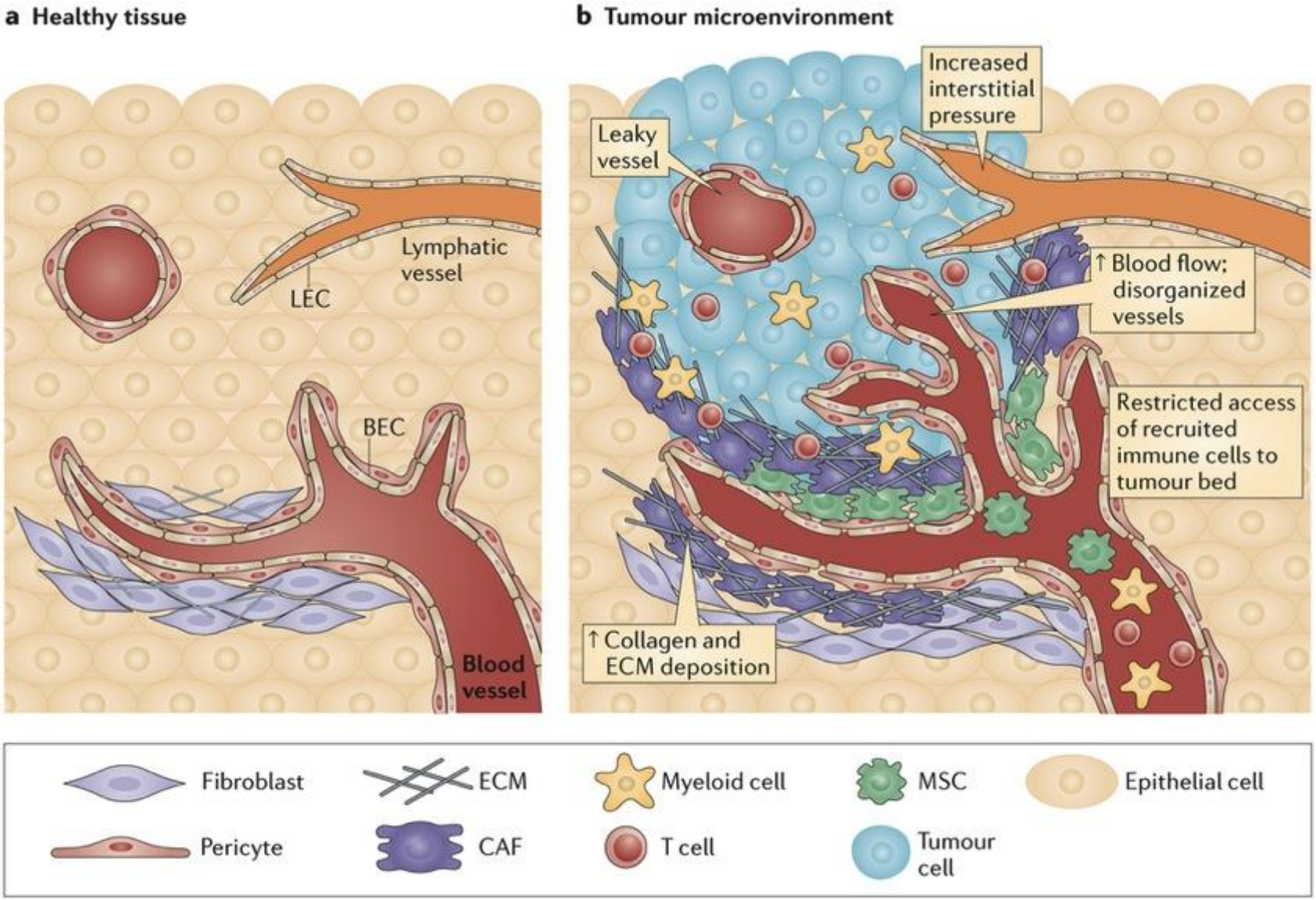
Macro dissection of neoplastic areas from mounted sections

DNA extraction

DNA diagnostic test


Selective pressures
Tx
a Ecosystem 1
Ecosystem 2
Ecosystem 4
Recurrence
Single
founder
cell (stem or
progenitor)
Ecosystem 3
Confined
(CIS)
Diffuse
Metastases
Subclones with unique
genotype / 'driver' mutations


Intra-patient
variation
Intra-tumour
variation


a Healthy tissue
b Tumour microenvironment
Lymphatic vessel
LEC
BEC
Blood vessel
Increased interstitial pressure
Leaky vessel
↑ Blood flow; disorganized vessels
Restricted access of recruited immune cells to tumour bed
↑ Collagen and ECM deposition
Fibroblast
ECM
Myeloid cell
MSC
Epithelial cell
Pericyte
CAF
T cell
Tumour cell

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

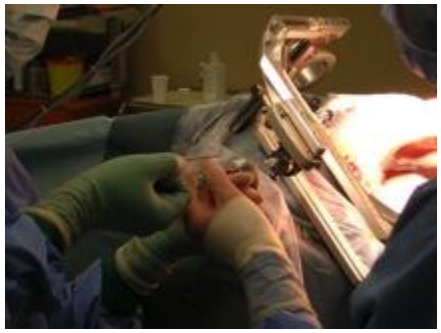
WGS:- Potential to detect all Cancer mutations (CNS, indels, SV, CNVS) – exome and intron

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.



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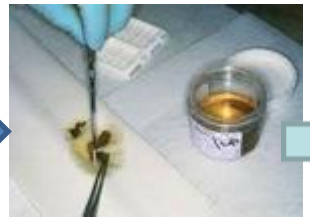

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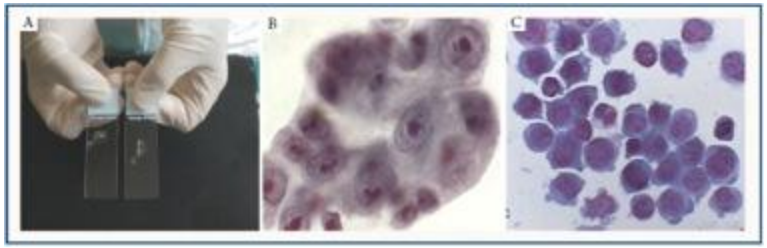
DNA diagnostic te

Current clinical pathway for DNA diagnostics from biopsy/resection material

Fresh Frozen

DNA extraction



Macro dissection of neoplastic areas from mounted sections

Microscopic examination to identify neoplastic areas

Validation

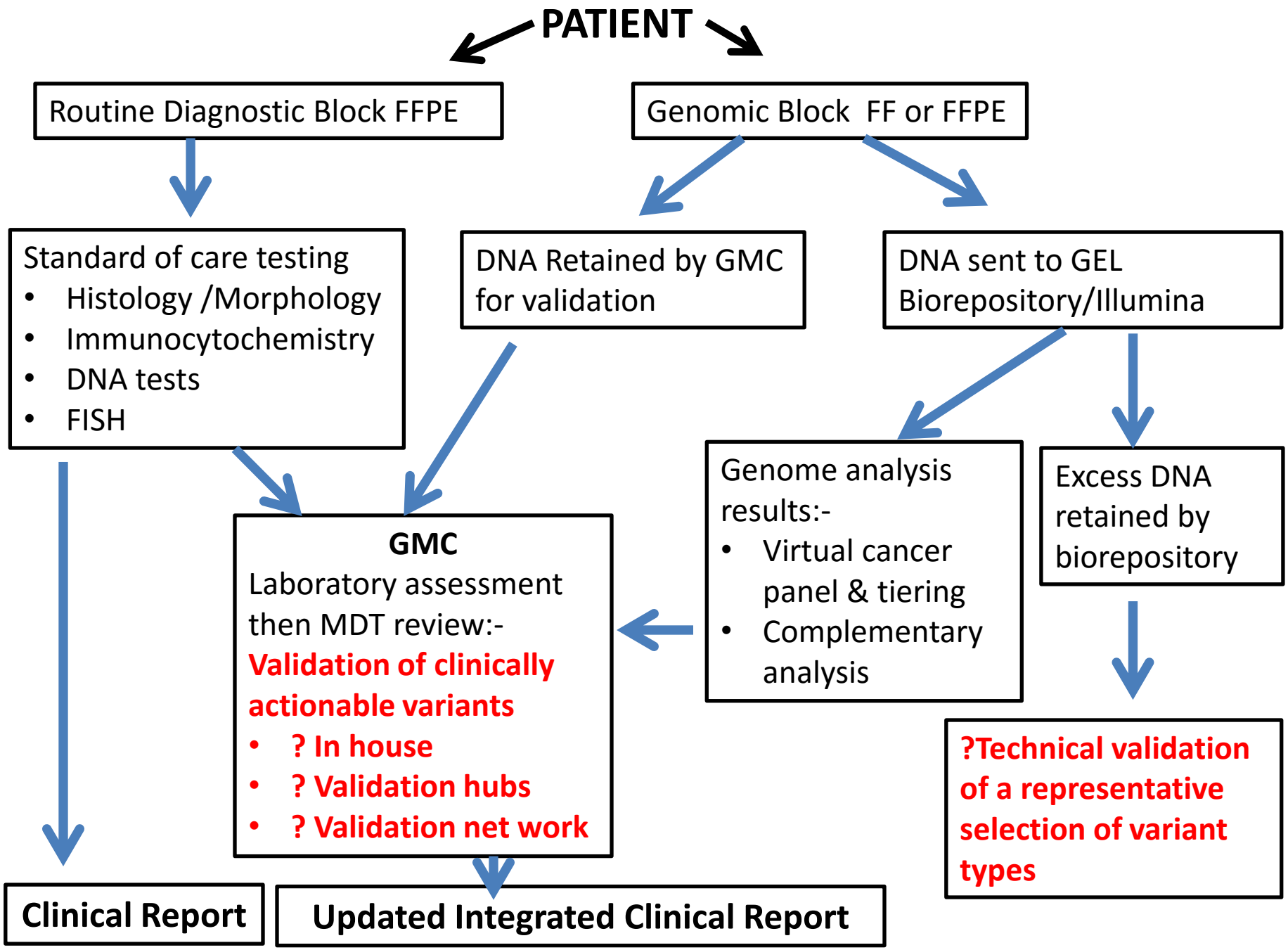
- GEL WGS pathway not accredited
- Fresh frozen preferred but there will always be occasions when this is not possible. FFPE will be the only option for some patients/tumours
- Confirmatory test used will need to have been validated for FF and FFPE (UKAS requirement)

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.

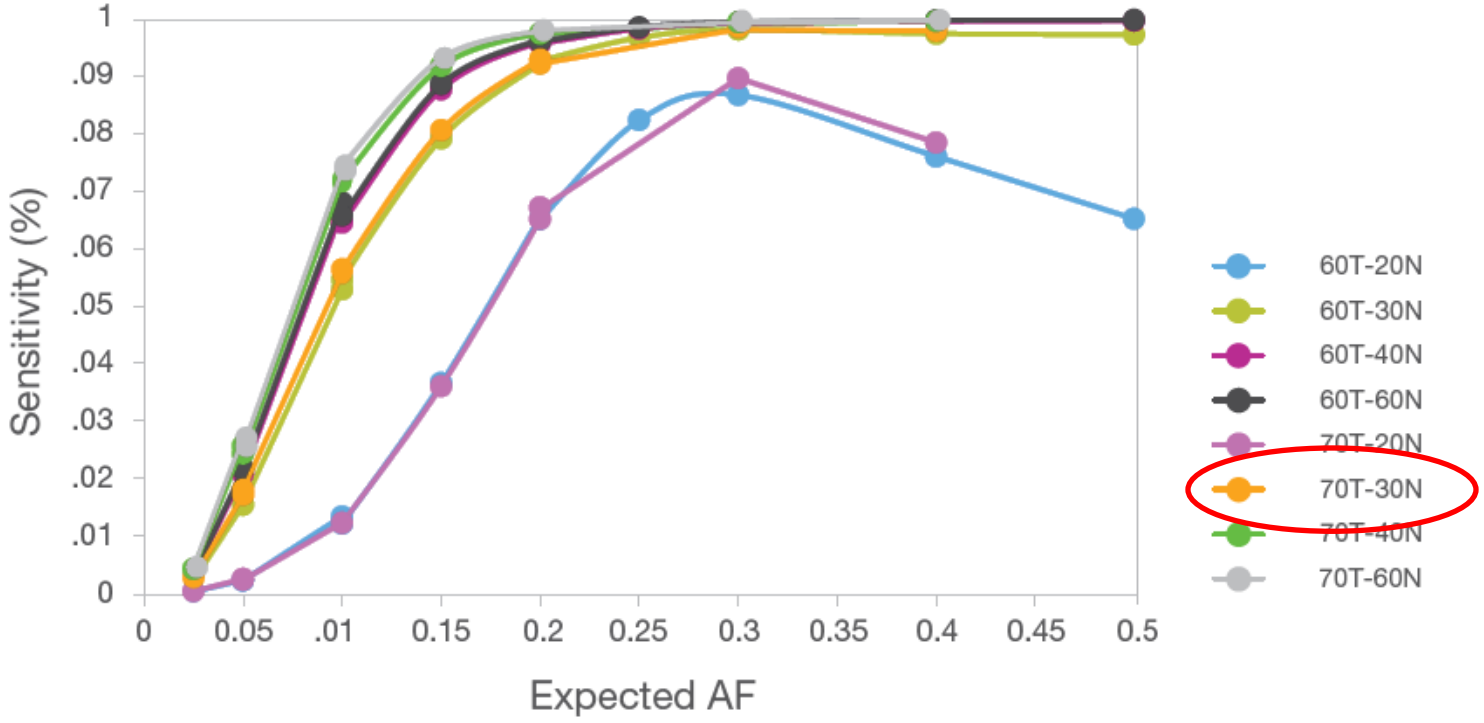

PATIENT
Routine Diagnostic Block FFPE
Genomic Block FF or FFPE
Standard of care testing
• Histology /Morphology
• Immunocytochemistry
• DNA tests
• FISH
DNA Retained by GMC for validation
DNA sent to GEL Biorepository/Illumina
Genome analysis results:-
• Virtual cancer panel & tiering
• Complementary analysis
Excess DNA retained by biorepository
GMC
Laboratory assessment then MDT review:-
Validation of clinically actionable variants
• ? In house
• ? Validation hubs
• ? Validation net work
?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

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