100,000 Genomes Project: Cancer Programme Validating WGS for clinical use

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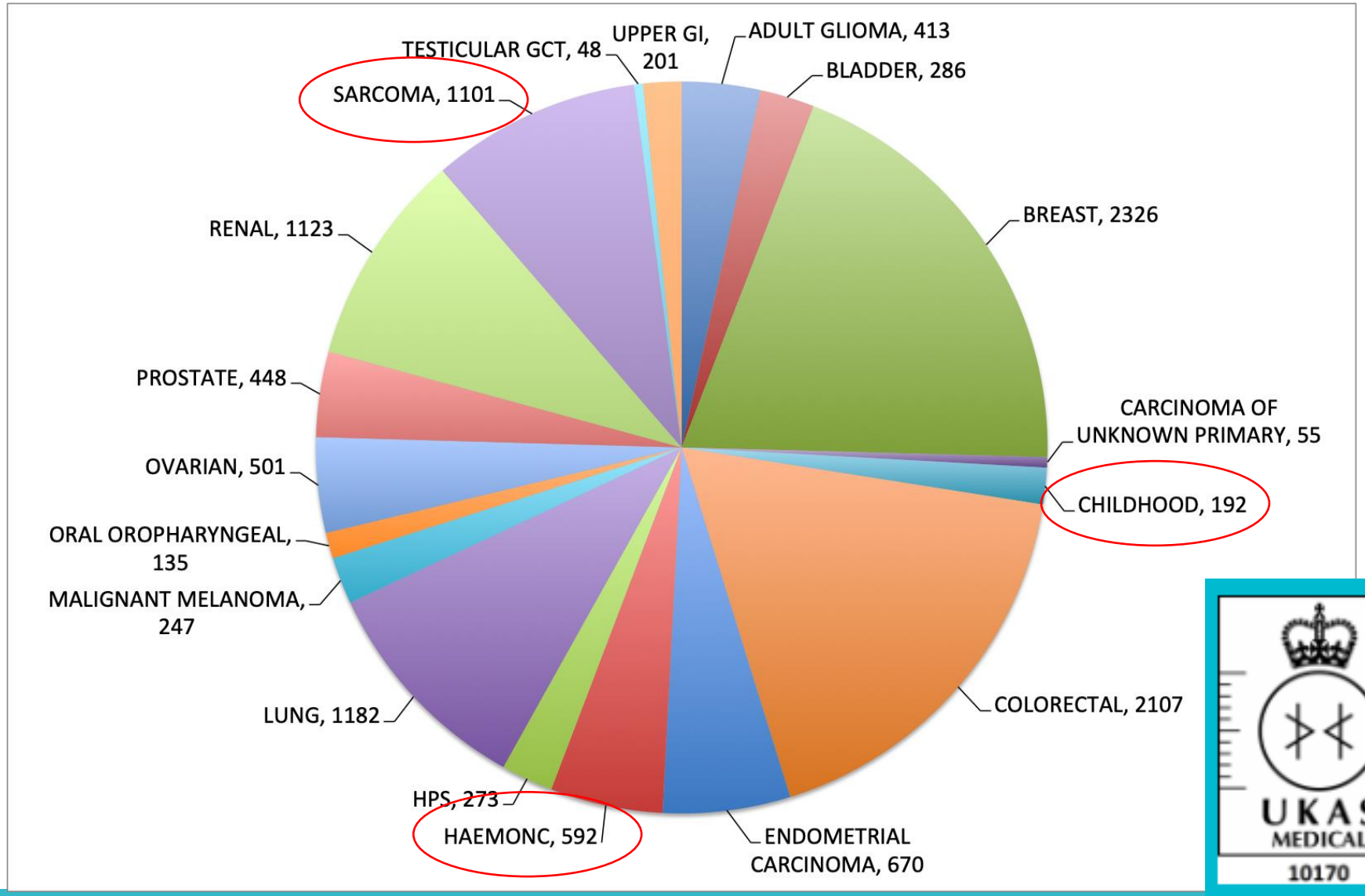
Whole Genome Sequencing results returned by June, 2019 – 12K patients



Return of results: somatic small variants

Gene	GRCh38 coordinates ref/alt allele	Transcript	CDS change and protein change	Predicted consequences	Population germline allele frequency (1KG|gnomAD)	VAF	Alt allele/total read depth	COSMIC ID	Gene-level actionability	Variant-level actionability	Gene mode of action
JAK2	9:5073770 G>T	ENST00000381652	c.1849G>T p.(Val617Phe)	missense_variant	- |0.0004	0.46	56/121	COSM12600		Trial (ET) Trial (ET) Trial (ET) Trial (PV) Trial (PV) Trial (PV) Trial (PMF) Prognostic (ET) Prognostic (MDS/MPN) Prognostic (MPN) Prognostic (PV) Prognostic (PMF) Prognostic (RARS-T)	oncogene
TP53	17:7673806 C>T	ENST00000269305	c.814G>A p.(Val272Met)	missense_variant	- |0.0000	0.38	37/98	COSM10891 COSM99950 COSM3388172 COSM1645249	Trial (AML) Trial (AML)	Therapeutic (MDS) Prognostic (MDS)	both
TP53	17:7674858 C>G	ENST00000269305	c.672+1G>C	splice_donor_variant	- | -	0.43	50/116	N/A	Therapeutic (MDS) Trial (AML) Trial (AML) Prognostic (MDS)		both

TP53 mutations in AML patients are associated with a worse overall survival

Papaemmanuil et al. NEJM (2016)

Validation of WGS against standard of care: panel testing

- 96 patients
- 3 Genomic Medicine Centres
- Lung and colorectal tumours
- Well-studied cancer genes
- Qiagen Human Clinically Relevant Tumour Panel, Thermofisher Oncomine Focus assay, Thermofisher Ion Ampliseq hot spot cancer panel (two out of three are ISO-accredited tests)
- The same DNA was used by both tests

- Additional validation experiment was performed against high-confidence high-depth exome data (TRACERx study):
  - SNV > 10% VAF: 99% recall, 90% precision
  - Indel > 10% VAF: 95% recall, 85% precision

Small variants validation

Confusion matrix	NGS panel positive	NGS panel negative
WGS positive	**True positive 155 variants**	**False positive 1 variants**
WGS negative	**False negative 2 variants**	**?**

- Positive percentage agreement, PPA = 155/157 = **98.7%**
- Positive predictive value, PPV = 155/156 = **99.4%**
- All discrepant variants had support < 5% in WGS
- There were too few indels to calculate the accuracy of indel calling separately

Return of results: somatic Structural Variants and Copy Number Aberrations

Non-redundant list of structural variants

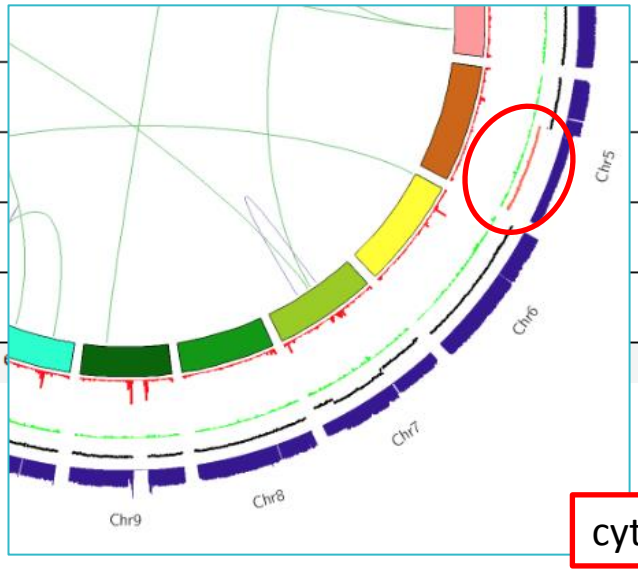
When connected to the Internet columns can be sorted by clicking on colu
Click to collapse/expand

Search: del

AML 5q- syndrome with common deleted region at 5q31-q32

Genomic coordinates	Type	Cytological bands
3:11775-90719661	LOSS(1)	del(3)(p26.3;p11.1)
5:65357263-181288262	LOSS(1)	del(5)(q12.3;q35.3)
6:154481294 6:154481365	DEL	6q25.2
7:84086471 7:84086527	DEL	7q21.11
17:18000253 17:18000357	DEL	17p11.2
19:38275666 19:38275970	DEL	19q13.2
19:38602868 19:38602924	DEL	19q13.2

Showing 1 to 7 of 7



cytogenetic analysis was not performed due to myelofibrosis

Myelodysplastic disorders carrying both isolated del(5q) and JAK2(V617F) mutation: Concise review, with focus on lenalidomide therapy *Musto et al. Onco Targets Ther, (2014)*

Return of results: somatic Structural Variants and Copy Number Aberrations

Genomics england

ETV6-RUNX1 fusion is associated with a favourable outcome and has been associated with late relapse in childhood B-ALL

Gene-centered view of structural variants

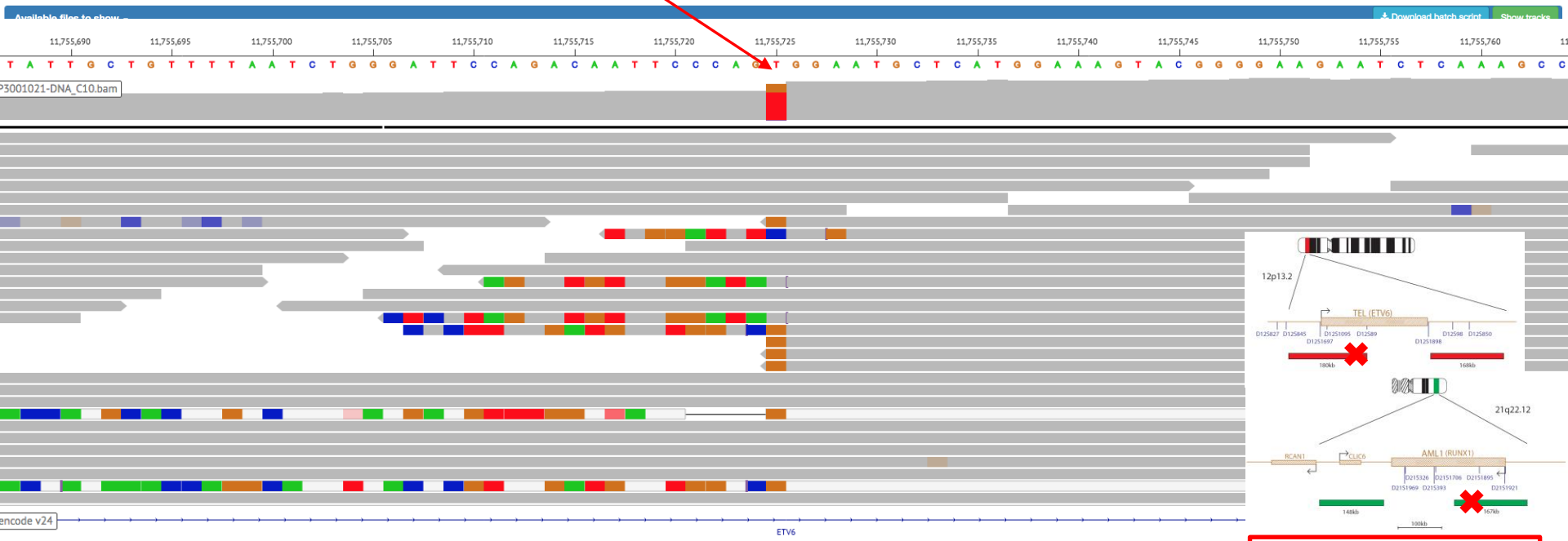
When connected to the Internet columns can be sorted by cl

Click to collapse/expand

Search: ETV6

Gene	Transcript	Genomic coordinates	Type	Size	Cytological bands	CGC	Role in cancer
ETV6 RUNX1	ENST00000396373 ENST00000437180	12:11755726 [21:34935336[GG	BND	N/A	t(12;21)(p13.2;q22.12)	CGC, CGC	tumour suppressor, oncogene/tumour suppressor
ETV6 RUNX1	ENST00000396373 ENST00000437180	12:11872062 G]21:34935312]	BND	N/A	t(12;21)(p13.2;q22.12)	CGC, CGC	tumour suppressor, oncogene/tumour suppressor

Showing 1 to 2 of 2 entries (filtered from 480 total entries)



Negative FISH test

Validation of WGS against standard of care: FISH testing

- 46 patients
- 5 Genomic Medicine Centres
- Patients with pediatric ALL, AML, CLL, multiple myeloma, lymphoma
- Broad spectrum of variants: ABL2, BCR-ABL1, BCL6, CDKN2A, CSF1R, ETV6-RUNX1, IGH-MYC, IGH-BCL2, HLF, KMT2A, MECOM, MYC, PDGFRB, STIL-TAL1, TLX1, TLX3, TCL1, TCF3-PBX1, TRG, TRB, TRAD, ATM, TP53, FLT3 ITD, PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11, CBFB-MYH11

- Additional data available: 80 ALL samples with FISH, SNP arrays, karyotypes, MLPA; FISH data for sarcoma patients; HER2 status for breast cancer patients

CNV validation

Confusion matrix	FISH positive	FISH negative	
WGS positive	**True positive 46 variants**	**False positive 1 variant**	47
WGS negative	**False negative 10 variants**	**True negative 13 variants**	23
	56	14	

- CNVs: Positive percentage agreement, PPA = 46/56 = 82%
- CNVs: Positive predictive value, PPV = 46/47 = 98%
- CNVs: False Positive Rate, FPR = 1/14 = 7%
- Discrepancies are due to: sub-clonal variants with < 15% VAF (3), low quality sample (3), low tumour purity sample (2)

SV validation

Confusion matrix	FISH positive	FISH negative	
WGS positive	**True positive 25 variants**	**False positive 5 variants**	30
WGS negative	**False negative 1 variants**	**True negative 123 patients**	124
	26	128	

- SVs: Positive percentage agreement PPA = 25/26 = 96%
- SVs: Positive predictive value PPV = 25/30 = 83%
- SVs: False Positive Rate, FPR = 5/128 = 4%

FLT3 ITD

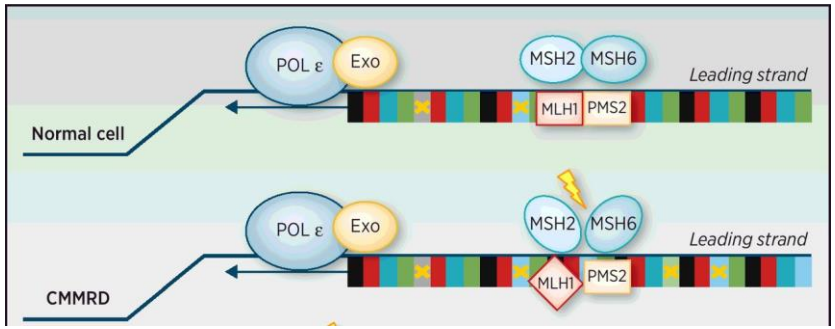
Confusion matrix	FISH positive	FISH negative	
WGS positive	**True positive 5 variants**	**False positive 0 variants**	5
WGS negative	**False negative 1 variants**	**True negative 7 patients**	8
	6	7	

- CNVs: Positive percentage agreement, PPA = 5/6 = 83%
- CNVs: Positive predictive value, PPV = 5/5 = 100%
- CNVs: False Positive Rate, FPR = 0/7 = 0%
- Discrepancy is due to sub-clonal variant with < 5% VAF

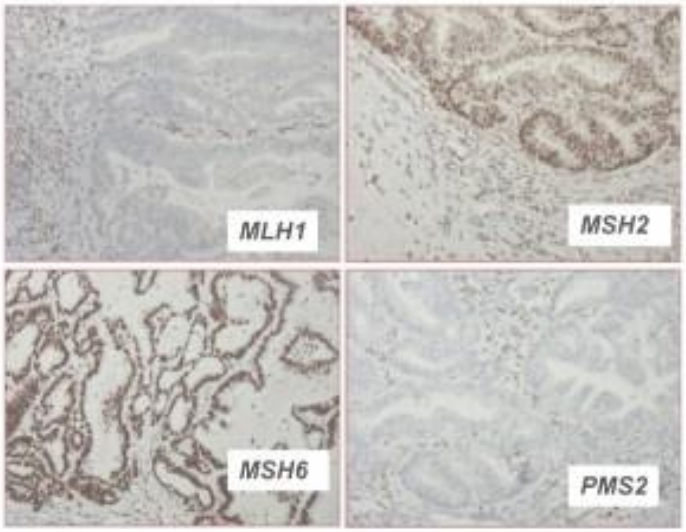
Return of results: pan-genomic markers

Genomics england

Genomes with deficiency in DNA mismatch repair (MMR) machinery

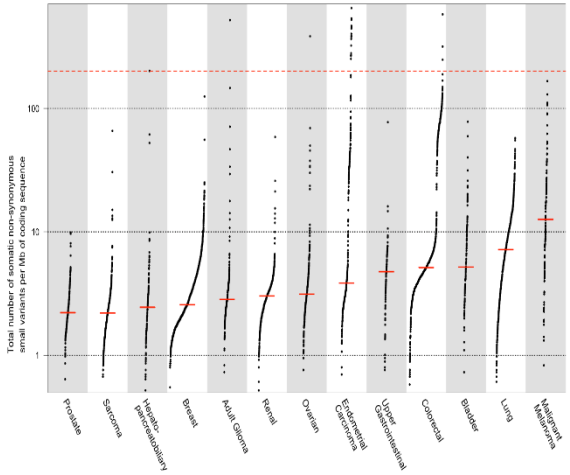


Standard of care test:
IHC for MMR genes MLH1, MSH2, MSH6 and PMS2

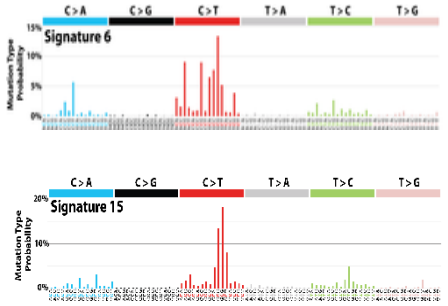


Pan genomic markers

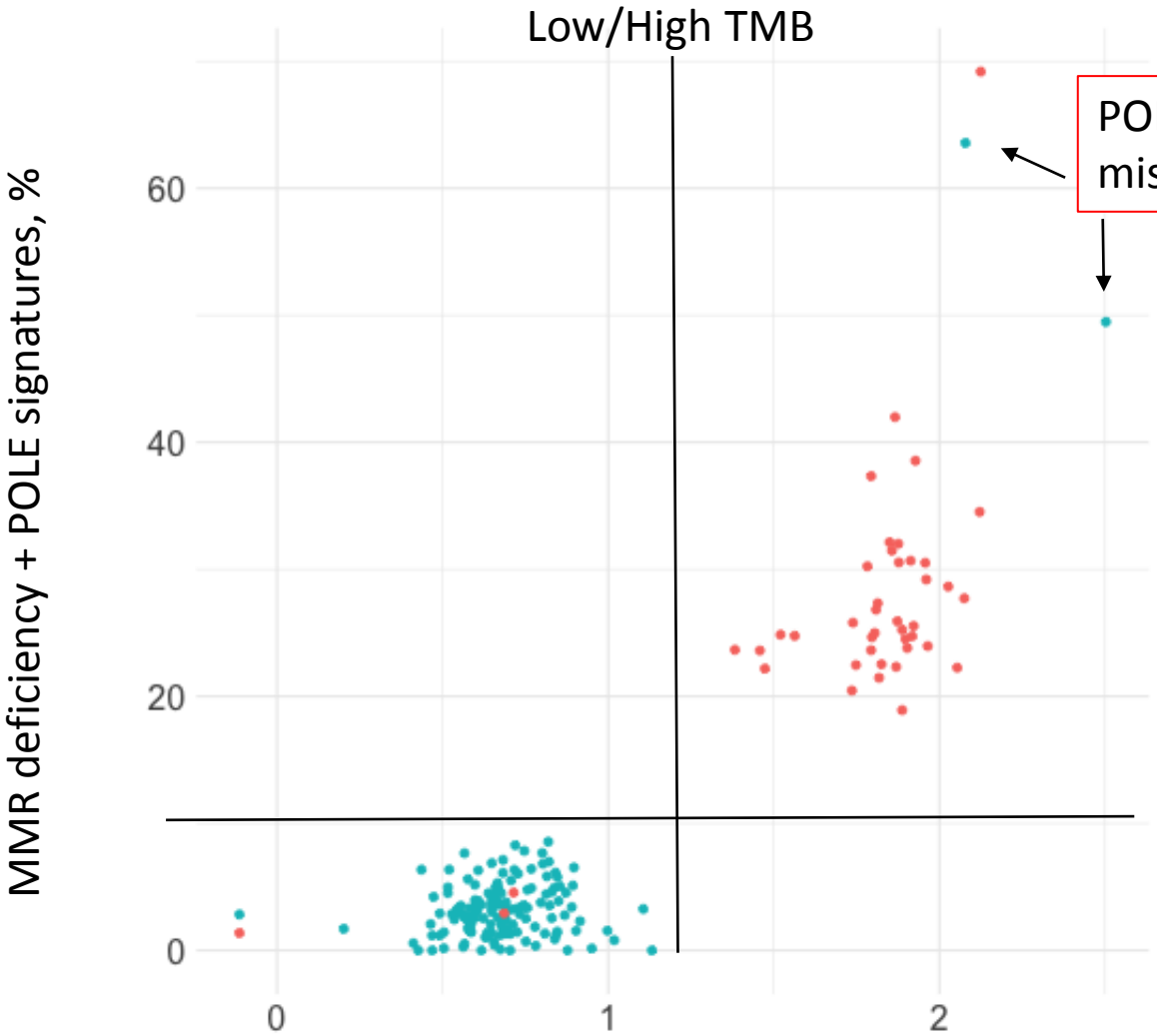
Mutation burden



Mutational signatures



Validation of WGS against standard of care: IHC test for MMR genes



POLE somatic variant that are missed by IHC

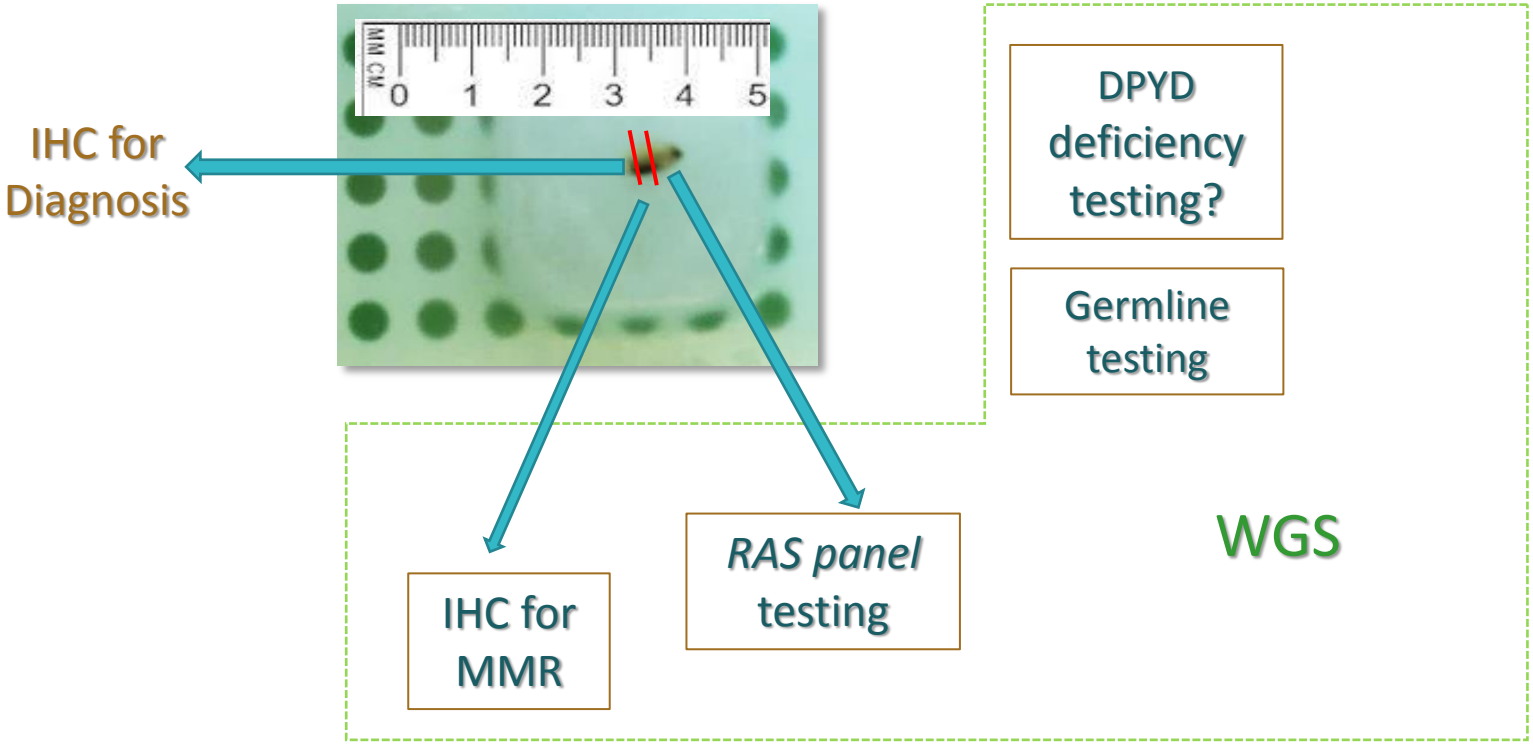
IHC for MMR genes: MLH1, MSH2, MSH6 and PMS2

- Abnormal
- Normal

High/low MMR + POLE signatures

Total 200 cases
45 abnormal MMR IHC

Impact of WGS: molecular testing for colorectal tumours



By Nirupa Murugaesu

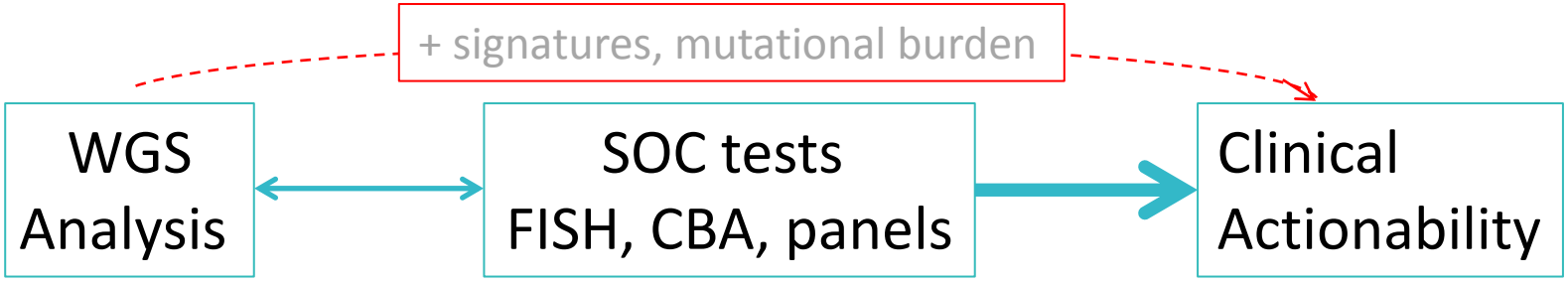
Strategy for utilising WGA

Current:

1. Enhance recruitment to existing clinical trials
   - Better curation of existing national stratified oncology trials: FOCUS-4, MATRIX/SMP2
   - Enhance recruitment to Phase 2 targeted therapy trials

Next steps:

2. Pan genomic markers
   - Utilise WGA for stratification as a companion diagnostic
   - Better molecular characterisation of tumours/patients
3. Designing clinical trials
   - Utilise WGA for stratification
   - To better identify potential biomarkers for stratification
   - Better molecular characterisation of tumours/patients



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