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Clinical interpretation of germline cancer predisposition

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Outline

- How is germline cancer predisposition diagnosed through genetic testing on a blood or saliva specimen.
- Can we detect germline pathogenic variants by tumor sequencing?
- What additional information on germline cancer susceptibility does tumor/normal sequencing provide.

Germline testing for cancer patients

Cancer patient referred to clinical genetics practice

↓

Detailed personal and family history is obtained

↓

Clinical geneticists determine what genetic testing a patient is eligible for based on clinical guidelines

↓

Genetic testing is ordered for genes that may be causative of the patient's cancer

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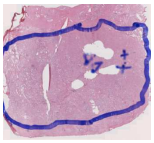
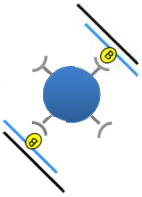
MSK-IMPACT

Integrated Mutation Profiling of Actionable Cancer Targets

DNA from **FFPE Tumor** and **Normal** cells



Capture DNA for **505 cancer genes**



Next-gen Sequencing (500 - 1000 x)

Align to genome and analyze

Cheng, Mitchell, Zehir, Shah, Benayed, *et al.*, *J Mol Diagn*, March 2015

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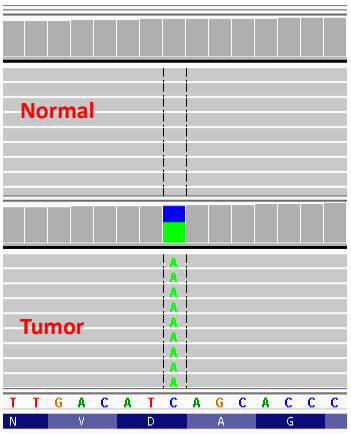
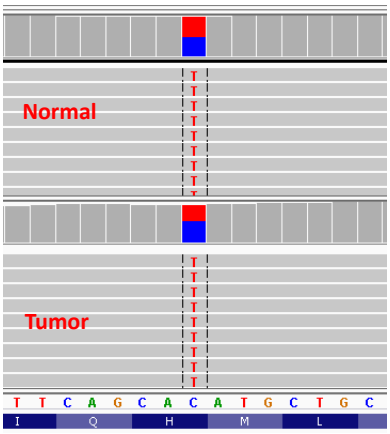
Tumor-Normal sequencing distinguishes somatic and germline variants

Somatic variant



Germline variant



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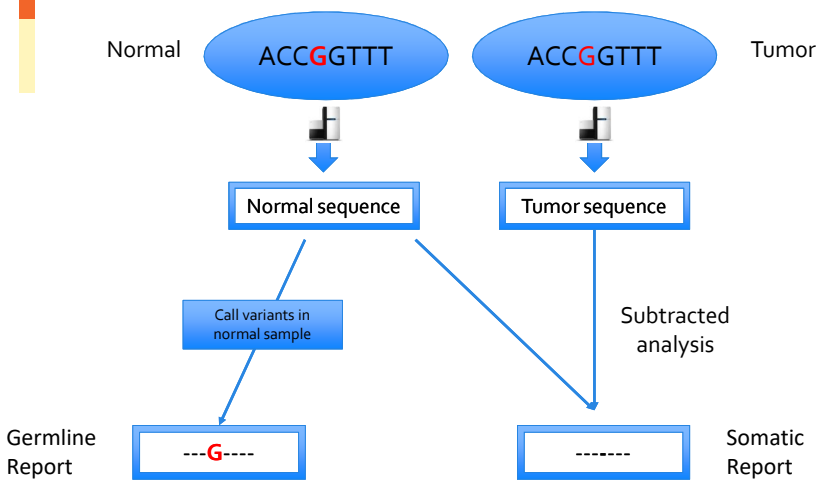
Generating somatic and germline reports from tumor-normal sequencing



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FOUNDATIONONE

Patient Name [redacted] Report Date [redacted] Tumor Type Liver cholangiocarcinoma

Date of Birth
Sex
FMI Case #
Medical Record #
Specimen ID

[redacted]

ABOUT THE TEST:

FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related gen

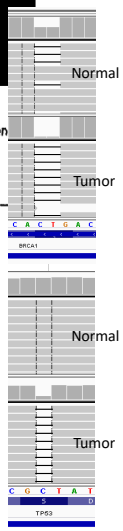
PATIENT RESULTS

- 9 genomic findings
- 3 therapies associated with potential clinical benefit
- 0 therapies associated with lack of response
- 8 clinical trials

TUMOR TYPE: LIVER CHOLANGIOCARCINOMA

Genomic Alterations Identified†

BRCA1 Q804fs*5 Germline
BRIP1 N196S Germline
PTEN loss Somatic
ARID2 L370*Somatic
CDKN2A p16INK4a P11fs*26Somatic
ERBB4 R711C Somatic
MAP2K4 D263fs*4Somatic
PREX2 R463C
TP53 S185fs*62 Somatic



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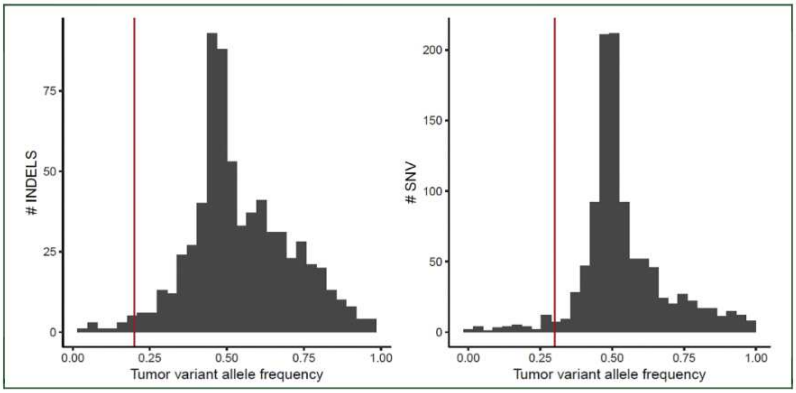
VAF of true germline pathogenic variants in tumor



Setting VAF thresholds at 0.2 for indels and 0.3 for SNVs removed 54% of somatic pathogenic variants and filtered out only 3.5% of true germline pathogenic variants (50/1492 variants)

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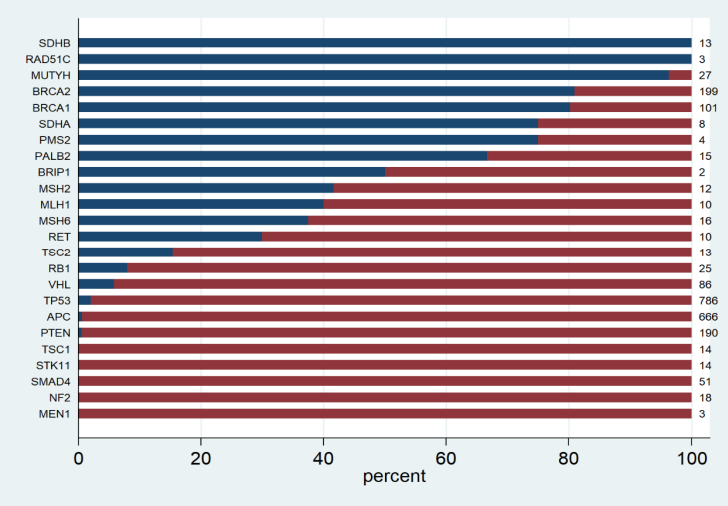
Germline vs. Somatic frequencies in 17,000 IMPACT cases



Red=somatic

Blue-= germline

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Mandelker et al. Annals of oncology 2019

Recommendations for triggering of laboratory confirmation in a germline sample

	Pan-Tumor		On-Tumor
All Ages	BRCA1	RAD51C	FLCN
	BRCA2	RAD51D	FH
	BRIP1	RET	BAP1
	MLH1	SDHA	POLE
	MSH2	SDHAF2	CDKN2A
	MSH6	SDHB	
	MUTYH	SDHC	
	PALB2	SDHD	
	PMS2	TSC2	
	VHL		
Age<30 only	RB1		TP53
	APC		NF1

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Mandelker et al. Annals of oncology 2019

What proportion of germline pathogenic variants are not detected by tumor sequencing?

- Reasons for inability to detect germline pathogenic variants via tumor sequencing:
  - High homology
  - Intronic variants
  - Repetitive element insertions (Alu)
  - Exon level copy number variants

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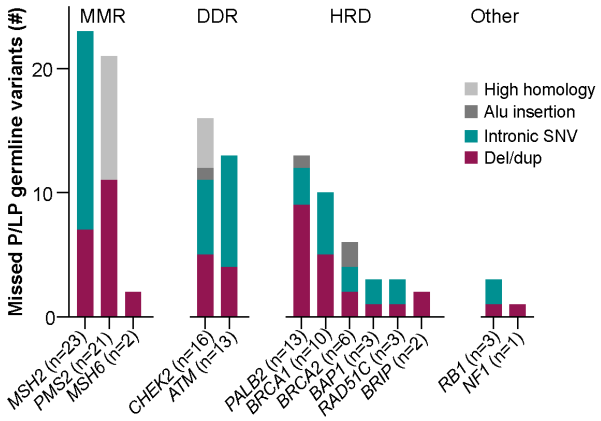
Genomic context for variants not detected by tumor sequencing



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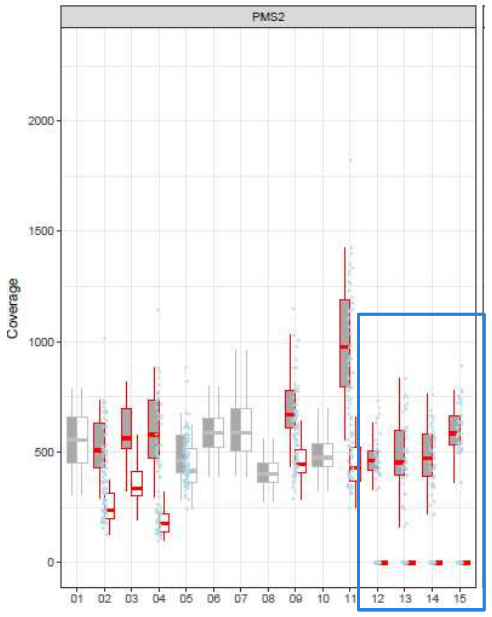
Standard quality metrics do not allow for detection of variants in high homology regions



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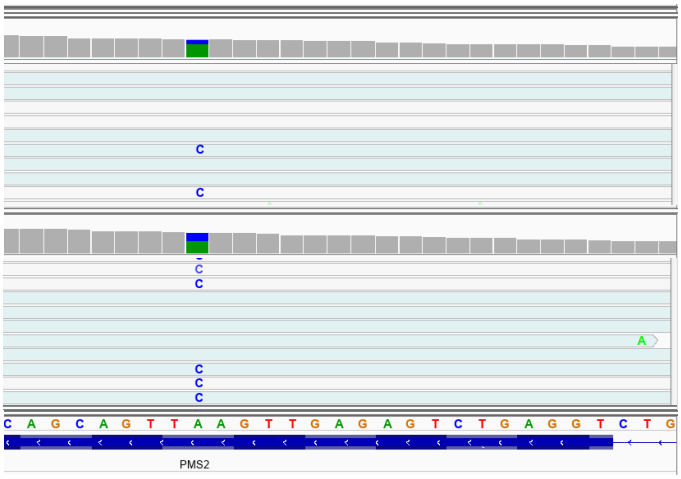
Variants in regions with high homology will have low mapping quality



Blood

Tumor

PMS2 c.2192T>G p.Leu731* pathogenic variant in exon 13 only detected in low mapping quality reads.

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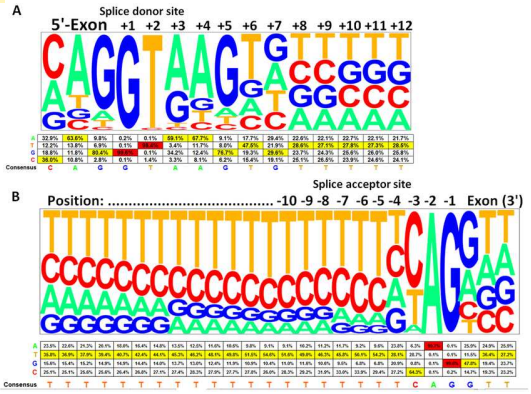
Intronic Variants



Example: **MSH2 c.942+3A>T**: Recurrent pathogenic germline variant that causes skipping of MSH2 exon 5

In MSK cohort 16/108 (15%) MSH2 germline pathogenic variants are this MSH2 c.942+3A>T

Because tumor sequencing only reports +/-1 and 2, this variant would not be reported on tumor sequencing reports.

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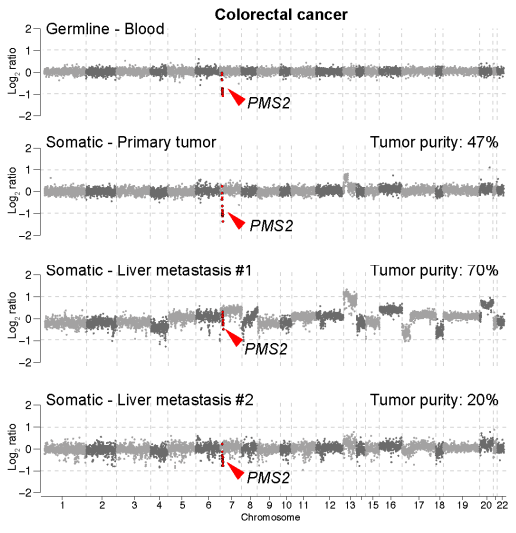
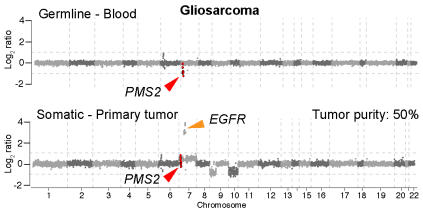
Germline CNV detected in tumor sample affected by sample quality and somatic copy number changes





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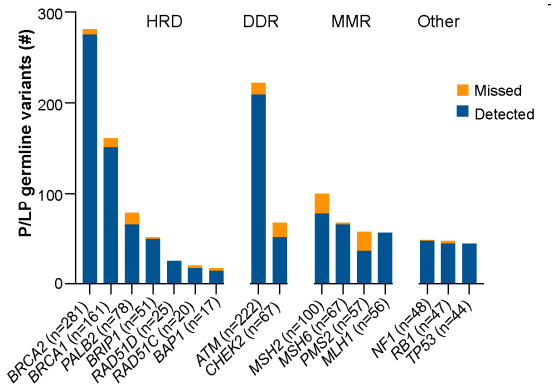
Variants not detected by tumor sequencing by biological pathway



For 16 cancer susceptibility genes tested, 10.2% of pathogenic germline variants were not detected

Variants not detected: 7.3% 12.8% 16.4%

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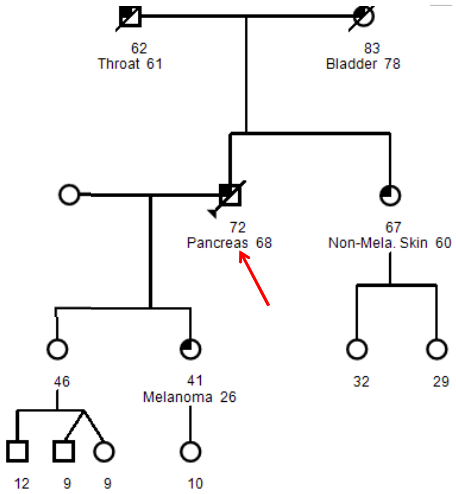
Case Presentation



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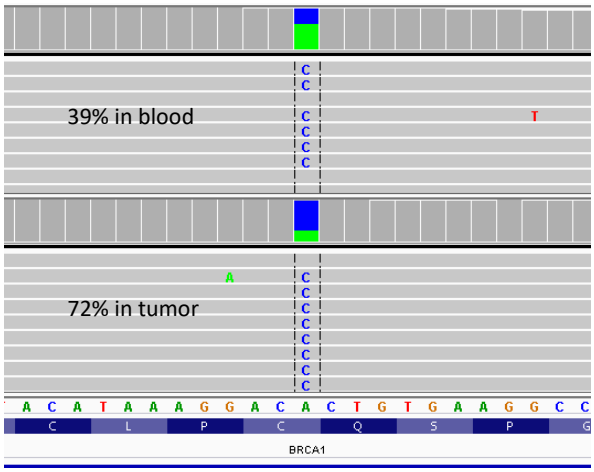
Having sequencing traces of tumor and normal allow for evaluation of loss of heterozygosity



Pathogenic BRCA1 c.181T>G (p.Cys61Gly) variant detected

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Cancer Risk in Carriers of Germline Mutations in BRCA1 and BRCA2

Risk of Cancer in Individuals Wth a BRCA1 or BRCA2 Mutation			
Cancer Type	General Population (No Mutation)	Individuals With Mutation	
		BRCA1	BRCA2
Breast	12%	50-80%	40-70%
Ovarian	1-2%	24-40%	11-18%
Male Breast	0.10%	1-2%	5-10%
Prostate	15% (N. Europe Origin)	up to 30%	up to 39%
	18% (African American)		
Pancreatic	0.50%	1-3%	2-7%

Petrucelli et al 2013

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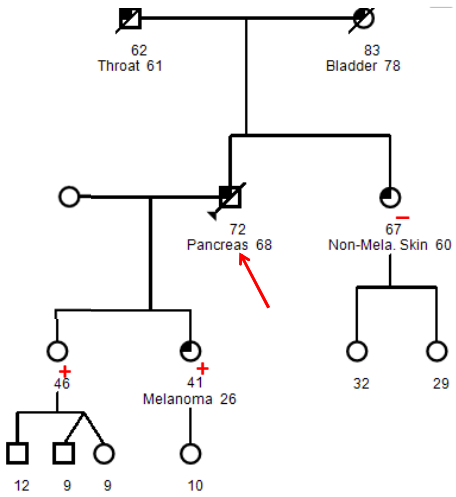
Follow-up



Both of the proband's daughter's tested positive for the pathogenic BRCA1 variant. Both have undergone prophylactic oophorectomies, and are undergoing breast cancer screenings.

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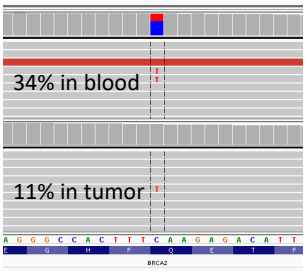
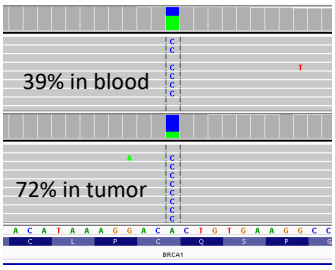
Not all variants in blood are germline in origin

- 84 yo man with pancreatic cancer at age 82 and a history of melanoma at age 58.
- BRCA2 c.9466C>T p.Gln3156* pathogenic variant found at 34 % VF in blood





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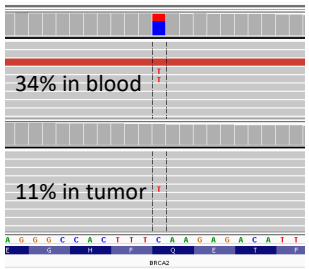

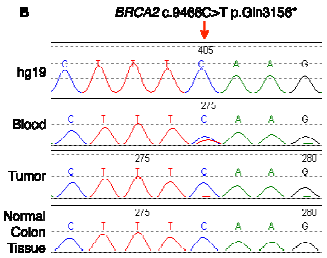

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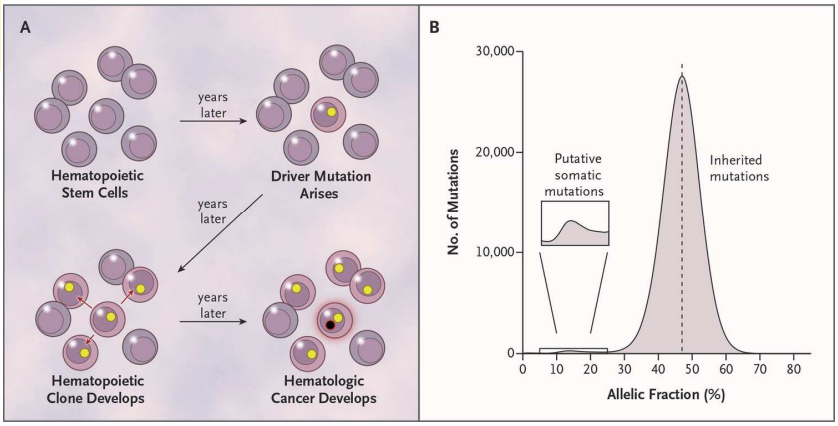
Clonal Hematopoiesis



2.4% (407/17,000) of cancer patients in our cohort had clonal hematopiesis variant in their blood at a germline variant frequency (>25%).

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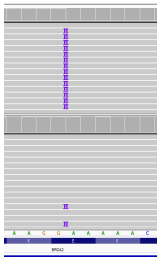
Genovese et al NEJM 2014

63 year old female diagnosed with ovarian cancer at age 51, currently with metastatic disease. Prior negative genetic testing

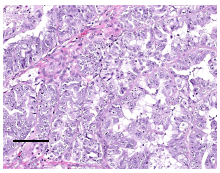
BRCA2 p.698Nfs*33

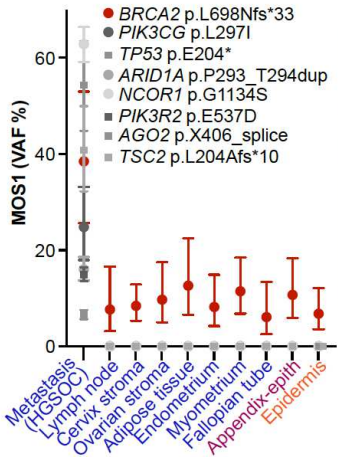
Tumor VAF=47%

Blood VAF=8%

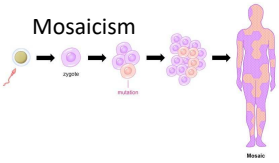


HGSOC





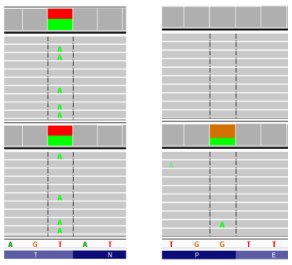
Mosaicism



5 variant possibilities distinguished by tumor normal sequencing

Blood

Tumor



Germline Somatic

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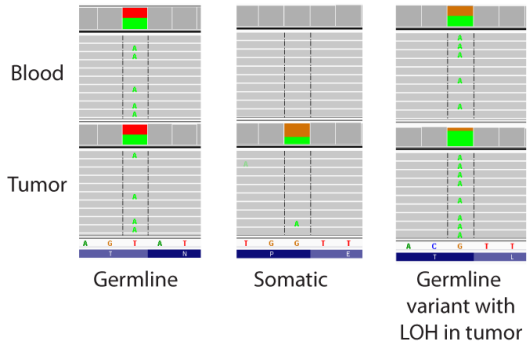
5 variant possibilities distinguished by tumor normal sequencing



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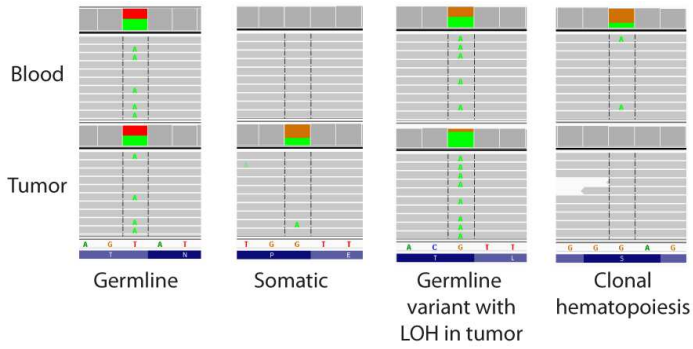

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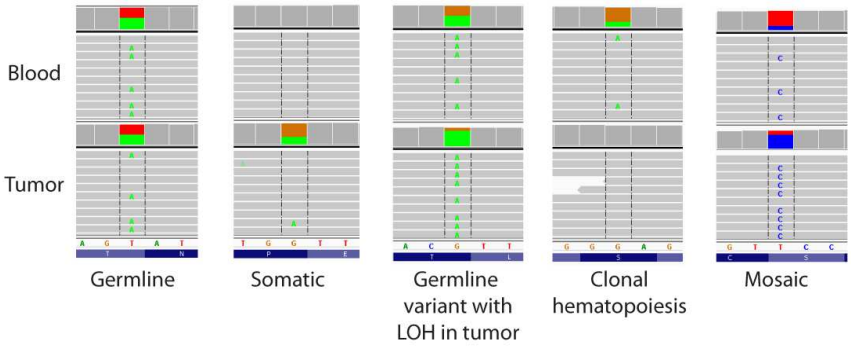

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Conclusions

- While detecting germline pathogenic variants in tumor sequencing is largely possible, it is substandard compared to clinical germline testing.
- While barriers such as cost and collecting two samples remain for tumor/normal sequencing, this methodology represents an efficient method to simultaneously detect somatic alterations and identify hereditary cancer predispositions.

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