Germline Variant Calling in Formalin-fixed Paraffin-embedded Tumours

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Overview

Overview

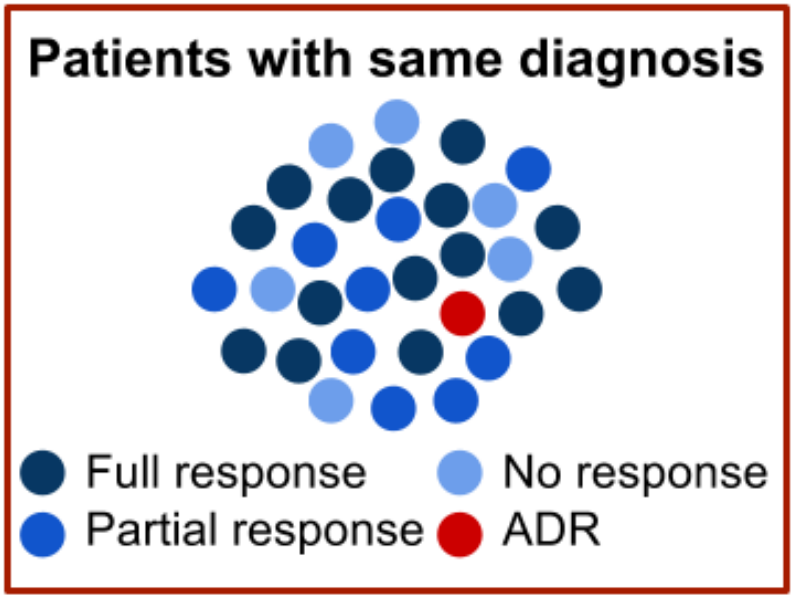
Germline variants have important clinical implications

Cancer Predisposition

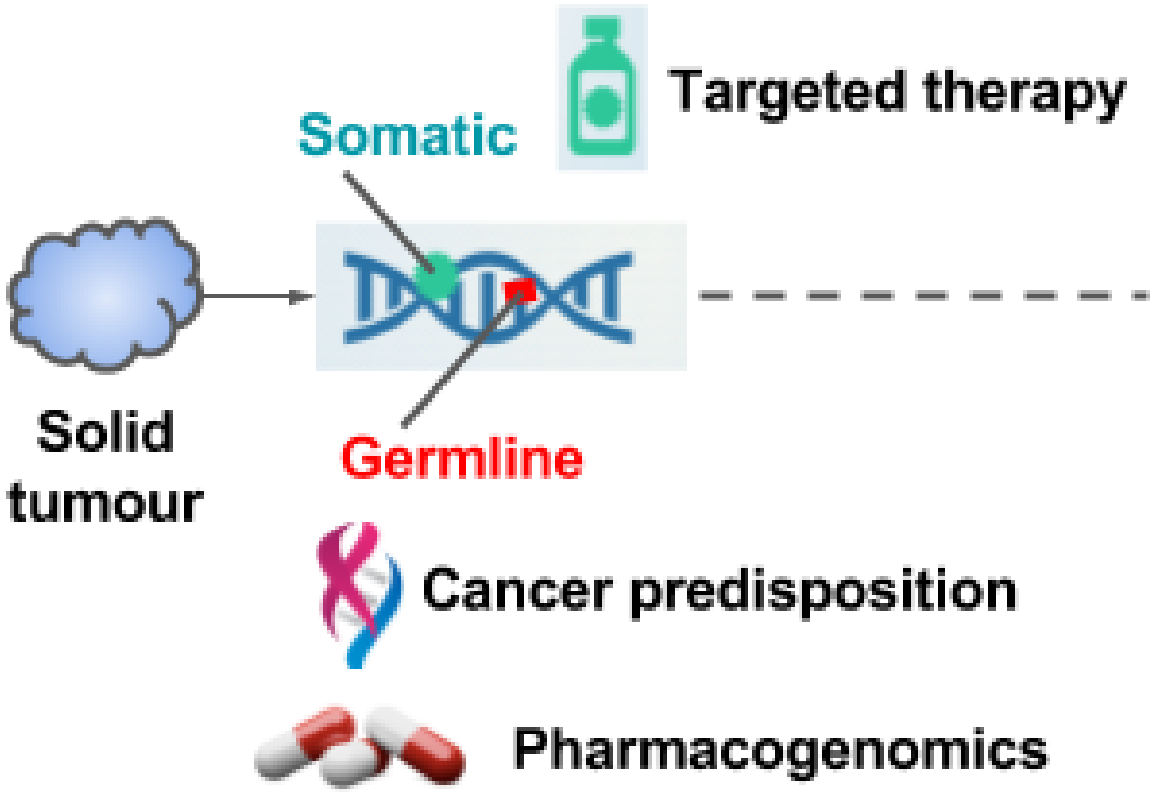
- Preventive measures
- Sibling testing

Pharmacogenomics

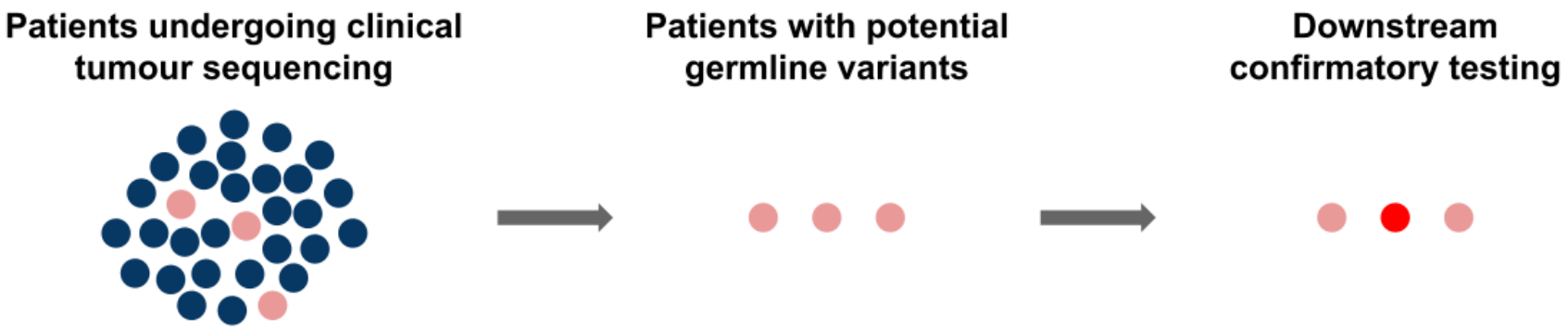
- Treatment tolerance
- Adverse drug reaction (ADR)



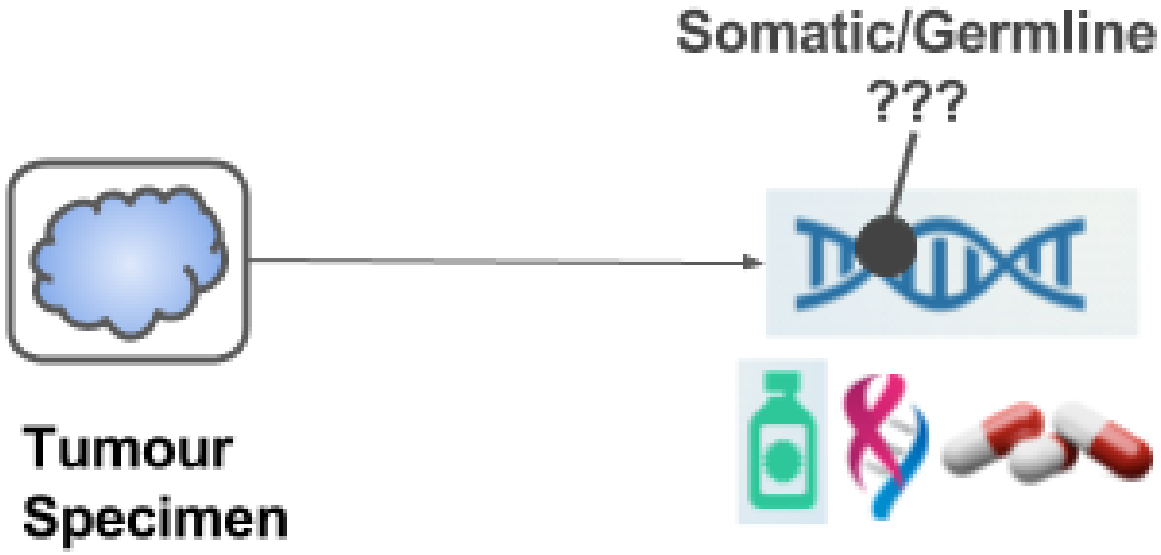
The tumour genome contains germline information



Clinical tumour sequencing could be a practical, cost-effective approach to provide germline testing



Challenge: Distinguishing between germline and somatic variants in the tumour genome



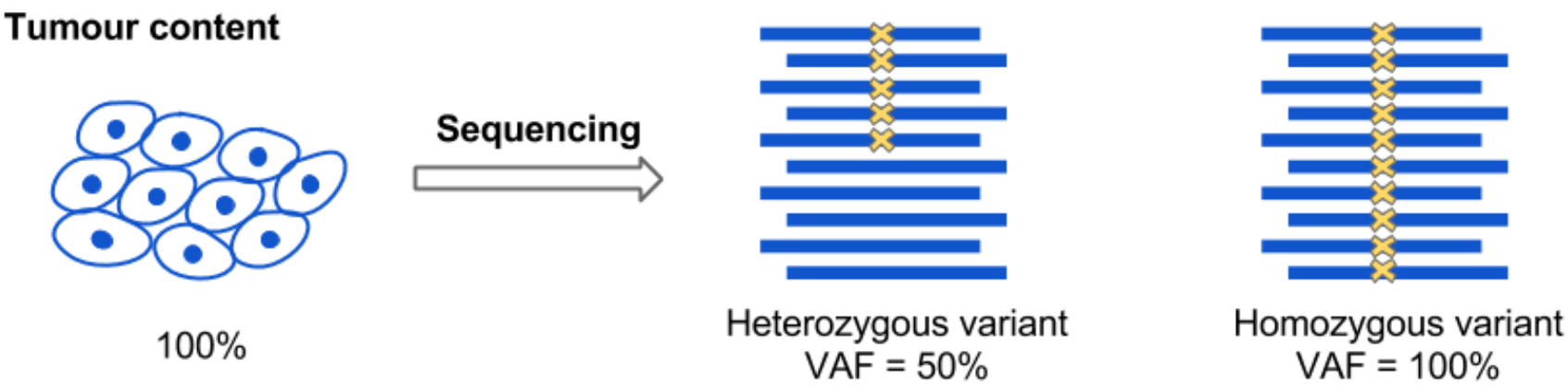
Overview

Research question

Can we accurately identify germline variants in tumour genomes?

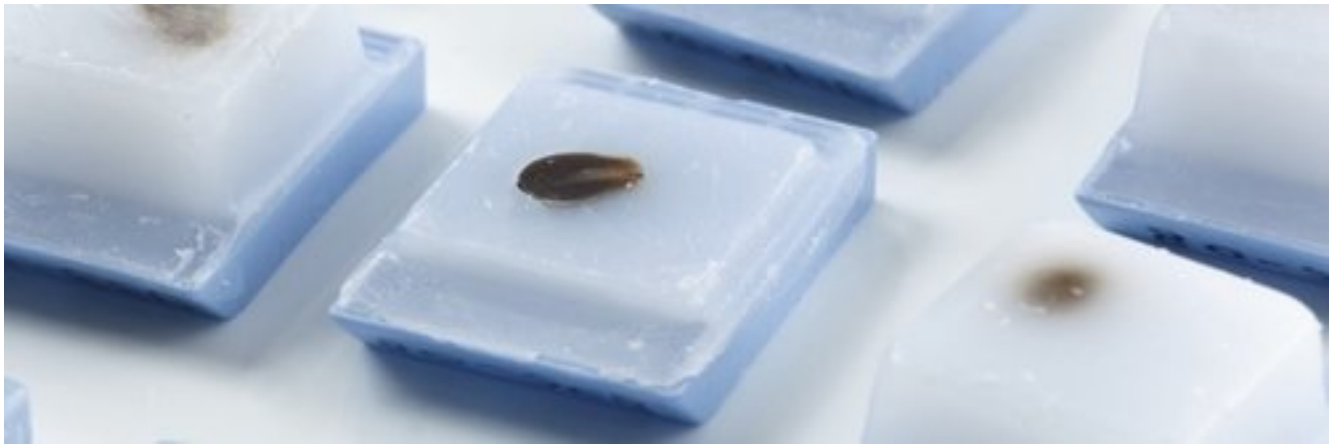
Overview

Variant allele frequency (VAF)



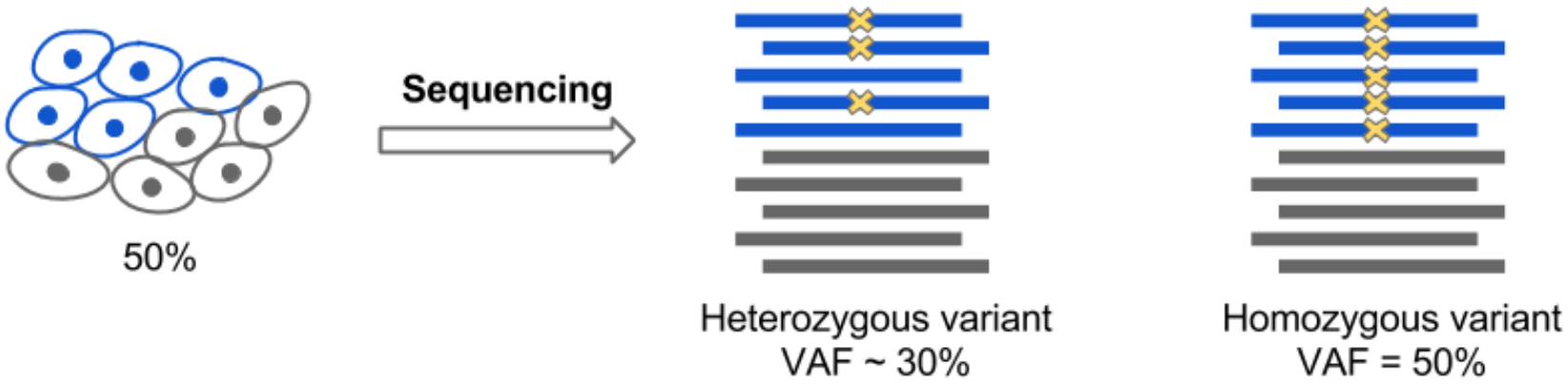
VAF in tumour specimens can deviate from diploid zygosity

DNA damage induced by formalin (e.g. fragmentation and sequence artifacts)



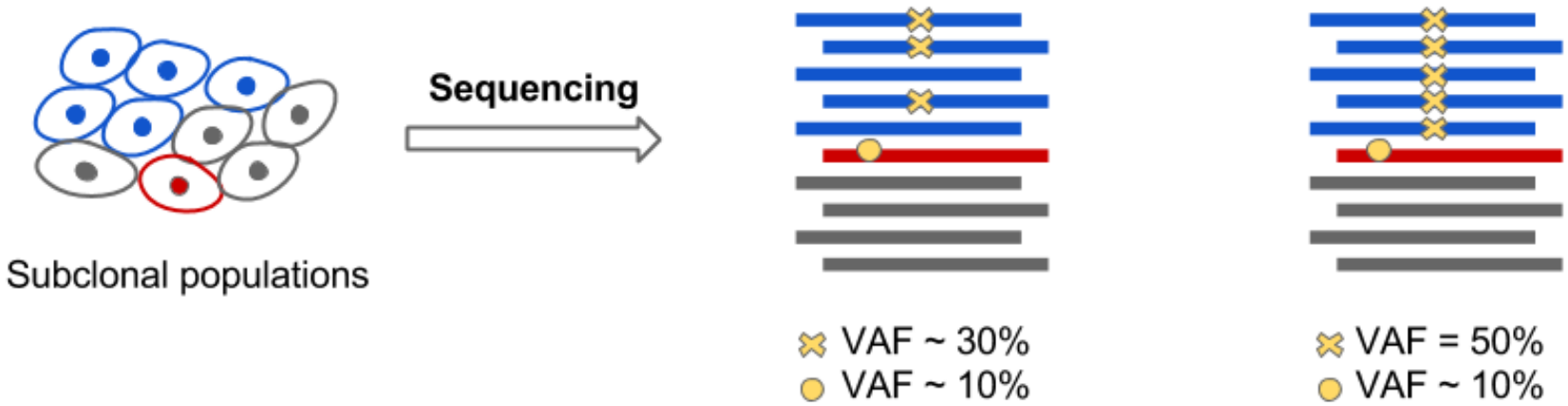
Somatic VAF in tumour specimens can deviate from diploid zygosity

Mixture of tumour and normal cells

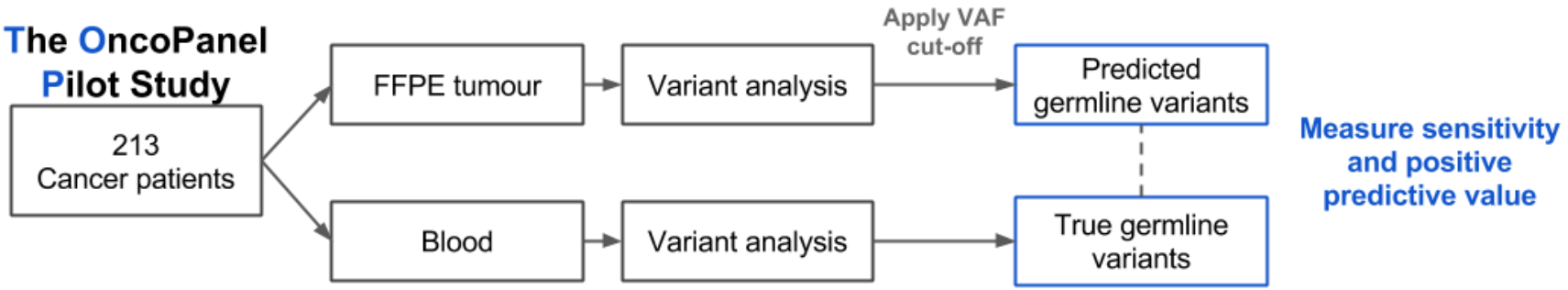


Somatic VAF in tumour specimens can deviate from diploid zygosity

Tumour heterogeneity



Study Design



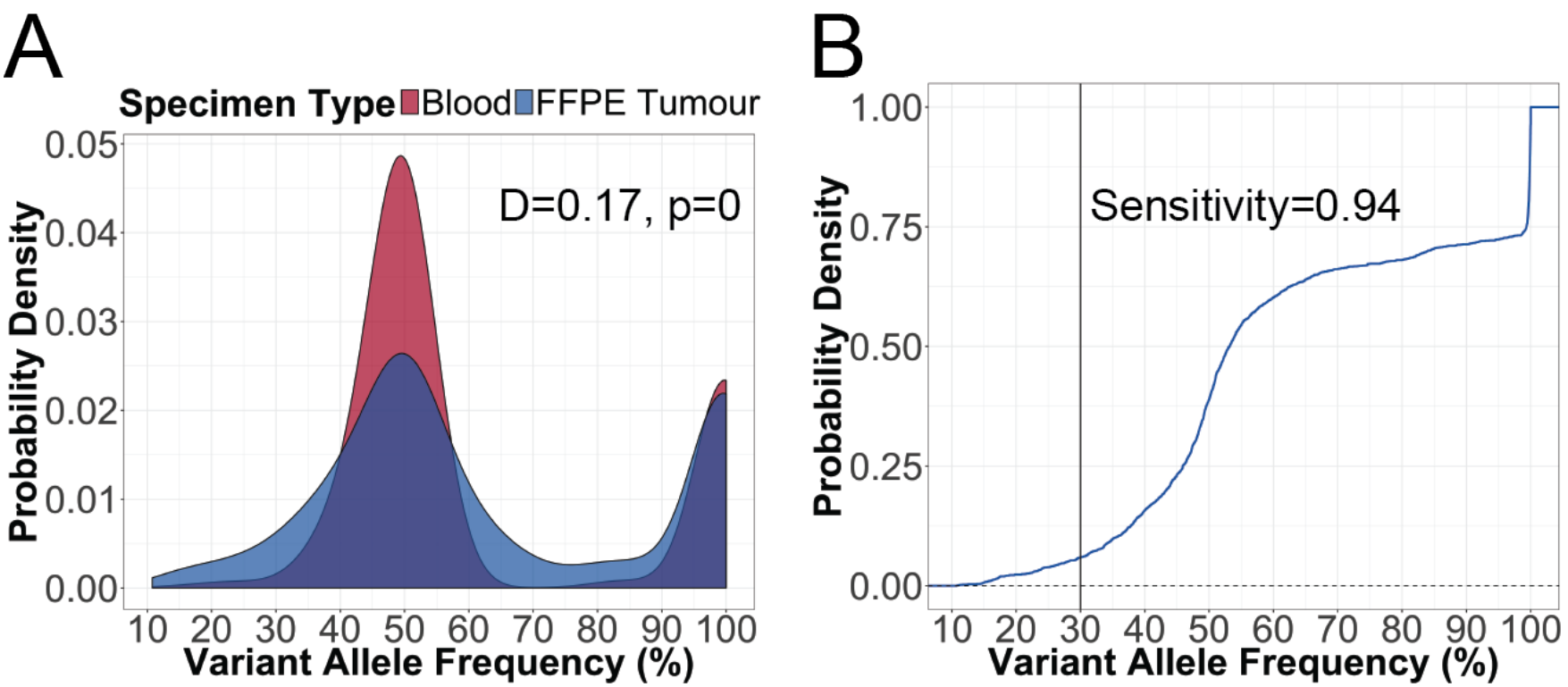
Detection in matched blood	Predicted variant status: Germline	Predicted variant status: Somatic
Present	True positive	False negative
Absent	False positive	True negative

Sensitivity = TP / (TP + FN)

PPV = TP / (TP + FP)

Overview

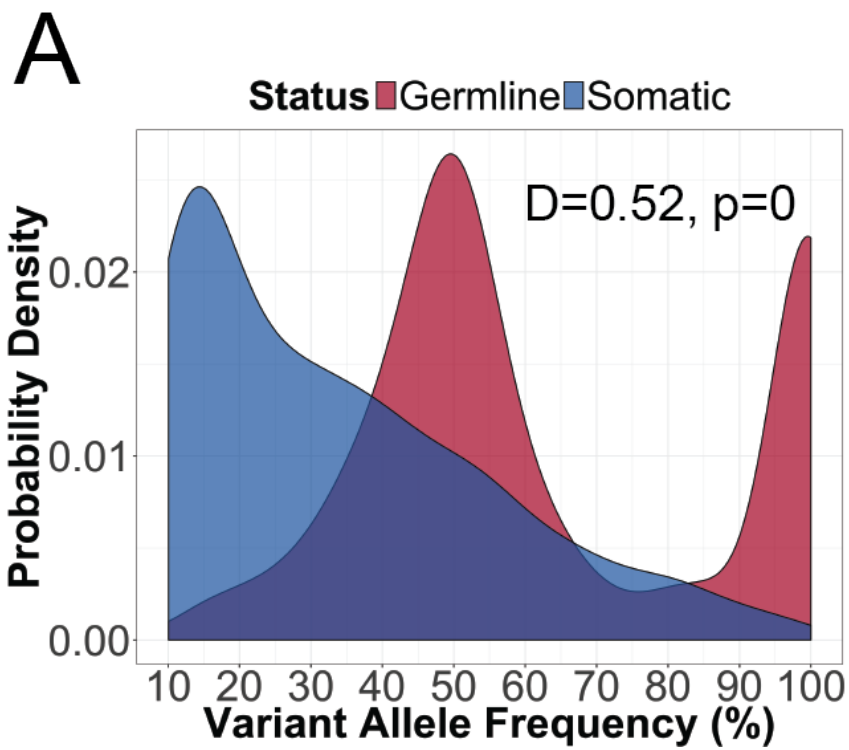
VAF of germline variants in blood and FFPE tumours

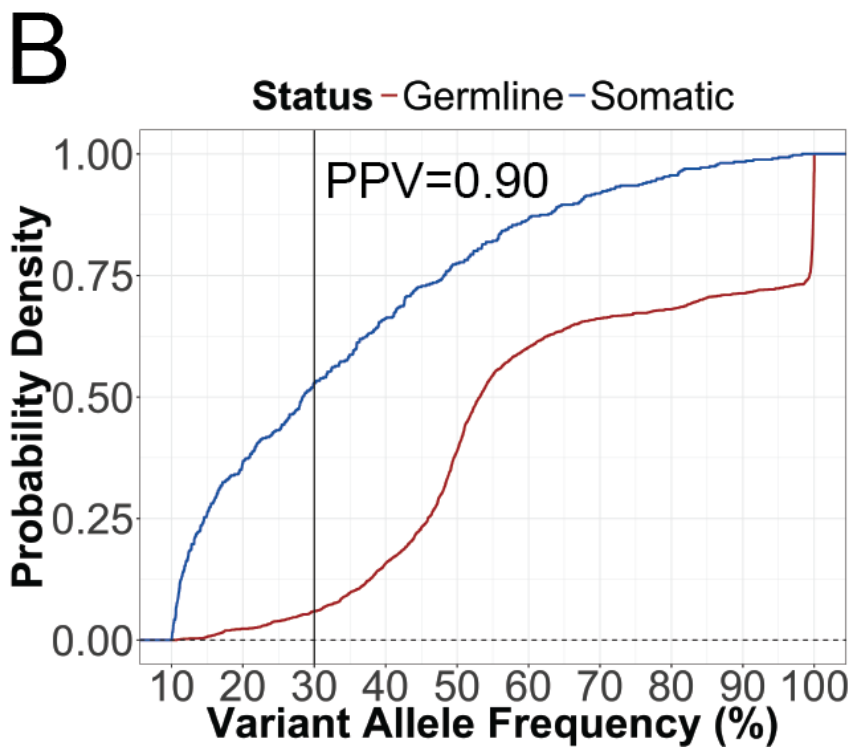


Sensitivity in detection of germline variants at different VAF thresholds

VAF (%)	False Negative	True Positive	Sensitivity	95% CI
10	0	1981	1.0	1.0–1.0
15	13	1968	0.99	0.99-1.0
20	46	1935	0.98	0.97–0.98
25	77	1904	0.96	0.95–0.97
30	117	1864	0.94	0.93–0.95
35	192	1789	0.90	0.89–0.92
40	313	1668	0.84	0.83–0.86
45	458	1523	0.77	0.75–0.79

VAF of germline and somatic variants in FFPE tumour





High positive predictive value can be achieved for referral of germline variants to downstream confirmatory testing

VAF (%)	False Positive	True Positive	Total Calls	PPV	95% CI
10	431	1981	2412	0.82	0.81–0.84
15	319	1968	2287	0.86	0.85–0.87
20	273	1935	2208	0.88	0.86–0.89
25	245	1904	2149	0.89	0.87–0.90
30	203	1864	2067	0.90	0.89–0.91
35	178	1789	1967	0.91	0.90–0.92
40	146	1668	1814	0.92	0.91–0.93
45	118	1523	1641	0.93	0.91–0.94

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Conclusions

1. A VAF approach demonstrates high sensitivity and precision at separating between germline and somatic variants.

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2. At 30% VAF threshold, sensitivity for detection of germline variants in FFPE tumour is 0.94 and the positive predictive value for referral to downstream confirmatory testing is 0.90.

Conclusions

1. A VAF approach demonstrates high sensitivity and precision at separating between germline and somatic variants.
2. At 30% VAF threshold, sensitivity for detection of germline variants in FFPE tumour is 0.94 and the positive predictive value for referral to downstream confirmatory testing is 0.90.
3. Germline variants could be accurately identified in FFPE tumour sequencing.

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