Shifting from DNA-Based to RNA-Informed Somatic Mutation Detection: A Systematic Benchmark Across Different Tumor Types

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1. Context

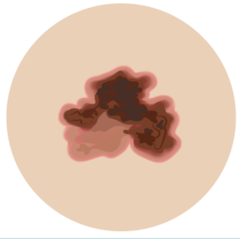
Benefits of tumor-only RNA variant calling

- cost-effective
- **actionable mutations**
- multi-omics approach
- no need for matched normal

Diffuse Large B-Cell Lymphoma (DLBCL)

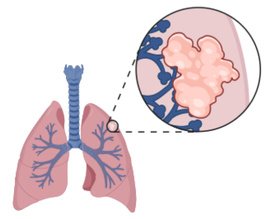
- most common subtype of **Non-Hodgkin Lymphoma**
- most prevalent lymphoid malignancy in adults
- **150.000** new diagnoses annually worldwide

Skin Cutaneous Melanoma (SKCM)



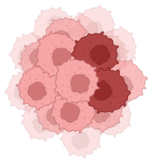
- most severe type of **skin cancer** but representing only 1 % of all types
- responsible for 80 % of skin cancer related deaths
- **325.000** new diagnoses annually worldwide

Lung Adenocarcinoma (LUAD)



- most common histological subtype of **lung cancer**
- 40 % of all lung cancer types
- **542.000 & 717.000** new diagnoses anually worldwide (male and female respectively)

What makes mutation calling in RNA "easier"?



Tumor purity

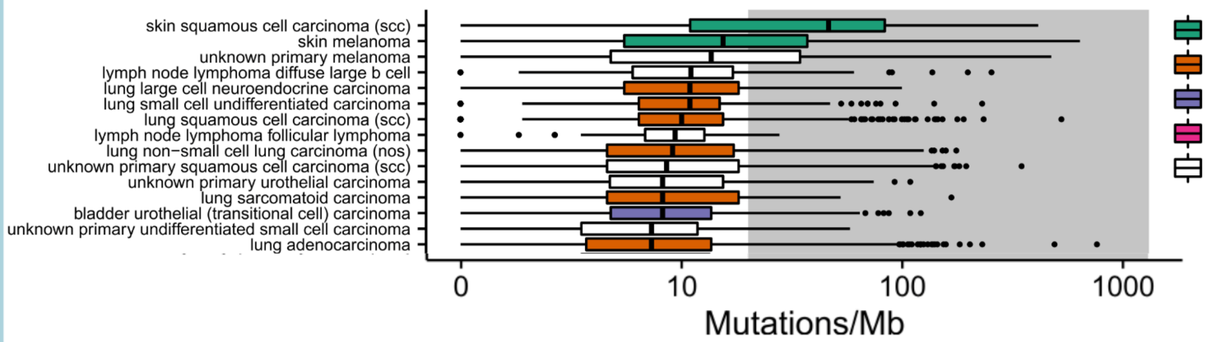
Tumor vs normal DNA

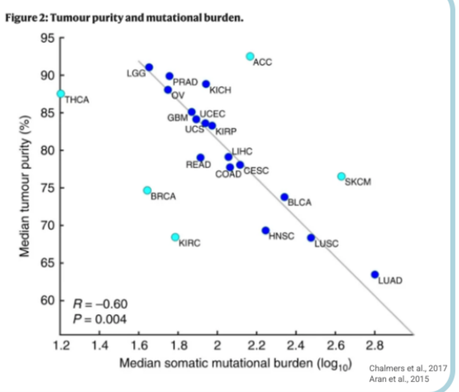
Variant Allele Frequency (VAF)

$$\frac{\text{Variant reads}}{\text{Total reads}} * 100$$

Tumor mutational burden (TMB)

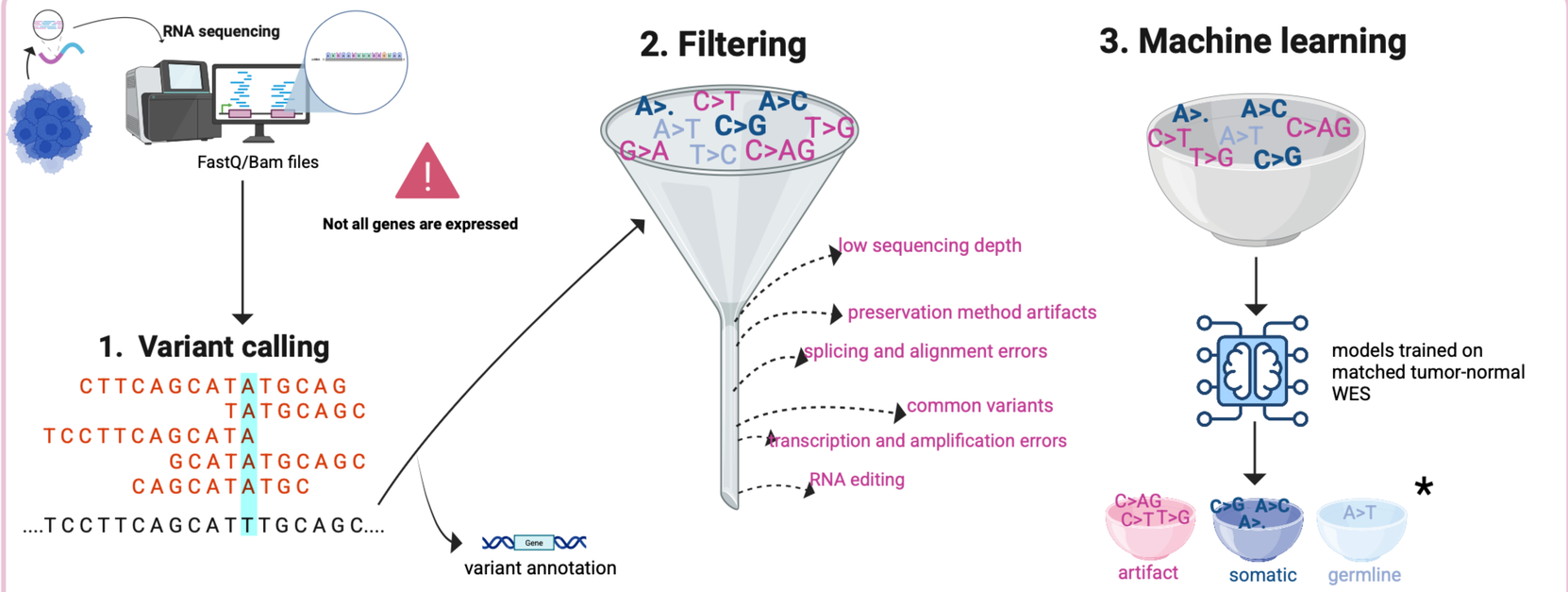
Total number of non-silent somatic mutations within a cancer's cell genome

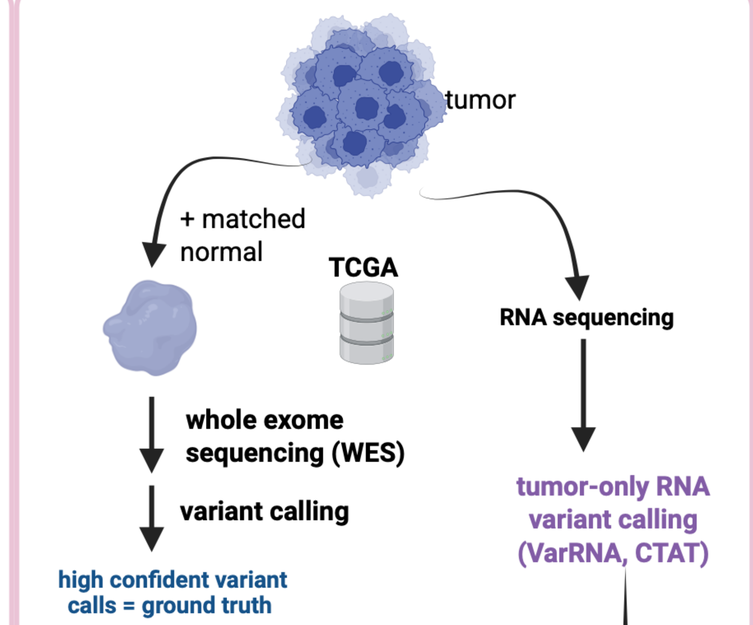




RNA variant calling in tumor-only samples enables the detection of expressed, functionally active alterations, facilitating precision medicine approaches

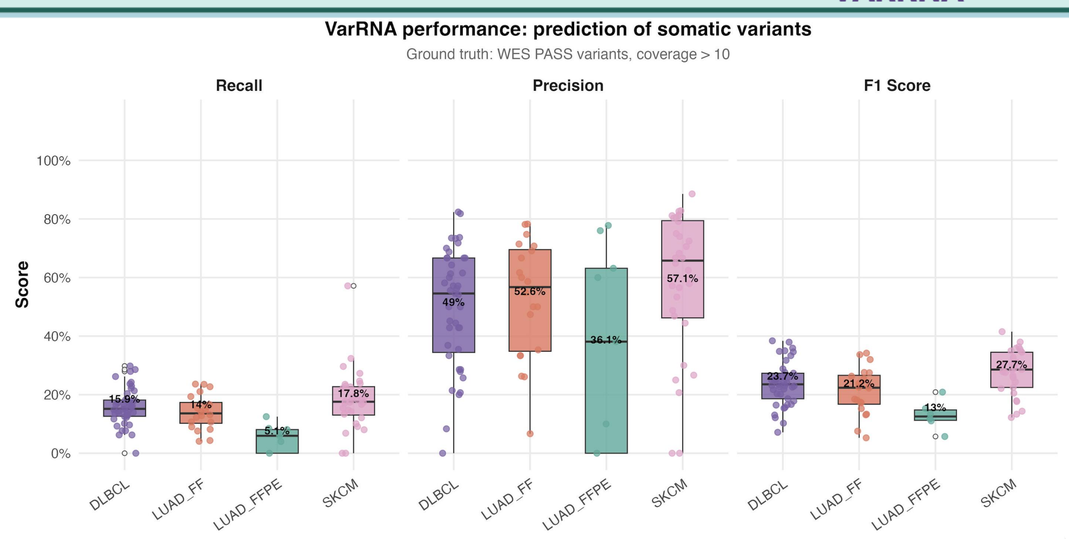
2. Methodology

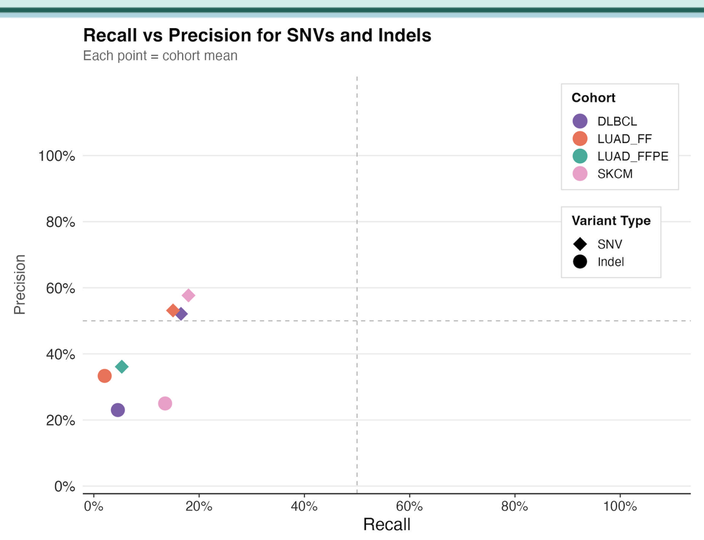


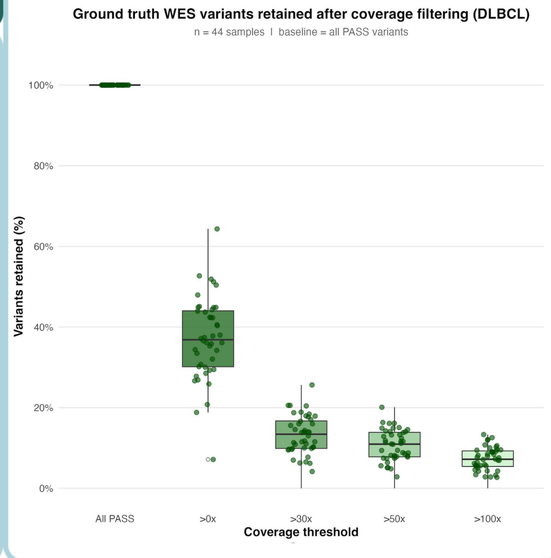


3. Results

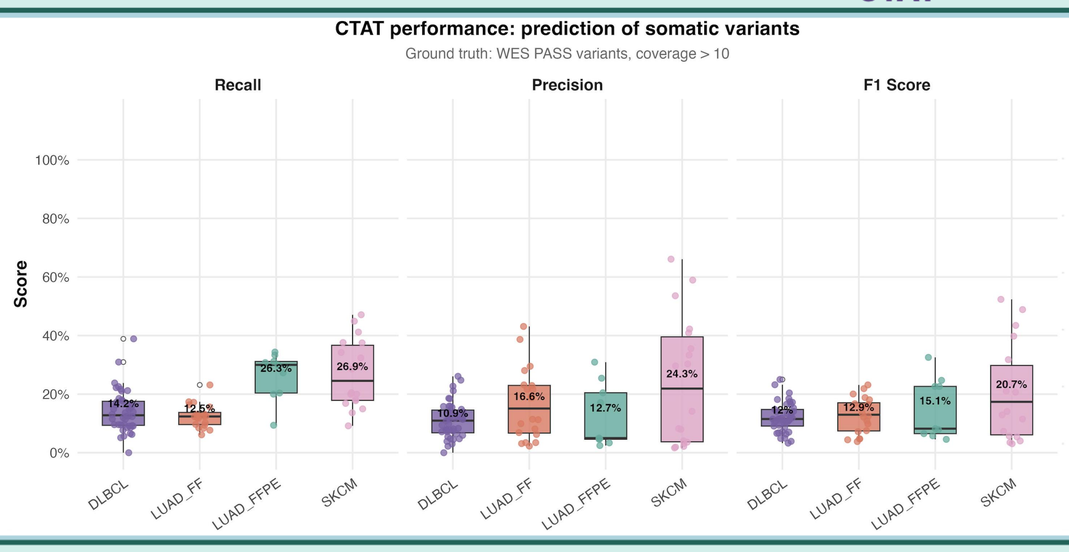
VARRNA

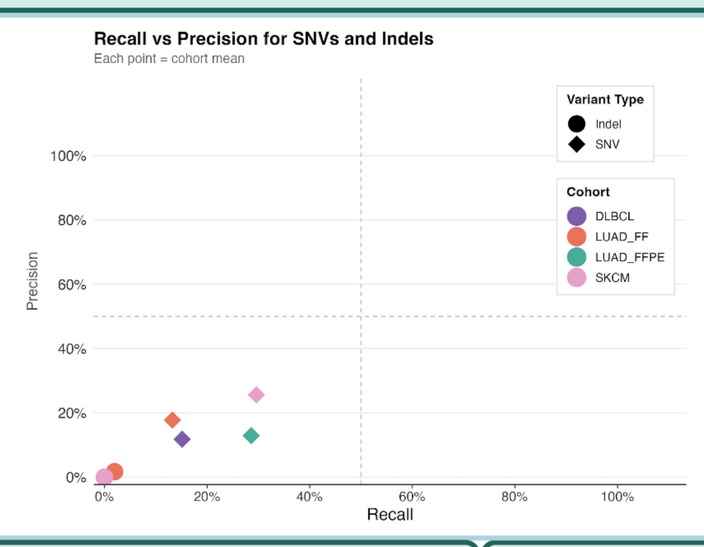


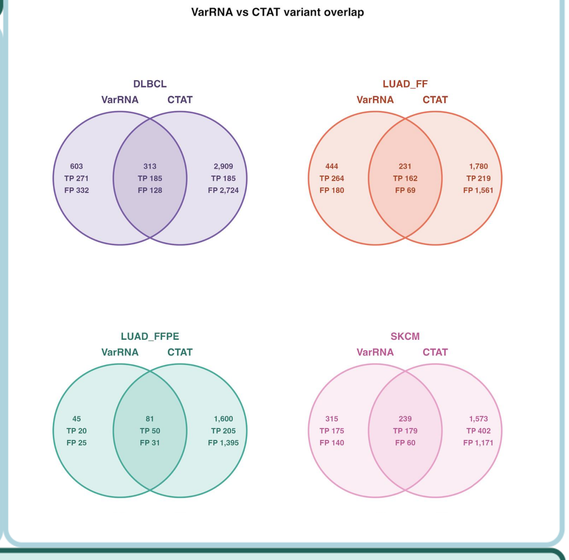




CTAT







4. Conclusions & future directions

- **VarRNA outperforms CTAT across cohorts**, with diminished performance in FFPE samples driven by reduced recall
- Both methods achieve **higher accuracy for SNVs than for indels**
- **FFPE samples show overall reduced performance**, likely driven by artefacts such as cytosine deamination
- **Limited concordance between variant calling tools highlights substantial inter-tool variability in detected mutations**

- **Optimal VarRNA performance requires cohort-specific model training** to account for heterogeneity in cancer types, preservation methods, library preparation, and sequencing technologies
- **A third, independent variant calling pipeline is required to enable robust benchmarking**

5. References

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