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Algorithmic genomic alteration filtering and circulating tumor DNA (ctDNA) quantification in serial liquid biopsy (LBx) from patients with prostate cancer

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BACKGROUND

Several approaches exist to quantify ctDNA in LBx, with many using genomic alteration variant allele frequencies (VAFs) as a proxy for ctDNA tumor fraction (TF). Genomic alterations from non-tumor sources such as **clonal hematopoiesis** and **germline** can confound TF estimates.

We evaluate the contribution of algorithmic filtering of non-tumor genomic alterations to ctDNA quantification over time and the impact of **clonal hematopoiesis** on detection of targetable alterations.

MATERIALS AND METHODS

- Serial real-world LBx ordered during the course of clinical care from 337 patients with prostate cancer were analyzed. ctDNA TF on FoundationOne®Liquid CDx (F1LCDx) was quantified by aneuploidy and VAF of genomic alterations as previously described (PMID: 38526394; Rolfo et al, CCR 2024).
- Patients with tissue-based comprehensive genomic profiling results in our data set were excluded to avoid overlap with future algorithm validation.
- A machine learning algorithm, trained and validated using cell free DNA from plasma and DNA from buffy coat sequenced with F1LCDx, was used for Variant Origin Prediction (VOP), i.e., **germline**, **clonal hematopoiesis**, or **tumor somatic** (probabilities sum to 100%). Probability cutoffs were selected from crossfold validation leveraging sequencing results from paired buffy coats such that positive predictive value for **tumor somatic**/**germline** predictions was 99% and 95% for **clonal hematopoiesis** predictions. Variants which did not meet at least one cutoff were excluded. For variants detected across serial liquid biopsy, concordance of VOP was assessed. Structural rearrangements detected via liquid biopsy were not evaluated for VOP.
- Maximum VAF (maxVAF) was iteratively calculated for each specimen,

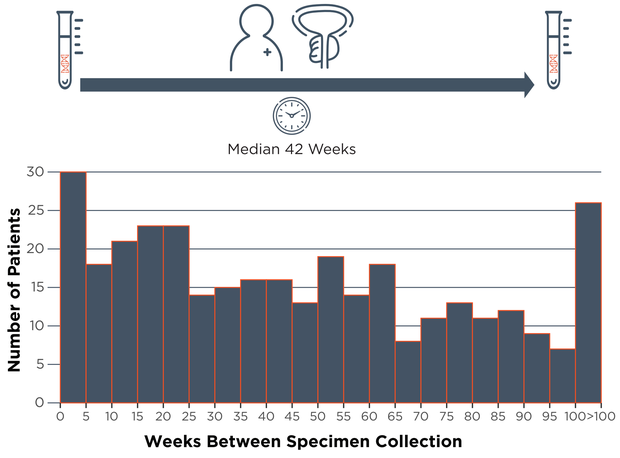

FIGURE 1: Timing of serial liquid biopsy collection. Median time between liquid biopsies was 42 weeks (IQR: 19 – 72). The median patient age at first liquid biopsy was 72.

RESULTS – VARIANT ORIGIN PREDICTIONS

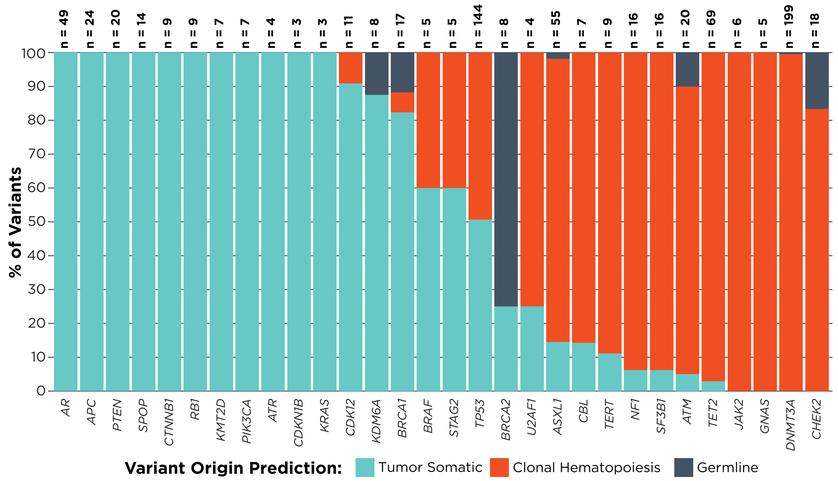

FIGURE 2: Variant origin predictions for known and likely pathogenic coding variants from top 30 most commonly altered genes in patients first liquid biopsy. Genes are sorted in order of frequency of **tumor somatic** mutations then total number of variants.

RESULTS – VARIANT ALLELE FREQUECY BY VARIANT ORIGIN PREDICTION

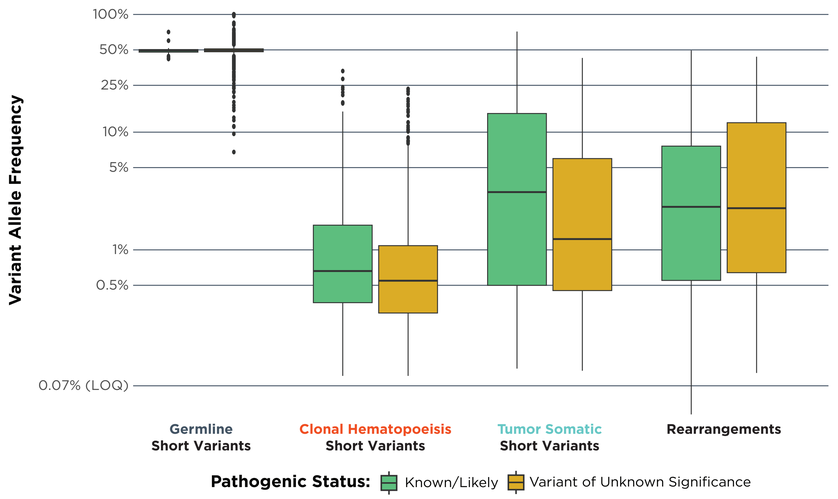

FIGURE 3: Distribution of variant allele frequencies by variant origin prediction grouped by pathogenic status. Variant origin predictions were made for short variants only. The limit of quantification (LOQ) is 0.07% variant allele frequency for short variants.

RESULTS – MAXIMIUM VAF ACCURACY IMPROVES WITH OPTIMIZED VARIANT FILTERING

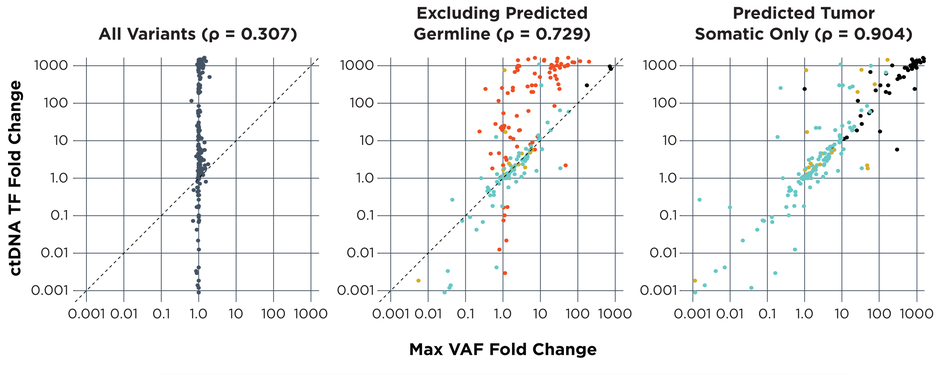

FIGURE 4: Correlation between ΔmaxVAF and ΔctDNA TF increased with each additional variant filter. When including germline variants, 100% of pairs used a germline variant for maxVAF in the first LBx. When only CH and tumor somatic variants were used, 43% of cases used a CH variant for maxVAF in the first LBx.

RESULTS – LONGITUDINAL VARIANT ORIGIN PREDICTION CONCORDANCE

Tumor Somatic

2nd:	1st: S	1st: G/C
S	423	64
G/C	39	3,980

PPA: 91.6%
OPA: 97.7%

Clonal Hematopoeisis

2nd:	1st: C	1st: G/S
C	751	30
G/S	53	3,672

PPA: 93.4%
OPA: 98.2%

Germline

2nd:	1st: G	1st: S/C
G	3,226	12
S/C	14	1,254

PPA: 99.7%
OPA: 99.4%

FIGURE 5: All genomic alterations classified as germline (n = 3,240) in the first specimen were detected in the second specimen. For genomic alterations detected in both specimens, PPA was 99.7% for germline (3,226/3,240) and 93.4% (751/804) for clonal hematopoeisis classifications. **S** = **Tumor Somatic**; **C** = **Clonal Hematopoesis**; **G** = **Germline**

CONCLUSIONS

- We demonstrate filtering of germline and clonal hematopoeisis is critical to quantify ctDNA change over time and that an algorithmic approach in the absence of paired buffy coat sequencing is feasible.
- Additionally, algorithmic filtering identifies high rates of CH in a subset of actionable genomic alterations in prostate cancer, highlighting the need to identify CH to avoid incorrect therapy selection.