Equal-depth sequencing of white blood cells (wbc) and plasma from prostate cancer (pca) liquid biopsies (lbx) and association with clonal hematopoiesis (ch) confounders in clinically relevant genes.

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Abstract

Background: CH results from fitness-enhancing mutations in hematopoietic stem cells that accumulate with age. Deep sequencing of LBx, a standard of care for guiding therapy selection in advanced PCa, can be confounded by detection of variants arising from both ctDNA and CH-derived DNA.

Objective: We report the prevalence of CH variants in PCa and other cancers and describe an algorithmic method for accurately distinguishing CH from tumor variants.

Methods: Plasma cell-free DNA from 1813 patients with cancer (n = 270 PCa) was sequenced using FoundationOneLiquid CDx (F1LCDx) in a CLIA lab setting. In parallel, DNA from buffy coats was extracted, sheared, and sequenced on F1LCDx to identical depth, to establish ground truth for CH variant identity. This cohort was used to train a machine learning variant origin prediction (VOP) model incorporating fragmentomics and other sequencing features to assign probabilities of origin (germline, tumor, or CH) to all short variants across the 300+ genes baited on the assay. Only pathogenic variants were considered.

Results: 1247/1813 (69%) of LBx detected ≥1 CH variant, including 209/270 (77%) of PCa LBx (mean 1.8 variants/sample). CH contributed confounding variants in clinically relevant genes including CHEK2, ATM, BRAF, TP53, and BRCA2 in 90/270 (33%) of PCa LBx.. Median VAF of CH variants in these genes was 0.3% in both LBx and WBC. . In the pan-cancer cohort, the VOP algorithm identified CH variants with 95.2% (2613/2745) sensitivity, 94.1% (3741/3975) specificity, and 91.8% (2613/2847) positive predictive value (PPV). For CH variants with VAF<1%, VOP had 95.0% (1622/1707) sensitivity, 1021/1169 (87.3%) specificity, and 91.6% (1622/1770) PPV.

Conclusions: Equal-depth DNA sequencing of plasma and matched WBC reveals higher CH prevalence in LBx than previously reported: 77% of PCa LBx with a CH variant, including 33% with a CH variant in clinically relevant genes. Many potentially actionable variants have low VAF in LBx, and filtering CH via shallower depth WBC sequencing than that of LBx risks leaving low VAF CH variants to be mistaken for tumor variants. Equal-depth WBC sequencing or an algorithmic method validated using such sequencing is required for accurate identification of low VAF CH confounders in LBx.

Do you have any conflicts of interest?

Yes, I have a conflict of interest.

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