

When your sample is **negative** for actionable findings, **ctDNA tumor fraction** is a must

True positive results stand out, but negative results are harder to interpret.

ALK AR BRAF BRCA1 BRCA2 EGFR
ESR1 FGFRI FGFRI2 FGFRI3 KRAS NTRK1
NTRK2 NTRK3 PIK3CA ROS1



Negative Result from a Liquid Biopsy Test
Without ctDNA tumor fraction

Is the blood sample negative for actionable variants?
Without ctDNA tumor fraction, a negative liquid biopsy result is unclear.

BIOMARKER FINDINGS

Blood Tumor Mutational Burden -
4 Muts/Mb

ctDNA tumor fraction
Low (< 1.0%)

Microsatellite status -
MSI-High Not Detected

THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Biomarker Findings section.

Low ctDNA Tumor Fraction. This result does not compromise confidence in any reported alterations. See Biomarker Finding Summary.

MSI-High not detected. No evidence of microsatellite instability in this sample (See Appendix section).

FoundationOne®Liquid CDx reports both ctDNA tumor fraction and variant allele frequencies (VAF) in the Professional Services section.

ctDNA Tumor Fraction* =

ctDNA

cfDNA

Reported on FoundationOne®Liquid CDx as <1% or numerical value if ≥1%.



Clonal hematopoiesis (CH) variants can cloud ctDNA assessment based on VAF alone.
VAF of potential germline and CH variants may overestimate VAF-based calculations.



- Foundation Medicine's **ctDNA tumor fraction** value accounts for **aneuploidy, variant allele frequency**, fragment length information, **clonal hematopoiesis predictions (CH)**, and known tumor-associated alterations.
- The combination of these components ensures a **more accurate determination** for consistency of the **amount of ctDNA** in a sample than VAF alone.³

Are your negative liquid biopsy results really negative?

When you have positive findings, VAF is helpful

Variant Allele Frequency (VAF)

VAF supports an understanding of how prevalent a particular variant is in a particular sample.

- **VAF can inform treatment selection** as a measure of tumor heterogeneity, but it's not ctDNA tumor fraction.^{3,4}
- Reported on FoundationOne®Liquid CDx as individual variant measurements.
- May be used to calculate median or mean VAF or maximum somatic allele frequency (MSAF).
- **VAF alone is not sufficient** to determine the **ctDNA level** in a sample.³

How does VAF appear on FoundationOne®Liquid CDx reports?

HISTORIC PATIENT FINDINGS (Genomic Findings)		VAF%
EGFR	● exon 19 deletion (E746_A750del)	8.2%
DNMT3A	● M880V	5.5%
GNAS	● R201C	0.29%
TP53	● R175H	5.9%

Table included with both ctDNA tumor fraction and Variant Allele Frequency within a FoundationOne®Liquid CDx report under Professional Services Section.

VAF % =

sequencing reads where the variant is found

the total number of sequencing reads at that location

Note: VAF is not a substitute for germline testing

FoundationOne®Liquid CDx is for prescription use only and is a qualitative next-generation sequencing based *in vitro* diagnostic test for advanced cancer patients with solid tumors. The test analyzes 324 genes utilizing circulating cell-free DNA and is FDA-approved to report short variants in 311 genes and as a companion diagnostic to identify patients who may benefit from treatment with specific therapies (listed in Table 1 of the Intended Use) in accordance with the approved therapeutic product labeling. Additional genomic findings may be reported and are not prescriptive or conclusive for labeled use of any specific therapeutic product. Use of the test does not guarantee a patient will be matched to a treatment. A negative result does not rule out the presence of an alteration. Patients being considered for eligibility for therapy based on detection of *NTRK1/2/3* and *ROS1* fusions should only be tested if tissue is unavailable. Patients who are negative for other companion diagnostic mutations should be reflexed to tumor tissue testing and mutation status confirmed using an FDA-approved tumor tissue test, if feasible. For the complete label, including companion diagnostic indications and complete risk information, please visit <http://www.FILCDxLabel.com>.

* Approximated

† Sensitivity is high for short variants and fusions when ctDNA tumor fraction is ≥1%. Copy number amplifications and deletions of exons or genes are detectable across a range of ctDNA tumor fraction values and reflexing to FoundationOne CDx may provide additional findings when these types of alterations report negative. (Refer to the FoundationOne Liquid CDx product label for additional information.)

ctDNA tumor fraction is reported as a laboratory professional service that is not reviewed or approved by the FDA. Patients who are negative for companion diagnostic mutations should be reflexed to tumor tissue testing and mutation status confirmed using an FDA-approved tumor tissue test, if feasible.

References

1. Chan HT, Nagayama S, Chin YM, et al. Clinical Significance of Clonal Hematopoiesis in the Interpretation of blood Liquid Biopsy. *Mol Onc.* 2020; 14(8): 1719-1730. <https://doi.org/10.1002/1878-0261.12727>.
2. Husain H, Pavlick DC, Fendler BJ, et al. Tumor Fraction Correlates with Detection of Actionable Variants Across >23,000 Circulating Tumor DNA Samples. *JCO Precis Oncol.* 2022 Oct;6:e2200261. doi: 10.1200/PO.22.00261.
3. Rolfo CD, Madison R, Pasquina LW. Utility of ctDNA Tumor Fraction to Inform Negative Liquid Biopsy (LBx) Results and Need for Tissue Reflex in Advanced Non-Small Cell Lung Cancer(aNSCLC). *J Clin Onc.* 2023; 41(16): 9076-9076. DOI: 10.1200/JCO.2023.41.16_suppl.9076.
4. Bielo LB, Trapani D, et al. Variant allele frequency: a decision-making tool in precision oncology? *Trends in Cancer* VOLUME 9, ISSUE 12, P1058-1068, DECEMBER 2023 doi: 10.1016/j.trecan.2023.08.011

