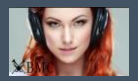
Liquid Biopsy in Clinical Practice: FAQs

October 12, 2022



M-ES-00006918

FOUNDATION MEDICINE

Liquid Biopsy

Examples of Frequently Asked Questions

- What are the clinical applications of liquid biopsies?
- Factors influencing amount of ctDNA in blood?
- When to draw a liquid biopsy?
- When to use liquid vs tissue biopsy?
- What is the concordance between liquid and tissue biopsy?
- How to interpret tumor fraction, blood TMB, VAF in liquid biopsies?
- What are the limitations of liquid biopsy?

VAF: Variant Allele Frequency
TMB: Tumor mutational burden
ctDNA: circulating tumor DNA

FOUNDATION MEDICINE

Session agenda

Liquid Biopsy FAQ

1. Review of FAQ on liquid biopsy
2. Panel discussion and open Q&A

FOUNDATION MEDICINE

Poll Question - first

What is the main applicability of Foundation Medicine's F1Liquid CDx test?

A. Early disease detection

B. Monitoring residual disease

C. Identification of potential targeted treatments in metastatic setting

D. Identification of acquired resistance variants to guide treatment decisions

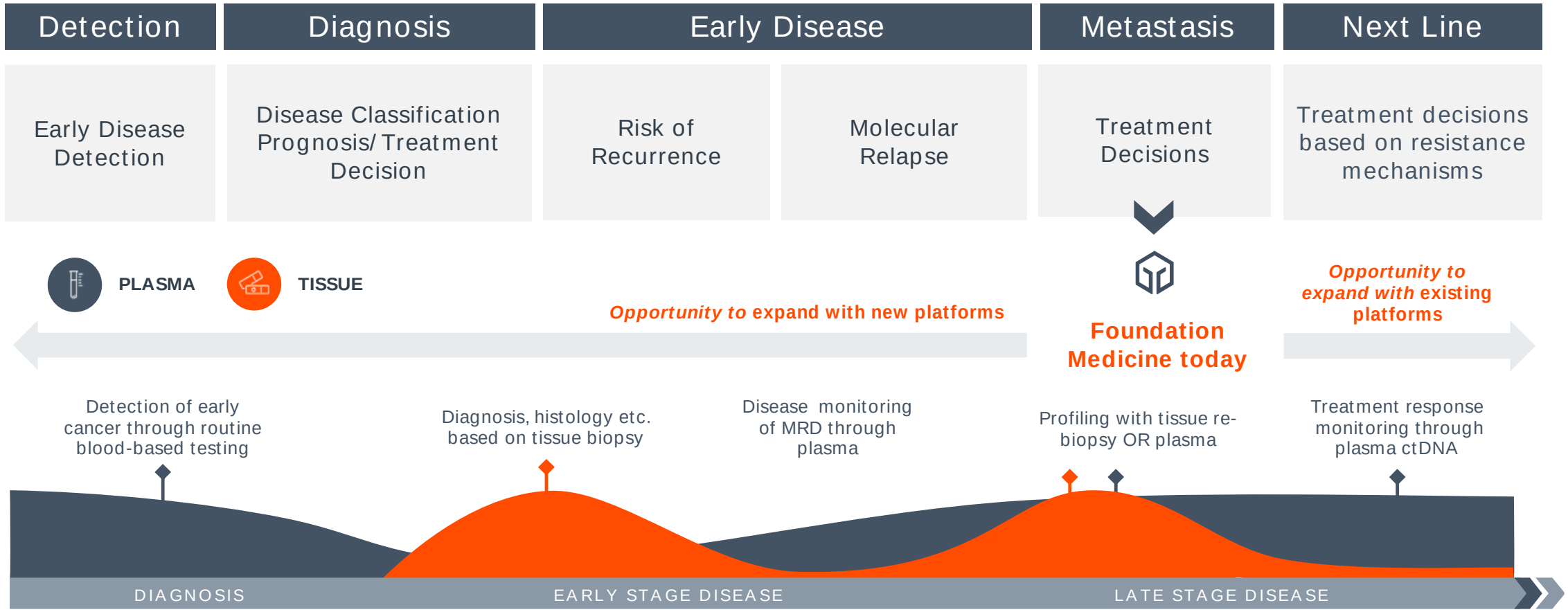
E. All of the above

F. C and D

G. A and B

FOUNDATION MEDICINE

Foundation Medicine's Role Across the Patient Journey



ctDNA = circulating tumor DNA, MRD = minimal residual disease.

FOUNDATION MEDICINE

Poll Question

What are the main factors impacting the level of ctDNA in the blood?

A. Tumor stage

B. Type of treatment prior to blood collection

C. Tumor burden

D. Tumor type

E. All of the above

FOUNDATION MEDICINE

When do I Choose Liquid Biopsy versus Tissue?

1

Patients in whom traditional biopsy is inaccessible or impractical

- Anatomically inaccessible/unacceptable risk[1]
- Resource-limited settings (e.g. leading to delayed biopsy)[2]
- Time sensitivity (acutely ill)[1]

2

Patients in whom traditional biopsy is insufficient

- Tissue exhausted by immunohistochemistry, PD-L1 or other single marker testing, hotspot testing, or smaller tumor-specific panels[1]
- Highly necrotic tumor with inadequate nucleated tumor cells[3]
- Low tumoral content in specimen[1]

3

Patients who have disease progression or relapse on targeted therapies[1]

- Detection of suspected resistance mutations
- To consider new therapy options including clinical trials

1. Rolfo C, et al. *J Thorac Oncol.* 2018;13(9):1248-1268; 2. Anderson B, et al. *Lancet Oncol.* 2011;12(4):387-398; 3. Sepulveda AR, et al. *J Clin Oncol.* 2017;35(13):1453-1486.

FOUNDATION MEDICINE

When do I Draw a Liquid Biopsy?

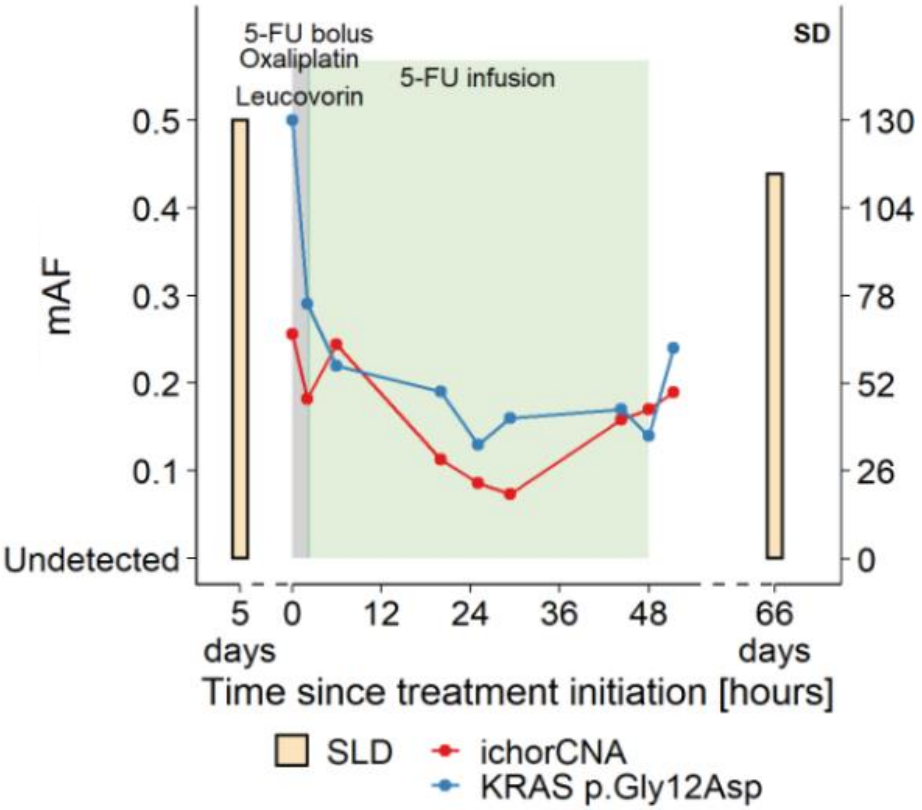
Timing and type of last therapy or procedure

- Surgeries to remove tumor or debulking procedures will decrease ctDNA levels.[1]
- Chemotherapy can reduce the overall tumor burden and impact the ability to detect ctDNA in blood.[2,3]
- Studies in NSCLC have shown cfDNA is higher 24 hours after radiotherapy and continues to increase over the course of a week following treatment.[4]

Other considerations

- Blood Transfusion or Transplant: DNA derived from the donor is inadvertently transfused into the recipient, thereby falsely increasing total cfDNA and complicating NGS interpretation[5]

ctDNA levels in plasma from 2 patients under chemotherapy[3]



cfDNA = cell-free DNA, ctDNA = circulating tumor DNA, 5-FU = fluorouracil, ichorCNA = ichor copy number alteration algorithm, mAF = mutant allele frequency, SLD = sum of longest diameter.

1. Pereira E, et al.. *PLoS One*. 2015;10(12):e0145754; 2. Said R, et al. *Oncotarget.* 2020;11(2):188-211; 3. Moser T. et al. Abstract presented at AACR Virtual Meeting II 2020. Abstract 2881, 4. Kageyama SI, et la. *Oncotarget.* 2018;9(27):19368-19378; 5. Ruhaak LR, et al. *Clin Chem.* 2018;64(4):749-751.

FOUNDATION MEDICINE

How do we explain discordant results between different sequencing tests?

FOUNDATION MEDICINE

Poll Question

Most studies have demonstrated high concordance between tumor and liquid biopsy for detection of genomic alterations. What are some factors that can contribute to discordance?

A. Amount of ctDNA in the blood specimen

B. Time between tissue and blood specimen collection

C. Tumor fraction

D. Tumor heterogeneity

E. All of the above

FOUNDATION MEDICINE

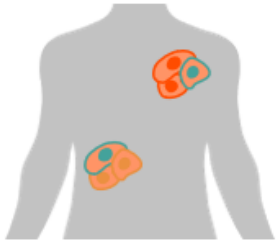
Tissue biopsy may not capture the genomic landscape of a patient's entire tumour burden

Intratumour *heterogeneity*

The genomic landscape **within a single tumour manifestation** may not be uniform

Tissue biopsy may not capture subclonal populations of tumour cells with distinct alterations

Intrapatient *heterogeneity*



The genomic landscape may **differ between tumour sites** within a patient

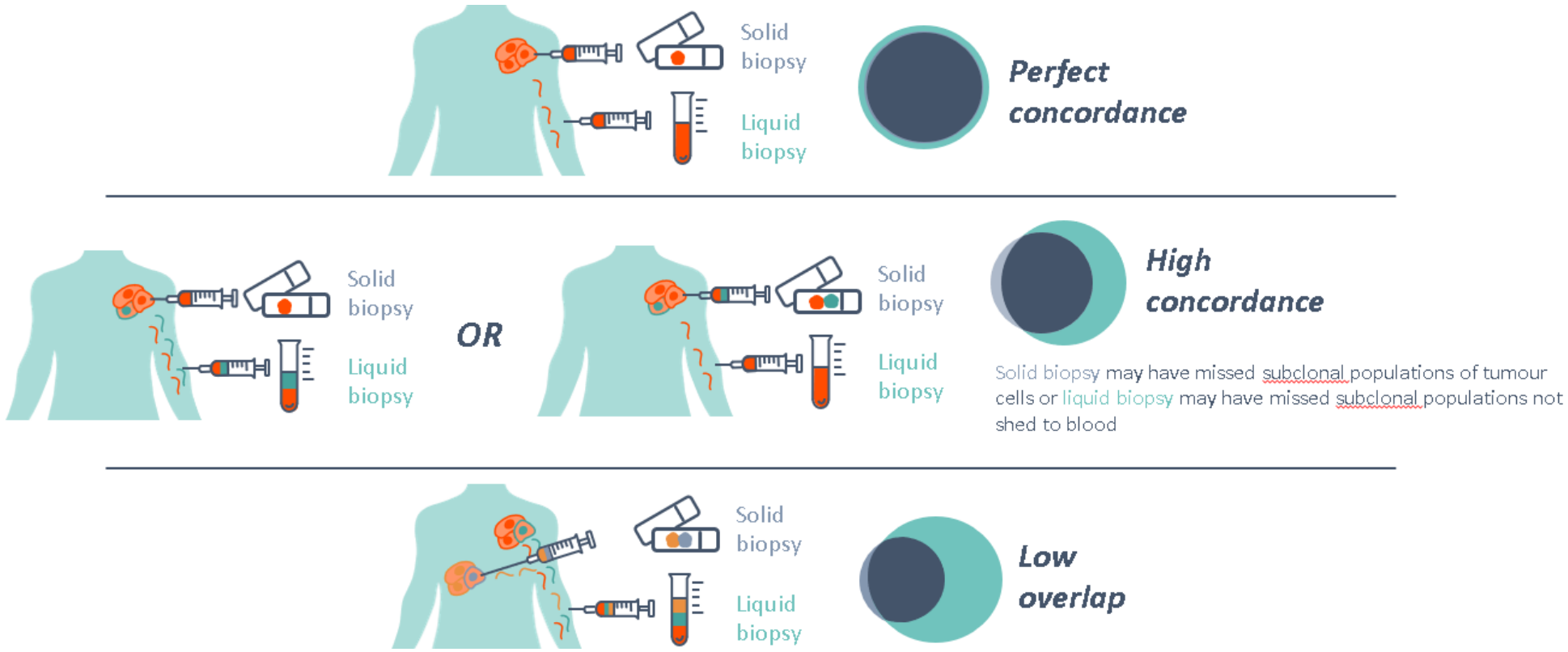
Tissue biopsy from a single lesion will miss alterations unique to other lesions

Keep in mind: The genomic landscape of a cancer evolves over time, hence archival tissue may not fully represent the tumour genotype at progression

Scherer, F. (2020) in *Recent Results in Cancer Research*: *Tumor Liquid Biopsies*. Springer

FOUNDATION MEDICINE

ctDNA-based analysis captures intrapatient heterogeneity, which can lead to low concordance with tissue



ctDNA: circulating tumour DNA.
Figures adapted from Jahangiri, L and Hurst, T. (2019) *Cancers (Basel)* 11:1938 and Scherer, F. (2020) in *Recent Results in Cancer Research*: *Tumor Liquid Biopsies*. Springer

FOUNDATION MEDICINE

What is tumor fraction?

How is tumor fraction calculated?

How can tumor fraction be useful?

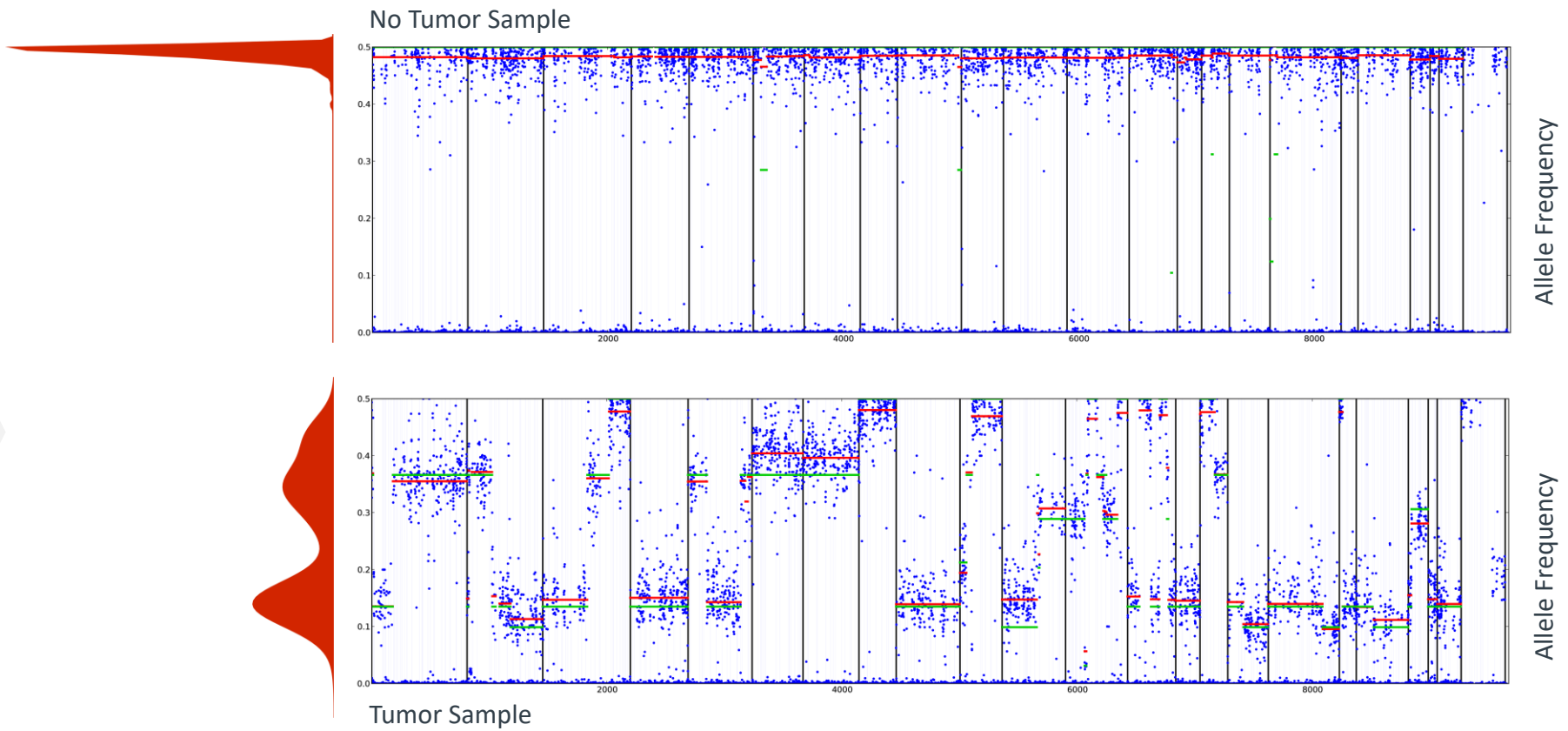
FOUNDATION MEDICINE

Aneuploidy Can Be Used to Inform Tumor Fraction in Cell Free DNA

Distribution of SNP alleles (SNP variance) is different in tumor vs. non-tumor, thus SNP measurement can estimate % tumor DNA present

This method relies on including sufficient SNPs in the baitset

Observed aneuploidy can estimate tumor fraction down to a level of ~10%



SNP = single nucleotide polymorphism. Data on File, Foundation Medicine, Inc., 2020.

FOUNDATION MEDICINE

High Tumor Fraction → High Concordance with Tissue

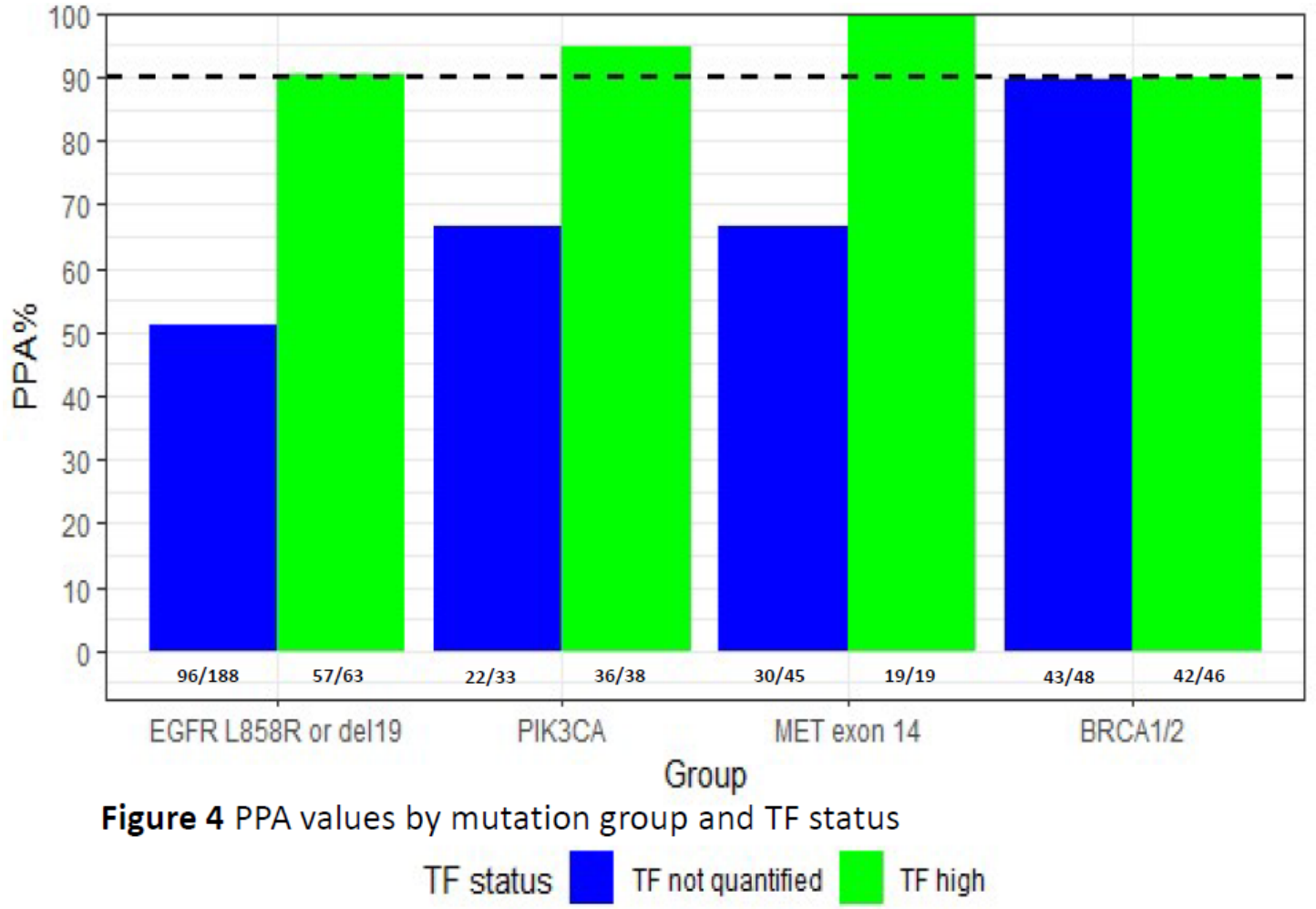


Figure 4 PPA values by mutation group and TF status

Li H, et al. Presented at AACR 2021.

FOUNDATION MEDICINE

Poll Question

The variant allele frequency of a driver and targetable alteration impacts response to the respective targeted drug.

A. True

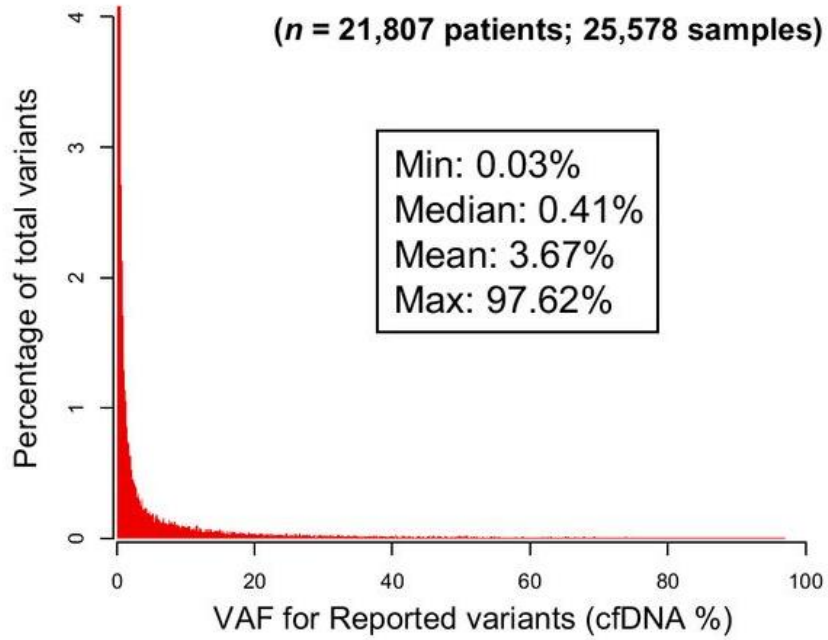
B. False

FOUNDATION MEDICINE

Variant Allele Frequency (VAF)

VAF for liquid biopsies is expected to be lower than in tissue testing

- Variant allele frequency is the percentage of reads containing a particular variant relative to the NGS coverage (total number of reads) at the specific locus.
- VAF is not normalized to the tumor content of the specimen.
- For FoundationOne Liquid CDx, VAF is reported for short variants and indels as well as for rearrangements.



- 21,807 advanced cancer patients VAF distribution of all somatic SNVs, indels and fusions detected by cfDNA testing
- Median VAF 0.41%

VAF = variant allele fraction; cfDNA = cell-free DNA; SNV = single nucleotide variant.

Zill OA, et al. *Clin Cancer Res.* 2018;24(15):3528-3538.

FOUNDATION MEDICINE

What is the Significance of Variant Allele Frequency?

Case Report – NSCLC with no EGFR GA identified by local tissue testing

GENOMIC SIGNATURES
Blood Tumor Mutational Burden - 3 Muts/Mb
Microsatellite status - MSI-High Not Detected
Tumor Fraction - Cannot Be Determined

GENE ALTERATIONS		VAF %
EGFR -	L858R	0.48%

10 Trials see p. 11

THERAPY AND CLINICAL TRIAL IMPLICATIONS
No therapies or clinical trials. See Genomic Signatures section
MSI-High not detected. No evidence of microsatellite instability in this sample (see Appendix section).
Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.

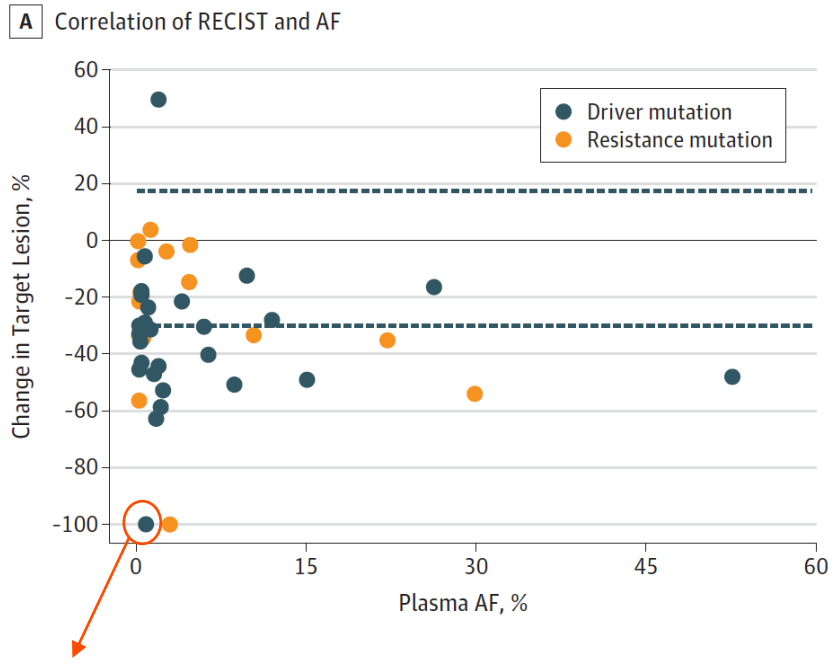
THERAPIES APPROVED IN THE EU (IN PATIENT'S TUMOR TYPE)		THERAPIES APPROVED IN THE EU (IN OTHER TUMOR TYPE)
Afatinib	1	None
Dacomitinib	1	
Erlotinib	1	
Gefitinib	1	
Osimertinib	1	

1 NCCN category

GENE ALTERATIONS WITH NO REPORTABLE THERAPEUTIC OR CLINICAL TRIALS OPTIONS

For more information regarding biological and clinical significance, including prognostic, diagnostic, germline, and potential chemosensitivity implications, see the Gene Alterations section.

TET2 - E1786fs*1, F1689fs*6 ..... p. 6

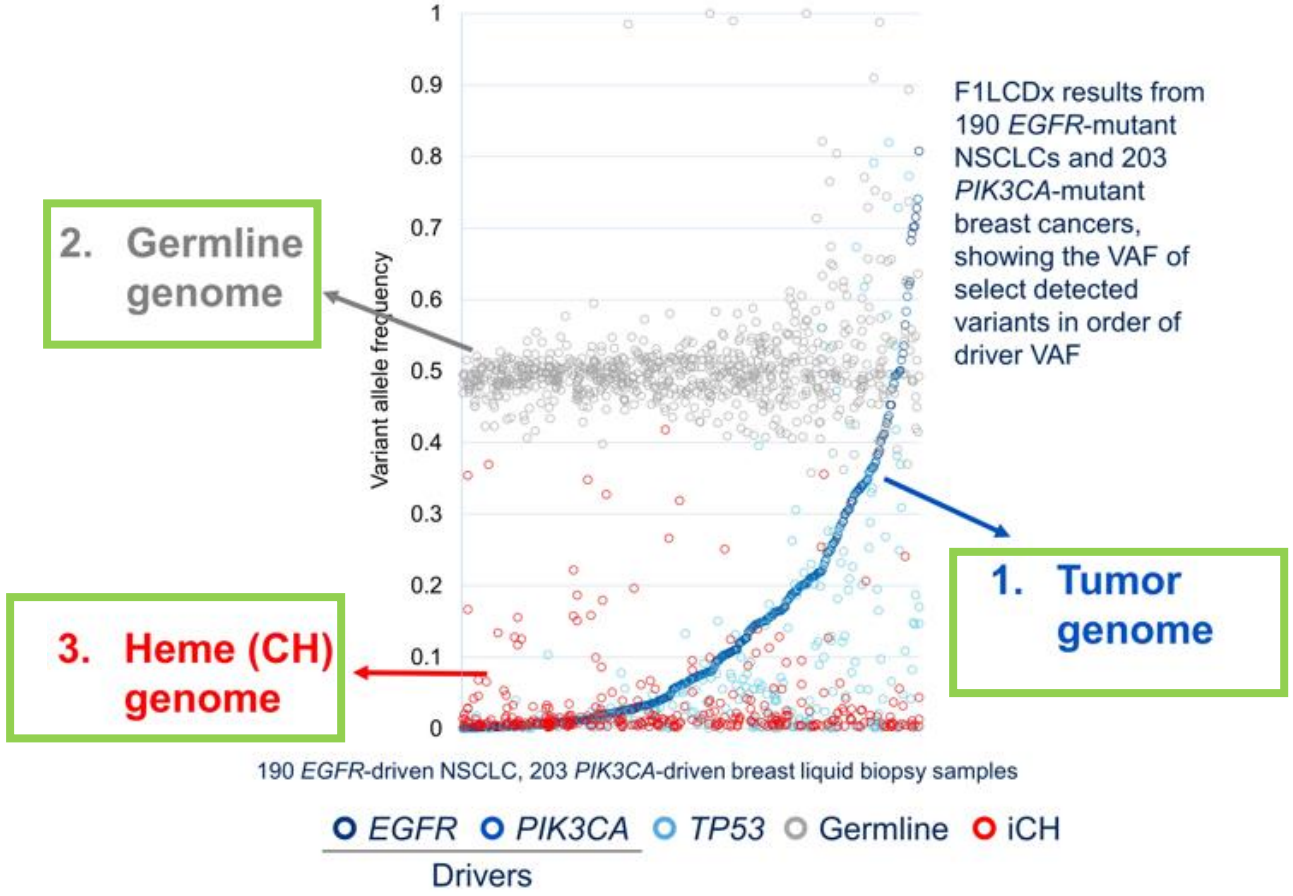


EGFR L858R VAF 0.9% - **CR**

Aggarwal C, et al. JAMA Oncol. 2019;5(2):173–180.

FOUNDATION MEDICINE

What is the Significance of Variant Allele Frequency?



What characterizes CH variants?

Antonarakis E, et al. Presented at ASCO 2021.

FOUNDATION MEDICINE

Poll Question

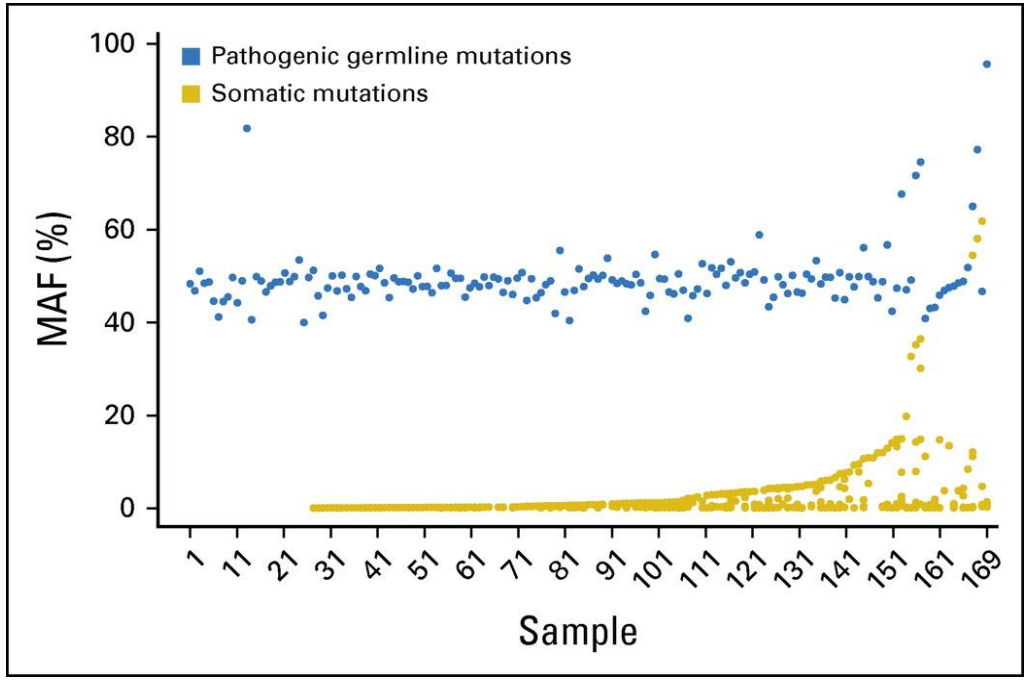
Germline mutations can be detected in liquid biopsies even when ctDNA levels are low.

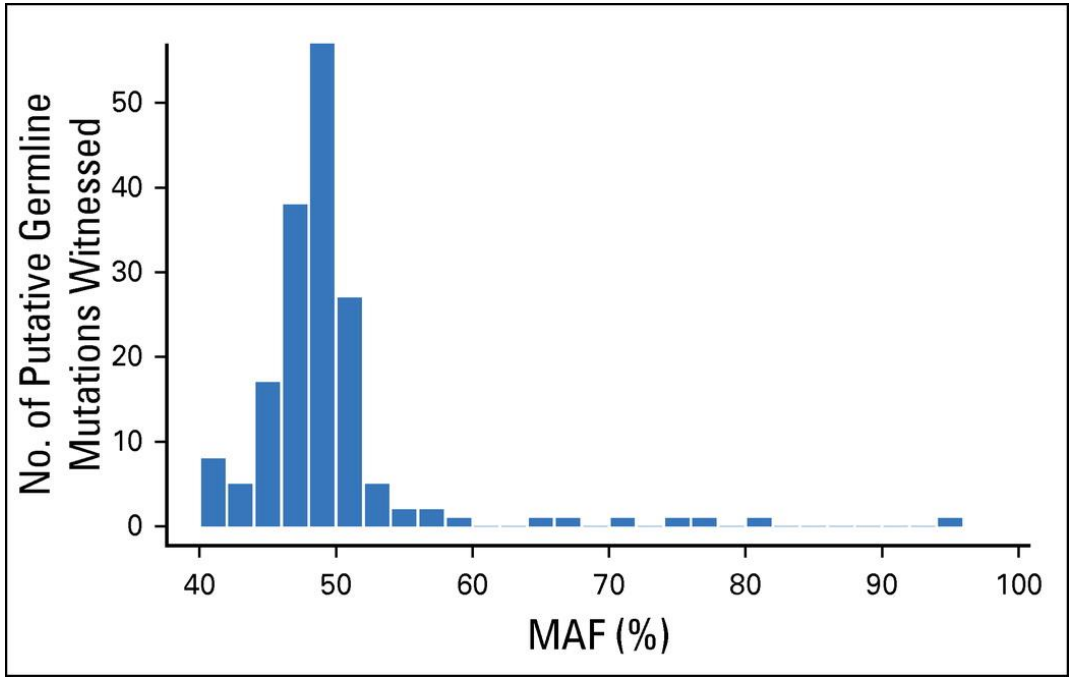
A. True

B. False

FOUNDATION MEDICINE

What is the Significance of Variant Allele Frequency?





- 11,681 cfDNA samples from 10,888 patients with advanced solid tumors
  - Mean VAF of putative germline findings was 48.7% with low variance
- Deviation from VAF ~50% often related to copy number

1. Slavin TP, et al. *J Clin Oncol.* 2018;36(35):3459-3465

FOUNDATION MEDICINE

Poll Question

Detection of copy number changes is more challenging in liquid vs tissue biopsies.

A. True

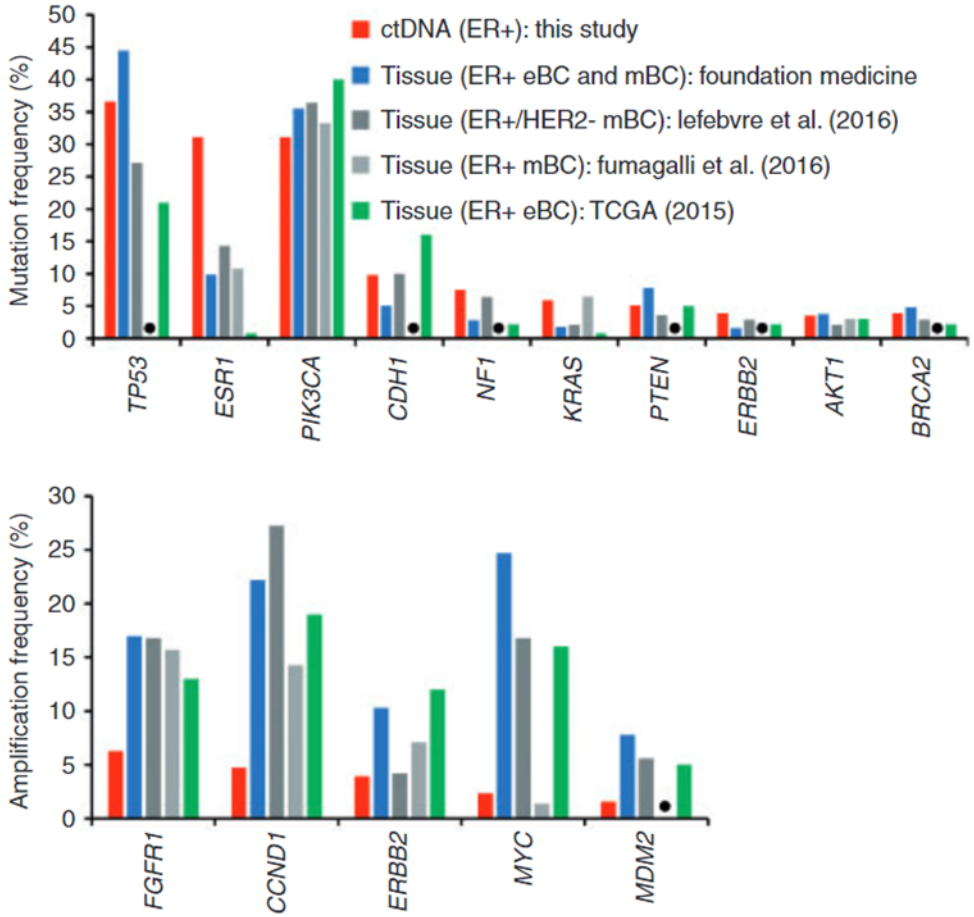
B. False

FOUNDATION MEDICINE

FoundationOne®Liquid CDx Validation:

Limits of Detection (LoD) for Different Types of Alterations

Alteration type	Number of variants analyzed	Bait set region	Median LoD*	Quartile 1 to Quartile 3 Range
Short variants	269	Enhanced	0.40% VAF	0.33%-0.50%
	595	Standard	0.82% VAF	0.70%-0.98%
Rearrangements	7	Enhanced	0.37% VAF	0.26%-0.47%
	1	Standard	0.90% VAF	NA
Copy number amplifications	8	NA	21.7% TF	19.8%-25.2%



- LoD for each variant type established by processing a total of 1,069 sample replicates across 10 contrived (enzymatically fragmented cell-line gDNA) samples representing short variants, rearrangements, and copy number alterations
- LoD defined as lowest level at which a ≥95% detection rate is observed

*The accuracy of %VAF/%TF has not been analytically validated.

FoundationOne Liquid CDx Technical Information. Foundation Medicine; 2020. Accessed January 11, 2021. https://www.FoundationMedicine.com
Chung, et al. Ann Oncol. 2017.

FOUNDATION MEDICINE

Poll Question

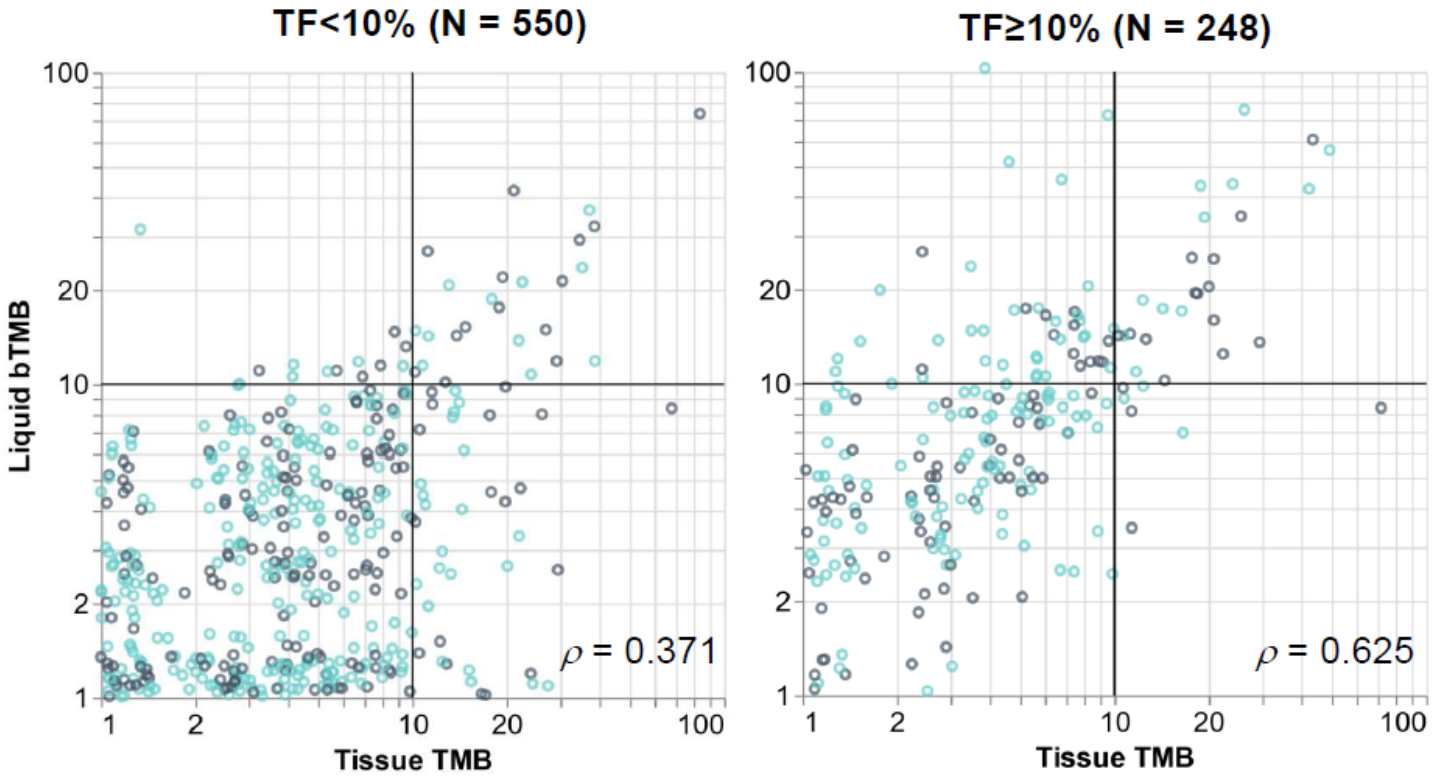
Concordance between blood and tissue Tumor Mutational Burden is dependent on amount of shed ctDNA.

A. True

B. False

FOUNDATION MEDICINE

How is Blood TMB Related to Tissue TMB?



Time between collection	N	ρ
<90 days	215	0.479
≥90 days	335	0.283

Time between collection	N	ρ
<90 days	98	0.758
≥90 days	150	0.590

1. Yoshino T, et al. Presented at ASCO 2021.

FOUNDATION MEDICINE

Poll Question

What are the limitations of liquid biopsy for solid tumors?

A. May not represent the complete genomic profile of the tumor

B. Possibility of false negative based on frequency of variants in the peripheral blood

C. Possibility of false positive due to presence of age-related acquired genomic variants in non-cancer cells (clonal hematopoiesis)

D. High failure rate in the presence of active tumors (i.e. with radiographic progression)

E. A, B and C

F. All of the above

FOUNDATION MEDICINE

Conclusion

Added clinical value through capturing genomic heterogeneity

Discordance between mutation profiles from solid and liquid biopsies is generally common because…

Liquid biopsy

…can capture the genomic heterogeneity of all cancerous lesions, thus, ctDNA can detect mutations that may not be identified in one single biopsy

…but may not detect mutations identified in tissue samples due to a low content of ctDNA in cfDNA (e.g. in cancers with low tumour burden)

Thus combining all high-confidence somatic mutations present either in solid or liquid biopsy samples for the generation of a final report is recommended

FOUNDATION MEDICINE

Session agenda

Liquid Biopsy FAQ

1. Review of FAQ on liquid biopsy
2. Panel discussion and open Q&A

FOUNDATION MEDICINE