Introduction to ctDNA-based Liquid Biopsy

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March 18th, 2026

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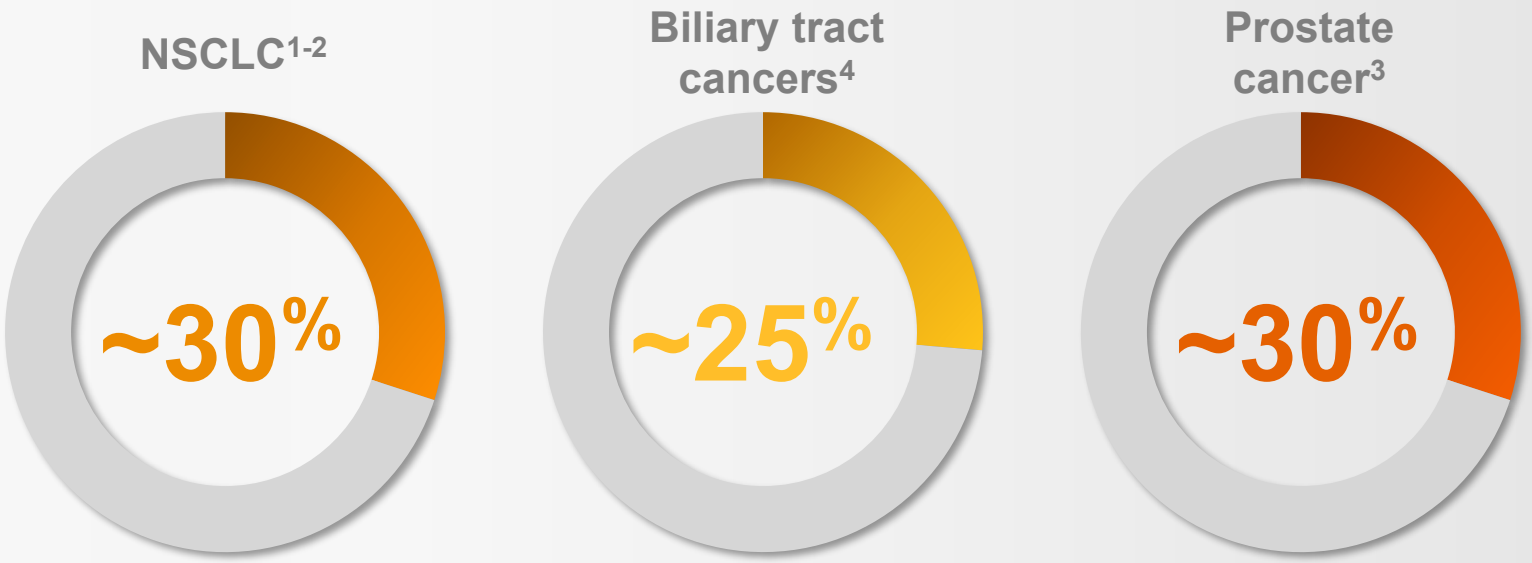
Missed opportunities for precision medicine

Frequency of tissue insufficiency



~20% of the time in other advanced cancers, including breast, colorectal, gynecological, and gastrointestinal cancers[5,6]

Insufficient tissue *quantity* or *quality* is a barrier to NGS

Factors influencing QNS:

- Biopsy procedure[7]
- Tumor percentage[8]
- Tissue handling and fixation[9]
- Prior testing[10]

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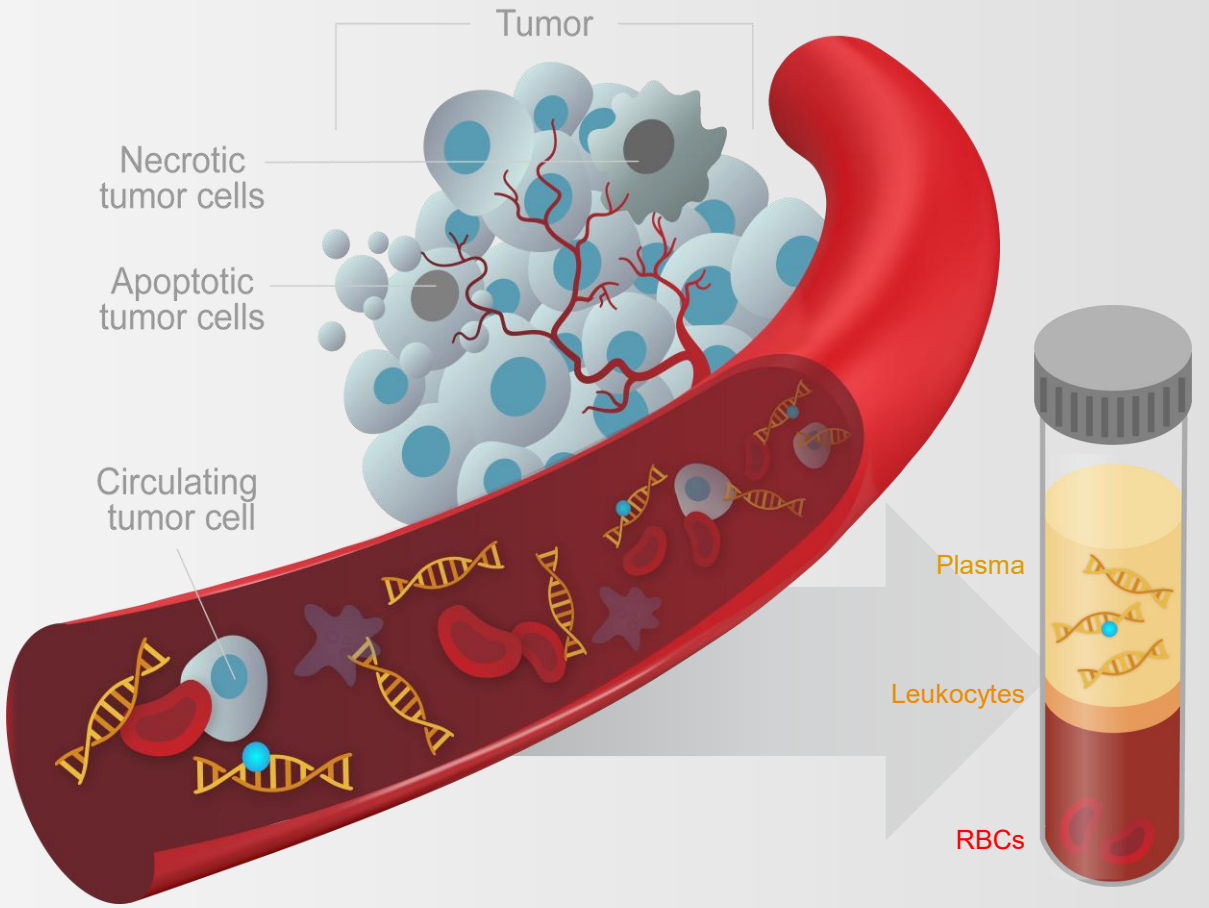
NGS=next-generation sequencing; NSCLC=non-small cell lung cancer; QNS=quantity/quality not sufficient

1. Hagemann IS, Devarakonda S, Lockwood CM, et al. Cancer. 2015;121(4):631-639;2. Aggarwal C, Thompson JC, Black TA, et al. JAMA Oncol. 2019;5(2):173-180; 3. Hussain M, Corcoran C, Sibilla C, et al. Clin Cancer Res. 2022 Apr 14;28(8):1518-1530; 4. Lamarca A, Kapacee Z, Breeze M, et al. J Clin Med. 2020;9(9):2854; 5. Sholl LM, Do K, Shivdasani P, et al. JCI Insight. 2016;1(19):e87062; 6. Meric-Bernstam F, Brusco L, Shaw K, et al. J Clin Oncol. 2015;33(25):2753-2762; 7. Mata DA, Harries L, Williams EA, et al. Arch Pathol Lab Med. 2023;147(3):338-347; 8. Goswami RS, Luthra R, Singh RR, et al. Am J Clin Pathol. 2016;145(2):222-237; 9. Hussain M, Corcoran C, Sibilla C, et al. Clin Cancer Res. 2022;28(8):1518-1530; 10. Drilon A, Wang L, Arcila ME, et al. Clin Cancer Res. 2015;21(16):3631-3639

Background: circulating tumor DNA (ctDNA)

Cell-free DNA (cfDNA) are small DNA fragments shed into the circulation during necrosis or apoptosis. cfDNA is found in healthy individuals and those with cancer

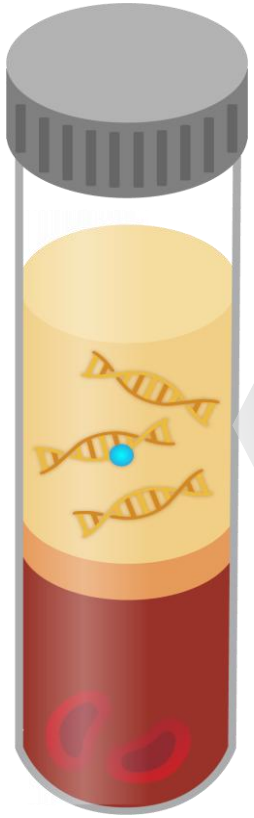
Circulating tumor DNA (ctDNA) is the fraction of cfDNA coming from a tumor



ctDNA generally accounts for <1% of cfDNA levels in blood

Elazezy M, Joosse SA. Comput Struct Biotechnol J. 2018;16:370-378

Major genomic variant types are detectable with liquid biopsy

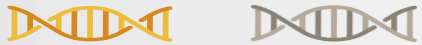


Single/short nucleotide changes

Chromosomal rearrangements

Copy number aberrations

Methylation variants



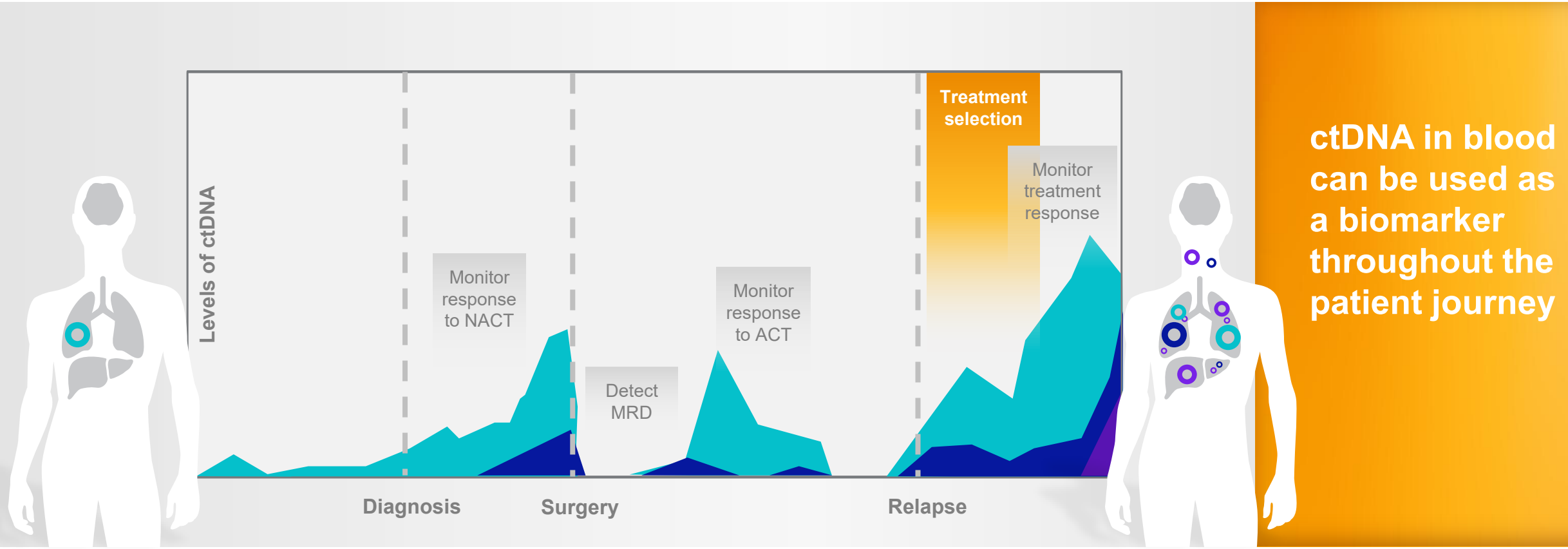
Liquid biopsies can assay DNA and RNA

Liquid biopsies can be performed using blood, CSF, urine, saliva, and pleural effusion fluid

CSF=cerebrospinal fluid

Keller L, Belloum Y, Wikman H, Pantel K. Br J Cancer. 2021;124(2):345-358

ctDNA assays can be applied across the cancer continuum



ctDNA in blood can be used as a biomarker throughout the patient journey

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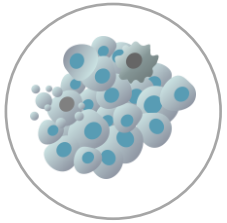
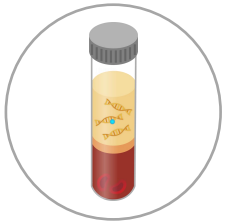
ACT=adjuvant chemotherapy; ctDNA=circulating tumor DNA; MRD=minimal residual disease; NACT=neoadjuvant chemotherapy.
Adapted from Wan JCM, Massie C, Garcia-Corbacho J, et al. Nat Rev Cancer. 2017;17(4):223-238

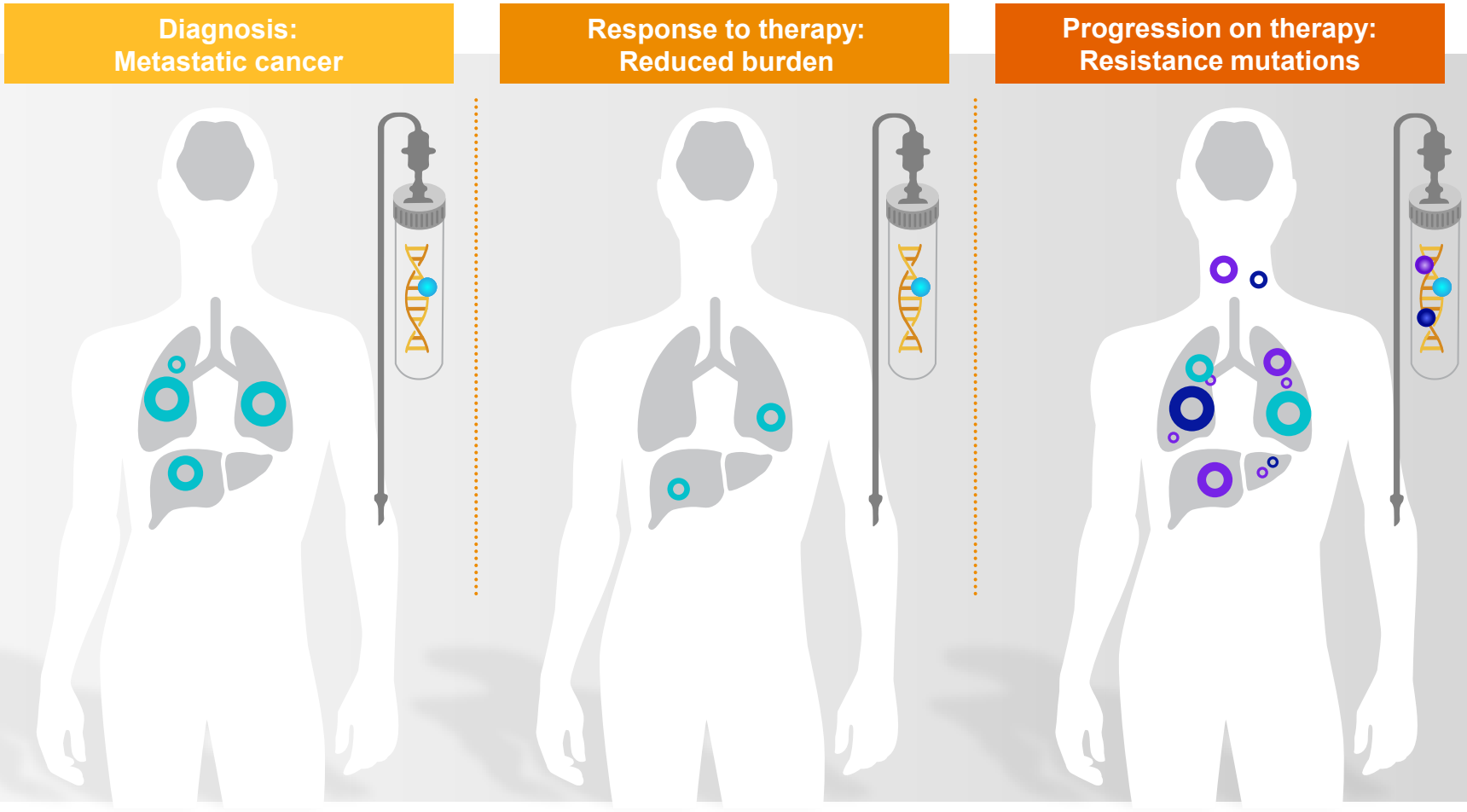
Comparison of liquid vs tumor tissue biopsy for biomarker testing

	Advantages	Limitations
Tumor tissue 	• Pathology information • PD-L1 assessment • High sensitivity • RNA	• Invasive • Represents snapshot of tumor genomics • Longer TAT • Re-biopsy not always feasible
Liquid biopsy 	• Less invasive, easy serial testing • More representative of tumor heterogeneity • Rapid TAT	• Sensitivity depends on tumor shed rate • Result interference by CH • Lower sensitivity for specific types of alterations

CH=clonal hematopoiesis of indeterminate potential; PD-L1=programmed cell death ligand 1; TAT=turnaround time.
Rolfo C, Mack P, Scagliotti GV, et al. J Thorac. Oncol. 2021;16(10):1647-1662.

Liquid biopsies can capture intra- and intertumoral heterogeneity

ctDNA from blood can represent clones from a single primary tumor as well as multiple metastatic sites

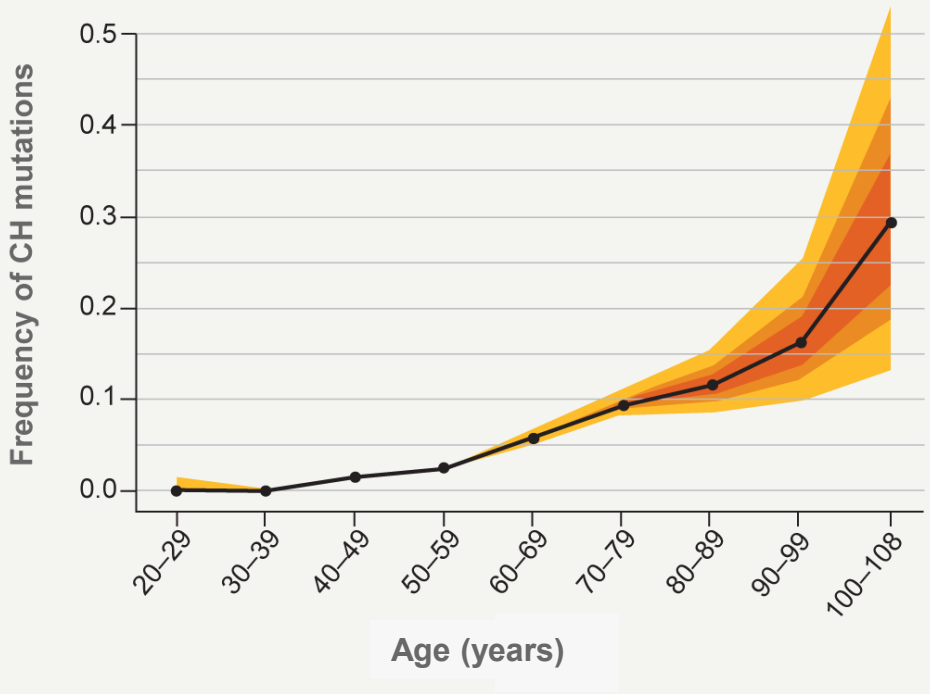


ctDNA=circulating tumor DNA

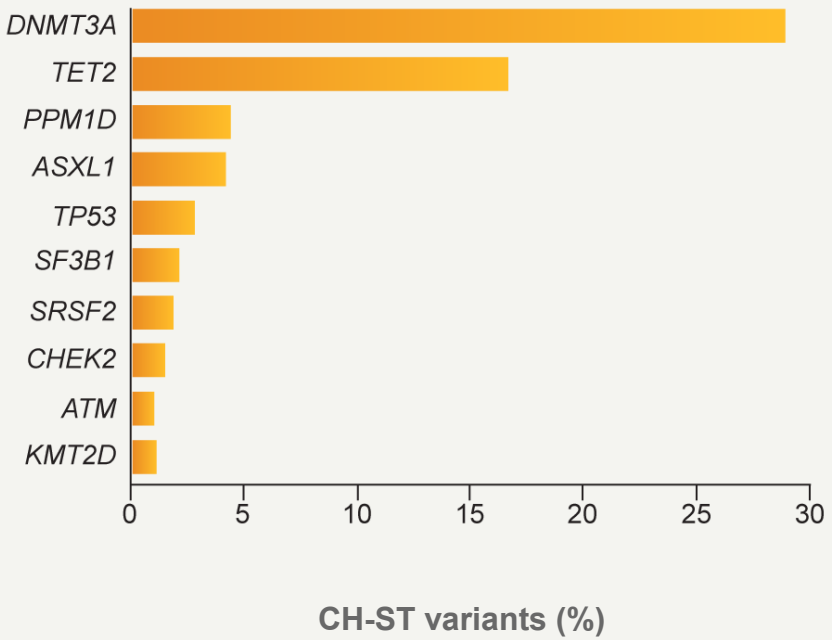
Rapisuwon S, Vietsch EE, Wellstein A. Comput Struct Biotechnol *J*. 2016;14:211-222

Clonal hematopoiesis (CH) is a source of biological background noise in liquid biopsies

Prevalence of somatic mutations by age[1]



Most frequent somatic mutations[2]



CH, the clonal expansion of mutated hematopoietic stem and progenitor cells, is a common aging process[3]

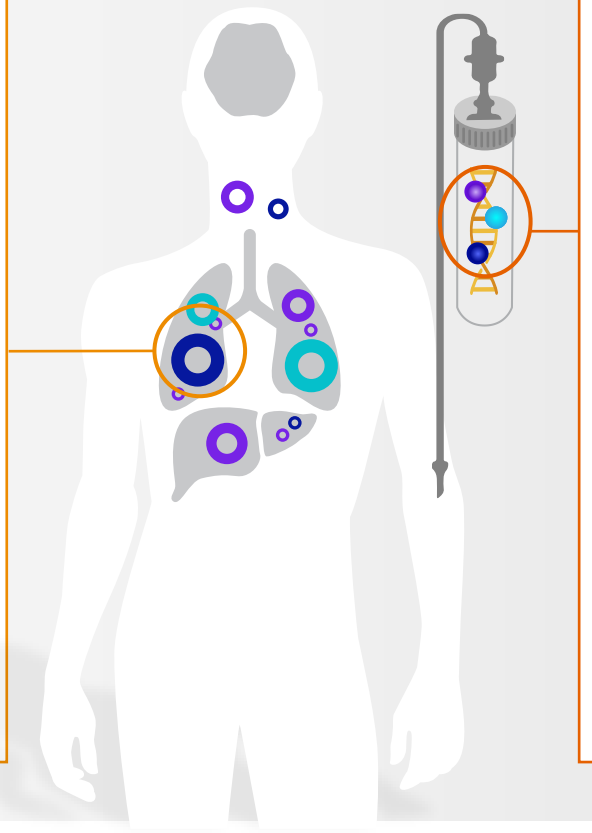
CH=clonal hematopoiesis; CH-ST=clonal hematopoiesis in solid tumor

1. Jaiswal S, Fontanillas P, Flannick J, et al. N Engl J Med. 2014;371(26):2488-2498; 2. Ptashkin RN, Mandelker DL, Coombs CC, et al. JAMA Oncol. 2018;4(11):1589-1593; 3. Chan ICC, Wiley BJ, Bolton KL. Front Oncol. 2022;12:794021

Factors influencing ctDNA levels and detection

Disease characteristics

- Tumor type
- Tumor burden and staging
- Disease status
  (stable vs progressive disease)
- Tumor microenvironment
  (stroma and vascularization)



Other factors

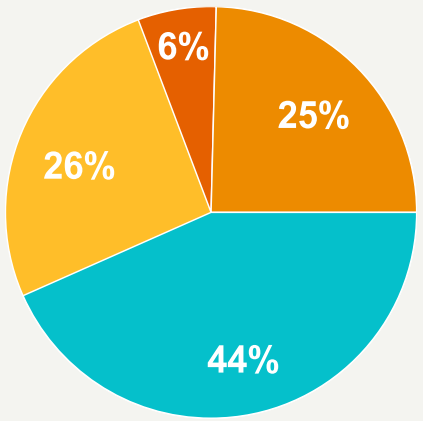
- Time of sampling relative to treatment or procedures
- Sample acquisition, transport, and processing procedures
- Type of variant
- Assay sensitivity

Not all patients may be candidates for liquid biopsy at a given time

ctDNA=circulating tumor DNA

Vivancos A, Tabernero J. Nat Med. 2022;28(11):2255-2256

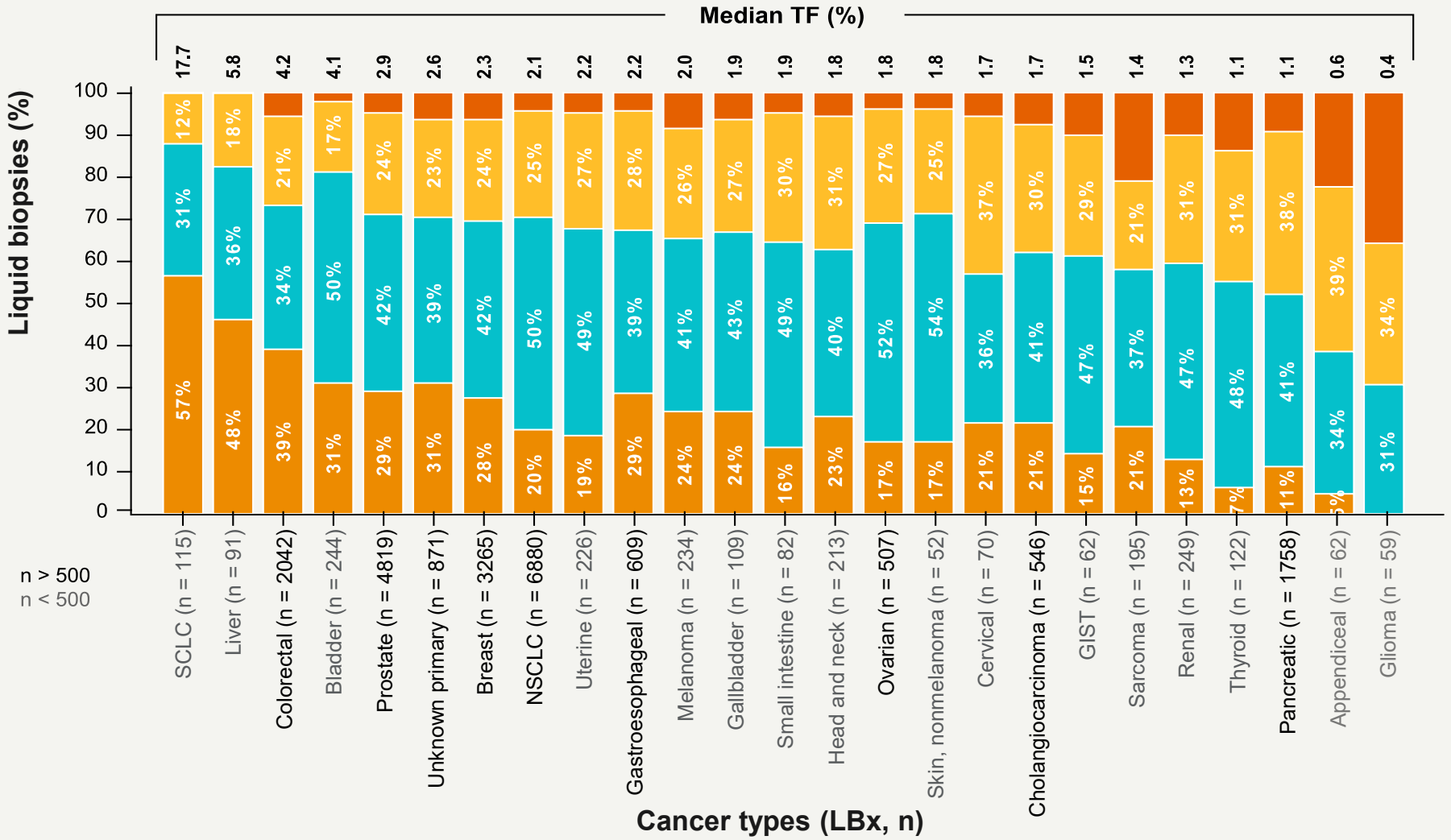
ctDNA detection rates vary across tumor types



Entire cohort (23,482)

ctDNA fraction

- ≥ 10%
- (1%–10%)
- 0%–1%
- Not detected

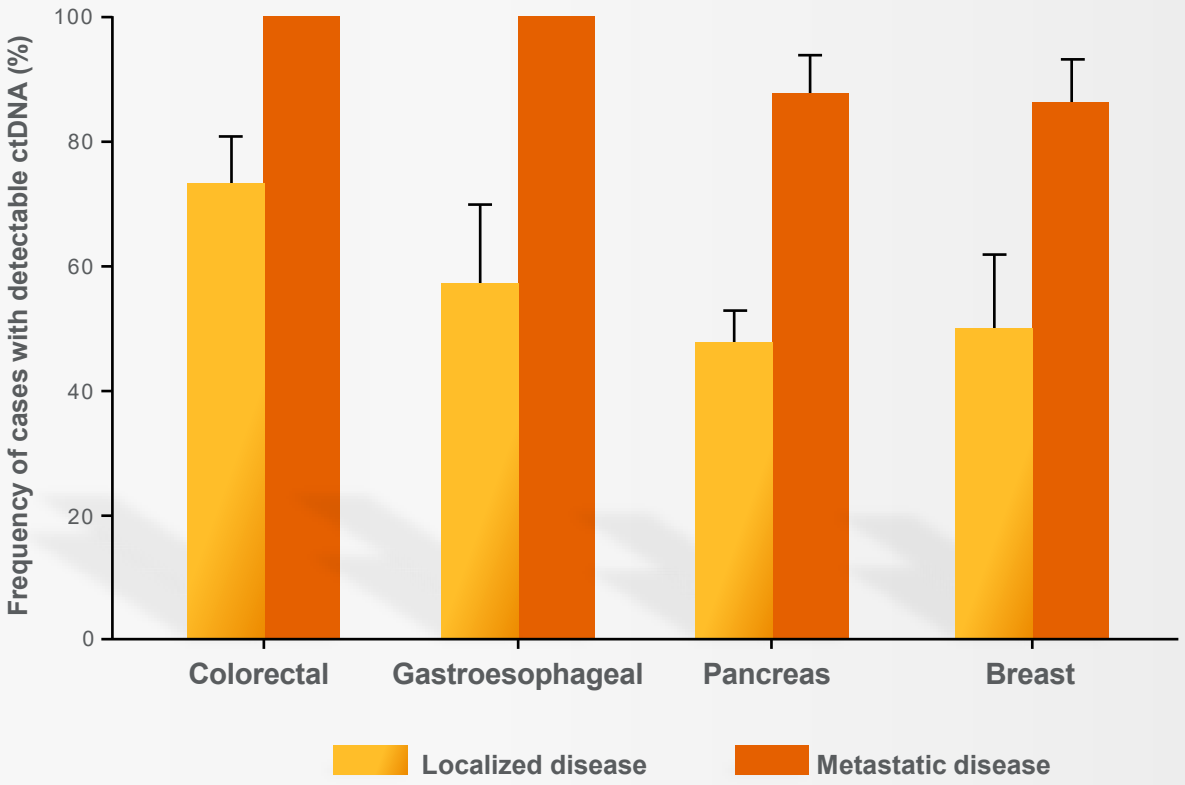


ctDNA=circulating tumor DNA; GIST=gastrointestinal stromal tumor; LBx=liquid biopsy; NSCLC=non-small cell lung cancer; SCLC=small cell lung cancer; TF=tumor fraction

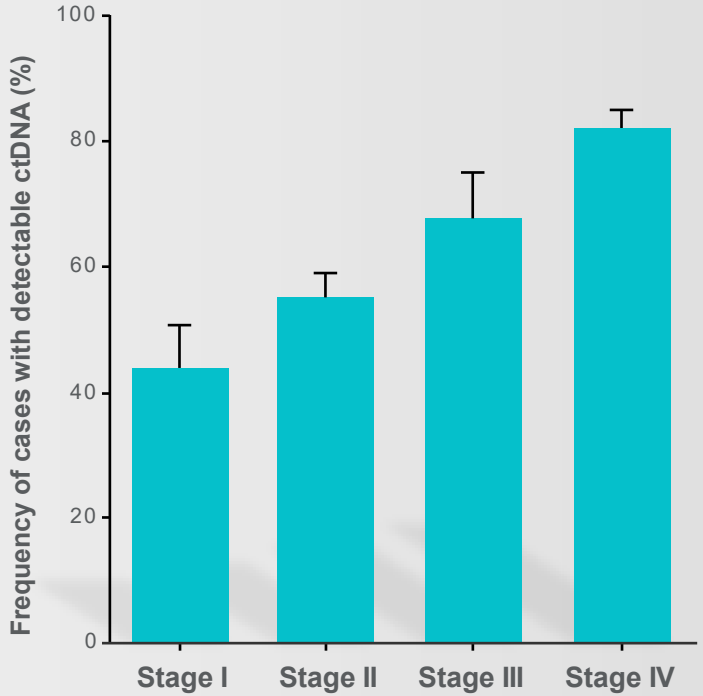
Husain H, Pavlick DC, Fendler BJ, et al. JCO Precis Oncol. 2022;6:e2200261

ctDNA detection rates increase with stage

Fraction of patients with detectable ctDNA



Detectable ctDNA by stage

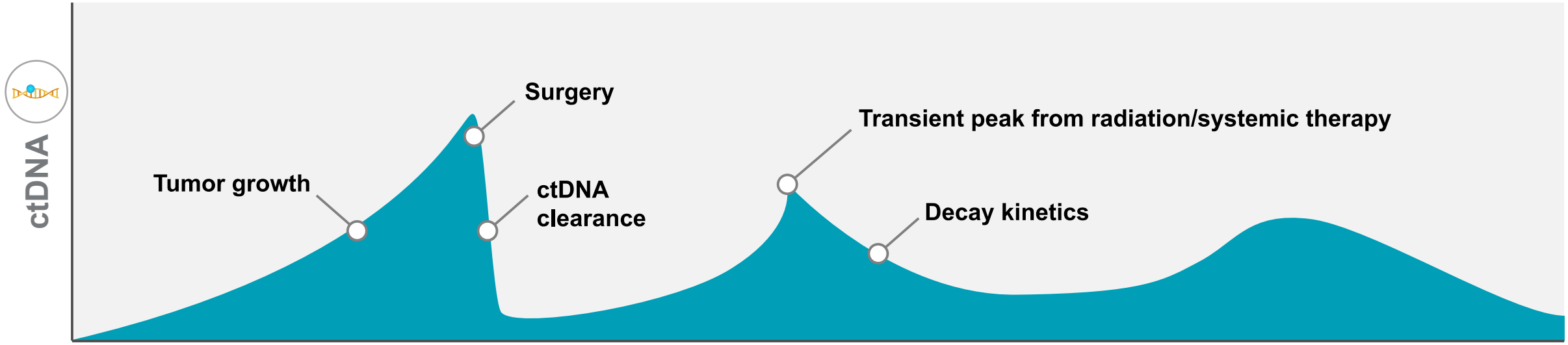


Late-stage and metastatic tumors shed more ctDNA

ctDNA=circulating tumor DNA

Bettegowda C, Sausen M, Leary RJ, et al. Sci Transl Med. 2014;6(224):224ra24

Timing of blood sampling in relation to treatment



Surgery	Radiation	Systemic therapy
Substantial decrease in ctDNA observed following surgical intervention	Transient peak in ctDNA fraction in the days following initiation of radiotherapy, followed by a steady decline over the course of treatment, measurable by weeks 2 and 3	ctDNA fraction typically declines within the first few days of treatment regardless of clinical response

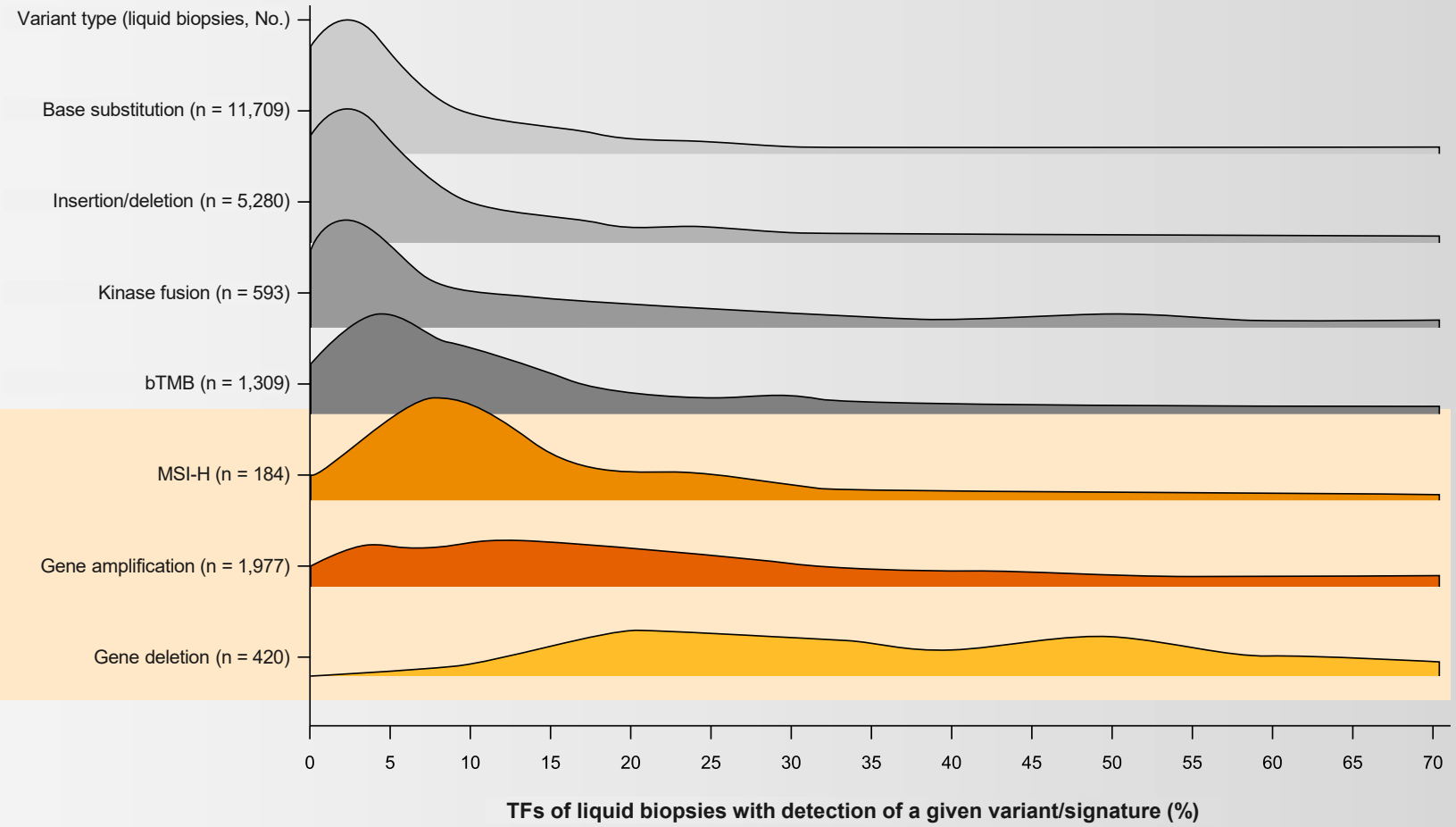
ctDNA=circulating tumor DNA

Sanz-Garcia E, Zhao E, Bratman SV, Siu LL. *Sci Adv*. 2022;8(4):eabi8618

Detection of variant types and genomic signature depends on ctDNA fraction

Detection of genomic biomarkers such as MSI and CNVs require a higher ctDNA fraction

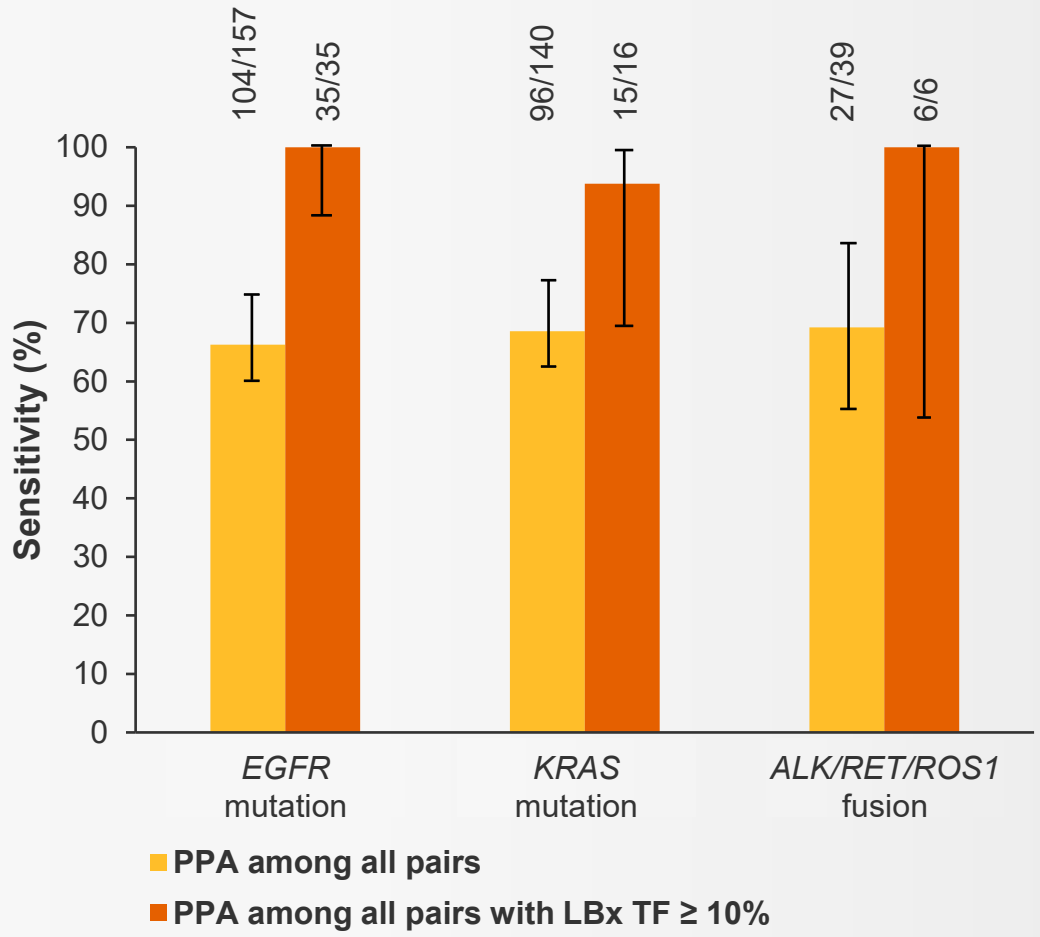
Interpretation of *ERBB2* or *MET* amplifications or *BRCA1/2* loss can be challenging in samples with low ctDNA

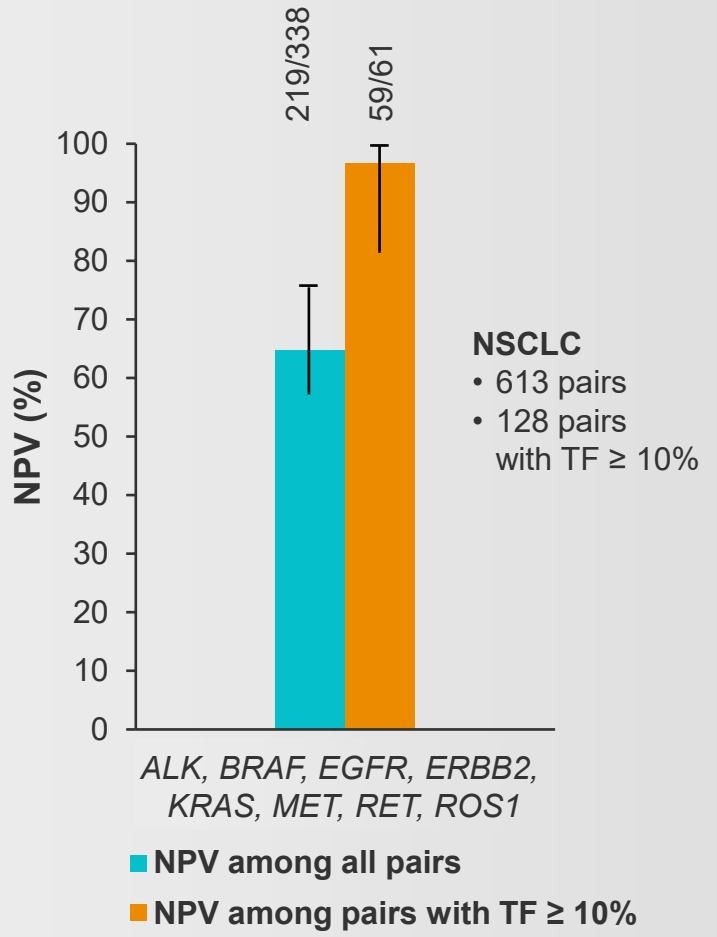


bTMB=blood tumor mutational burden; CNV=copy number variation; ctDNA=circulating tumor DNA; MSI=microsatellite instability; MSI-H=microsatellite instability-high; TF=tumor fraction

Husain H, Pavlick DC, Fendler BJ, et al. JCO Precis Oncol. 2022;6:e2200261

Sensitivity and specificity increase with higher ctDNA fractions





Reflexing to tissue may be considered to confirm negative results if TF is low

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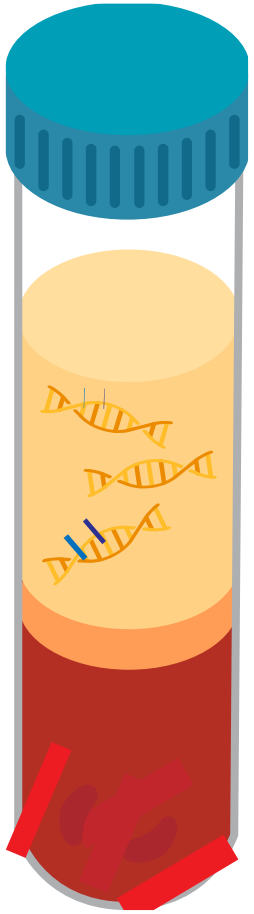
ctDNA=circulating tumor DNA; LBx=liquid biopsy; NPV=negative predictive value; PPA=percent positive agreement; TF=tumor fraction

Husain H, Pavlick DC, Fendler BJ, et al. JCO Precis Oncol. 2022;6:e2200261

Methods for measuring ctDNA fraction within a specimen



Methods for quantifying ctDNA fraction vary by:

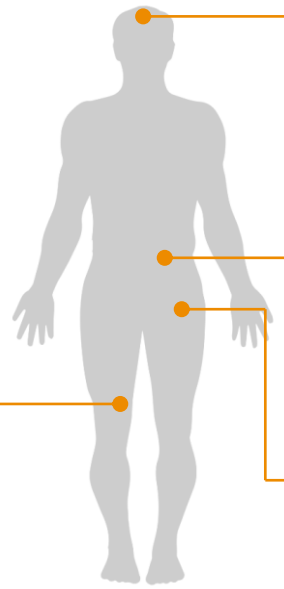
- Platform used, e.g., PCR vs NGS
- Bioinformatic pipeline used
- Assessment of somatic variants/MSAF, aneuploidy, or methylation signature

cfDNA=cell-free DNA; MSAF=maximum somatic allele frequency; NGS=next-generation sequencing; PCR=polymerase chain reaction

Hasenleithner SO, Speicher MR. Mol Cancer. 2022;21(1):81

Comparison of multiple tumor biopsies vs liquid biopsy in *BRAF V600E* CRC

Tissue biopsies



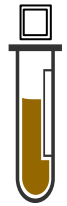
BRAF V600E 54.7% VAF
EGFR amp

BRAF V600E 36.4% VAF
KRAS G12S 6.4% VAF
NRAS Q61R 3.1%. VAF

BRAF V600E 45.4% VAF
KRAS G12S 0.2% VAF

BRAF V600E 61.6% VAF
KRAS G12S 22.4% VAF

Liquid biopsy



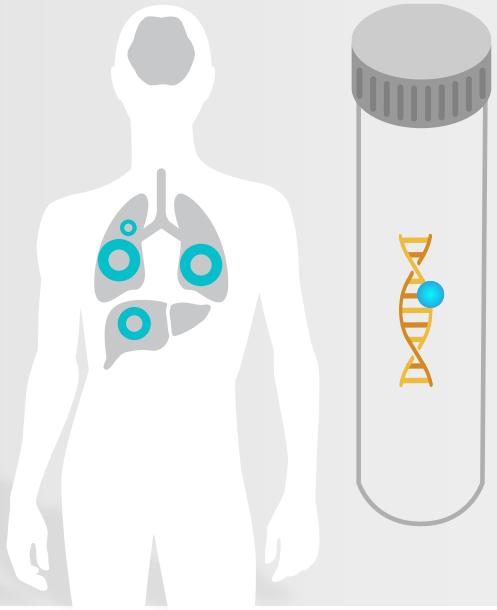
- *BRAF* V600E 24% VAF
- *KRAS* G12S 2.1% VAF
- *NRAS* Q61R 0.6% VAF
- *EGFR* amp

Postprogression ctDNA analysis showed:

- The original *BRAF* V600E
- The emergent *KRAS* G12S
- *NRAS* Q61R
- Low-level *EGFR* amplification

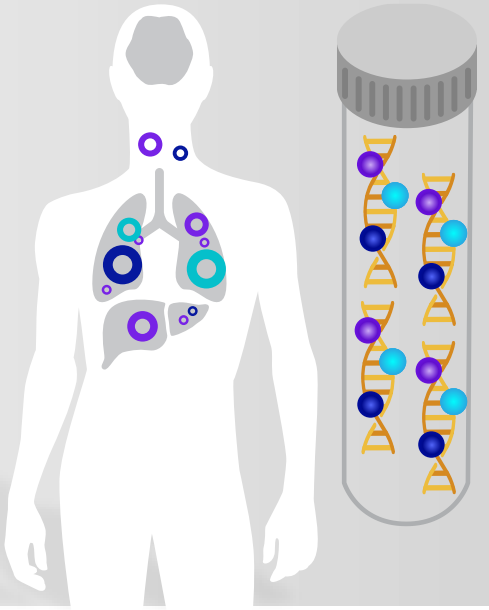
CRC=colorectal cancer; VAF=variant allele frequency
Parikh AR, Leshchiner I, Elagina L, et al. Nat Med. 2019 Sep;25(9):1415-1421

Summary of liquid biopsy and ctDNA dynamics



Low ctDNA shedding tumor
Low ctDNA fraction[1]
Local or stable disease[2]
Patient responding to treatment[4]
Less confident in negative findings[3]
May need to consider reflexing to tissue[4]

High ctDNA shedding tumor
High ctDNA fraction[1]
Advanced/metastatic disease[2]
Patient with refractory or progressive disease[3]
More confident in negative findings[3]
May not need to reflex to tissue[4]



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ctDNA=circulating tumor DNA

1. Hemming ML, Klega KS, Rhoades J, et al. JCO Precis Oncol. 2019;2019:PO.18.00235; 2. Bettegowda C, Sausen M, Leary RJ, et al. Sci Transl Med. 2014;6(224):224ra24; 3. Wan JCM, Massie C, Garcia-Corbacho J, et al. Nat Rev Cancer. 2017;17(4):223-238; 4. Husain H, Pavlick DC, Fendler BJ, et al. JCO Precis Oncol. 2022;6:e2200261

Liquid biopsy testing is increasingly recommended for biomarker profiling in multiple solid tumor types

ESMO Recommendations			
Treatment Selection At Diagnosis	**When Tissue is Not Available**	**When Fast Turnaround Needed**	**Detection of Resistance Mechanism/Treatment Monitoring**
• Colorectal • NSCLC • Prostate	• Breast • Cholangiocarcinoma • Colorectal • Endometrial • Gastric • Hepatocellular • Ovarian • Pancreatic • Soft tissue sarcoma • Thyroid • Urothelial	• Cholangiocarcinoma • Colorectal • Gastric	• Breast • NSCLC

ESMO=European Society for Medical Oncology; NSCLC=Non-small-cell lung cancer.

Pascual F et al. *Annals of oncology : official journal of the European Society for Medical Oncology* vol. 33,8 (2022): 750-768. doi:10.1016/j.annonc.2022.05.520

Thank you for your attention!

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