The NTRKers: WHO WE ARE

- NTRKers Foundation, Inc. is a non-profit. We are a growing community of patients and care partners, who unite and support people affected by NTRK gene fusion cancer

- Our goal is for every adult and child with NTRK gene fusion cancer to have the best chance of living life to the fullest

WHAT THE NTRKers ARE DOING TO HELP

- We collaborate with medical experts and other cancer groups

- We host educational webinars and patient summits

- We share experiences and information within our closed Facebook group

NTRKers

NEVER ALONE: TESTING. KNOWLEDGE. SUPPORT

The NTRKers: WHERE TO FIND US

- Find us online: NTRKers.org
- Email us: Info@NTRKers.org
- Find us on Twitter: @NTRKers
- Find us on Facebook: NTRK Support Group

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NEVER ALONE: TESTING. KNOWLEDGE. SUPPORT

Liquid Biopsy and *NTRK* fusion Detection

Emily K. Dalton MS, CGC

Associate Director, Medical Affairs

March 13, 2025

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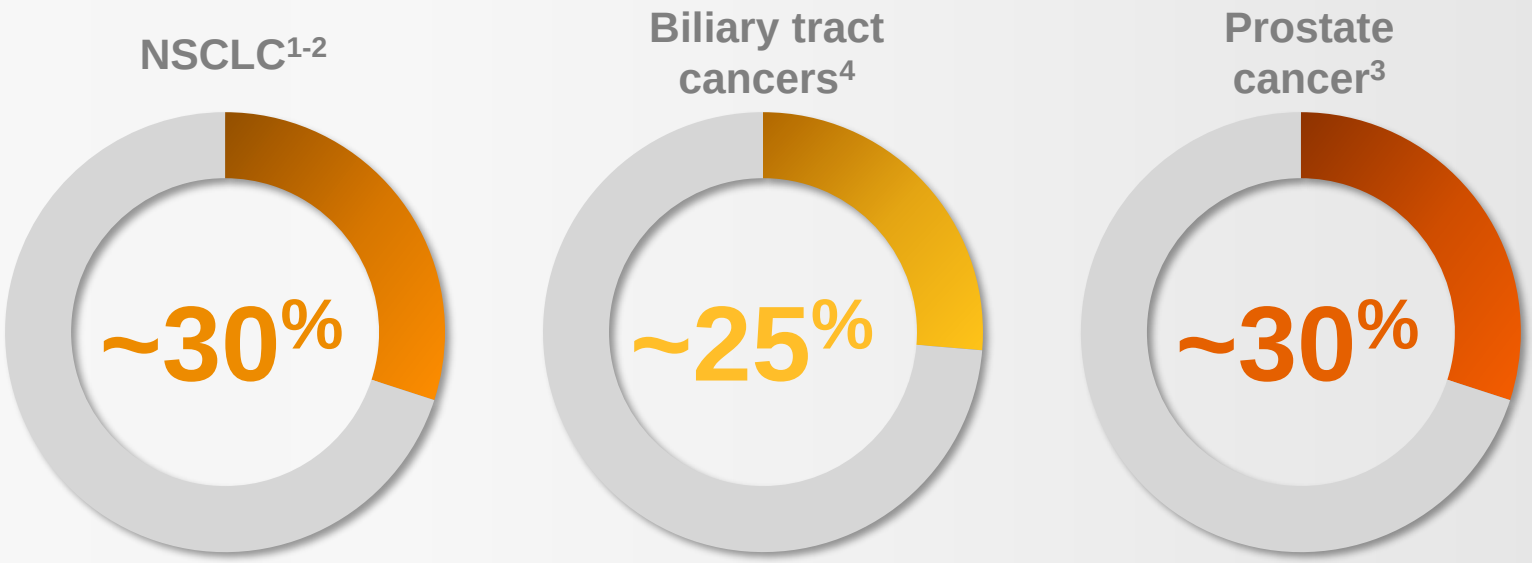
Disclaimer

We will not provide any medical advice or speak to individual cases during this event.

All information shared during this discussion is for general informational and educational purposes only. It is not a substitute for professional medical advice. Always consult with a qualified and licensed physician or other medical care provider and follow their advice without delay.

Opportunities for Precision Medicine are Missed Up to 30% of the Time, Risking Suboptimal Patient Outcomes

Frequency of tissue insufficiency



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

Tissue insufficiency due to *quantity or quality* is a barrier to biomarker testing

Factors influencing tissue:

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

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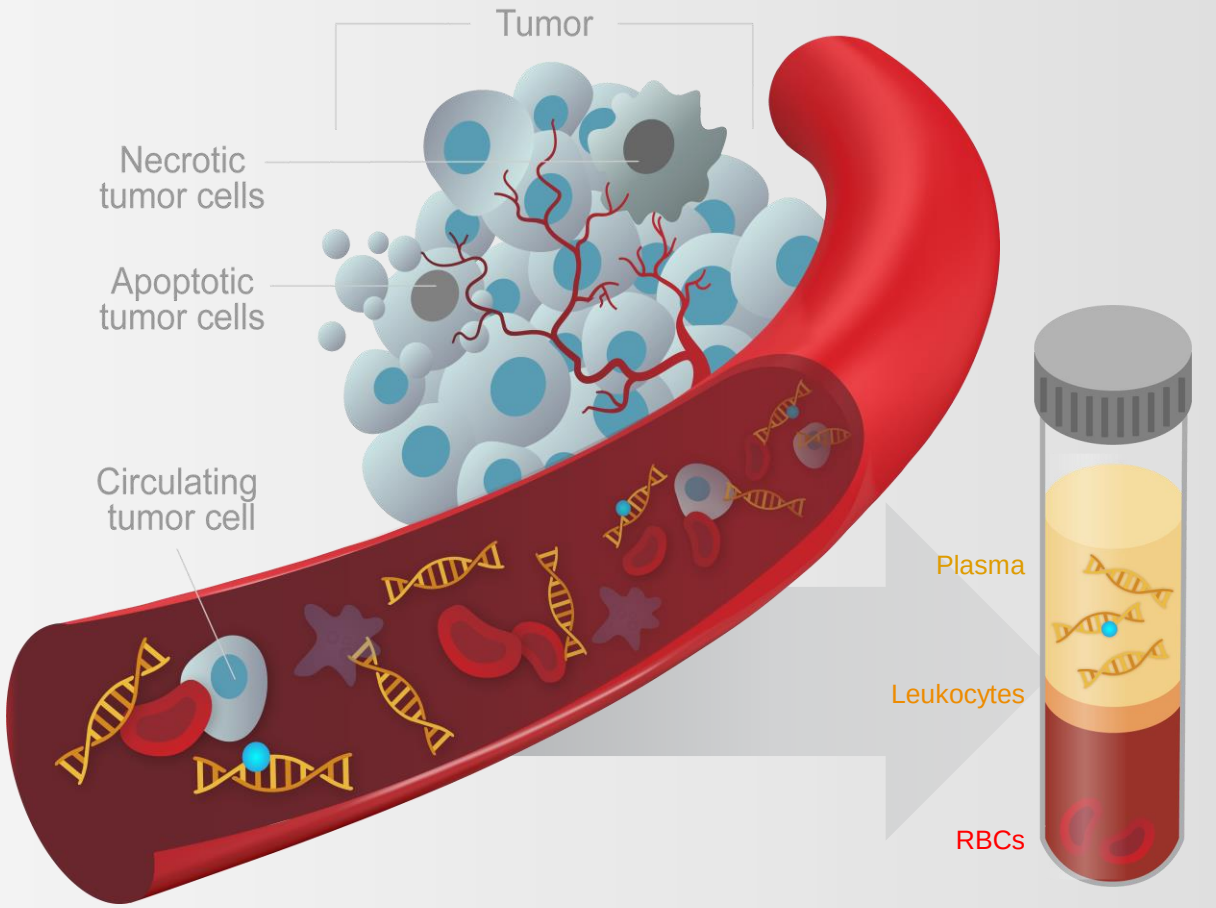
NGS=next-generation sequencing; NSCLC=non-small cell lung cancer; QNS=quantity not sufficient

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

Cell-free DNA (cfDNA) are small DNA fragments that are shed into the blood stream. cfDNA is found in both healthy individuals and those with cancer

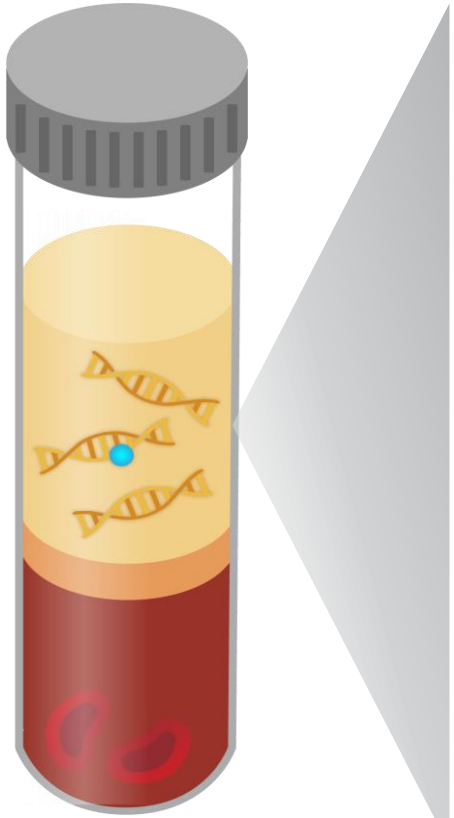
Circulating tumor DNA (ctDNA) is the fraction of circulating DNA coming from the tumor

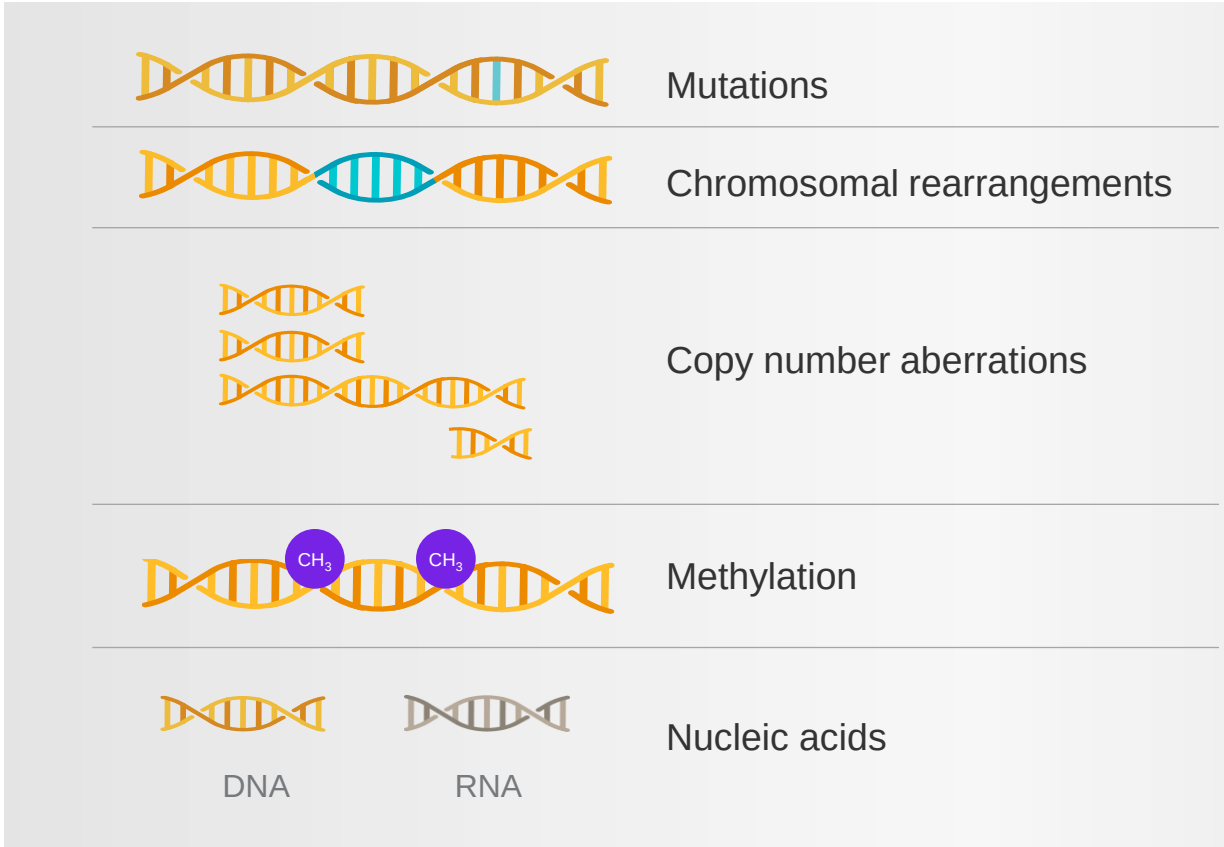


ctDNA generally accounts for < 1% of cfDNA levels in the bloodstream

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

Major Genomic Variant Types can be Detected with Liquid Biopsies



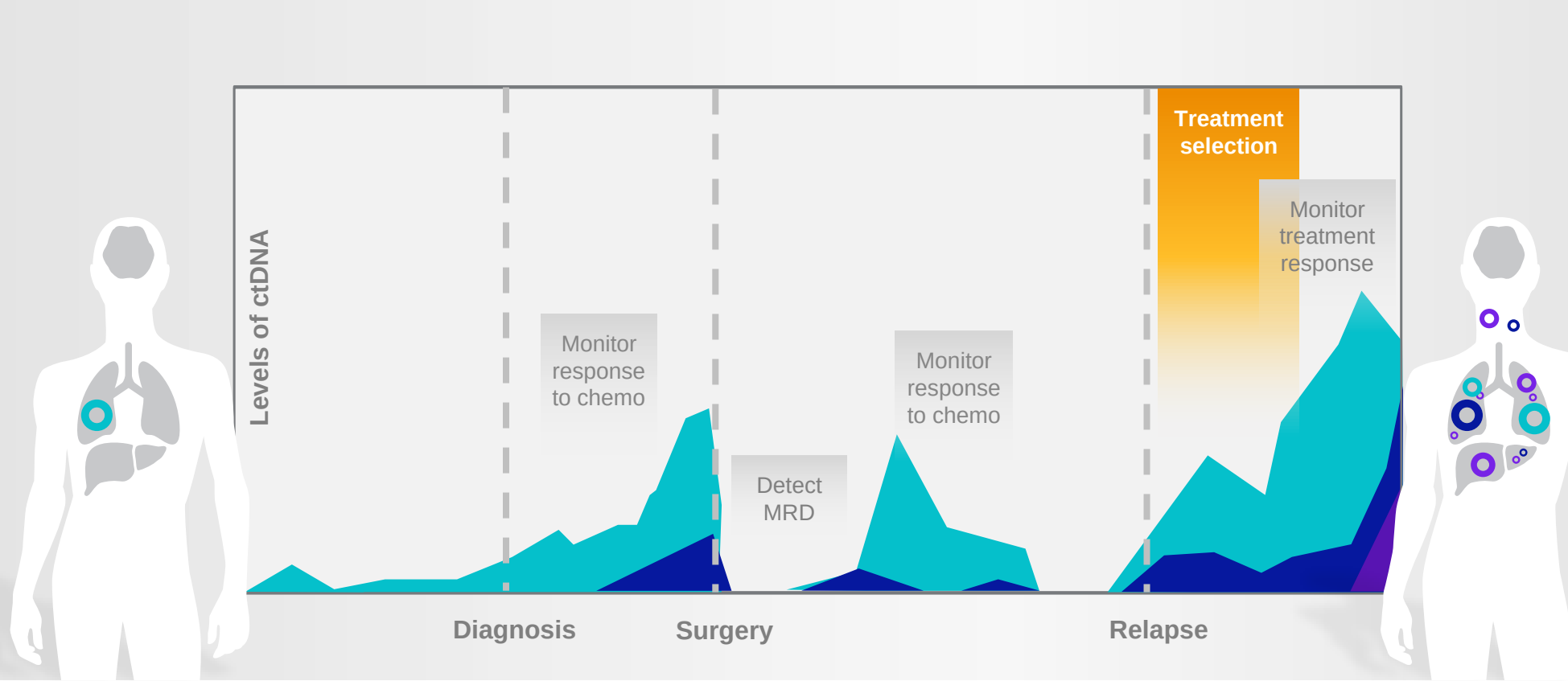


Liquid biopsies can be performed using a variety of specimen types, including CSF, urine, saliva, and pleural effusion fluid

CSF=cerebrospinal fluid

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

Applications of ctDNA Assays Across the Cancer Continuum



ctDNA in the bloodstream can be used as a biomarker at multiple time points in the patient journey

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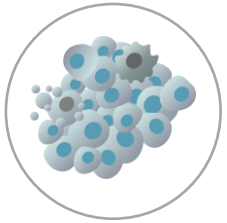
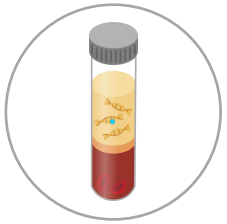
ACT=adjuvant chemotherapy; ctDNA=circulating tumor DNA; MRD=minimal residual disease; NACT=neoadjuvant chemotherapy.
Adapted from Wan JCM, Massie C, Garcia-Corbacho J, et al. Nat Rev Cancer. 2017;17(4):223-238

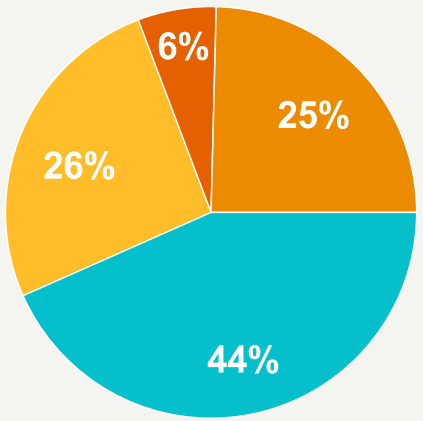
Liquid vs Tumor Tissue Biopsy for Biomarker Testing

	Advantages	Limitations
Tumor tissue 	• Pathology information • Other lab tests • High sensitivity • RNA	• Invasive • Represents snapshot of tumor genomics • Longer TAT • Rebiopsy not always feasible
Liquid biopsy 	• Less invasive, easy sequential testing • More representative of tumor heterogeneity • Rapid TAT	• Sensitivity depends on tumor shed rate • Result interference by other conditions • Lower sensitivity for specific types of alterations

CHIP=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.

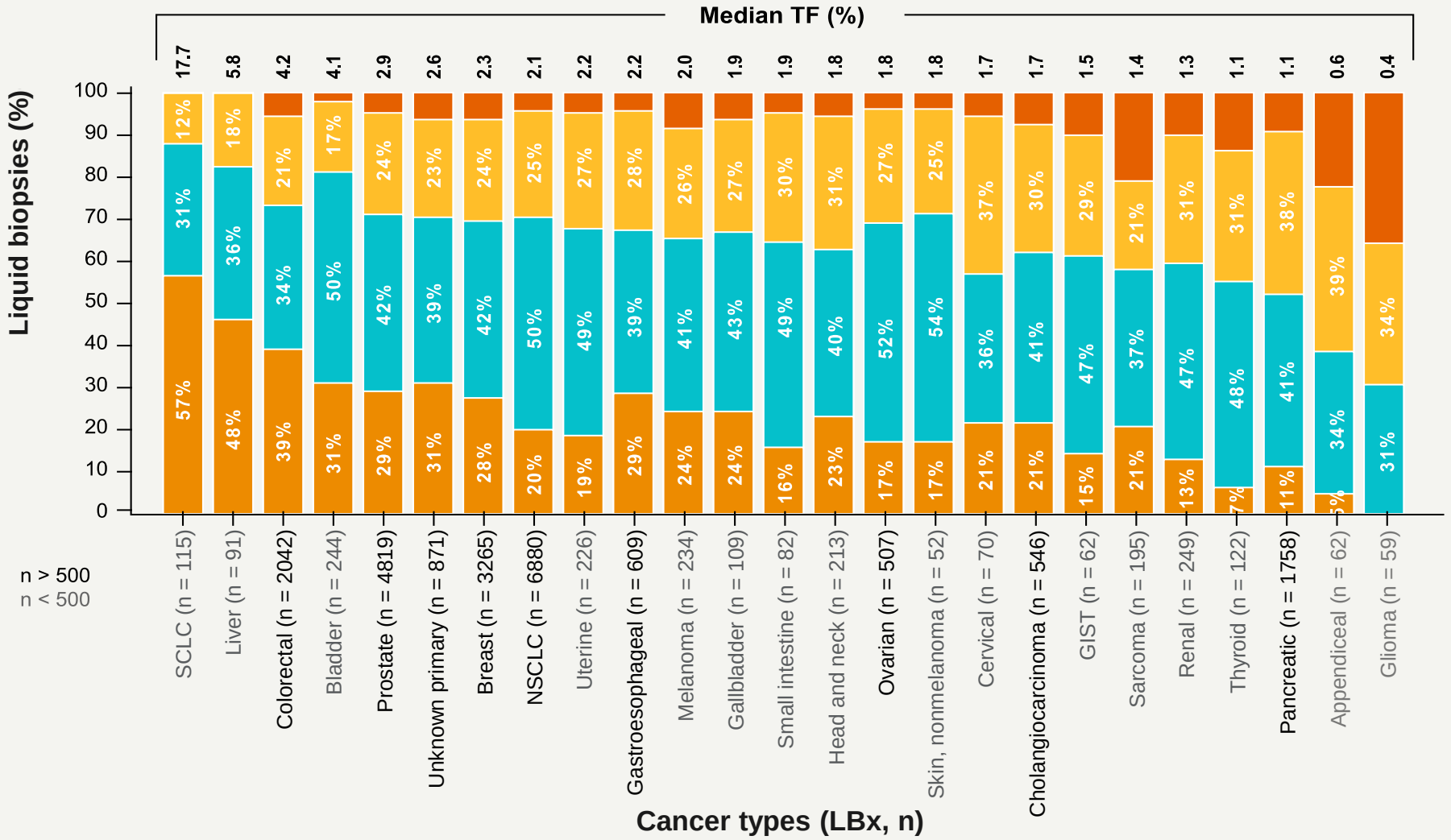
ctDNA Detection Rate Varies across Tumor Types



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

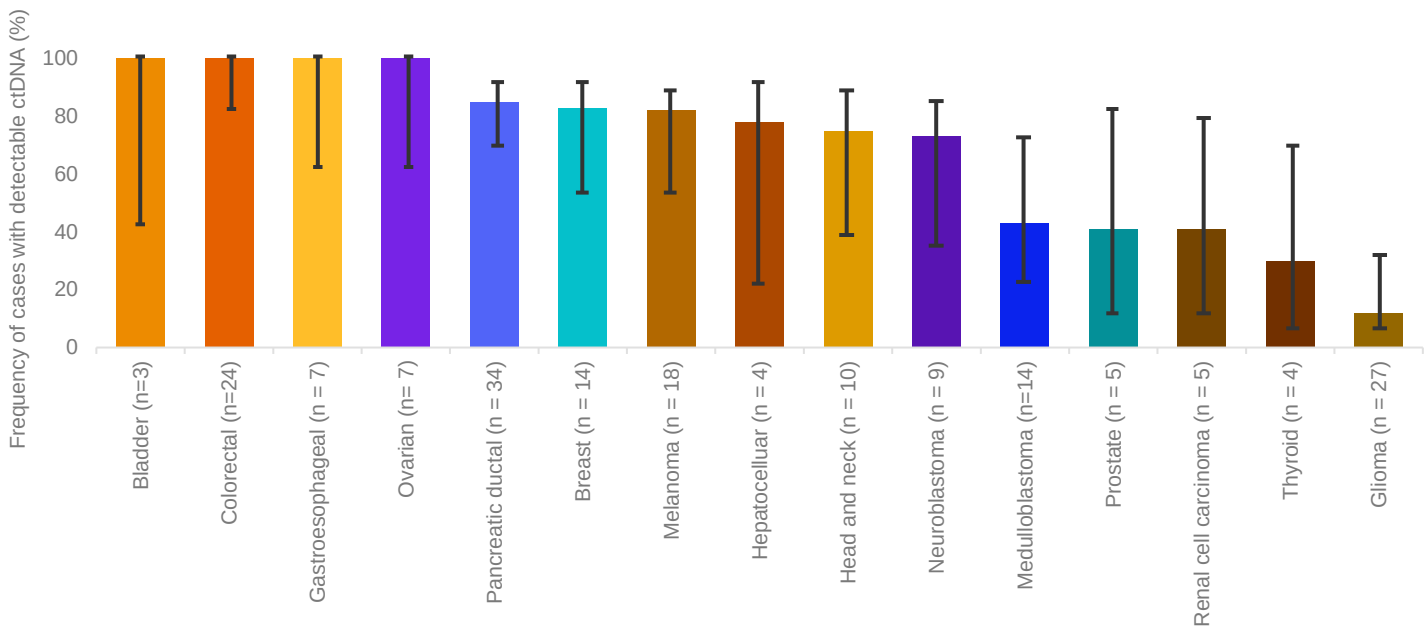
Husain H, Pavlick DC, Fendler BJ, et al. JCO Precis Oncol. 2022;6:e2200261

Not All Cancers Shed ctDNA Equally

Factors affecting ctDNA levels and detection:

- Disease sites
- Tumor burden
- Timing of blood sampling
- Disease status
  (stable vs progressive disease)
- Type of variant

Detection rate varies across tumor types

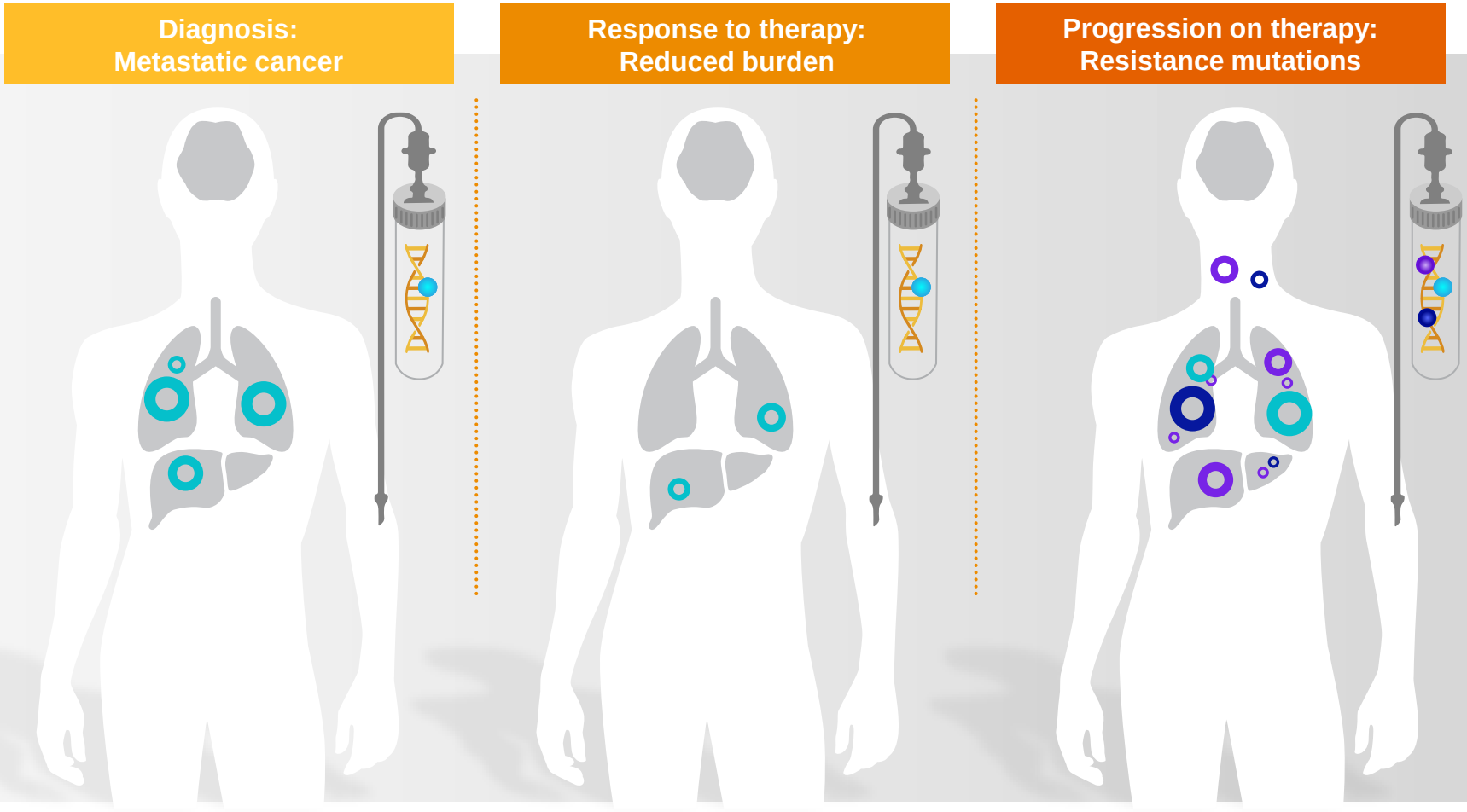


ctDNA=circulating tumor DNA

Bettegowda C, Sausen M, Leary R, et al. Science Translational Medicine 2014; 6:224, pp 224re24

Liquid Biopsies can Capture Intratumoral and Intertumoral Heterogeneity

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



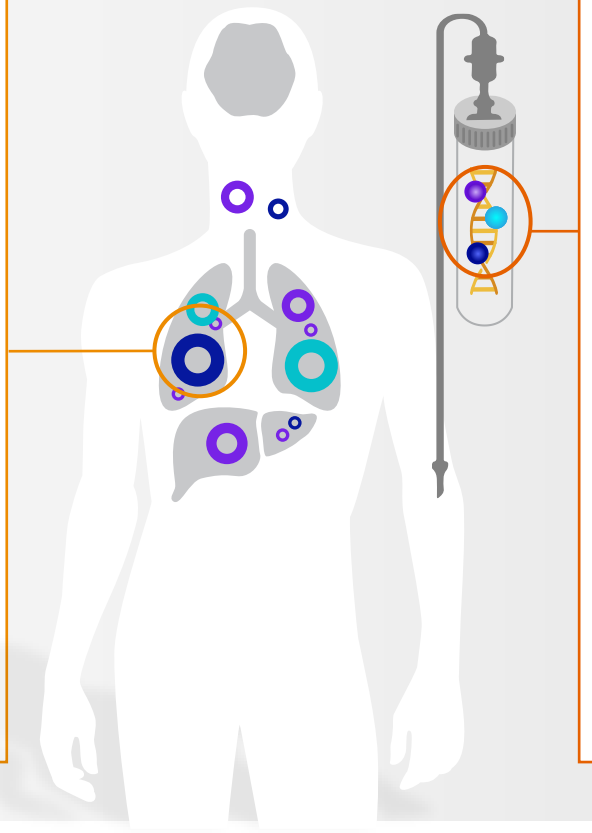
ctDNA=circulating tumor DNA

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

Factors Influencing ctDNA Levels and Detection

Disease characteristics

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



Other factors

- Sample timing related 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

NTRK Fusions with Large Introns are Missed with DNA-Only Sequencing Tests

Testing both DNA and RNA is the most sensitive way to identify fusions

76 patients
with *NTRK* fusions identified by genomic testing

46 patients
tested with both DNA and RNA assays

30 patients
received either:
DNA alone
RNA alone
FISH

***NTRK* missed by DNA**

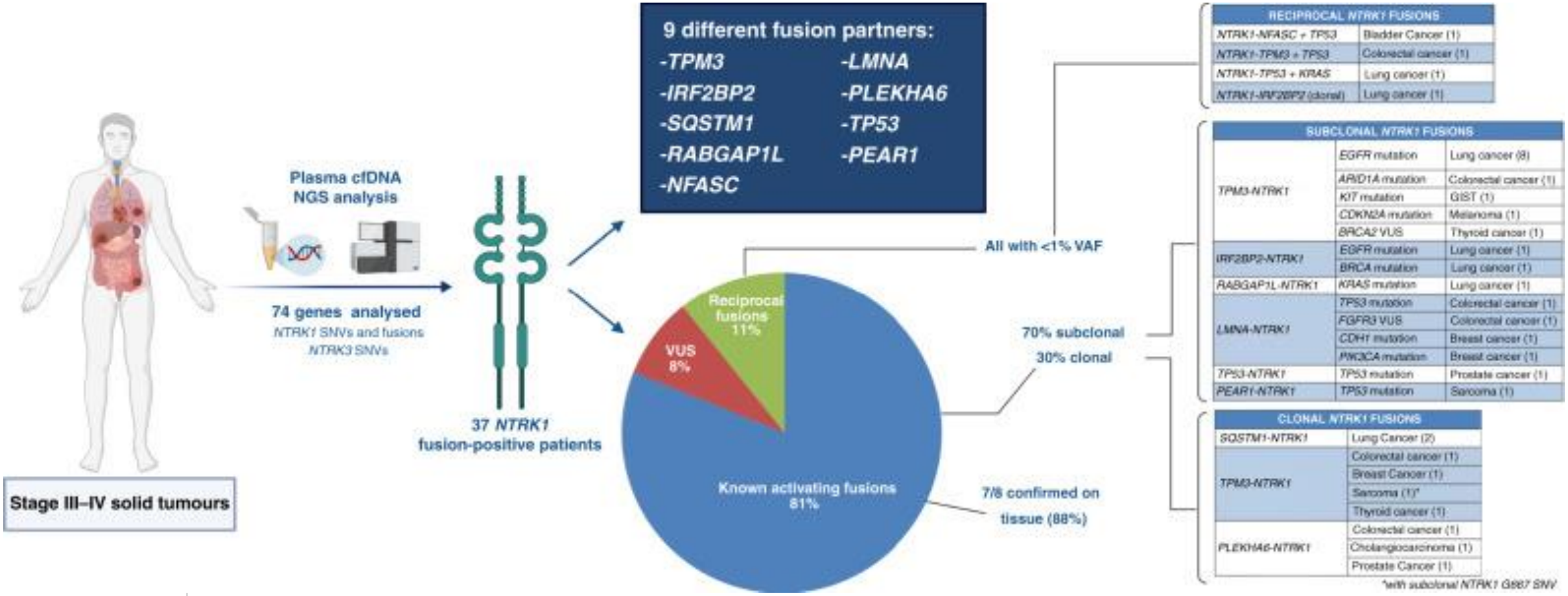
12 patients (26%)
NTRK fusions missed* by DNA testing were subsequently detected by RNA testing

***NTRK* identified by DNA**

34 patients (73%)
In-frame *NTRK* fusions identified by DNA testing and confirmed by RNA testing

*Missed fusions were *NTRK2/3* introns that couldn't be analyzed by the DNA panel due to size
FISH=fluorescence in situ hybridization
Rosen EY, Goldman DA, Hechtman JF, et al. Clin Cancer Res. 2020;26(7):1624-1632

Clinical Utility of Liquid Biopsy in *NTRK*-fusion positive patients



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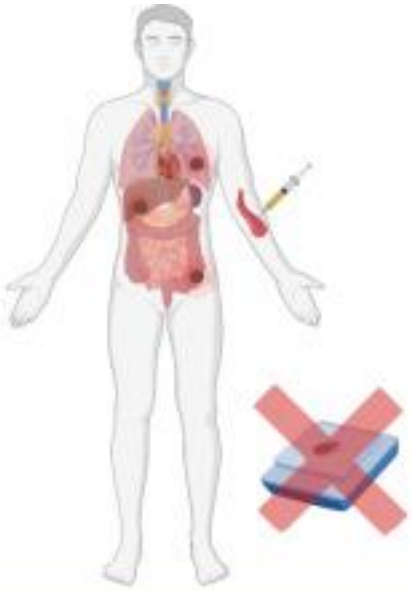
Drilon, A. Br J Cancer. 2021;126(3):514-520

Clinical Utility of Liquid Biopsy in *NTRK*-fusion positive patients



Identification of *NTRK* fusions in patients with insufficient tissue for comprehensive genotyping

Rescue of a significant proportion of undetected *NTRK* fusion-positive patients

Identification of the mechanisms of acquired resistance to TRK inhibitors as alternative to tissue re-biopsy

Secondary *NTRK* mutations off-target mechanisms

Identification of *NTRK* fusions as mechanisms of resistance to EGFR TKIs and other targeted therapies

Clinical trial enrollment Tailored strategies

Drilon, A. Br J Cancer. 2021;126(3):514-520

Key Takeaways