18th Annual

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LUNG CANCER SYMPOSIUM

Liquid Biopsy: Current and Future Applications

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Disclosure information

Valsamo Anagnostou

I have the following financial relationships to disclose:

Current/within the past 5 years: Grant/Research support (to Johns Hopkins) from: Astra Zeneca, Bristol-Myers Squibb, Personal Genome Diagnostics/Labcorp and Delfi Diagnostics; advisory board member for: Astra Zeneca and Neogenomics, honoraria: Foundation Medicine, Personal Genome Diagnostics

My additional financial relationship disclosures are:

I am an inventor on patent applications (63/276,525, 17/779,936, 16/312,152, 16/341,862, 17/047,006 and 17/598,690) submitted by Johns Hopkins University related to cancer genomic analyses, ctDNA therapeutic response monitoring and immunogenomic features of response to immunotherapy that have been licensed to one or more entities. Under the terms of these license agreements, the University and inventors are entitled to fees and royalty distributions.

-and-

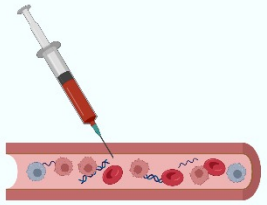
I will not discuss off label use and/or investigational use in my presentation.

Comprehensive genomic profiling (CGP)

- Single NGS assay that assesses a variety of genomic alterations
- Single nucleotide variants, indels, splice variants, copy number variation, fusions
- Genomic signatures, tumor mutation burden, microsatellite instability, homologous recombination deficiency

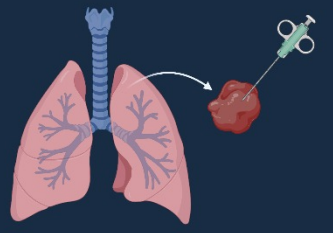
ctDNA CGP

- Minimally invasive
- Fast turn around time
- Higher false negative rate
- Higher false positive rate (CH, aging)
- Varying LOD based on alteration
- Captures tumor heterogeneity
- Captures tumor evolution and new targets
- May alleviate health disparities in genomic testing

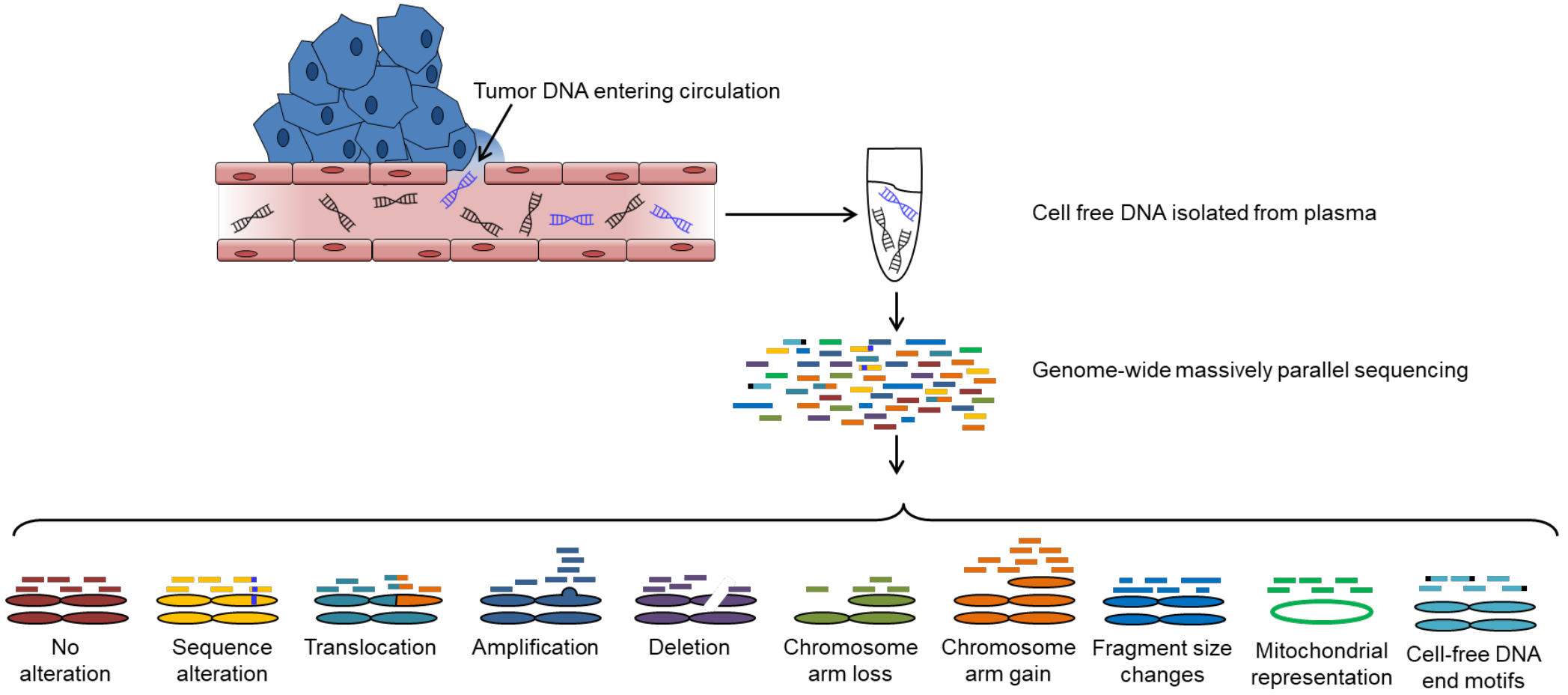


Tumor tissue CGP

- Gold standard for tumor genotyping
- Invasive
- Longer turnaround time
- Higher rate of failed or missing tumor tissue sequencing
- Higher false negative rate when tumor purity is low
- Does not capture tumor heterogeneity or evolution
- Disparities in tumor CPG

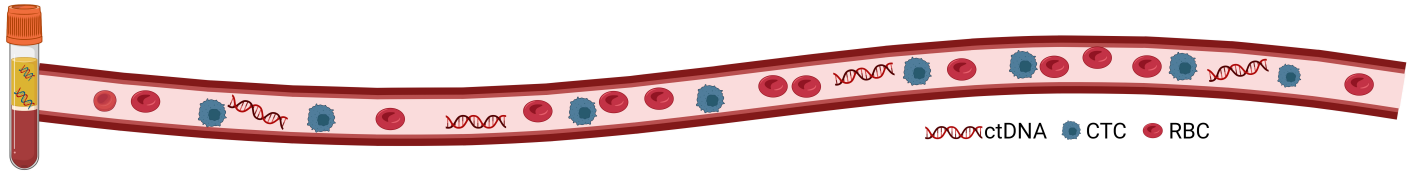


Liquid biopsy approaches for sensitive and specific detection of cancer

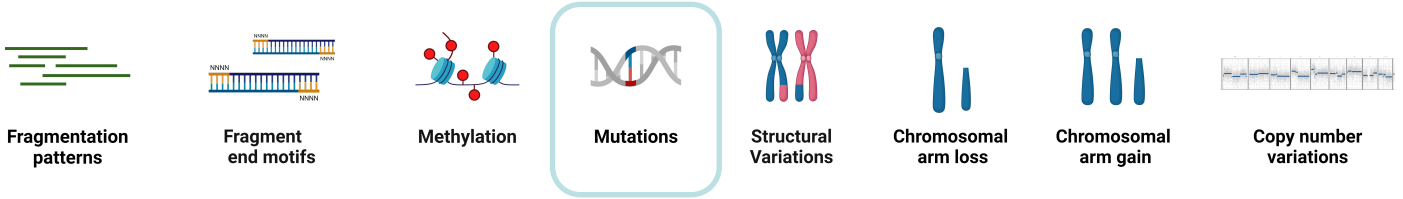


Bruhm et al., *Nat Genet*, 2023, Mattox et al., *Cancer Discov*, 2023, Wang et al., *PNAS*, 2023, Sivapalan et al., *Clin Can Res*, 2023, Foda et al., *Cancer Discov*, 2023
Cohen et al., *Nat Biotechnol*, 2021, Cristiano et al., *Nature*, 2019, Anagnostou et al., *Can Res*, 2019, 2020, Cohen et al., *Science*, 2018, Phallen et al., *Science TM*, 2017

Liquid biopsy methods for ctDNA detection



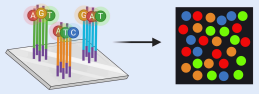
Compendium of genomic and epigenetic alterations in ctDNA



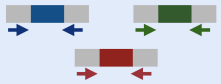
Tumor Informed

1. Tissue biopsy & blood

2. Tumor tissue sequencing (panel NGS, WES, WGS)



3. Personalized ctDNA assay



Advantages

- Higher Sensitivity
- Higher Specificity
- Low false positive rate

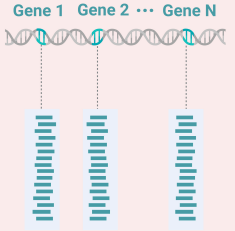
Disadvantages

- Requires invasive biopsy
- Higher failure rate
- Costly
- Time consuming

Tumor Agnostic

1. Blood only

2. cfDNA next-gen sequencing
± matched WBC NGS
± cfDNA targeted methylation



Advantages

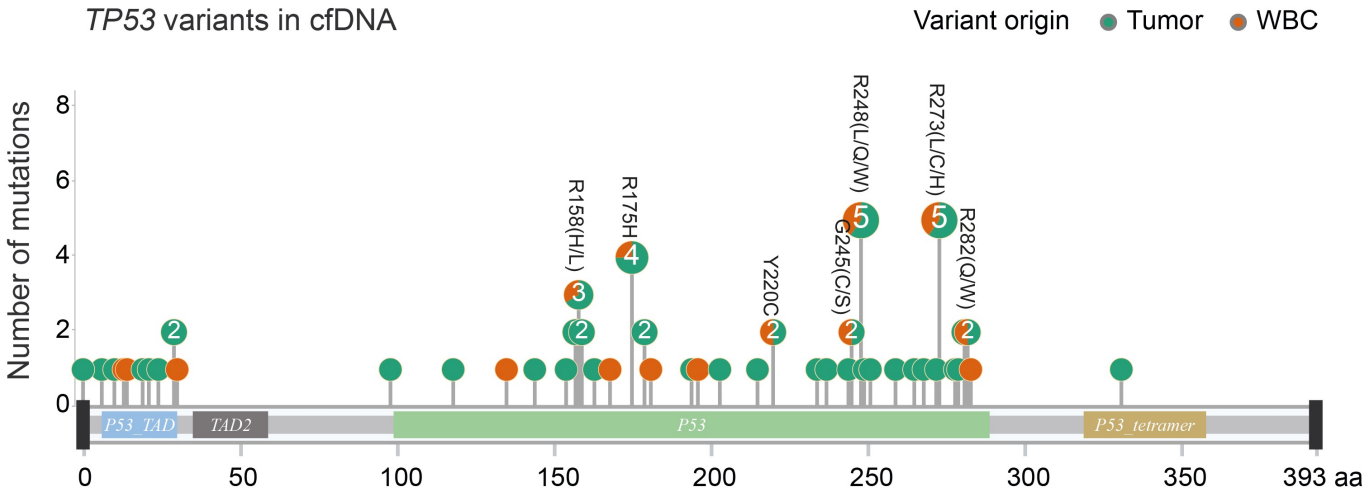
- Feasible
- Cost effective
- Fast turnaround time
- Minimally invasive
- Captures tumor heterogeneity
- Captures tumor evolution and new targets

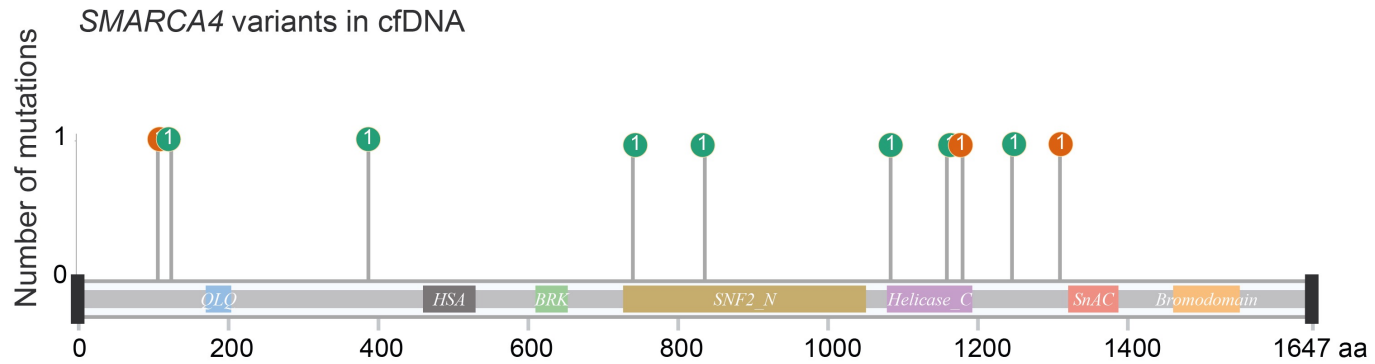
Disadvantages

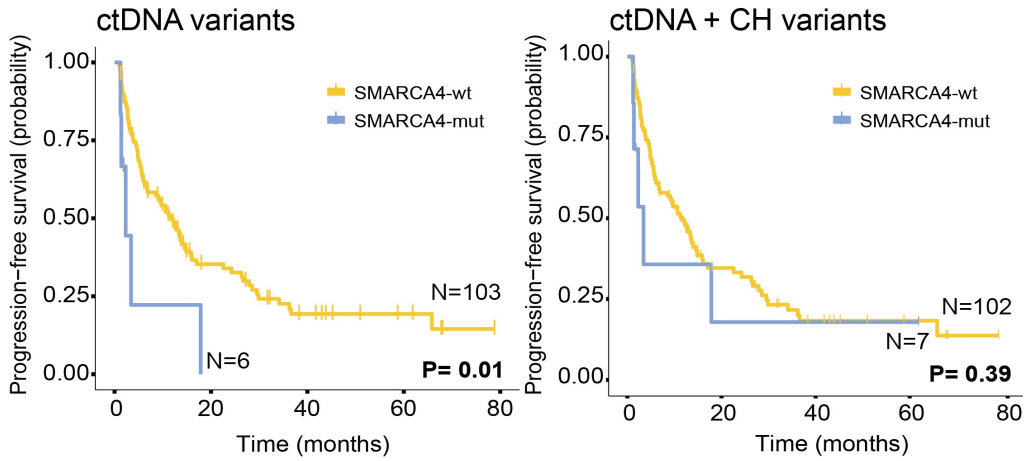
- Higher false negative rate (suboptimal LOD)
- Clonal hematopoiesis contamination (if plasma only approach)

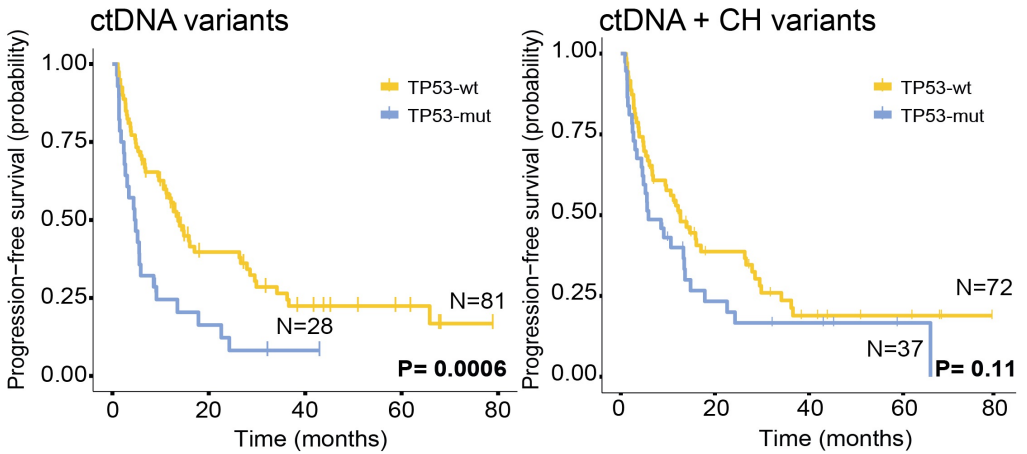
Landon et al., *International Review of Cell and Molecular Biology* , in press

The confounding effect of clonal hematopoiesis variants in clinical outcome prediction



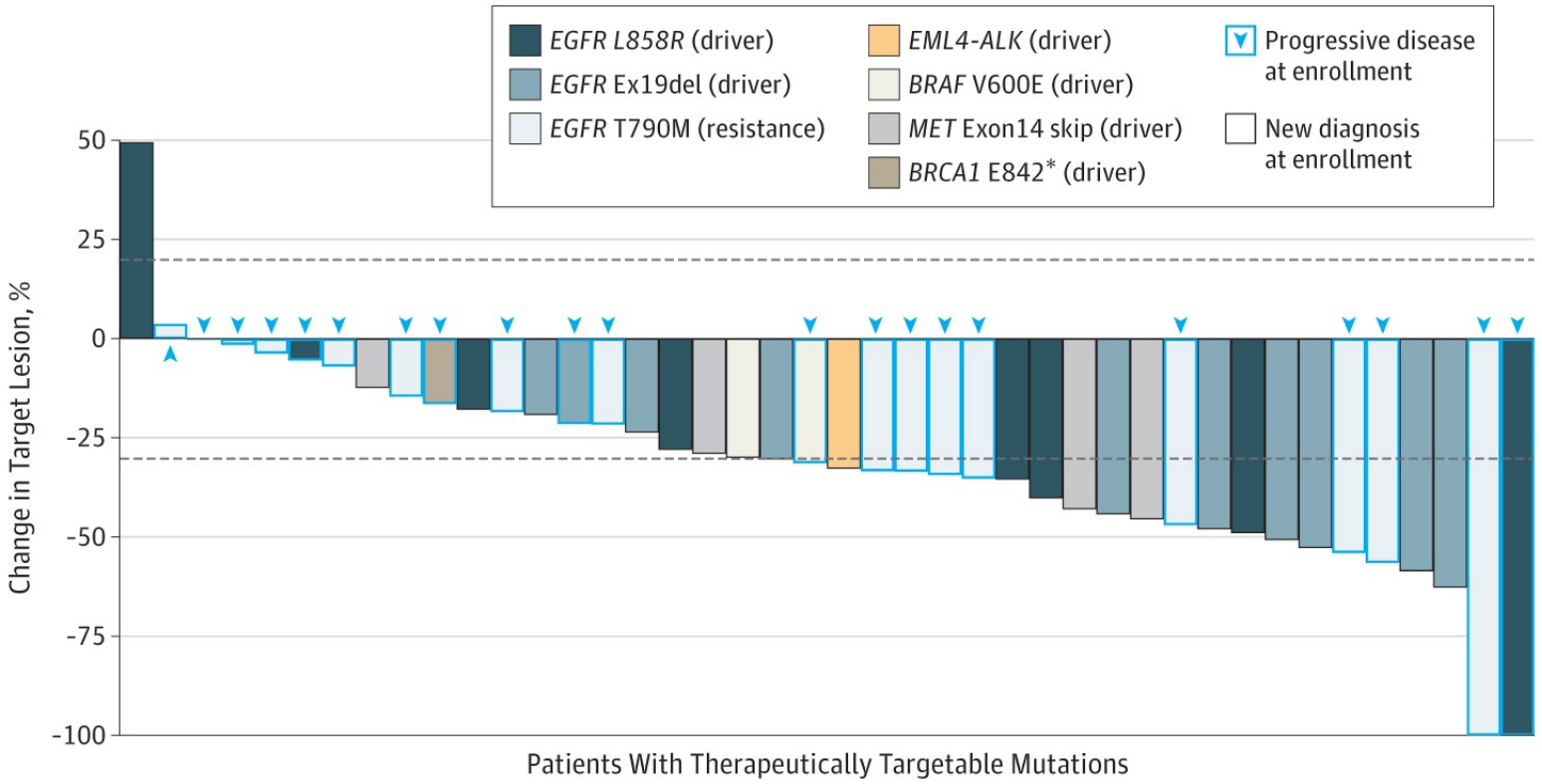


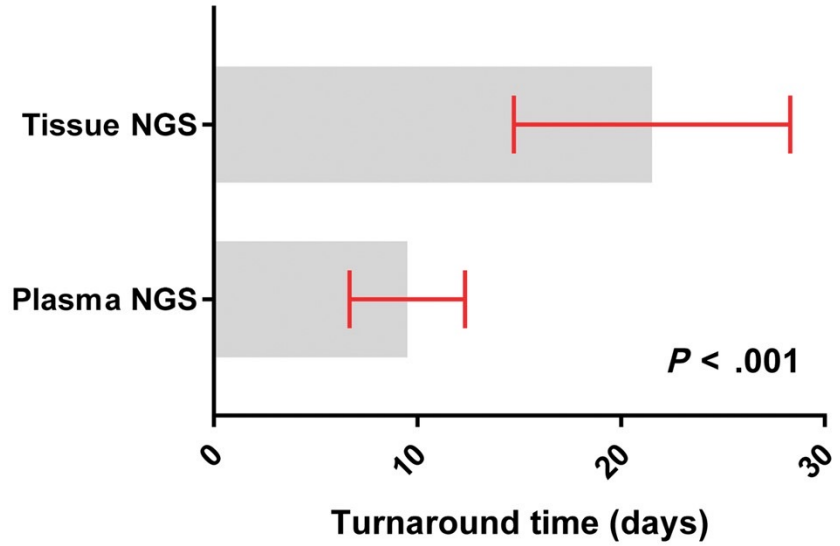




Sivapalan et al., *presented at AACR24*

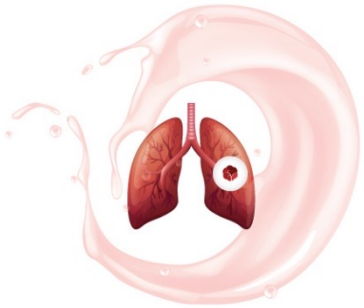
ctDNA CGP may overcome challenges with tissue CGP



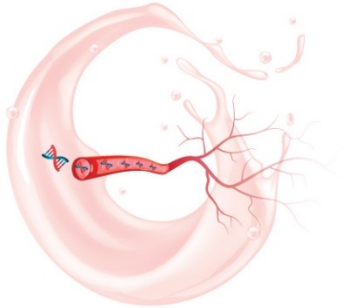


Aggarwal et et al., *JAMA Oncol*, 2019, Sabari et al., *J Natl Cancer Inst*, 2019

Capturing residual disease through liquid biopsies



Tumor arises.



Tumor cells shed ctDNA in the blood stream.

ctDNA is isolated from serial blood draws.

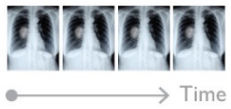
Mutations in ctDNA are detected by NGS.

Detection of Residual Disease

Liquid biopsies allow for minimal residual disease detection after surgery.

Detection of ctDNA early after definitive therapy may be a robust biomarker for minimal residual disease.

Monitoring Therapeutic Response

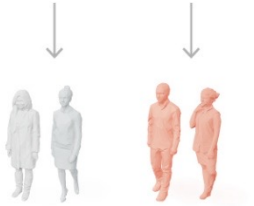


Imaging is inadequate to capture clinical response in the setting of immunotherapy.

Liquid biopsies allow for minimally invasive, real time tracking of circulating tumor burden during therapy.

Interventional ctDNA Trials

Interventional ctDNA trials rely on molecular response assessments during therapy.



Patients are stratified by ctDNA response, followed by treatment escalation or de-escalation.

Sivapalan et al., *JITC Special Review Series on Liquid Biopsies,* 2023, Anagnostou and Velculescu, *Cancer Discovery,* 2024

Landmark ctDNA MRD predicts outcomes for NSCLC

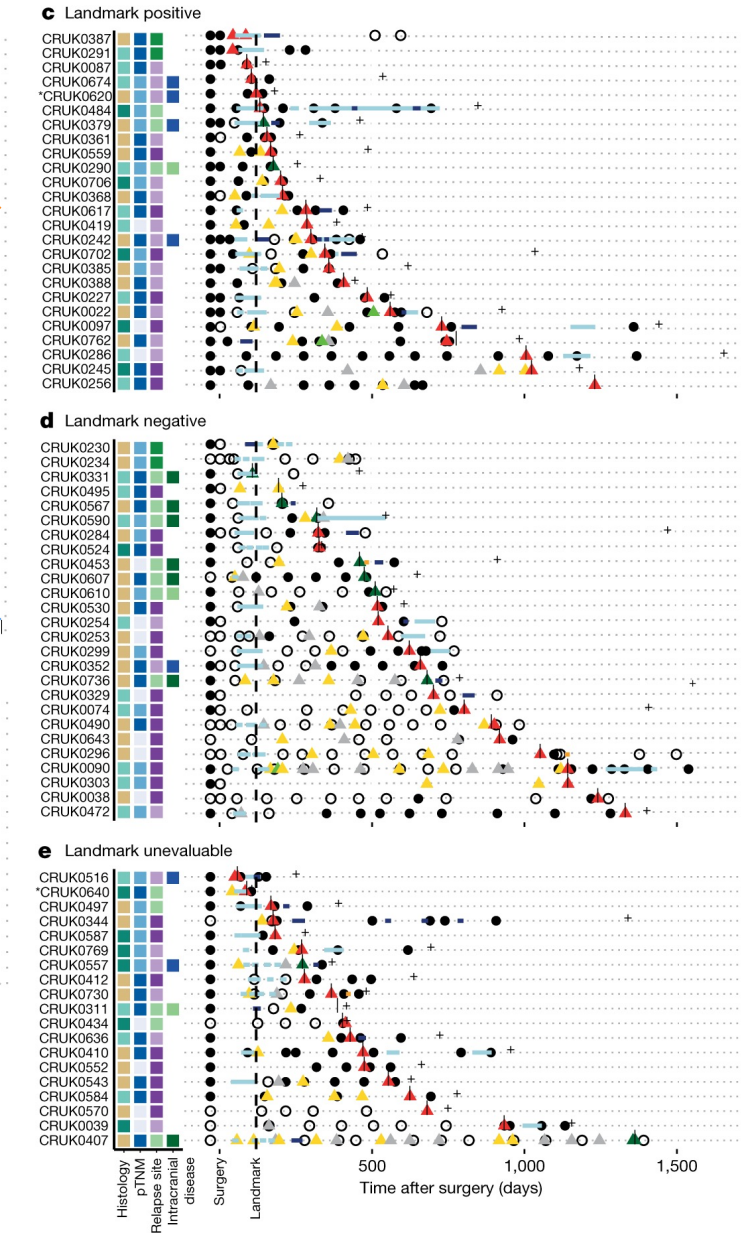
ctDNA MRD+
Recurrence +
(n=25)

Clinical sensitivity 49%

ctDNA MRD-
Recurrence +
(n=26)

51% discordance

ctDNA in-evaluable
Recurrence +
(n=19)



- Tumor-informed anchored multiplex PCR enrichment
- Assay sensitivity using a 50-variant panel at 0.01% VAF was > 90% at DNA input > 20 ng
- LOD 95 VAF 0.008% (80 PPM)
- **Landmark ctDNA MRD assessed within 120 days of surgery: 25% ctDNA MRD+**
- **Clinical sensitivity 49% (fraction of ctDNA MRD+ among those who recurred)**
- Landmark ctDNA MRD+ patients had a hazard ratio of 5.3 for OS and a hazard ratio of 6.8 for freedom from relapse relative to MRD- (P<0.001)
- Landmark-positive patients had the longest lead times (228 days)
- Patients relapsing in the first year of surgery are more likely to be MRD positive

Abbosh et al., *Nature*, 2023

What is the role of ctDNA MRD in navigating the evolving therapeutic landscape of early-stage NSCLC?

- Numerous treatment options are currently available to reduce the risk of recurrence in patients with early-stage resectable non–small-cell lung cancer (NSCLC), including neoadjuvant and adjuvant immunotherapy, which have been shown to improve outcomes in patients undergoing surgery.

- Major pathological response (MPR; ≤10% viable tumor cells remain after surgery) or pathological complete response (pCR; no viable tumor cells remaining after surgery) have been shown to predict disease-free survival post neoadjuvant immune checkpoint blockade and are commonly used as surrogate endpoints.

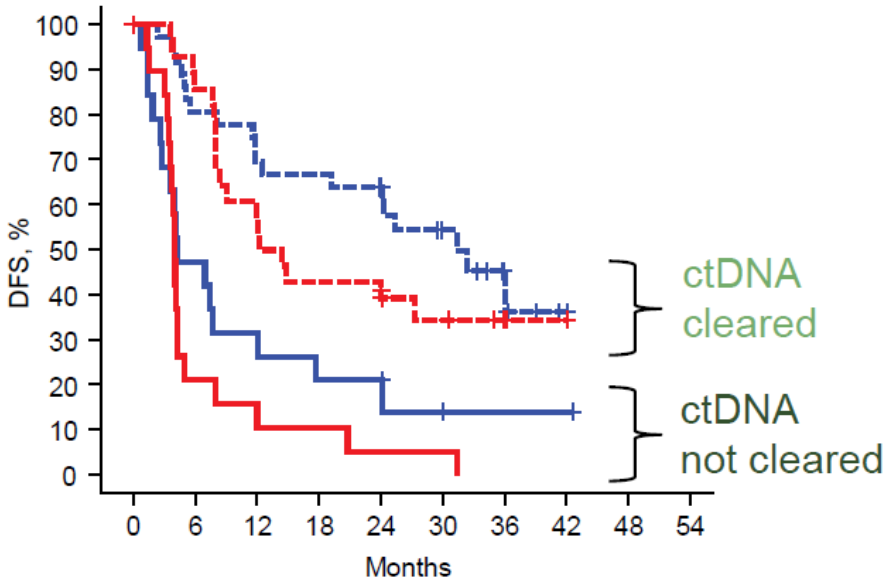
- Despite the expanding field, we are limited in our ability to **risk-stratify** patients for neoadjuvant immunotherapy or **personalize adjuvant therapy** following neoadjuvant immunotherapy.

- It is imperative to employ **ultra-sensitive ctDNA approaches** to define the subset of patients who will benefit most from additional therapy in the neoadjuvant/adjuvant space that is the pre-requisite stepping stone for the **design of ctDNA interventional clinical trials for patients with resectable NSCLC**.

Felip E et al., *Lancet,* 2021, O'Brien M et al., *Lancet Oncol,* 2022, Cascone T et al., *N Engl J Med,* 2024, Heymach JV et al., *N Engl J Med,* 2023, Forde PM et al., *N Engl J Med,* 2022, Lu S et al., *JAMA*, 2024, Wakelee H et al., *N Engl J Med*, 2023

IMpower-010: Adjuvant immunotherapy may delay ctDNA MRD emergence

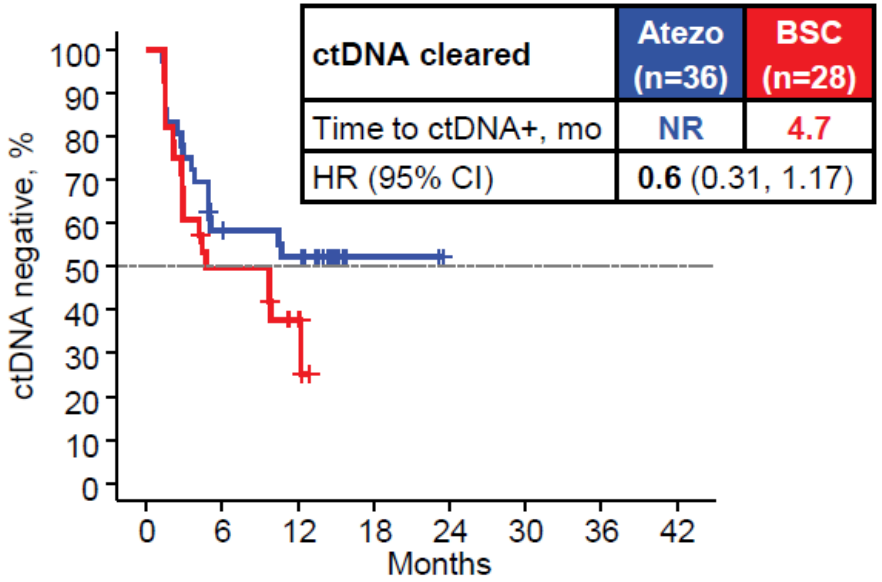
DFS by treatment arm and post-chemo ctDNA clearance status



ctDNA cleared	Atezo (n=36)	BSC (n=28)
mDFS, mo	31.3	13.3
HR (95% CI)	**0.7** (0.37, 1.34)	

ctDNA not cleared	Atezo (n=19)	BSC (n=20)
mDFS, mo	4.2	3.9
HR (95% CI)	**0.67** (0.34, 1.32)	

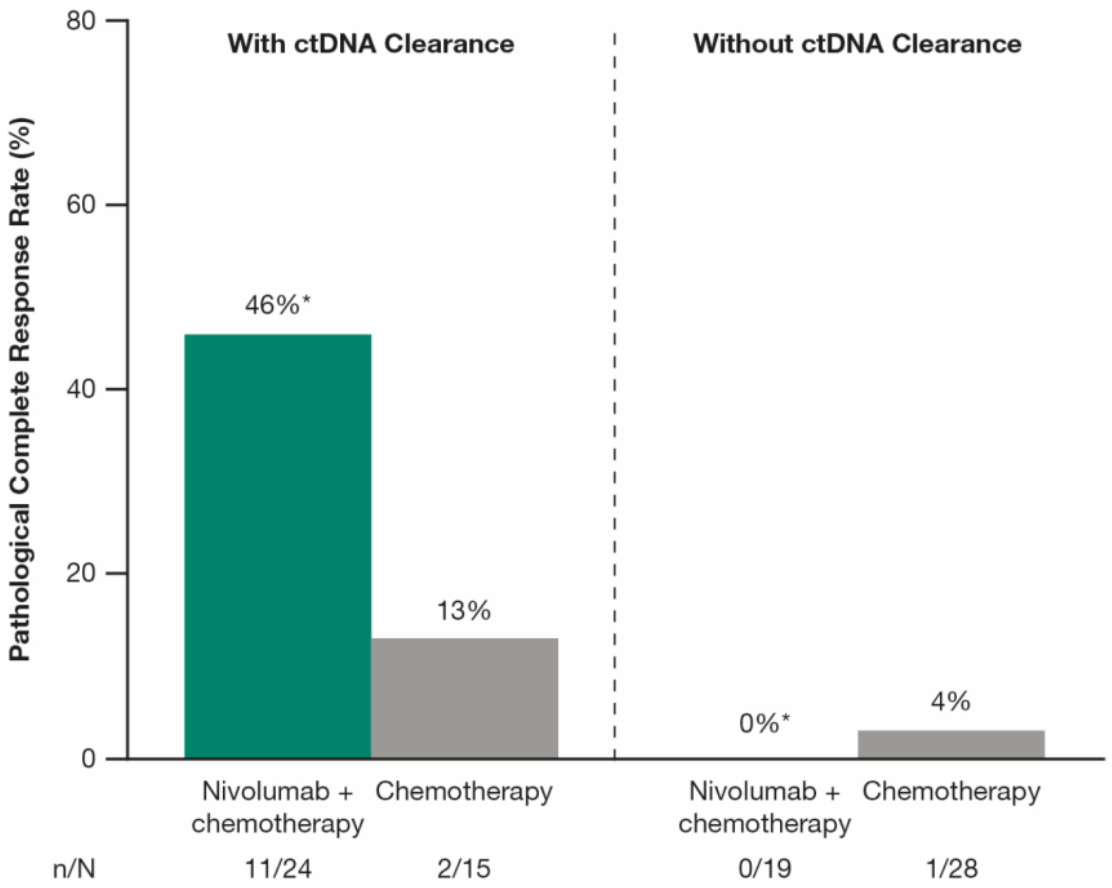
Time to ctDNA+ by treatment arm



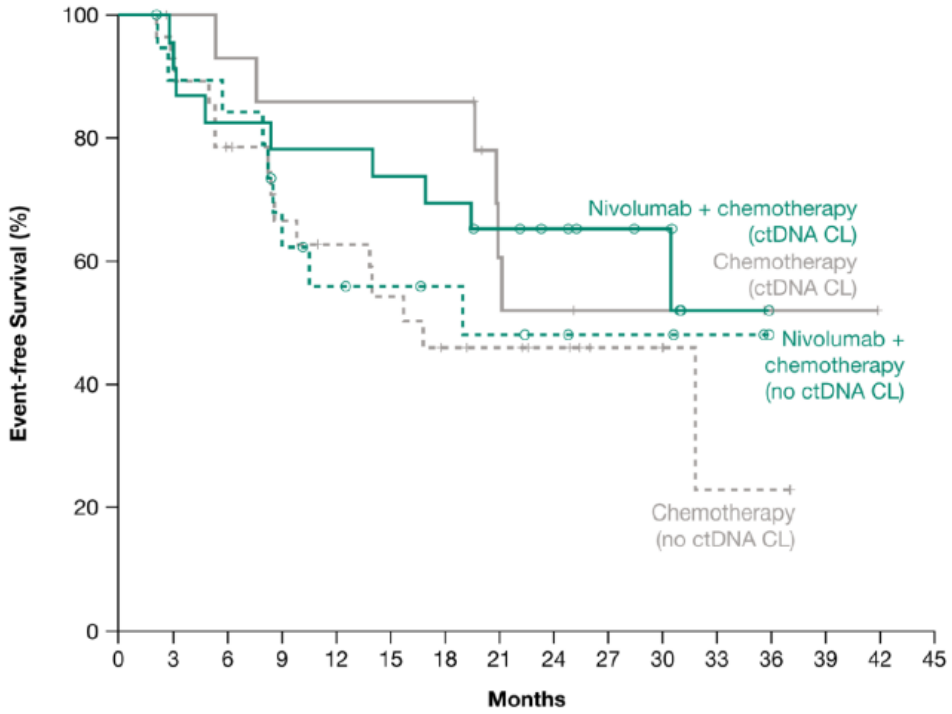
ctDNA cleared	Atezo (n=36)	BSC (n=28)
Time to ctDNA+, mo	NR	4.7
HR (95% CI)	**0.6** (0.31, 1.17)	

Felip et al., presented at *ESMO Immuno-Oncology*, 2022

Pre-op ctDNA clearance predicts pCR and EFS with neoadjuvant chemo-immunotherapy

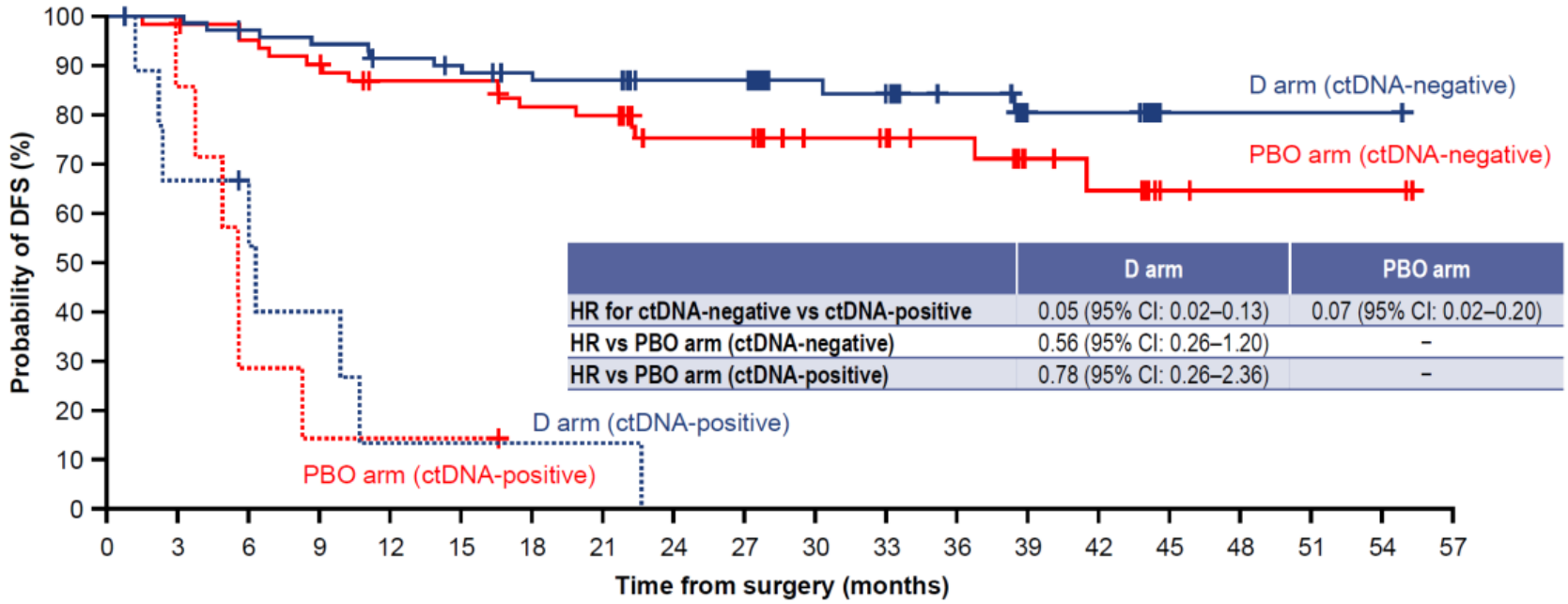


	Nivolumab + chemotherapy		Chemotherapy	
	ctDNA CL (n=24)	No ctDNA CL (n=19)	ctDNA CL (n=15)	No ctDNA CL (n=28)
Median EFS, mo (95% CI)	NR (16.8–NR)	18.9 (8.3–NR)	NR (19.6–NR)	16.8 (8.3–NR)
HR (95% CI)	0.60 (0.20–1.82)		0.63 (0.20–2.01)	



Forde et al., *NEJM*, 2022

Post-op ctDNA clearance predicts DFS (AEGEAN trial)



	D arm	PBO arm
HR for ctDNA-negative vs ctDNA-positive	0.05 (95% CI: 0.02–0.13)	0.07 (95% CI: 0.02–0.20)
HR vs PBO arm (ctDNA-negative)	0.56 (95% CI: 0.26–1.20)	–
HR vs PBO arm (ctDNA-positive)	0.78 (95% CI: 0.26–2.36)	–

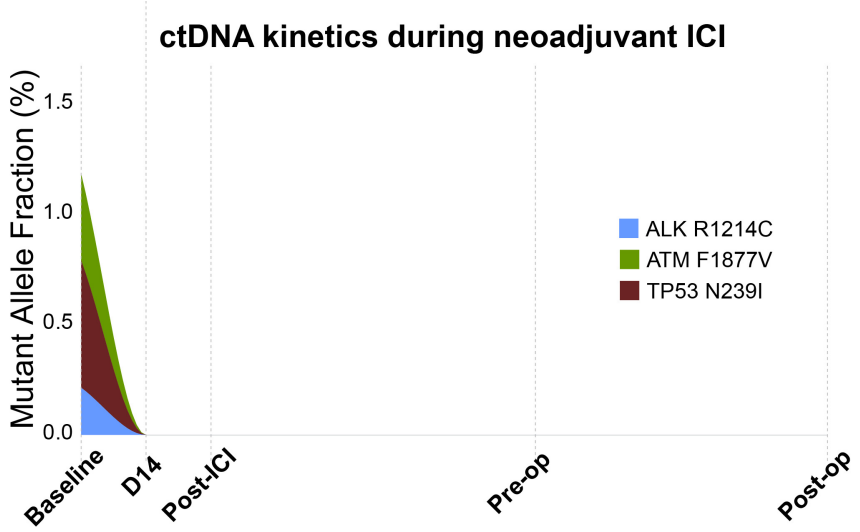
No. at risk

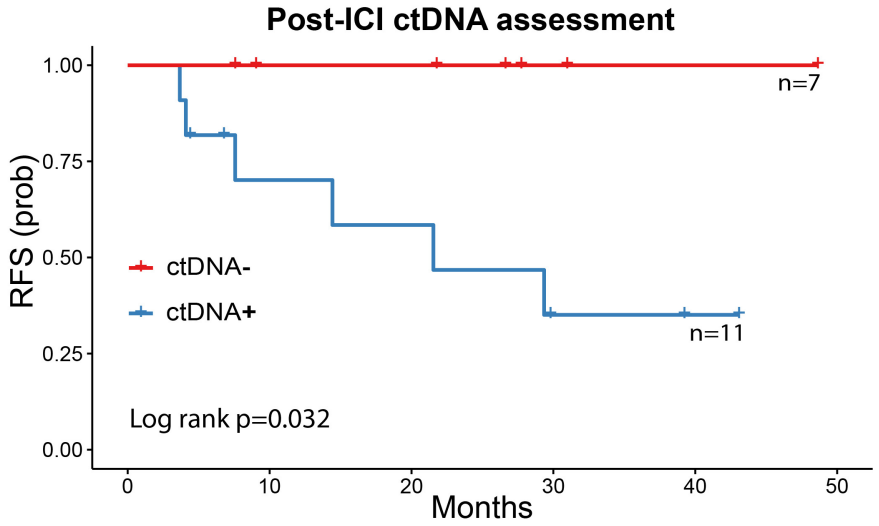
D arm (ctDNA-negative)	72	71	68	66	63	61	58	56	48	48	32	30	24	14	14	1	1	1	1	0
D arm (ctDNA-positive)	9	6	5	3	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
PBO arm (ctDNA-negative)	62	61	58	55	50	50	46	45	32	32	25	24	18	12	10	3	2	2	2	0
PBO arm (ctDNA-positive)	7	6	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

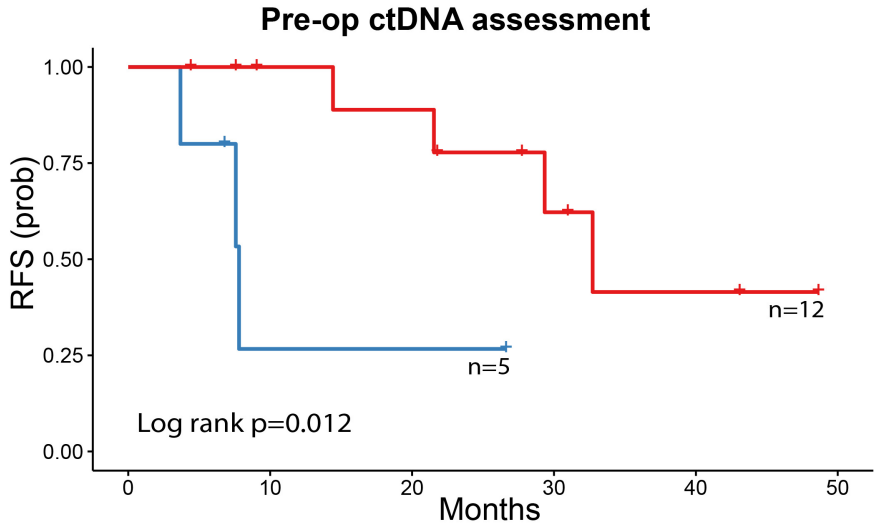
- In the AEGEAN clinical trial, patients with ctDNA detected at adjuvant C1D1 had the poorest disease-free survival outcomes compared to ctDNA-negative patients in both treatment arms.

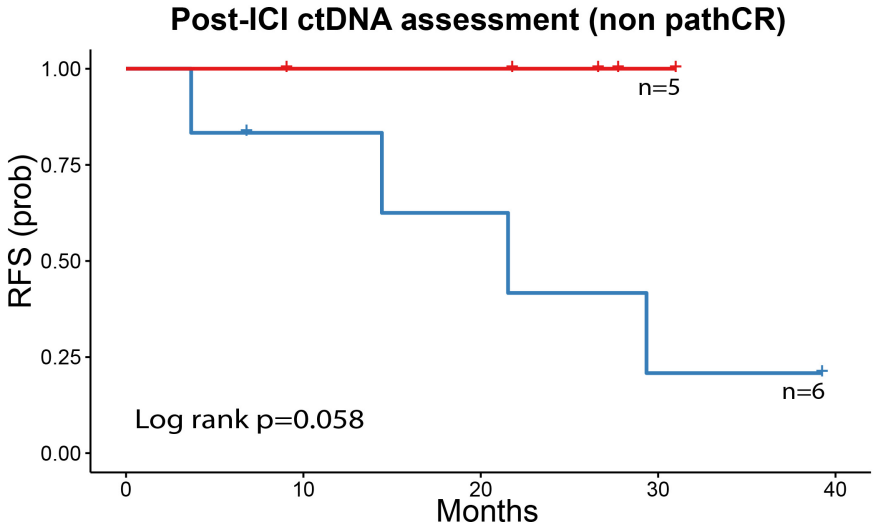
Reck M et al., *presented at ESMO* 2024

ctDNA assessment may help refine the heterogeneity of non pCR



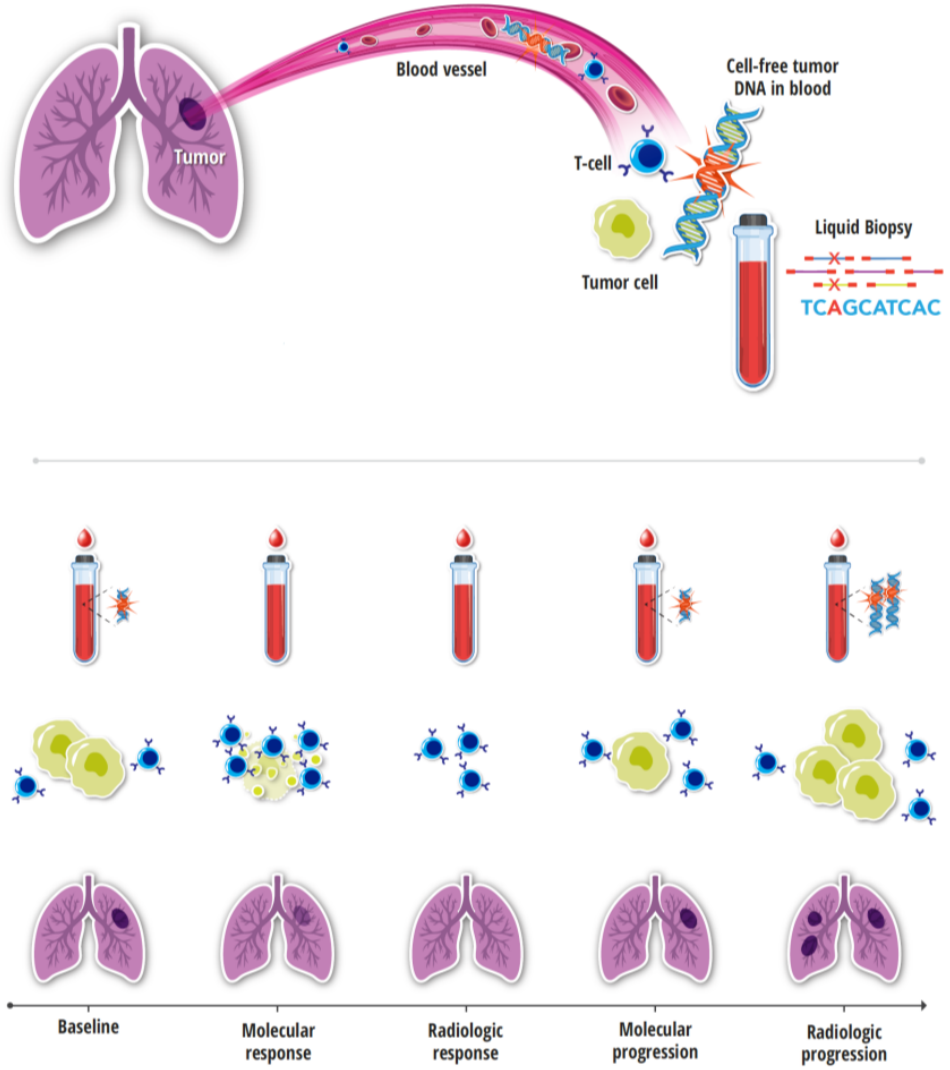


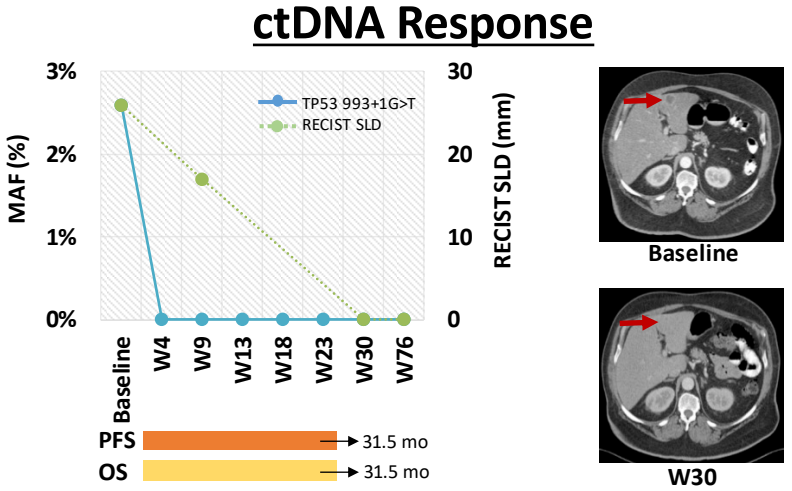


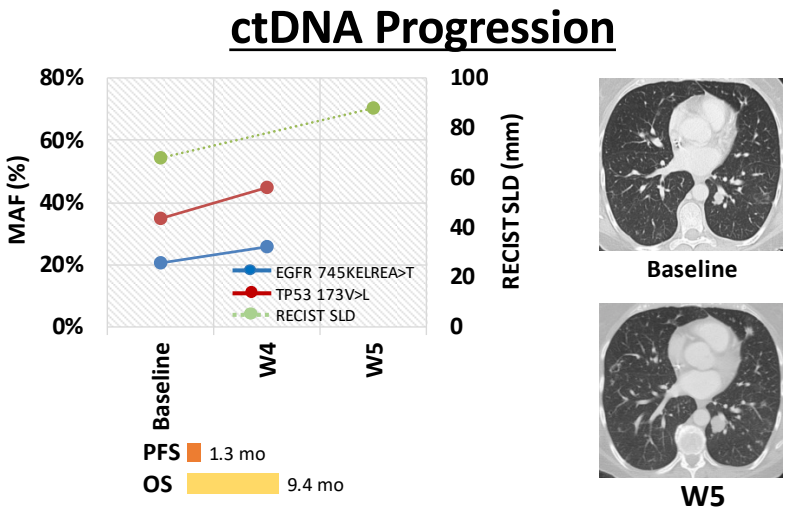


Kelly, Landon et al., *Nat Med,* 2024

ctDNA dynamics during immune checkpoint blockade

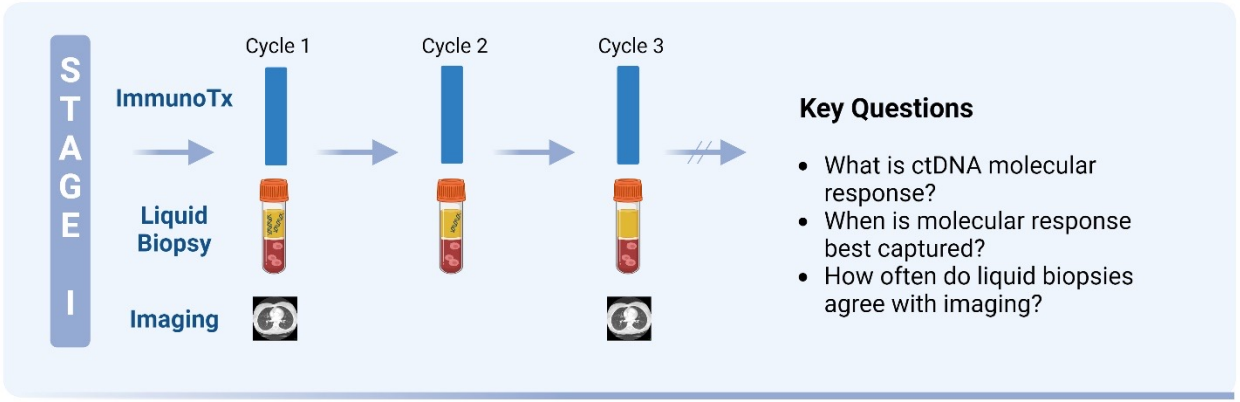




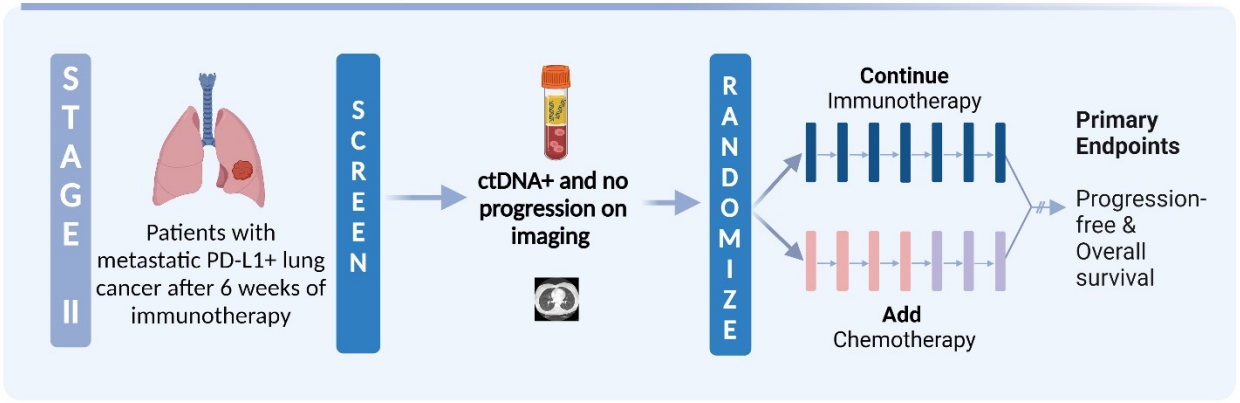


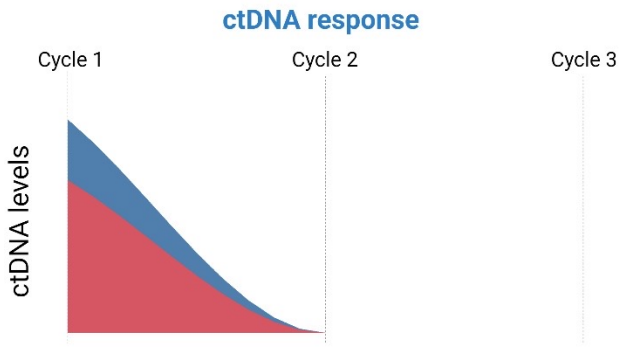
Anagnostou, Forde et al., *Cancer Res*, 2019, Huang et al., *J Immunother Cancer*, 2022, Sivapalan et al., *Clin Can Res*, 2023, Murray, Sivapalan et al., *Clin Can Res*, 2024

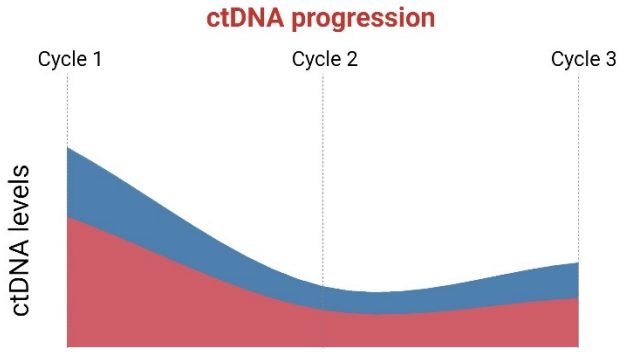
BR.36: A ctDNA-directed phase II/III study of molecular response adaptive immunotherapy in NSCLC

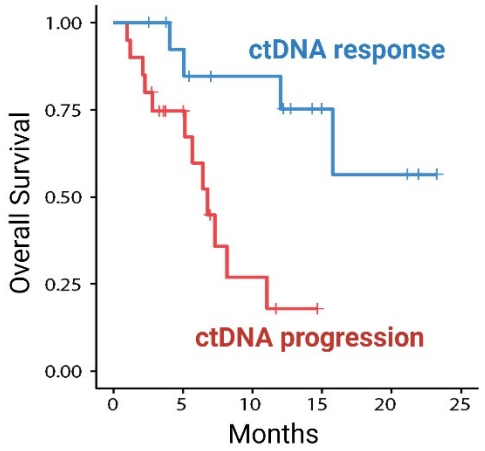


Individuals with ctDNA progression on pembrolizumab can be rapidly and accurately identified followed by treatment escalation







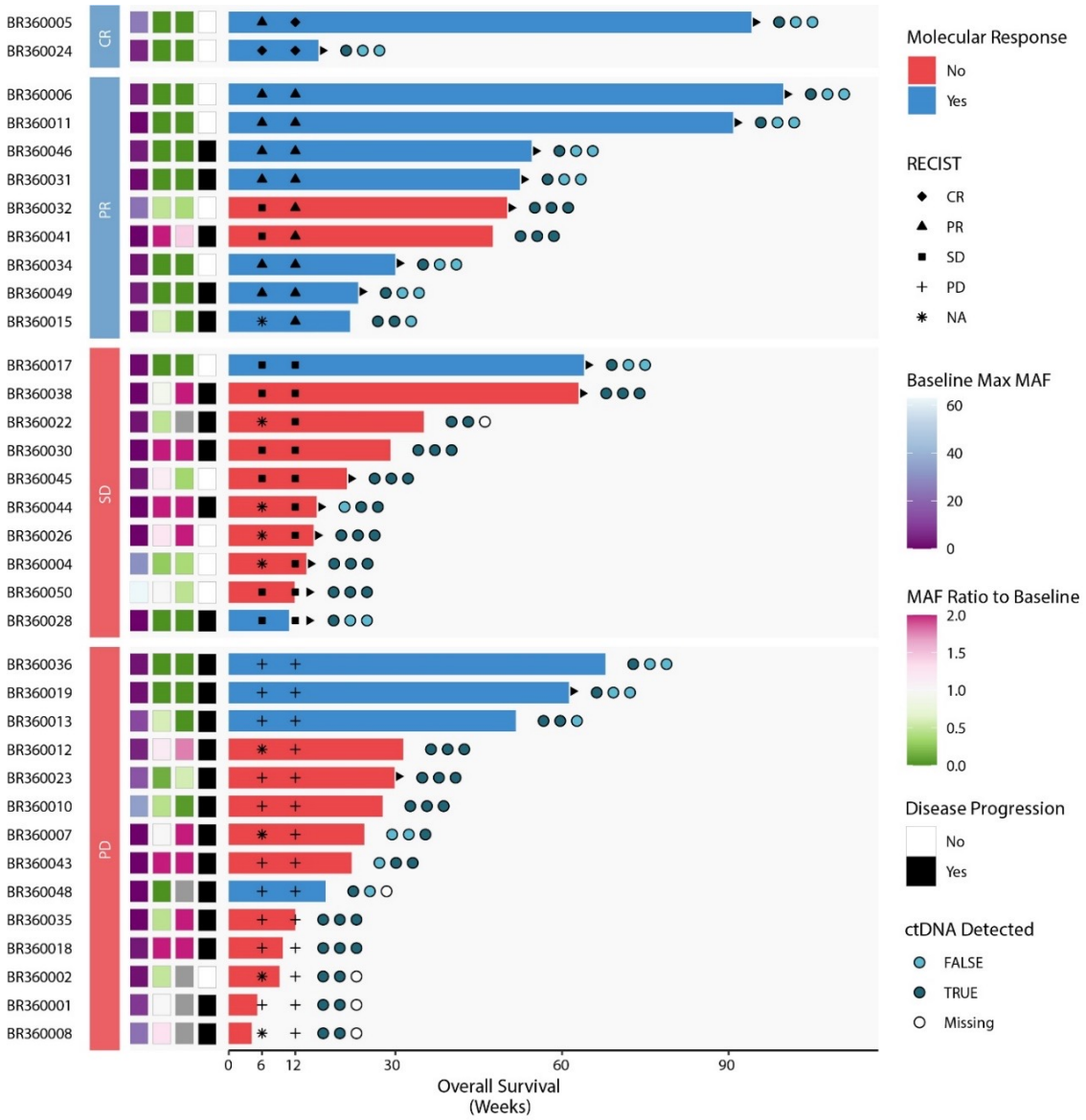


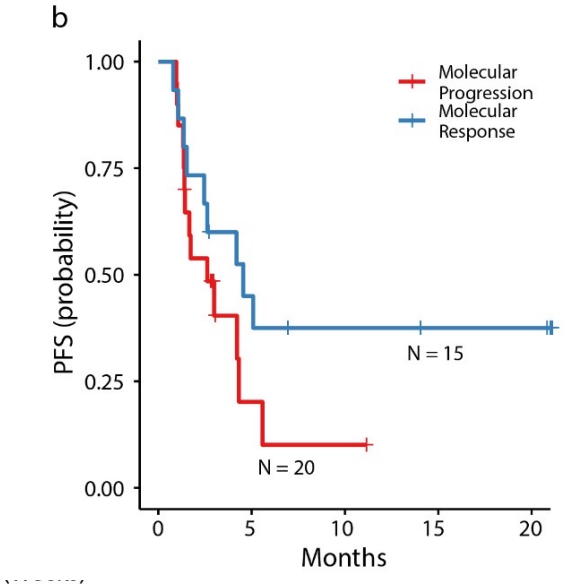
Core Conclusions (BR.36 stage 1)

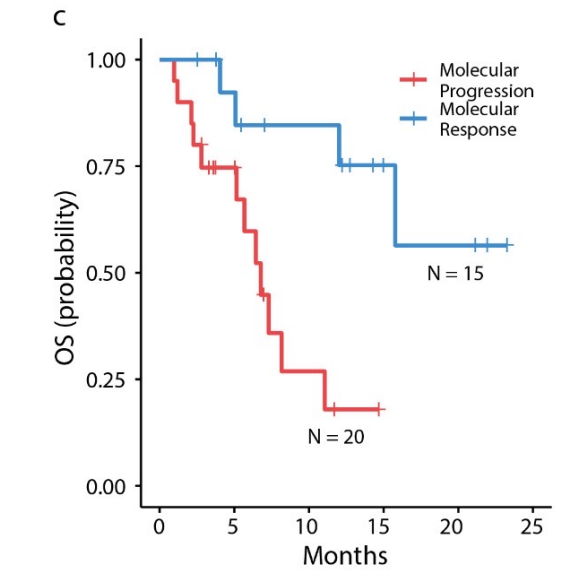
- Undetectable ctDNA 6 weeks on pembrolizumab provides an early measure of clinical response.
- Molecular responses overall agree with imaging (82% sensitivity).
- Liquid biopsies better predict overall survival.

CCTG BR.36, NCT04093167, Anagnostou et al., *Nat Med*, 2023

ctDNA-RECIST response concordance



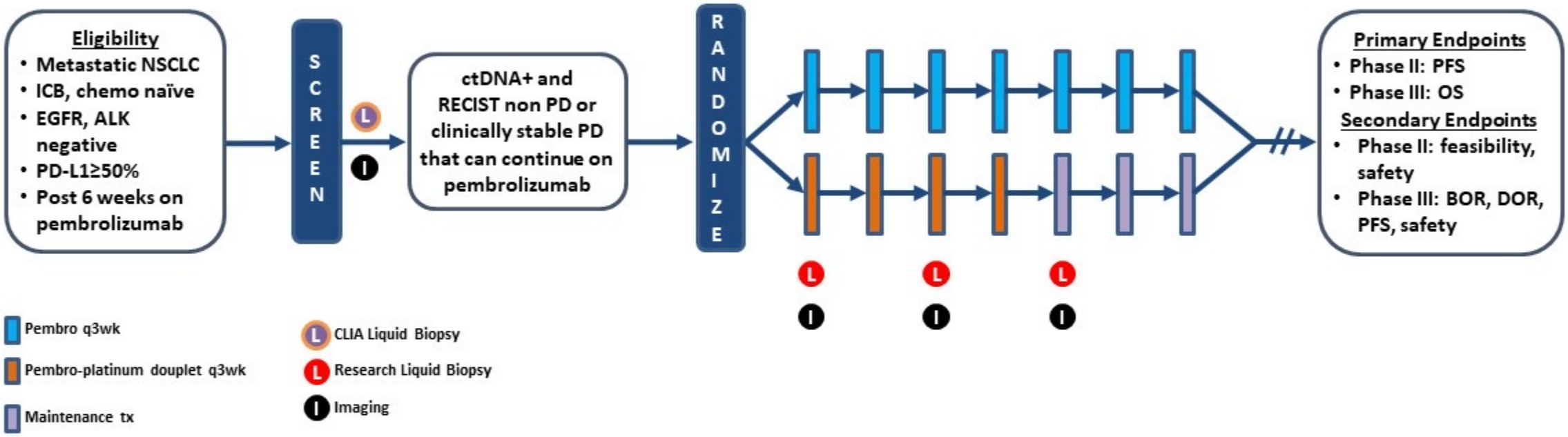




- **BR.36 stage 1 met its primary endpoint.**
- **The sensitivity of molecular response for RECIST best overall response was 82%**, (90% CI: 52% - 97%), **specificity was 75%** (90% CI: 56.5% - 88.5%).
- Median time to ctDNA response 2.1 months
- Patients with mR attained longer PFS and OS.

Anagnostou et al., *Nat Med*, 2023

BR.36 stage 2-A Biomarker-Directed, Open Label, Multi-Center Phase II/III Study of Molecular Response Adaptive Immuno-Chemotherapy in Patients with NSCLC



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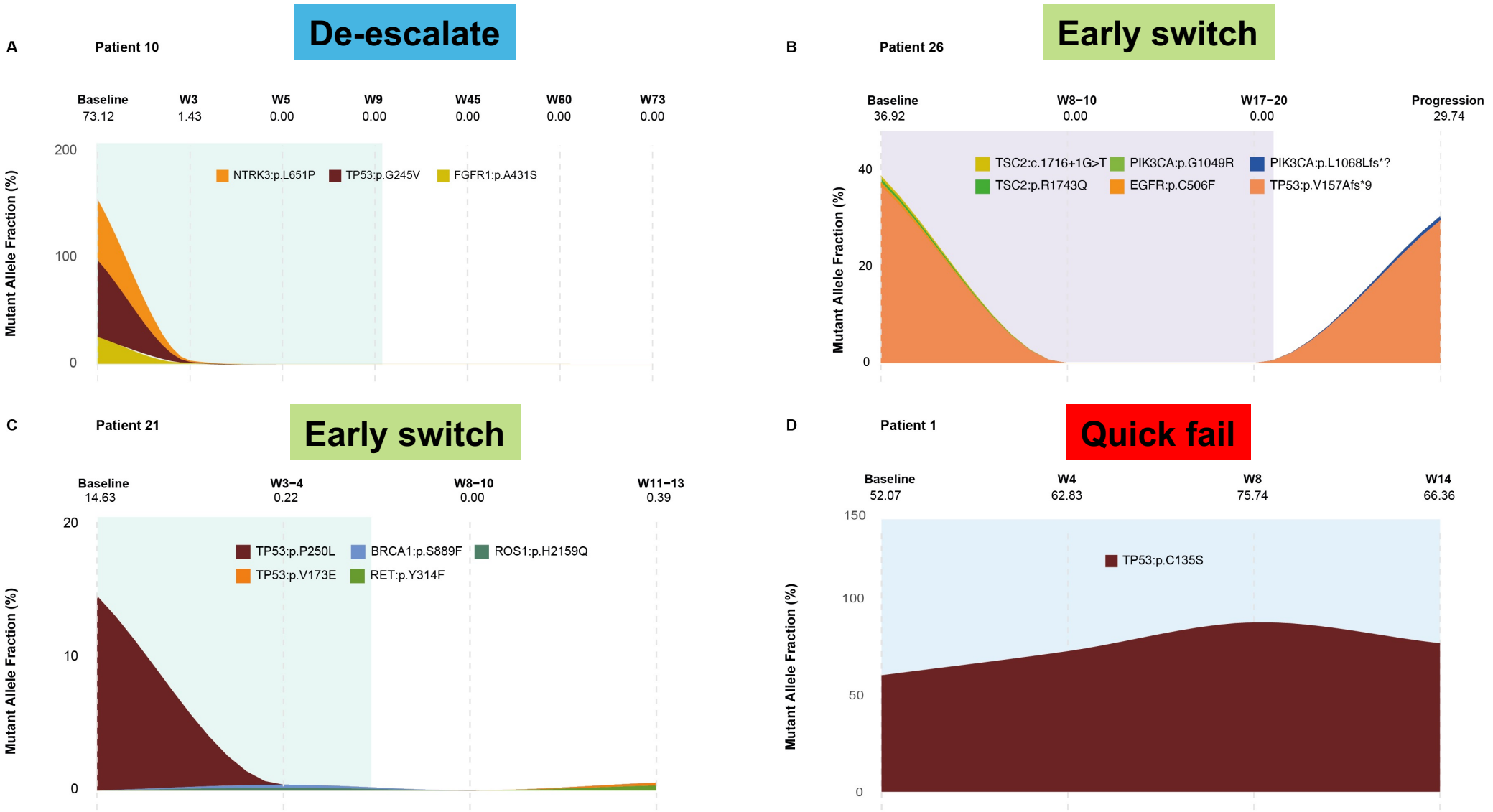
Bridging scientific discovery with clinical cancer care through ctDNA-adaptive trials



Adapted from Sivapalan et al., *Clin Cancer Res*, 2023, Sivapalan et al., *J Immunother Cancer*, 2023

Take-home messages

- Liquid biopsy approaches can be implemented in clinical diagnostic applications in precision-oncology.
- ctDNA CGP complements tissue CGP and has advantages in capturing tumor evolution and emerging therapeutic targets.
- Technical performance and biologic noise in ctDNA CPG represent challenges.
- Serial ctDNA measurements and molecular response are promising early endpoints.
- ctDNA molecular response adaptive clinical trials will establish the role of ctDNA as an early endpoint of immunotherapy response.
- ctDNA MRD can be informative in navigating the expanding therapeutic options for patients with early stage NSCLC

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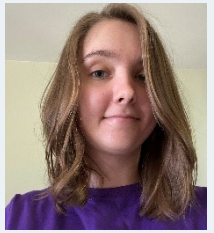
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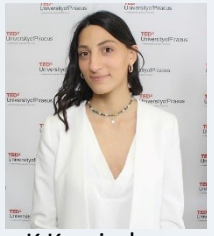
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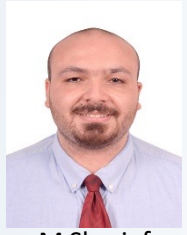
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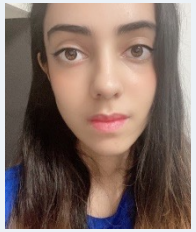
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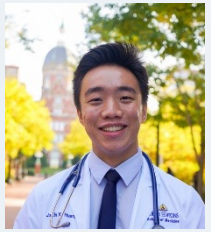
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