Moving ctDNA Utilization Beyond Predictive Biomarkers: The Next Frontier

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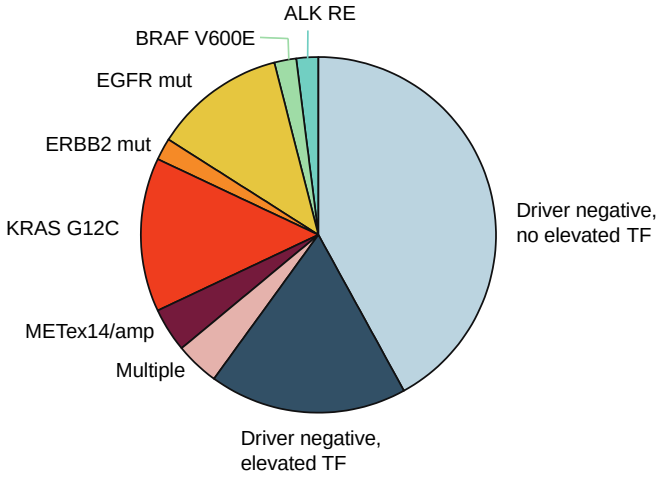
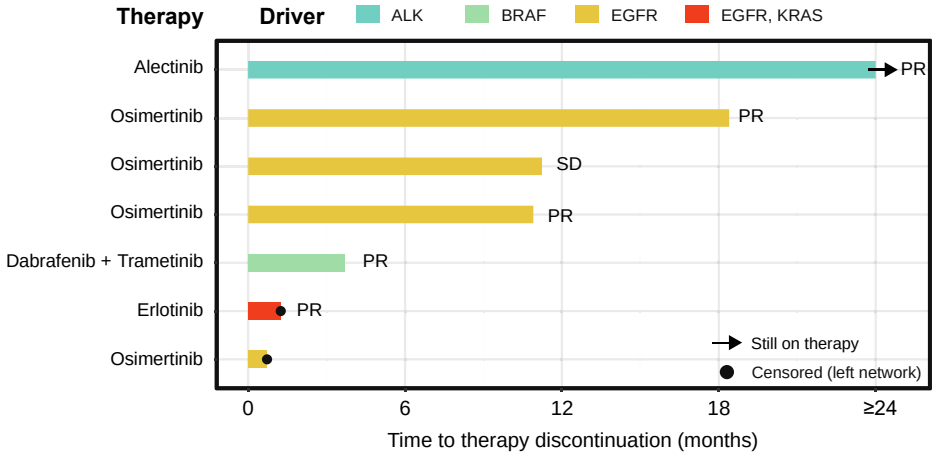
ISLB
INTERNATIONAL SOCIETY OF LIQUID BIOPSY

36% of early LBx samples were positive for an actionable NCCN driver





9 (18%) of patients were NCCN driver negative with estimated tumor fraction (TF) ≥ 10% (presumed true negatives)

7 driver+ patients received a 1L matched targeted therapy with a median TTD of 11 months and real-world response (PR) in majority of patients

Russo A. et al (Rolfo C.) JCO PO, Feb 2024

ctDNA tumor fraction informs the relative benefit from reflex to TBx CGP

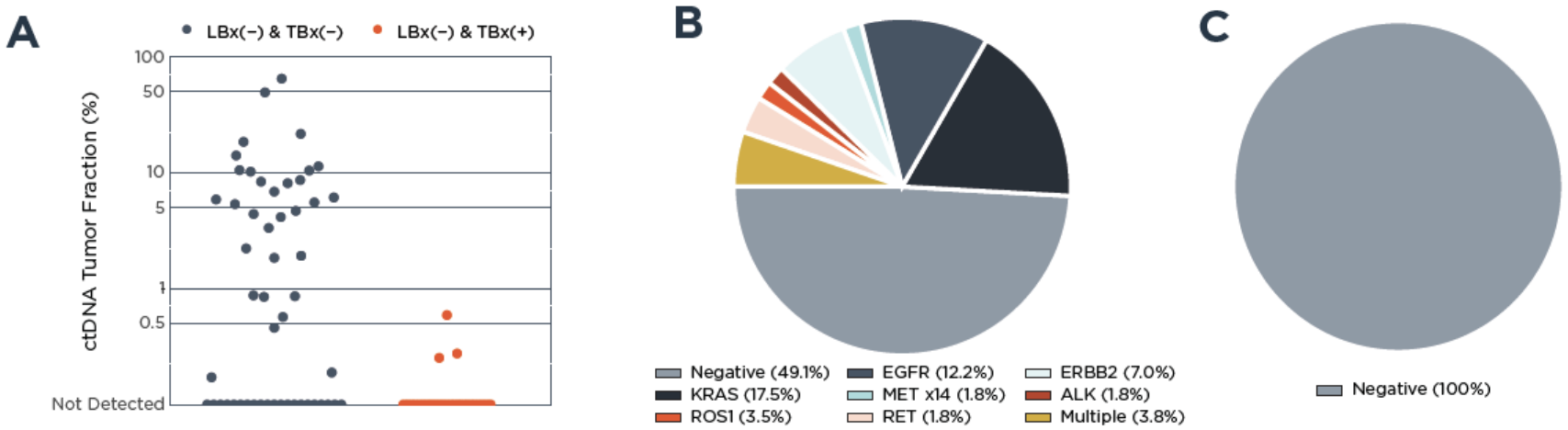
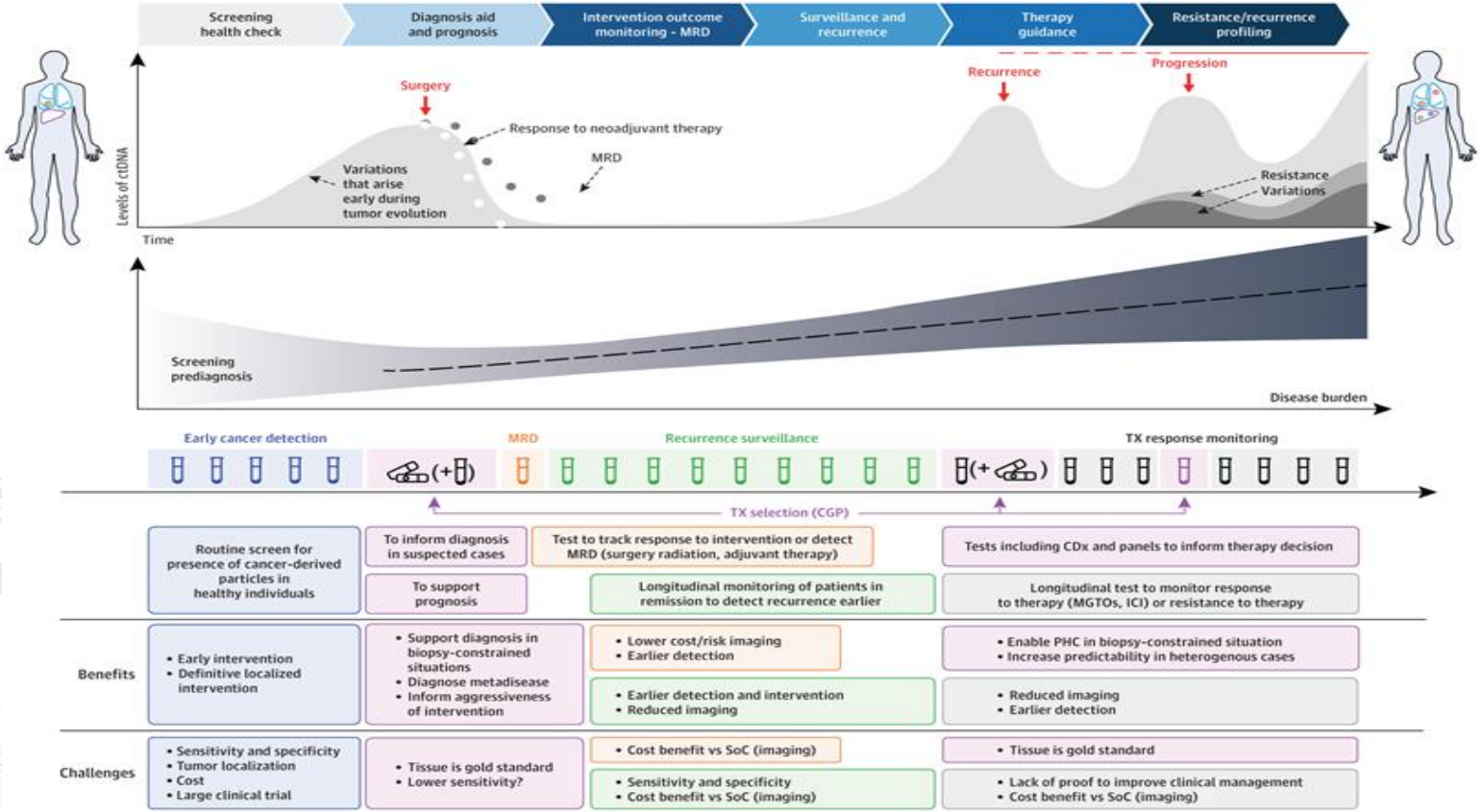


Figure 7: A) 24/81 (30%) patients with reflex TBx after negative LBx had a LBx TF ≥1% and, given high NPV for driver alterations, might have avoided reflex to confirmatory TBx. **B)** Amongst patients with TF <1%, 51% (29/57) of patients had a driver mutation detected on TBx reflex while **C)** no driver mutations (0%) were seen for patients with TF ≥1%

Rolfo C et al, Clin Cancer Research, April 10, 2024



Krebs M. Malapelle..(Rolfo) et al, JAMA Oncology October 2022

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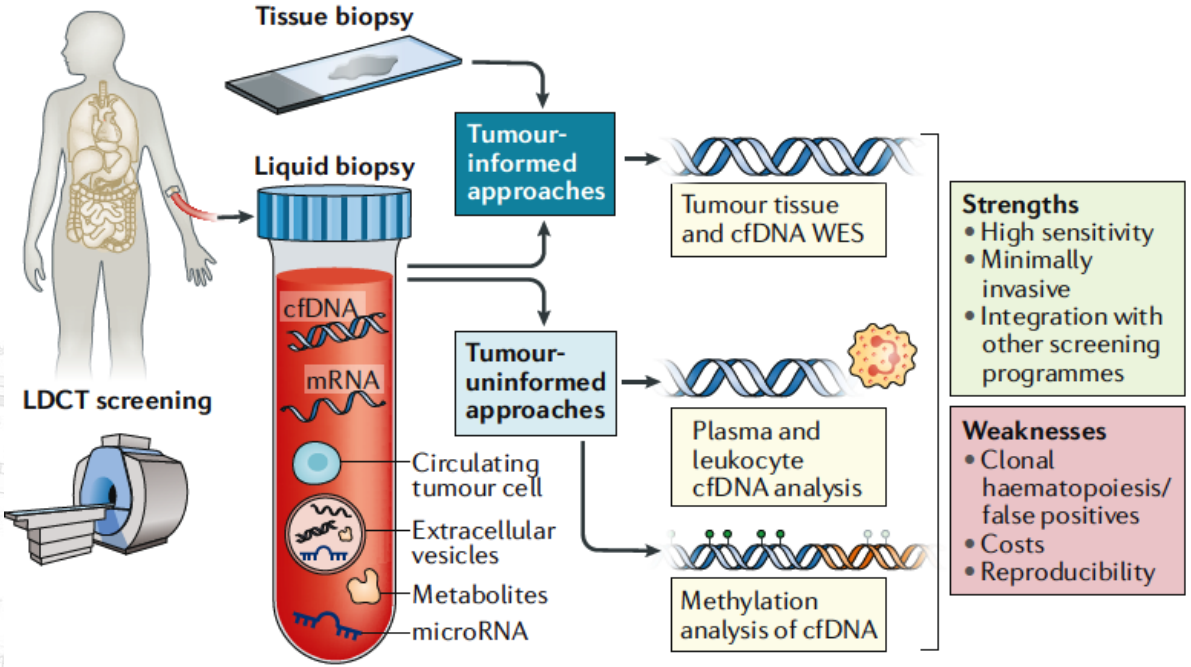
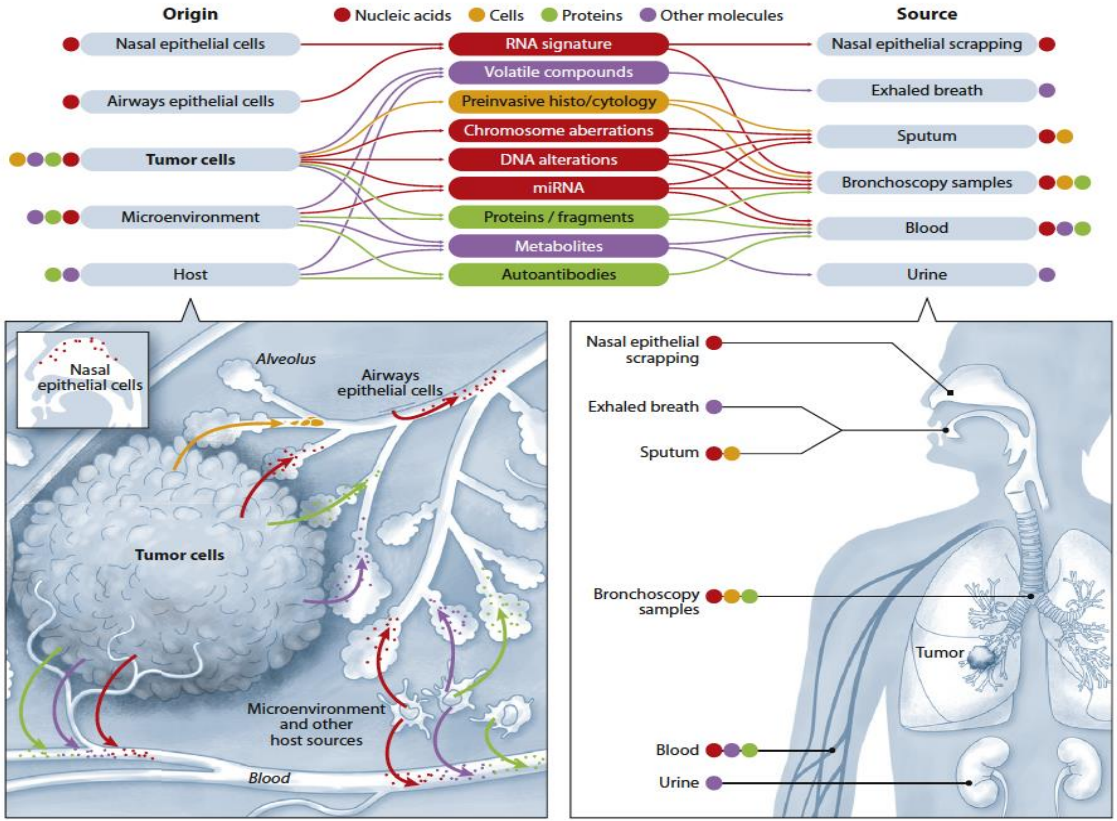
Liquid biopsy & early detection: Strengths and weaknesses of currently used approaches



Rolfo C & Russo A. Nat Rev Clin Oncol 2020;17:523-524.

Currently explored biomarker candidates for lung cancer screening



Seijo LM, et al. J Thorac Oncol 2019

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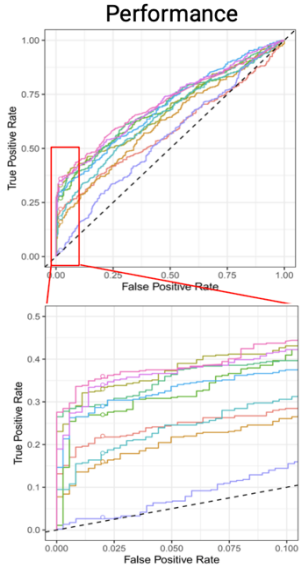
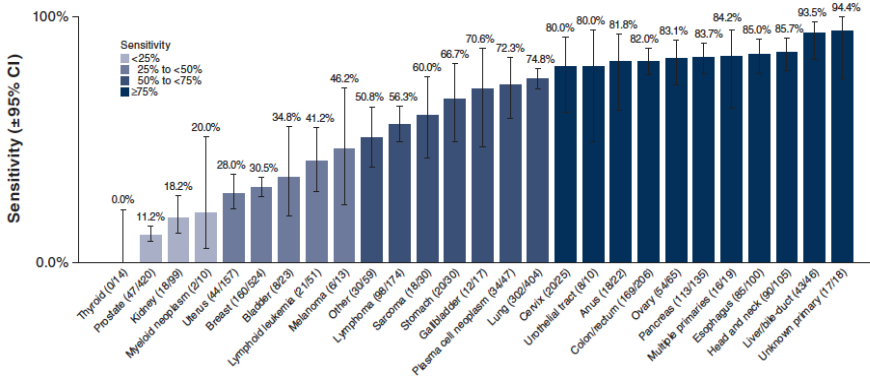
MCED test performance for cancer signal detection

Sensitivity at 98% specificity	WGBS	WG methylation	34% (30%-39%)	158/464



	Cancer	Non-cancer	Total
	2823	1254	4077
Test positive	1453	6	1459
Test negative	1370	1248	2618
	Sensitivity = 1453/2823 51.5% (49.6%-53.3%)	Specificity = 1248/1254 99.5% (99.0%-99.8%)	

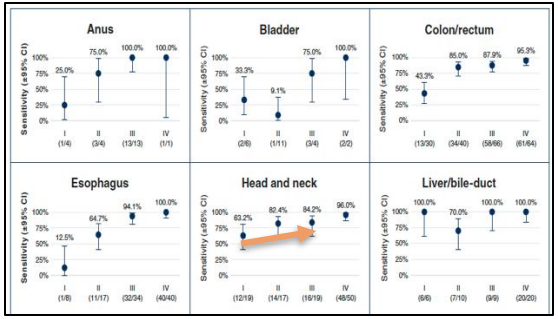
Two-sided 95% Wilson confidence intervals were calculated.

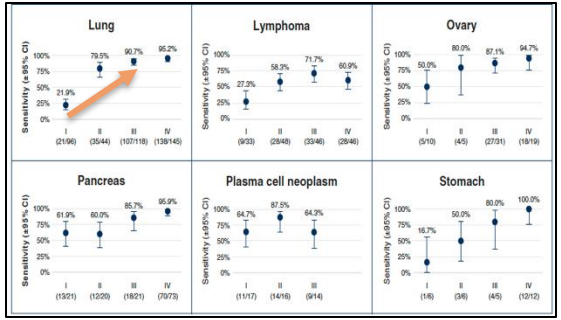


Confirmed status analysis set, *n* = 4077
cancer, *n* = 2823; non-cancer, *n* = 1254

Clinical cancer stage, *n* (%)	
I	849 (30.1)
II	703 (24.9)
III	566 (20.0)
IV	618 (21.9)

Klein EA, et al. Ann Oncol 2021

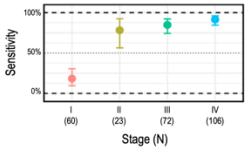




Clinical stage	Total *N*	Test positive	Sensitivity % (95% CI)[a]
All	2823	1453	51.5 (49.6% to 53.3%)
I	849	143	16.8 (14.5% to 19.5%)
II	703	284	40.4 (36.8% to 44.1%)
III	566	436	77.0 (73.4% to 80.3%)
IV	618	557	90.1 (87.5% to 92.2%)
I-II	1552	427	27.5 (25.3% to 29.8%)
I-III	2118	863	40.7 (38.7% to 42.9%)
I-IV	2736	1420	51.9 (50.0% to 53.8%)
III-IV	1184	993	83.9 (81.7% to 85.9%)
Not expected to be staged	67	23	34.3 (24.1% to 46.3%)
Missing	20	10	50.0 (29.9% to 70.1%)

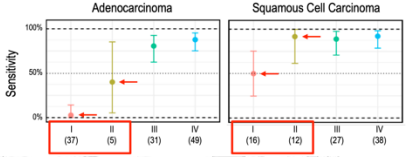
MCED: All subtypes have the same sensitivity?

Lung Cancer Detection Varies by Subtype at 99.4% Specificity

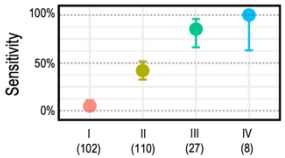


- Overall lung cancer sensitivity: 71.6% (95% CI: 65.8-77.0%)

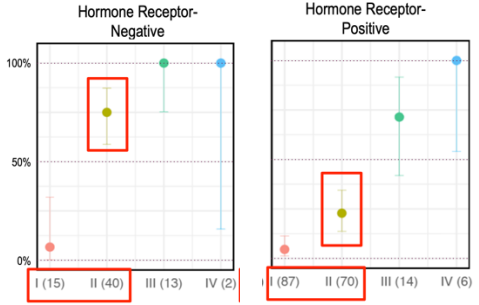
- Detection rate affected by early-stage adenocarcinomas
  - Detection higher in squamous cell carcinoma
- Consistent with prior report showing ctDNA detection was higher in squamous cell carcinoma than adenocarcinoma[1]



Breast Cancer Detection Varies by Subtype at 99.4% Specificity



- Overall breast cancer sensitivity: 33.2% (95% CI: 27.4-39.4%)



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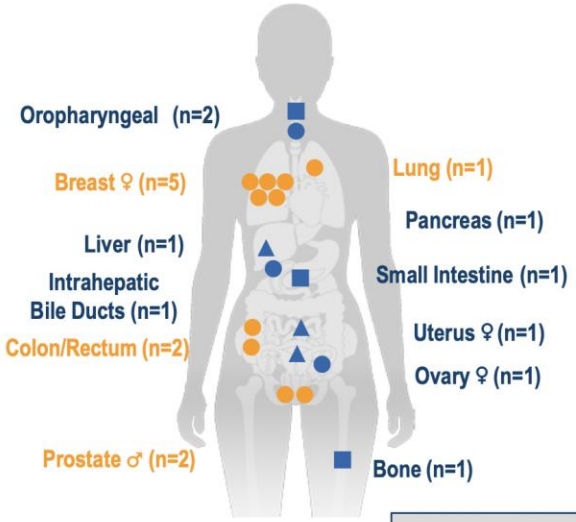
Oxnard et al, ESMO 2019, Klein EA, et al. Ann Oncol 2021

Cancers Diagnosed After a True Positive MCED Signal

19 Solid Tumors

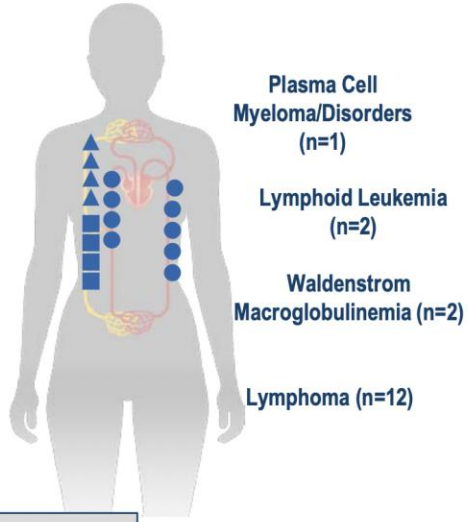


35 people were diagnosed with 36 cancers

- 24 in high-risk cohort
- 11 in not-high-risk cohort

- 7 recurrent cancers
- 14 early-stage cancers
- 26 cancers lacking standard screening

17 Hematologic Cancers



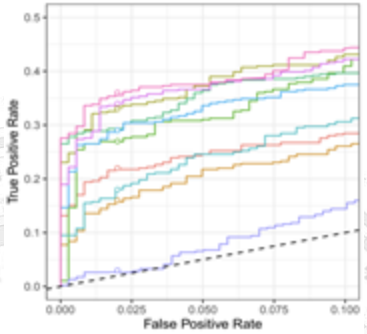
AJCC Staging: ▲Stage I ■Stage II ●Stage III/IV/No Stage/Recurrent

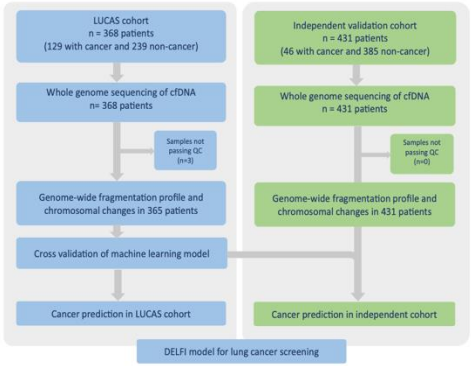
Available Screening: USPSTF cancer screening or No standard screening

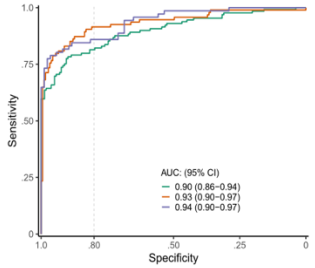
Fragmentomics in a Single-tumor test

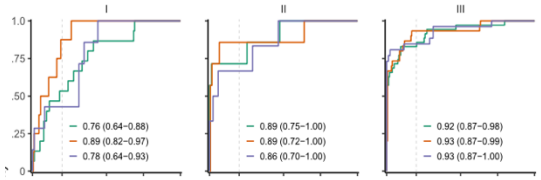
Sensitivity at 98% specificity			
	Fragment endpoints	18% *** (15%-22%)	84/464
	Fragment lengths	29% * (25%-34%)	135/464

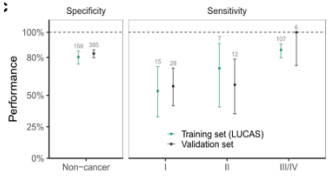
Non-cancer individuals: 236
Patients with Lung cancer 129











Cristiano S, et al. Nature 2019

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DELFI–Lung Cancer Training Study (DELFI-L101)

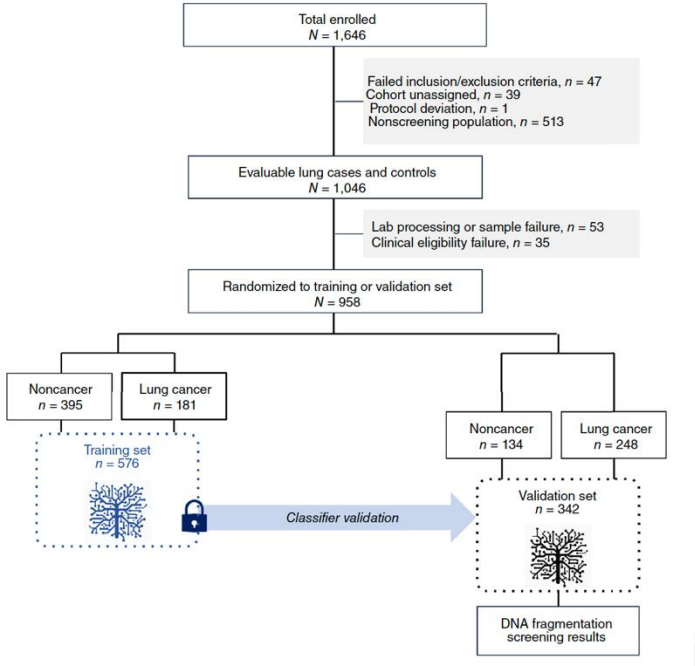
DELFI L101 – A case–control study for training and validation of a lung cancer screening test

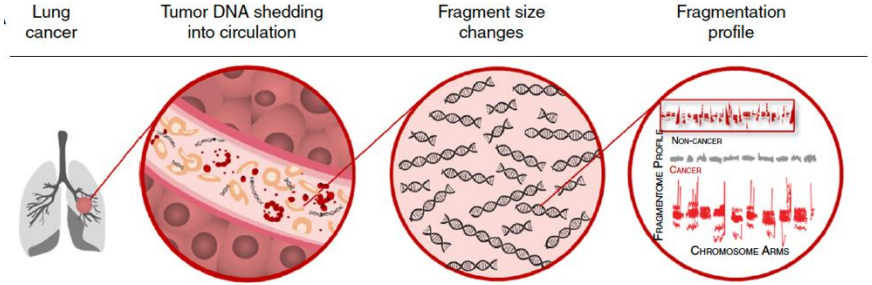
Lung cases: Participants with pathologically confirmed, untreated lung cancer
Noncancer Controls: Participants without a lung cancer diagnosis

All participants were required to meet the following:

Inclusion criteria:
- Age ≥50 years
- Current / former smoker ≥ 20 pack years
- Planned or recent thoracic CT imaging

Exclusion criteria:
- Treatment for cancer
- History of cancer within 1 year, prior heme malignancy or myelodysplasia





Mothios D, et al. Nature Communications 2021

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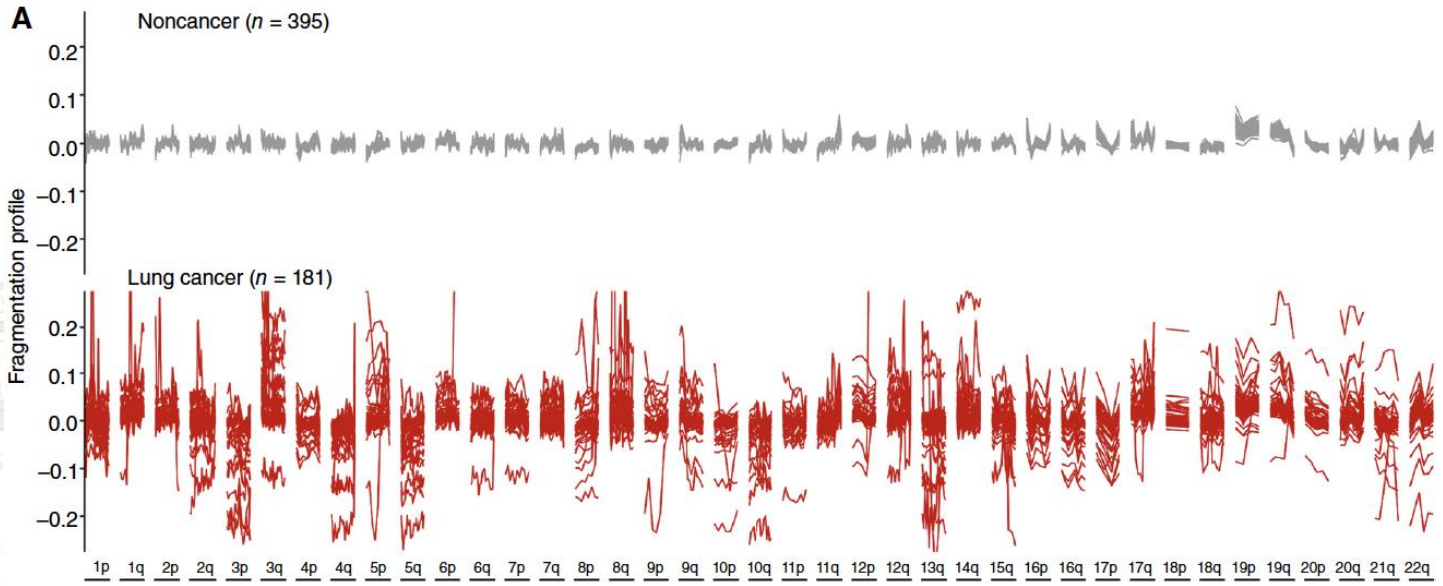
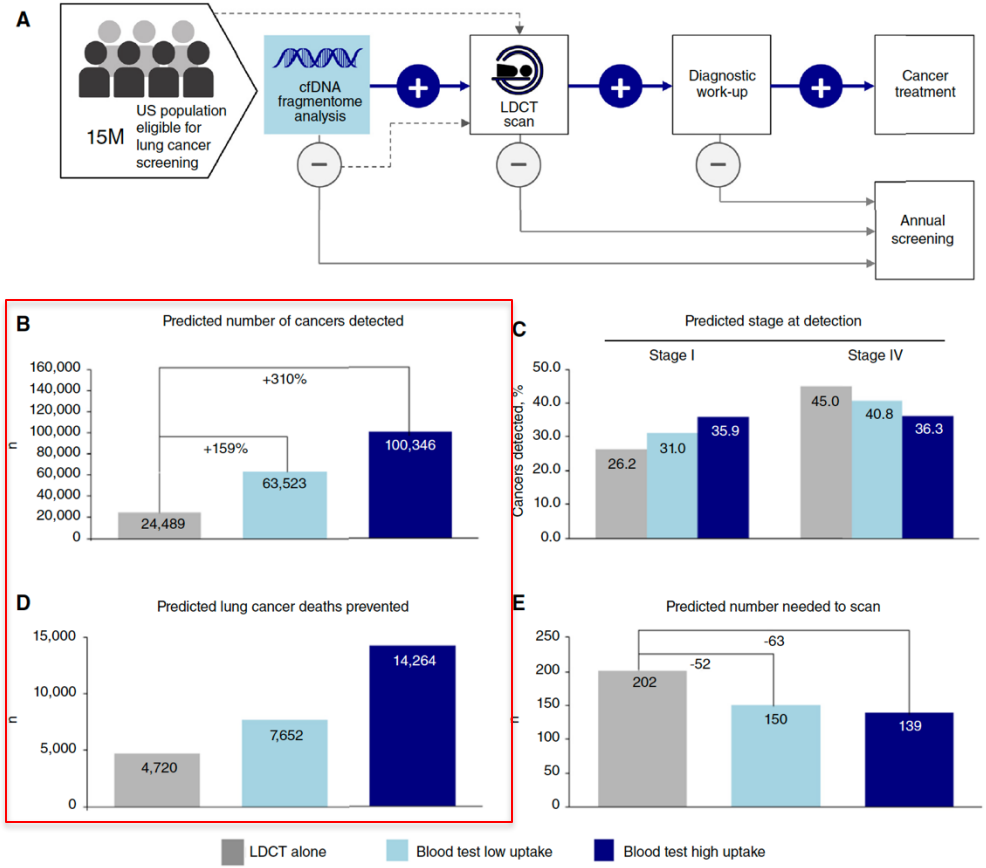
The noncancer individuals had similar fragmentation profiles, whereas patients with lung cancer exhibited significant variation



Mazzone PJ, et al. Cancer Discov 2024



Mazzone PJ, et al. Cancer Discov 2024

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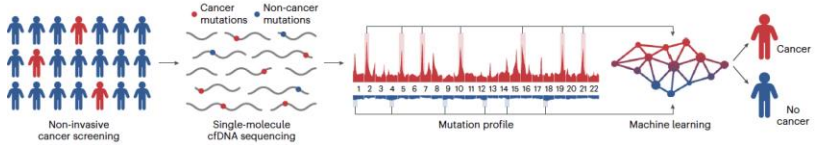
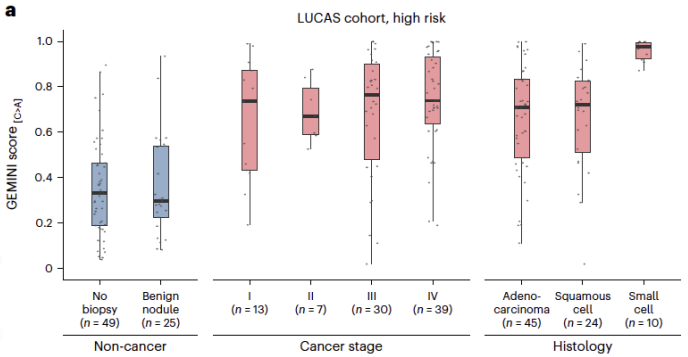
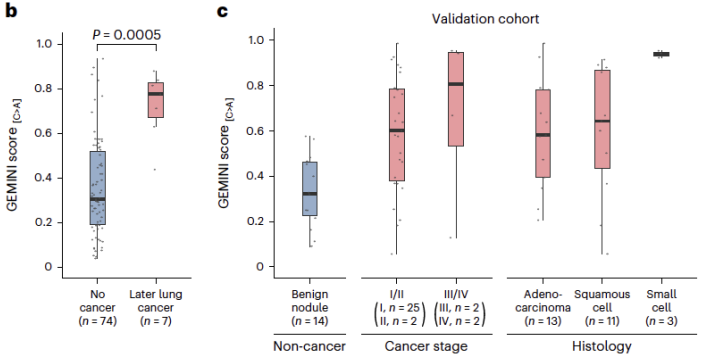
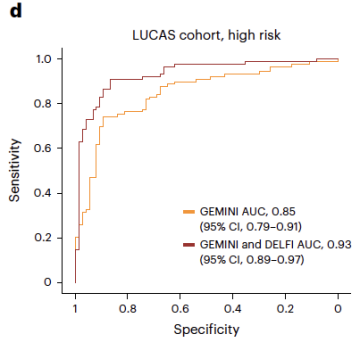
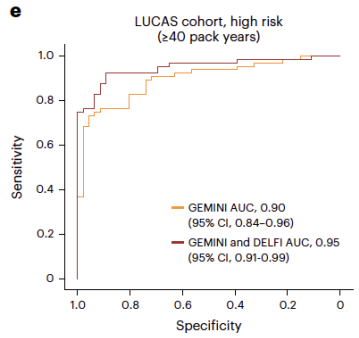
GEnome-wide Mutational Incidence for Non-Invasive detection of cancer (GEMINI)











Mazzone PJ, et al. Cancer Discov 2024

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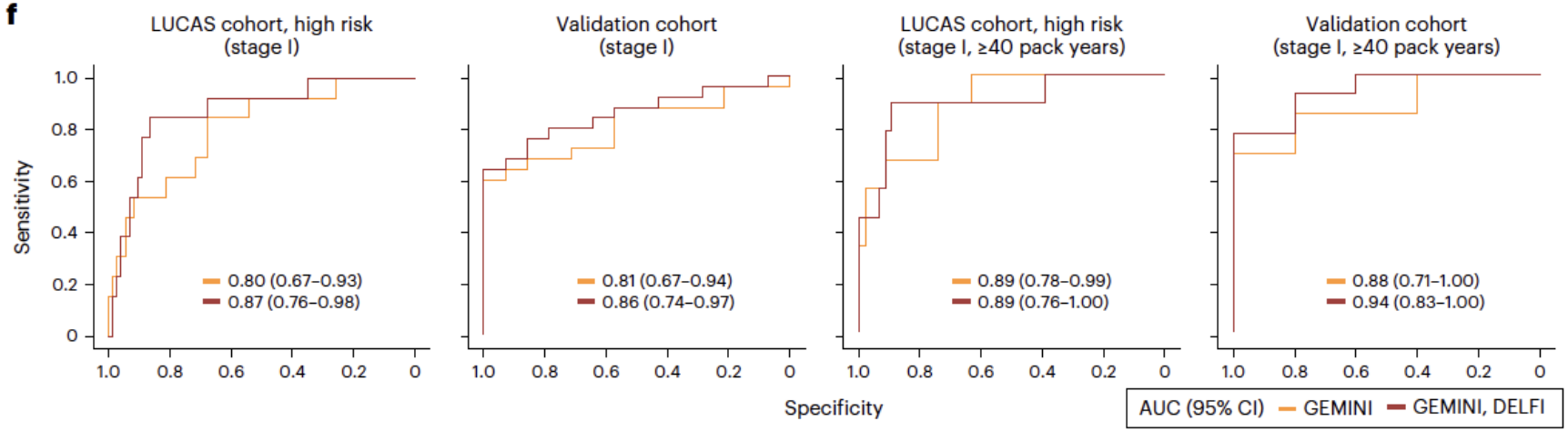
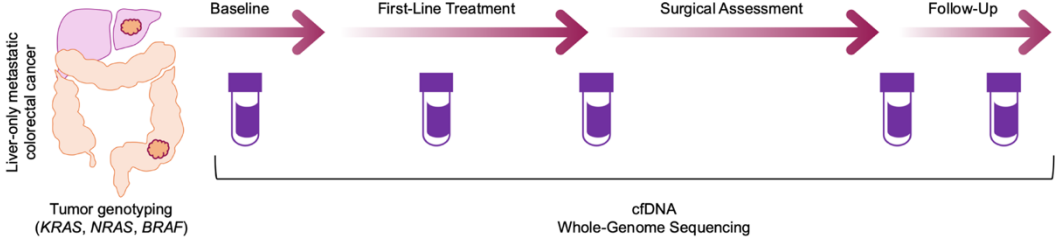
Detection of lung cancer using GEMINI and a combined GEMINI–DELFI approach.

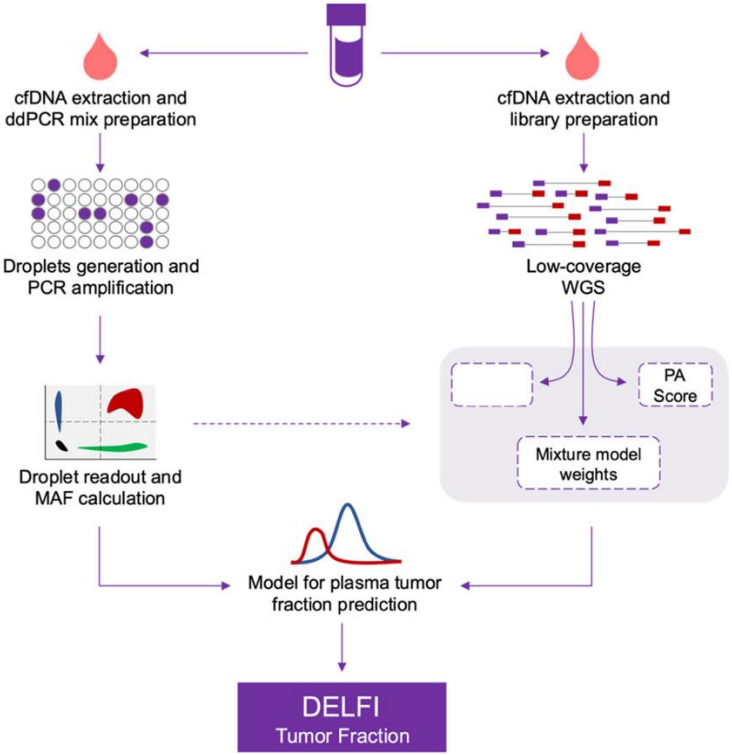


A machine-learning model using genome-wide mutational profiles combined with other features and followed by CT imaging detected >90% of patients with lung cancer, including those with stage I and II disease

Bruhm DC (Velculescu), et al. Nat Genet 2023

Cancer treatment monitoring using cell-free DNA fragmentomes



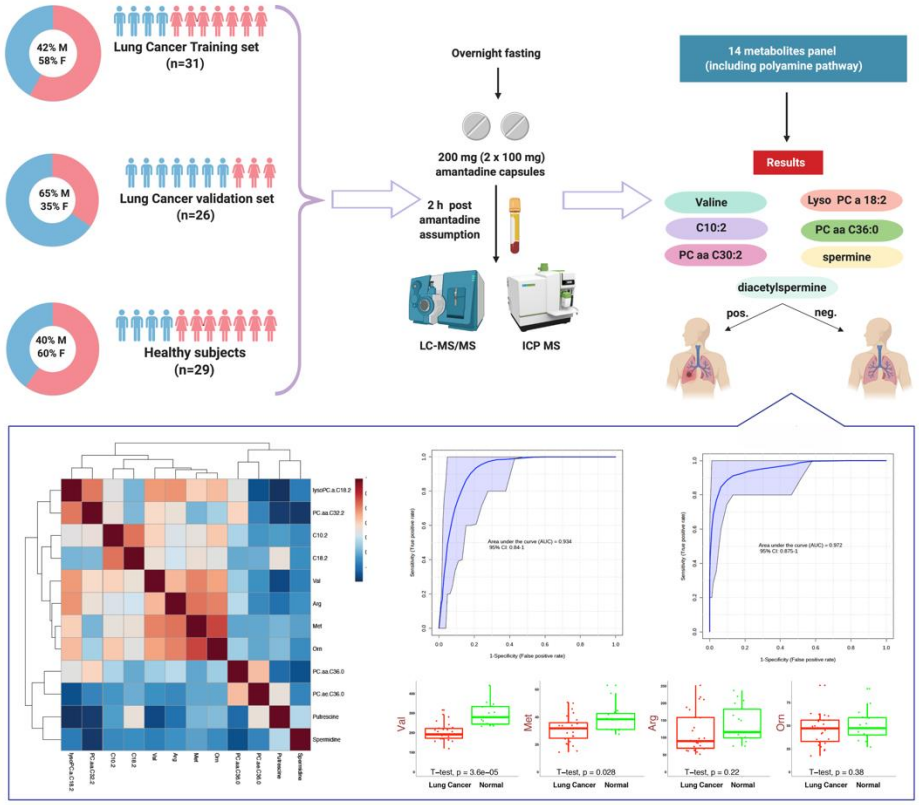


✓Here, we develop a tumor-independent and mutation-independent approach (DELFI tumor fraction, DELFI-TF) using low-coverage whole genome sequencing to determine the cfDNA tumor fraction and validate the method in two independent cohorts of patients with colorectal or lung cancer.

van 't Erve I, et al. Nat Commun 2024

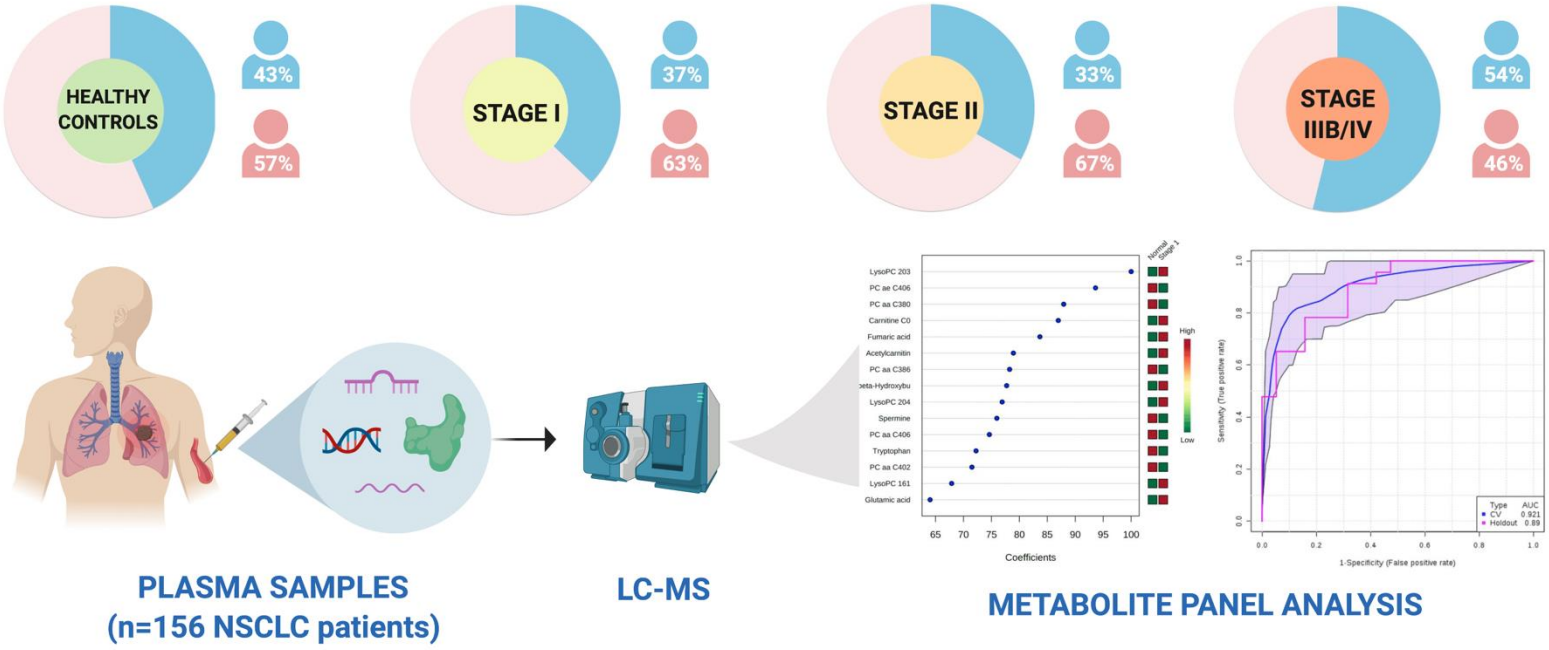
Liquid biopsy in lung cancer screening: The contribution of metabolomics. Results of a pilot study

- ✓A panel consisting of 14 metabolites, which included 6 metabolites in the polyamine pathway, was identified that correctly discriminated lung cancer patients from controls with an AUC of 0.97 (95% CI: 0.875-1.0).
- ✓When used in conjunction with the SSAT-1/polyamine pathway, these metabolites may provide the specificity required for diagnosing lung cancer from other cancer types and could be used as a diagnostic and treatment monitoring tool



Cancers 2019, 11, 1069Cancers 2019, 11, 1069

A High-Performing Plasma Metabolite Panel for Early-Stage Lung Cancer Detection



Zhang L ..,Rolfo C (Wishart), et al. Cancers 2020

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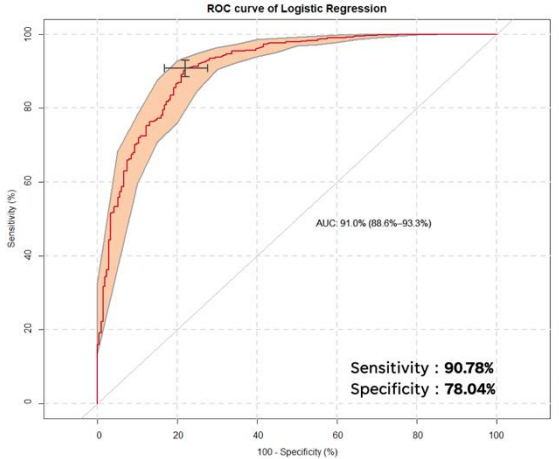
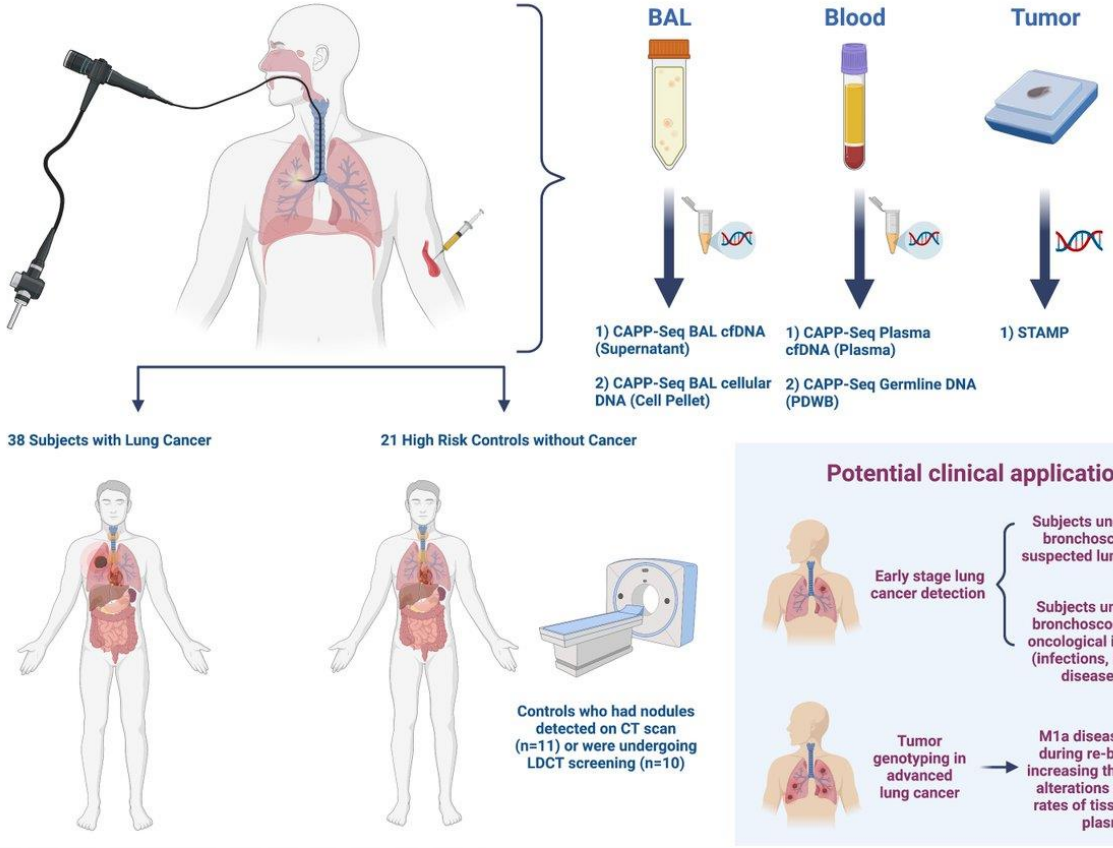
Metabolomic Biomarkers for resectable lung cancer detection and risk assessment

Cases	Controls
N=586	N=214
275 NSCLC Adeno (Stage I & II)	91 healthy
141 NSCLC Squamous (Stage I & II)	31 asthma
50 NSCLC Advanced Stages	46 COPD
120 Pulmonary neuroendocrine tumors (NETs)	8 Bronchiectasis
	38 COVID

Lung Cancer	Stage I	Stage II
AUC : 91% Se /Sp 91% / 78%	AUC : 91% Se /Sp 94% / 75%	AUC : 93% Se /Sp 92% / 81%
NSCLC	**Advanced Stage**	
AUC : 89% Se /Sp 87% / 74%	AUC : 93% Se /Sp 82% / 91%	

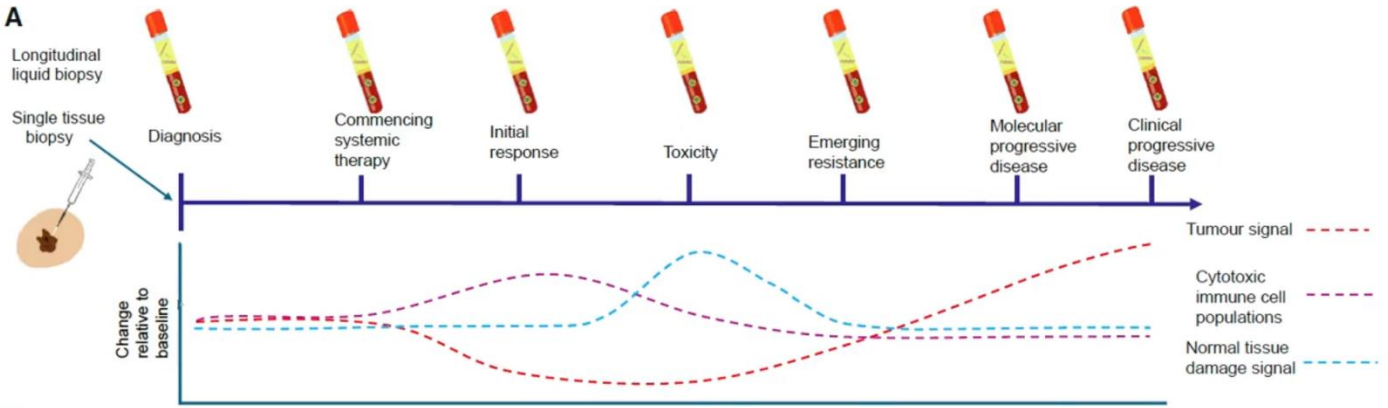


- Separation of lung cancer patients from controls can be observed in the 2D scores plot using a panel of 9 metabolites.
- Linear regression model using metabolites and smoking status yielded an overall AUC of 0.91 with sensitivity of 91% and specificity over 78%.



Rolfo C, Malapelle U & Russo A, Cancer research, August 2022

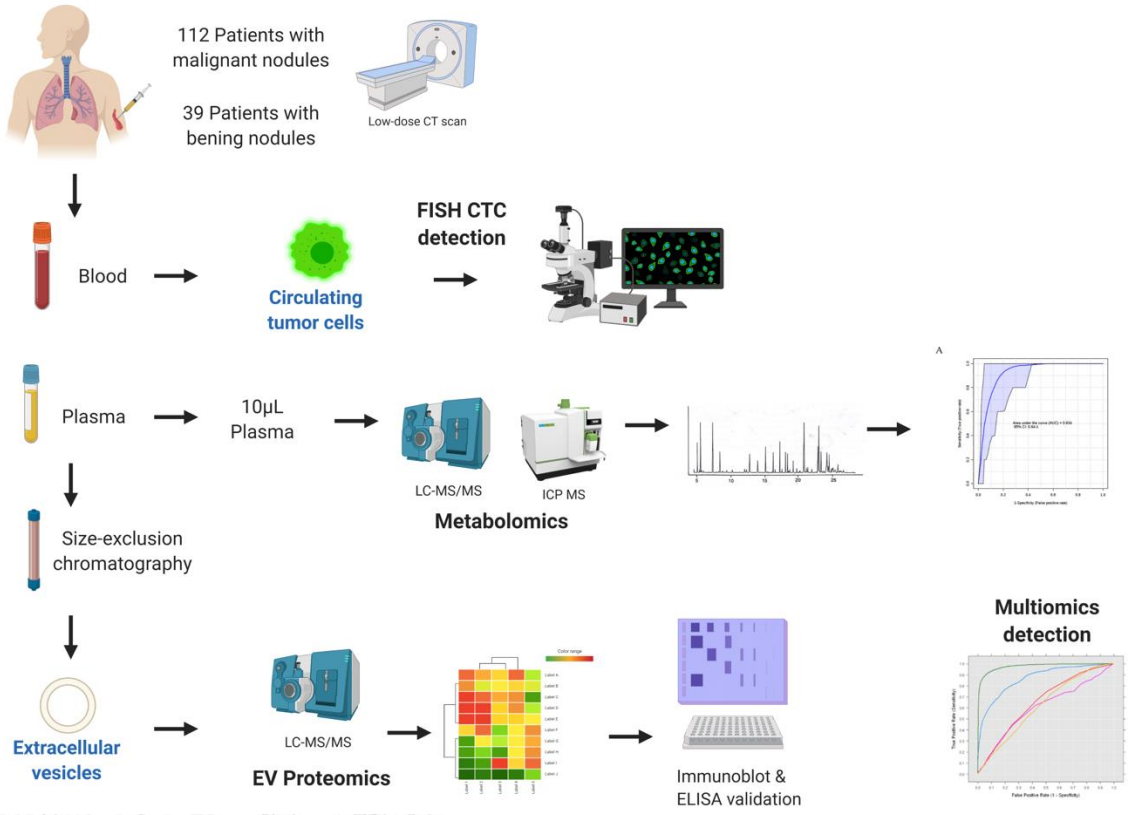
Potential omic applications for LBx monitoring



B

Potential 'Omic Applications	Screening programmes for early detection	Determining tissue of origin	Identifying actionable mutations/fusions	Monitoring for minimal residual disease	Monitoring for tumour response on therapy	Exploring immune cfDNA changes on therapy	Identifying and monitoring normal tissue damage	Detecting and characterising emerging resistance	Monitoring for progressive disease
Genomic	●		●	●	●			●	●
Methylation	●	●		●	●	●	●	●	●
Fragmentomic	●	●		●	●	●	●		●
Transcriptomic		●	●			●			

Tivey A, et al. Cell Reports Medicine 2024



lBratulic S. et al, PNAS , Dec 2022

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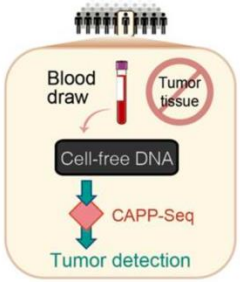
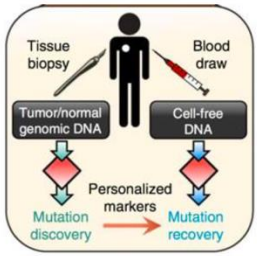
Two strategies for ctDNA analysis

Tumor naive

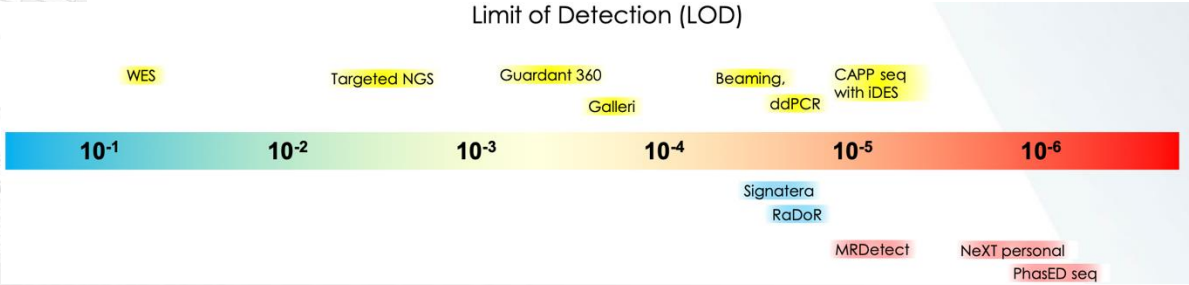


- Methods: SNV, methylation, and/or fragmentomics
- Applications: genotyping, screening

Tumor informed



- Methods: SNV tracking tracking of known mutations
- Applications: MRD, response assessment



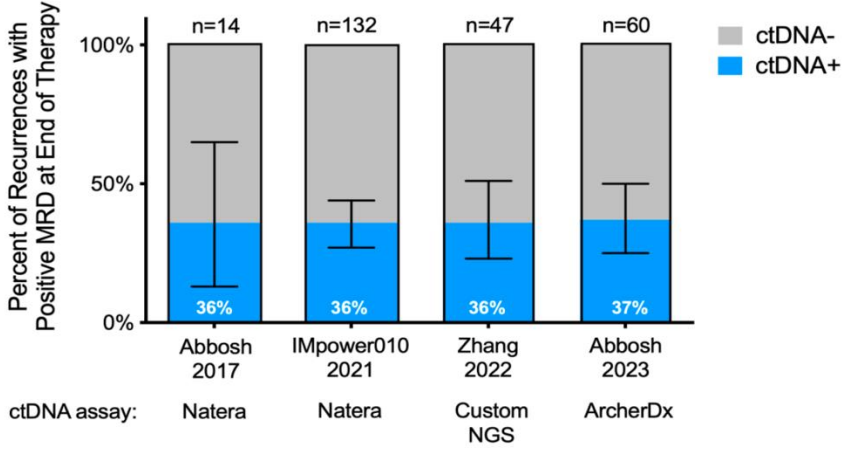
Siravegna Nature Rev Clin Oncol 2017, Semenkovich JITC 2023

The James

The Ohio State University
Comprehensive Cancer Center

Key limitation of 1st Generation ctDNA MRD assays: High False Negative Rate

- 1st generation ctDNA MRD assays **track SNVs** found by sequencing tumor tissue (i.e. "tumor-informed")
- MRD detection with 1st generation assays has **high positive predictive value** for recurrence
- However, **sensitivity is suboptimal** and ~2/3rd of ultimate recurrences are initially false negative

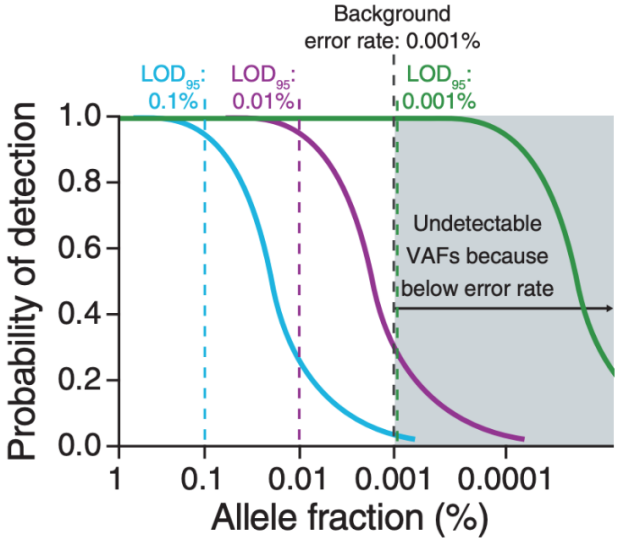


Abbosh et al. Nature 2017; Zhou et al. ESMO I-O Meeting 2021; Zhang et al. Cancer Discovery 2022; Abbosh et al. Nature 2023

Increasing Sensitivity for MRD Detection

Approaches for improving LOD of MRD assays:

1. Track more mutations
2. Decrease background error rate

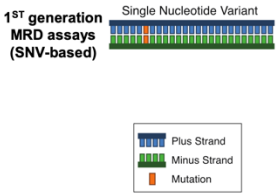


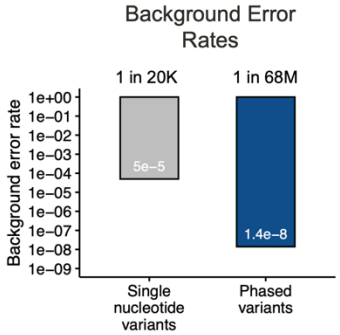
	Mutations	cfDNA Input	Depth
—	+	+	+
—	++	++	++
—	+++	+++	+++

Moding et al. Cancer Discov. 2021

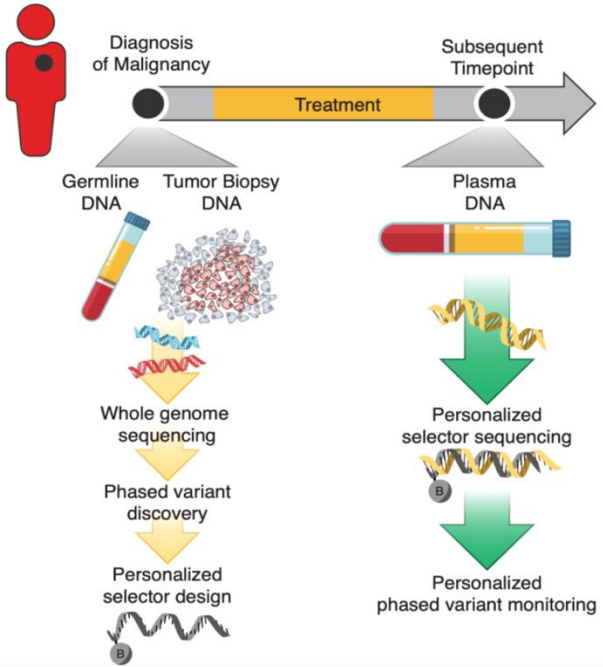
Decreasing background error rates: Phased Variant Sequencing (PhasED-Seq)

phased variant enrichment and detection sequencing (PhasED-seq), a method that uses multiple somatic mutations in individual DNA fragments to improve the sensitivity of ctDNA detection.



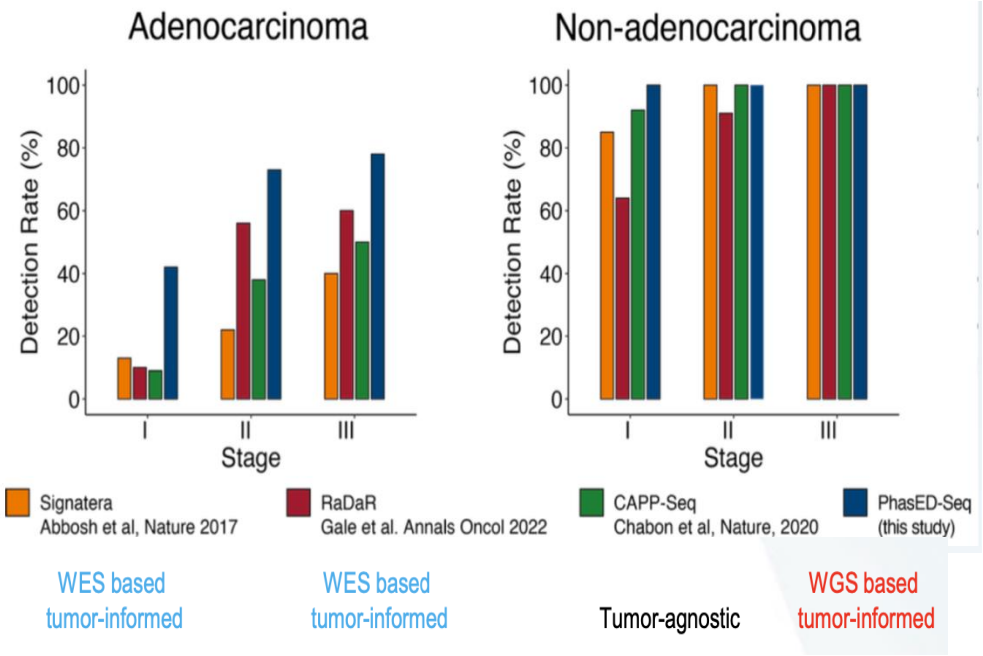


Prior methods aimed at lowering the LOD have focused on somatic variants detected on both of the complementary strands of parental DNA duplexes. Using Phased variants' (PVs), where two or more mutations occur in cis (that is, on the same strand of DNA.



Kurtz et al. Nature Biotechnol 2021; Isbell et al. AACR Annual Mtg 2023; Kurtz et al. Nature Biotechnol 2021

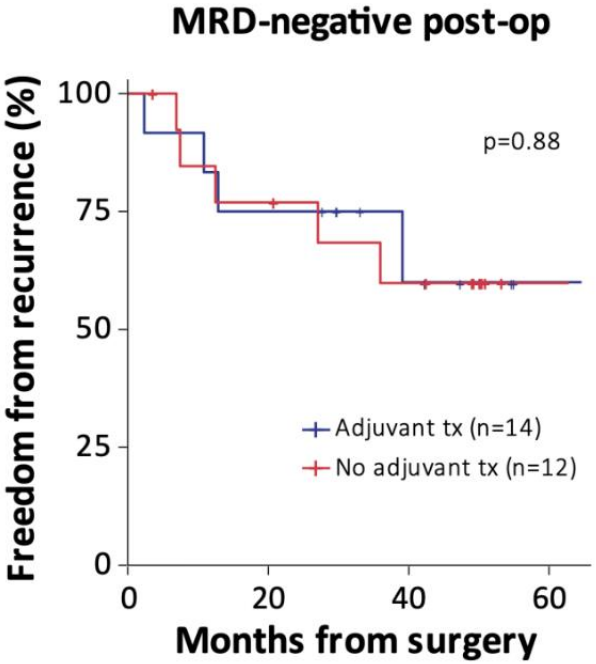
Pre-operative(baseline) detection of ctDNA

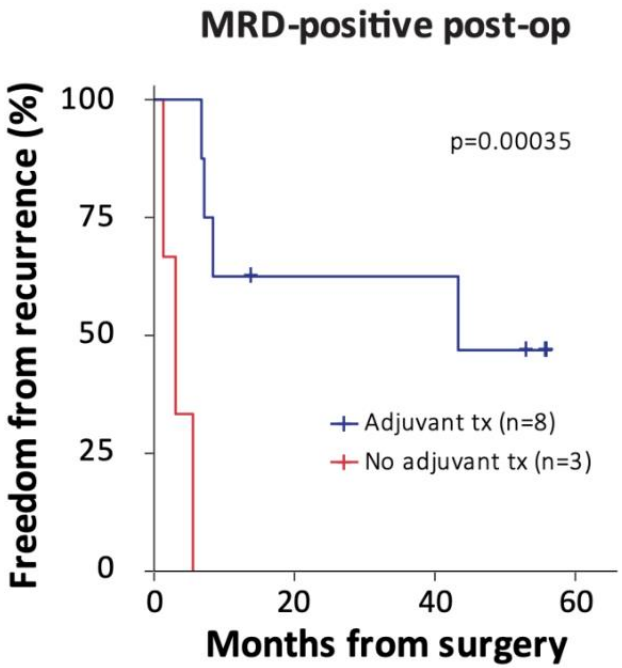


PhasED-Seq improved disease detection as compared with SNV-based approaches (for example, CAPP-seq), including tumor fractions as low as ~1 in 1,000,000.

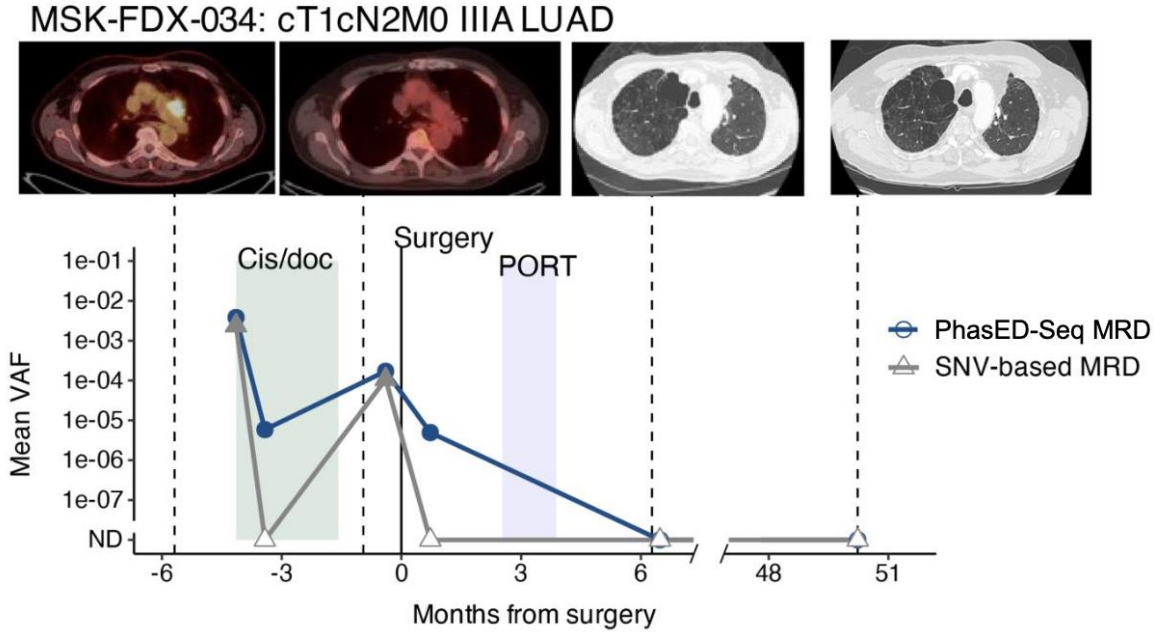
Isbell, AACR 2023 Max Diehn ESMO 2024

Effect of Adjuvant Therapy Based on MRD-status post-op





Accurate Assessment of Response to Neoadjuvant Treatment and Postoperative Radiotherapy



EVIDENCE Study Design: phase III randomized adjuvant study in resected EGFR-mutant NSCLC

Completely resected stage II-IIIA NSCLC

- 18 years to 70 years old
- ECOG PS 0-1
- Confirmed EGFR mutation (L858R or Exon 19 deletion)
- No Previous systemic anti-tumor therapy

R 1:1

Icotinib (125mg TID), 24 months

N=322

Standard chemotherapy, 4 cycles

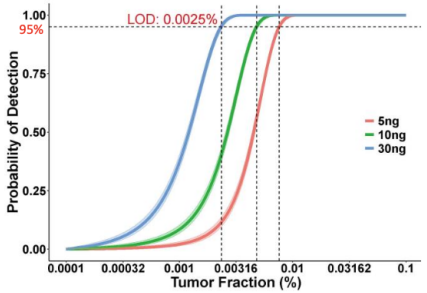
Primary endpoint: disease-free survival (DFS)

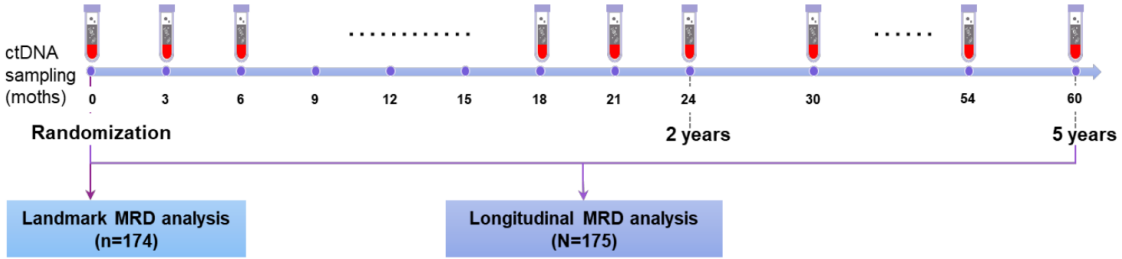
Treatment continued until:
- Disease relapse
- Treatment completion
- Unacceptable toxic effects
- Death

Follow-up: every 12 weeks for the first 2 years after random assignment and every 24 weeks in years 3–5

Exploratory endpoint: DFS defined by ctDNA status

Analytical validation of MinerVa Prime assay showing an LOD of 0.0025% at 30ng of DNA input

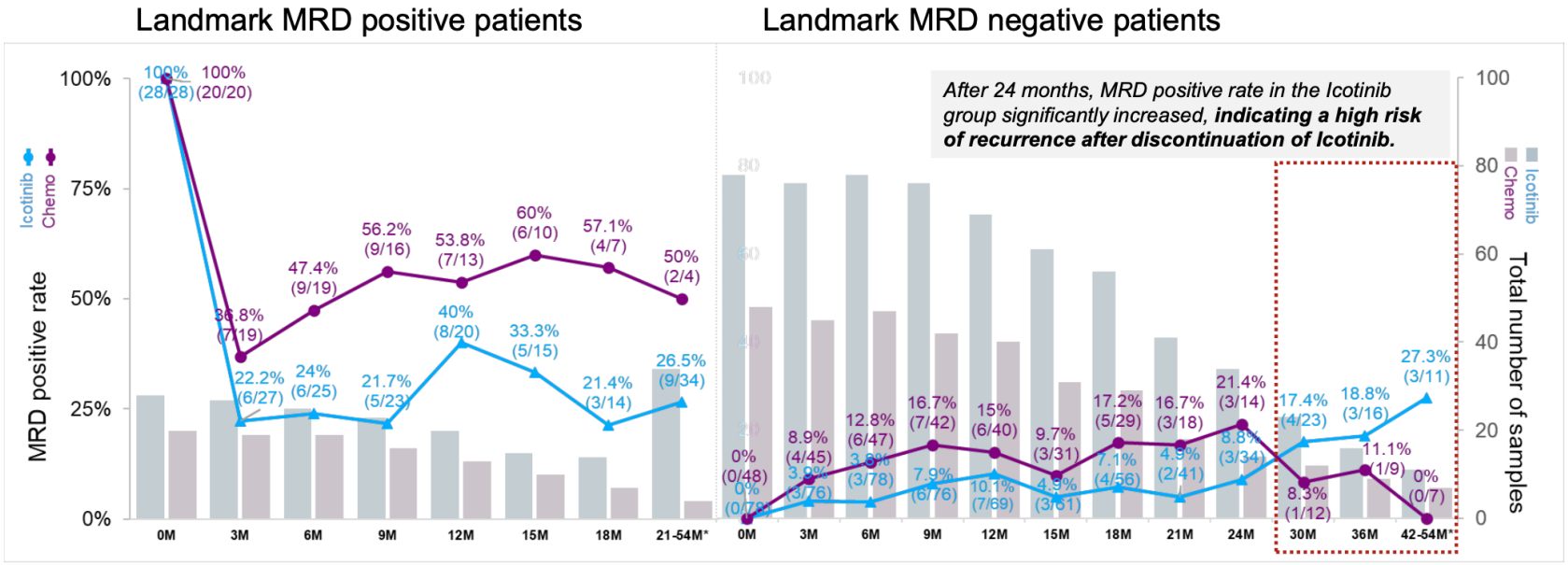




Samples were retrospectively tested using **Genecast MinerVa Prime assay**, a tumor-informed MRD method tracking up to 50 plasma ctDNA alterations identified in tumor tissue by whole-exome sequencing, which had been pre-validated to have an LoD of 0.0025% and a specificity of 99.5% at 30ng of input cfDNA.

MRD positive rate at each detection time point

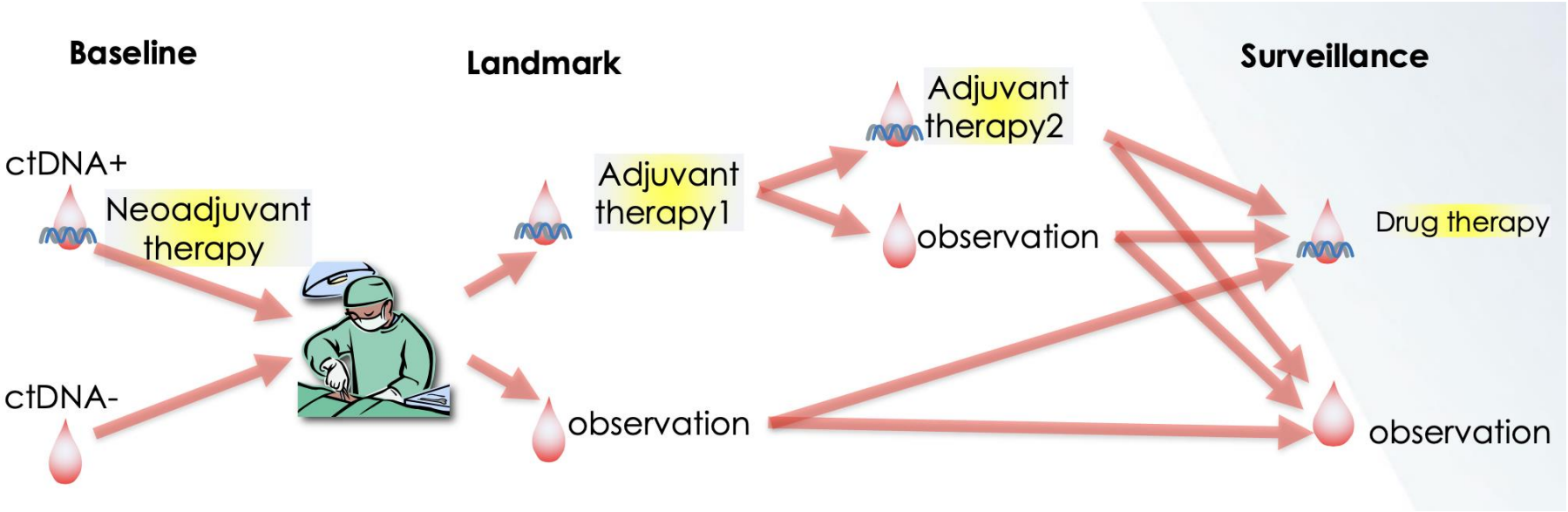
*Compared with the chemotherapy group, patients in the **Icotinib group** showed **higher MRD clearance rates** and **lower MRD relapse rates** at subsequent time points.*



*Cumulative sample size and positivity rate within the designated time frame.

These results coincide with the observed superior DFS in the Icotinib group, regardless of landmark MRD status.

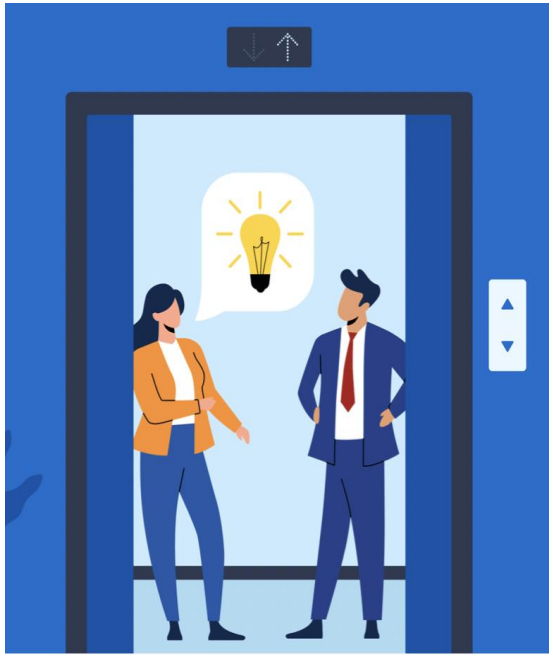
DNA guided perioperative management in the future



Modified from Soh, Hamada Fujino, and Mitsudomi, Cancers (Basel) . 2021

Take Home message… My elevator pitch

- Early detection is crucial to increase survival rates in cancer
- We need methods to complement the screening programs
- Do we need Multi-cancer detection or single tumor?
- Important to include risk populations in trials
- Role of Multiomics!
- Detecting MRD is crucial to improve survival and disease control rates (knowing differences between assays and sensitivity it's also crucial!)



Acknowledgements team and collaborations

Rolfo Lab

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

The James
THE OHIO STATE UNIVERSITY COMPREHENSIVE CANCER CENTER

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Icahn School of Medicine at Mount Sinai

INSTITUTO NACIONAL DE CANCEROLOGÍA México

UNIVERSITÀ DEGLI STUDI DI MESSINA

UPMC HILLMAN CANCER CENTER

UNIVERSITY of MARYLAND SCHOOL OF MEDICINE

UHN Princess Margaret Cancer Centre

Genyo
CENTRO PFIZER-UNIVERSIDAD DE GRANADA-JUNTA DE ANDALUCÍA DE GENÓMICA E INVESTIGACIÓN ONCOLÓGICA

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