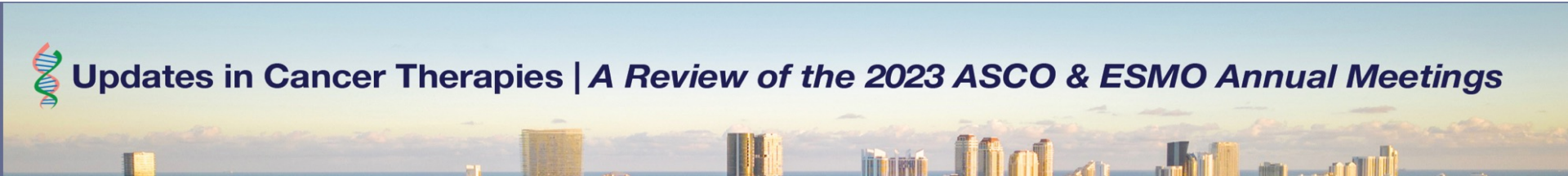
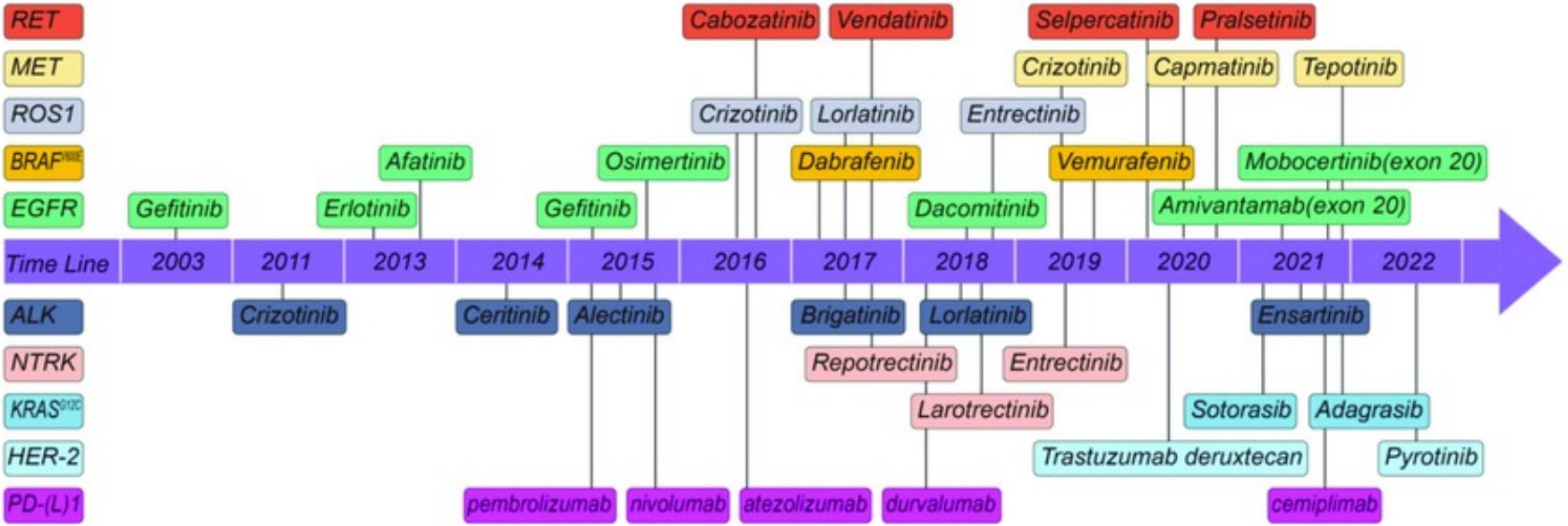
Novel Frontiers in the Use of cDNA in Oncology

Christian Rolfo, MD, PhD, MBA, Dr.hc
Professor in Medicine
Icahn School of Medicine, Mount Sinai
Associate Director of Clinical Research
Center for Thoracic Oncology
The Tisch Cancer Institute
Mount Sinai, New York, NY, US

Mount Sinai *Tisch Cancer Center*

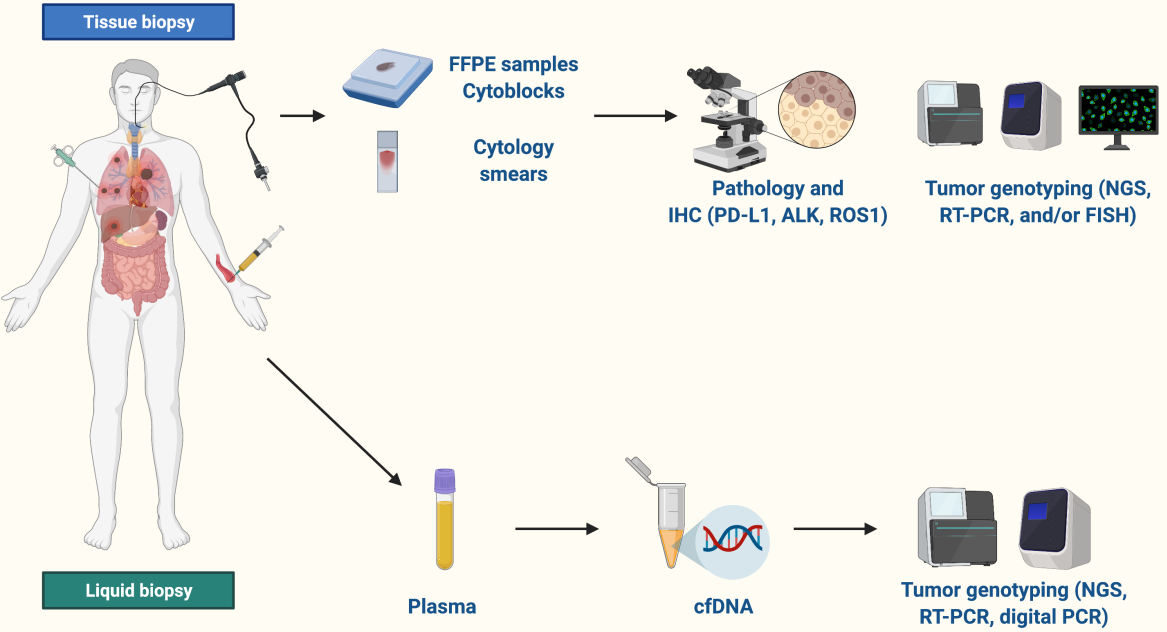
ISLB INTERNATIONAL SOCIETY OF LIQUID BIOPSY





Gou et al, Cells 2022, 11, 3200

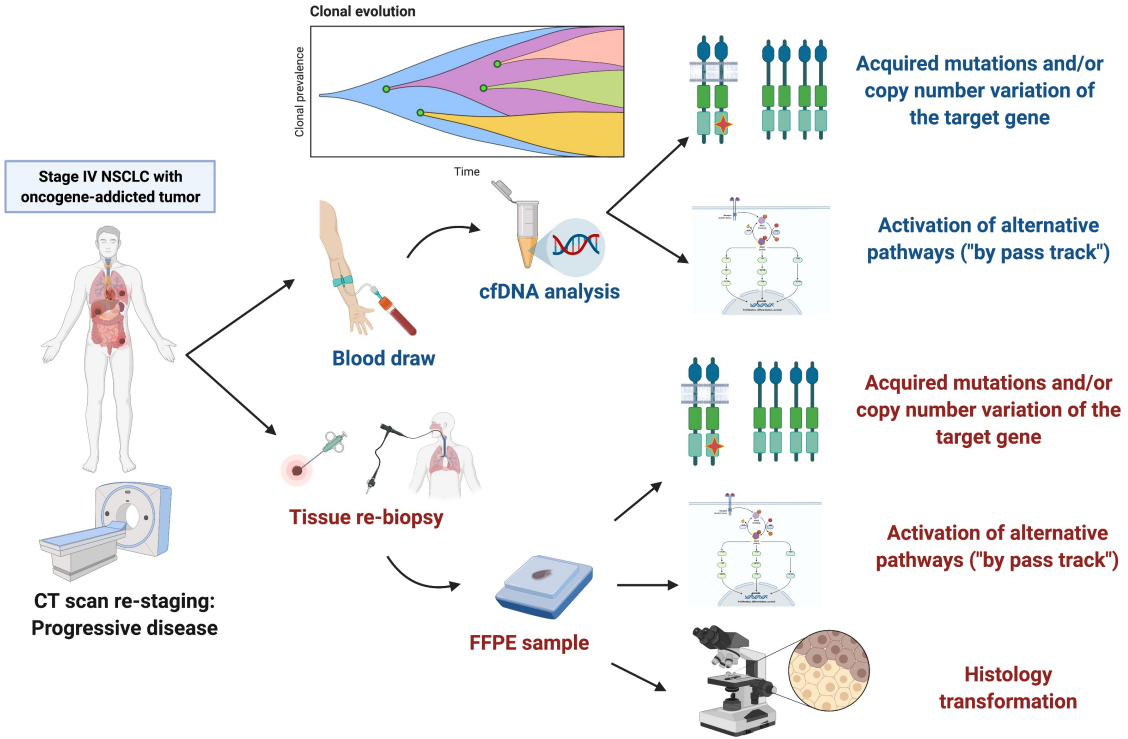
Tissue vs. Liquid biopsy



	Advantages	Disadvantages
Tissue biopsy	✓Pathology information ✓Assessment of DNA and non-DNA biomarkers ✓PD-L1 assessment	✓Longer TAT ✓Limited tissue quantities ✓Invasive ✓At PD, re-biopsy not always feasible ✓Tumor heterogeneity
Liquid biopsy (cfDNA)	✓High concordance rate ✓ Rapid TAT ✓Minimally invasive ✓Repeatable over time ✓Better capture tumor hetereogenity and clonal evolution	✓Non-DNA biomarkers not evaluable ✓Increased costs if used concurrently with tissue testing ✓False negatives

Rolfo C, et al. J Thorac Oncol 2021;16(10):1647-1662.

Identifying the mechanisms of acquired resistance: Tissue vs. Liquid biopsy



Main liquid biopsy techniques used

NGS-based approaches:
- ✓High sensitivity
- ✓Multiplex
- ✓Gene rearrangements
- ✓Gene amplifications

PCR-based approaches:
- ✓Variable sensitivity
- ✓Single gene testing
- ✓Only for mutations

Main techniques used for tumor tissue

NGS-based approaches:
- ✓High sensitivity
- ✓Multiplex
- ✓Gene rearrangements
- ✓Gene amplifications

FISH:
- ✓Gene rearrangements & amplifications

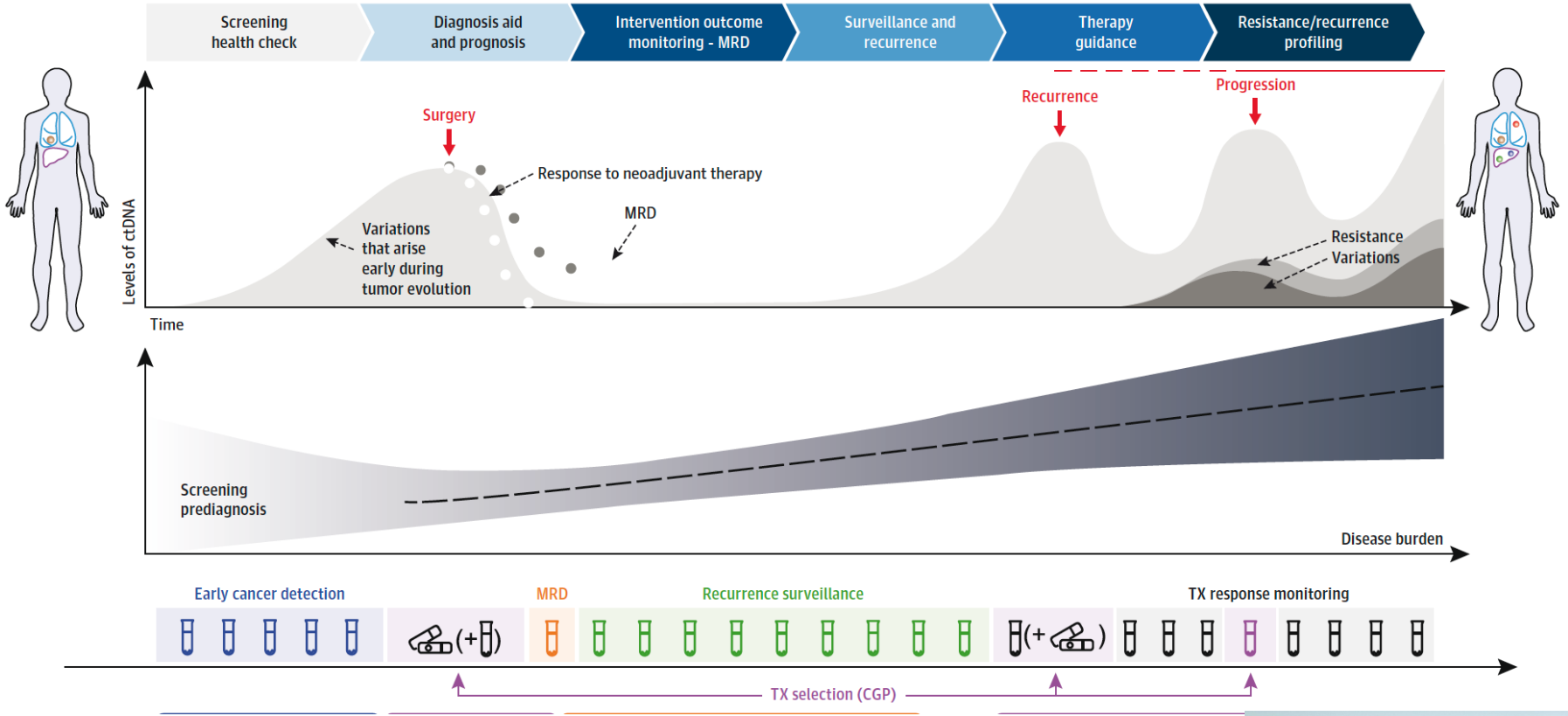
PCR-based approaches:
- ✓Variable sensitivity
- ✓Single/Multiplex gene testing
- ✓Only for mutations

IHC:
- ✓Protein expression

Rolfo C, et al. J Thorac Oncol 2021

Updates in Cancer Therapies

Liquid biopsy during lung cancer patient journey



Krebs MG (Rolfo C) et al. JAMA Oncol 2022

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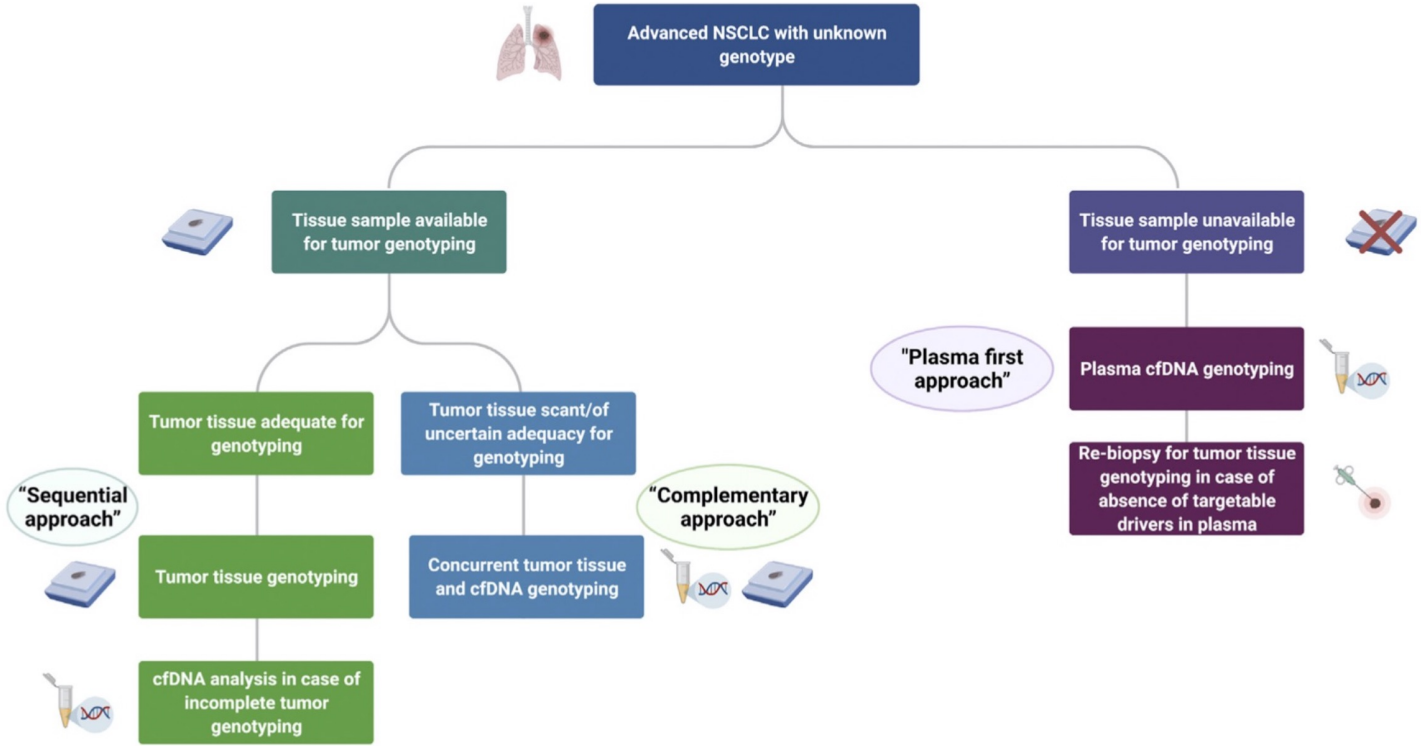
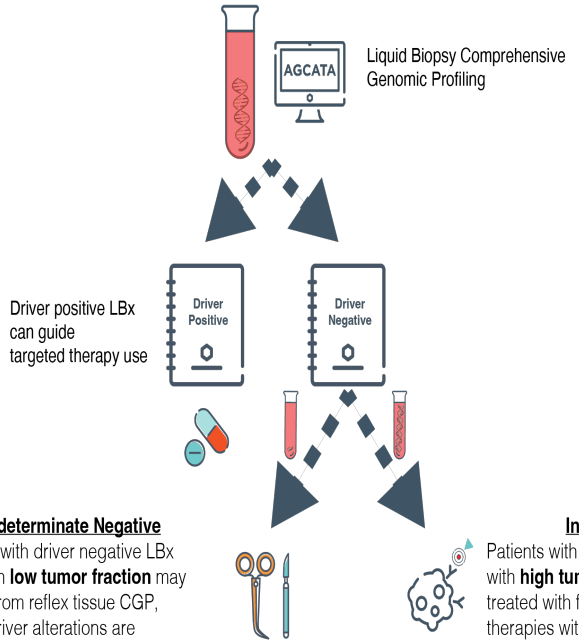
Updates in Cancer Therapies

Why is important the approach we use in liquid biopsy?



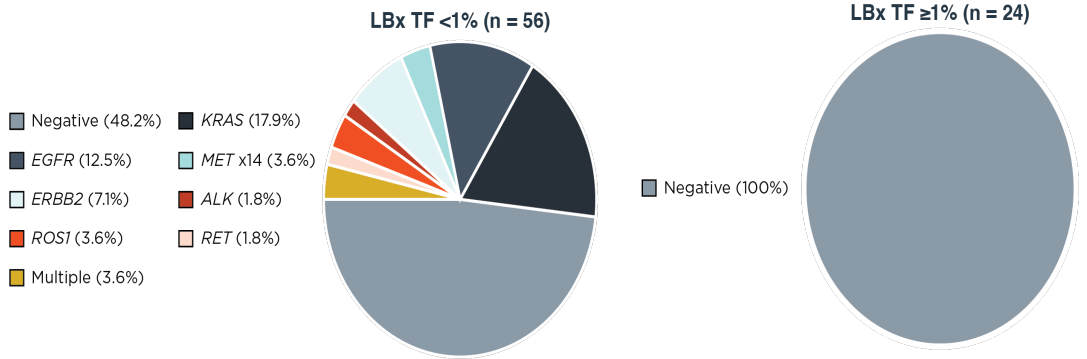
Rolfo C, et al. J Thorac Oncol 2021

Measurement of ctDNA Tumor Fraction Identifies Informative Negative Liquid Biopsy Results with Reduced Value of and Need for Tissue Confirmation



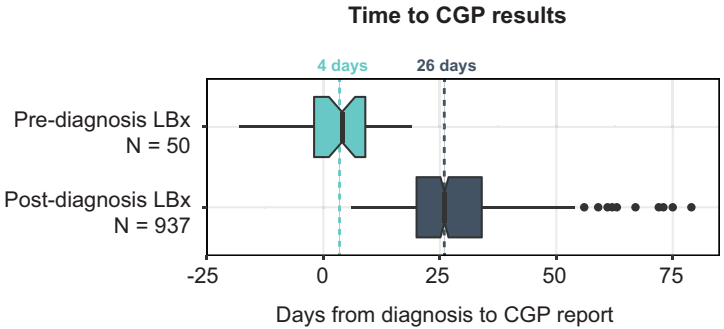
Indeterminate Negative
Patients with driver negative LBx CGP with **low tumor fraction** may benefit from reflex tissue CGP, where driver alterations are frequently detected

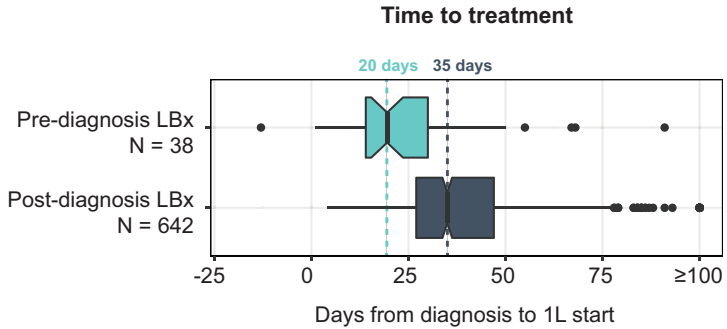
Informative Negative
Patients with driver negative LBx CGP with **high tumor fraction** can be treated with first line standard of care therapies without reflex to tissue biopsy



Rolfo C, et al. ASCO 2023

Liquid biopsy of lung cancer prior to pathological diagnosis is associated with shorter time to treatment

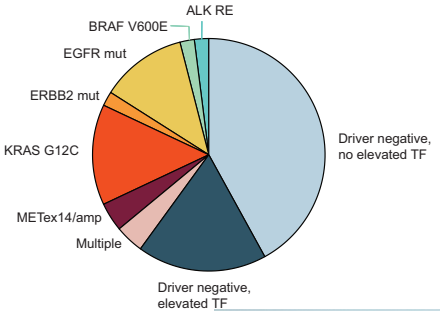




Average time from diagnosis to 1L for pre-diagnosis LBx orders (N = 38) was 20 days compared to 35 days for post-diagnosis LBx orders (N = 642) ($p < 0.001$).

Abbreviated time to CGP result return and faster therapy initiation

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

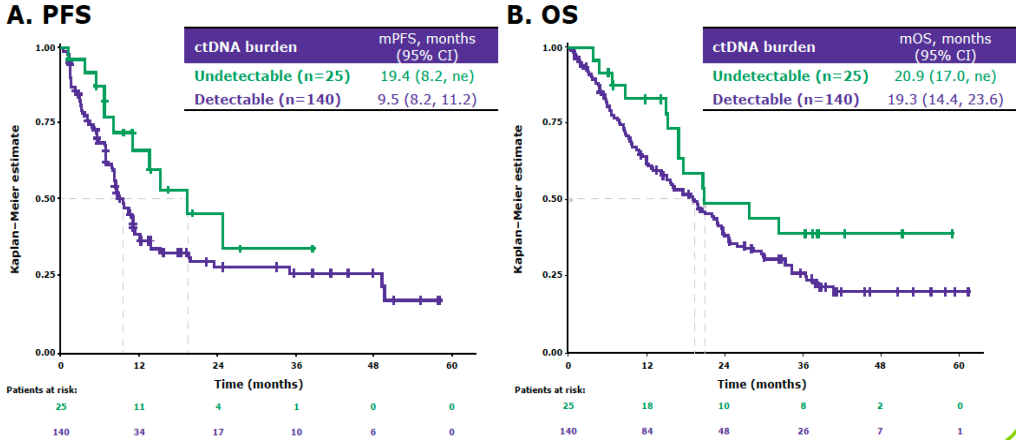


Rolfo C, et al. ISLB 2022 (OP.08)

Updates in Cancer Therapies

Liquid biopsies (LBx) and tissue biopsies (TBx) for identifying *MET*exon 14 skipping (*MET*ex14) in advanced NSCLC: Analyses from the Phase II VISION study of tepotinib

IRC	1L		+2L	
	T+/L– (n=52)	T+/L+ (n=42)	T+/L– (n=54)	T+/L+ (n=32)
ORR, % (95% CI)	57.7 (43.2, 71.3)	64.3 (48.0, 78.4)	44.4 (30.9, 58.6)	53.1 (34.7, 70.9)
mDOR, months (95% CI)	ne (10.4, ne)	19.4 (7.6, ne)	12.6 (5.1, 20.8)	9.9 (4.4, 15.4)
mPFS, months (95% CI)	22.1 (14.8, ne)	12.1 (7.8, 49.7)	13.8 (8.2, 24.9)	8.2 (5.5, 13.7)
mOS, months (95% CI)	32.7 (15.3, ne)	28.5 (14.2, ne)	20.8 (15.6, 32.5)	19.8 (10.0, 26.5)



Tepotinib had robust, durable activity in 1L and +2L, with a more favorable treatment outcome when METex14 skipping was undetectable in blood (L–)

- TBx and LBx are both suitable and complementary for detecting METex14 skipping,1but LBx may select patients with a worse prognosis, potentially due to greater disease burden

Rolfo et al, ESMO 2023

Updates in Cancer Therapies

Liquid biopsy can capture the dynamic evolution of resistance mechanisms to EGFR TKIs

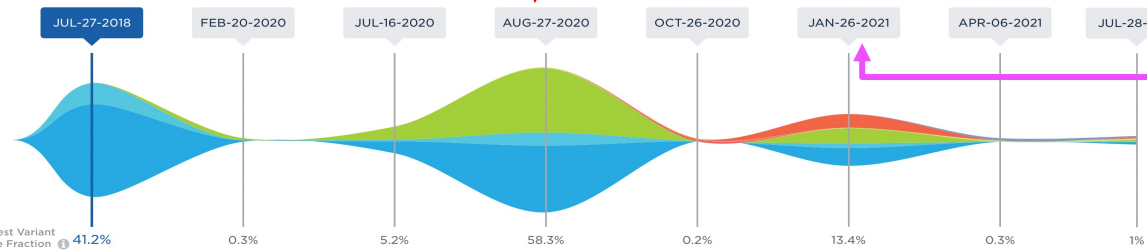
Osimertinib start with an intracranial CR and extracranial PR

After 18 mos oligo-PD (LN mets) → SBRT and continued osi

Further disease progression → osimertinib stopped; switch to erlotinib

PET/CT in Nov. 2020: CR

52-year-old never smoker female; diagnosed in July 2018 with cT4 N3 M1c lung adenocarcinoma (stage IVB).



Erlotinib discontinuation → platinum-based chemo start

Genetic Alteration	% cfDNA or amplification							
EGFR E746_A750del	41.2%	0.2%	4.7%	58.3%	ND	13.4%	ND	1%
EGFR C797S	ND	0.3%	5.2%	55.6%	ND	10.7%	ND	0.7%
ARID1A Q456Q	ND	ND	ND	0.2%	ND	0.2%	0.3%	0.6%
EGFR T790M	ND	ND	ND	ND	ND	9.6%	ND	0.4%
TP53 C275Y	ND	ND	ND	ND	ND	ND	0.1%	0.2%
ARID1A F1728F	ND	ND	ND	ND	ND	ND	0.3%	0.2%
TP53 S127F	6.5%	ND	0.4%	7.6%	ND	2.6%	ND	0.2%
***BRAF* Amplification**	2.2%	ND	ND	ND	ND	ND	ND	ND
***CDK6* Amplification**	2.2%	ND	ND	ND	ND	ND	ND	ND
***EGFR* Amplification**	3.4%	ND	ND	4.2%	ND	ND	ND	ND
***NTRK2* L699L**	-	-	-	-	0.2%	ND	ND	-
***EGFR* N338N**	ND	ND	ND	ND	0.1%	ND	ND	ND
***FGFR1* V795I**	ND	ND	ND	ND	ND	ND	0.1%	ND

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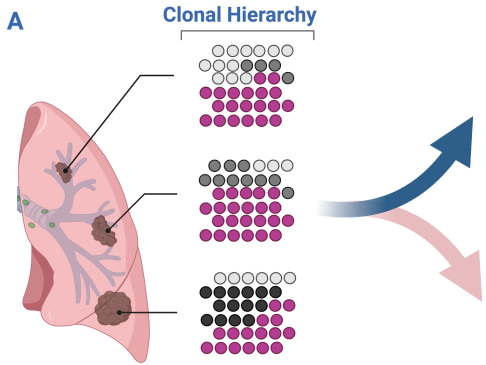
Russo A (Rolfo C) et al. Clin Lung Cancer 2023

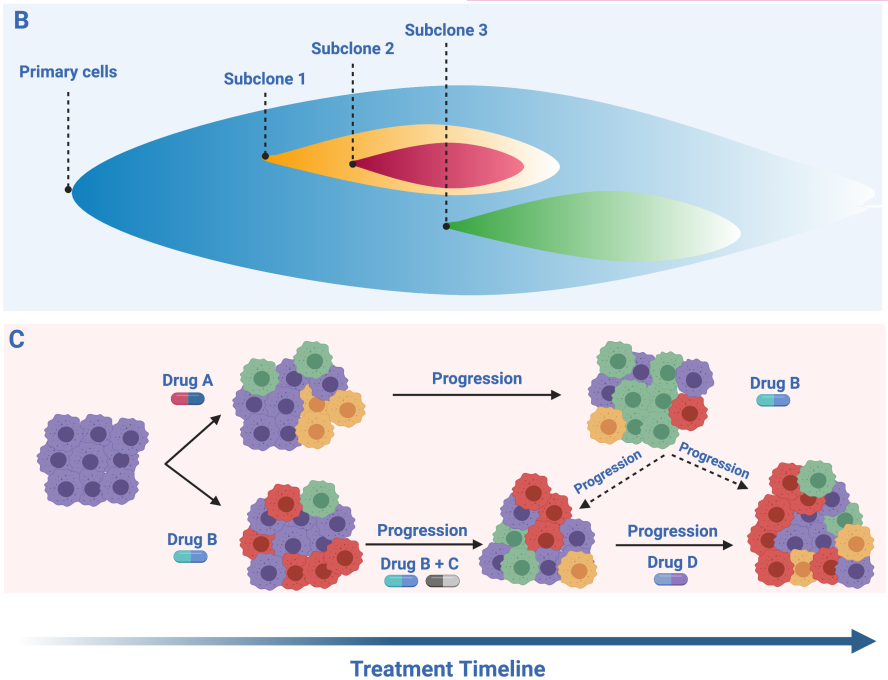
Acquired resistance is a dynamic process

Mechanisms of acquired resistance might be heterogenous and multiple mechanisms can simultaneously occur in the same patient, reflecting the clonal heterogeneity of the tumor



Tracking the clonal evolution of the tumor over time might allow the implementation of tailored therapeutic approaches

The clonal evolution of the tumor under the selective pressure of anticancer therapies



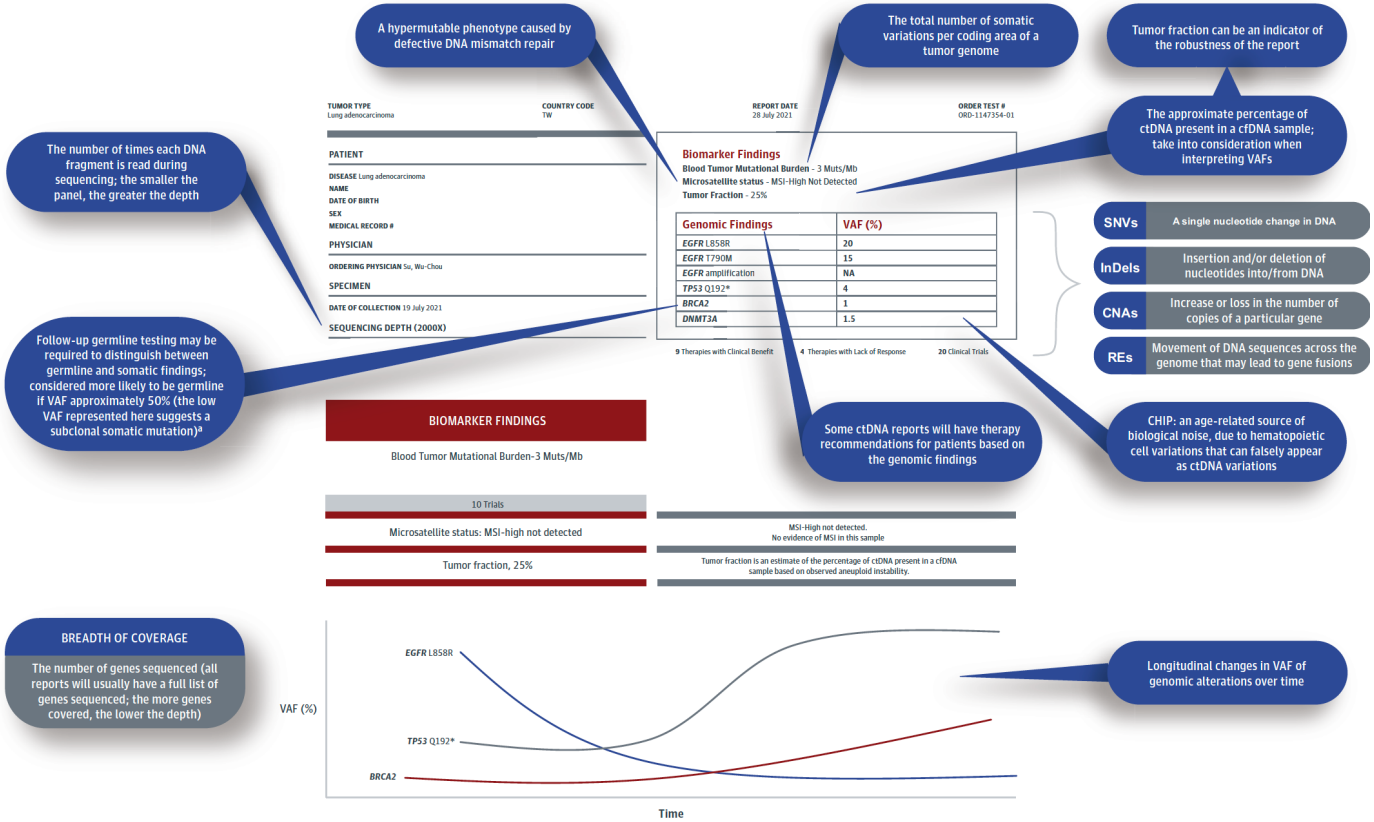
Passaro A (Rolfo C), et al. ESMO Open 2020

Updates in Cancer Therapies

Acquired Resistance Is Oncogene and Drug Agnostic

Resistance Mechanisms	MET	EGFR/ HER2/ HER3	AXL	IGF1-R	FGFR 1/2/3	NTRK 1/2/3	KIT	RAS (KRAS, NRAS)	BRAF	MAP2K1
Therapeutic Targets	EGFR	EGFR	EGFR	ALK	EGFR	EGFR	ALK	EGFR	EGFR	ALK
	ALK	ALK	ALK				ROS1	ALK	ALK	BRAF
	NTRK	ROS1	RET					NTRK	NTRK	NTRK
		RET	BRAF					ROS1		
		BRAF						RET		

Doebele RC. Cancer Cell 2019



Krebs MG (Rolfo C) et al. JAMA Oncol 2022

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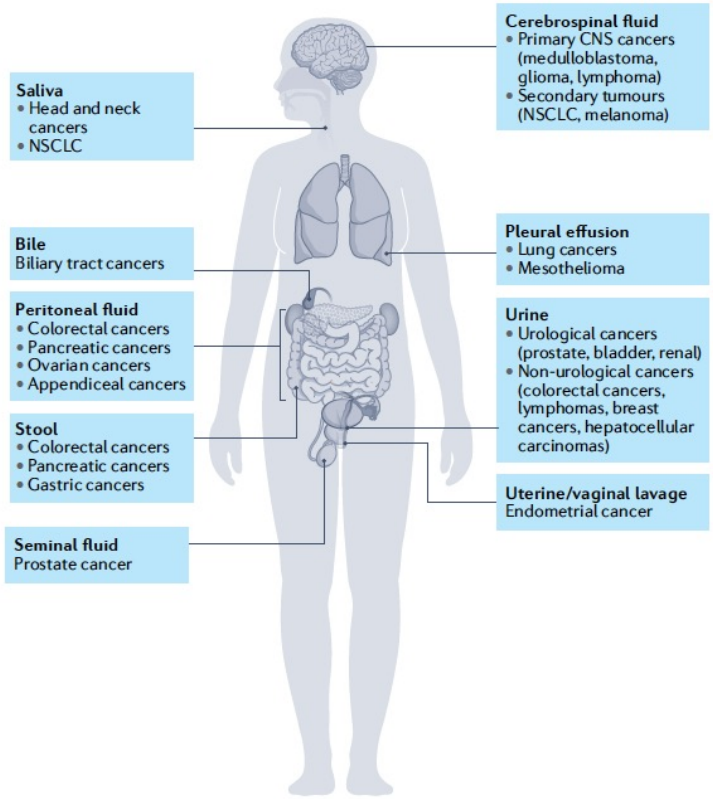
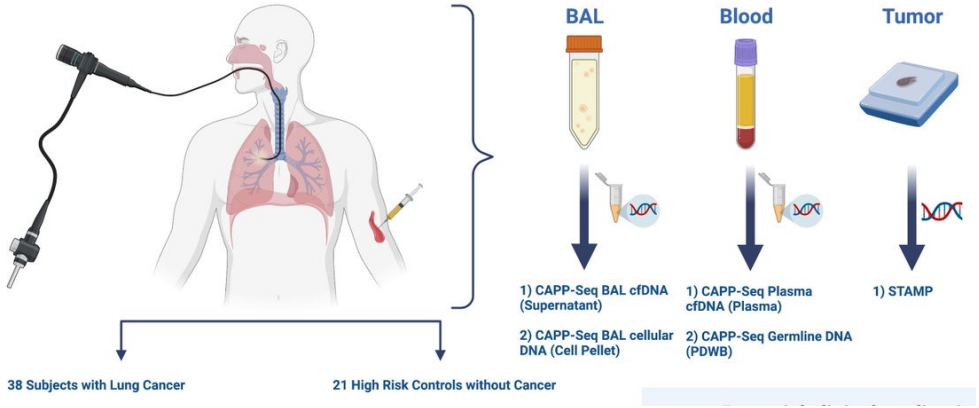
Updates in Cancer Therapies

Non-blood sources for liquid biopsy



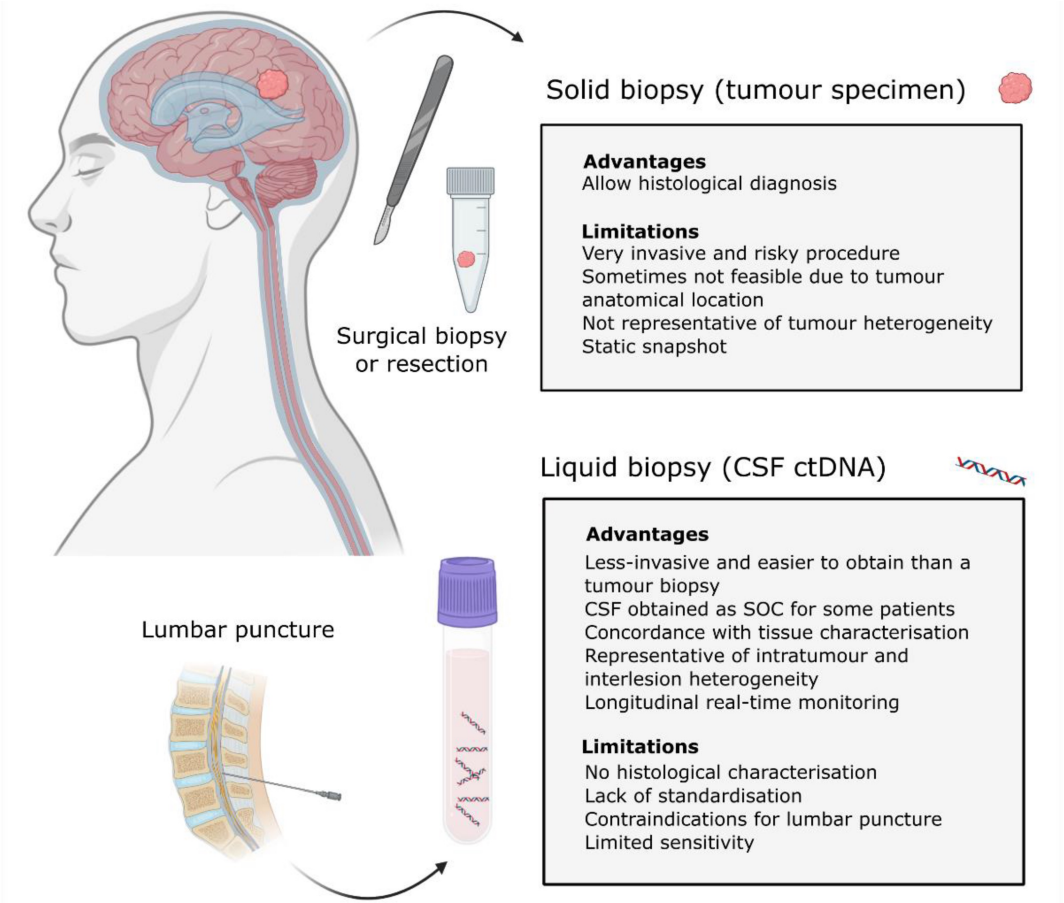


Hybrid-capture, targeted deep sequencing of lung cancer mutational burden in cell-free BAL fluid identifies more tumor-derived mutations with increased allele frequencies compared with plasma cell-free DNA.

Rolfo, Malapelle, Russo, Cancer Res (2022) 82 (16): 2826–2828

Nair et al (M. Diehn) Cancer Res 2022;82:2838–47

Tivey A, et al. Nat Rev Clin Oncol 2022



Solid biopsy (tumour specimen)

Advantages
Allow histological diagnosis

Limitations
Very invasive and risky procedure
Sometimes not feasible due to tumour anatomical location
Not representative of tumour heterogeneity
Static snapshot

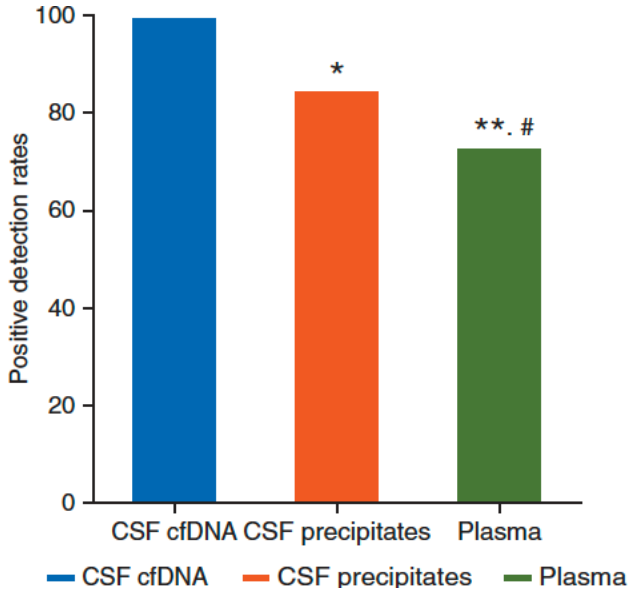
Liquid biopsy (CSF ctDNA)

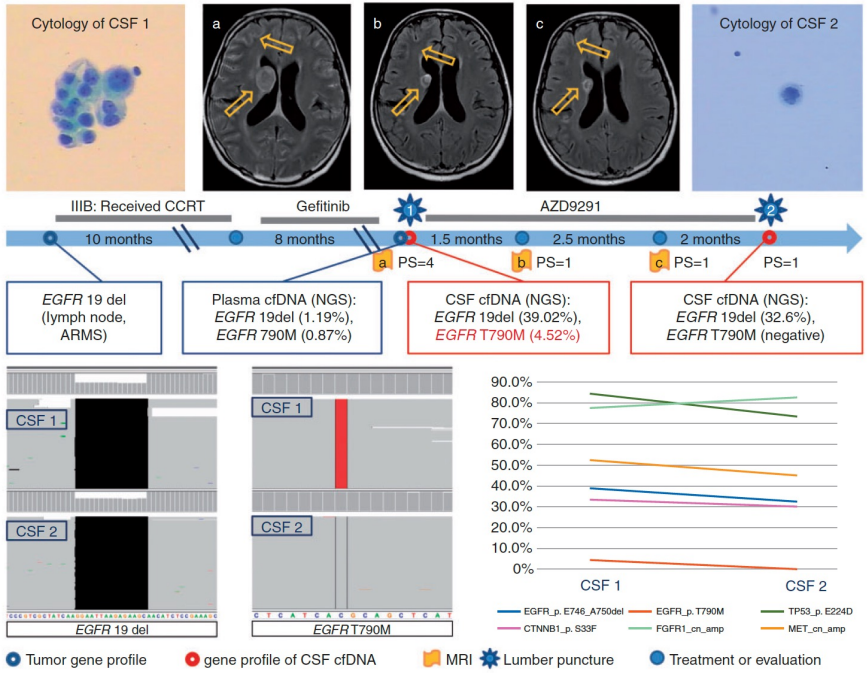
Advantages
Less-invasive and easier to obtain than a tumour biopsy
CSF obtained as SOC for some patients
Concordance with tissue characterisation
Representative of intratumour and interlesion heterogeneity
Longitudinal real-time monitoring

Limitations
No histological characterisation
Lack of standardisation
Contraindications for lumbar puncture
Limited sensitivity

Escudero et al, Cancers **2021**, *13*(9), 1989

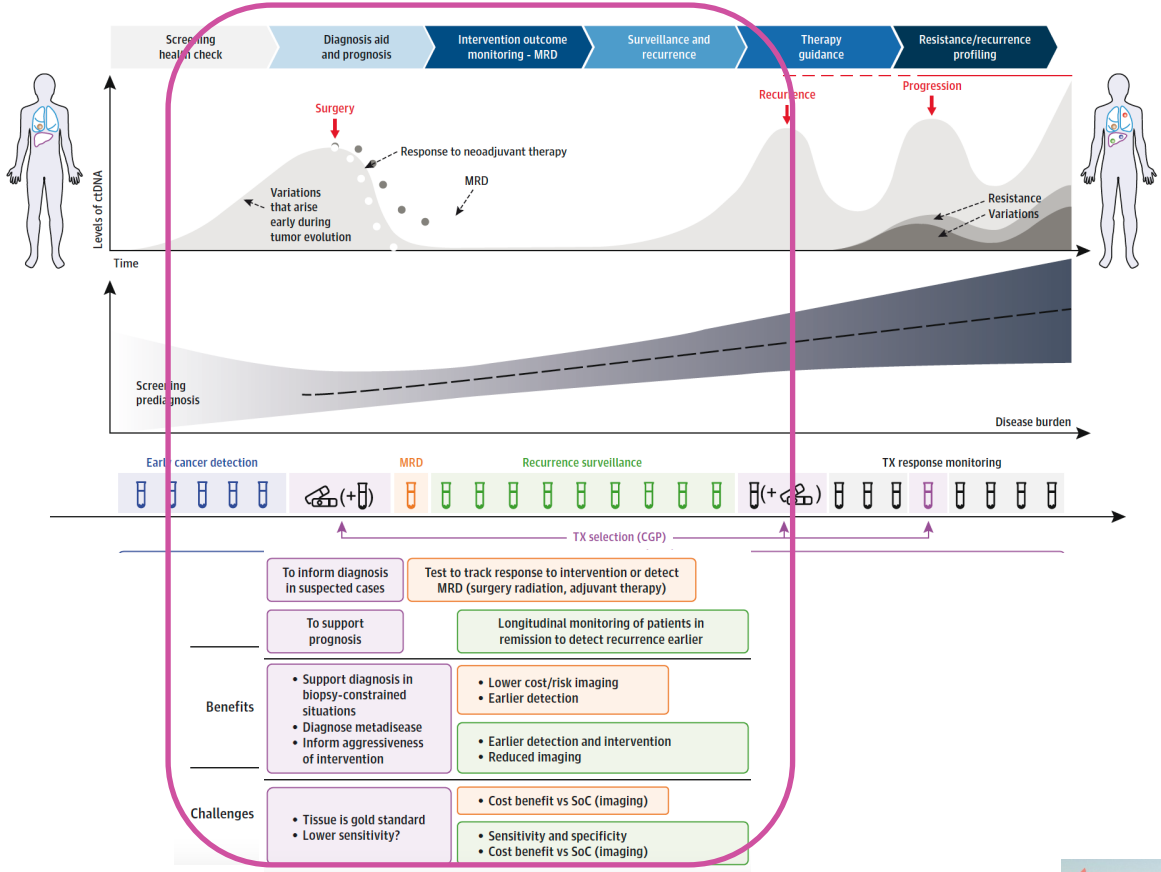
CSF for EGFR mutations detection in patients with leptomeningeal metastases





Li YS, et al. Ann Oncol 2018

Liquid biopsy during lung cancer patient journey



Krebs MG (Rolfo C) et al. JAMA Oncol 2022

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Updates in Cancer Therapies

Retrospective Data From ~900 NSCLC Patients demonstrate that pre- and post-treatment MRD is a strongly prognostic biomarker

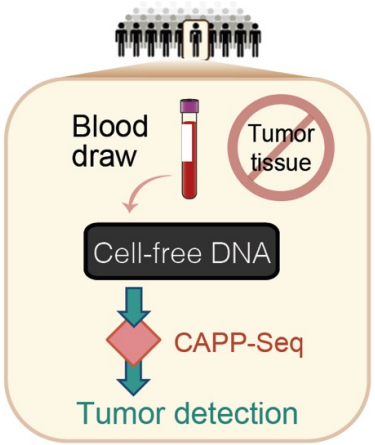
Study	N	Stage	Treatment(s)	ctDNA assay
Chaudhuri *Cancer Discov* 2017	37	IB-IIIB	RT and/or surgery +/- chemo	CAPP-Seq
Abbosh *Nature* 2017	24	IA-IIIB	Surgery +/- chemo	Natera
Chen *CCR* 2019	25	I-III	Surgery +/- chemo	cSMART
Moding *Cancer Discov* 2020	48	IIB-IIIB	chemoRT +/- IO	CAPP-Seq
Abbosh *AACR* 2020	88	I-III	Surgery +/- chemo	ArcherDx
Zviran *Nat Med* 2020	22	I-III	Surgery +/- chemo	MRDetect
Waldeck *Mol Oncol* 2021	16	IA-IIIB	Surgery +/- chemo, RT	Custom NGS
Xia *CCR* 2021	329	I-III	Surgery +/- chemo	Custom NGS
Gale *Ann Oncol* 2022	59	I-III	RT and/or surgery +/- chemo	Inivata
Zhang *Cancer Discov* 2022	245	I-III	Surgery +/- chemo, IO, TKI	Custom NGS

***Several studies including different population, treatment and assays

Slide Courtesy Dr. Natasha Leighl

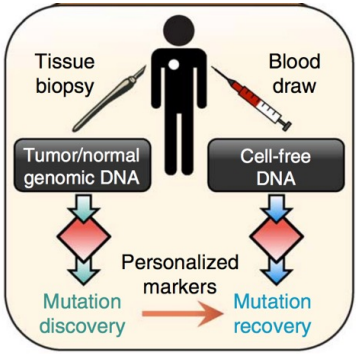
Different types of ctDNA MRD Assays

Tumor-naive



- Genotyping with no knowledge of tumor mutations (“off the shelf”)
- Faster, less expensive
- Limit of detection ~0.1%

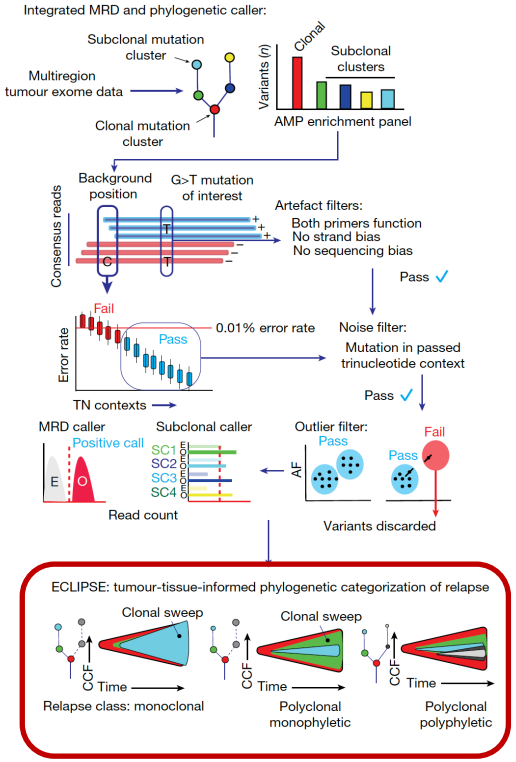
Tumor-informed



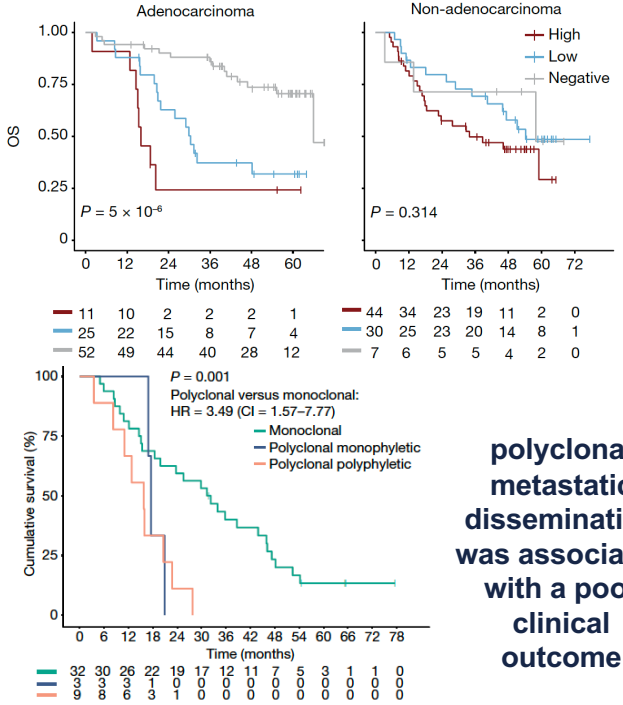
- Tracking multiple known mutations (bespoke or personalized)
- Requires tumor tissue, time, $$
- Limit of detection ~0.01%

Courtesy Dr. Natasha Leighl

Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA (WES)

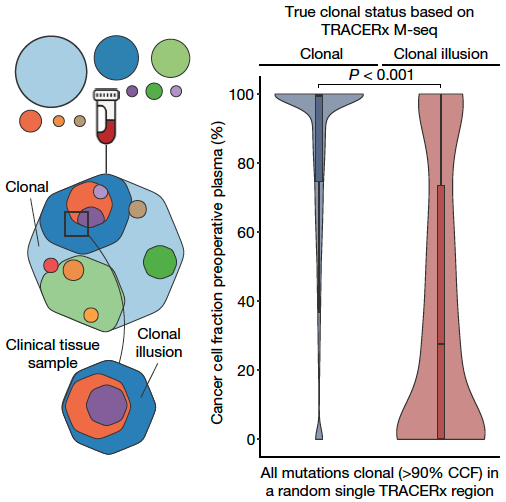


ctDNA can identify MRD and predict survival



polyclonal metastatic dissemination was associated with a poor clinical outcome

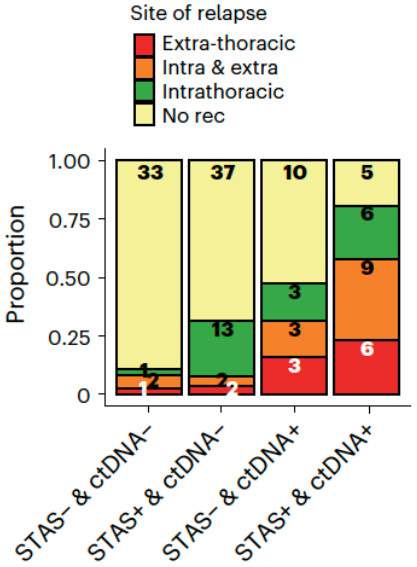
LB can overcome sampling bias from a single tissue sample

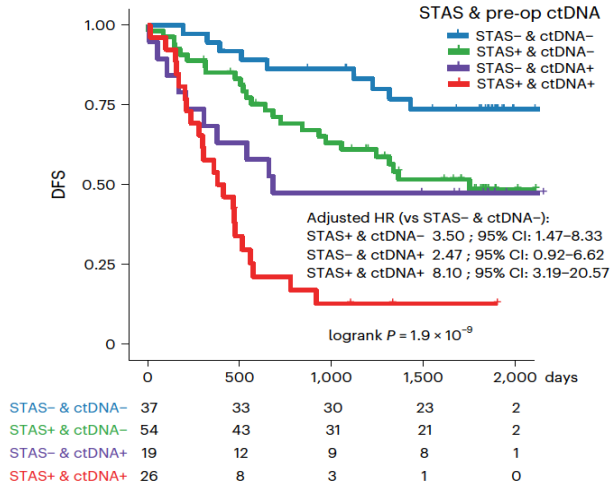


Abbosh C, et al.(Swanton) Nature 2023

Integration of liquid biopsy and pathology: The TRACERx study

STAS: Spread Through Air Spaces



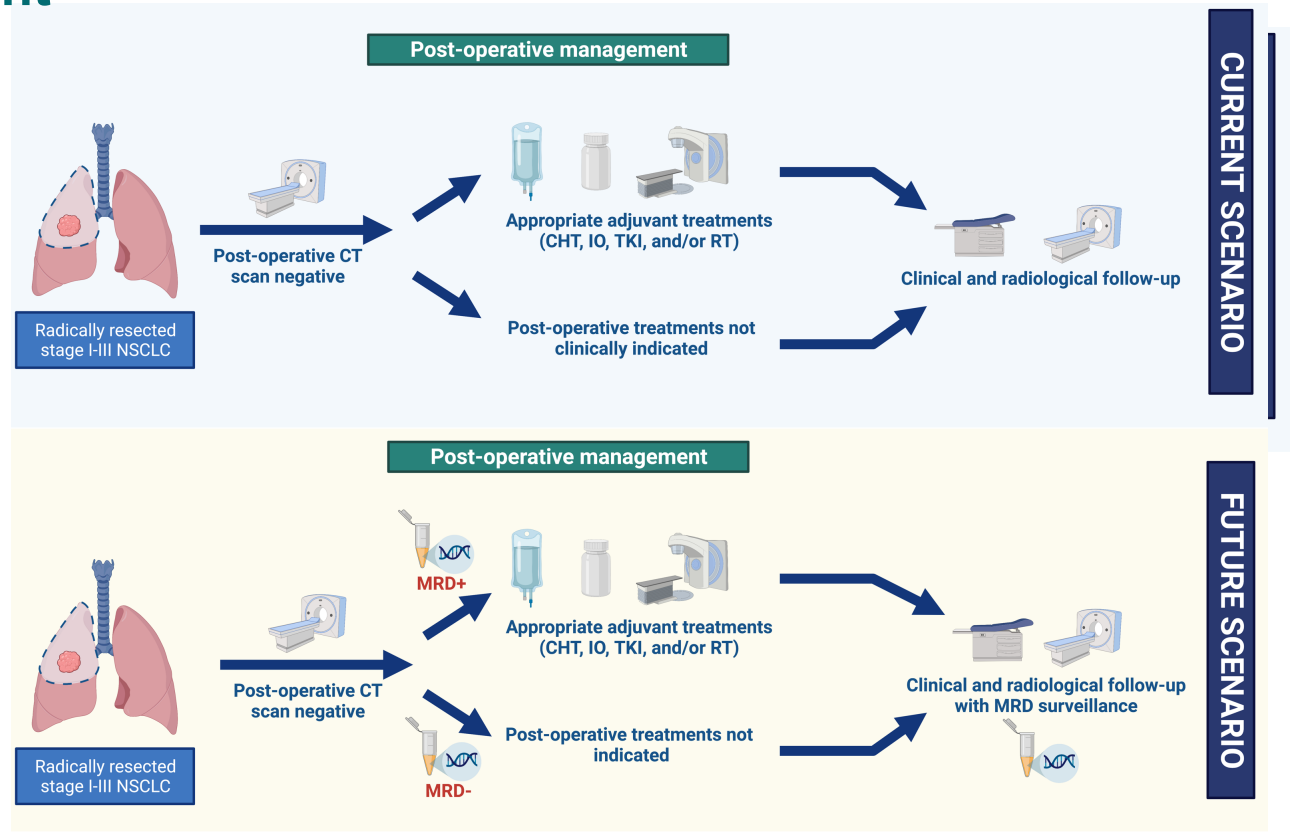


High-grade patterns	**Micropapillary**	**Solid/cribriform**
Pathological features	**STAS+**	**Necrosis+** High Ki-67
Genomic features	High clonal diversity (lack of large recent clonal expansion)	**Pre-op ctDNA+** High CIN Low clonal diversity (presence of large recent clonal expansion)
Relapse site	Intra-thoracic	Extra-thoracic

"…These data provide insights into the relationship between LUAD morphology, the underlying evolutionary genomic landscape, and clinical and anatomical relapse risk…"

Karasaki T, et al. Nat Med 2023

Liquid biopsy can potentially revolutionize post-operative management



Rolfo C, Russo A, Malapelle U. eBioMedicine 2023

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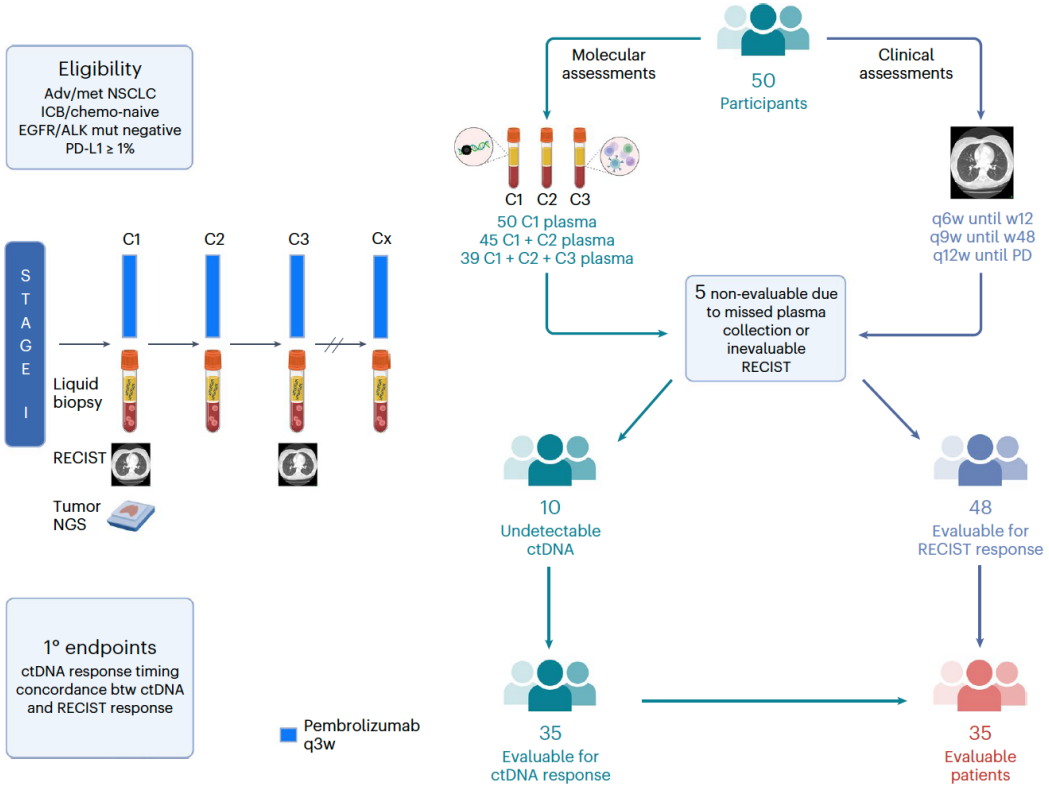
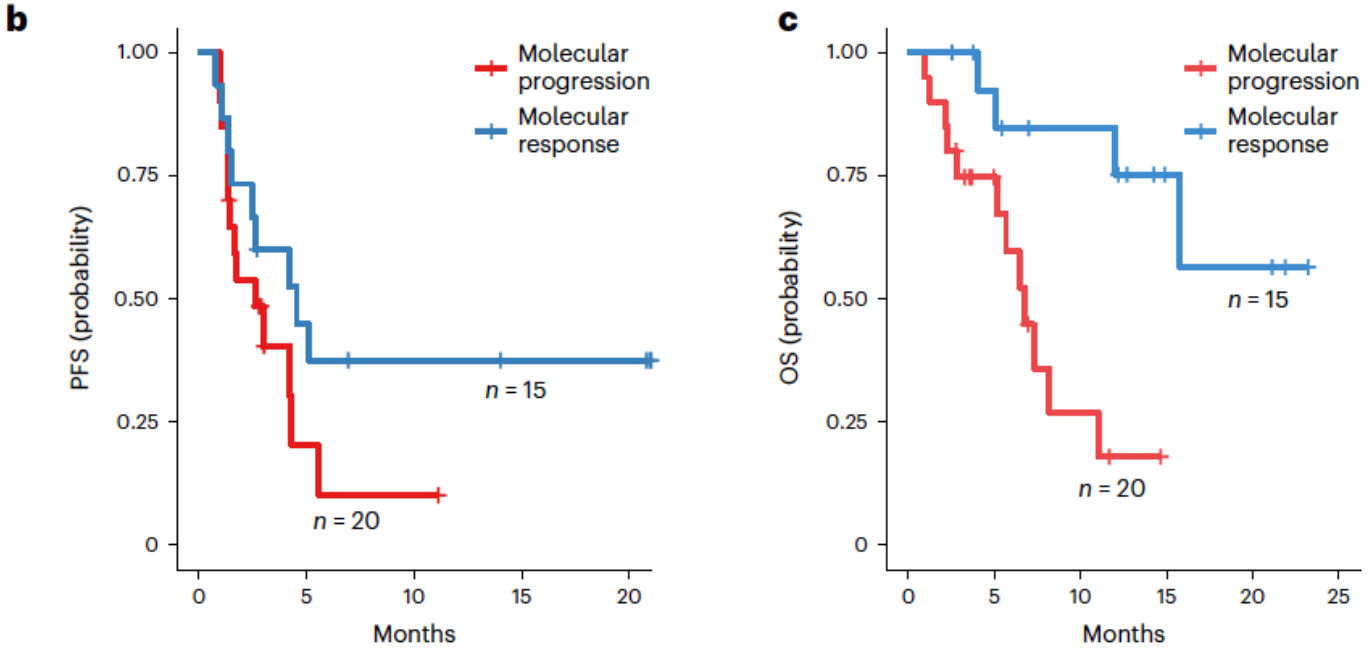
Updates in Cancer Therapies

BR.36 study design



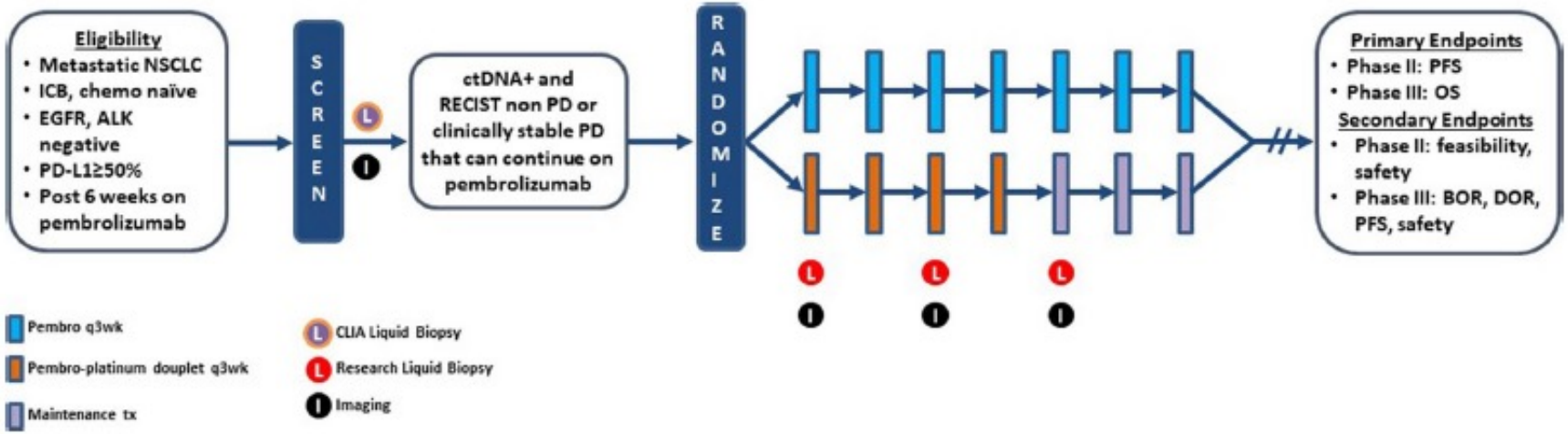
Anagnostou V, et al. Nat Med 2023

Molecular response and IO outcomes



Anagnostou V, et al. Nat Med 2023

Trial schema for the interventional second stage of the BR.36 study.



Anagnostou V, et al. Nat Med 2023

ADMIRO Trial

Principal investigators: Besse & Remon

- Stage II NSCLC (8th TNM)
- ECOG PS 0-1
- EGFR-negative
- PET-CT and brain MRI/CT

Surgery

Plasma

ctDNA post-surgery (14–42 days)

Tumour uninformed assay

R 1:1

+14 to +60 days

ctDNA guided

Plasma ctDNA positive

Plasma ctDNA negative

Adjuvant CT, 4 cycles, q3w +/- ICB according to SoC

Observation

Pathologic guided

Plasma ctDNA + & -

Standard management

Primary endpoint: DFS at 2 years (ctDNA-guided versus pathologic-guided)

Secondary endpoint:

- DFS ctDNA positive versus negative in ctDNA guided arm
- MRD-free interval in ctDNA negative
- Overall survival

Stratification:

- Histology: squamous versus non-squamous
- Nodal status: N0 versus N1
- Site

These blood samples will be collected and stored

Plasma — **Week 6**

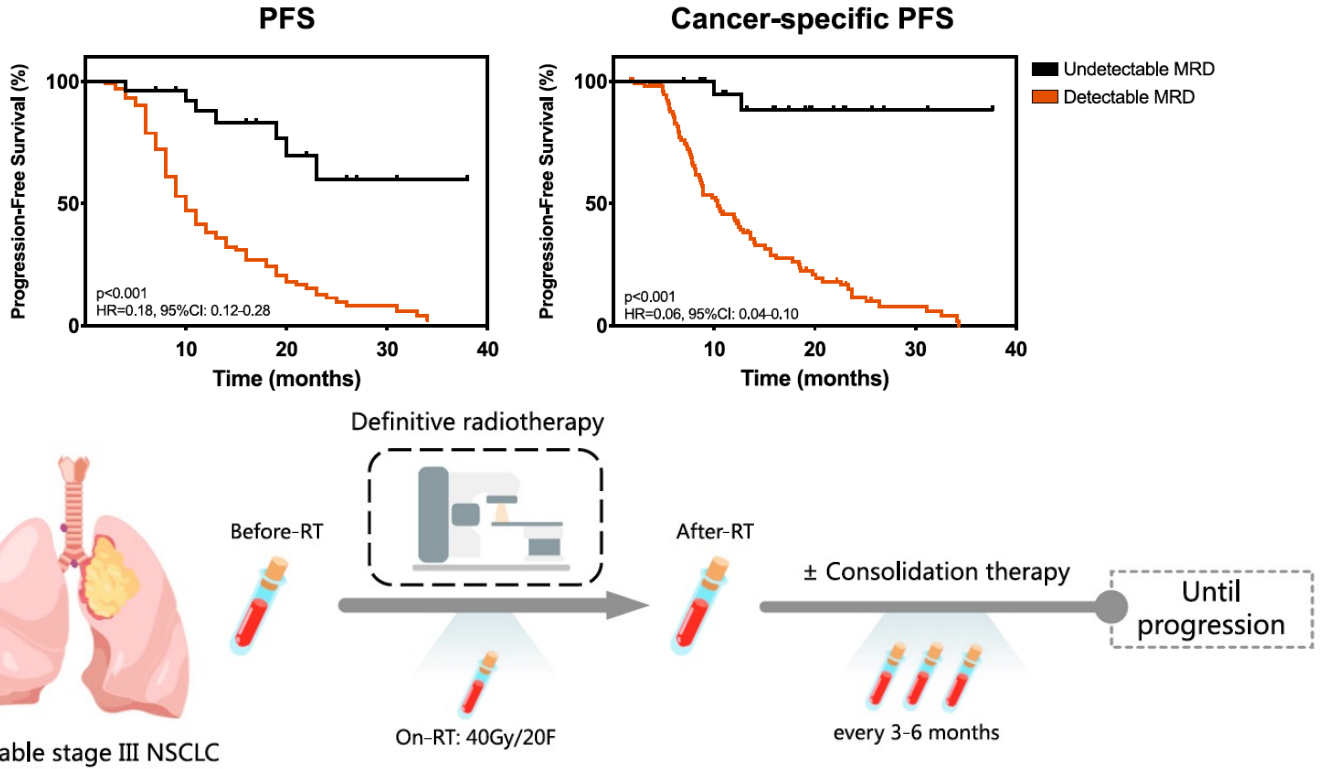
Plasma — **Week 12**

Plasma — **Every 6 months for 3 years**

Plasma — **At PD**

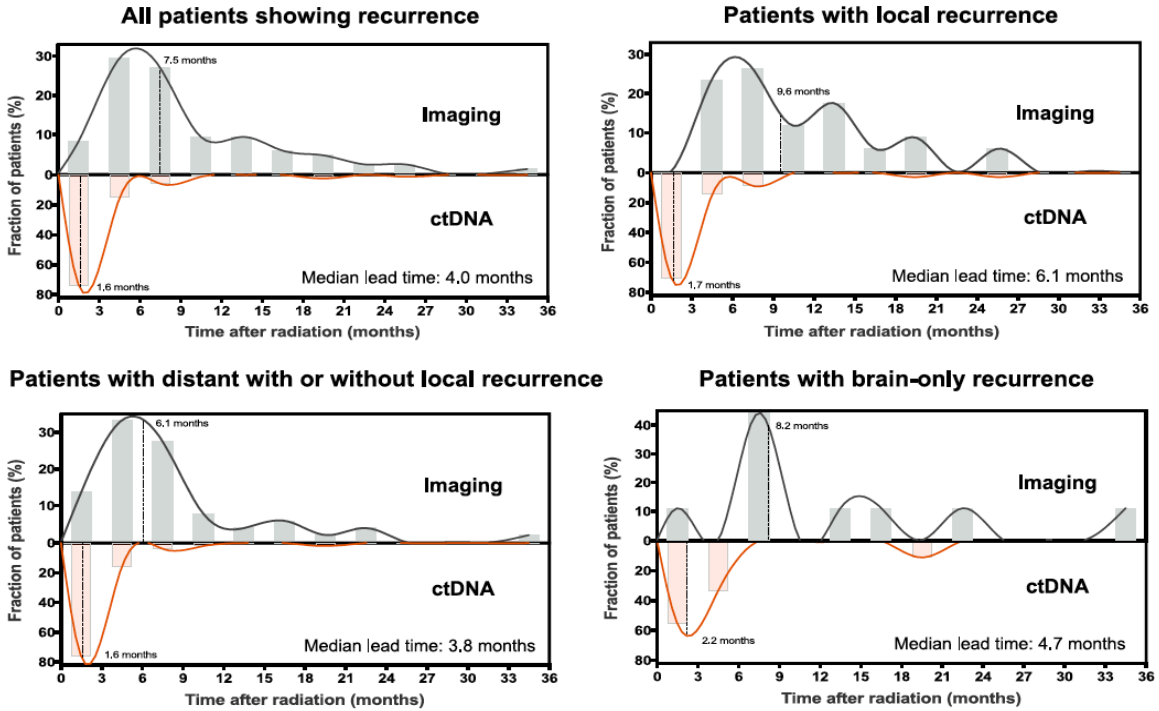
Information provided by Dr Natasha Leighl and Dr Jordi Remon.

Dynamic circulating tumor DNA during chemoradiotherapy predicts clinical outcomes for LA-NSCLC patients



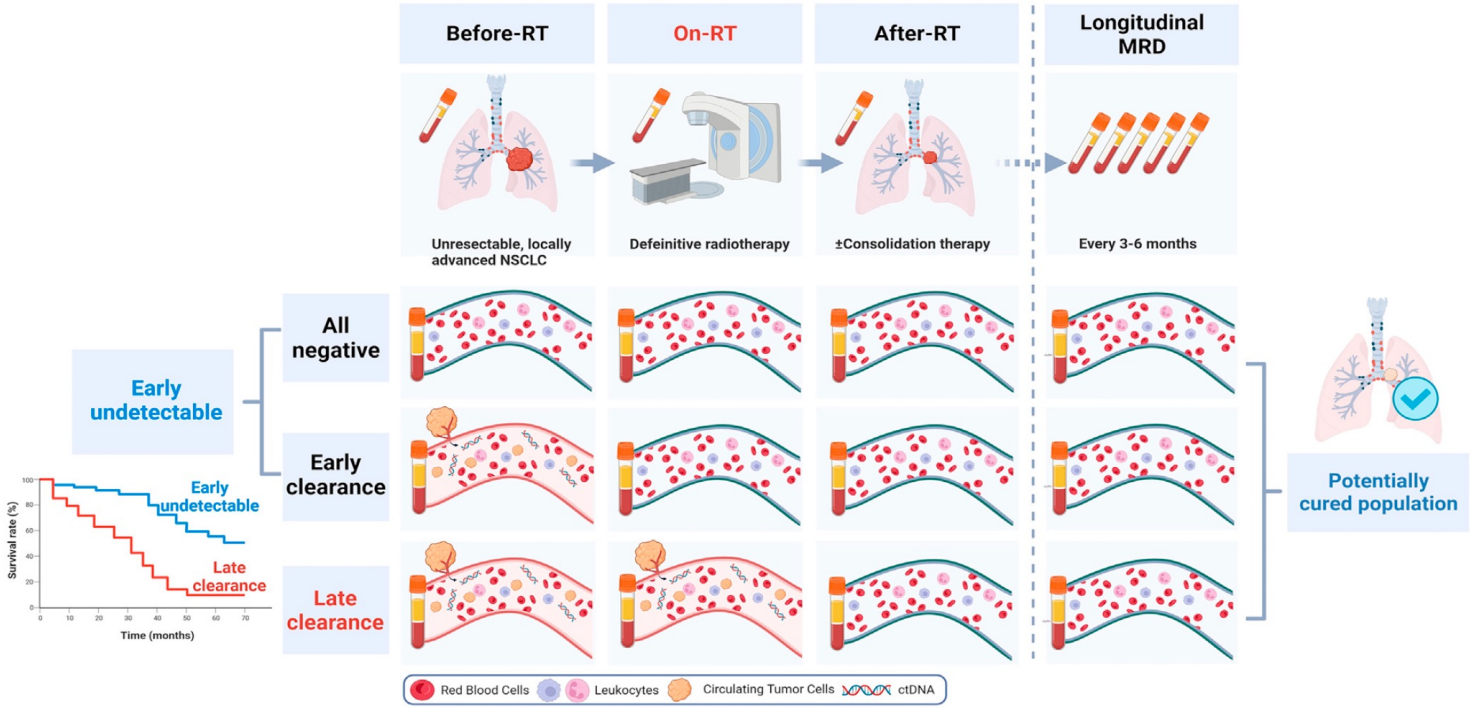
Pan Y, et al. Cancer Cell 2023

Longitudinal MRD detection rate of patients with different progression patterns



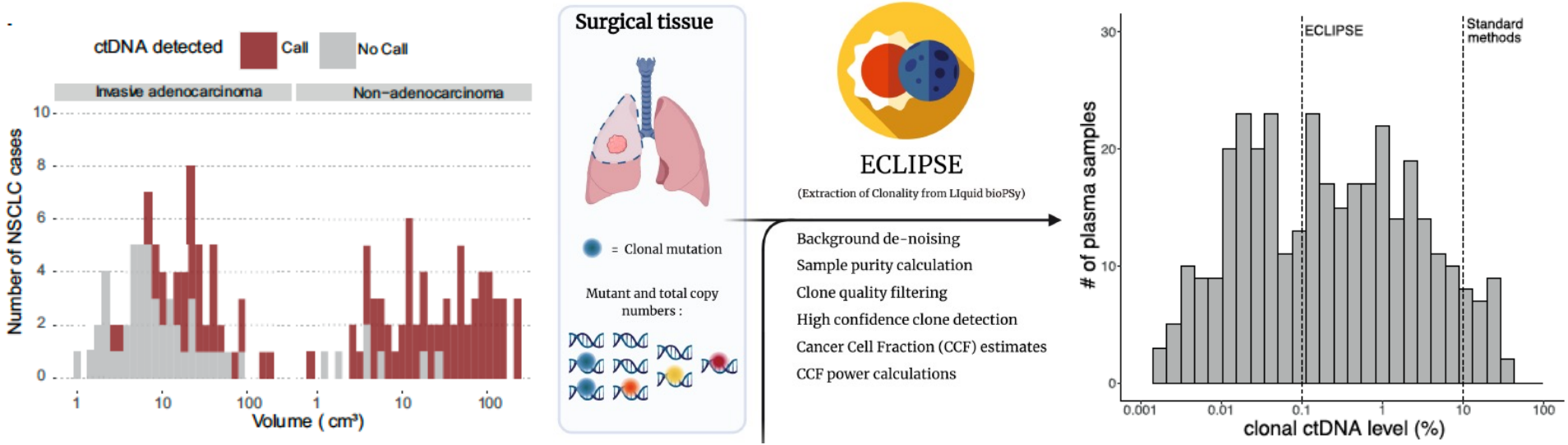
Pan Y, et al. Cancer Cell 2023

The growing significance of longitudinal MRD in NSCLC clinical practice



Li Z, et al. Cancer Cell 2023

Detecting MRD via Subclonal Populations and AI

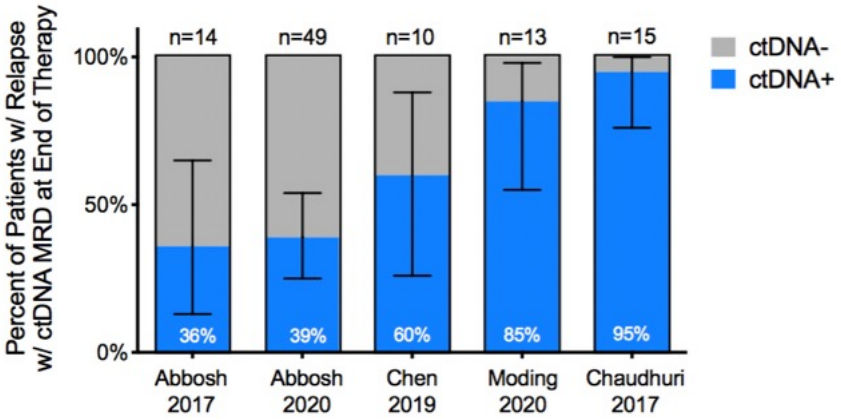


Abbosh C, et al. *Nature*. 2023;616:553–562.

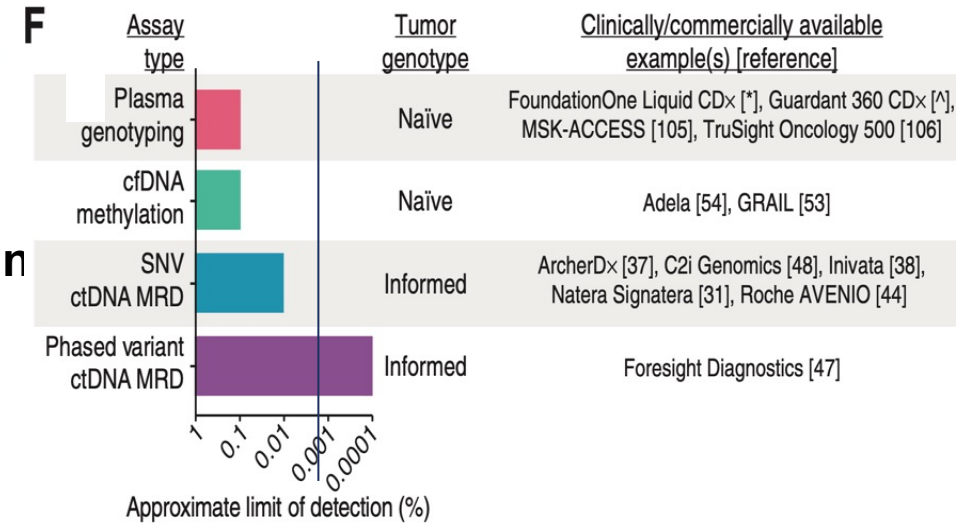
Slide Courtesy of Dr. Natasha Leighl

Updates in Cancer Therapies

Sensitivity of MRD Assay: A Challenge



Summary of assay types, tumour genotyping requirements and approximate LODs[2]



Chin R-I, et al. Mol Diagn Ther. 2019;23:311–31. Moding EJ, et al. Cancer Discov. 2021;1:2968–86.
Slide Courtesy of Dr. Natasha Leighl

Take Home Message

- Liquid Biopsy is a perfect tool for monitoring in advance disease and MRD
- Integrating liquid biopsy in clinical trials is a necessity
- Real time monitoring in patients with high risk of recurrence requires improved technology in liquid biopsy

Thanks!

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See You at

ISLB 2024

in Chicago, USA!