Update of Liquid Biopsy from Advanced to early disease

ISLB
INTERNATIONAL SOCIETY OF LIQUID BIOPSY

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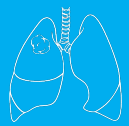

Center for Thoracic Oncology

Mount Sinai
The Tisch Cancer Institute

Disclosures

Research grants	Lung Cancer Research Foundation-Pfizer Grant 2019 American Cancer Society NIH SBIR, NCI SeroNet 2020
Personal financial interests	Speaker: MSD, Astra Zeneca, Roche, GuardantHealth
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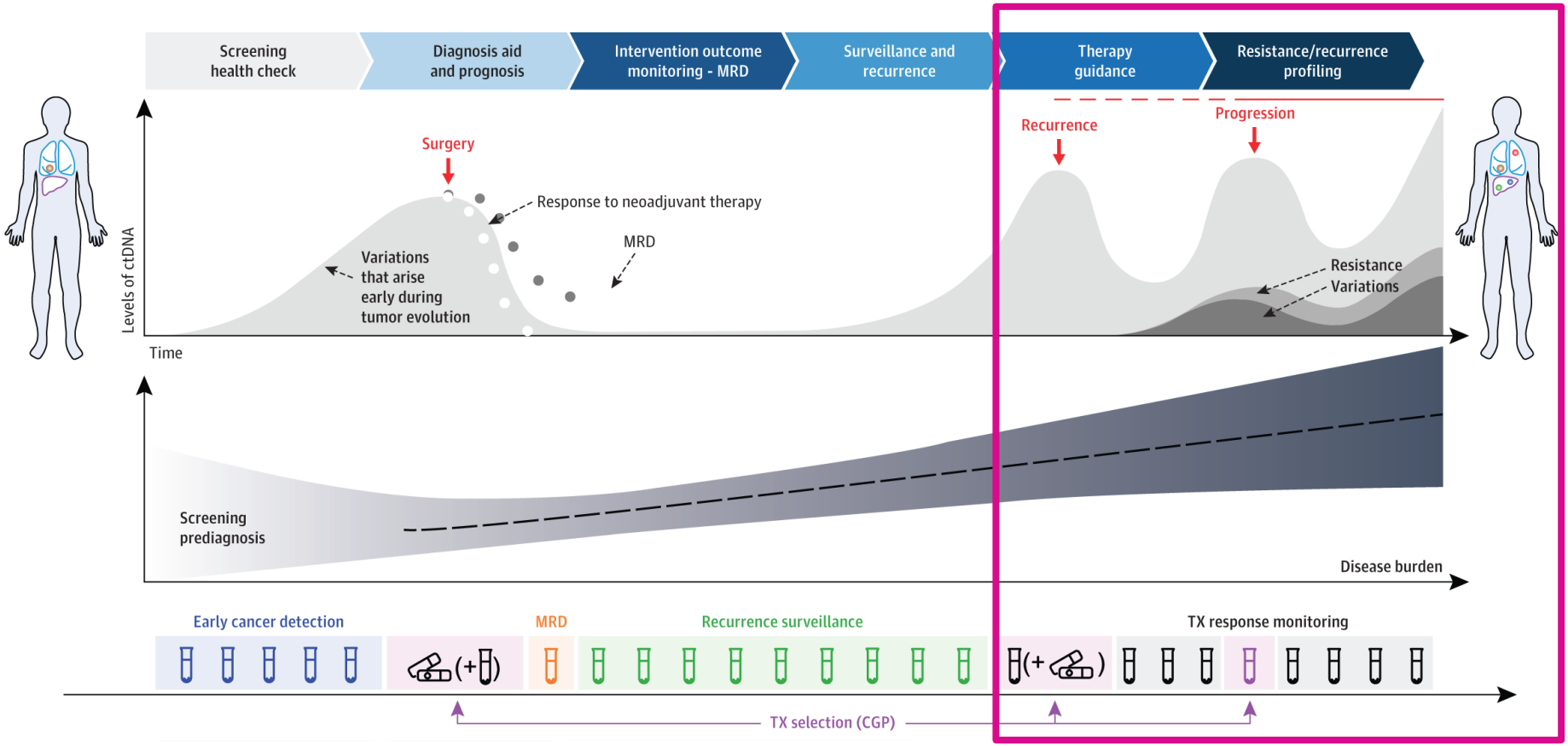
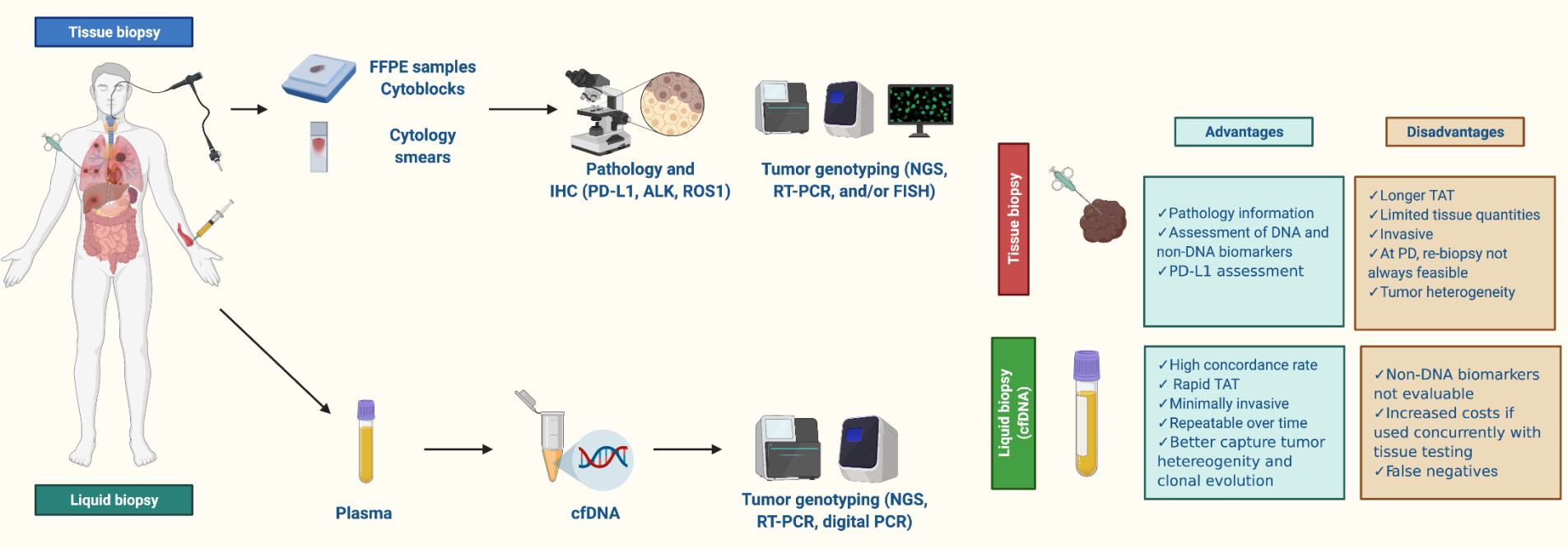
Non-financial interests	Research Collaboration: GuandantHealth (UMB)
Leadership roles	Chair Educational Committee IALSC - President ISLB (International Society of Liquid Biopsy) - Educational Chair: OLA Oncology Latin American Association Scientific Committee Member at ESO (European School of Oncology).



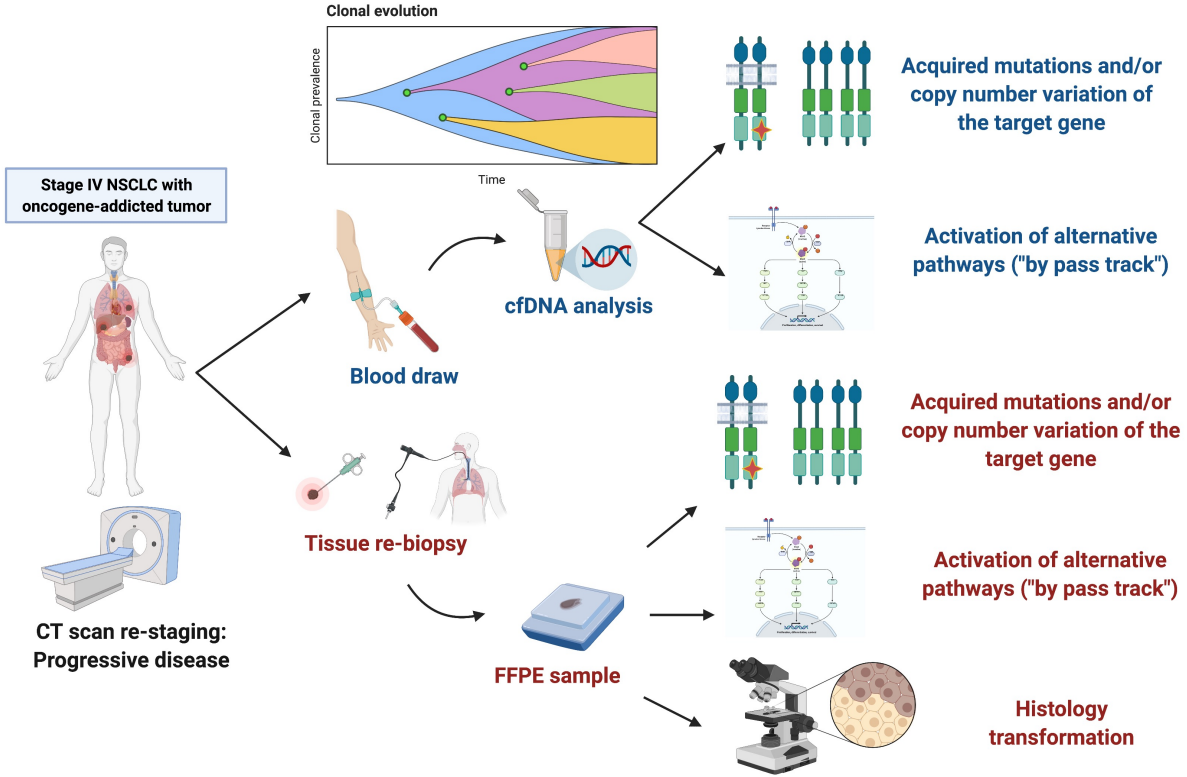
Krebs et al (Rolfo), JAMA Oncology OCT 2022

Tissue vs. Liquid biopsy



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

Clinical utility of liquid biopsy in oncogene-addicted NSCLC



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

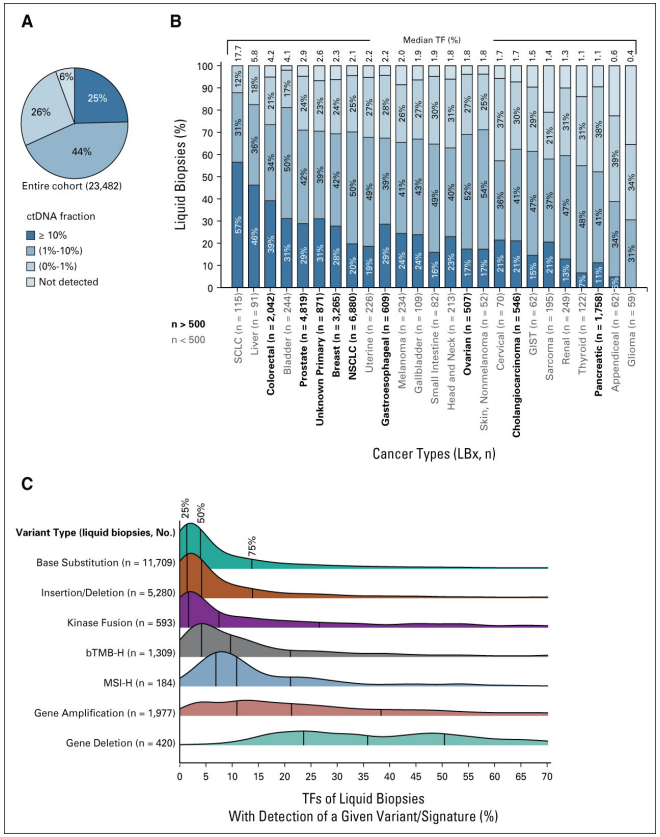
FISH:
- ✓Gene rearrangements & amplifications

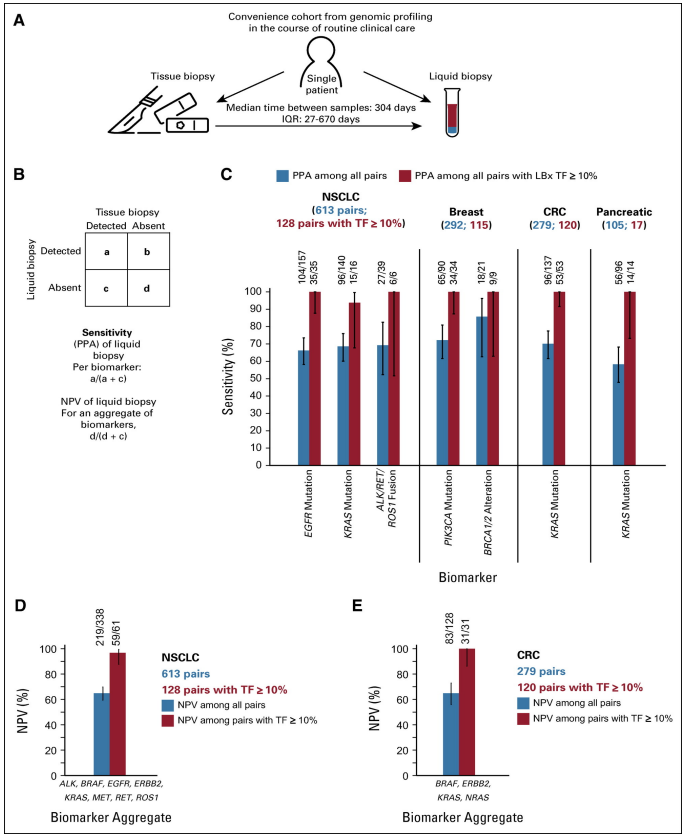
PCR-based approaches:
- ✓Variable sensitivity
- ✓Single/Multiplex gene testing
- ✓Only for mutations

IHC:
- ✓Protein expression

Rolfo C, et al. J Thorac Oncol 2021

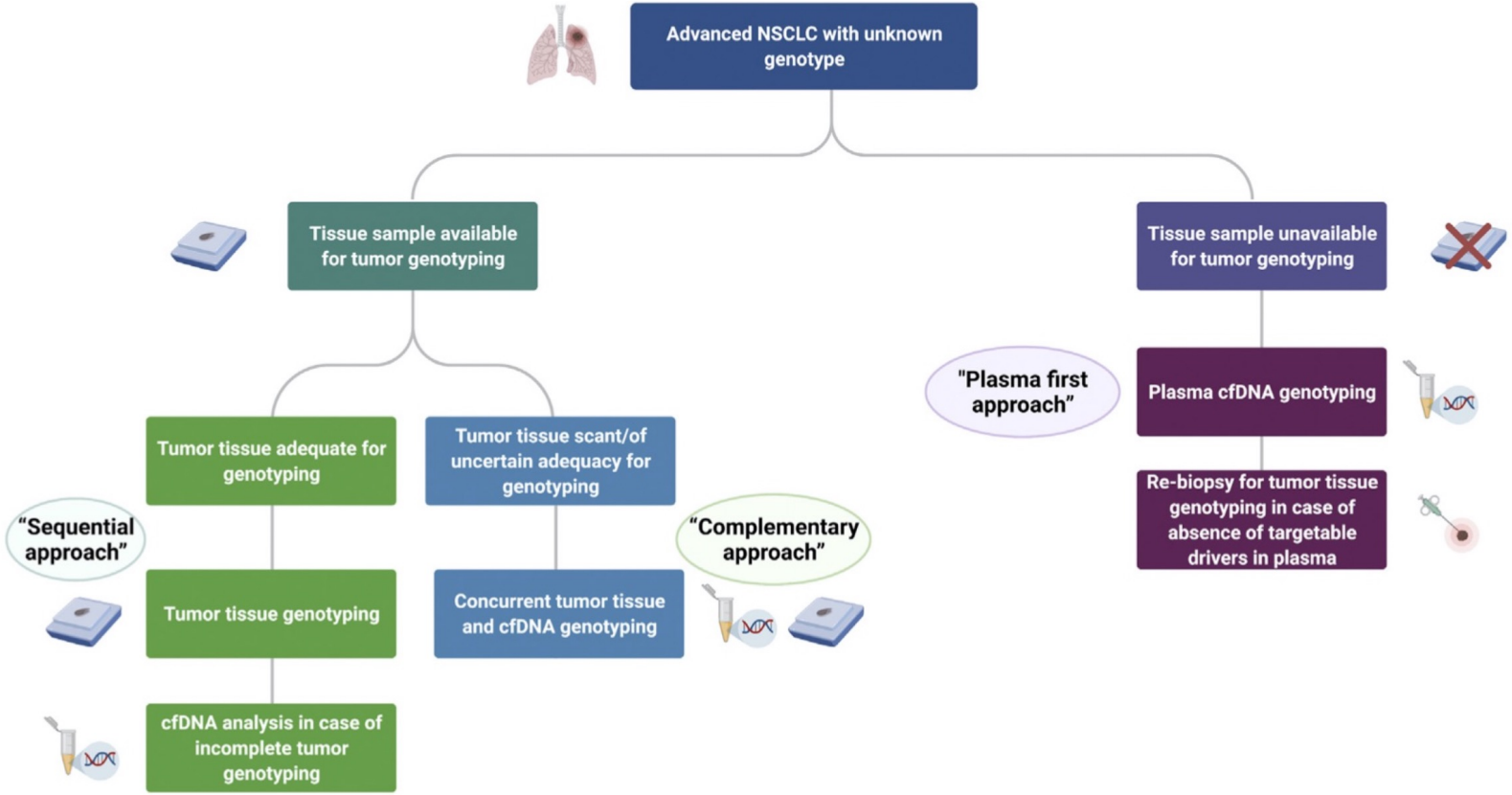
Tumor Fraction Correlates With Detection of Actionable Variants Across > 23,000 Circulating Tumor DNA Samples





- Elevated ctDNA shed is associated with both high sensitivity and negative predictive value for detection of actionable Genomic Alterations .
- The presence of elevated TF suggests adequate tumor profiling and may reduce the value of subsequent reflex to confirmatory tissue testing in patients with negative LBx results.

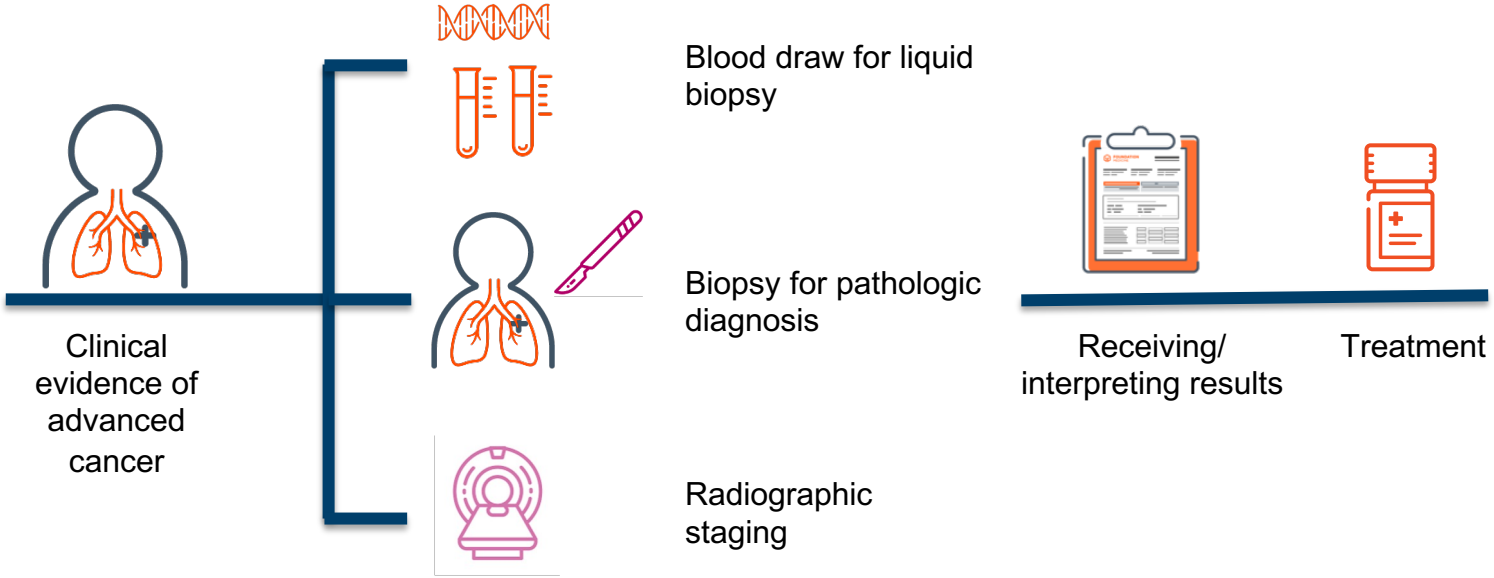
Husain at al, JCO PO, OCT 2022



Rolfo C, et al. J Thorac Oncol 2021

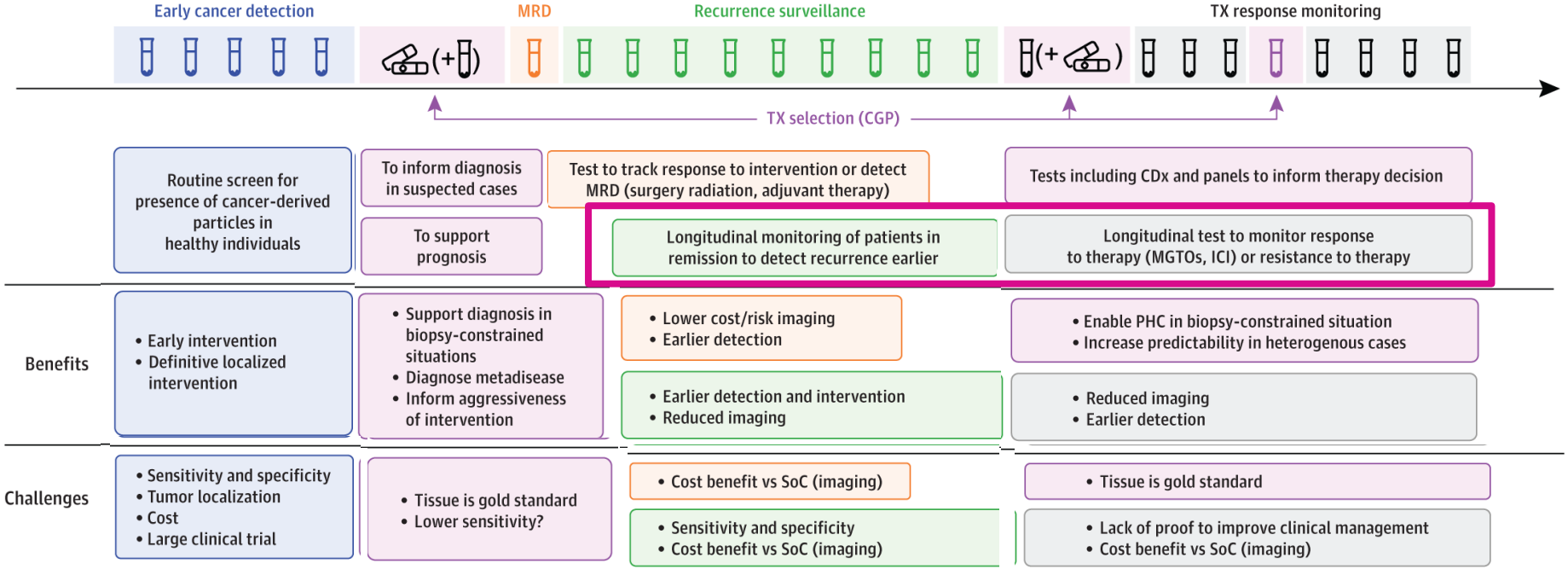
Expedited diagnostic odyssey

Stacking diagnostic steps may be able to shorten the diagnostic odyssey



Rolfo et al, in preparation

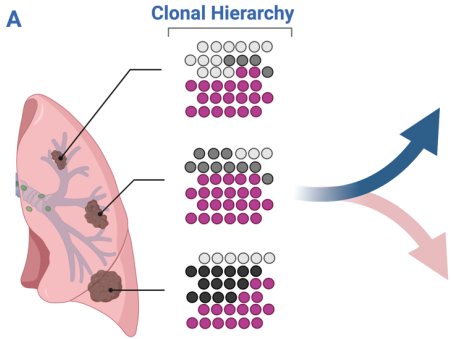
Benefits and challenges of LB in the Cancer Journey



Krebs et al (Rolfo), JAMA Oncology OCT 2022

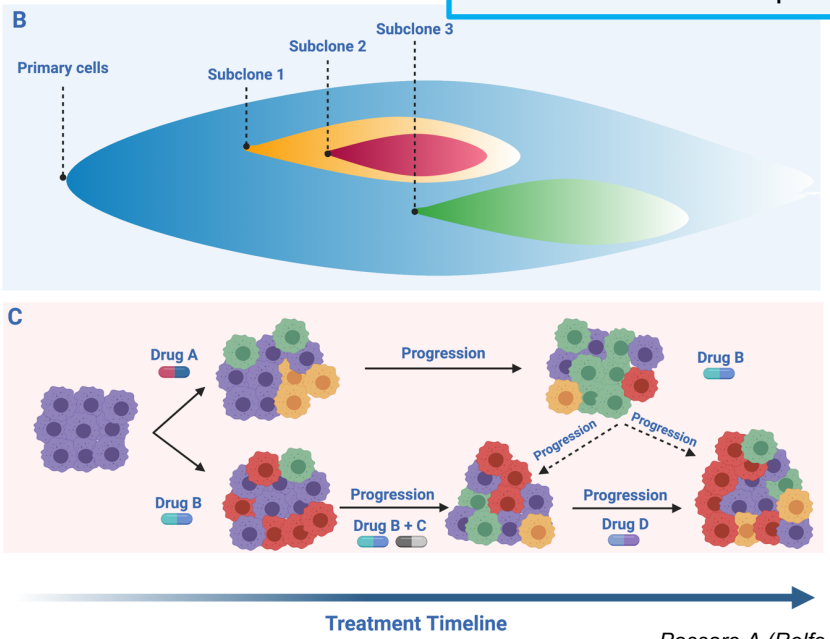
Acquired resistance is a dynamic process

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

Tailoring treatment with Liquid Biopsy

Osimertinib start with an intracranial CR and extracranial PR

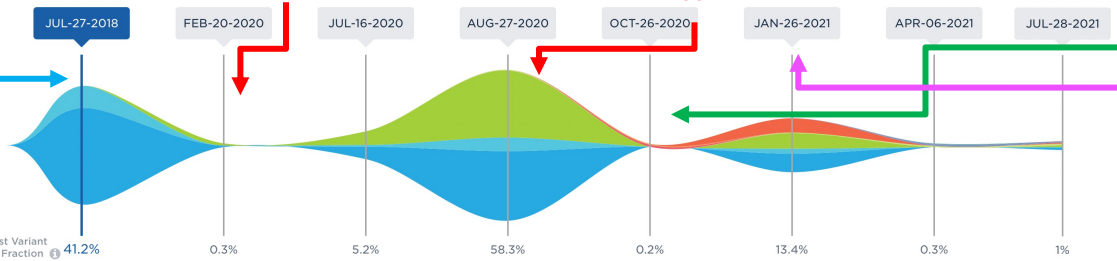
52-year-old never smoker female; diagnosed in July 2018 with cT4 N3 M1c lung adenocarcinoma (stage IVB).

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

Further disease progression → osimertinib stopped; switch to erlotinib

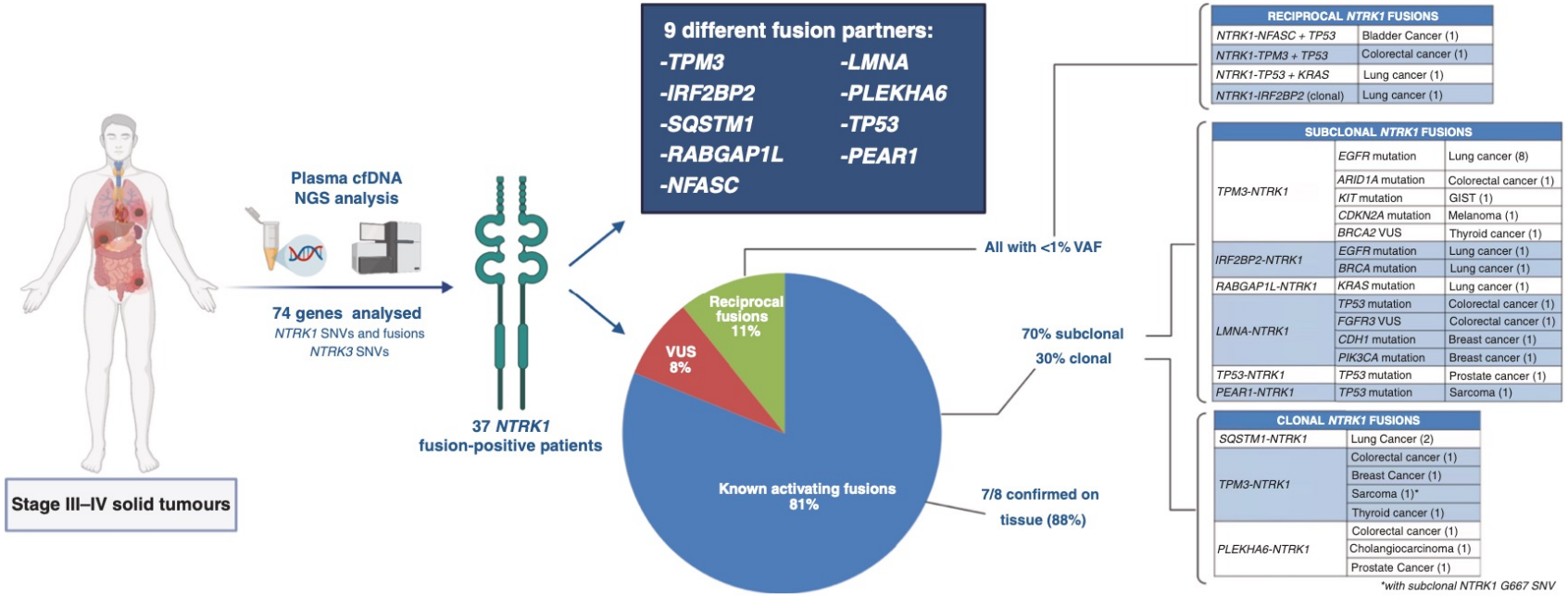
PET/CT in Nov. 2020: CR

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

NTRK1 Fusions identified by non-invasive plasma next-generation sequencing (NGS) across 9 cancer types



RECIPROCAL *NTRK1* FUSIONS	
NTRK1-NFASC + TP53	Bladder Cancer (1)
NTRK1-TPM3 + TP53	Colorectal cancer (1)
NTRK1-TP53 + KRAS	Lung cancer (1)
NTRK1-IRF2BP2 (clonal)	Lung cancer (1)

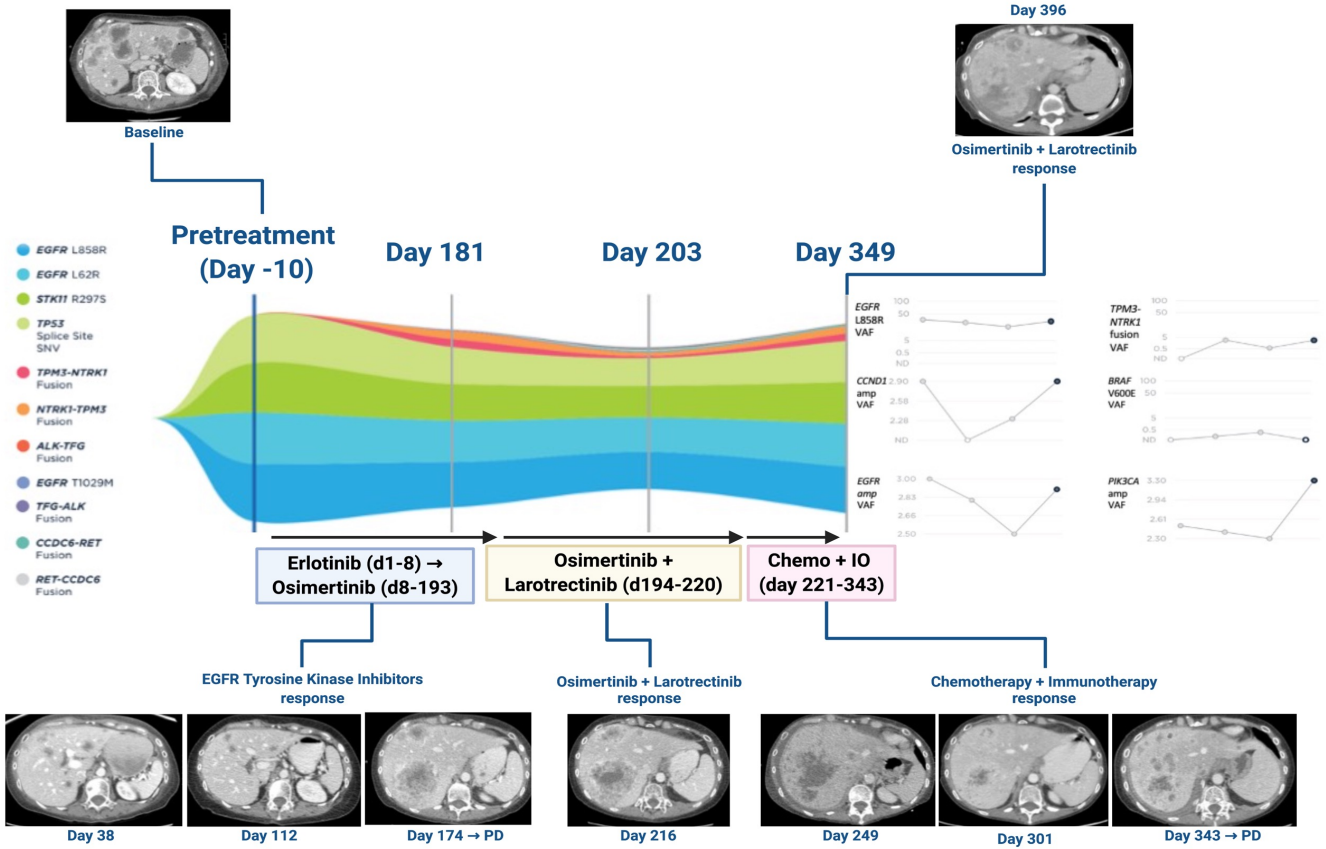
SUBCLONAL *NTRK1* FUSIONS		
TPM3-NTRK1	*EGFR* mutation	Lung cancer (8)
	ARID1A mutation	Colorectal cancer (1)
	KIT mutation	GIST (1)
	CDKN2A mutation	Melanoma (1)
	BRCA2 VUS	Thyroid cancer (1)
IRF2BP2-NTRK1	*EGFR* mutation	Lung cancer (1)
	BRCA mutation	Lung cancer (1)
RABGAP1L-NTRK1	*KRAS* mutation	Lung cancer (1)
LMNA-NTRK1	*TP53* mutation	Colorectal cancer (1)
	FGFR3 VUS	Colorectal cancer (1)
	CDH1 mutation	Breast cancer (1)
	PIK3CA mutation	Breast cancer (1)
TP53-NTRK1	*TP53* mutation	Prostate cancer (1)
PEAR1-NTRK1	*TP53* mutation	Sarcoma (1)

CLONAL *NTRK1* FUSIONS	
SQSTM1-NTRK1	Lung Cancer (2)
TPM3-NTRK1	Colorectal cancer (1)
	Breast Cancer (1)
	Sarcoma (1)*
	Thyroid cancer (1)
PLEKHA6-NTRK1	Colorectal cancer (1)
	Cholangiocarcinoma (1)
	Prostate Cancer (1)

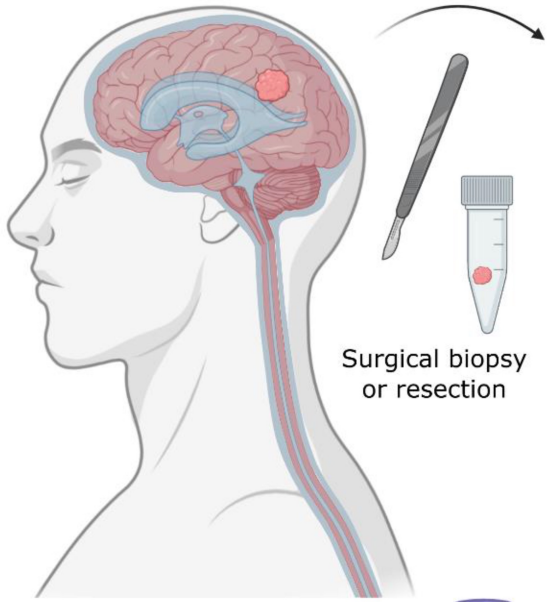
*with subclonal NTRK1 G667 SNV

Rolfo C et al, British Journal of Cancer, Sep 2021

NTRK fusions as mechanism of resistance



Rolfo C, et al, Br J Cancer 2021



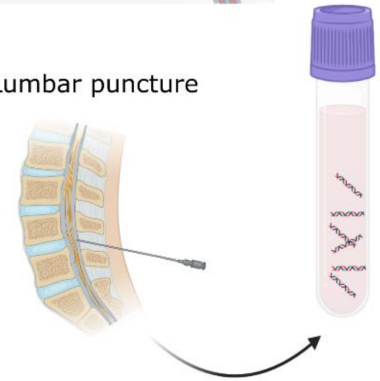
Surgical biopsy or resection

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

Lumbar puncture



Liquid biopsy (CSF ctDNA)

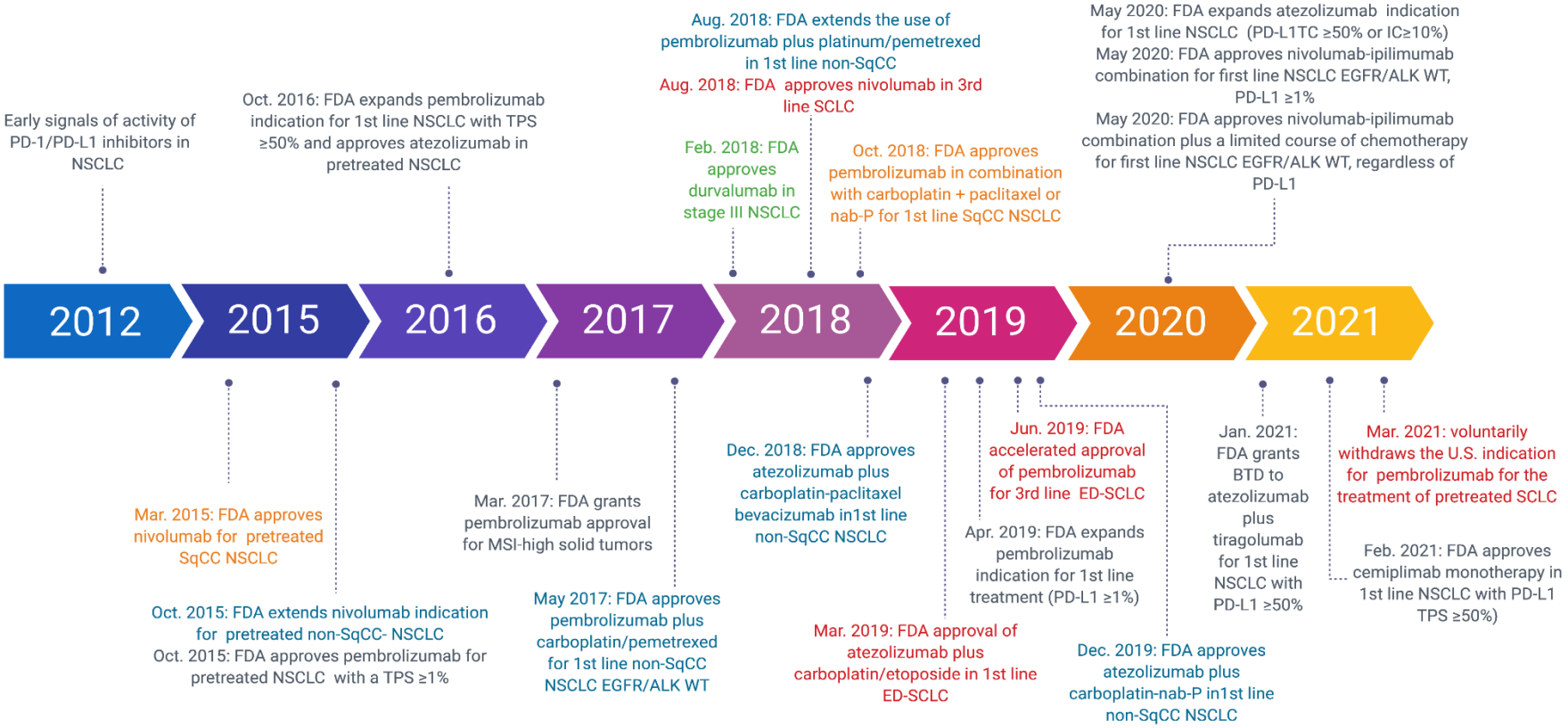
Advantages
Less-invasive and easier to obtain than a tumour biopsy
CSF obtained as SOC for some patients
Concordance with tissue characterisation
Representative of intratumour and interlesion heterogeneity
Longitudinal real-time monitoring

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

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

Immunotherapy:
The oncologists like a kid in a candy shop…

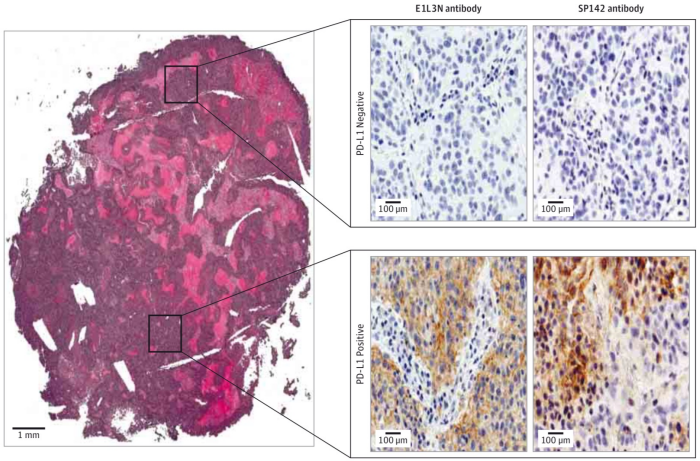
Milestones in Immunotherapy era in Lung Cancer

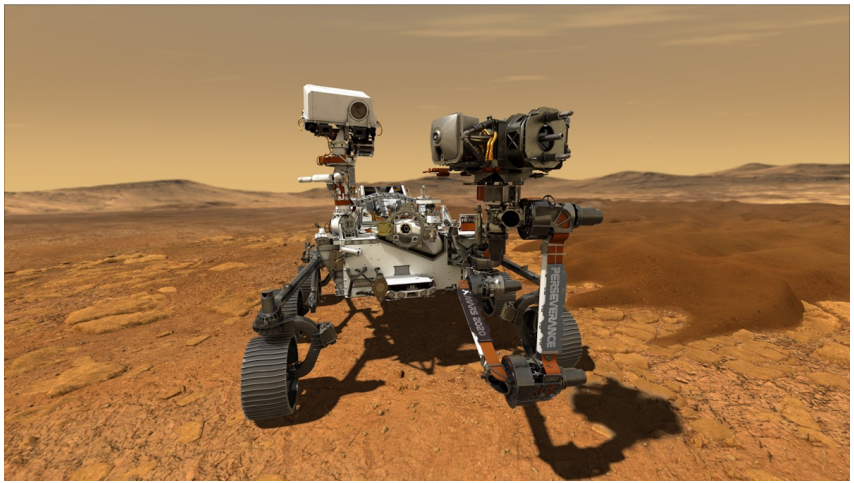


Adapted from Russo A (Rolfo C) et al. In: Naing A., Hajjar J. (eds) Immunotherapy. Adv Exp Med Biol 2020

Heterogeneity of PD-L1 Expression

An imperfect but useful biomarker

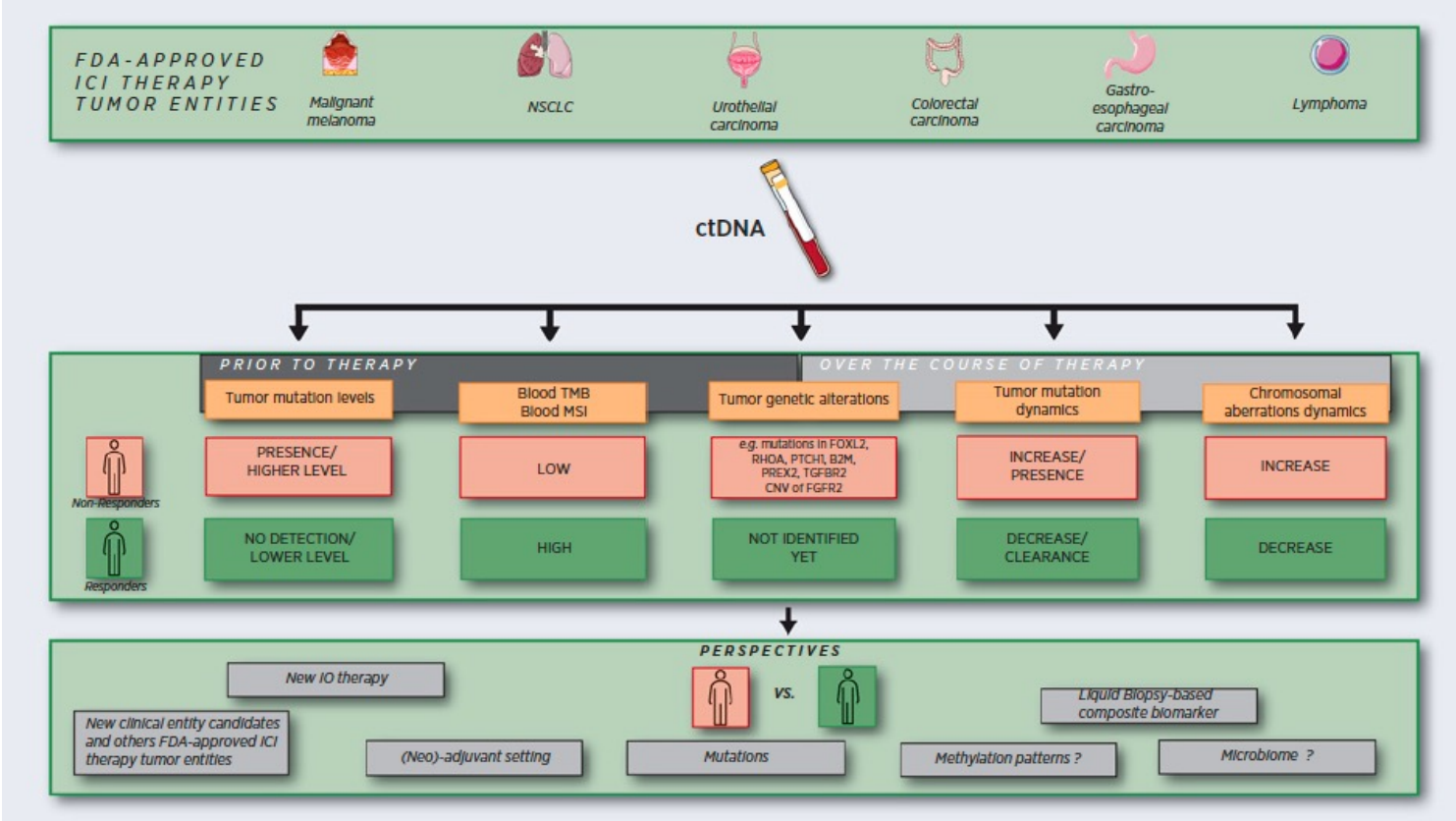




- **Intratumor** heterogeneity
- **Intrapatient** heterogeneity

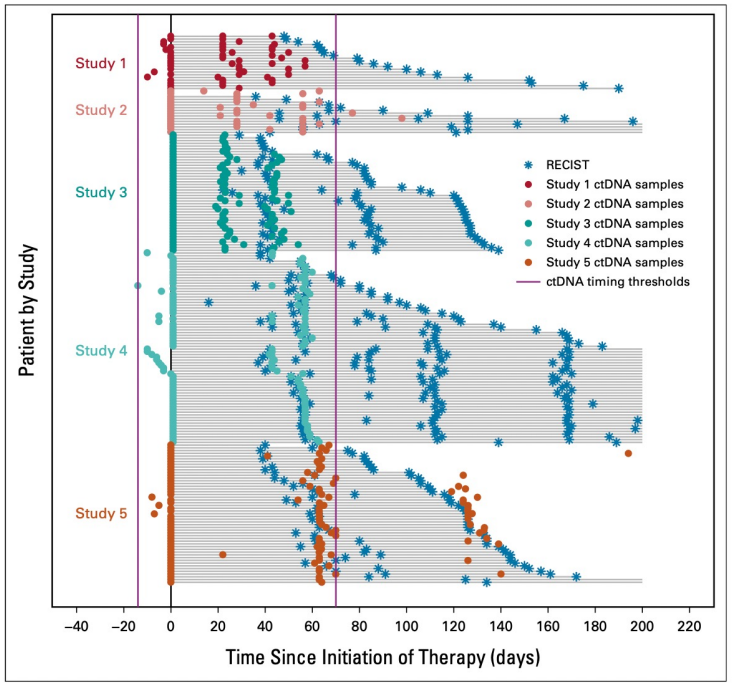
McLaughlin et al, JAMA Oncol. 2016;2(1):46-54

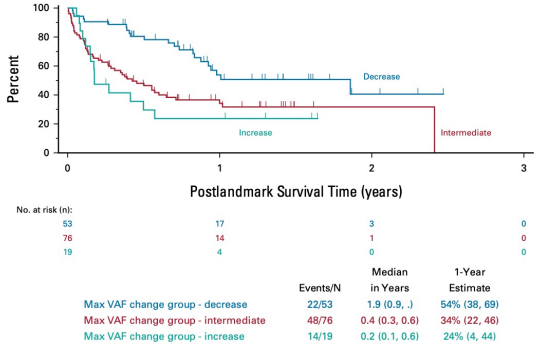
Use of Liquid Biopsy in Immunotherapy

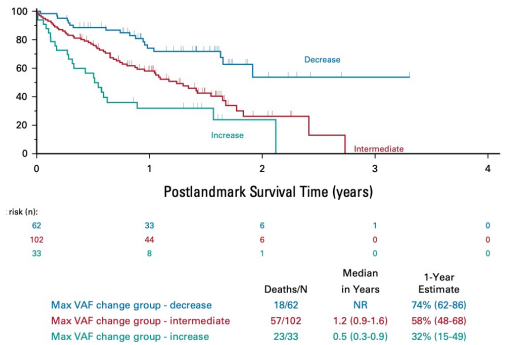


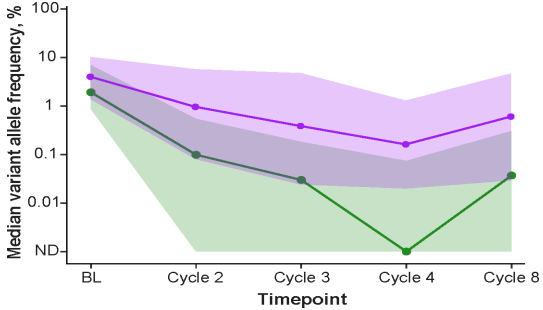
Stadler J, et al. Cancer Res 2022

Changes in Circulating Tumor DNA Reflect Clinical Benefit Across Multiple Studies of Patients With Non–Small-Cell Lung Cancer Treated With Immune Checkpoint Inhibitors







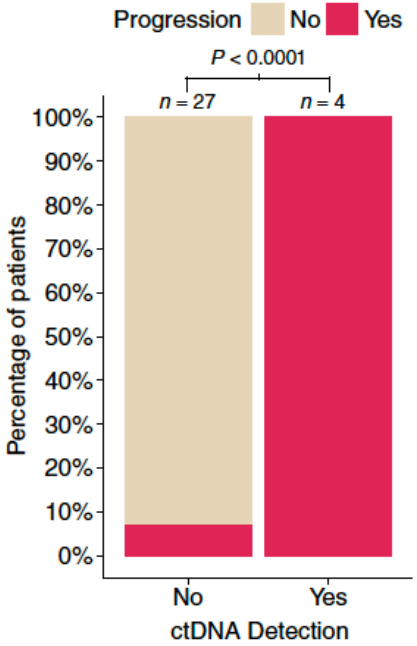


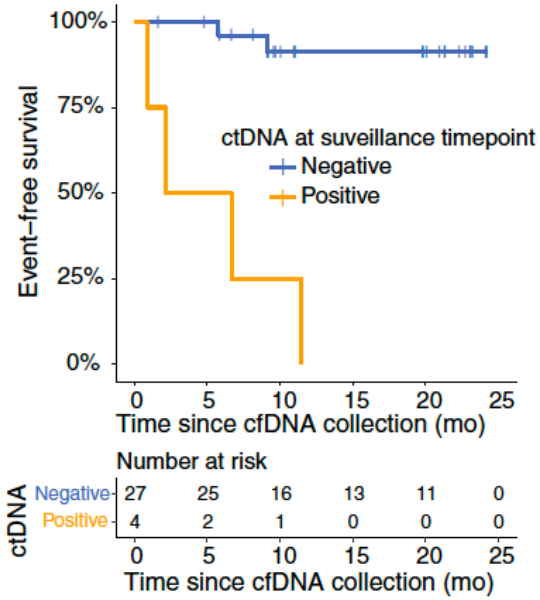
ctDNA dynamics reflect tumour burden (SLD) and response to therapy: IMpower150

CtDNA may serve as an important tool in clinical development and an early indicator of treatment benefit

Merino Vega et al (Allen J) JCO P), Aug 2022

ctDNA Analysis to Assess Risk of Progression to PD-(L)1 Blockade in NSCLC



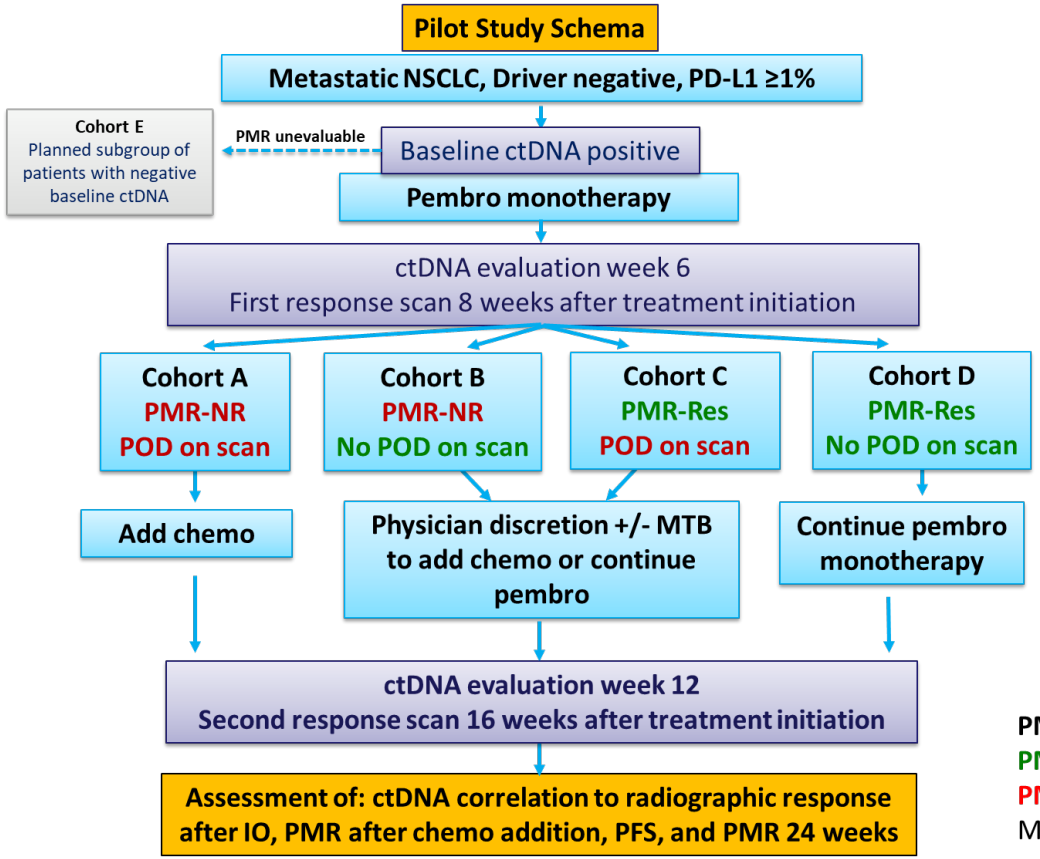


ctDNA analysis identifies patients at risk for eventual progression after long-term response to PD-(L)1 blockade.

Hellmann MD, et al. Clin Cancer Res 2020

CITAN: ctDNA-guided Immunotherapy-based Therapy in Treatment Naïve Advanced NSCLC

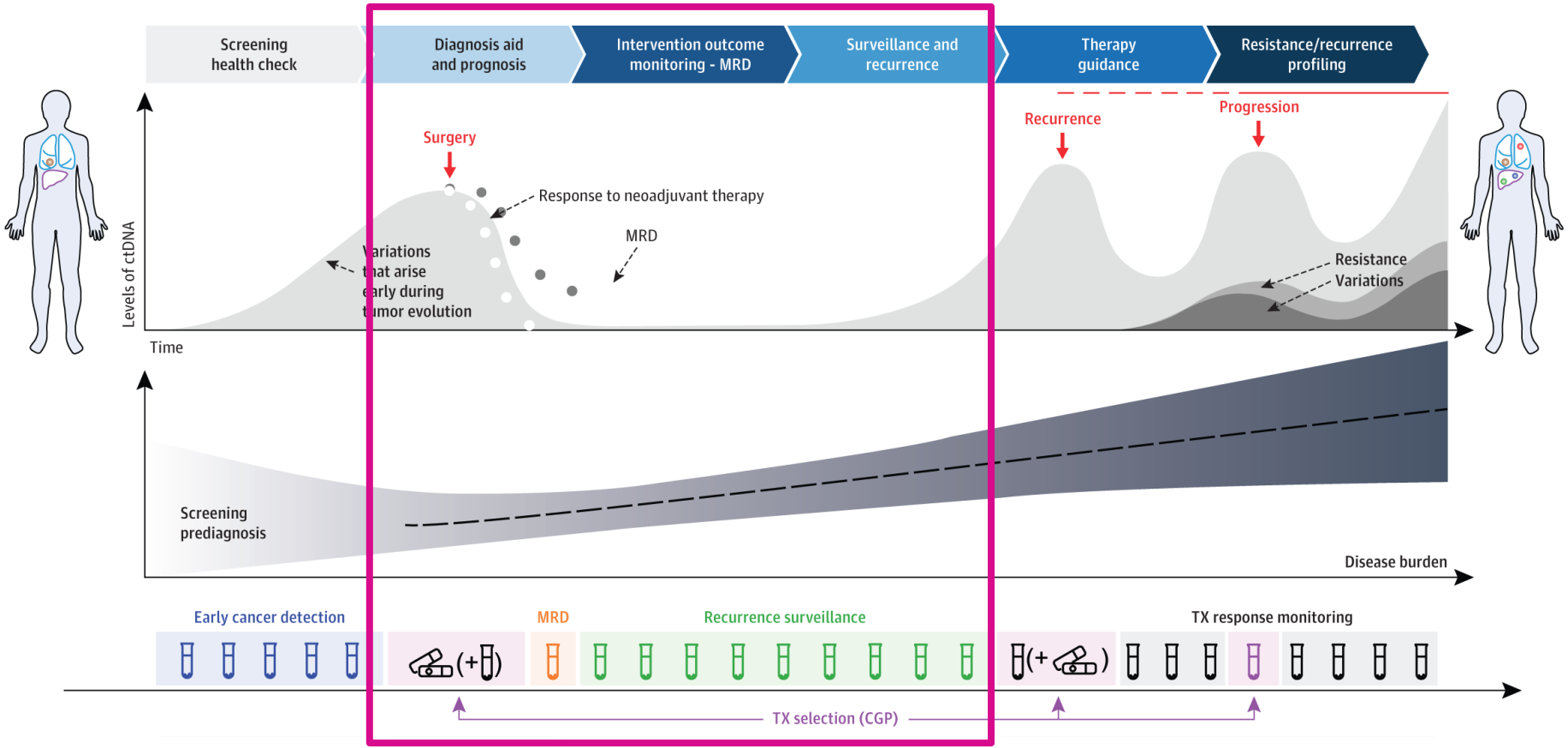
PI: Dr. Mack – Dr Rolfo



Translational Medicine Component

- **Baseline** Tissue, PBMCs
- **C2D1** ctDNA, PBMCs
- **C3D1** ctDNA, PBMCs
- **C4D1** ctDNA, PBMCs
- **Every 3 Cycles** ctDNA

PMR = Plasma Molecular Response
PMR-Res = ≥50% PMR (responsive)
PMR-NR = <50% PMR (non-responsive)
MTB = Molecular Tumor Board



Krebs et al (Rolfo), JAMA Oncology OCT 2022

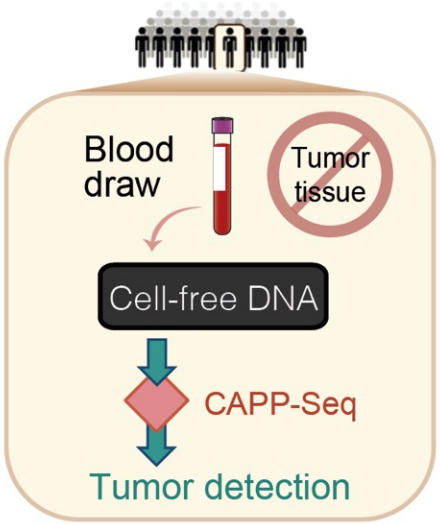
Retrospective Data From ~900 NSCLC Patients: Pre- and Post-treatment MRD strongly prognostic

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

Courtesy Dr. Max Diehn & Dr. Natasha Leigh

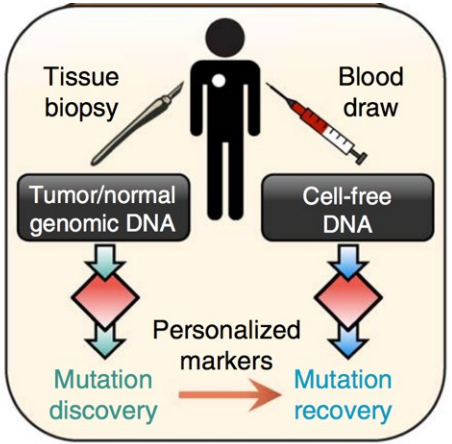
Different types of ctDNA MRD Assays

Tumor-naive



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

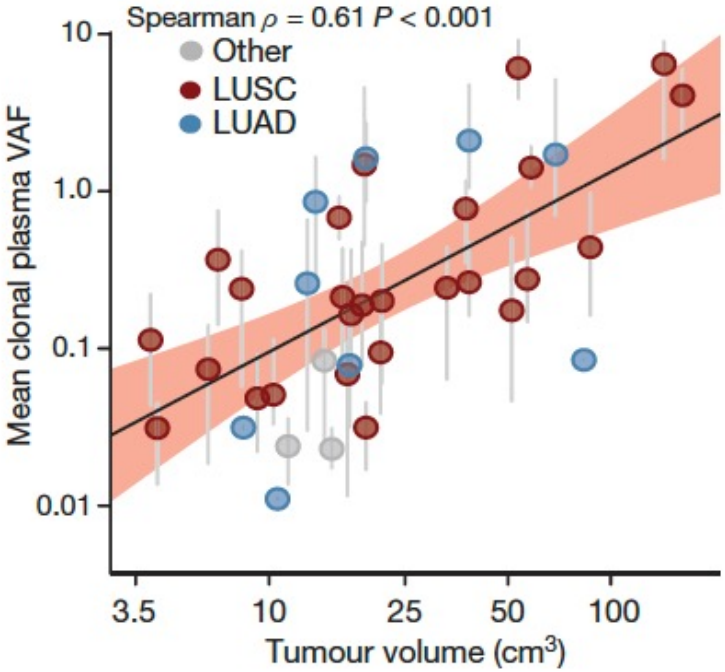
Tumor-informed



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

Courtesy Dr. Natasha Leighl

Tumour volume predicts plasma VAF



Tumour volume (cm^3) 1 10 100

Predicted VAF:

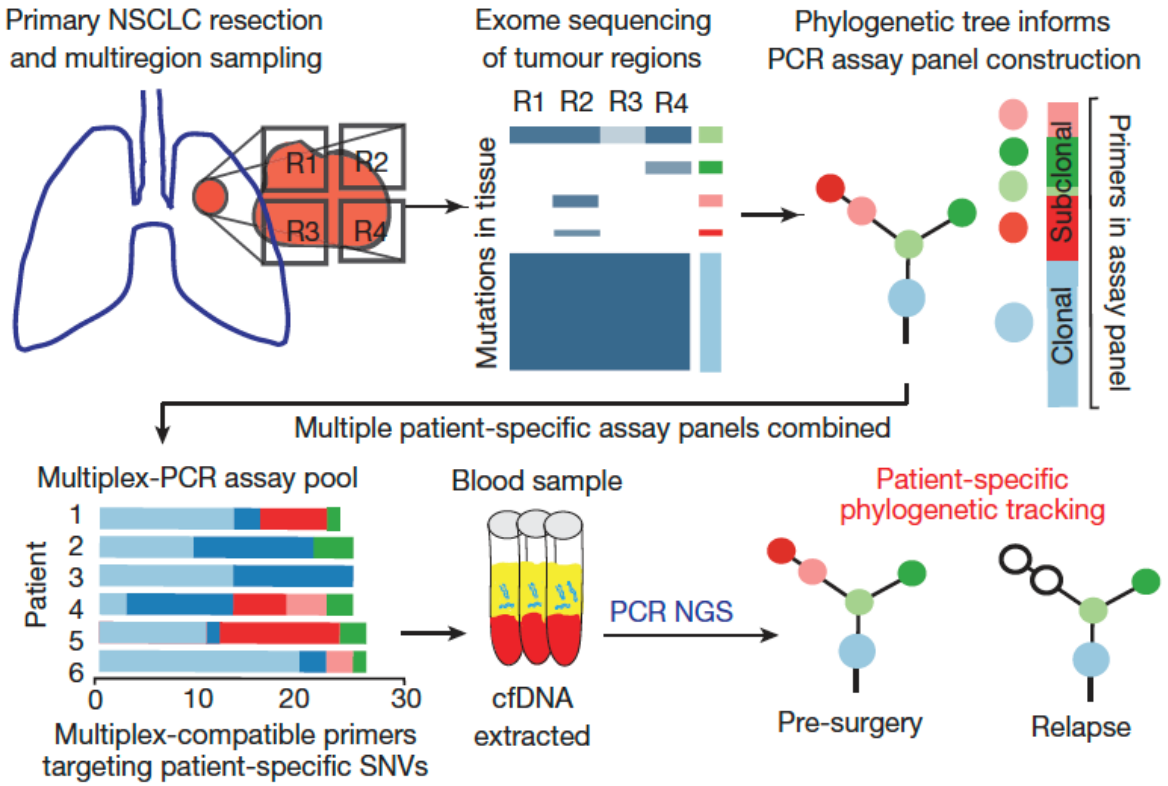
0.006% 0.1% 1.3%

95% CI (0.001–0.03%) (0.05–0.17%) (0.57–3.1%)

(Approximately 326 million malignant cells)

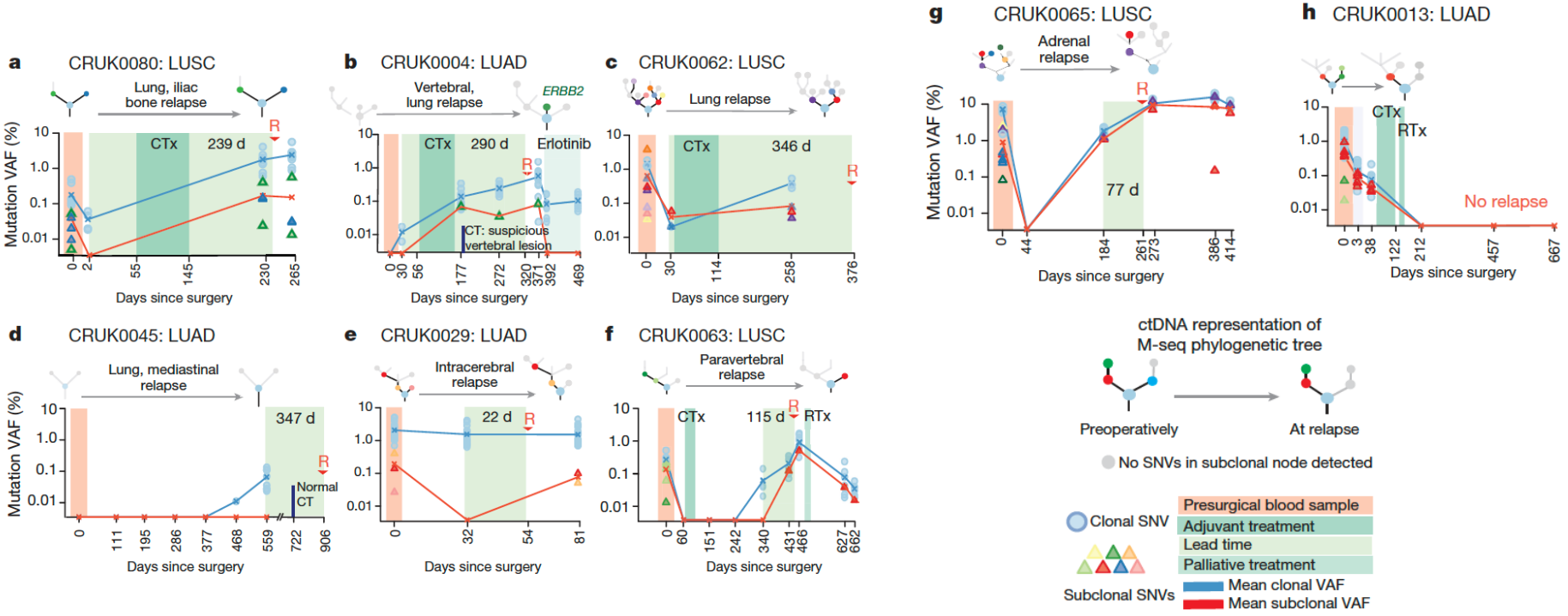
Abbosh C, *et al. Nature* 2017;545(7655):446-451.

Phylogenetic approach to profile the ctDNA – TRACERx Study



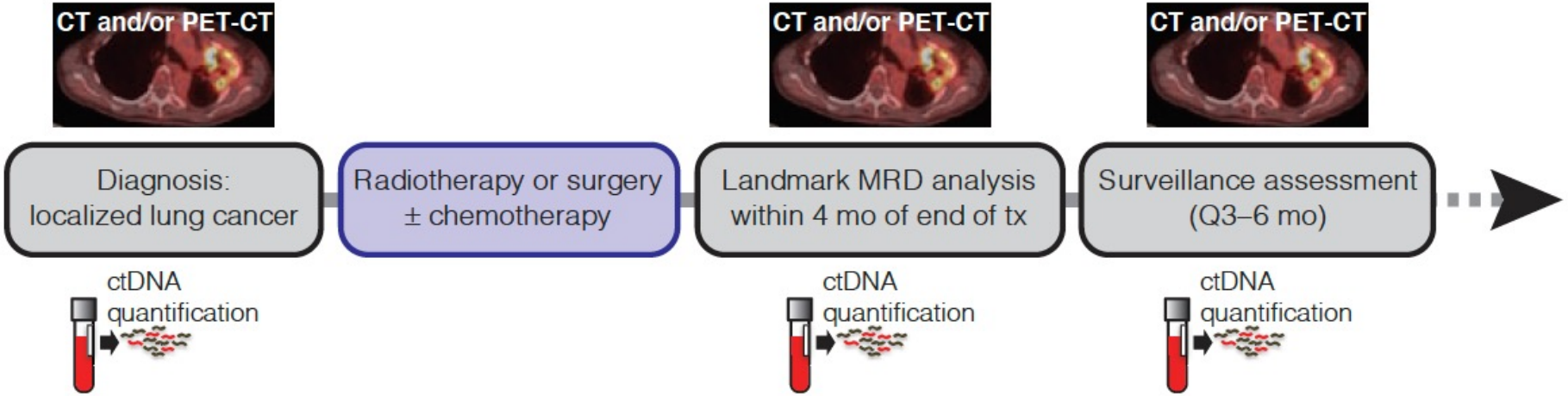
Abbosh C, *et al. Nature* 2017;545(7655):446-451.

Postoperative ctDNA detection predicts and characterises NSCLC relapse



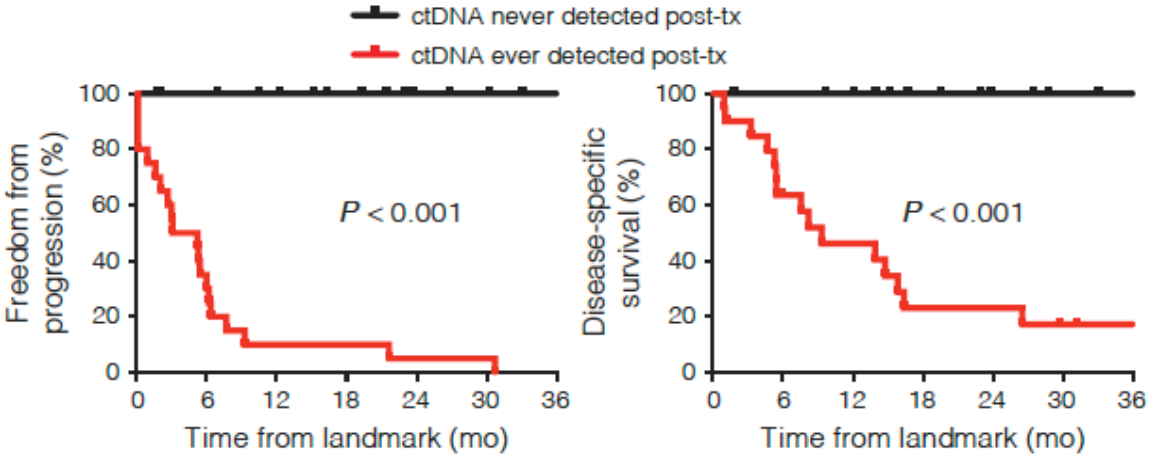
Abbosh C, *et al. Nature* 2017;545(7655):446-451.

Early Detection of MRD in Localised Lung Cancer by CAPP-Seq

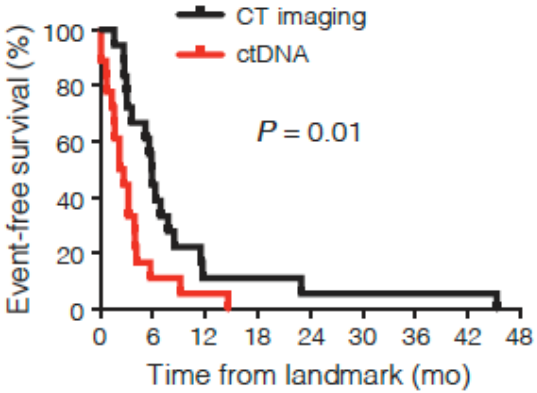


Chaudhuri AA, *et al. Cancer Discov* 2017;7(12):1394-1403.

Application of ctDNA analysis for posttreatment surveillance in patients with localised lung cancer



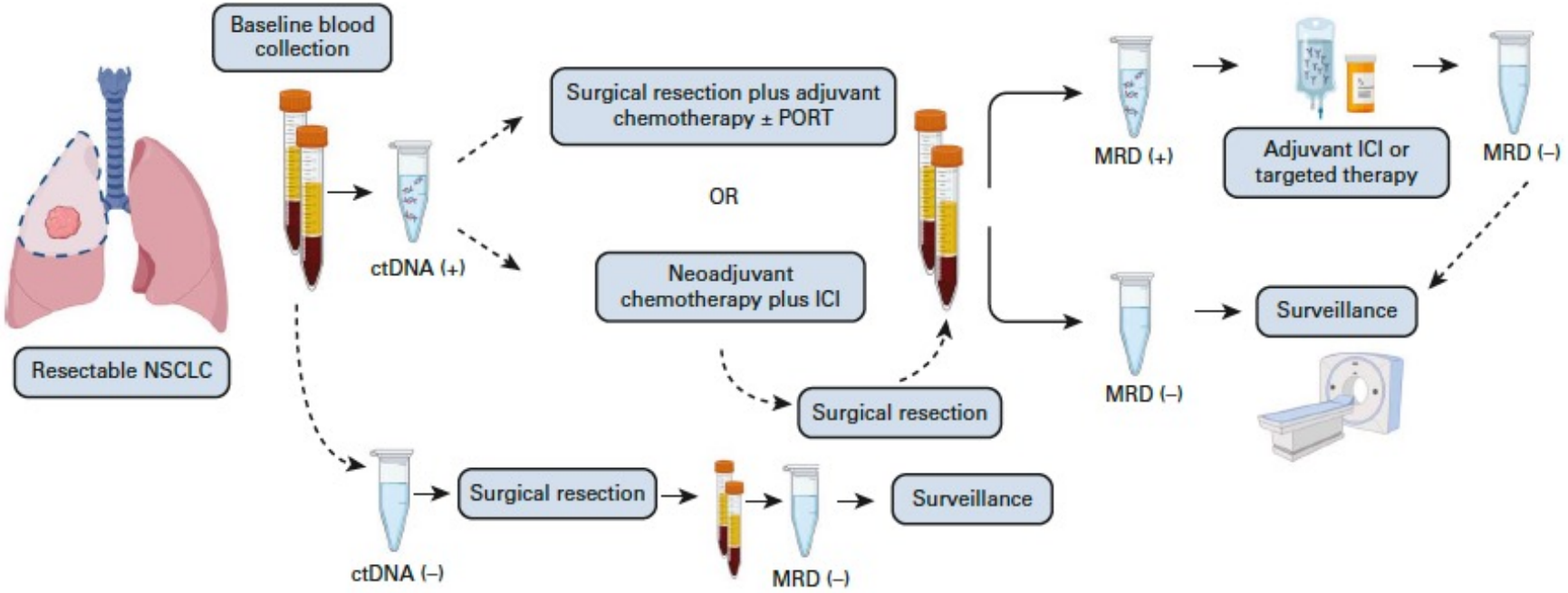
KM curves stratified by ctDNA detection status during posttreatment surveillance



ctDNA detection and time to imaging progression

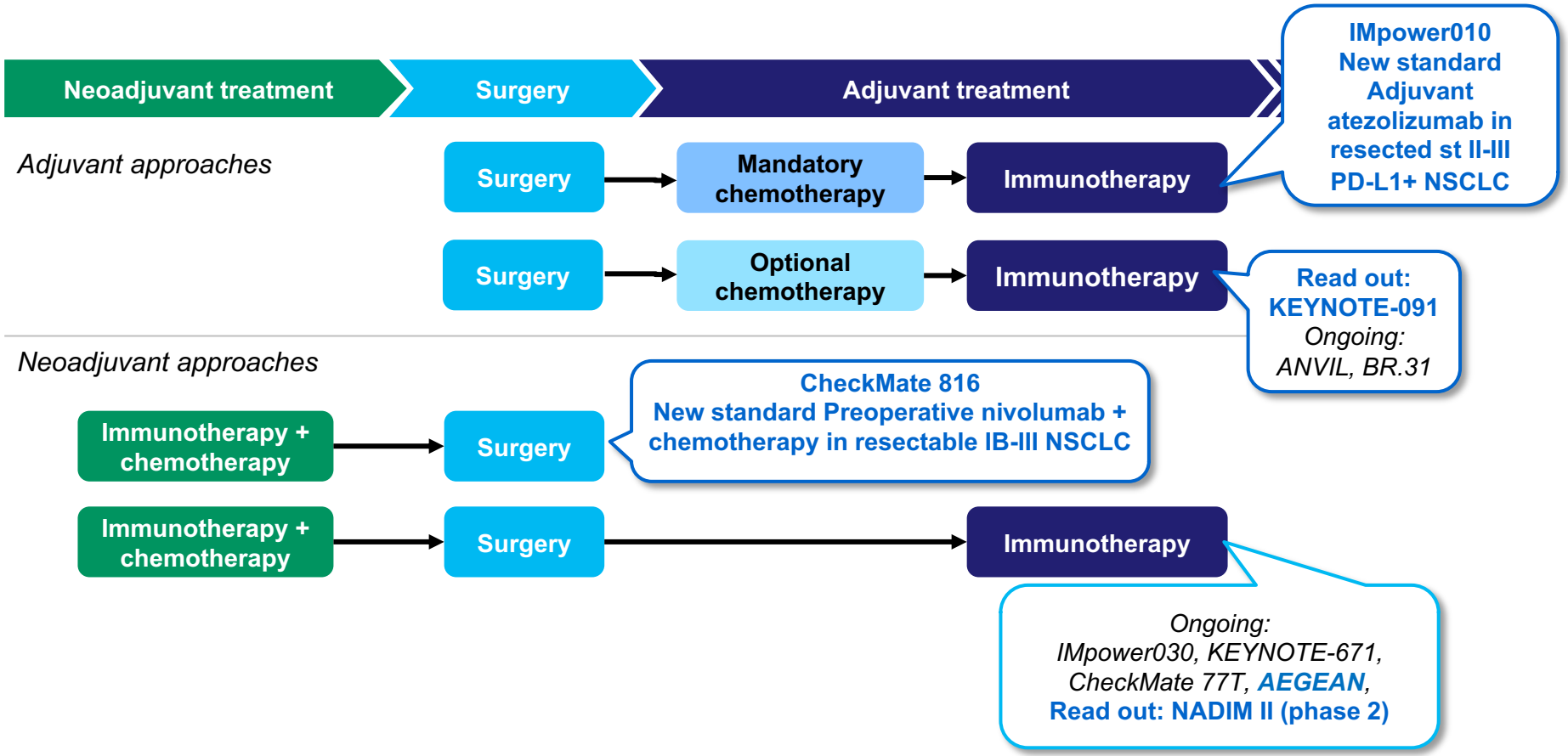
Chaudhuri AA, *et al. Cancer Discov* 2017;7(12):1394-1403.

Proposed clinical trial designs for early-stage NSCLC using ctDNA as a biomarker for treatment personalization



Pellini B & Chaudhuri AA. JCO 2022

Phase III studies in resectable NSCLC

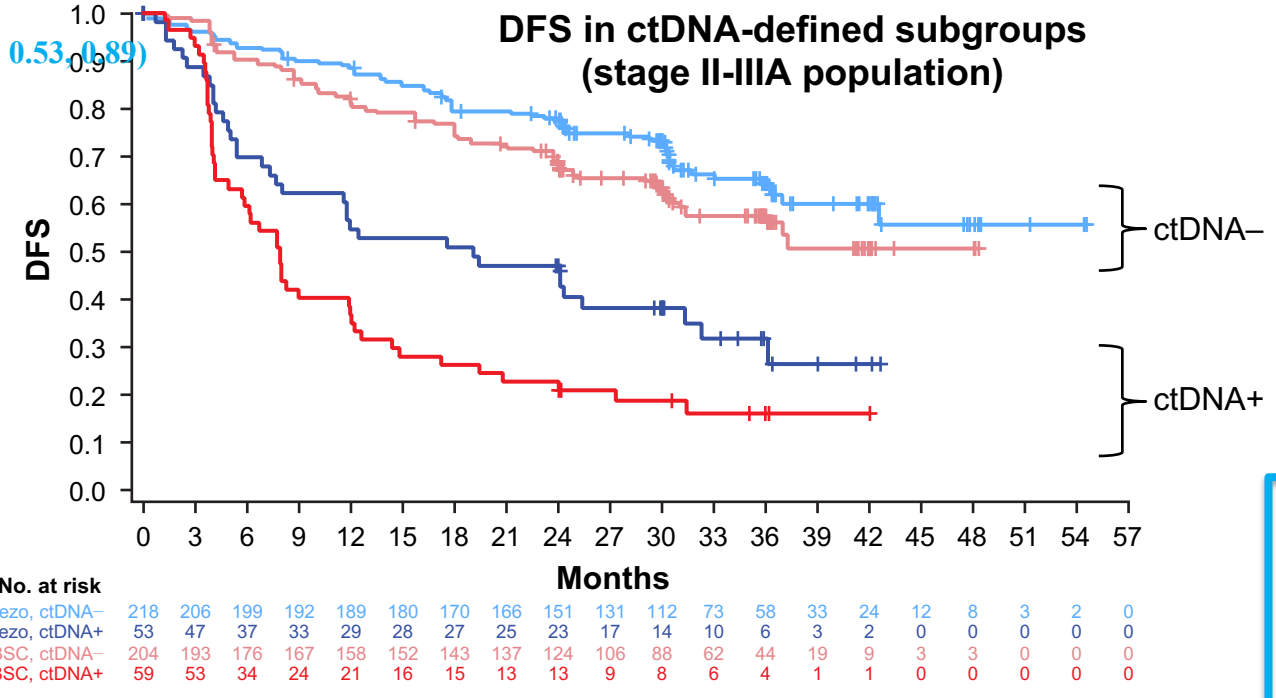


Modified from Dr. S. Peters, EPICS2022

Courtesy Dr. Natasha Leigh

ctDNA positivity was strongly prognostic, with DFS favouring atezo in both ctDNA+ and ctDNA– patients

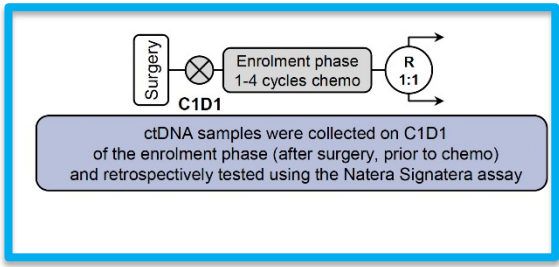
In all ctDNA-evaluable stage II-IIIA patients, mDFS was NR (atezo) vs 31.4 months (BSC), with an HR of 0.69 (95% CI:



ctDNA–	Atezo (n=218)	BSC (n=204)
mDFS, mo	NR	NR
HR (95% CI)	0.72 (0.52, 1.00)	

ctDNA+	Atezo (n=53)	BSC (n=59)
mDFS, mo	19.1	7.9
HR (95% CI)	0.61 (0.39, 0.94)	

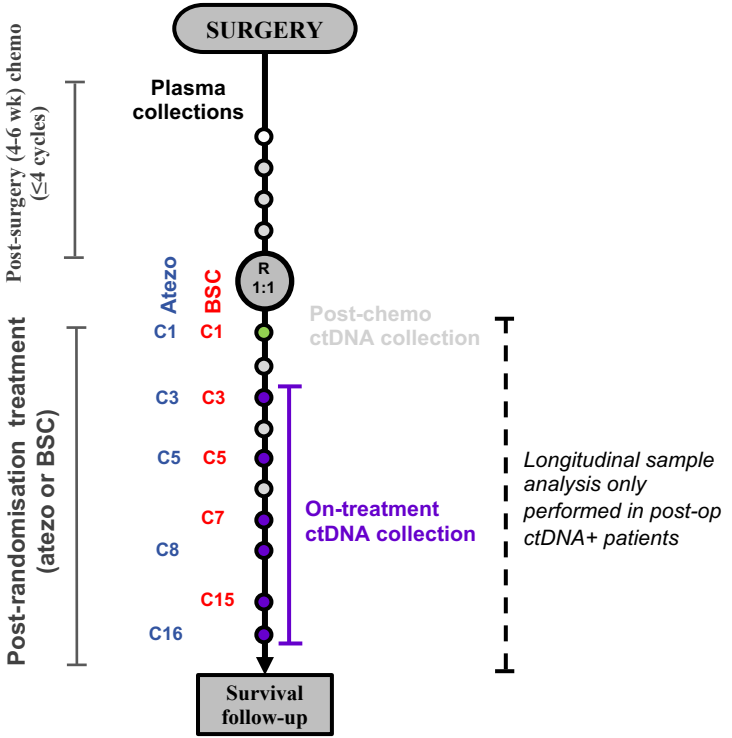
Plasma collection for ctDNA analysis

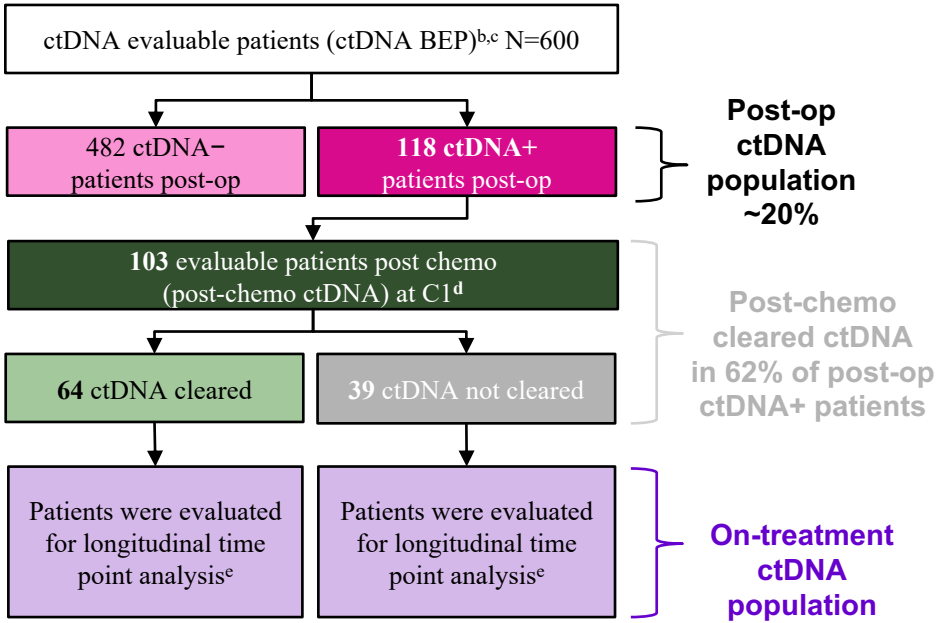


C. Zhou, ESMO 2021

Clinical cutoff: 21 January 2021. Unstratified HRs are shown.

Baseline and longitudinal plasma collection for ctDNA testing[a]



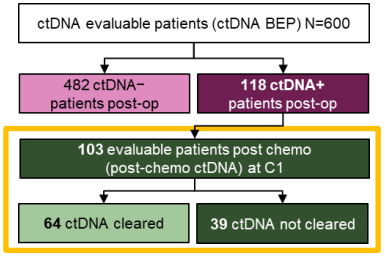


Chemo, chemotherapy; C, cycle. Clinical cutoff: 21 January 2021. [a] Using the Signatera (Natera) RUO test. [b] Treatment arms in the ctDNA BEP were balanced and comparable to the ITT population. [c] PD-L1 subgroup analyses conducted in the stage II-IIIA ctDNA BEP (n=532). [d] Samples in 15 patients were missing due to lack of consent or 4 mL plasma. [e] Patients with ≥1 on-treatment sample at C3, C5, C7/8 and C15/16. On-treatment analyses are shown on slides 9 (ctDNA cleared) and 10 (ctDNA not cleared) .

Modified from Dr. Felip, ESMO IO 2022

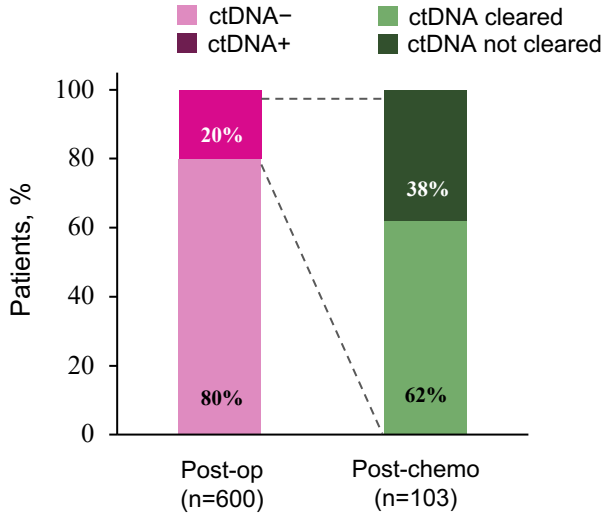
Courtesy Dr. Natasha Leigh

ctDNA clearance with adjuvant chemo in post-op ctDNA+ patients

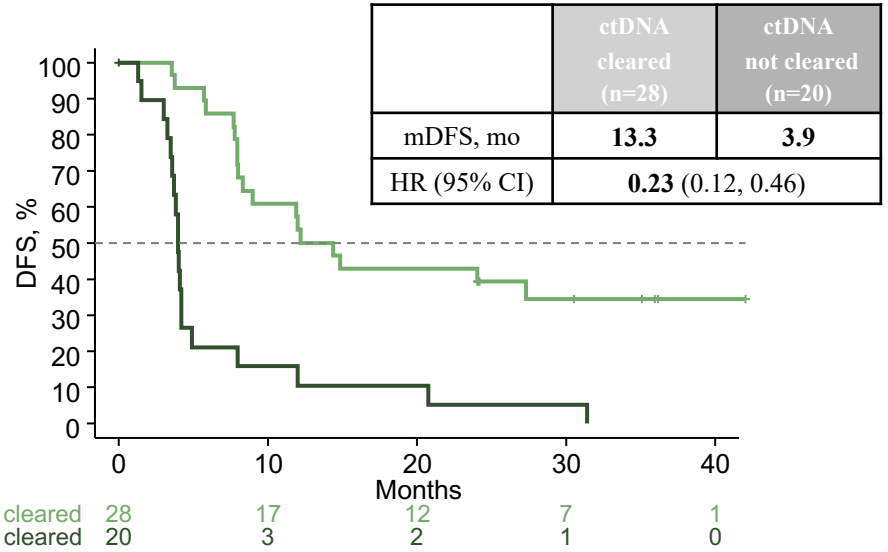


- Adjuvant chemo was effective in clearing ctDNA in ≈62% of post-op ctDNA+ patients
- Post-chemo ctDNA positivity was linked to poor DFS outcome

Impact of chemo on ctDNA clearance status



DFS by ctDNA clearance status in the BSC arm



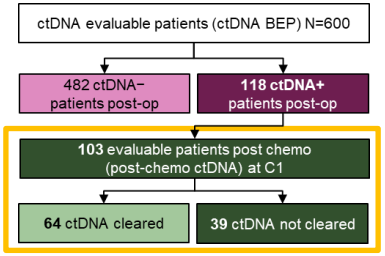
	ctDNA cleared (n=28)	ctDNA not cleared (n=20)
mDFS, mo	**13.3**	**3.9**
HR (95% CI)	**0.23** (0.12, 0.46)	

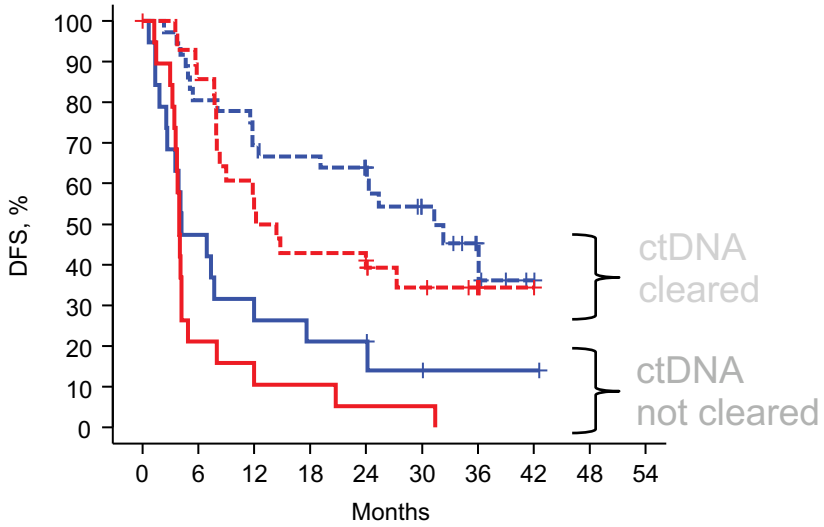
Clinical cutoff: 21 January 2021. Unstratified HRs are reported.

Courtesy Dr. Felip, ESMO IO 2022

Courtesy Dr. Natasha Leigh

DFS by treatment and post-chemo ctDNA clearance - all groups still appear to benefit from atezolizumab





Atezo, ctDNA cleared	36	35	29	28	25	24	24	23	21	17	12	10	5	2	1	0	0	0	0
Atezo, ctDNA not cleared	19	13	9	6	5	5	4	4	4	2	2	1	1	1	1	0	0	0	0
BSC, ctDNA cleared	28	28	24	18	15	12	12	12	12	8	7	6	4	1	1	0	0	0	0
BSC, ctDNA not cleared	20	16	4	3	2	2	2	1	1	1	1	0	0	0	0	0	0	0	0

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)	

Clinical cutoff: 21 January 2021.
Data are hypothesis generating and should be interpreted with caution due to the exploratory nature of the analysis and small sample size.

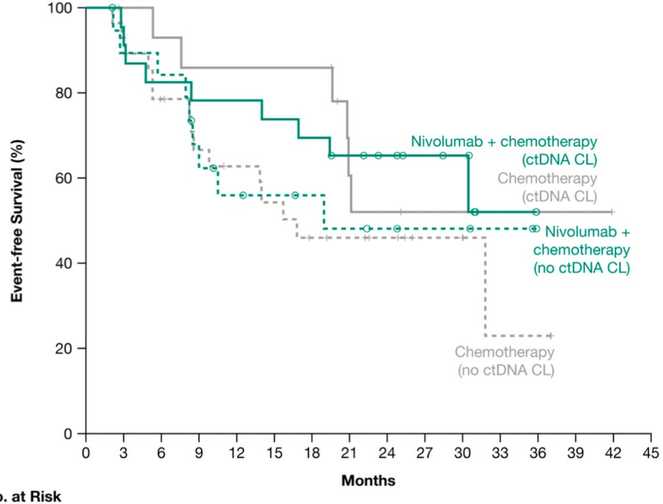
Modified from Dr. Felip, ESMO IO 2022

Courtesy Dr. Natasha Leigh

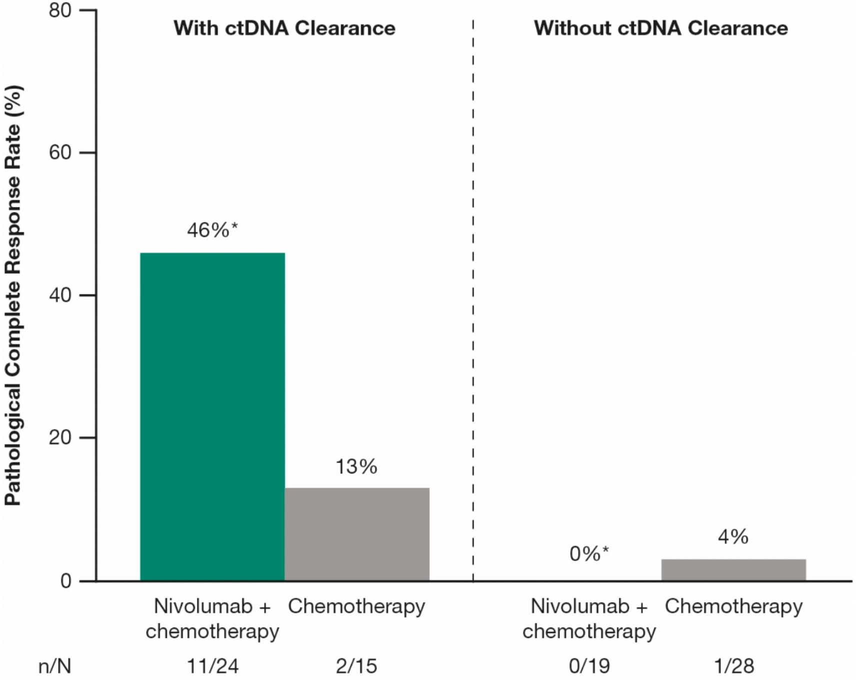
Liquid Biopsy in Neoadjuvant IO + chemo combination

WES ctDNA in 89 pts

	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)	



No. at Risk	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45
Nivolumab + chemotherapy (ctDNA CL)	24	21	19	18	18	17	16	13	11	8	7	1	0	0	0	0
Chemotherapy (ctDNA CL)	15	14	13	12	12	12	12	7	6	5	5	5	3	1	0	0
Nivolumab + chemotherapy (no ctDNA CL)	19	17	16	12	9	8	7	6	5	3	3	2	0	0	0	0
Chemotherapy (no ctDNA CL)	28	26	21	17	15	13	10	9	7	4	3	1	1	0	0	0

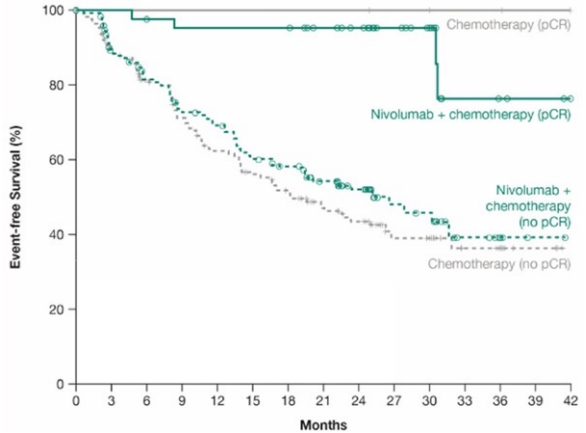


Forde P. et al, NEJM, AACR 2022

Courtesy Dr. Natasha Leigl

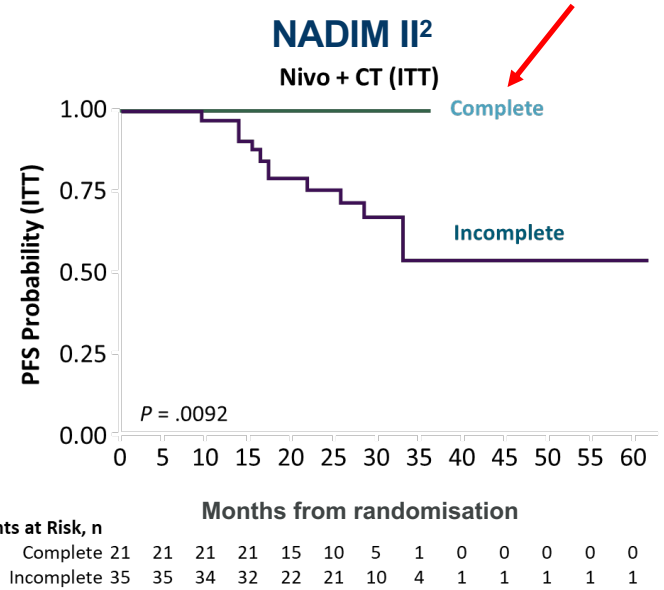
Pathologic complete response - a more promising surrogate endpoint

CheckMate 816[1]



	Nivo + CT		CT	
	pCR	No pCR	pCR	No pCR
mEFS, months	NR	26.6	NR	18.4
HR (95% CI)	0.13 (0.05, 0.37)		Not computed*	

NADIM II[2]



Courtesy of Dr. David Planchard and Dr. N. Leighl *1. Forde PM, et al. N Engl J Med 2022;386:1973–85; 2. Provencio M, et al. Presented at WCLC 2022 (Abstract PL03.12)

Current prospective interventional trials in early stage lung cancer

Number	Prior tx	Stage	N	ctDNA-positive intervention	ctDNA-negative intervention	Phase	Primary Endpoint	Site(s)
NCT04585477	Surgery or RT +/- chemo	I-III	80	Durvalumab	None	II	ctDNA change	Stanford
NCT04585490	chemoRT + several cycles durvalumab	III	48	Durvalumab + chemo	None	II	ctDNA change	Stanford
NCT04966663	Surgery	I	66	Nivolumab + chemo vs. No treatment	None	II	RFS	Toronto

Courtesy Dr. Natasha Leighl

DYNAMIC Study Design

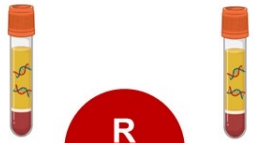
ACTRN12615000381583

Stage II Colon Cancer

- R0 resection
- ECOG 0 – 2
- Staging CT within 8 weeks
- Provision of adequate tumor tissue within 4 weeks post-op
- No synchronous colorectal cancer

Plasma Collections

Week 4 + 7 post-op



R
2:1

ctDNA-Guided Management

- **ctDNA-Positive → Adjuvant Chemo** (oxaliplatin-based or single agent FP)
- **ctDNA-Negative → Observation**

ctDNA-Positive = Positive result at week 4 and/or 7

Standard Management

Adjuvant treatment decisions based on conventional clinico-pathologic criteria

Endpoints

Primary
- RFS rate at 2 years

Key Secondary
- Proportion receiving adjuvant chemo

Secondary
- RFS by ctDNA status for ctDNA-guided arm
- TTR
- OS

Stratification Factors
- T stage (T3 vs T4)
- Type of participating center (metropolitan vs regional)

Surveillance:
- CEA → 3-monthly for 24M, then 6-monthly for 36M
- CT C/A/P → 6-monthly for 24M, then at 36M

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ASCO AMERICAN SOCIETY OF CLINICAL ONCOLOGY KNOWLEDGE CONQUERS CANCER

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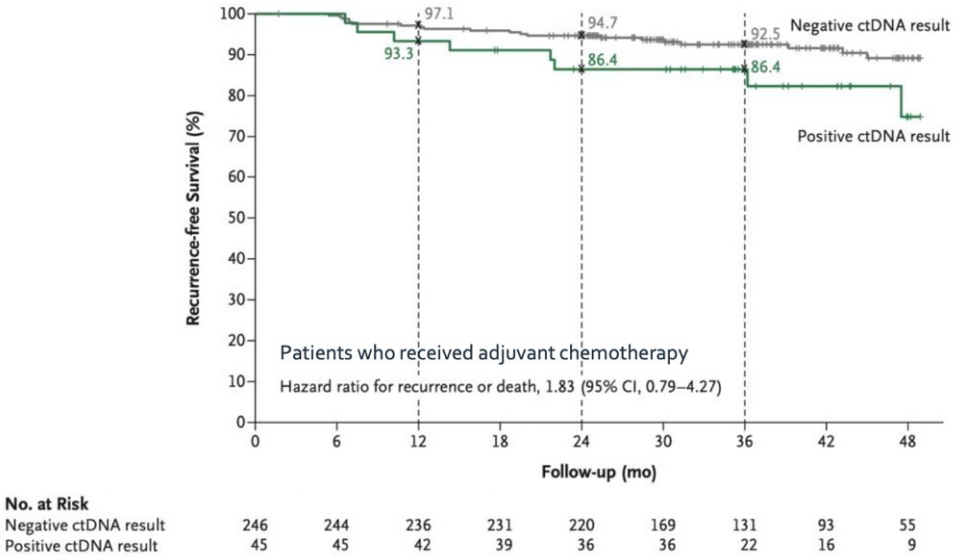
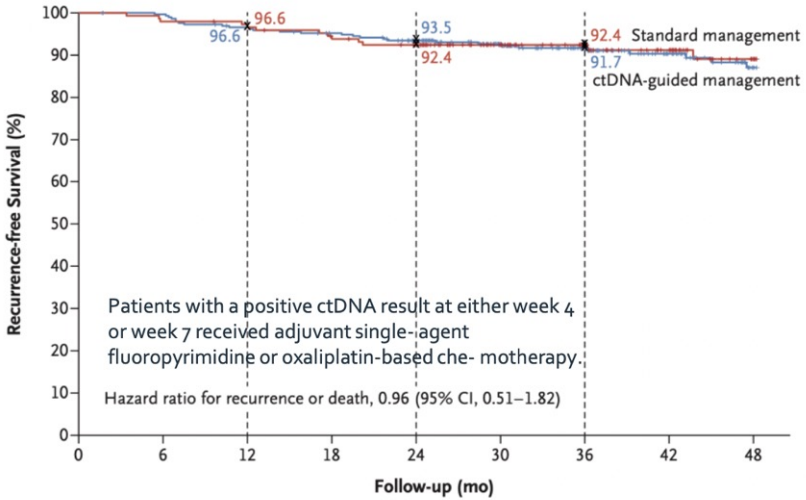
ctDNA-guided adjuvant therapy had similar outcomes to stage-directed treatment

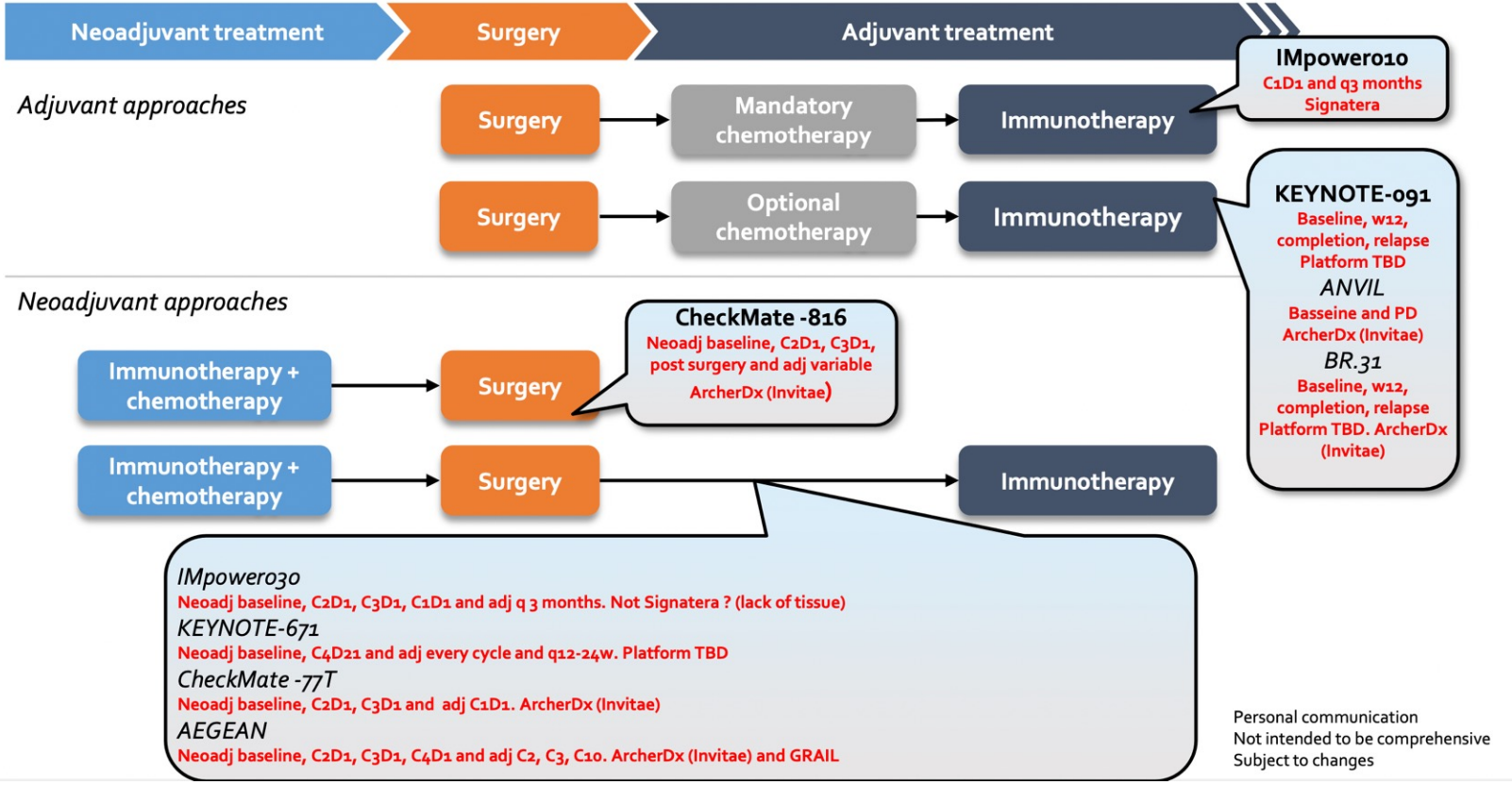




- 455 patients randomized, 302 were assigned to ctDNA-guided management and 153 to standard management
- 15% of patients in the ctDNA-guided group vs 28% in standard-management group received adjuvant chemotherapy
- ctDNA-guided management was noninferior to standard management
- Safe-Sequencing System tumor-informed personalized ctDNA assays (tumor-informed personalized approach)

Tie et al. New Engl J Med 2022; slide courtesy Dr. S. Peters ESMO 2022

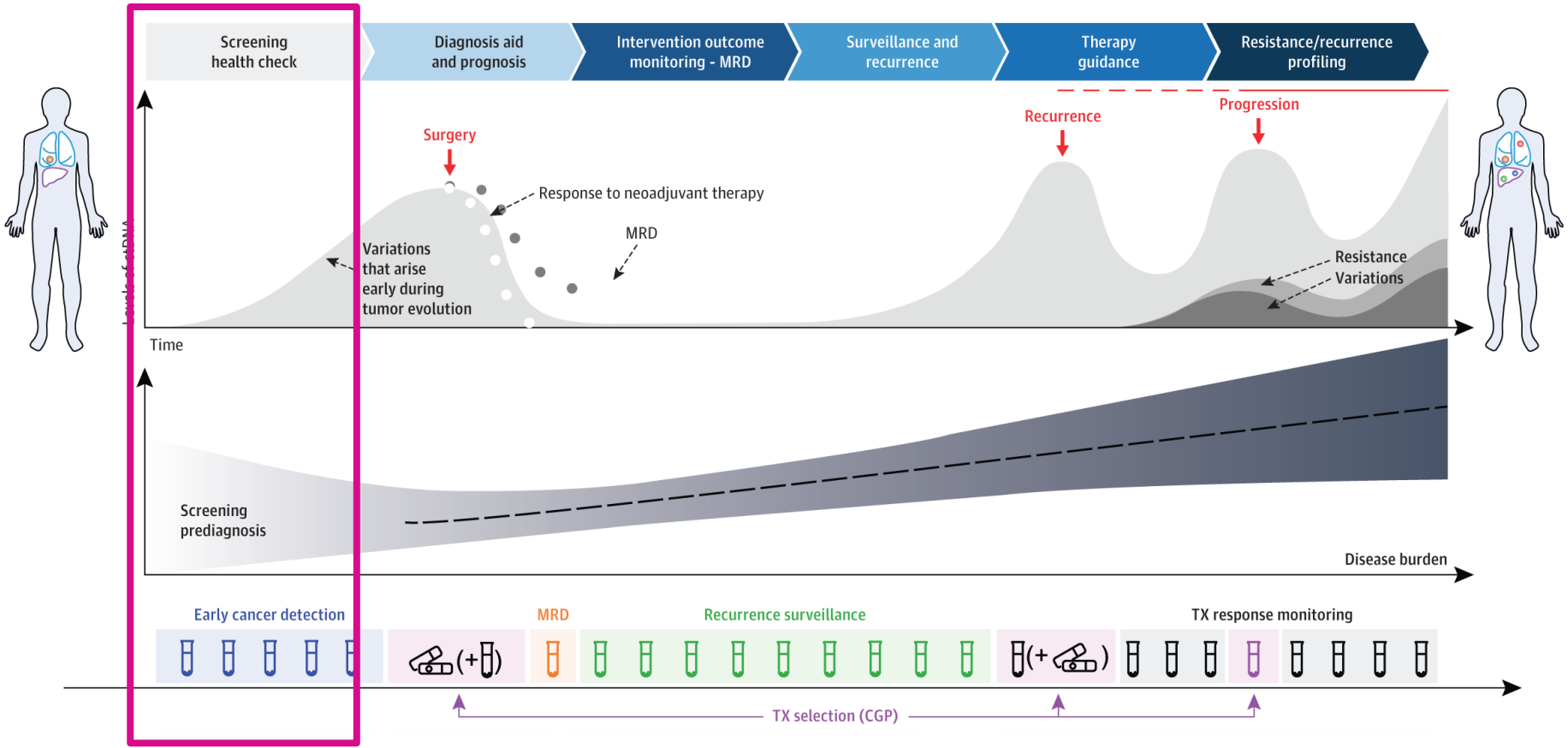
More data are on the way!



Courtesy: Dr. Solange Peters @ De. N. Leighl 2022

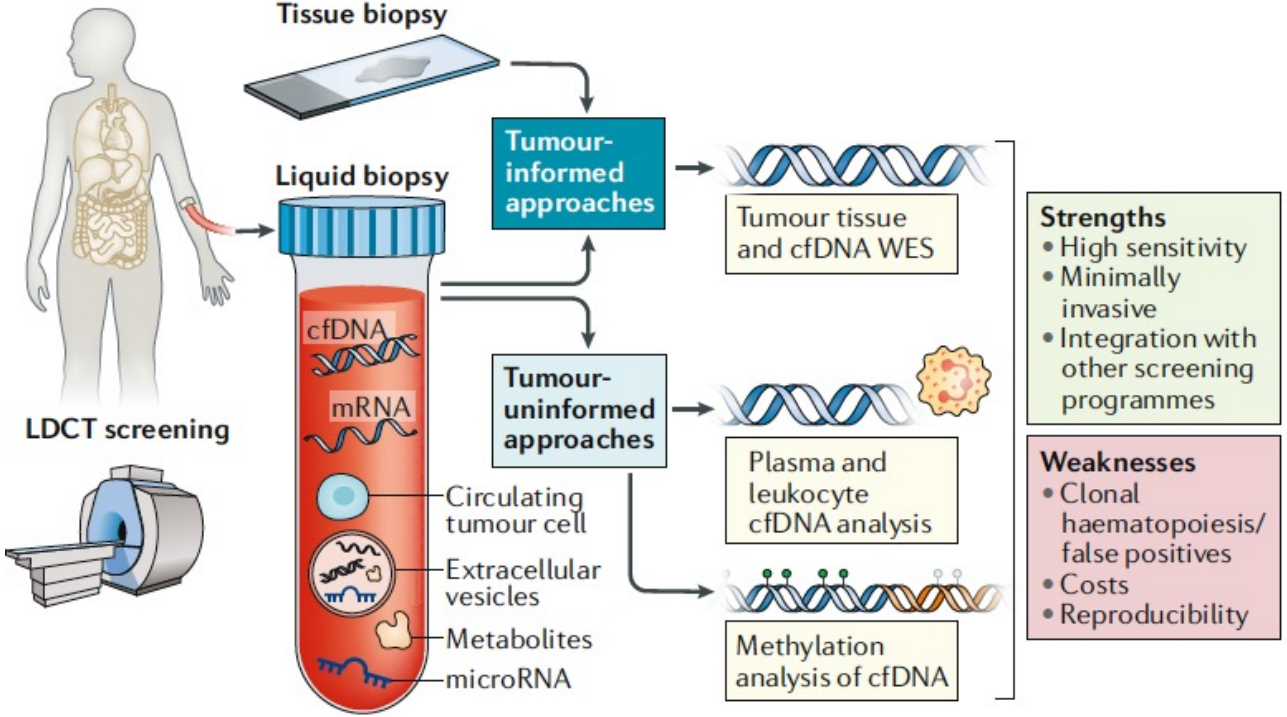
Take Home Message on MRD

- Detecting MRD is crucial to improve survival and disease control rates
- Liquid Biopsy is a perfect tool for MRD
- MRD at difference of early detection, counts with tissue and liquid biopsy as a source of information, increasing the possibilities
- 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
- Other analytes in liquid biopsy as exosomes or CTCs can go beyond cfDNA and offer opportunities in research and possible in clinical practice.



Krebs et al (Rolfo), JAMA Oncology OCT 2022

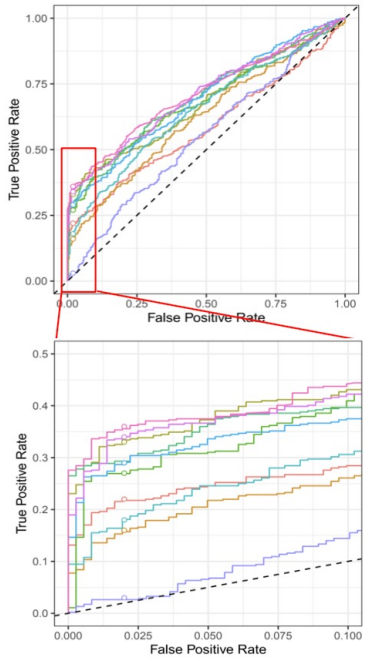
Liquid biopsy & early detection: Strengths and weaknesses of currently used approaches



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

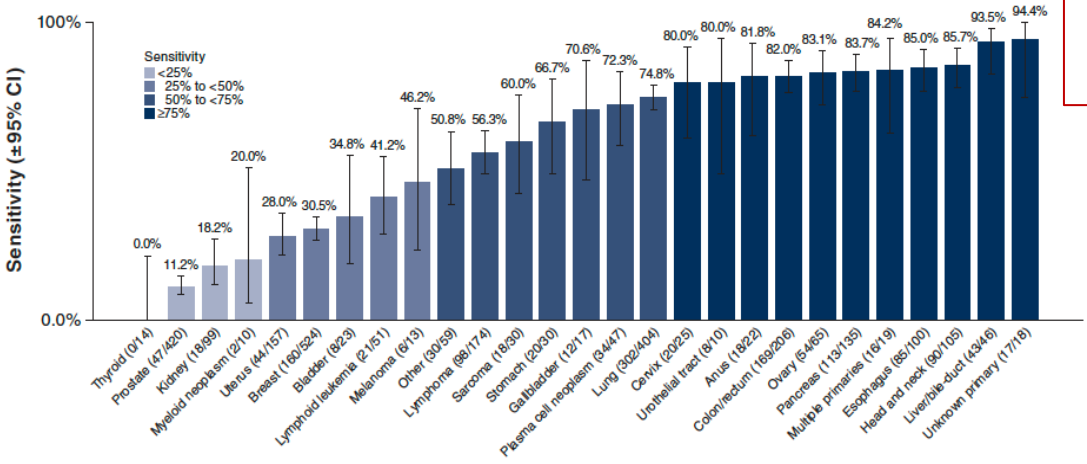
Sensitivity at 98% specificity	WGBS	WG methylation	34% (30%-39%)	158/464

Performance



	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%)	

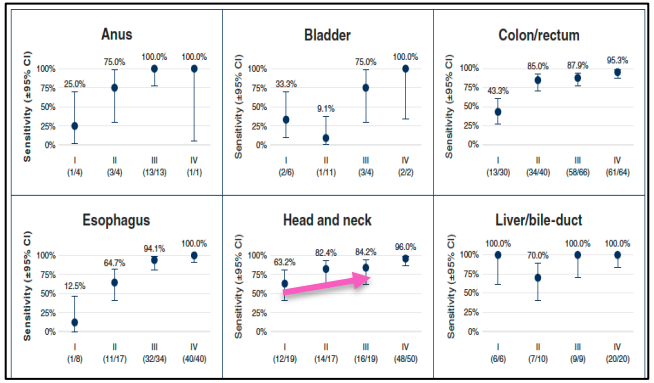
Two-sided 95% Wilson confidence intervals were calculated.

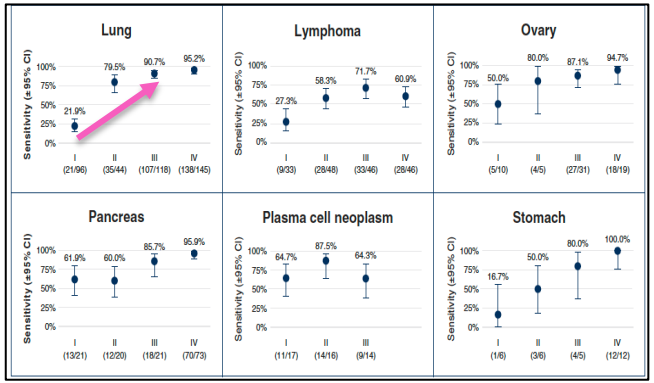


Confirmed status analysis set, *n* = 4077
cancer, *n* = 2823; non-cancer, *n* = 1254

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

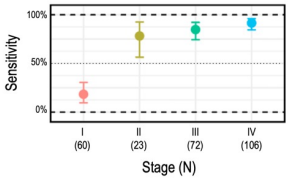




Clinical stage	Total *N*	Test positive	Sensitivity % (95% CI)[a]
All	2823	1453	51.5 (49.6% to 53.3%)
I	849	143	16.8 (14.5% to 19.5%)
II	703	284	40.4 (36.8% to 44.1%)
III	566	436	77.0 (73.4% to 80.3%)
IV	618	557	90.1 (87.5% to 92.2%)
I-II	1552	427	27.5 (25.3% to 29.8%)
I-III	2118	863	40.7 (38.7% to 42.9%)
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%)

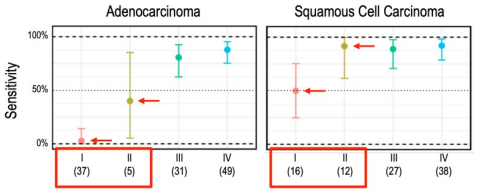
All subtypes have the same sensitivity?

Lung Cancer Detection Varies by Subtype at 99.4% Specificity

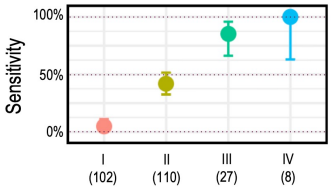


- Overall lung cancer sensitivity: 71.6% (95% CI: 65.8-77.0%)

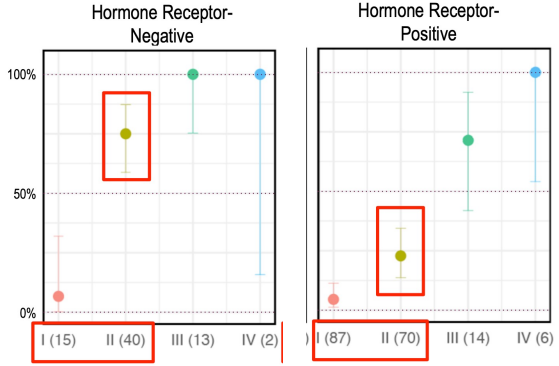
- Detection rate affected by early-stage adenocarcinomas
  - Detection higher in squamous cell carcinoma
- Consistent with prior report showing ctDNA detection was higher in squamous cell carcinoma than adenocarcinoma[1]



Breast Cancer Detection Varies by Subtype at 99.4% Specificity

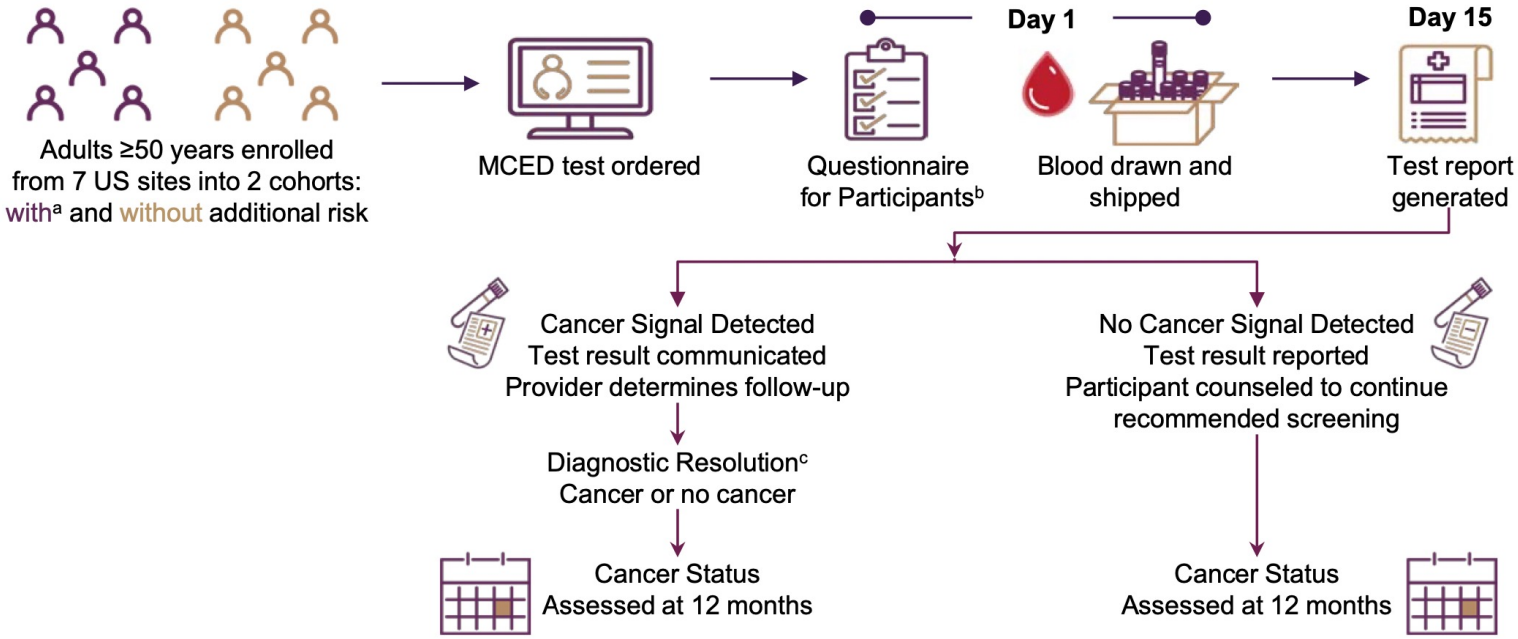


- Overall breast cancer sensitivity: 33.2% (95% CI: 27.4-39.4%)



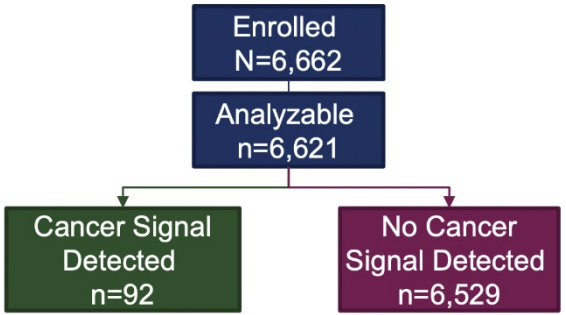
Oxnard et al, ESMO 2019, Klein EA, et al. Ann Oncol 2021

PATHFINDER: A Prospective Cohort Study to Return the Results of MCED Tests to Participants



Deb Schrag, ESMO 2022

	With Additional Risk[a] n = 3,681	Without Additional Risk n = 2,940	Total N = 6,621
Age[b], in years, mean (SD)	64.7 (8.7)	61.6 (8.1)	63.4 (8.6)
Female	65%	62%	63%
White, Non-Hispanic	93%	89%	92%
College Degree or Higher	59%	71%	65%
Up to Date With Standard Cancer Screening Prior to MCED Testing			
Colorectal Cancer[c]	91%	92%	92%
Breast Cancer[d]	78%	83%	80%

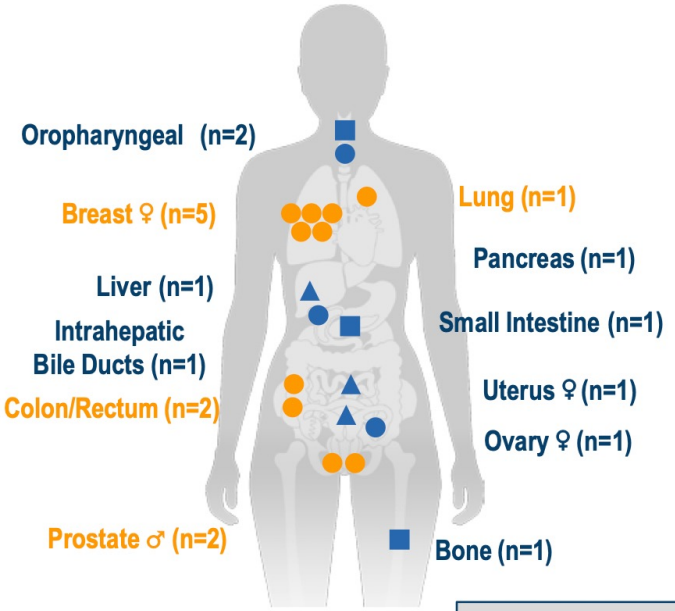


	With Additional Risk[a] n = 3,681	Without Additional Risk n = 2,940	Total N = 6,621
Signal Detected	1.5%	1.2%	**1.4%**
No Signal Detected	98.5%	98.8%	98.6%

Deb Schrag, ESMO 2022

Cancers Diagnosed After a True Positive MCED Signal

19 Solid Tumors

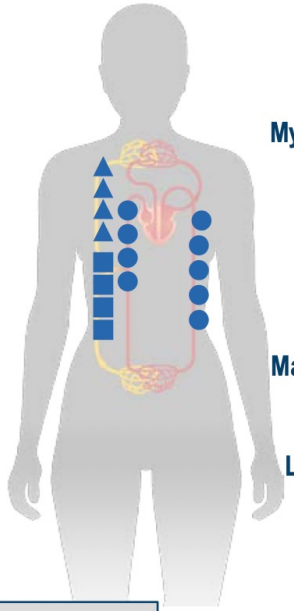


35 people were diagnosed with 36 cancers

- 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

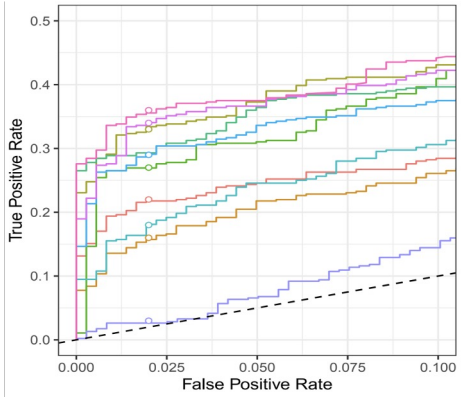
Available Screening: **USPSTF cancer screening** or **No standard screening**

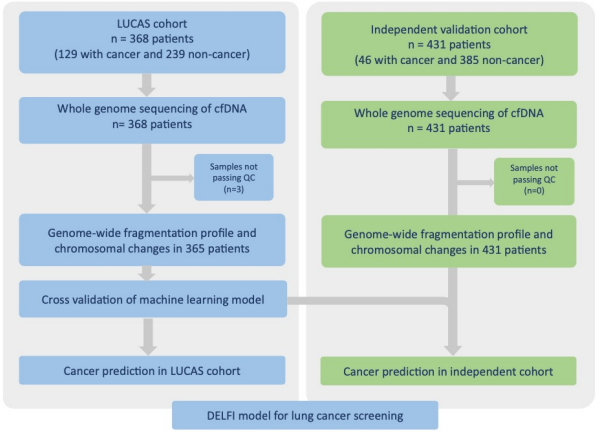
Deb Schrag, ESMO 2022

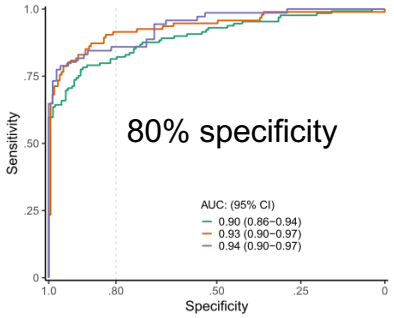
Fragmentomics in a Single-tumor test

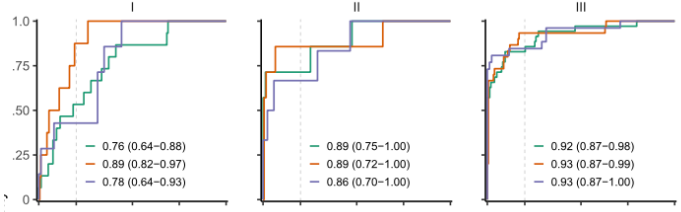
Sensitivity at 98% specificity			
	Fragment endpoints	18% *** (15%-22%)	84/464
	Fragment lengths	29% * (25%-34%)	135/464

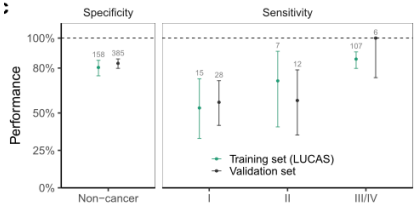
Non-cancer individuals: 236
Patients with Lung cancer 129





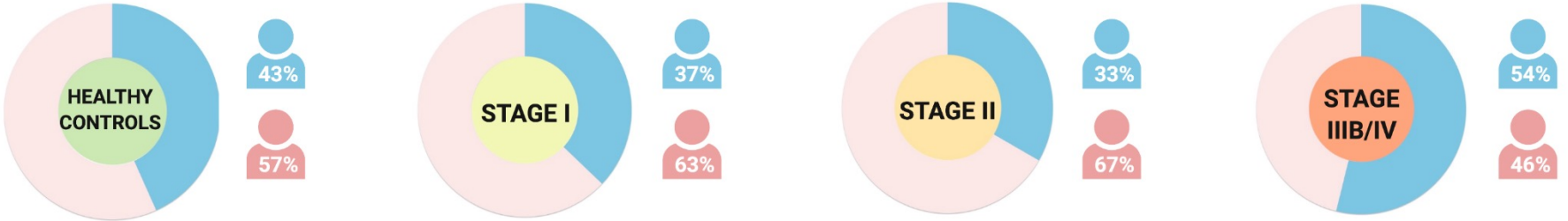




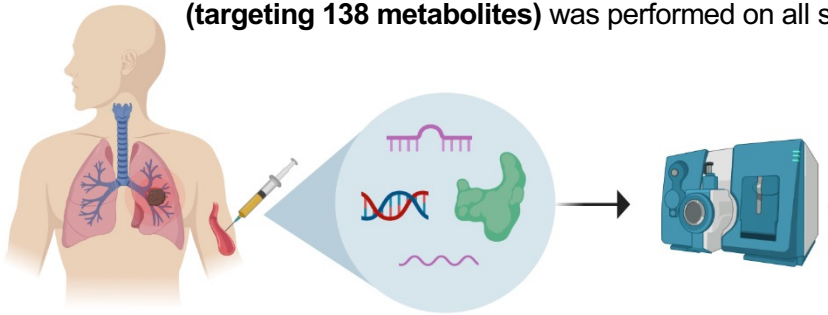


Cristiano S, et al. Nature 2019

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

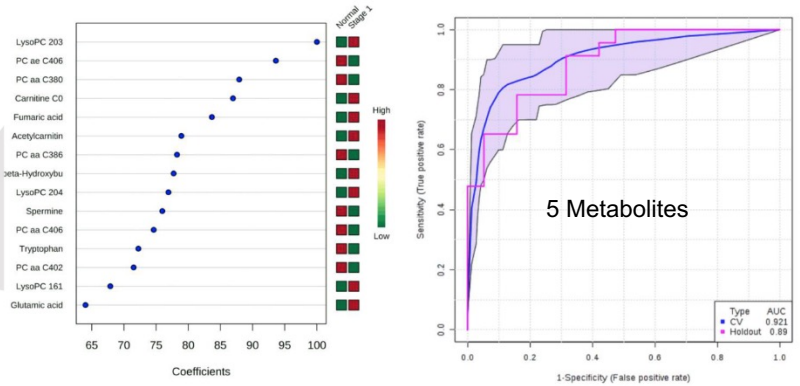


quantitative targeted mass spectrometry (MS) analysis **(targeting 138 metabolites)** was performed on all samples



PLASMA SAMPLES (n=156 NSCLC patients)

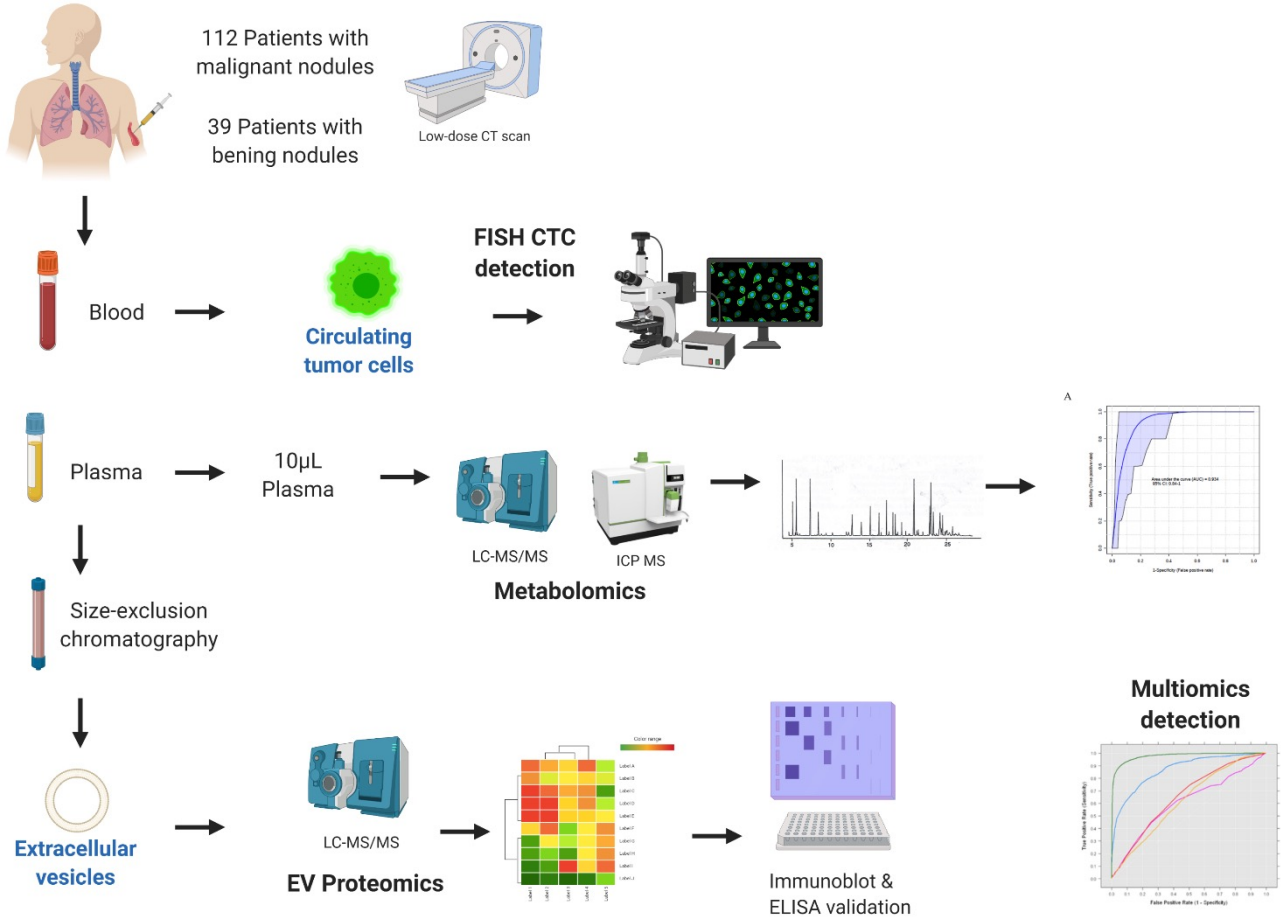
LC-MS



METABOLITE PANEL ANALYSIS

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

NSCLC Early detection



CENTER FOR THORACIC ONCOLOGY

CTO As facilitator of Multidisciplinary Expert Multi-omics approach in Lung Cancer Early Detection

Screening Program

Claudia Henscke

David Yankelevitz

Biomarkers Lab: Liquid & Tissue

Christian Rolfo

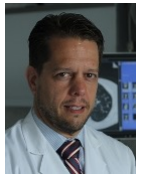
D. De Miguel

Phil Mack

Fred Hirsch

Pulmonology

Surgery



Javier Zulueta

David Steiger

Daniel Nicastri

Radiomics

Proteomics and RNAsq in EVs

cfDNA

RNAsq Tissue

Lung Nodule Clinic

External Collaboration

Statistician

Epidemiologist

Internal Support

CTCs

Metabolomics

Hsin-Hui (Vivien) Huang

Emanuela Taioli

Mount Sinai Innovation Partners

Take home message

- Early detection is crucial to increase survival rates in cancer
- Liquid Biopsy is an ideal tool to make it possible
- We need methods to complement the screening programs
- But also methods standing by their self
- Multi-cancer detection or single tumor? Not clear yet
- Important to include risk populations in trials
- We are not yet in the best scenario that we could be…
- But we are not far to get it!

@ChristianRolfo

Join us in 2023 Madrid, Spain

ISLB
INTERNATIONAL SOCIETY OF LIQUID BIOPSY

5TH ANNUAL CONGRESS
Liquid Biopsy
19 - 21 November 2023 | Madrid, Spain

Save the Date
19-21, November 2023