ctDNA in medical oncology: Clinical and Research Applications

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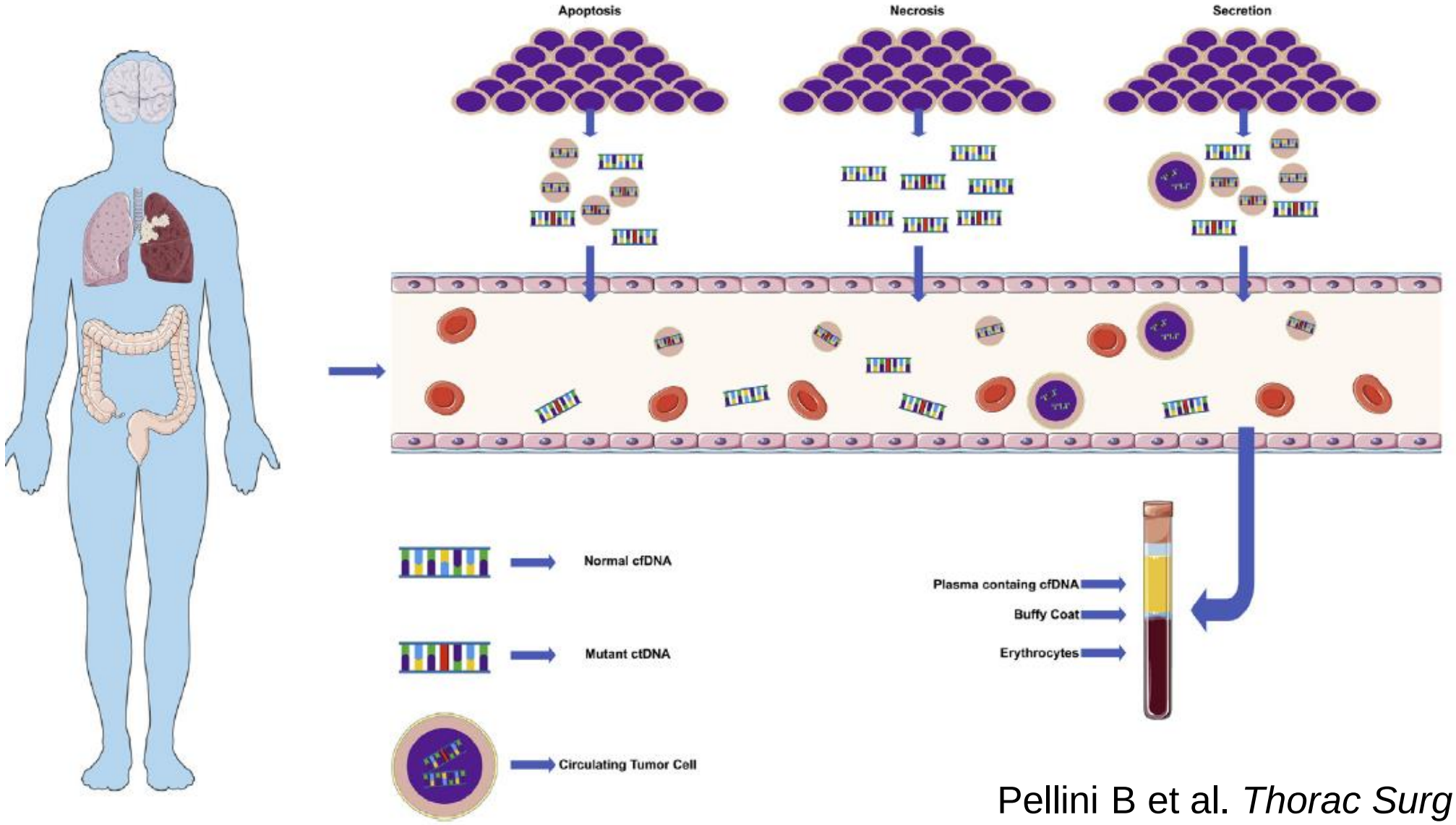
- Speaker Honoraria: MJH Life Science, Merck, Foundation Medicine, BioAscend, PlaytoKnow AG, Grupo Pardini, GBOT

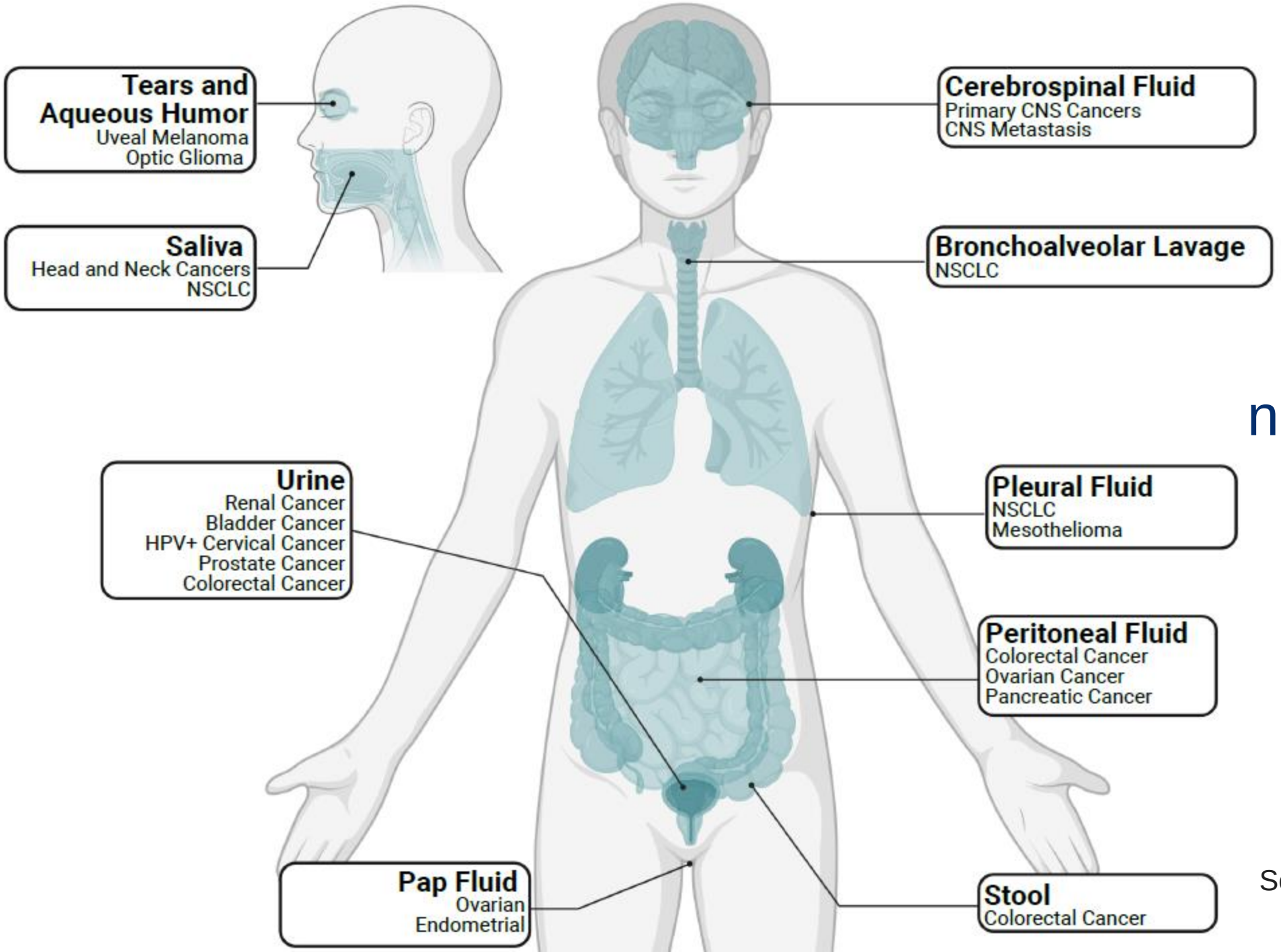
Outline

- ctDNA definition & sources of ctDNA
- ctDNA genotyping
- Tumor-informed vs. tumor-naïve assays
- ctDNA applications in oncology:
  - Molecular profiling
  - Treatment Monitoring
  - Minimal residual disease (MRD) detection

Tumor-derived fragments of nucleic acids identified in the blood are called circulating tumor DNA (ctDNA)



Pellini B et al. *Thorac Surg Clin*. 2020



Tumor-derived nucleic acids can be found in different biospecimens

Semenkovich NP et al. *JITC*. 2023

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Plasma genotyping

Test	Limit of detection (% ctDNA)	Advantages	Limitations
Allele-specific PCR	~0.1-1%	• Ease of use • Lower cost	• Lower sensitivity • Only tests small number of genomic positions
Digital PCR	~0.01%	• High sensitivity	• Only tests small number of genomic positions
Hybrid capture-based NGS	~0.001-0.5%	• High sensitivity • No need for personalization	• Less comprehensive than WES and WGS
WGS	~10%	• Broadly applicable • Entire genome is interrogated	• Expensive • Low sensitivity • Mostly limited to SCNA detection
WES	~5%	• Broadly applicable • Entire exome is interrogated	• Expensive • Low sensitivity

SCNA, somatic copy number alteration; NGS, next generation sequencing; WGS, whole genome sequencing; WES, whole exome sequencing

Adapted from Chaudhuri et al. Semin Radiat Oncol. 2015; Chin et al. Mol Diagn Ther. 2019

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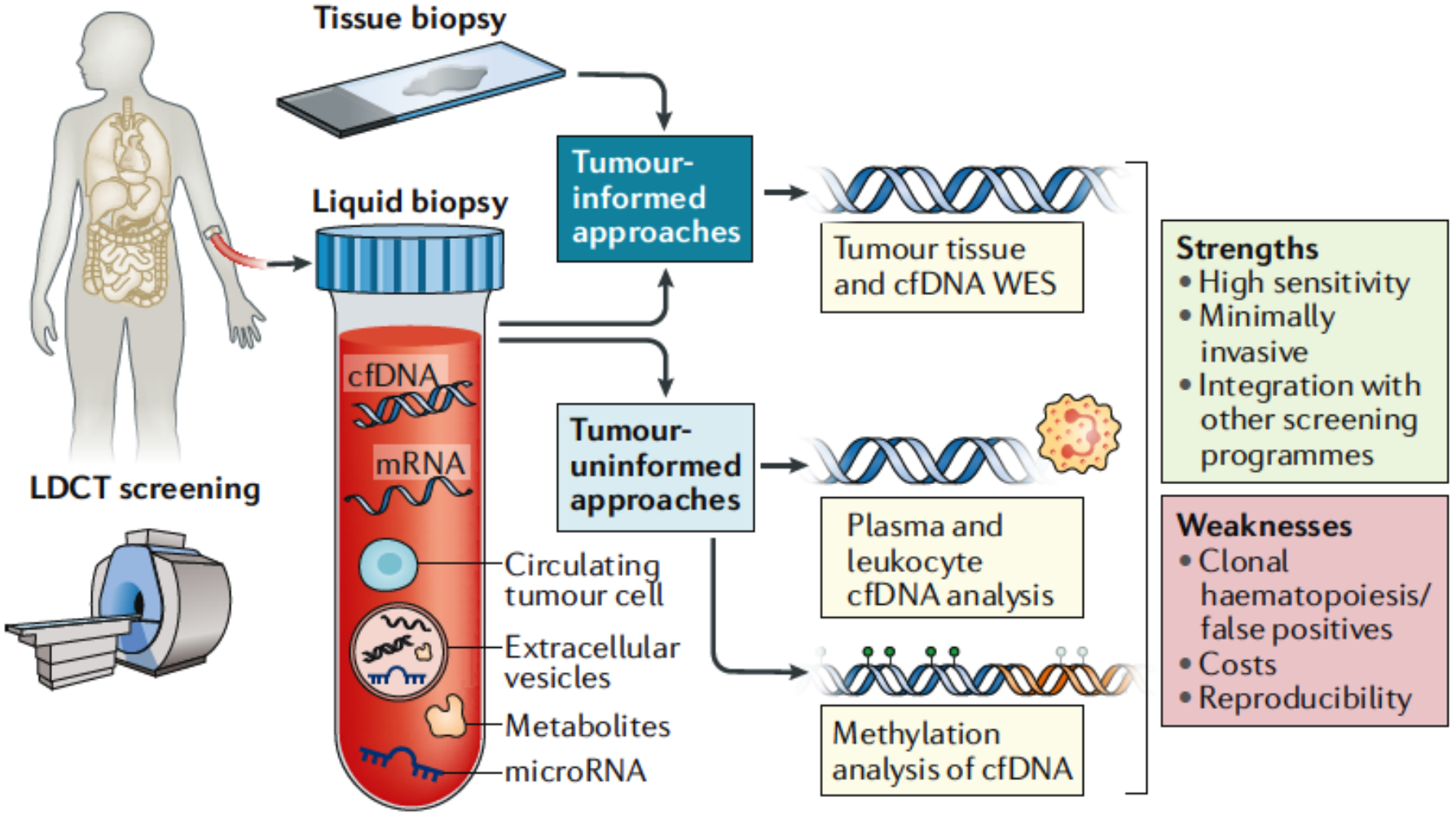
Tumor-informed vs. tumor-naïve assays

Tumor-Informed	Tumor-naïve
Requires tissue biopsy	No need for biopsy
Personalized assay	Off the shelf assay
Longer turnaround time	Shorter turnaround time
Does not account for tumor heterogeneity	Can detect clonal variants that emerge during follow-up
Potential for better sensitivity and specificity	Variable sensitivity and specificity

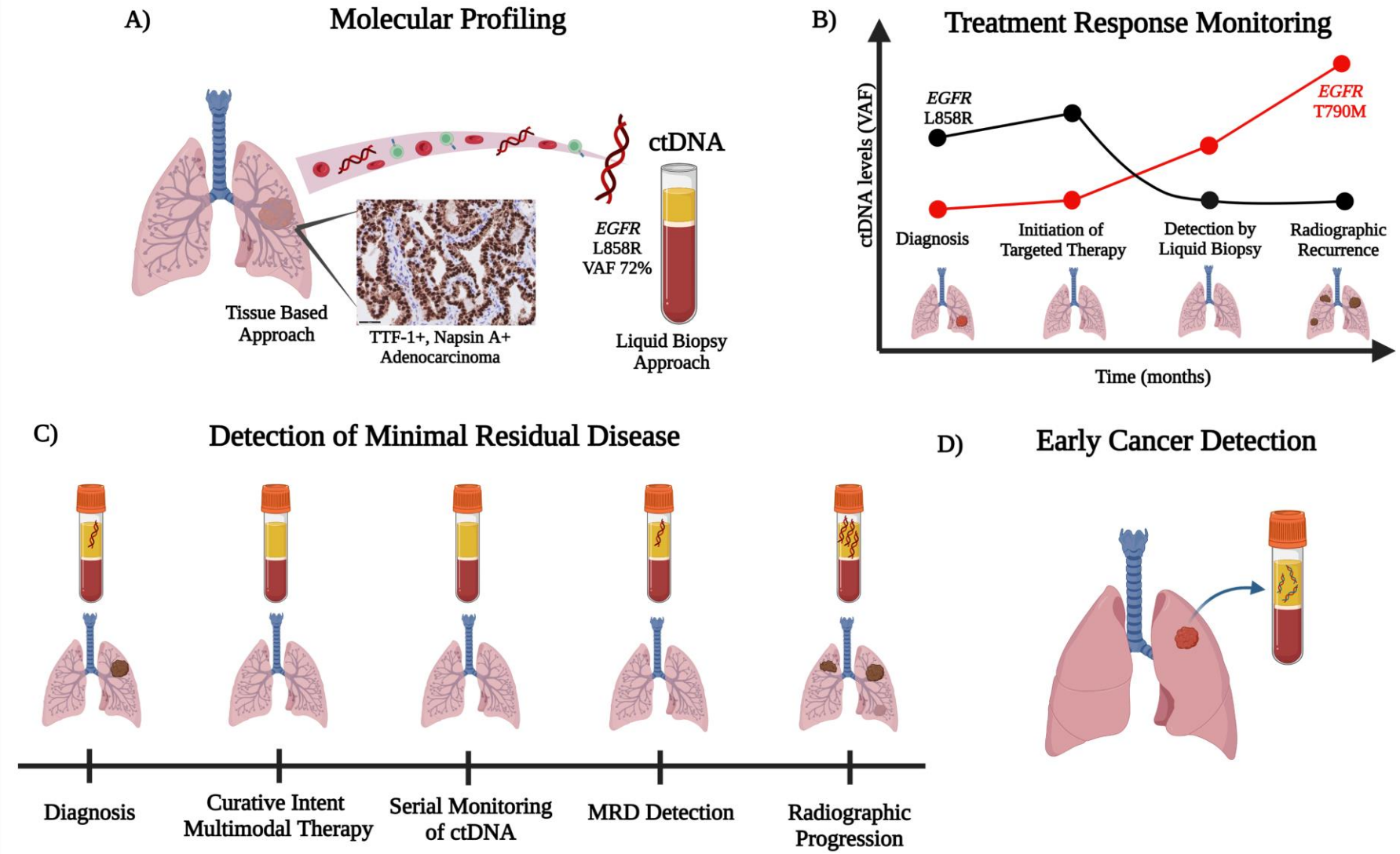
Pellini B and Chaudhuri A. *J Clin Oncol*. 2022

Challenges for ctDNA use in oncology



Rolfo C & Russo A. *Nat Rev Clin Oncol*. 2020

ctDNA applications in oncology



Shields M, Chen K…Pellini B. *Int J Mol Sci*. 2022

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ctDNA sequencing has high sensitivity and specificity to identify actionable genomic alterations

Table 3. Comparison of tissue versus cfDNA results for the guideline-recommended biomarkers in newly diagnosed metastatic NSCLC with FDA-approved therapies, *EGFR* exon 19 deletion and L858R, *ALK* fusion, *ROS1* fusion, and *BRAF* V600E

		Tissue+	Tissue−	Tissue not assessed	Tissue QNS	Total		
EGFR exon 19 del	cfDNA+	18	0	0	1	19	Sensitivity	81.8%
	cfDNA−	4	201	19	25	249	PPV	100.0%
	cfDNA TND	0	11	1	1	13	Specificity	100.0%
	cfDNA cancelled	0	0	1	0	1	NPV	98.0%
	Total	22	212	21	27	282	Concordance	98.2%
EGFR L858R	cfDNA+	9	0	0	2	11	Sensitivity	90.0%
	cfDNA−	1	213	19	24	257	PPV	100.0%
	cfDNA TND	0	11	1	1	13	Specificity	100.0%
	cfDNA cancelled	0	0	1	0	1	NPV	99.5%
	Total	10	224	21	27	282	Concordance	99.6%
ALK fusion (original)	cfDNA+	5	0	0	1	6	Sensitivity	62.5%
	cfDNA−	3	207	27	25	262	PPV	100.0%
	cfDNA TND	1	10	2	0	13	Specificity	100.0%
	cfDNA cancelled	0	1	0	0	0	NPV	98.6%
	Total	9	218	29	26	282	Concordance	98.6%
ALK fusion (reanalysis)	cfDNA+	6	0	0	1	7	Sensitivity	75.0%
	cfDNA−	2	207	27	25	261	PPV	100.0%
	cfDNA TND	1	10	2	0	13	Specificity	100.0%
	cfDNA cancelled	0	1	0	0	1	NPV	99.0%
	Total	9	218	29	26	282	Concordance	99.1%
ROS1 fusion	cfDNA+	0	0	0	0	0	Sensitivity	-
	cfDNA−	2	151	85	30	268	PPV	-
	cfDNA TND	0	7	5	1	13	Specificity	100.0%
	cfDNA cancelled	0	1	0	0	1	NPV	98.7%
	Total	2	159	90	31	282	Concordance	98.7%
BRAF V600E mutation	cfDNA+	2	0	0	0	2	Sensitivity	100.0%
	cfDNA−	0	90	158	18	266	PPV	100.0%
	cfDNA TND	0	5	8	0	13	Specificity	100.0%
	cfDNA cancelled	0	0	1	0	1	NPV	100.0%
	Total	2	95	167	18	282	Concordance	100.0%

NOTE: Overall concordance across all four genes was greater than 98.2%, with a PPV of 100%. With continuous assay improvements, one cfDNA result originally reported as a false-negative for *ALK* fusion was identified as positive.

Stage IV NSCLC
Tumor-naïve assay
(Guardant 360)

Leighl N et al. *Clin Cancer Res*. 2019

ctDNA sequencing has high sensitivity and specificity to identify actionable genomic alterations

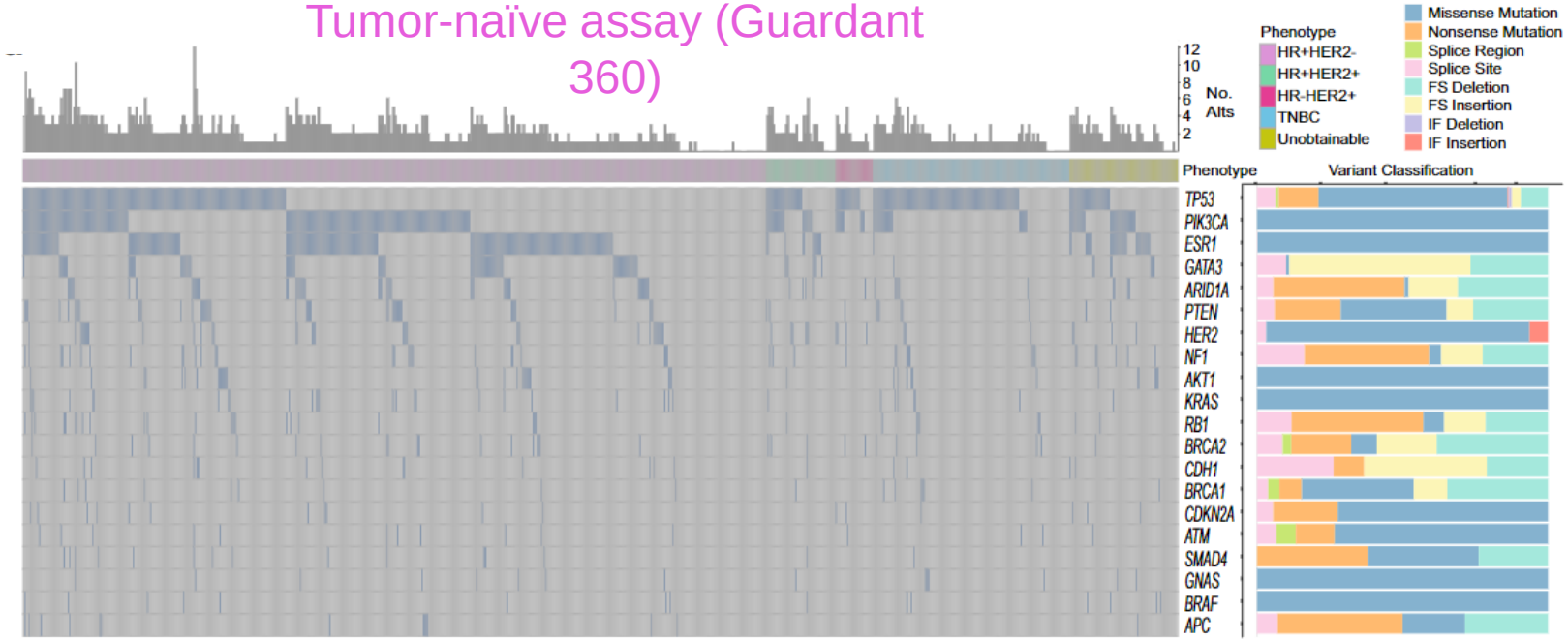
Stage IV NSCLC

Tumor-naïve assay (Resolution Bioscience)

93% concordance between **ctDNA** and **tissue NGS** to detect ***ALK* fusions**

Advanced Breast Cancer

Tumor-naïve assay (Guardant 360)

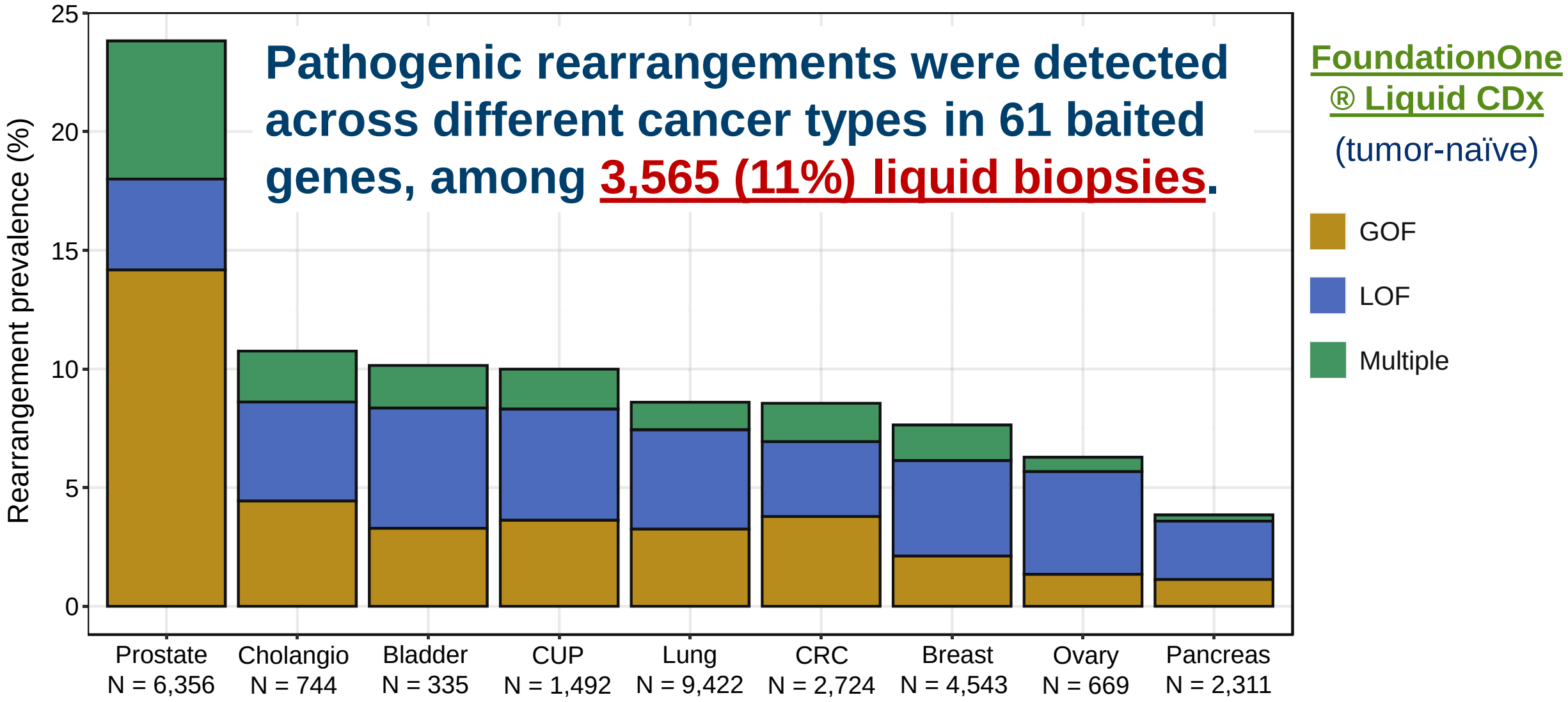


92% of **800 patients** were found to have **at least one ctDNA alteration**

Mondaca S et al. *Lung Cancer*. 2021

Kingston B et al. *Nat Commun*. 2021

Gene rearrangements can be detected using ctDNA



Kasi P et al. Abstract OP.02.Presented at ISLB 2022

Research Use

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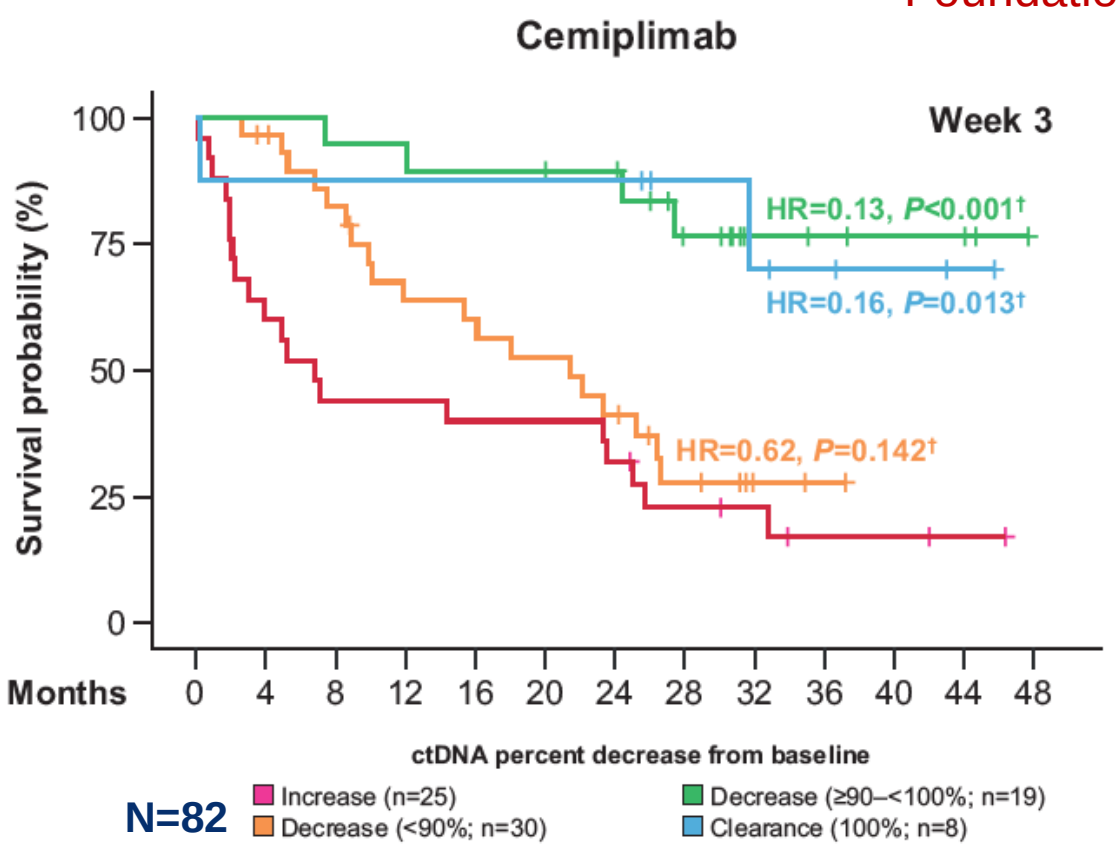
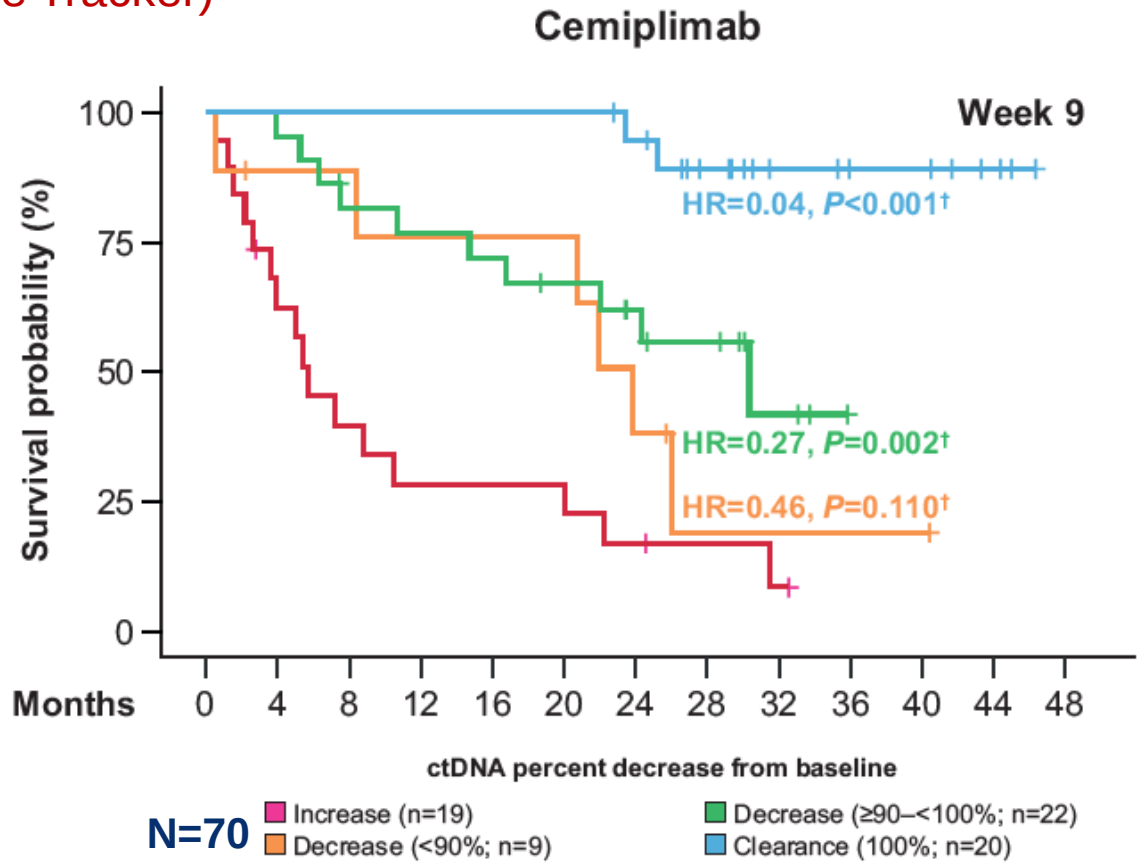
  - Minimal residual disease (MRD) detection

ctDNA decrease ≥90% at week 3 or 9 during cemiplimab treatment is associated with improved OS

Advanced NSCLC

Tumor-informed assay (Signatera™ & FoundationOne Tracker)

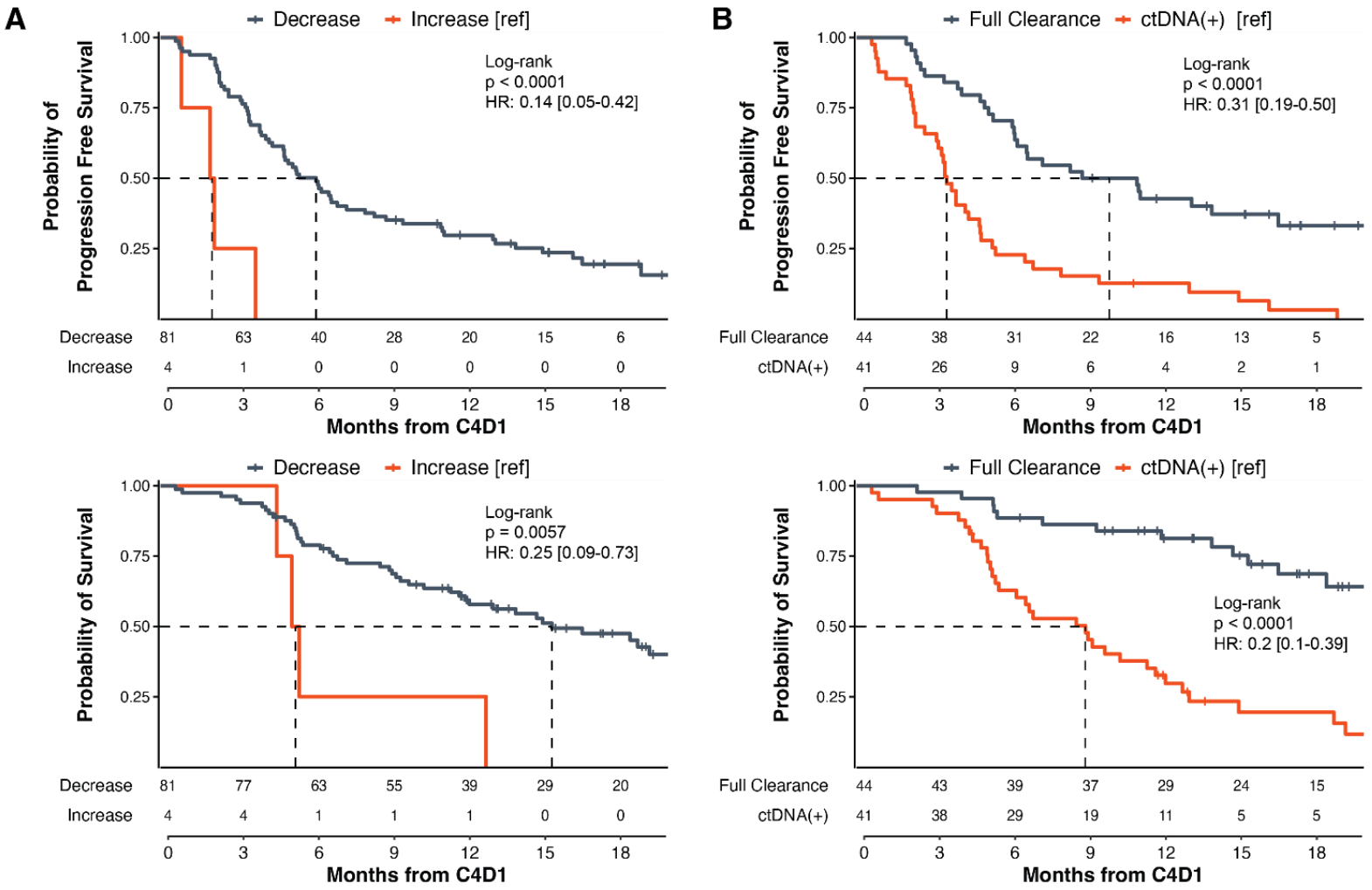




Vokes N et al. 2023 ASCO Annual Meeting.

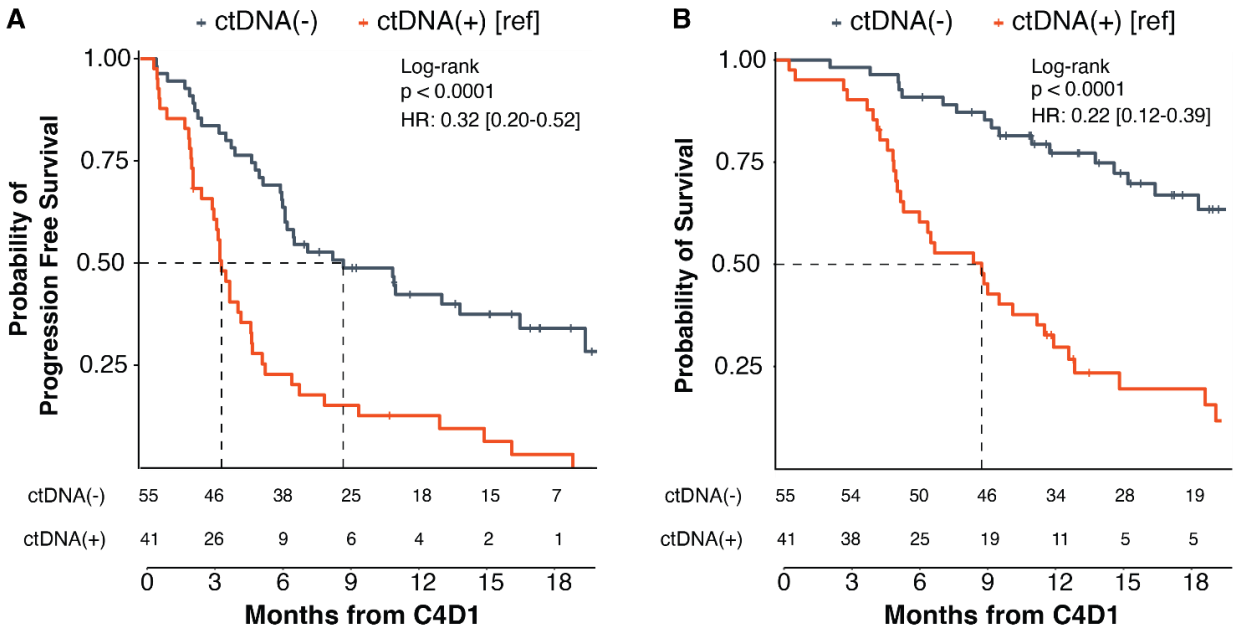
Circulating Tumor DNA Monitoring on Chemo-immunotherapy Informs Outcomes in Advanced Non-Small Cell Lung Cancer

Advanced NSCLC
Tumor-informed assay (FoundationOne Tracker)

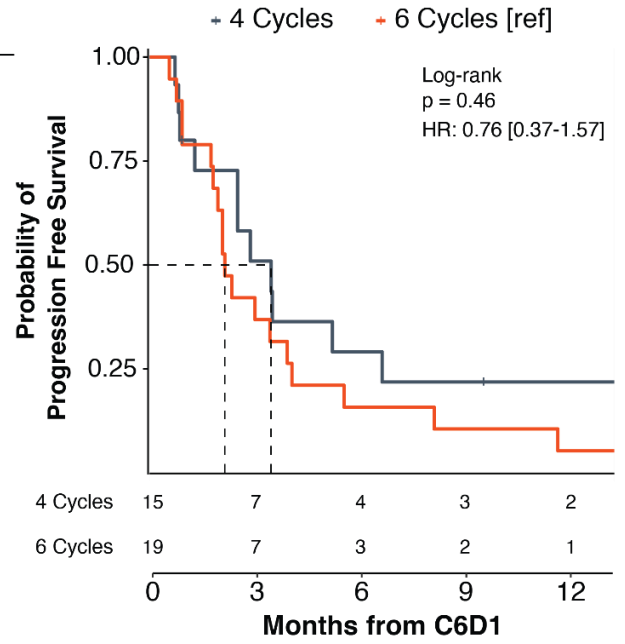


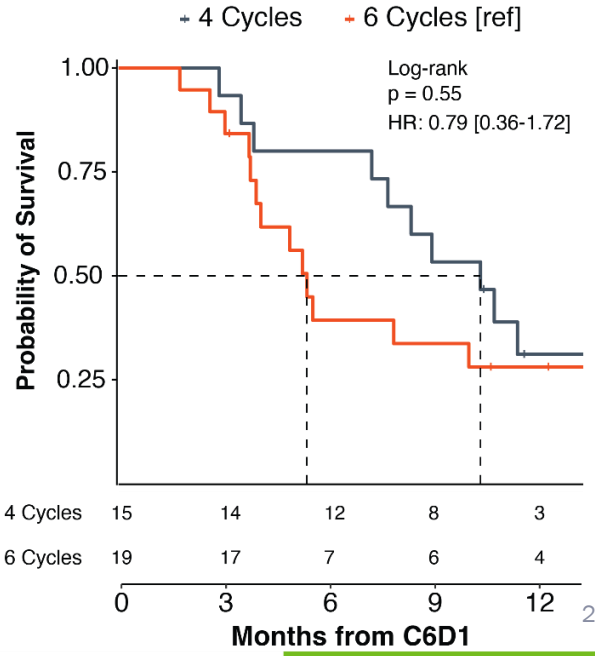
Pellini B et al. 2023 ASCO Annual Meeting.

ctDNA detection on chemoIO can risk stratify patients prior to IO maintenance start, even without baseline ctDNA analysis



Additional cycles of induction therapy are not associated with improved outcomes in patients with ctDNA detection at C4D1

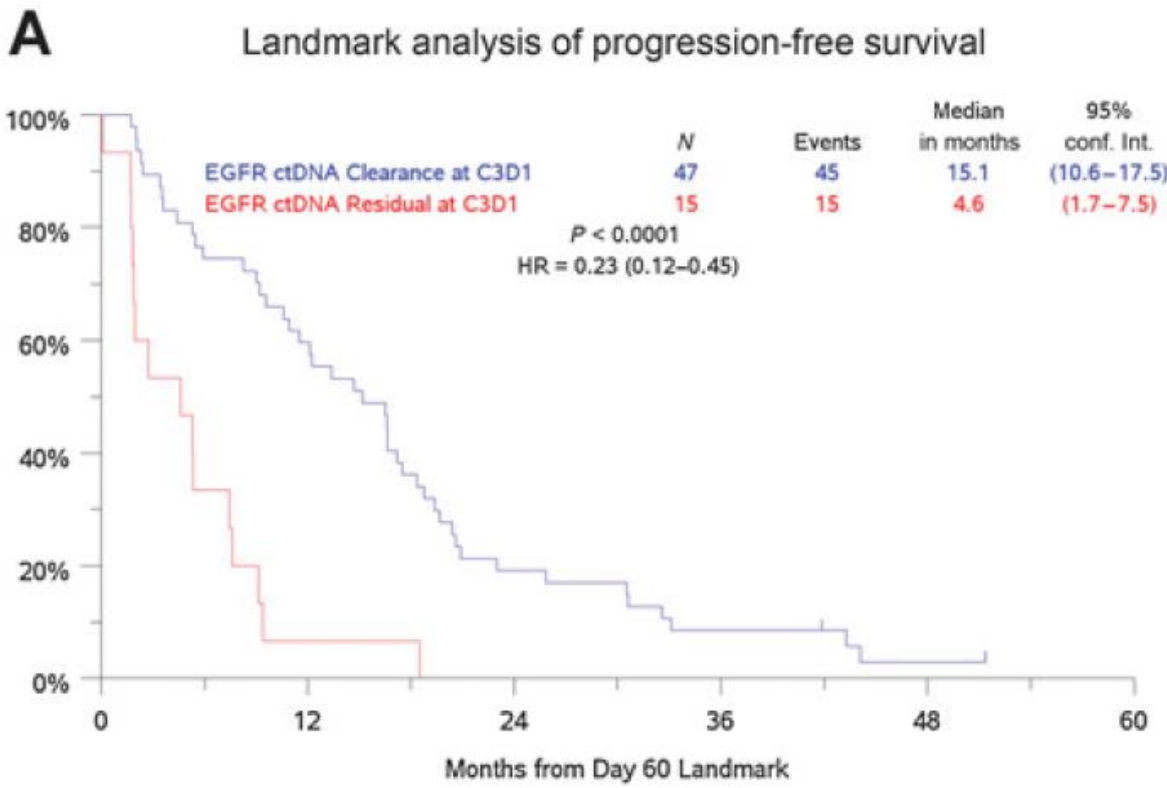


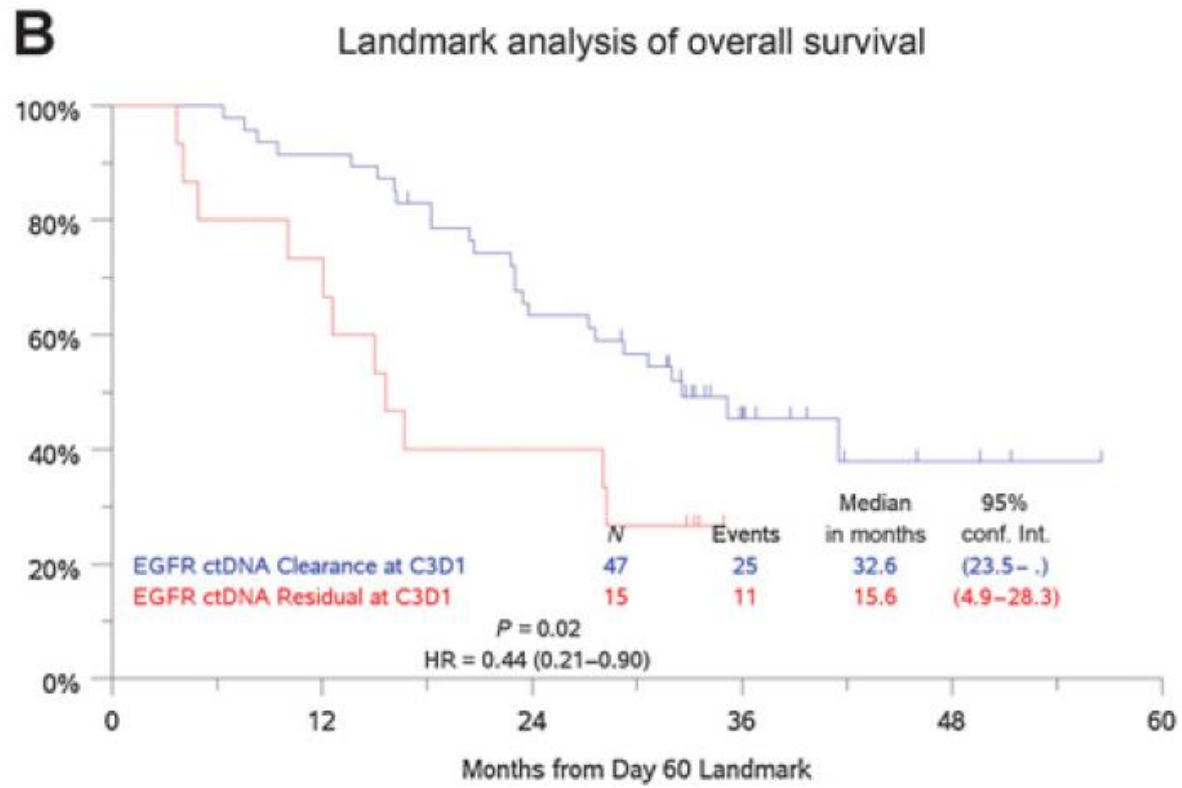


Pellini B et al. 2023 ASCO Annual Meeting.

Patients with undetectable *EGFR* 8 weeks after treatment start had better PFS and OS

Stage IV NSCLC
Tumor-naïve assay
(Guardant 360)



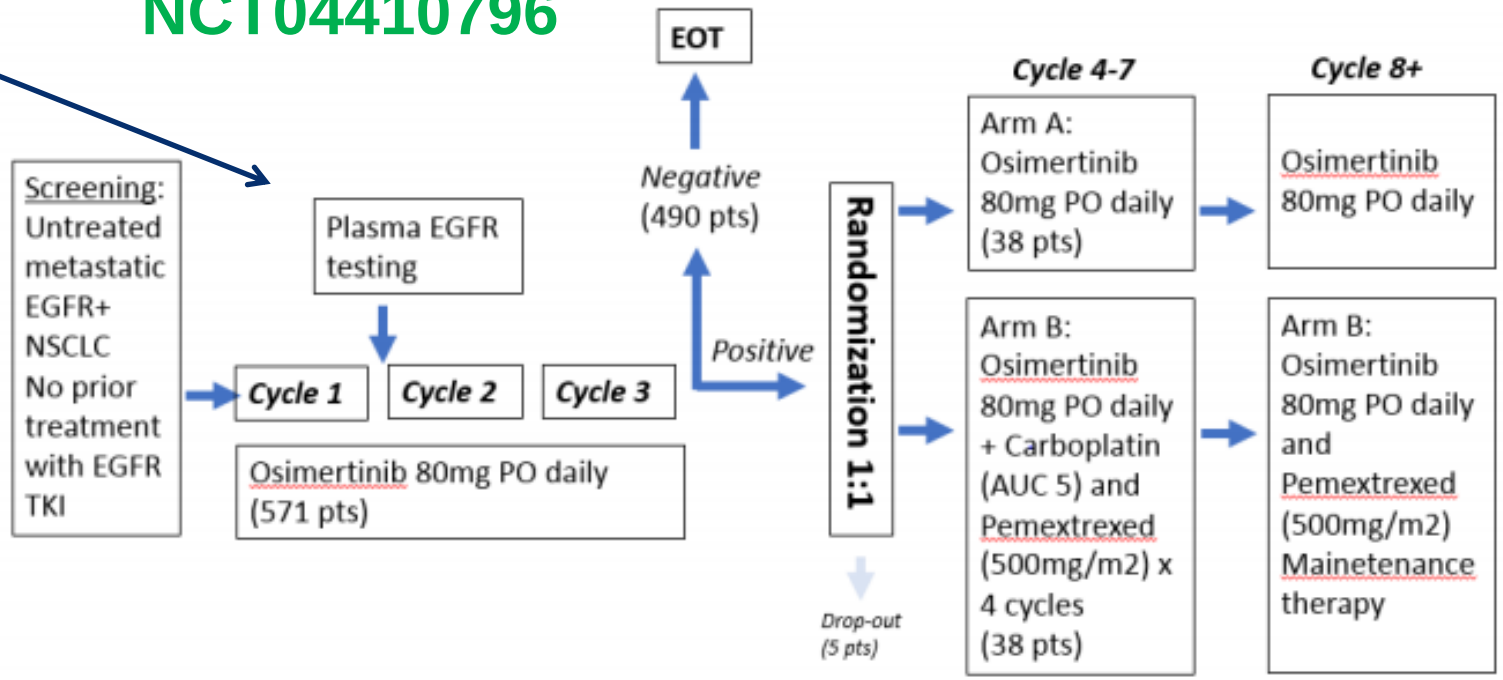


Mack PC et al. *Clin Cancer Res*. 2022

Treatment escalation based on ctDNA detection is under investigation for patients with *EGFR* mutations

3 weeks into therapy

NCT04410796



Treatment plan: All patients will receive osimertinib 80mg orally daily. Patients enrolled in Arm B will receive Carboplatin (AUC 5 IV q 3 weeks) and Pemetrexed (500mg/m2 IV q 3 weeks) for a total of 4 cycles followed by pemetrexed maintenance from cycle 8 onwards.

Total enrollment: Approximately 571 patients will be screened. 80 will be eligible for randomization and treatment consent. 76 will be randomized.

Time to completion: 5 years

National Study PI: Helena Yu, MD (MSKCC); Moffitt PI: Bruna Pellini, MD

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The DYNAMIC study demonstrated that a **ctDNA-guided approach** for patients with **stage II CRC** (treating only patients with a positive ctDNA after surgery) **did not compromise RFS** compared to standard-of-care

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

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

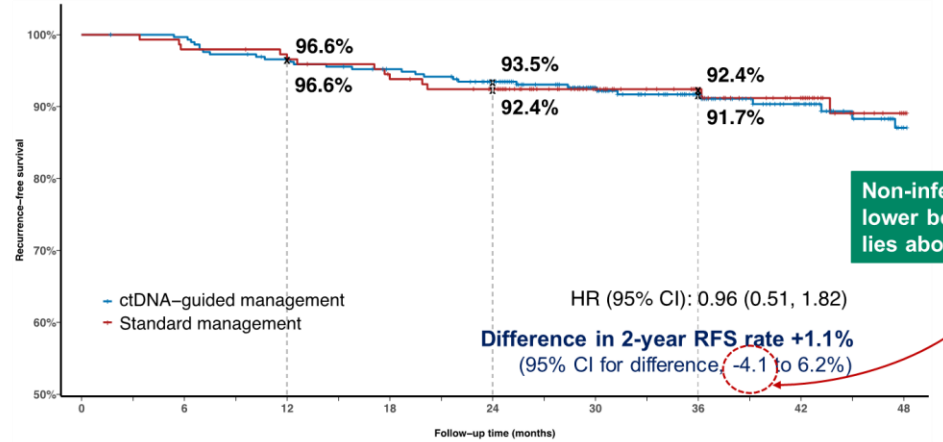
Stratification Factors

- 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

Recurrence-Free Survival

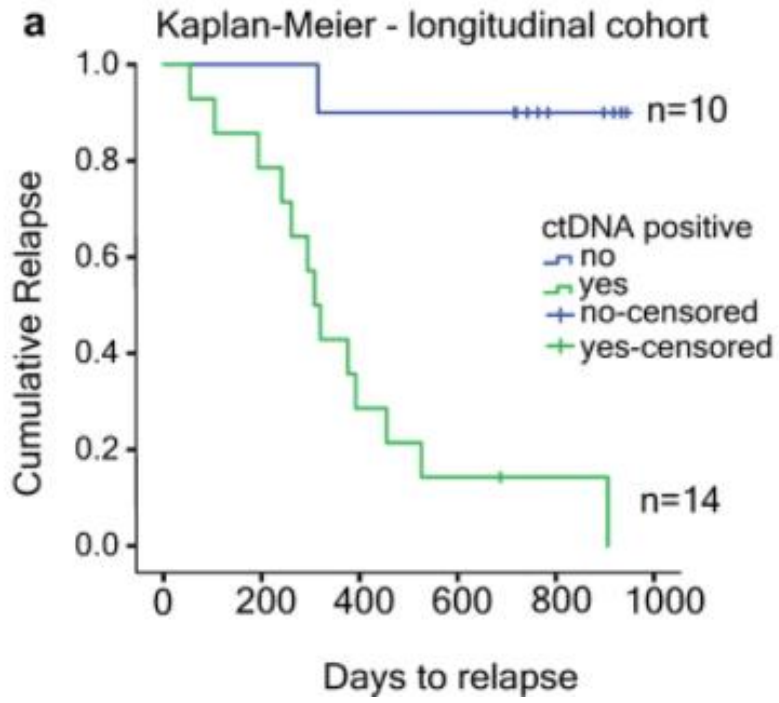


This approach substantially **reduced** the proportion receiving **adjuvant chemotherapy (28% → 15%)**

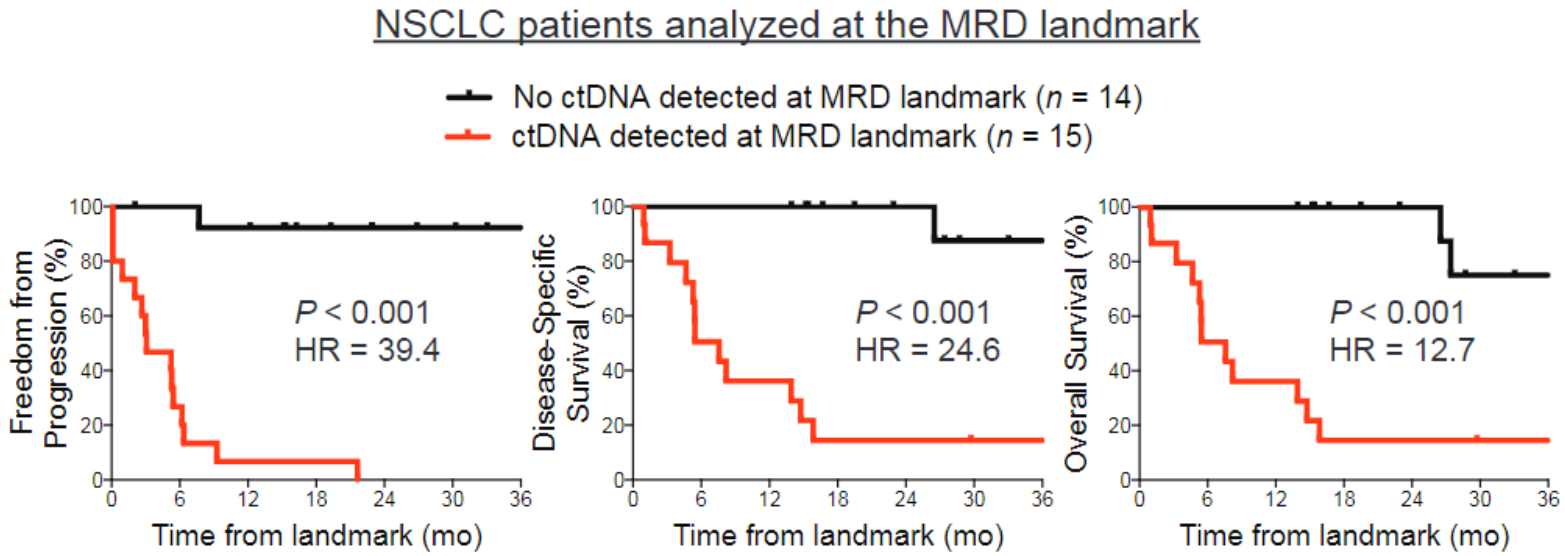
Tie J et al. *N Engl J Med*. 2022

ctDNA can detect minimal residual disease (MRD) and it is a prognostic biomarker

Stages I-III NSCLC
Tumor-informed assay
(Signatera™)



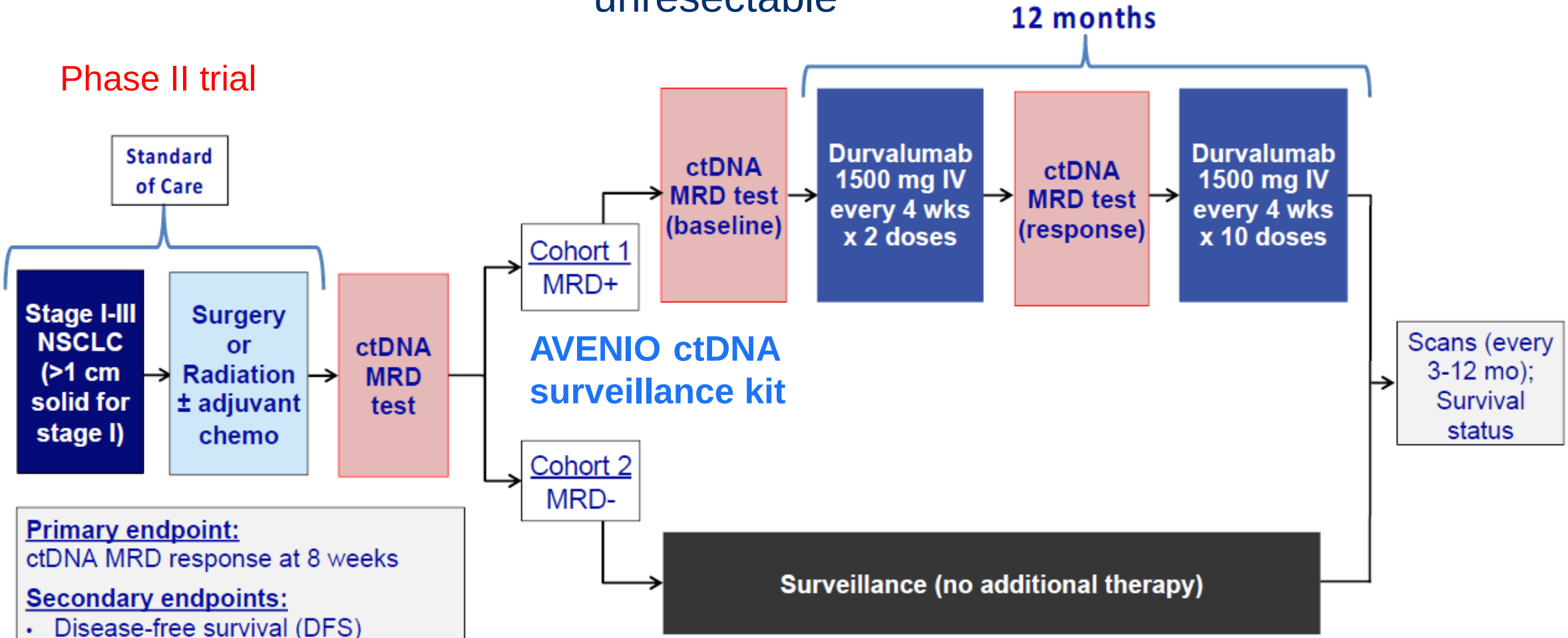
Stages I-III NSCLC
Tumor-naïve assay
(CAPP-Seq)



Abbosh C et al. *Nature*. 2017
Chaudhuri A et al. *Cancer Discov*. 2017

Prospective ctDNA MRD trial for patients with NSCLC stages I-III resectable & unresectable

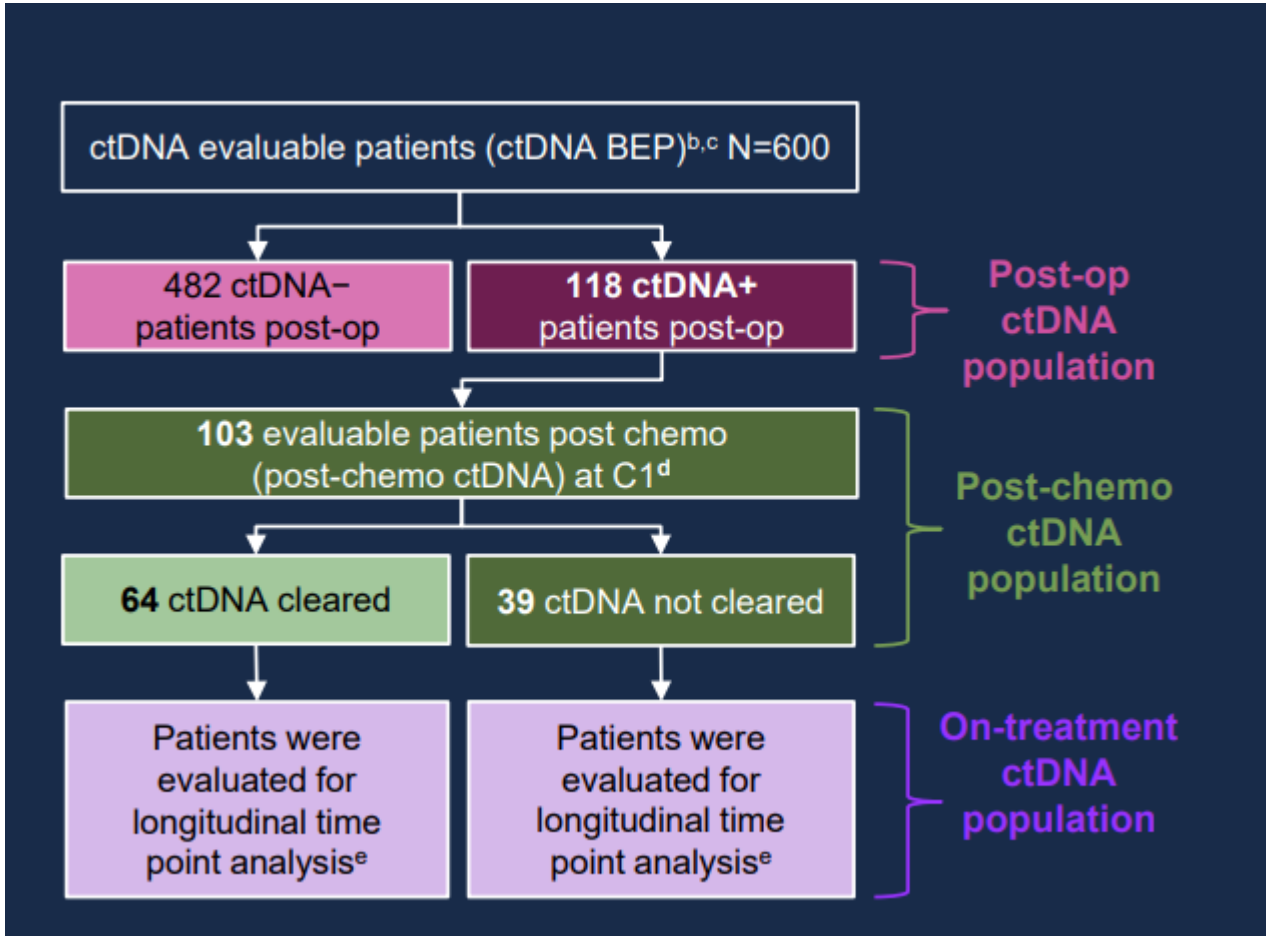
Phase II trial



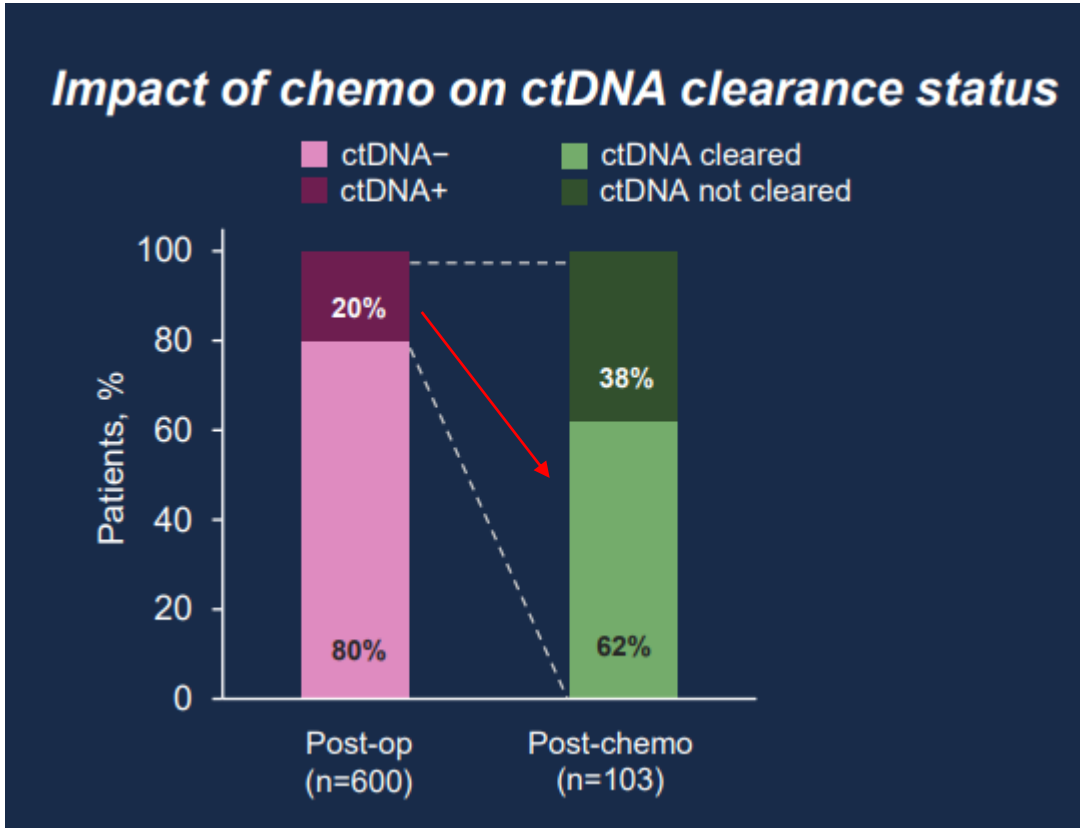
PIs: J. Neal & M. Diehn (NCT04585477)

Slide *adapted from* M. Diehn at TTLC 2022

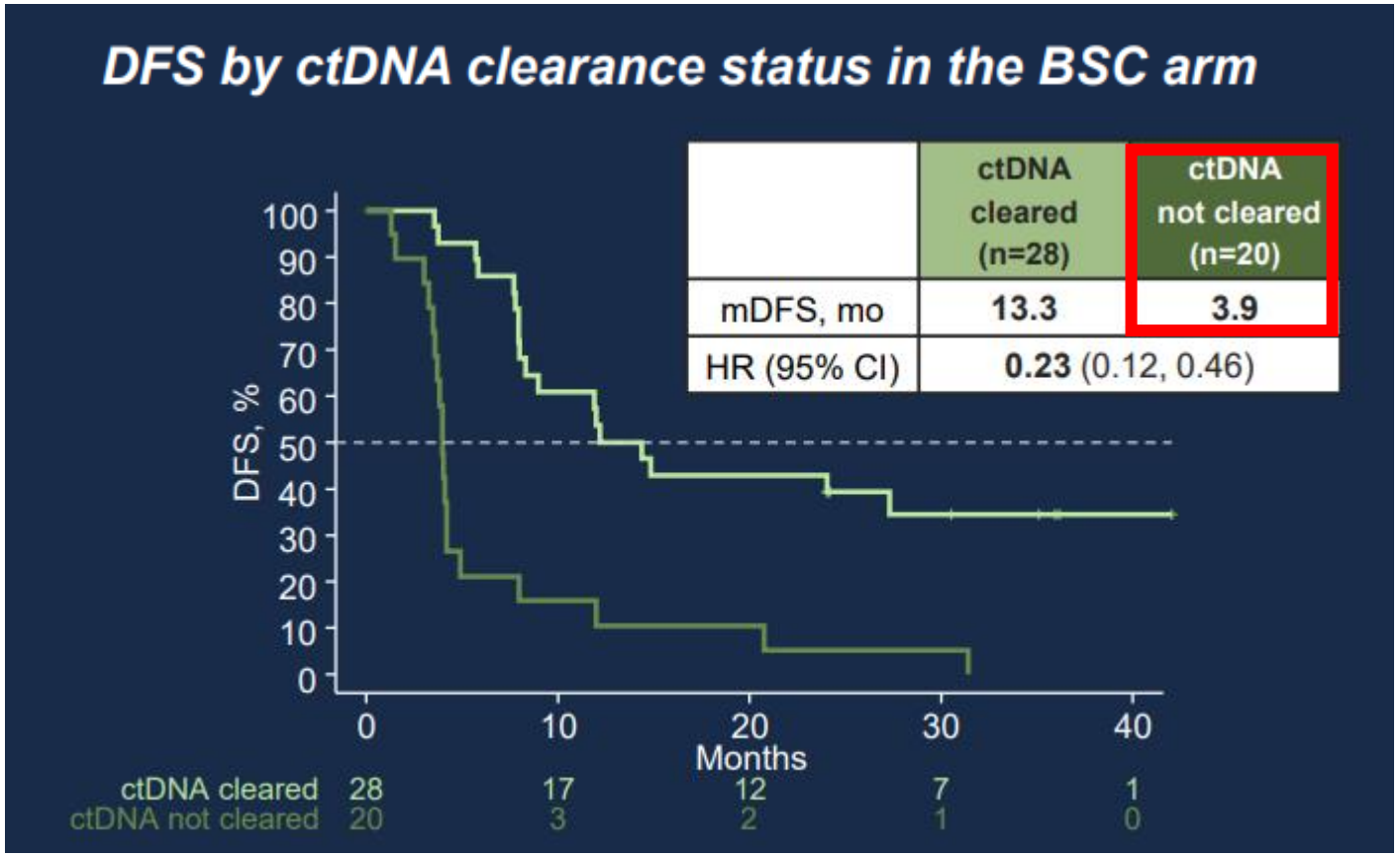
IMpower010: ctDNA Status in Patients With Resected NSCLC Who Received Adjuvant Chemotherapy Followed by Atezolizumab or Best Supportive Care



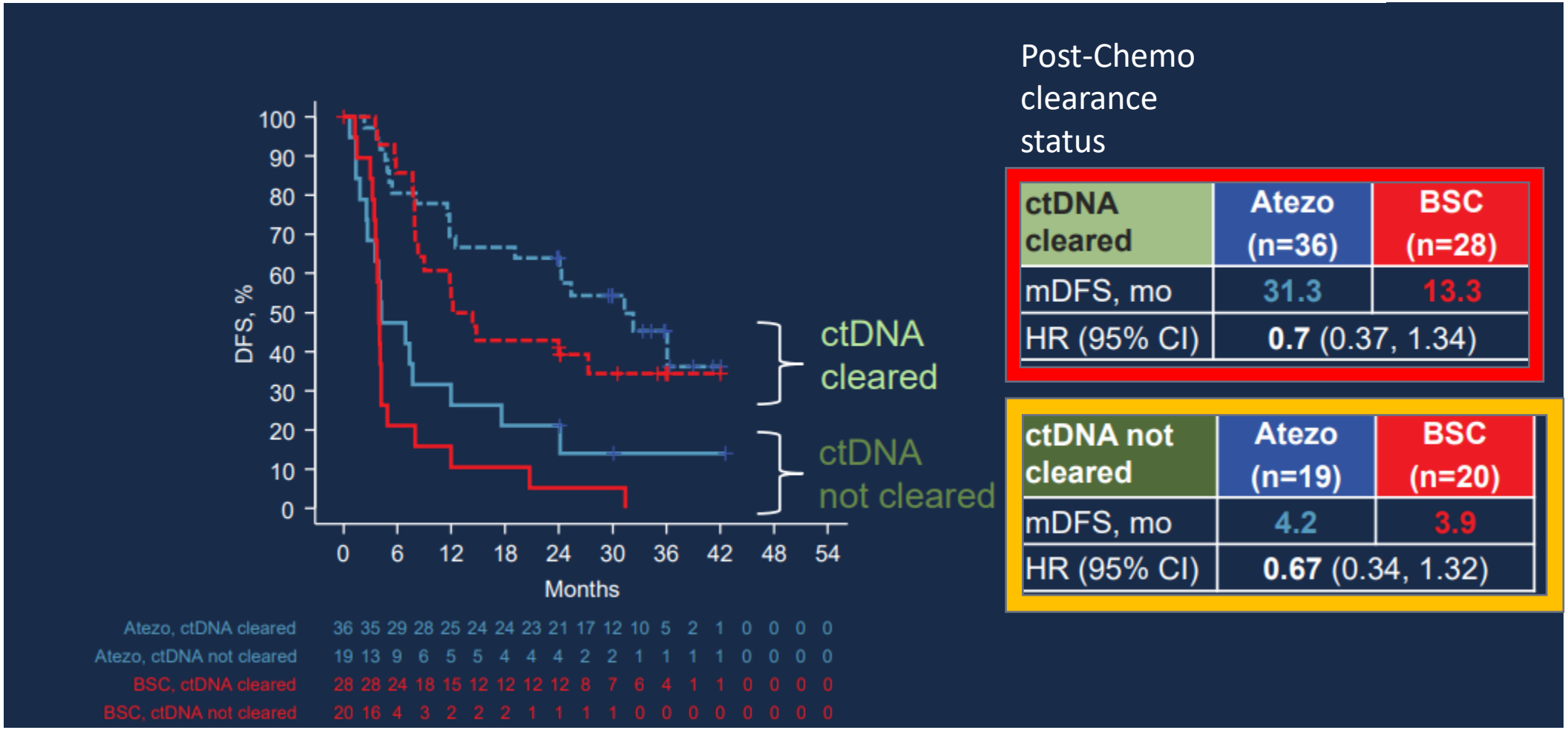
Adjuvant chemotherapy cleared ctDNA in ~62% of patients



IMpower-010: patients with detectable ctDNA MRD after adjuvant chemotherapy have worse prognosis



IMpower-010: data suggests adjuvant atezolizumab delays conversion to ctDNA +



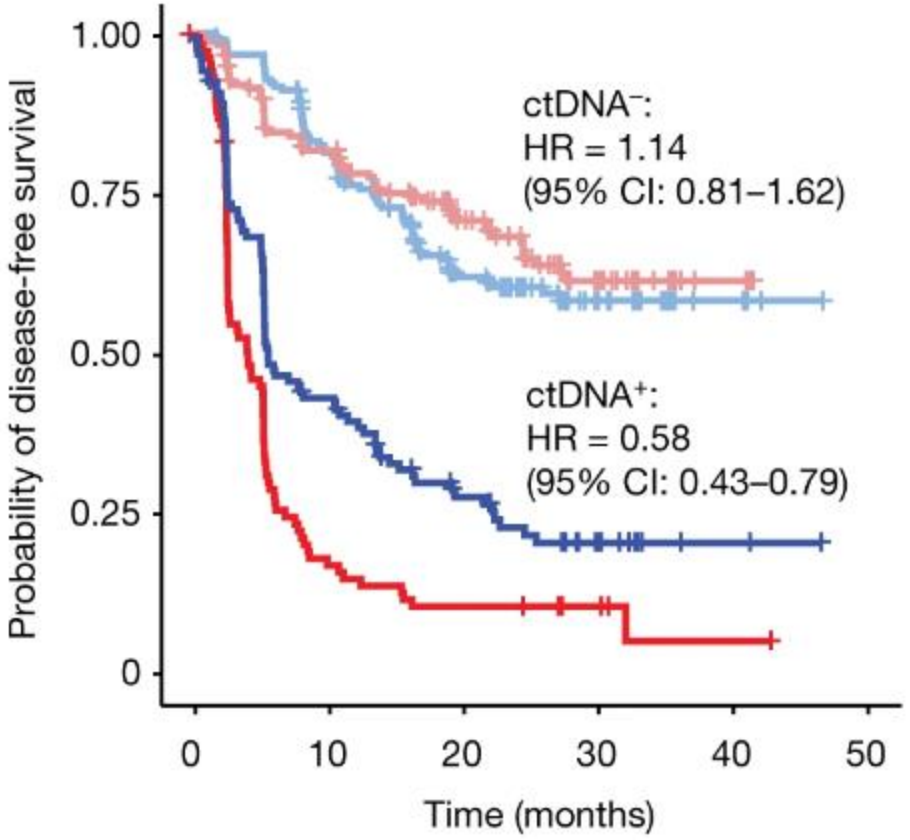
Post-Chemo clearance status

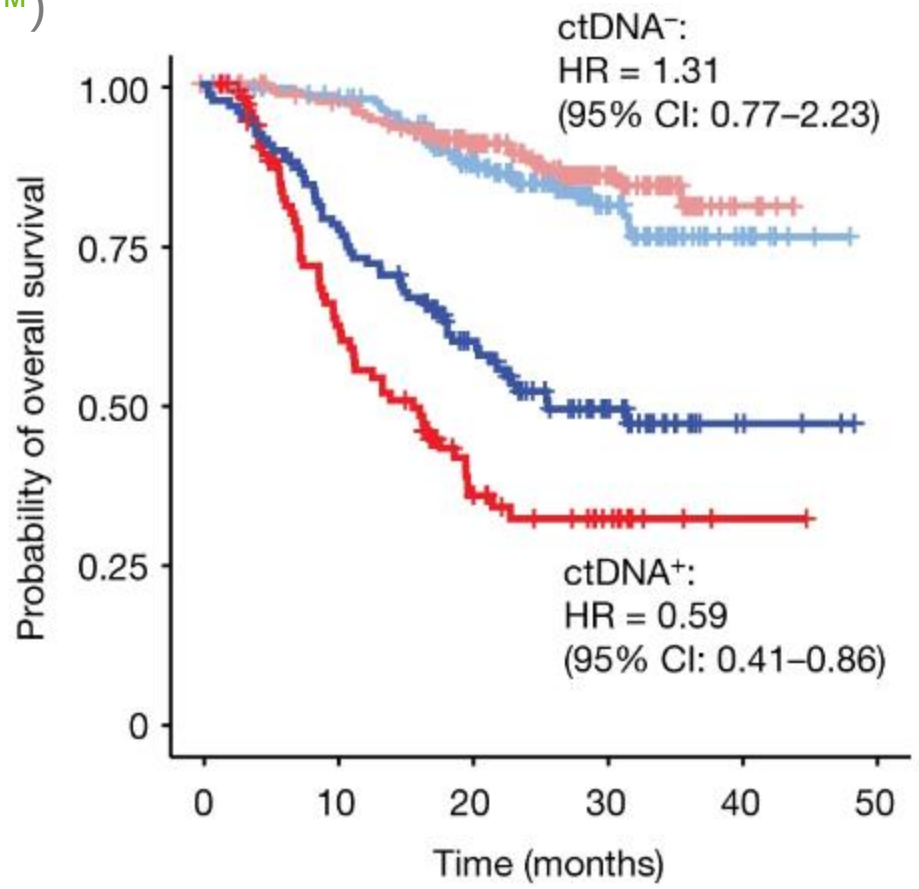
ctDNA cleared	Atezo (n=36)	BSC (n=28)
mDFS, mo	31.3	13.3
HR (95% CI)	**0.7** (0.37, 1.34)	

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

Patients with ctDNA MRD+ after surgery have better DFS and OS with adjuvant atezolizumab

Operable muscle-invasive urothelial cancer
Tumor-informed assay
(Signatera™)



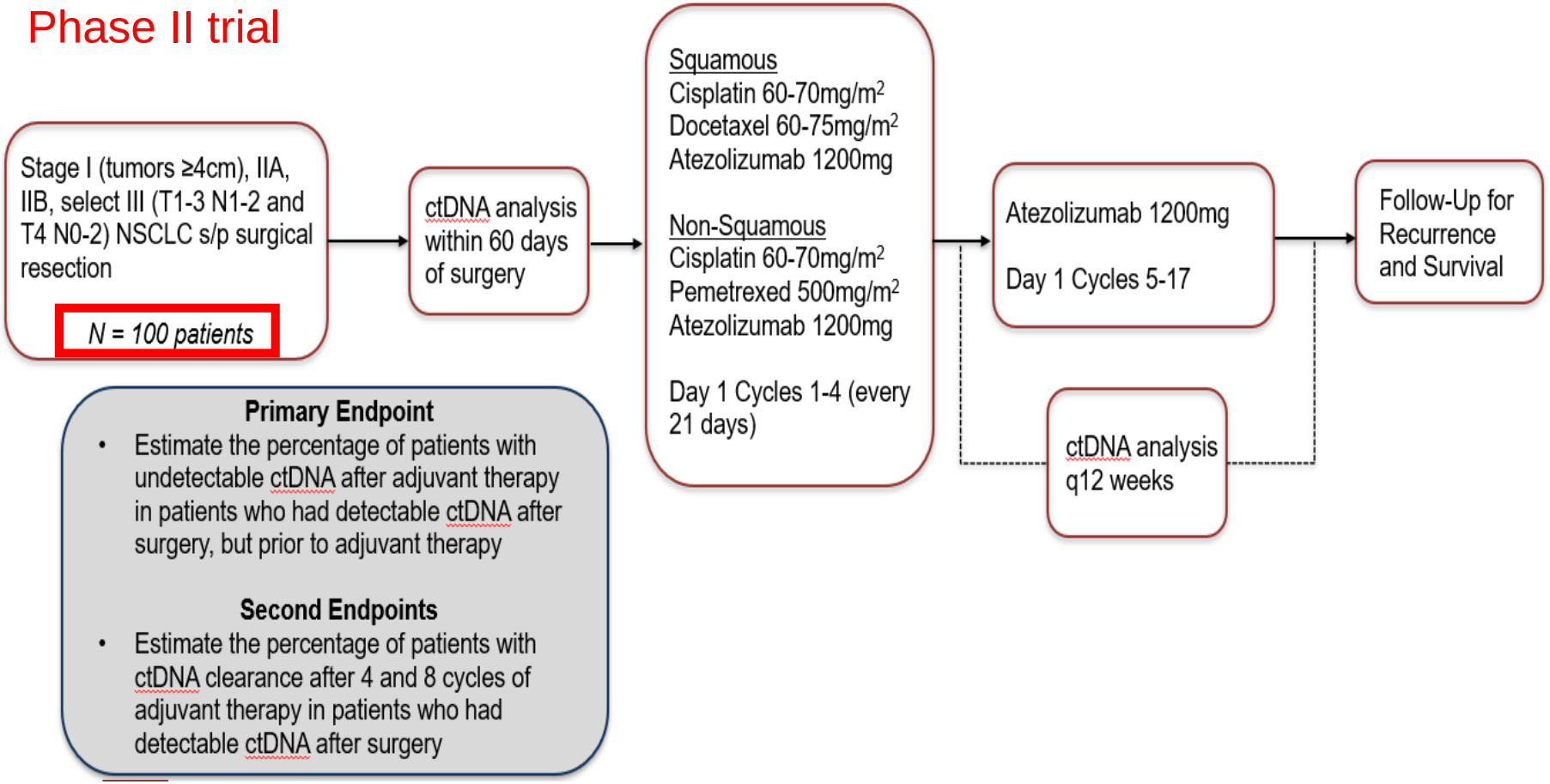


Powles T et al. *Nature*. 2021

Prospective ctDNA MRD trial for patients with resectable NSCLC stages I-III

BTCRC LUN19-396

Phase II trial

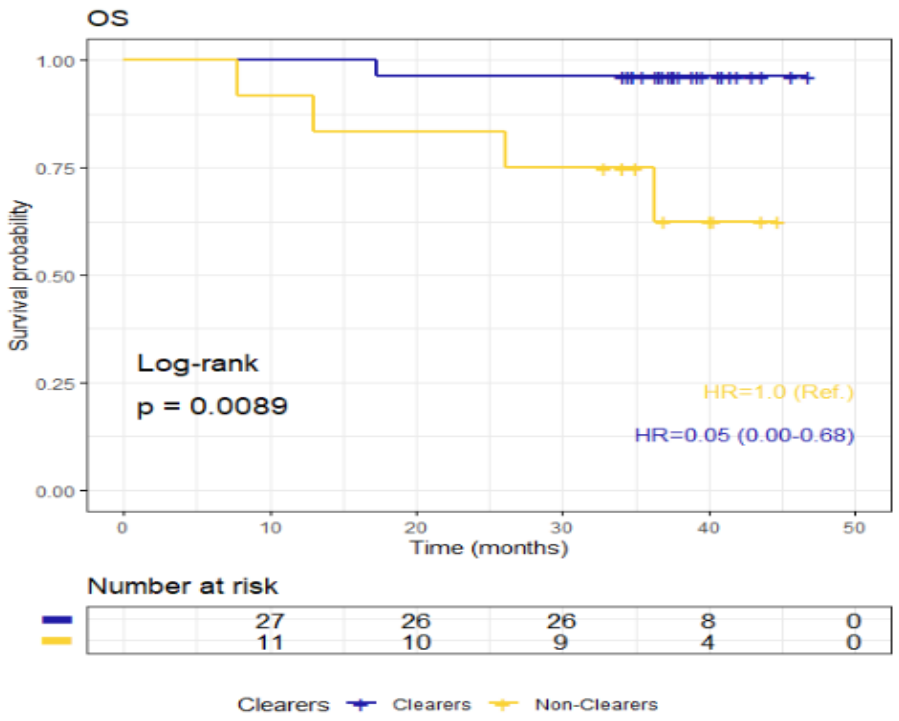


PI: Nasser Hanna; NCT04367311

Slide *adapted from* G. Durm at 2023 Hawaii Lung Cancer Summit.

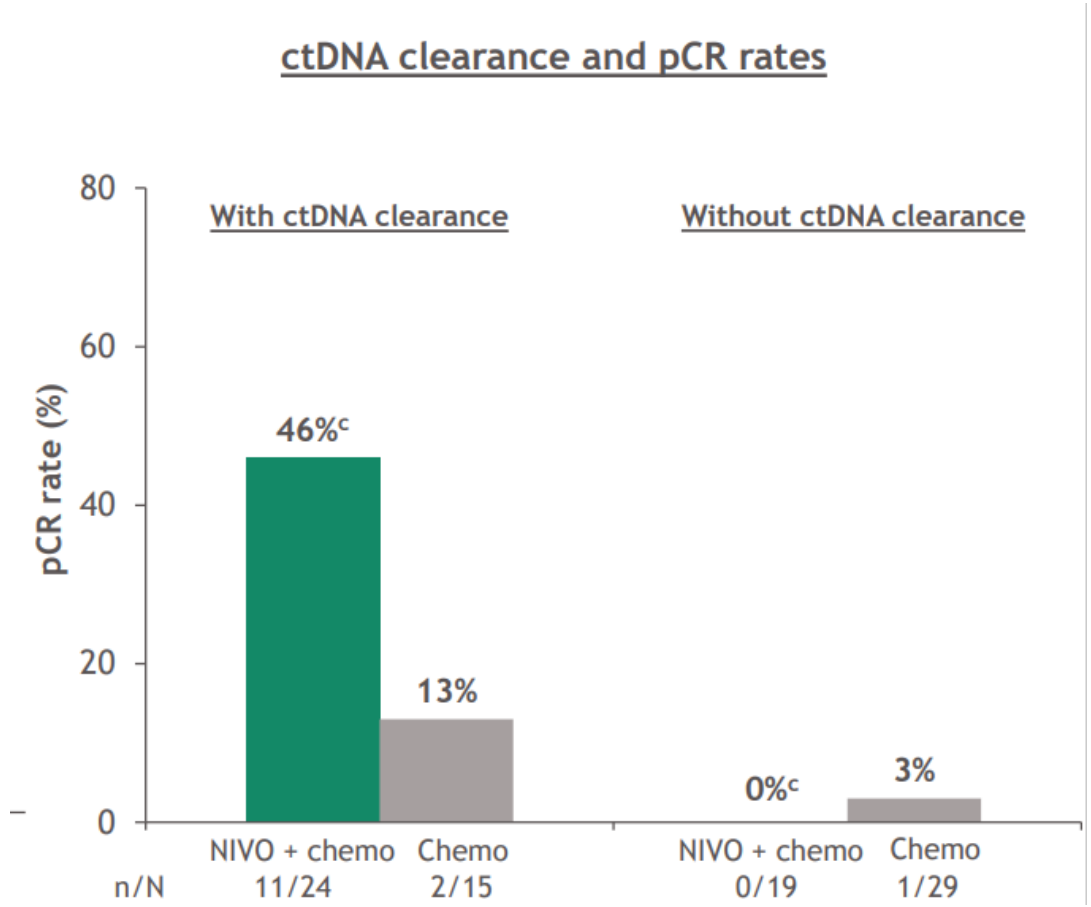
ctDNA clearance after neoadjuvant chemoIO correlates with clinical outcomes

NADIM



ctDNA clearance at the end of neoadjuvant treatment was associated with improved OS

CM816



Romero A et al. *J Thorac Oncol*. 2021:OA20.02

Forde P et al. *Cancer Res*. 2021: CT003

Take home points

- ctDNA can be used for molecular profiling in patients with advanced solid tumors to guide therapeutic decisions
- ctDNA can identify patients with advanced NSCLC who are responding to therapy (molecular response) at an early timepoint
- ctDNA can detect MRD and it is a strong prognostic biomarker
- Ongoing trials will inform if clinical decision-making can be guided by ctDNA and if that improves patients' outcomes

Thank you!

www.moffitt.org

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