Liquid Biopsies to Guide Targeted Therapy

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MOFFITT CANCER CENTER

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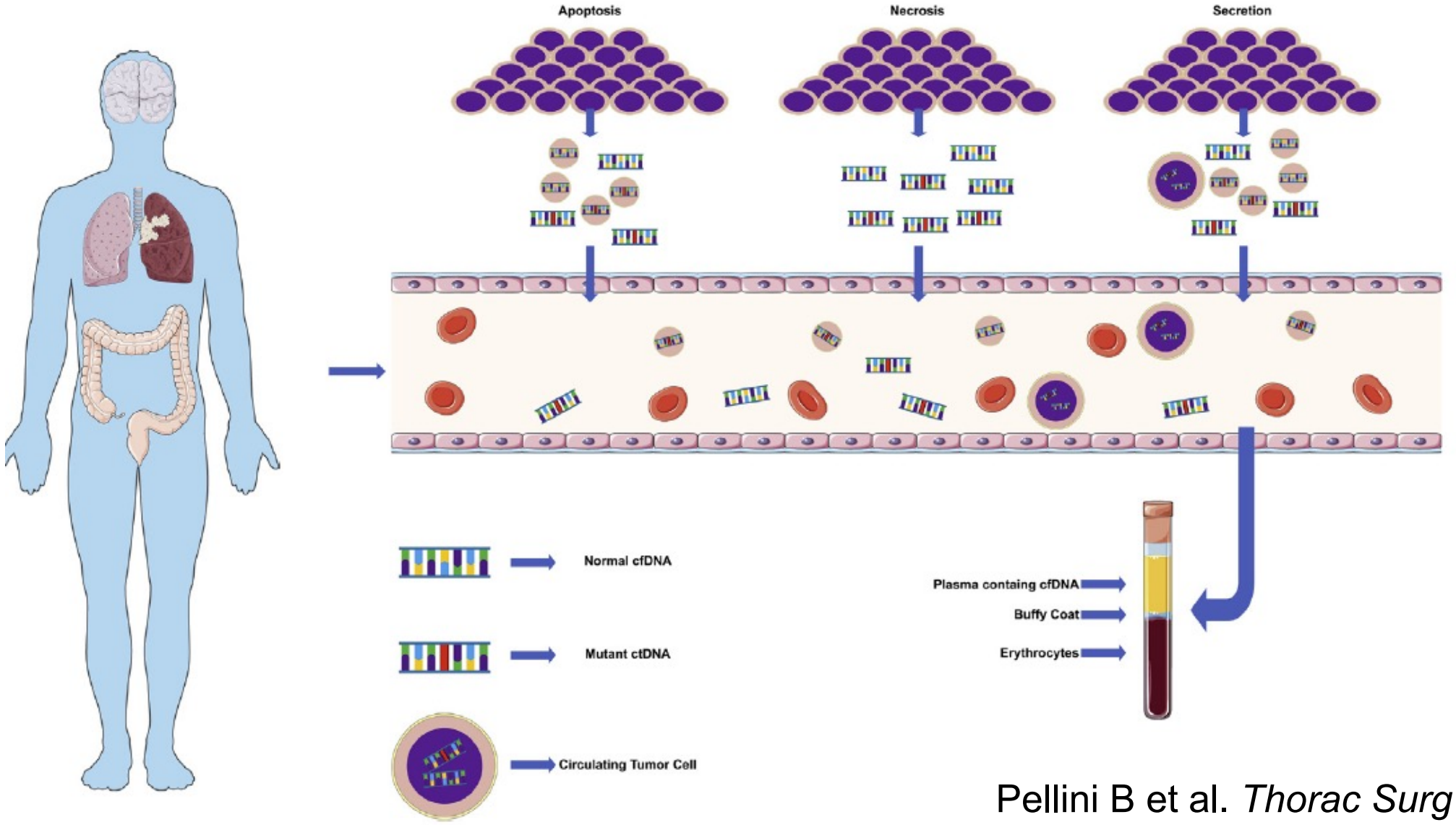
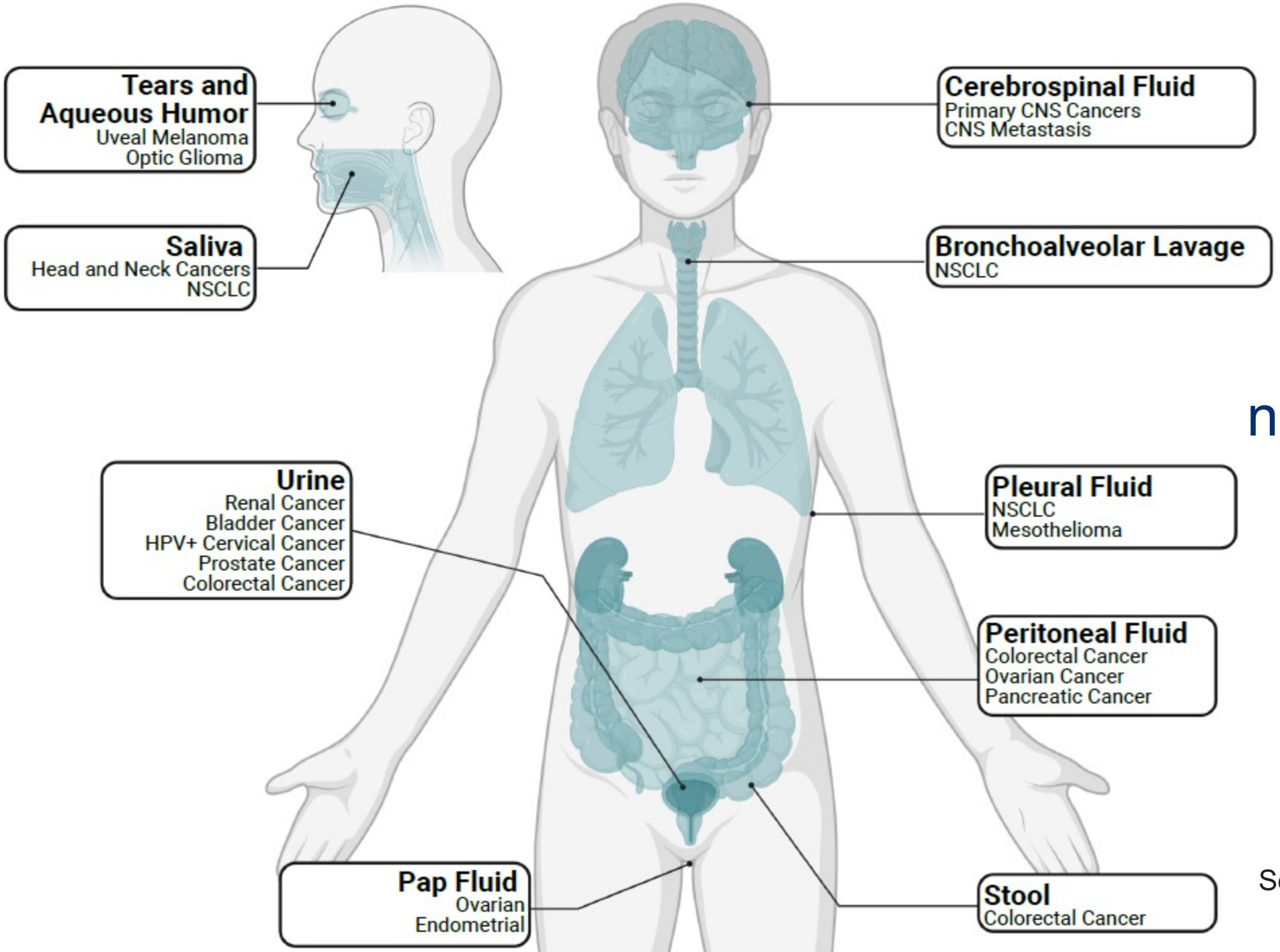
Outline

- ctDNA definition & sources of ctDNA
- Tumor-informed vs. tumor-naïve assays
- ctDNA applications in oncology:
  - Molecular profiling
  - Treatment Monitoring
  - Mechanisms of Resistance Detection
  - Future trial design discussion

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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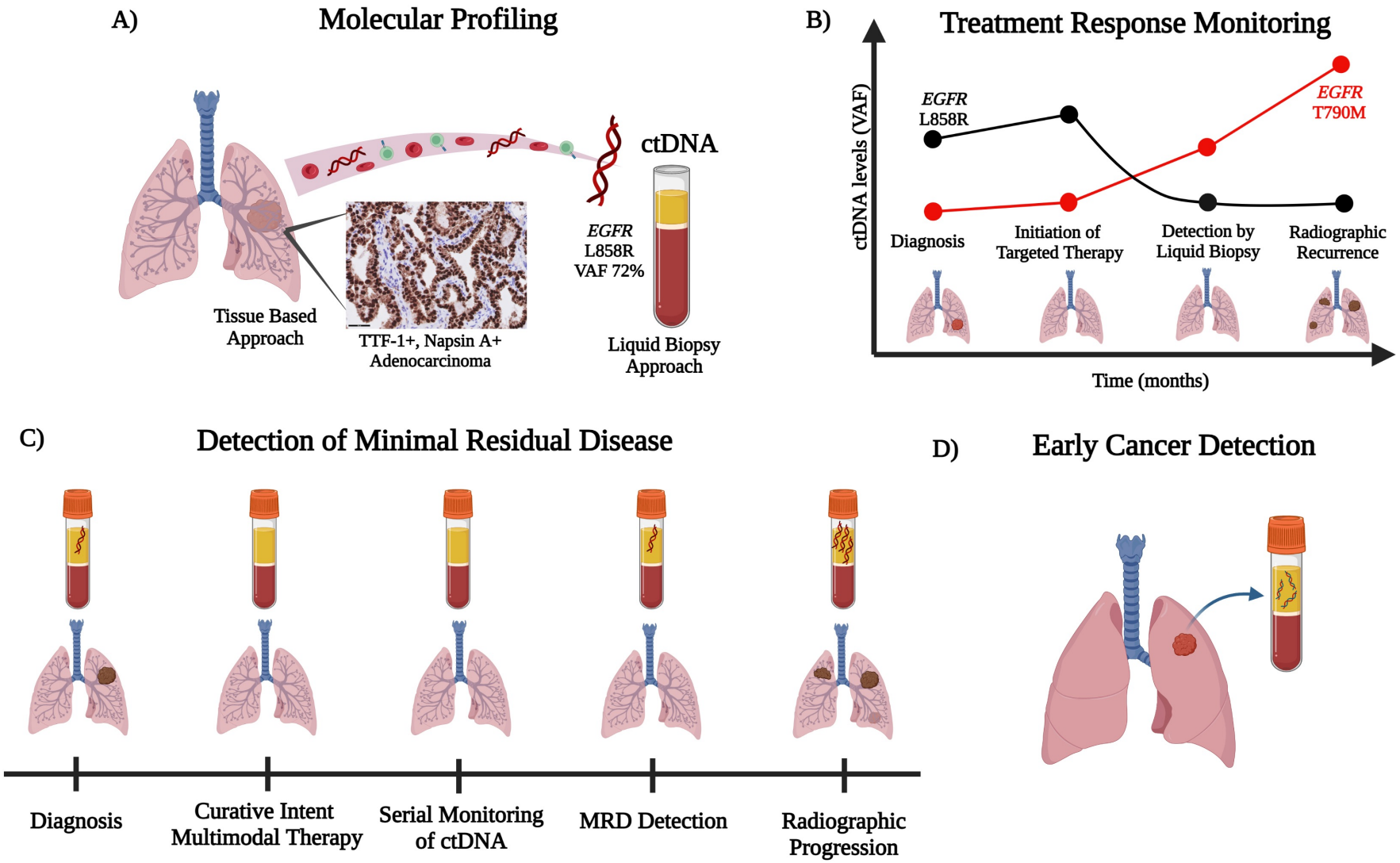
Tumor-informed vs. tumor-naïve assays

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

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%

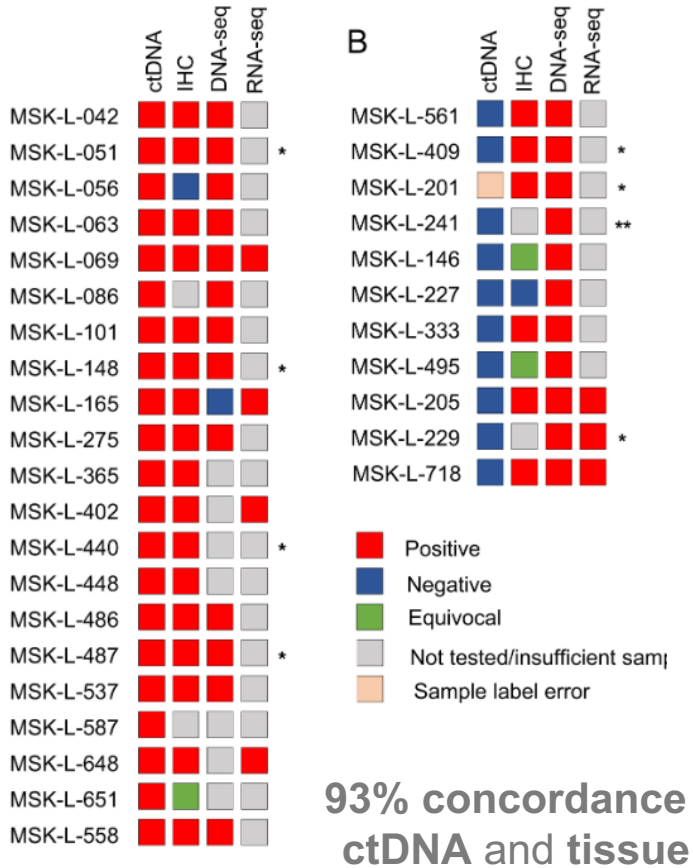
NOTE: Overall concordance across all four genes was greater than 98.2%, with a PPV of 100%. With continuous assay improvements, one cfDNA result originally reported as a false-negative for *ALK* fusion was identified as positive.

Stage IV NSCLC
Tumor-naïve assay
(Guardant 360)

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

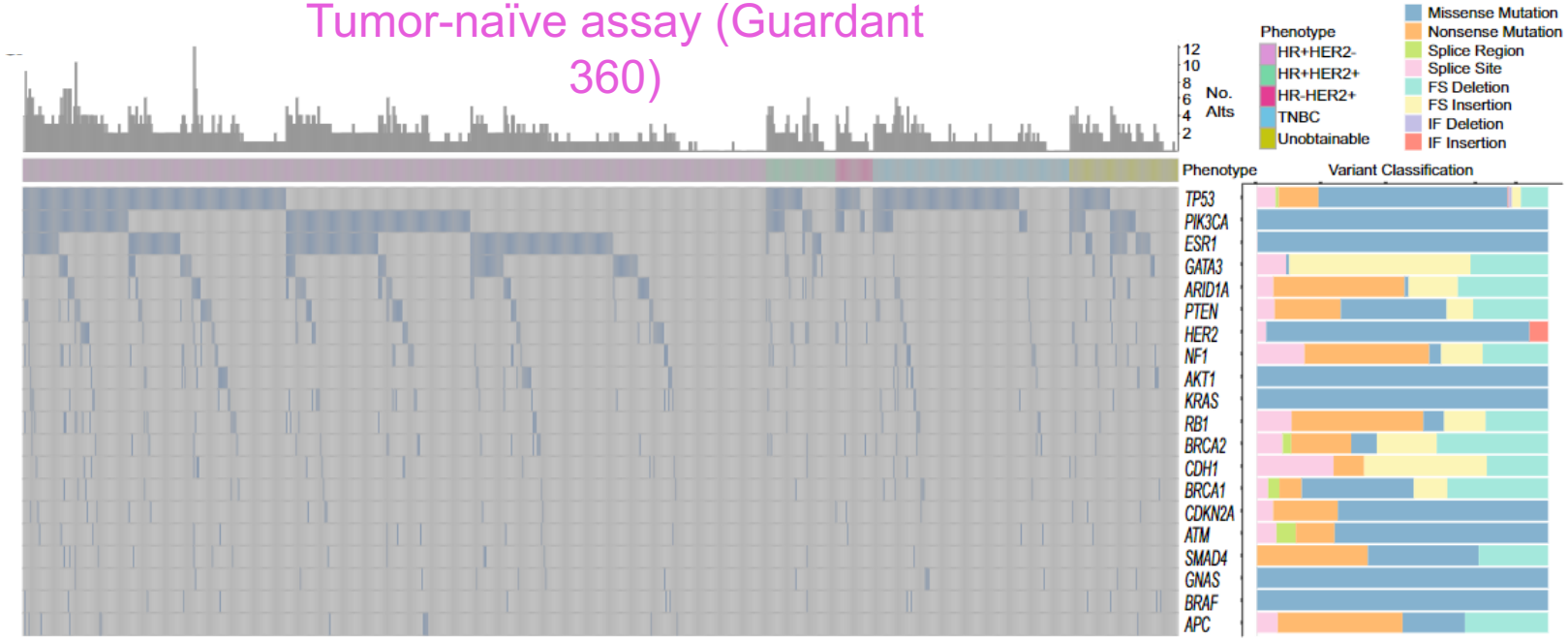
ctDNA sequencing has high sensitivity and specificity to identify actionable genomic alterations

Stage IV NSCLC
Tumor-naïve assay (Resolution Bioscience)



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

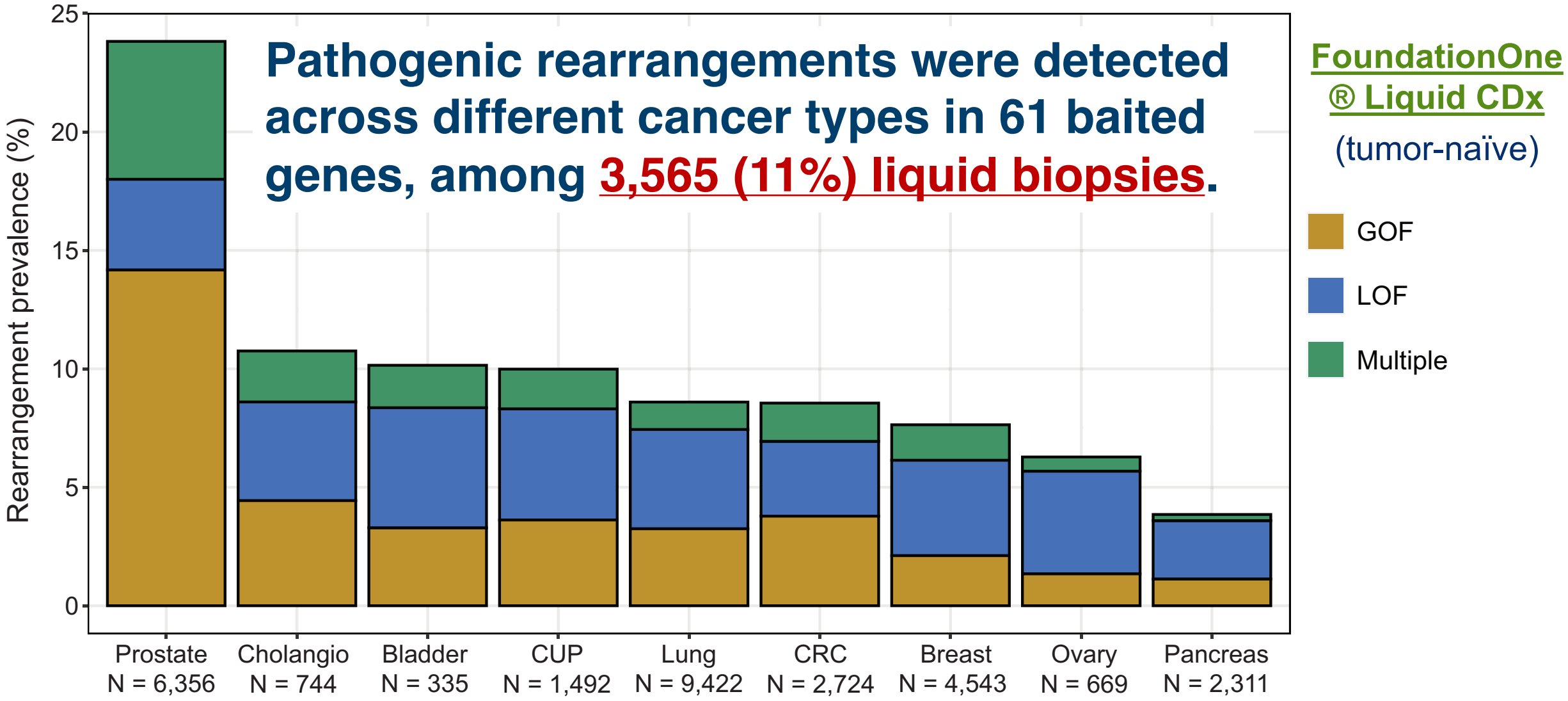
Advanced Breast Cancer
Tumor-naïve assay (Guardant 360)



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

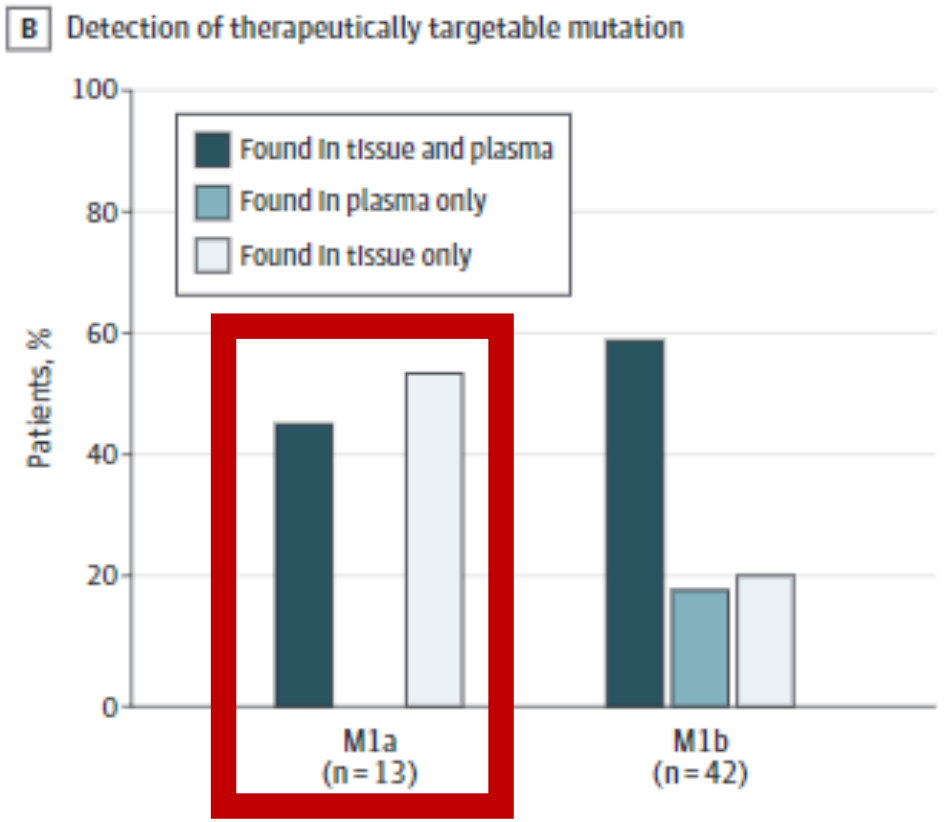
Mondaca S et al. *Lung Cancer*. 2021
Kingston B et al. *Nat Commun*. 2021

Gene rearrangements can be detected using ctDNA



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

Liquid biopsies may have false negative results, especially if metastasis are only present inside the thorax



M1a= metastasis in thorax
M1b= extra-thoracic metastasis

Aggarwal C et al. JAMA Oncol. 2019

Case 1

- 08/03/2022: 79-year-old, **never smoker** male, **left pleural effusion + consolidation on CT Chest**.
- Bronchoscopy and Thoracentesis: Adenocarcinoma of Lung Primary. Negative Brain MRI.
- **PD-L1** (IHC): Performed on pleural fluid = **TPS 70 %.**

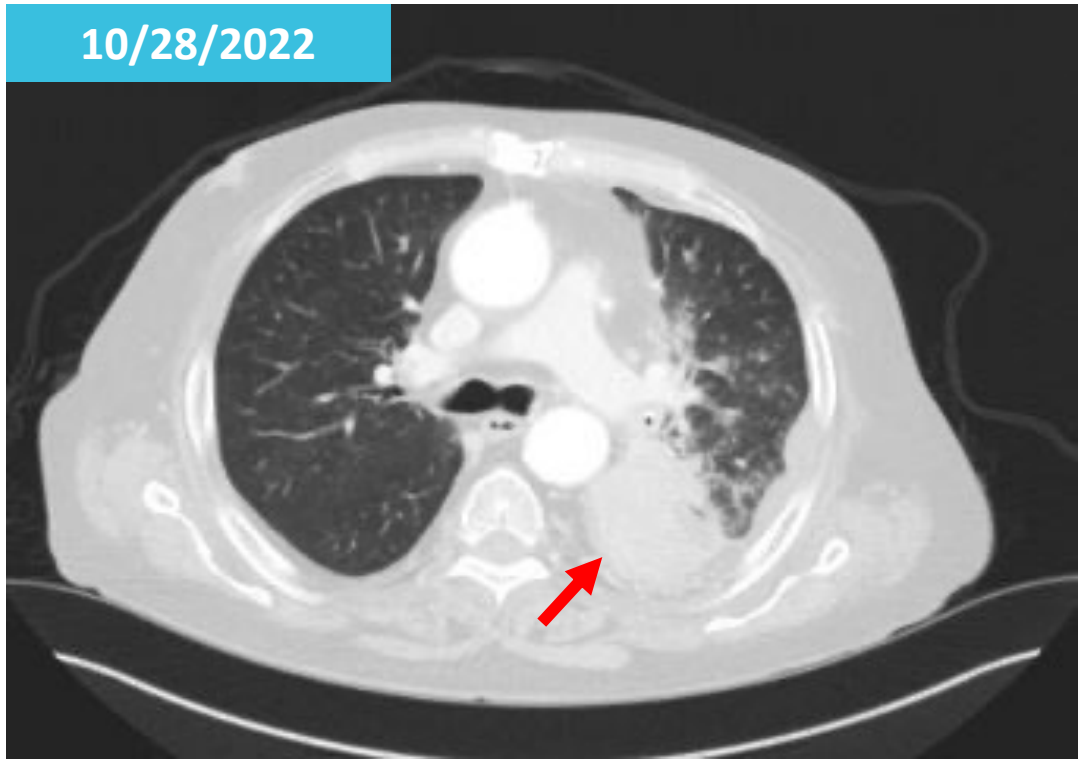
08/26/2022: **Liquid Biopsy = No actionable mutations** on the NGS panel.

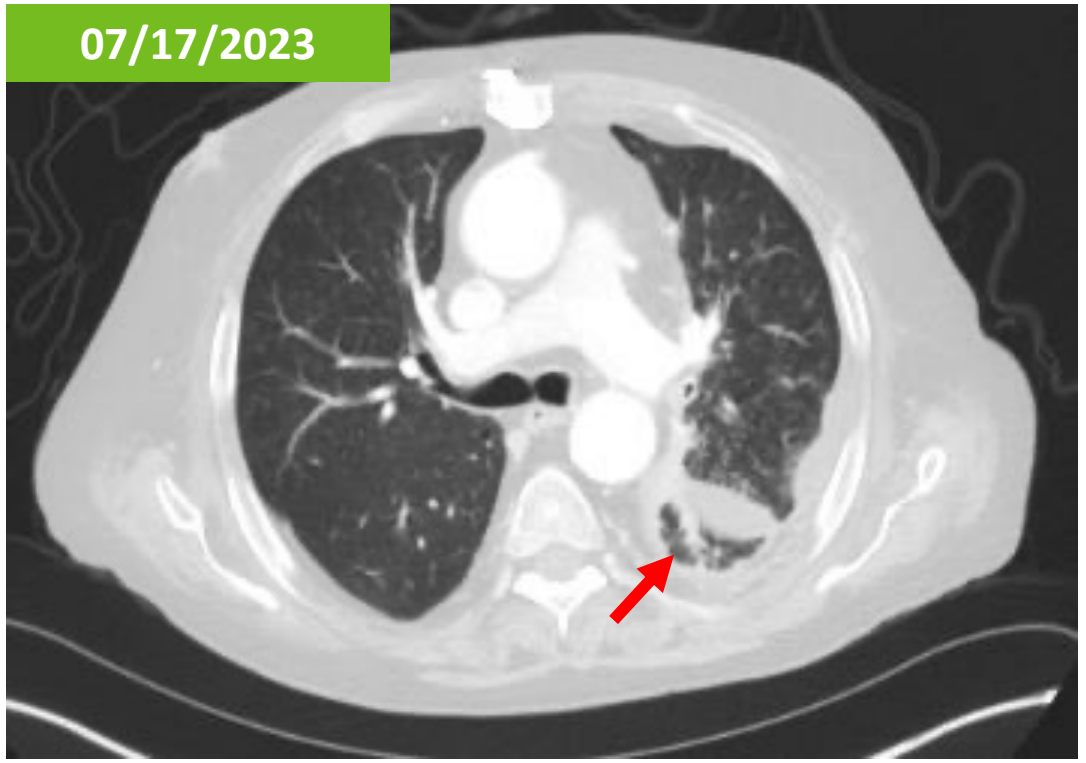
- **(08/23/2022 – 10/02/2022**): Patient treated with **single agent Pembrolizumab**
- **F/U CT TAP**: Increased lung mass + pleural effusion = **Disease progression.**
- **Patient referred to MCC for** second opinion/clinical trial consideration.

09/20/2022: **Pleural Fluid NGS** testing. **ERBB2/Her-2 Exon 20 insertion identified**

Case 1

- Patient was started on **Fam-trastuzumab deruxtecan on 11/10/2022**.
- **07/17/2023 - CT-TAP: Left lung scarring without** measurable tumor. **Improved left lower lobe aeration** with **decreased loculated effusion**. **No new metastases**.

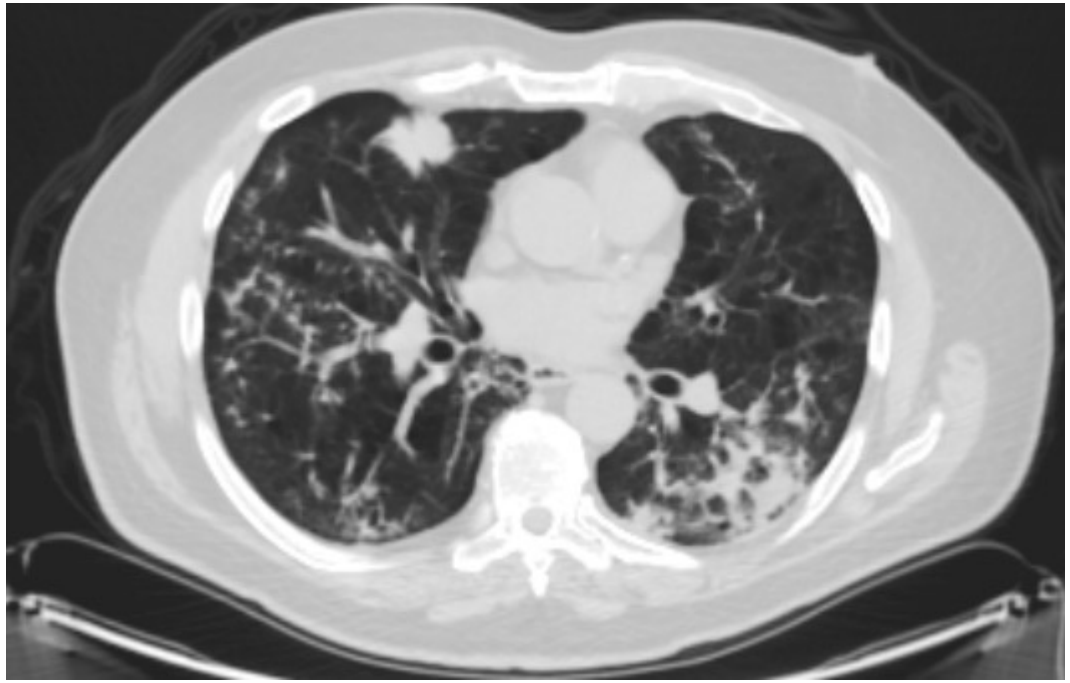




Case 2

- 71 yo man with a smoking history had a RUL nodule incidentally found during a CT A/P
- Diagnosed with adenocarcinoma of the lung in November/2023 with lung metastasis and lymphangitic spread

November/2023



Biomarker Findings

Blood Tumor Mutational Burden - 3 Muts/Mb
ctDNA Tumor Fraction - Low (< 1.0%)
Microsatellite status - MSI-High Not Detected

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix

DNMT3A E599fs*49
TET2 E330*

This assay tested >300 cancer-related genes, including the following 8 gene(s) routinely assessed in this tumor type: ***ALK, BRAF, EGFR, ERBB2, KRAS, MET, RET, ROS1.***

Report Highlights

- **Low ctDNA Tumor Fraction** was detected; in the absence of actionable driver alterations consider reflex testing to an FDA-approved tissue test, such as FoundationOne®CDx (p. 3)
- Variants that may represent **clonal hematopoiesis** and may originate from non-tumor sources: ***DNMT3A*** **E599fs*49** (p. 4), ***TET2*** **E330*** (p. 4)

NEW: To more easily navigate the content associated with patient results in an interactive format, physicians can access FoundationReport+ by visiting FMI-Portal.com

BIOMARKER FINDINGS	THERAPY AND CLINICAL TRIAL IMPLICATIONS
Blood Tumor Mutational Burden - 3 Muts/Mb	**No therapies or clinical trials.** See Biomarker Findings section
ctDNA Tumor Fraction - Low (< 1.0%)	**Low ctDNA Tumor Fraction. This result does not compromise confidence in any reported alterations. See Biomarker Finding Summary.**
Microsatellite status - MSI-High Not Detected	**MSI-High not detected. No evidence of microsatellite instability in this sample (see Appendix section).**

Case 2

- Started treatment with carboplatin and pemetrexed while awaiting tissue NGS
- NGS shows KRAS G12C mutation with co-mutations in STK11, KEAP1, TP53
- Pembrolizumab added with cycle 2 of treatment; scan after 2 cycles shows PR

Moffitt STAR

Molecular Report Summary

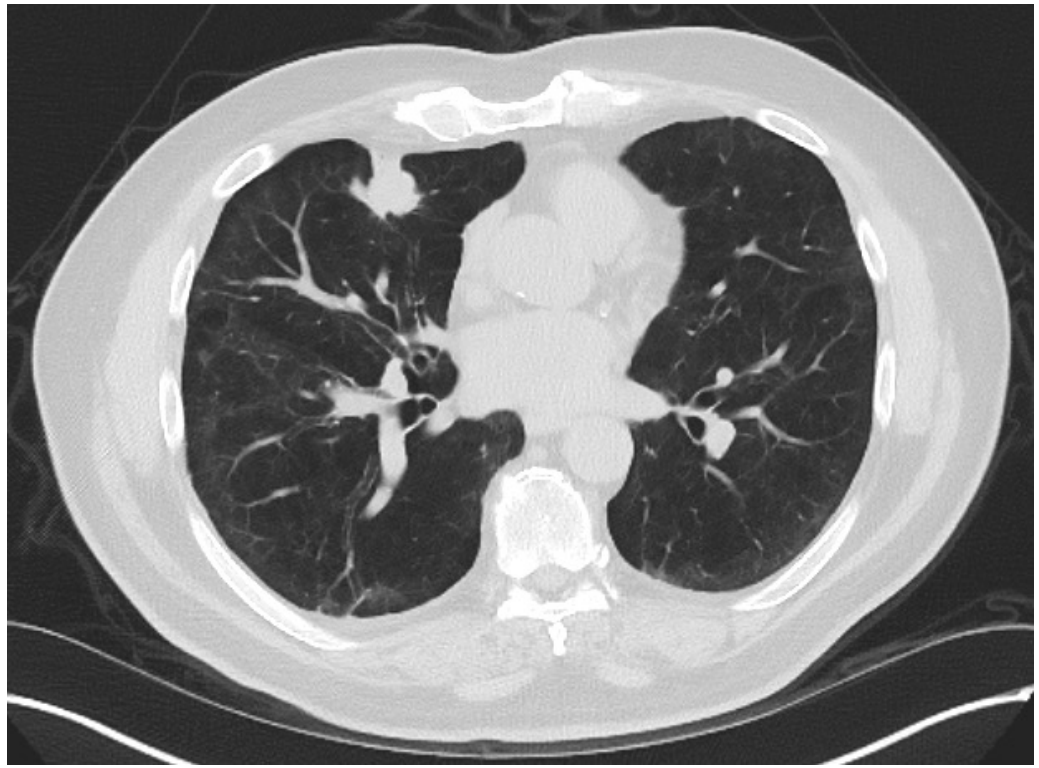
Genomic Alterations with Clinical Significance (see below for interpretation)

GENE	VARIANT	DNA CHANGE	FREQ	READS	CHR	POSITION
KRAS	p.G12C	c.34G>T	6.9%	568	12	25398285
TP53	p.Q165*	c.493C>T	1.1%	2000	17	7578437
CDKN2A	p.D84Y	c.250G>T	6.2%	1090	9	21971108
KEAP1	p.E343*	c.1027G>T	6.6%	1210	19	10602551
STK11	p.Y36_Q37delinsC*	c.107_109delinsGCT	5.1%	1695	19	1207019_1207021

Other Biomarkers

BIOMARKER	LEVEL	VALUE
TMB	High	14.1 muts/Mb
MSI	MS-Stable	1.6% Unstable Sites

January/2024



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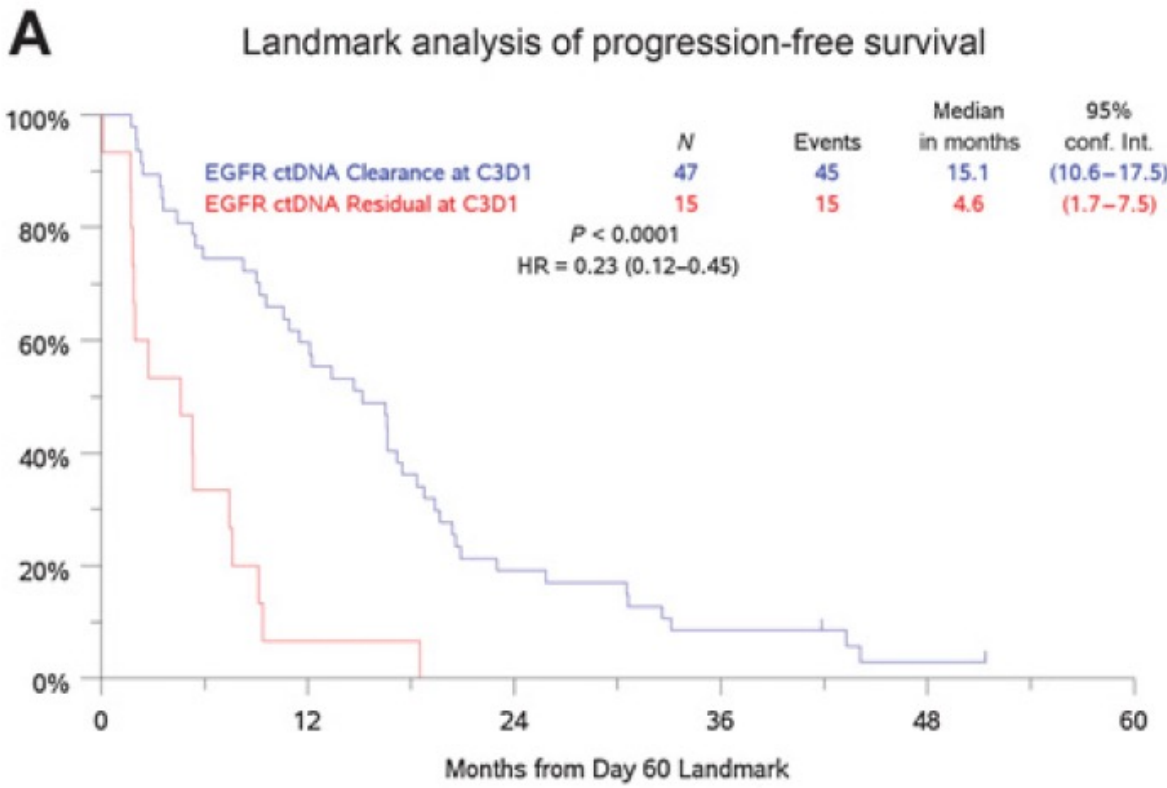
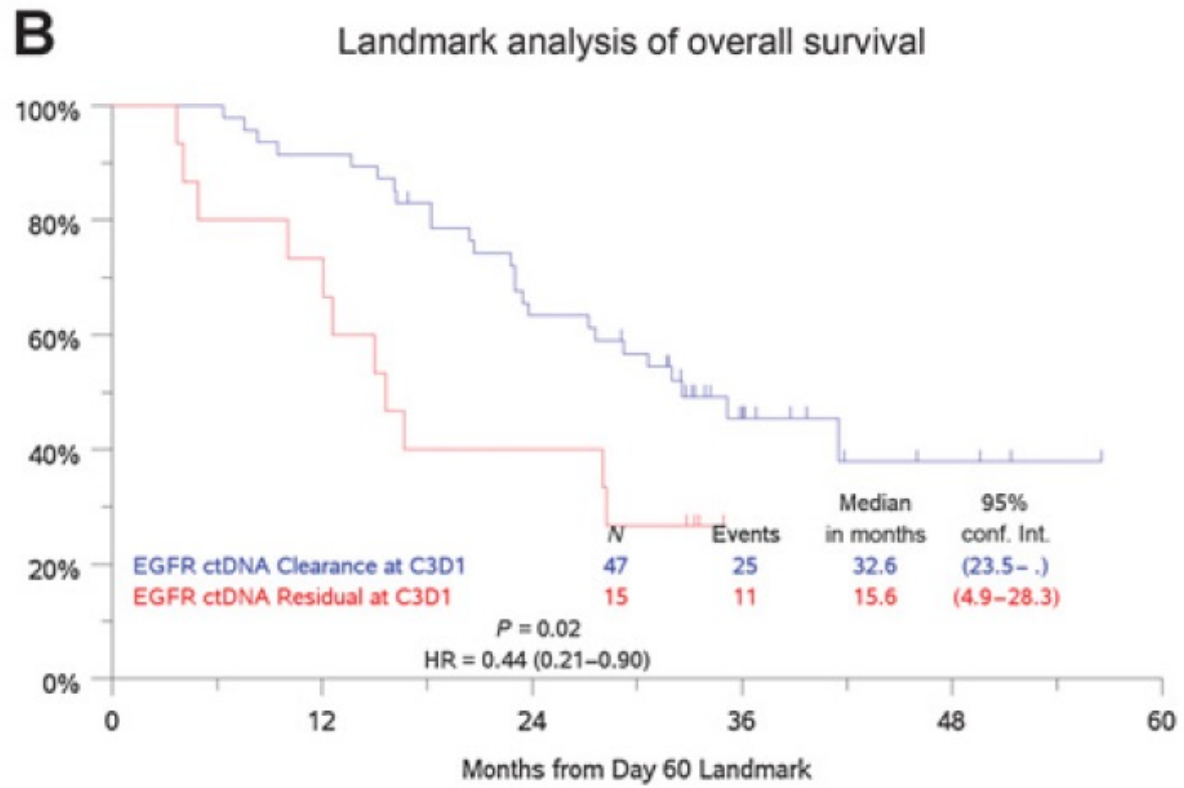
Patients with undetectable *EGFR* 8 weeks after treatment start had better PFS and OS

Stage IV NSCLC
Tumor-naïve assay
(Guardant 360)

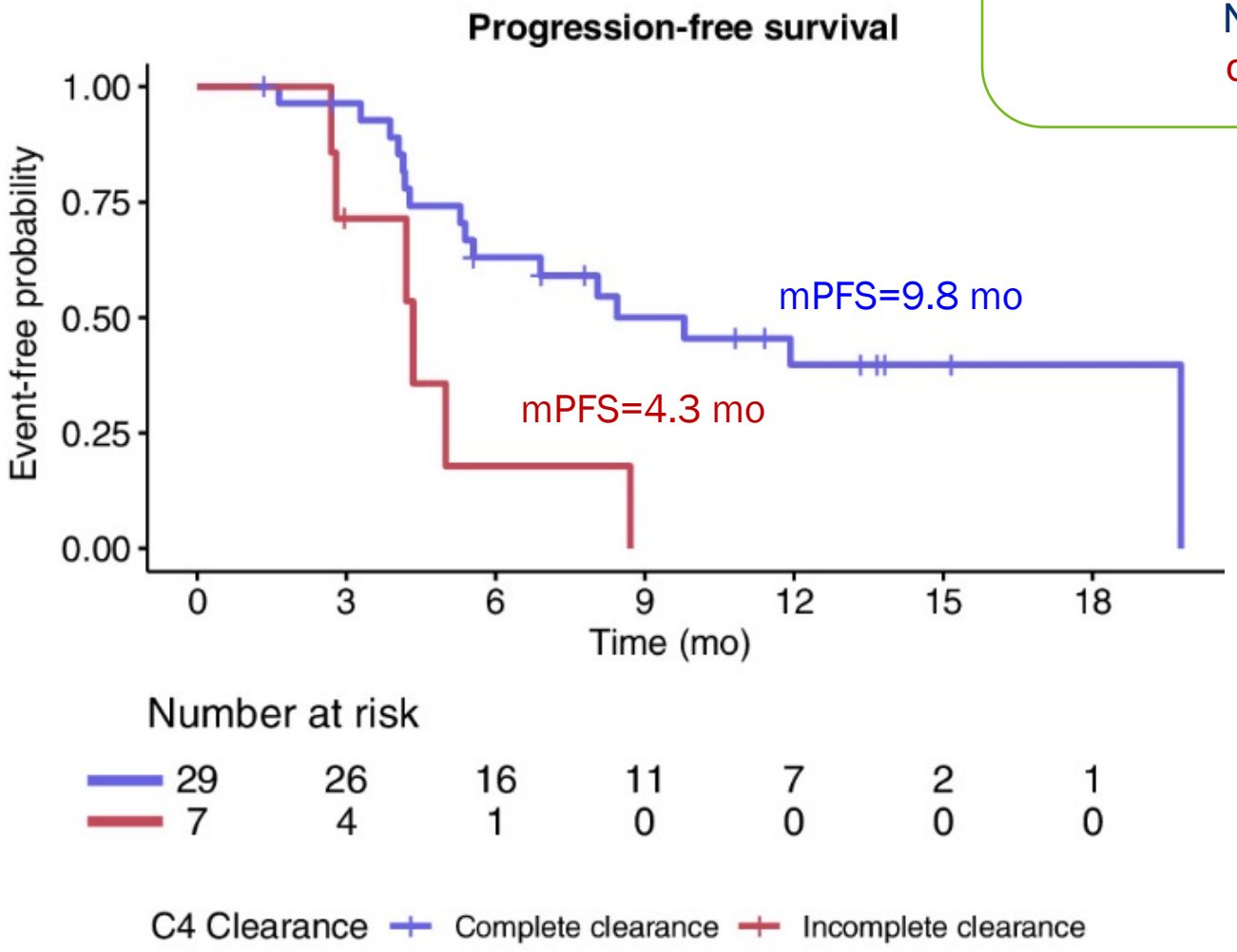


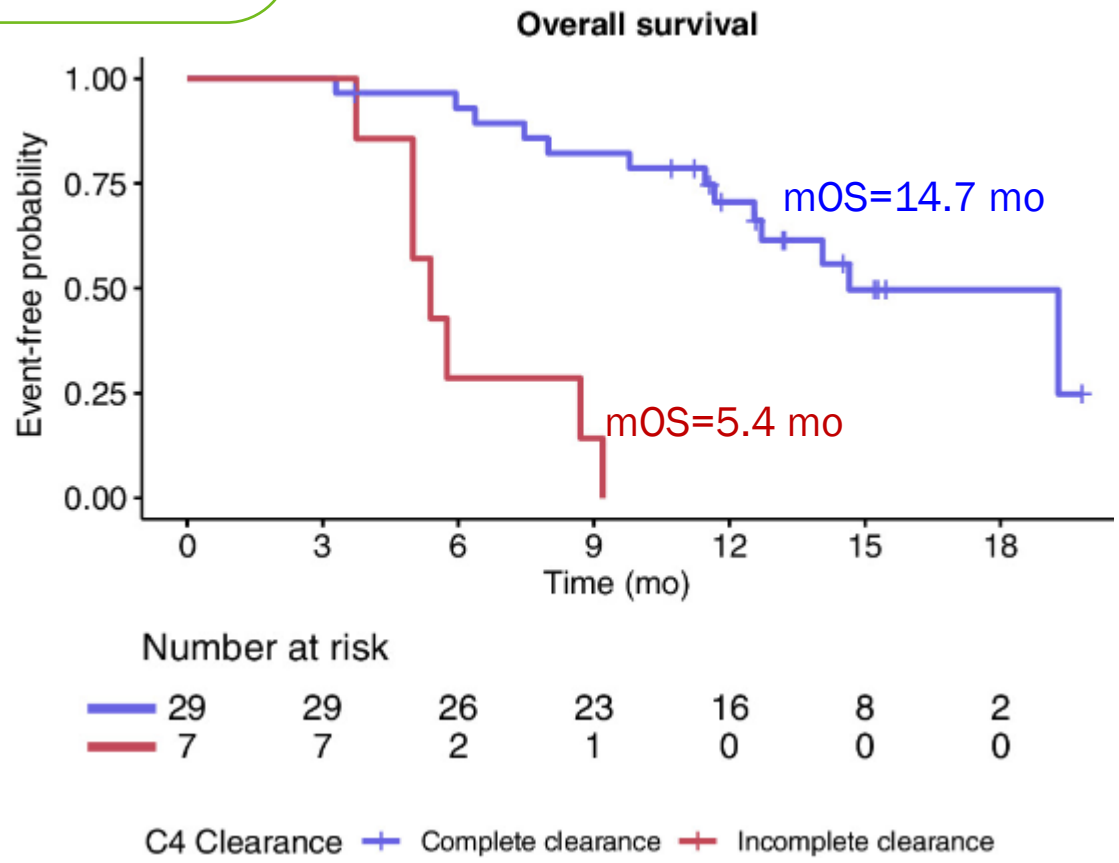


Mack PC et al. *Clin Cancer Res*. 2022

Complete ctDNA clearance at cycle 4 is associated with an improved PFS and OS

Stage IV KRAS G12C-mutant NSCLC
ddPCR



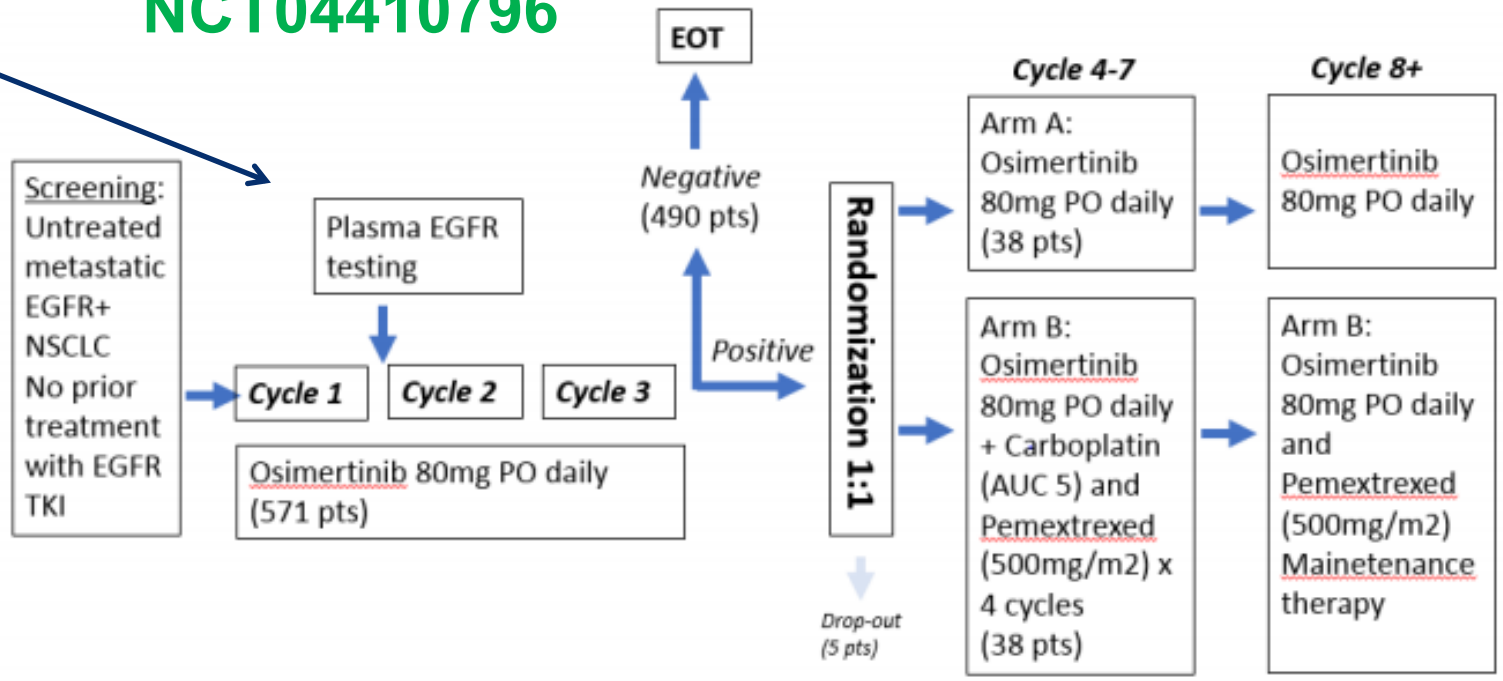


Paweletz CP et . *Clin Cancer Res*.2023

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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ctDNA can also be used to detect acquired mechanisms of resistance to targeted therapy

NSCLC (n = 67)*
23-gene Resolution Bioscience ctDx Lung test†

- With baseline tissue sample (n = 44; 66%)

CRC (n = 45)*
74-gene Guardant 360 ctDNA test†

- With baseline tissue sample (n = 32; 71%)

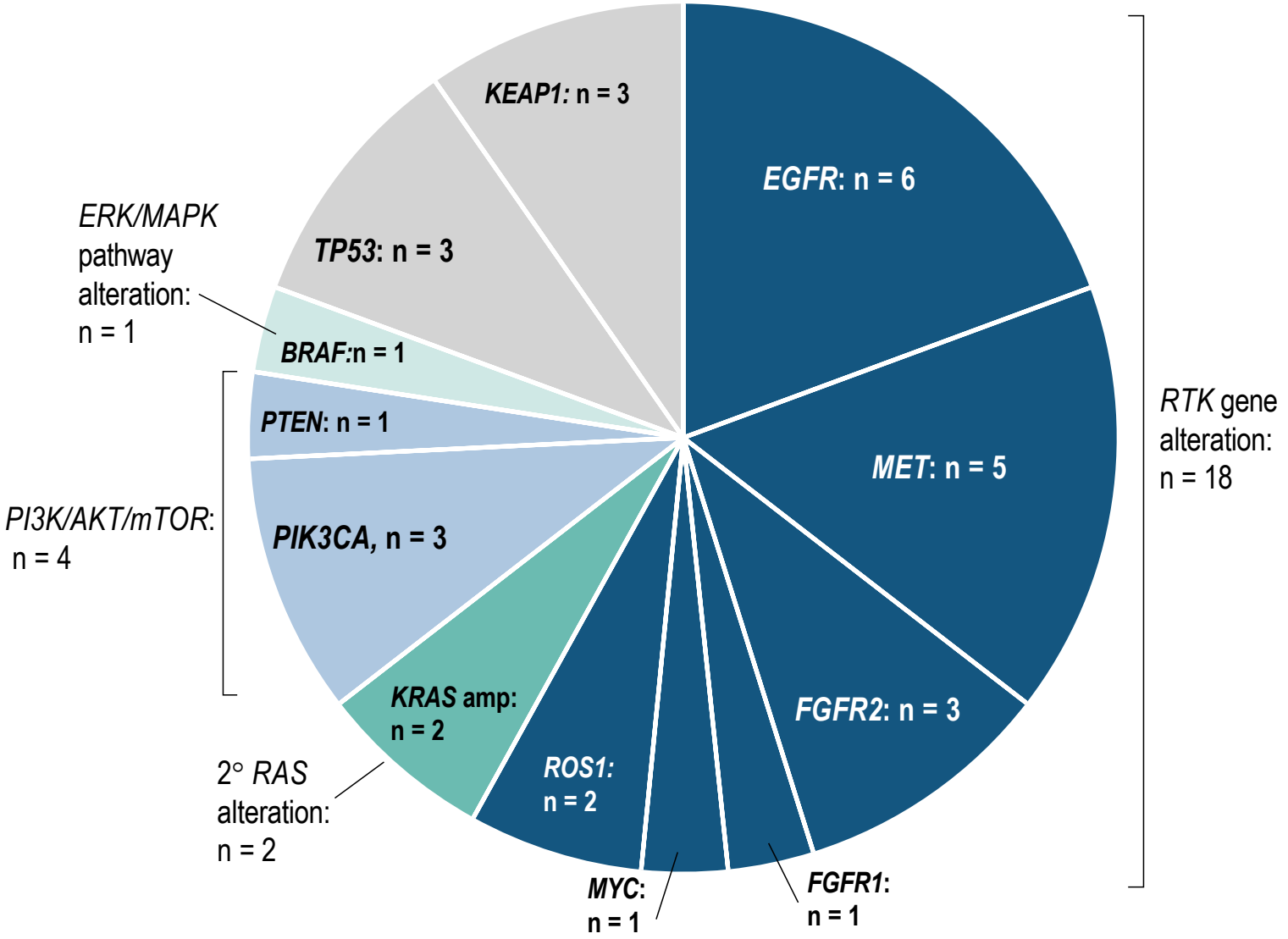
Analysis set

- **Acquired genomic alterations identified at disease progression**
  - **Absent at baseline (in plasma and tissue‡)**
  - **Present at progression**

Presented by Li BT et al. 2022 ASCO Annual Meeting.

Acquired Genomic Alterations Observed in 28% of Patients

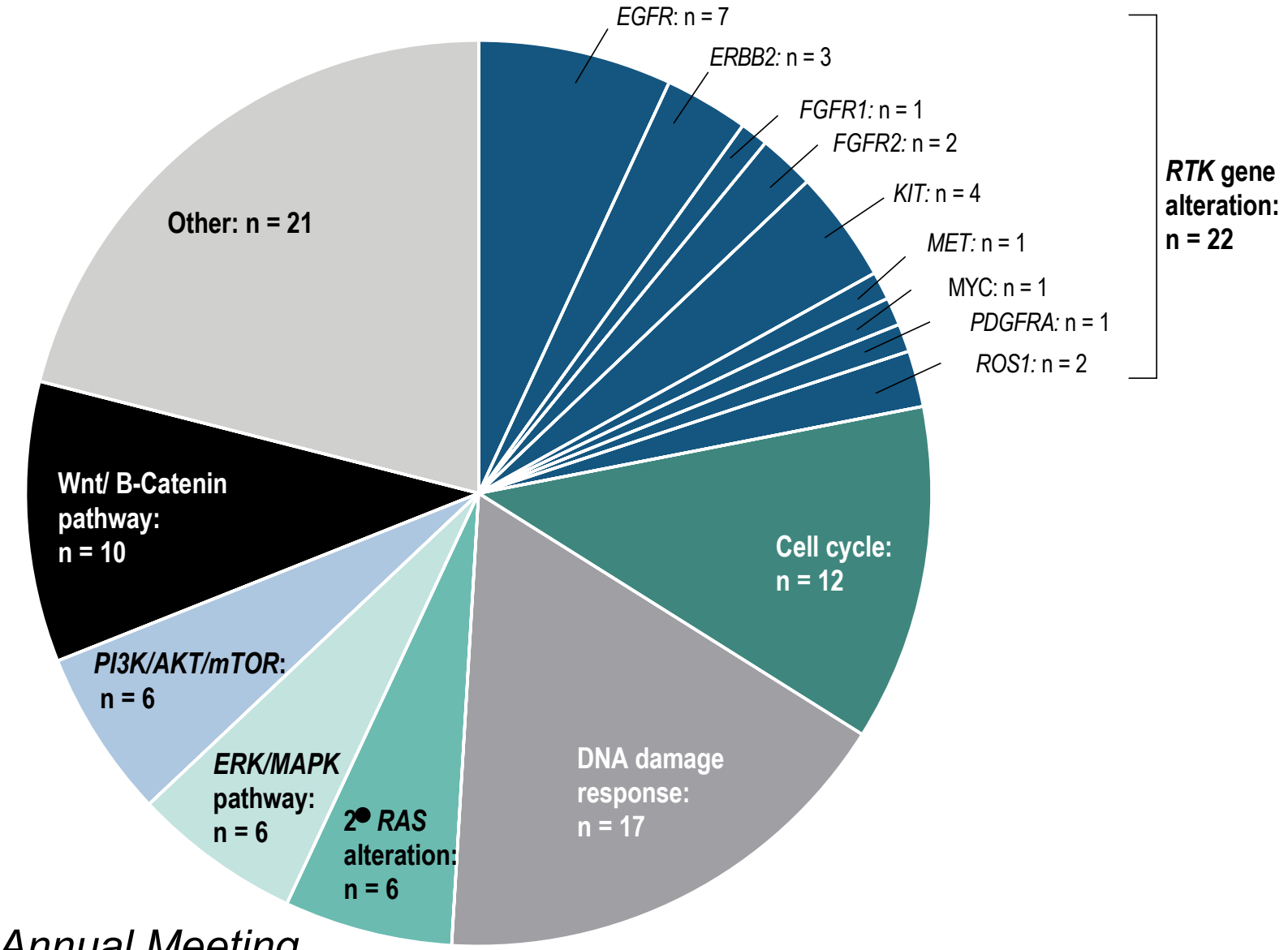
In total, 31 acquired alterations were detected in 19 patients with NSCLC



Presented by Li BT et al. 2022 ASCO Annual Meeting.

Acquired Genomic Alterations Observed in 71% of Patients

In total 100 acquired alterations were detected in 32 patients with CRC



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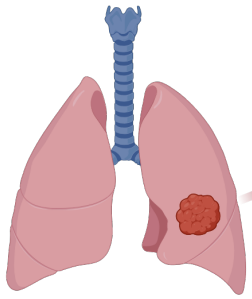
Proposed adaptive clinical trial design for EGFRm NSCLC



Resectable *EGFR*m NSCLC

Definitive Treatment
Surgery

Molecular Profiling and *EGFR* mutation detection

Adjuvant Chemotherapy
(if indicated)

MRD not detected

MRD detected

Adjuvant Osimertinib

MRD persistently not detected → Shorter duration of adjuvant osimertinib (1 year)

MRD not detected → Complete 3 years of adjuvant osimertinib

MRD persistently detected → Escalate adjuvant treatment (osimertinib +amivantamab/ ramucirumab/ chemotherapy)

Metastatic *EGFR*m NSCLC

Molecular Profiling and *EGFR* mutation detection

Standard Treatment
Osimertinib

Molecular Response Monitoring

ctDNA (-) Clearance → Continue osimertinib monotherapy

ctDNA (+) Persistence → **Treatment escalation** Chemotherapy/ Amivantamab/ Ramucirumab

Disease Progression

Detection of mechanisms of resistance

Figure 1

Desai A, …Pellini B. *Under review*.

Take home points

- ctDNA can be used for molecular profiling in patients with advanced solid tumors to guide therapeutic decisions
- Liquid biopsy false negative rates are higher when patients with NSCLC only have intra-thoracic metastasis (contralateral lung, pleura)
- Treatment can be started if an oncogenic driver alteration is identified on a liquid biopsy
- ctDNA can identify patients with advanced NSCLC who are responding to therapy (molecular response) at an early timepoint
- Liquid biopsies can be used to identify mechanisms of resistance to targeted therapy
- 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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