

# III JORNADA TRASLACIONAL DE ONCOLOGÍA DE PRECISIÓN:

A TRAVÉS DE LAS VÍAS DE SEÑALIZACIÓN  
SEVILLA, 12 Y 13 DE FEBRERO DE 2026

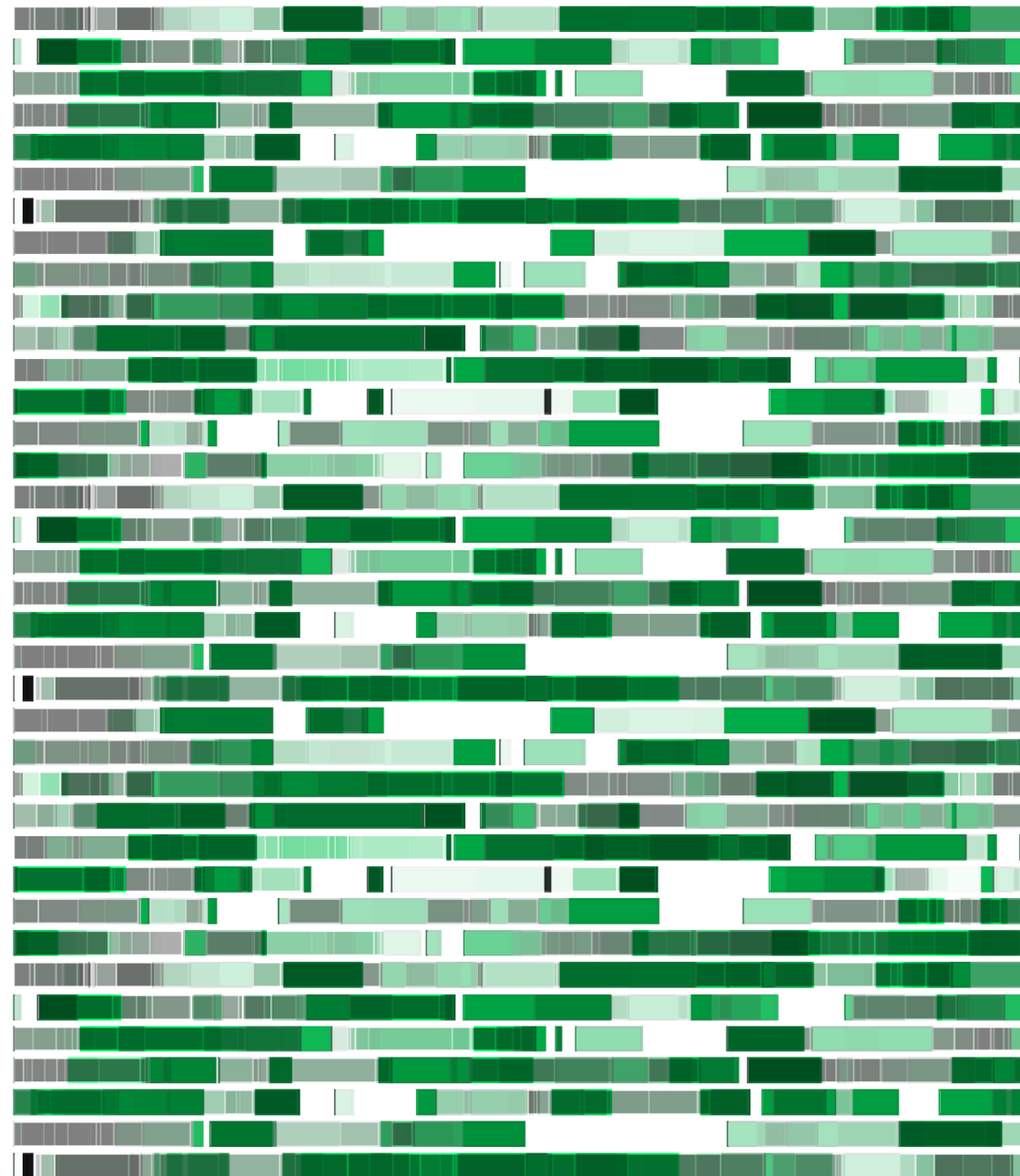
## TEST NO INVASIVO EN SANGRE PARA BIOPSIA LÍQUIDA

Atocha Romero

Hospital Universitario Puerta de Hierro

Organizador por:

**HENDERE HEALTHCARE**



III JORNADA TRASLACIONAL  
DE ONCOLOGÍA DE PRECISIÓN:

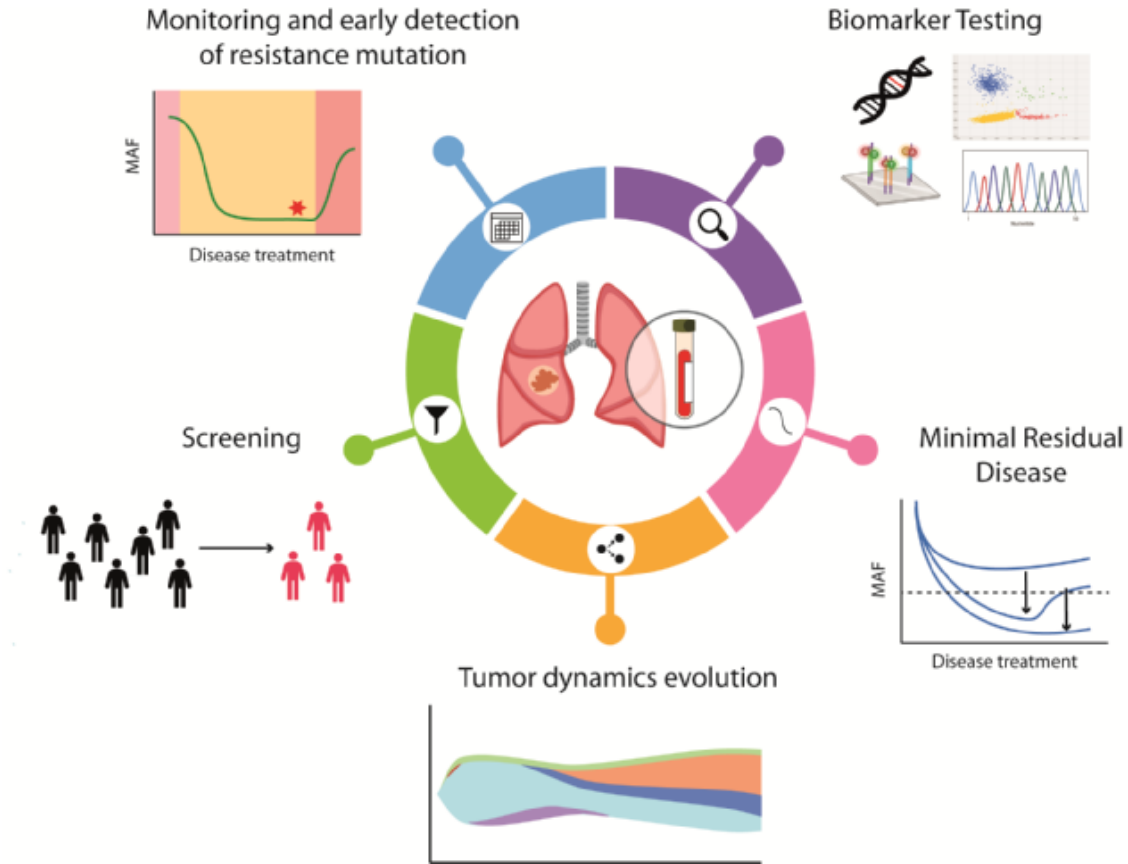
A TRAVÉS DE LAS VÍAS  
DE SEÑALIZACIÓN  
SEVILLA, 12 Y 13  
DE FEBRERO DE 2026



# TEST NO INVASIVO EN SANGRE PARA BIOPSIA LÍQUIDA



# CLINICAL UTILITY OF LIQUID BIOPSY



<b>T</b> Tumor Size	<b>N</b> Lymph Node	<b>M</b> Metastasis	<b>B</b> Blood
<b>T1</b> Tumor size/local invasion	<b>N0</b> Local nodes Distant nodes No regional lymph node invasion	<b>M0</b> No distant metastasis	<b>B0</b> ctDNA No ctDNA mutations in blood
<b>T2</b> Tumor size/local invasion	<b>N1</b> Local nodes Distant nodes Tumor spread to closest or small number of regional lymph nodes	<b>M1</b> Distant metastasis	<b>B1</b> ctDNA mutations in blood (can be further defined with more detailed quantification in the future)
<b>T3</b> Tumor size/local invasion	<b>N2</b> Local nodes Distant nodes Tumor spread to an extent between N1 and N3		
<b>T4</b> Tumor of any size that invades to other organs	<b>N3</b> Local nodes Distant nodes Tumor spread to more distant or regional numerous lymph nodes		



# NEOADJUVANT SETTING: BASELINE CTDNA IS OF PROGNOSTIC SIGNIFICANCE

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

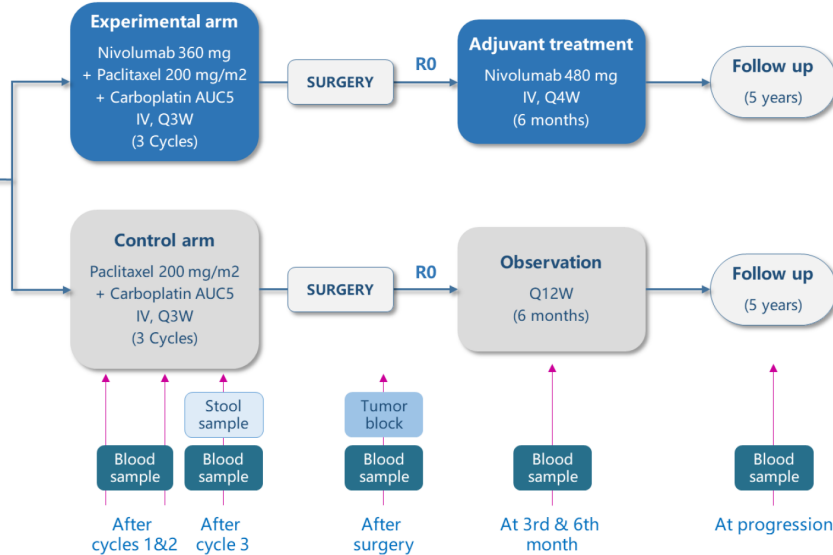
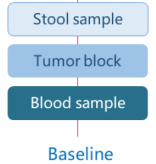
## Perioperative Nivolumab and Chemotherapy in Stage III Non-Small-Cell Lung Cancer

M. Provencio, E. Nadal, J.L. González-Larriba, A. Martínez-Martí, R. Bernabé, J. Bosch-Barrera, J. Casal-Rubio, V. Calvo, A. Insa, S. Ponce, N. Reguart, J. de Castro, J. Mosquera, M. Cobo, A. Aguilar, G. López Vivanco, C. Camps, R. López-Castro, T. Morán, I. Barneto, D. Rodríguez-Abreu, R. Serna-Blasco, R. Benítez, C. Aguado de la Rosa, R. Palmero, F. Hernando-Trancho, J. Martín-López, A. Cruz-Bermúdez, B. Massuti, and A. Romero

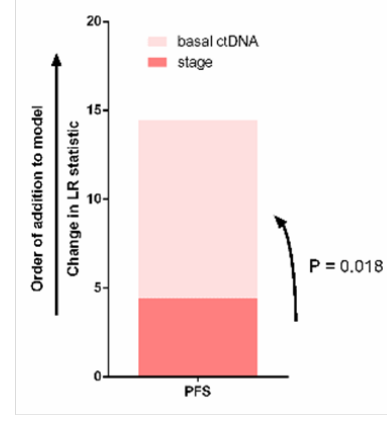
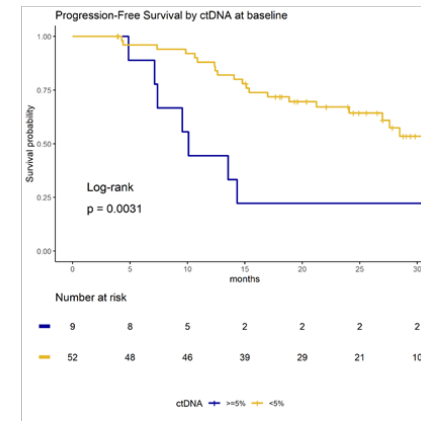
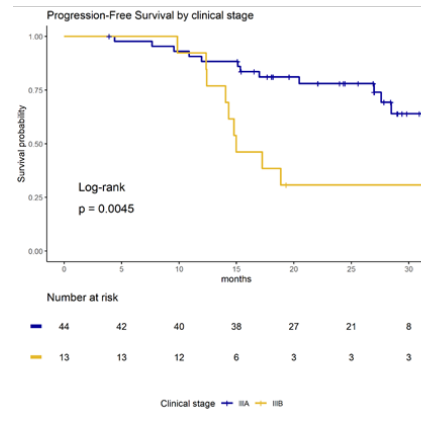
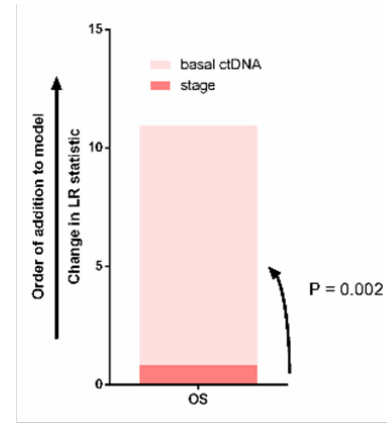
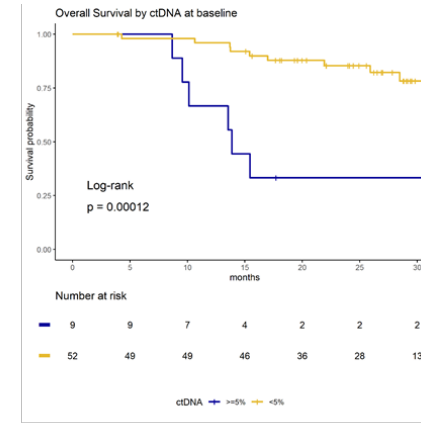
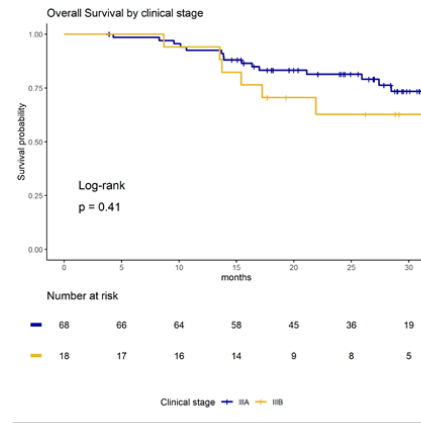


**NSCLC**  
Locally advanced  
Potentially resectable  
Stage IIIA-IIIIB  
(8<sup>th</sup> edition)  
EGFR/ALK excluded

Translational research

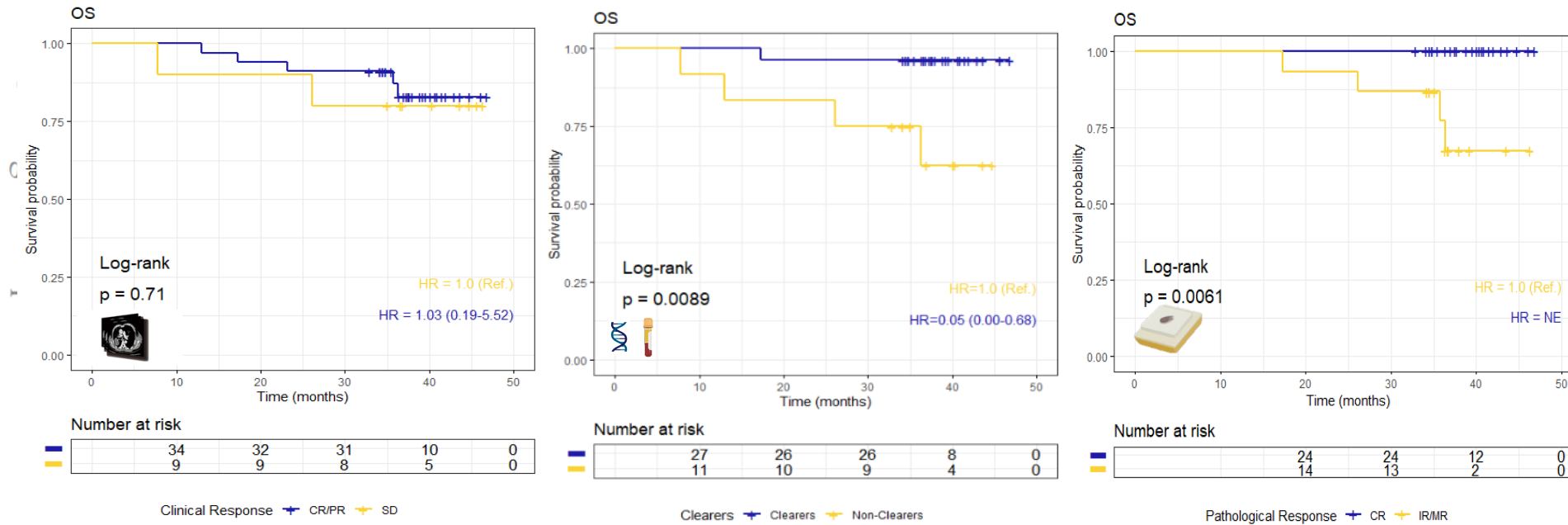


NADIM II (NCT03838159)





# CTDNA CLEARANCE AFTER NEOADJUVANT TREATMENT IS OF PROGNOSTIC SIGNIFICANCE



Survival surrogate	No.	HR (PFS) <sup>a</sup>	95% CI <sup>a</sup>	P <sup>a</sup>	C-index (PFS)	95% CI	HR (OS) <sup>a</sup>	95% CI <sup>a</sup>	P <sup>a</sup>	C-index (OS)	95% CI
Clinical response (CR plus PR v SD)	46	0.79	0.24 to 2.59	.698	0.62	0.47 to 0.77	0.87	0.20 to 3.75	.848	0.72	0.51 to 0.90
Pathologic response (pCR v major plus incomplete)	41	0.38	0.12 to 1.25	.111	0.63	0.47 to 0.78	0.24	0.04 to 1.33	.102	0.65	0.43 to 0.86
Undetectable ctDNA after treatment	40	0.26	0.07 to 0.93	.038	0.63	0.45 to 0.81	0.04	0.00 to 0.55	.015	0.82	0.61 to 1.00



# CTDNA CLEARANCE AFTER NEOADJUVANT TREATMENT IS OF PROGNOSTIC SIGNIFICANCE

nature communications



Article

<https://doi.org/10.1038/s41467-025-66097-w>

## Induction chemo-immunotherapy followed by chemo-radiotherapy and immunotherapy maintenance in stage III NSCLC (APOLO): a phase 2 trial

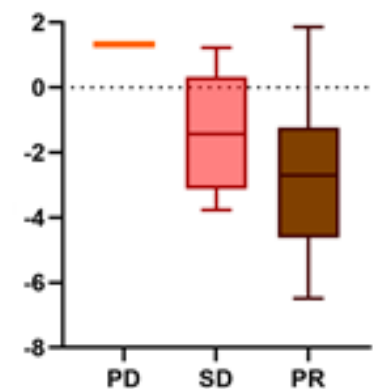
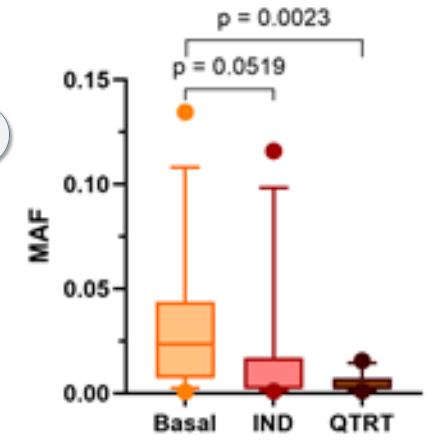
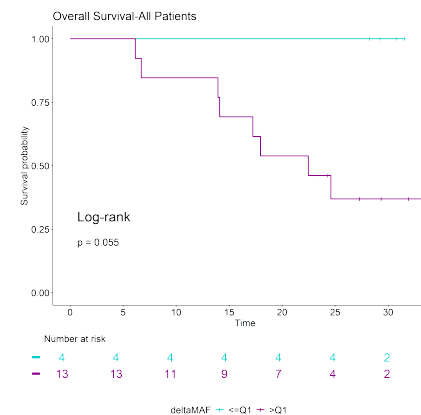
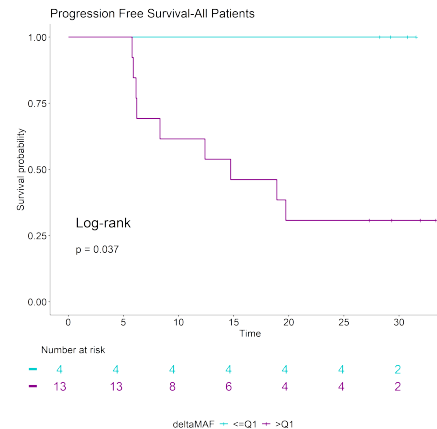
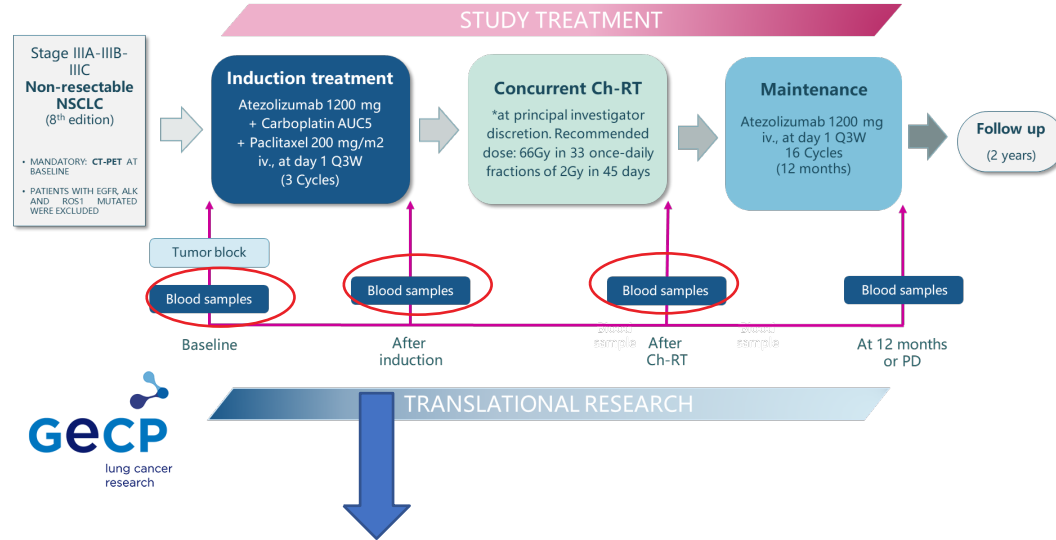
Received: 23 May 2025

Accepted: 28 October 2025

Published online: 24 December 2025

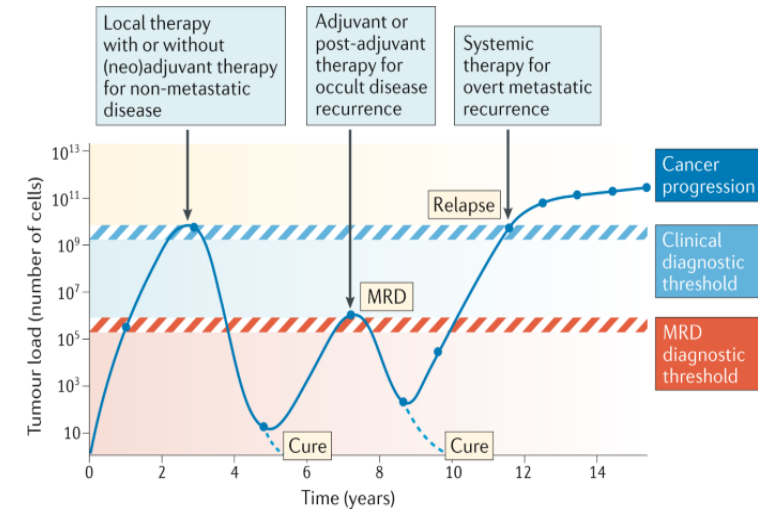
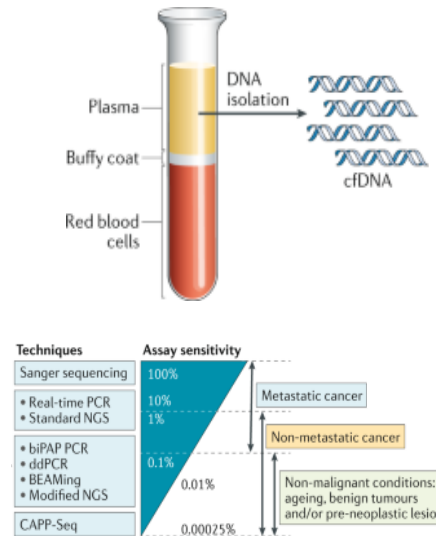
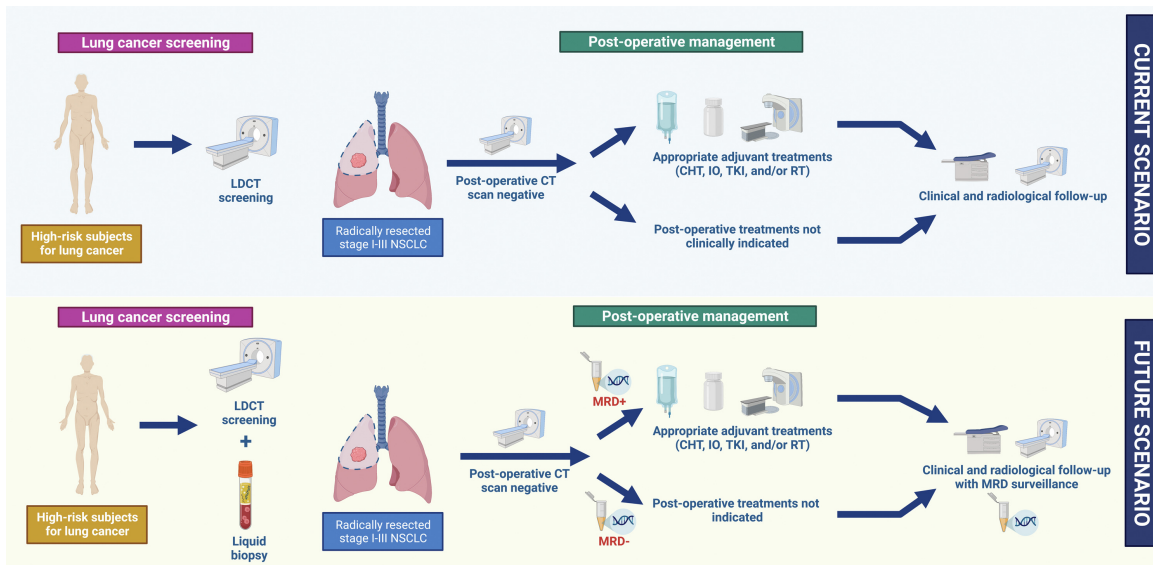
Check for updates

Mariano Provencio<sup>1</sup>, Begoña Campos<sup>2</sup>, María Guirado<sup>3</sup>, Laia Vila<sup>4</sup>, Rosario García Campelo<sup>5</sup>, Miriam Dorta<sup>6</sup>, Sergio Vázquez Estévez<sup>2</sup>, Asia Ferrández<sup>3</sup>, M. Ángeles Sala<sup>7</sup>, Ana Laura Ortega<sup>8</sup>, Ana Blasco<sup>9</sup>, Amelia Insa<sup>10</sup>, María Carmen Areses<sup>11</sup>, Ivana Sullivan<sup>12</sup>, Rafael Lopez<sup>13</sup>, Virginia Calvo<sup>1</sup>, Delvys Rodríguez-Abreu<sup>14</sup>, Joaquim Bosch-Barrera<sup>15</sup>, Ana López-Martin<sup>16</sup>, Raquel Marsé<sup>17</sup>, Laura Torrado<sup>2</sup>, Kirill Matskov<sup>3</sup>, Júlia Giner<sup>4</sup>, Manuel Fernández Bruno<sup>5</sup>, Emilio Sánchez Saugar<sup>6</sup>, Cristina Martínez-Toledo<sup>1</sup>, Pilar Mediavilla<sup>1</sup>, Atocha Romero<sup>1</sup> & Alberto Cruz-Bermúdez<sup>1</sup>





# ADJUVANT SETTING: ROLE OF MRD IN NSCLC



<https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964%2823%2900170-6/fulltext>

Pantel, K et al. Nat Rev Clin Oncol. 2019;16:409-424.



# TUMOR-INFORMED VS. TUMOR-NAÏVE ASSAYS

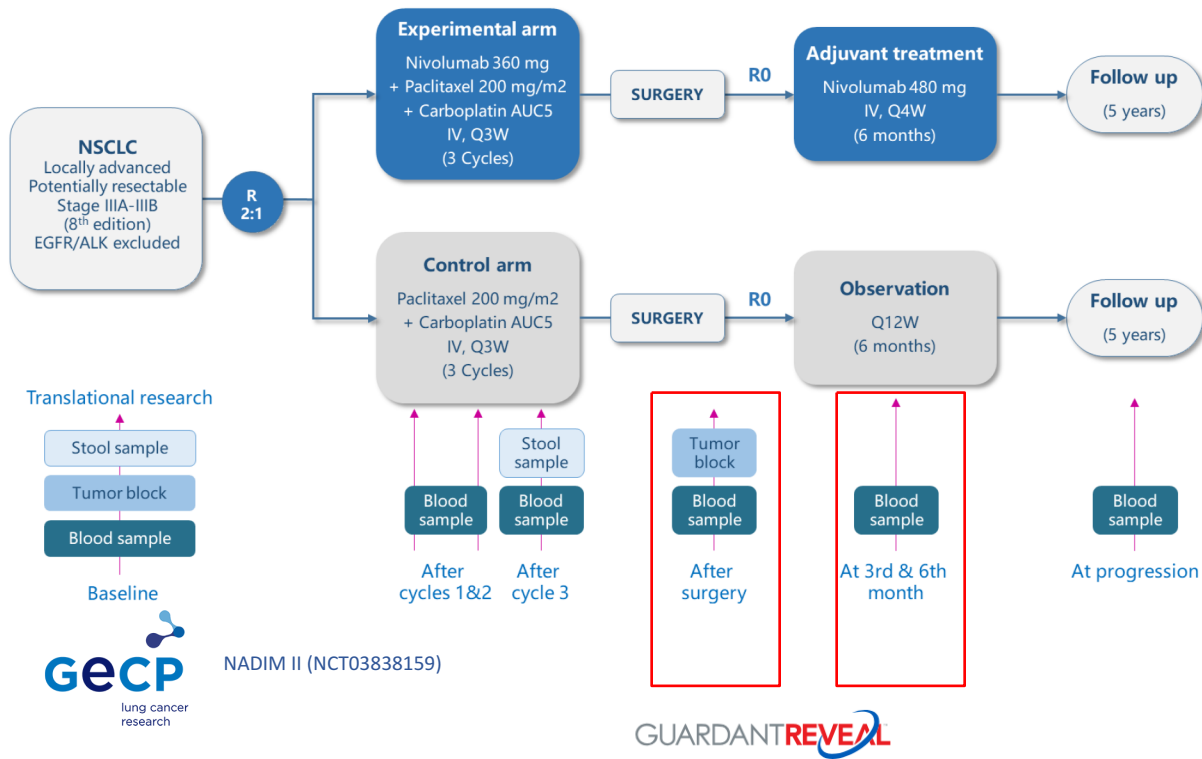
**Table 1. Studies evaluating circulating tumor DNA MRD in solid cancers**

Study	Tumor type	Methodology	Mutations monitored	Total patients	ctDNA MRD landmark		ctDNA surveillance	
					Sensitivity	Specificity	Sensitivity	Specificity
Peng et al. 2020 (111)	Lung	Amplicon NGS	Multiple	48	50.0%	88.9%	66.7%	72.2%
Chaudhuri et al. 2017 (62)	Lung	Capture NGS	Multiple	37	94.0%	100.0%	100.0%	100.0%
Abbosh et al. 2017 (31)	Lung	PCR NGS	Multiple	24	35.7%	90.0%	92.9%	90.0%
Moding et al. 2020 (90)	Lung	Capture NGS	Multiple	12	100.0%	100.0%	—	—
Garcia-Murillas et al. 2019 (113)	Breast	Digital PCR	Single	101	—	—	75.0%	92.1%
Coombes et al. 2019 (34)	Breast	Amplicon NGS	Multiple	49	55.6%	100.0%	88.9%	100.0%
Garcia-Murillas et al. 2015 (27)	Breast	Digital PCR	Single	43	50.0%	96.0%	80.0%	96.4%
Olsson et al. 2015 (112)	Breast	Digital PCR	Multiple	20	—	—	92.8%	100.0%
Tie et al. 2016 (91)	Colon	Amplicon NGS	Single	178	40.7%	98.0%	85.2%	98.0%
Tie et al. 2019 (92)	Colon	Amplicon NGS	Single	88	43.5%	92.3%	—	—
Wang et al. 2019 (114)	Colon	Amplicon NGS	Single	40	—	—	100.0%	90.6%
Reinert et al. 2019 (36)	Colorectal	Amplicon NGS	Multiple	94	41.2%	96.1%	87.5%	98.3%
Parikh et al. 2021 (52)	Colorectal	Methylation and Capture NGS	Multiple	72	55.6%	100.0%	69.0%	95.3%
Scholer et al. 2017 (28)	Colorectal	Digital PCR	Single	26	60.0%	100.0%	100.0%	100.0%
Diehl et al. 2008 (107)	Colorectal	Digital PCR	Single	20	100.0%	80.0%	—	—
Tie et al. 2018 (89)	Rectal	Amplicon NGS	Single	159	47.8%	94.1%	—	—
Khakoo et al. 2019 (109)	Rectal	Digital PCR	Multiple	23	75.0%	100.0%	—	—
Azad et al. 2020 (65)	Esophageal	Capture NGS	Multiple	15	55.6%	100.0%	—	—
Jiang et al. 2020 (110)	Pancreas	Capture NGS	Multiple	27	57.1%	92.3%	—	—
Sausen et al. 2015 (108)	Pancreas	Digital PCR	Single	20	69.2%	42.9%	—	—
Christensen et al. 2019 (35)	Bladder	Amplicon NGS	Multiple	66	21.4%	98.1%	100.0%	97.9%

NOTE: Clinical sensitivity (percentage of patients who relapsed in the follow-up period who were ctDNA positive) and clinical specificity (percentage of patients who did not relapse in the follow-up period who were ctDNA negative) were calculated for the first follow-up sample after completing definitive therapy (ctDNA MRD Landmark) or for repeated ctDNA analysis during follow-up (ctDNA Surveillance). Patients who received additional therapy after ctDNA analysis were excluded if noted in the study. —, not reported or unable to be calculated.



# ADJUVANT SETTING: ROLE OF MRD IN NSCLC



## CLINICAL CANCER RESEARCH

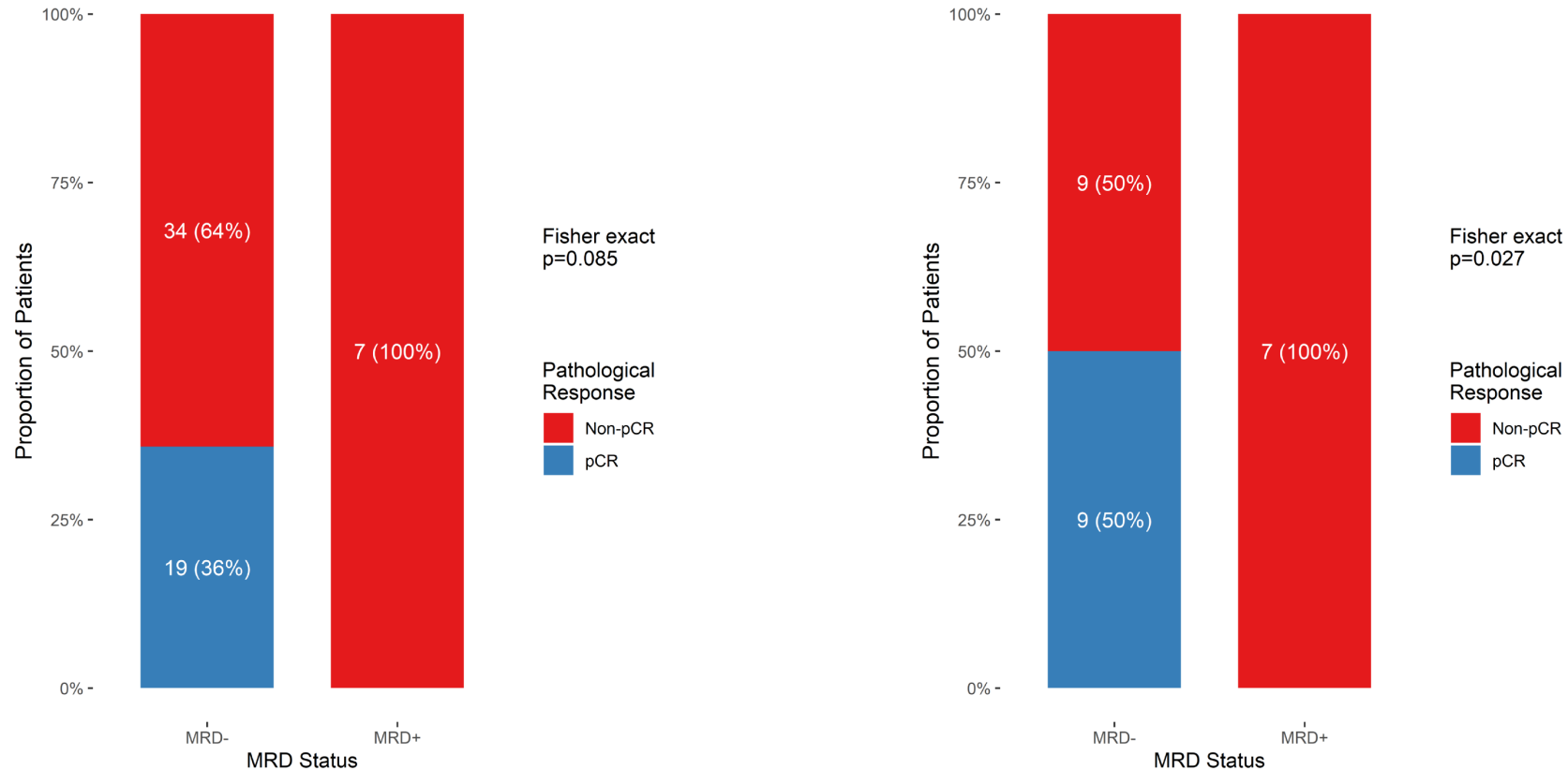
NOVEL BIOMARKERS AND PRECISION MEDICINE | JANUARY 20 2026

### Minimal Residual Disease Enhances Prognostic Stratification beyond Pathologic Response in Resectable Non-Small Cell Lung Cancer

Mariano Provencio ; Roberto Serna-Blasco ; Ernest Nadal ; José Luis González Larriba ; Alex Martínez-Martí ; Reyes Bernabé Caro ; Joaquim Bosch-Barrera ; Virginia Calvo ; Amelia Insa Molla ; Noemí Reguart ; Javier de Castro Carpeño ; Carlos Aguado de la Rosa ; Ramón Palmero Sanchez ; Florentino Hernando Tranco ; Javier Martín-López ; Alejandro Rodríguez-Festa ; Pilar Mediavilla-Medel ; Martín Lázaro ; Jim Hayes ; Alberto Cruz-Bermúdez ; Bartomeu Massutí ; Atocha Romero

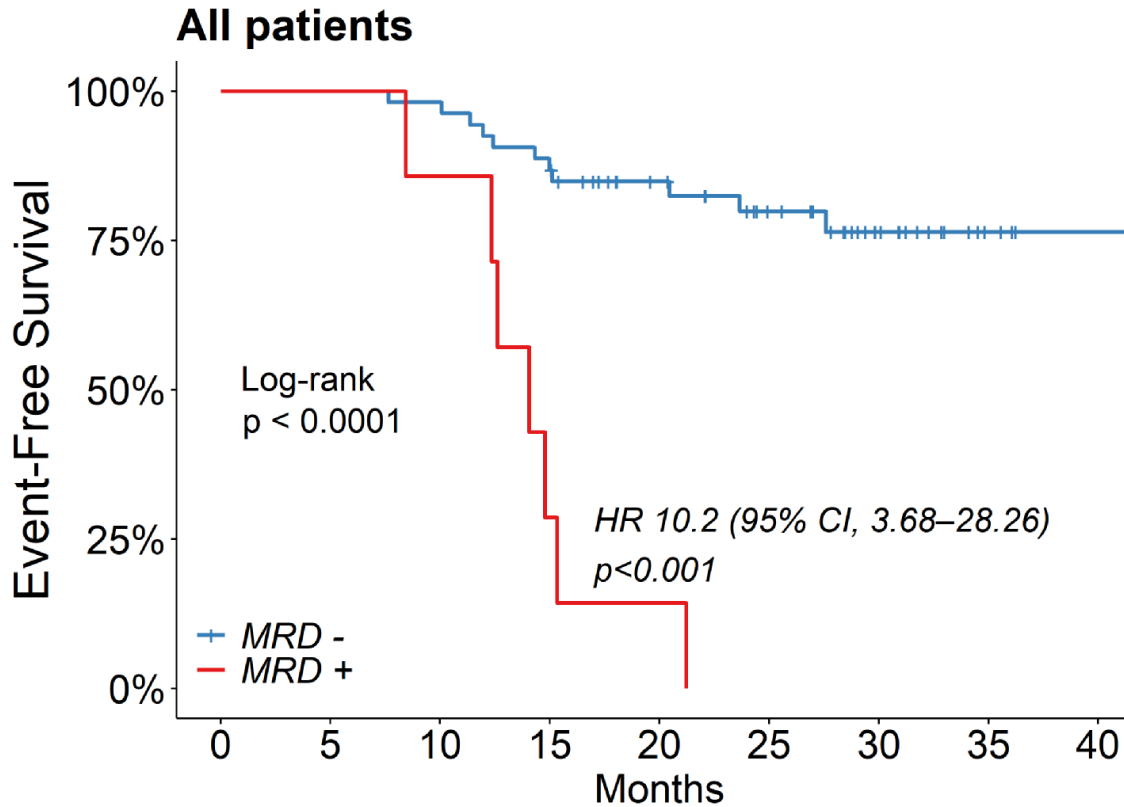


# ASSOCIATION OF PCR WITH MRD DETECTION



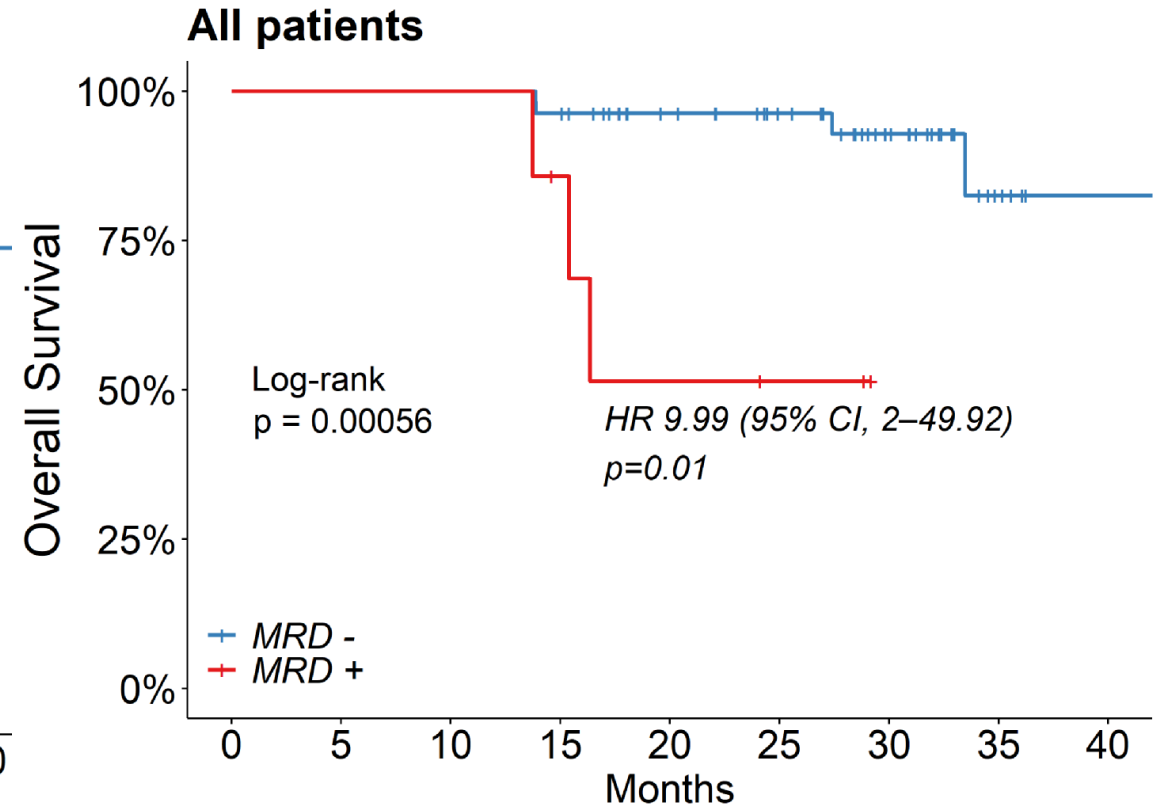


# EFS AND OS ACCORDING TO MRD STATUS



Patients at risk

MRD -	53	53	52	46	36	27	15	4	1
MRD +	7	7	6	2	1	0	0	0	0

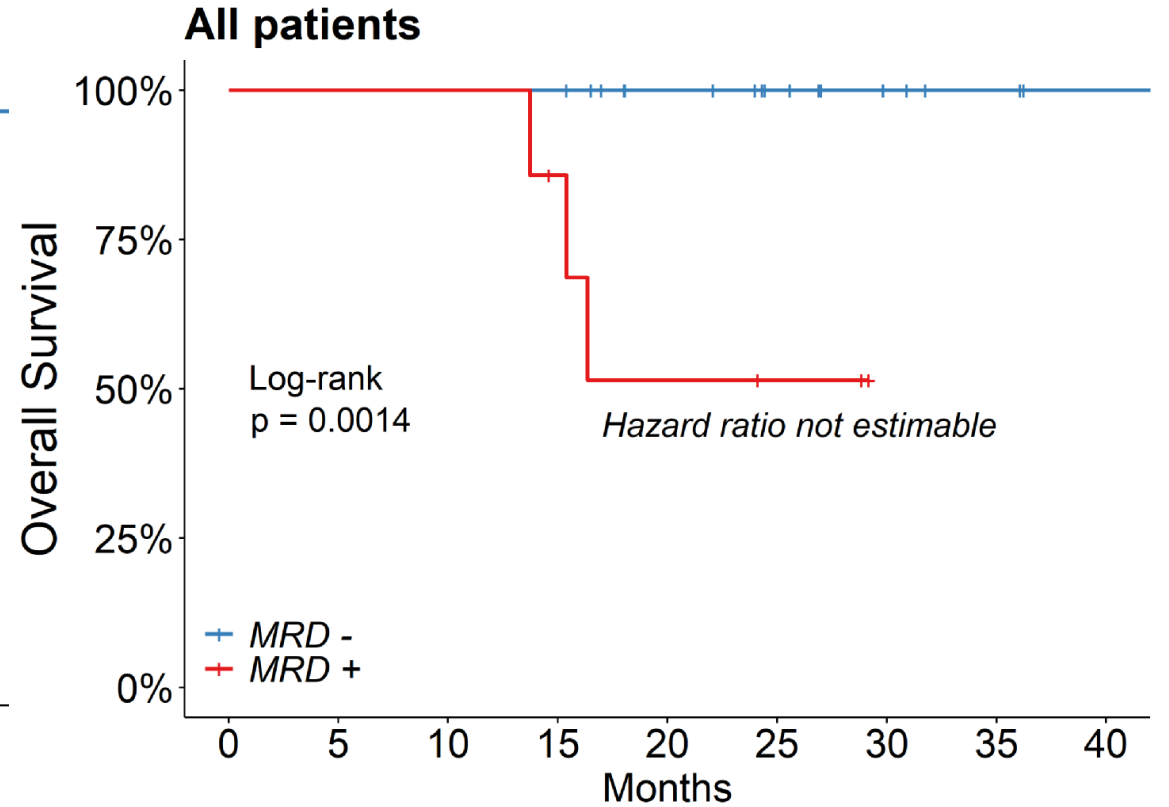
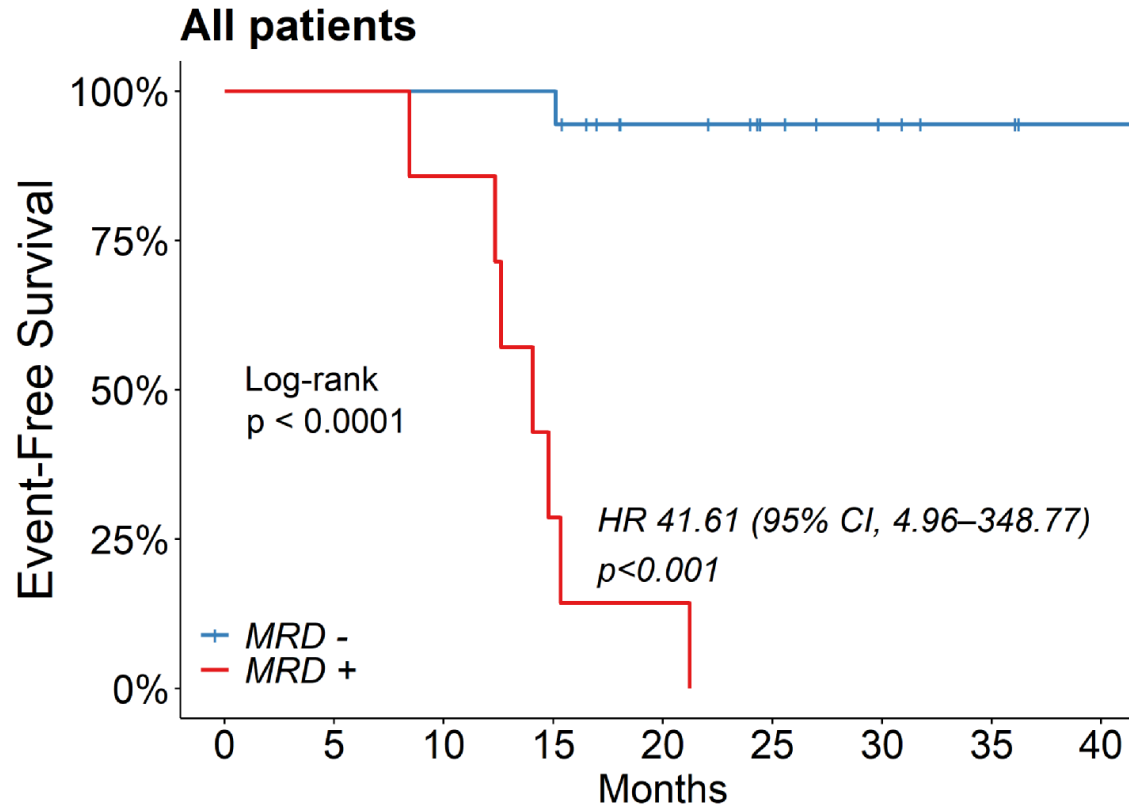
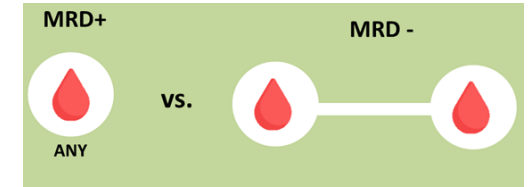


Patients at risk

MRD -	53	53	53	51	40	33	20	5	1
MRD +	7	7	7	5	3	2	0	0	0



# EFS AND OS ACCORDING TO MRD STATUS



Patients at risk

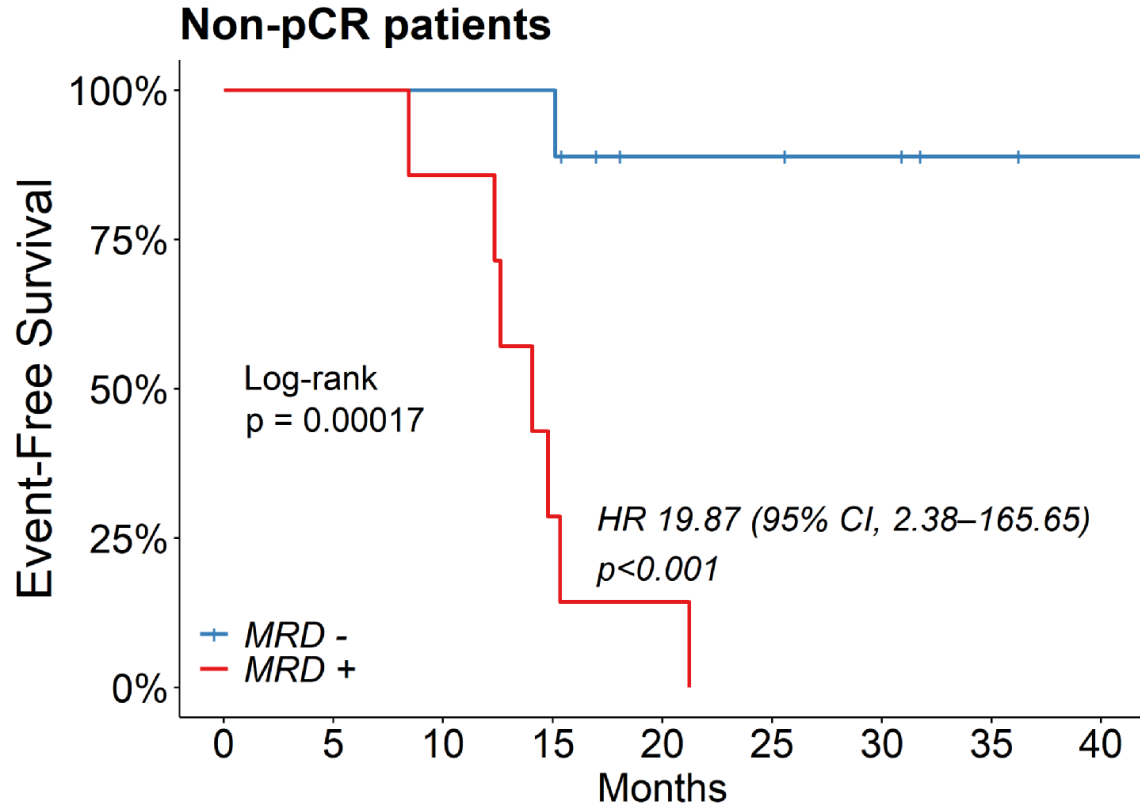
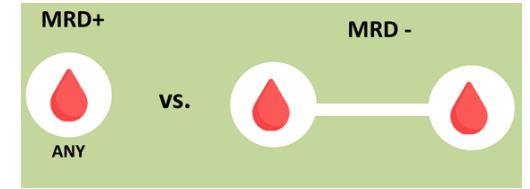
MRD -	18	18	18	18	12	8	5	3	1
MRD +	7	7	6	2	1	0	0	0	0

Patients at risk

MRD -	18	18	18	18	13	9	5	3	1
MRD +	7	7	7	5	3	2	0	0	0

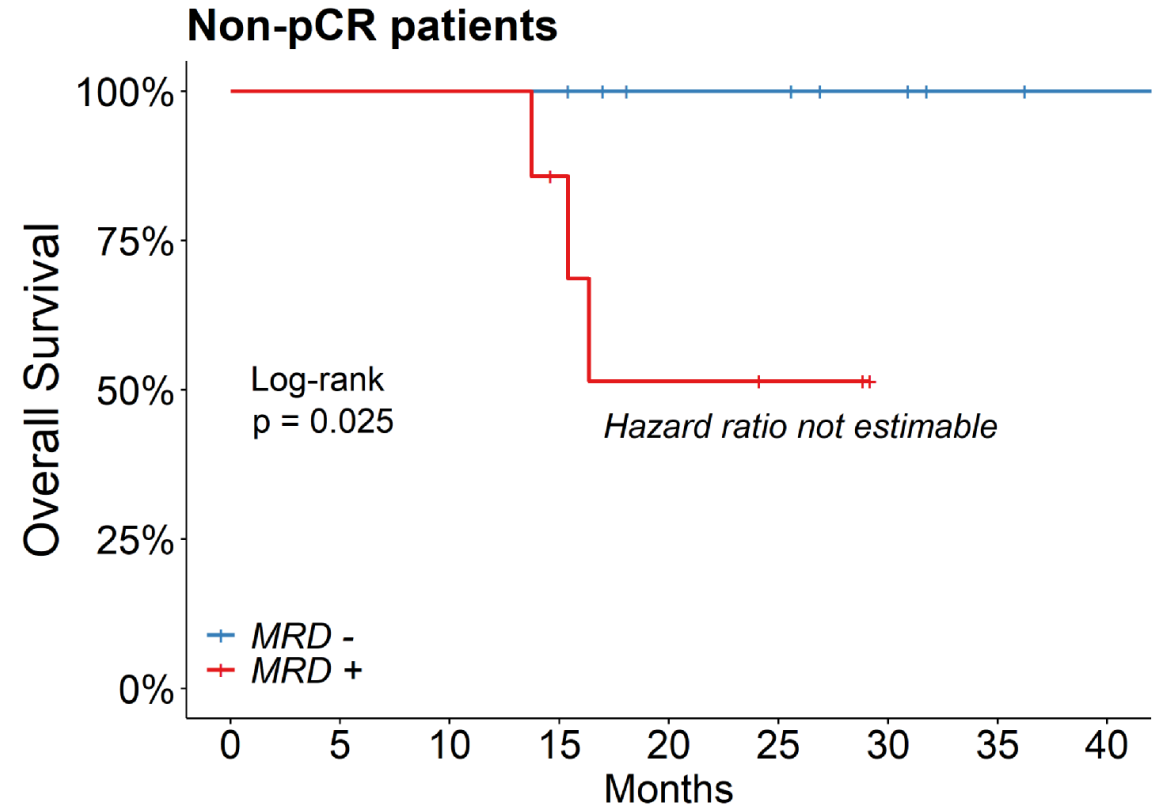


# EFS AND OS ACCORDING TO MRD STATUS IN NON PCR POPULATION



Patients at risk

MRD -	9	9	9	9	5	5	4	2	1
MRD +	7	7	6	2	1	0	0	0	0

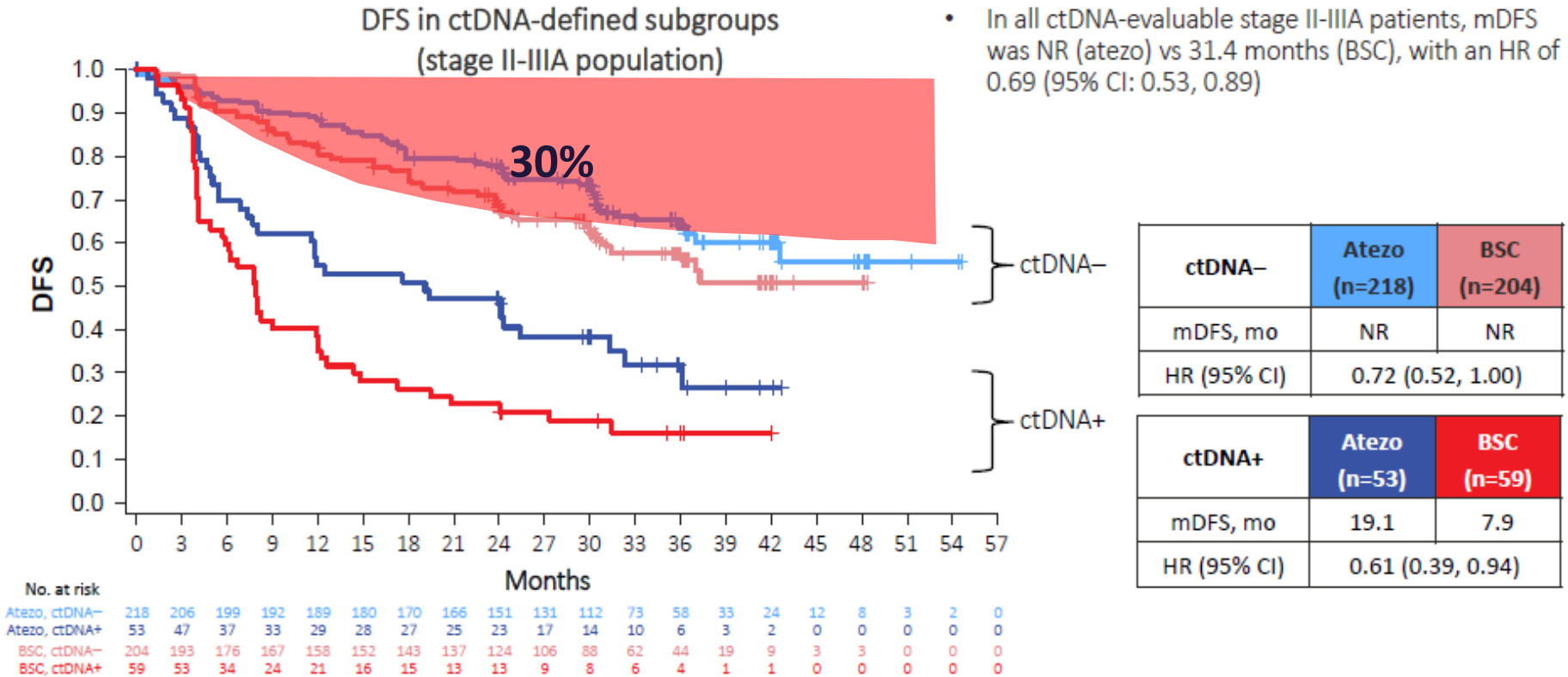


Patients at risk

MRD -	9	9	9	9	6	6	4	2	1
MRD +	7	7	7	5	3	2	0	0	0



## MRD IN IMPOWER010



*MRD post-surgery is prognostic but neither predictive nor sufficiently sensitive for de-escalation decisions*



# Use of Circulating Tumor DNA for Curative-Intent Solid Tumor Drug Development Guidance for Industry

*Additional copies are available from:*

Office of Communications, Division of Drug Information  
Center for Drug Evaluation and Research  
Food and Drug Administration  
10001 New Hampshire Ave., Hillendale Bldg., 4<sup>th</sup> Floor  
Silver Spring, MD 20993-0002  
Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353  
Email: [druginfo@fda.hhs.gov](mailto:druginfo@fda.hhs.gov)  
<https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs>

Office of Communication, Outreach and Development  
Center for Biologics Evaluation and Research  
Food and Drug Administration  
10903 New Hampshire Ave., Bldg. 71, Room 3128  
Silver Spring, MD 20993-0002  
Phone: 800-835-4709 or 240-402-8010  
Email: [ocod@fda.hhs.gov](mailto:ocod@fda.hhs.gov)  
<https://www.fda.gov/vaccines-blood-biologics/guidance-compliance-regulatory-information-biologics/biologics-guidances>

Office of Policy  
Center for Devices and Radiological Health  
Food and Drug Administration  
10903 New Hampshire Ave., Bldg. 66, Room 5431  
Silver Spring, MD 20993-0002  
Phone: 301-796-5900  
E-mail: [CDRH-Guidance@fda.hhs.gov](mailto:CDRH-Guidance@fda.hhs.gov)  
<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/default.htm>

U.S. Department of Health and Human Services  
Food and Drug Administration  
Oncology Center of Excellence (OCE)  
Center for Devices and Radiologic Health (CDRH)  
Center for Drug Evaluation and Research (CDER)  
Center for Biologics Evaluation and Research (CBER)  
November 2024

ctDNA MRD could be used for treatment optimization, to add on therapy for patients who are at higher risk of disease recurrence (i.e., ctDNA MRD positive) or to de-escalate therapy for patients with lower risk of disease recurrence (i.e., ctDNA MRD negative)

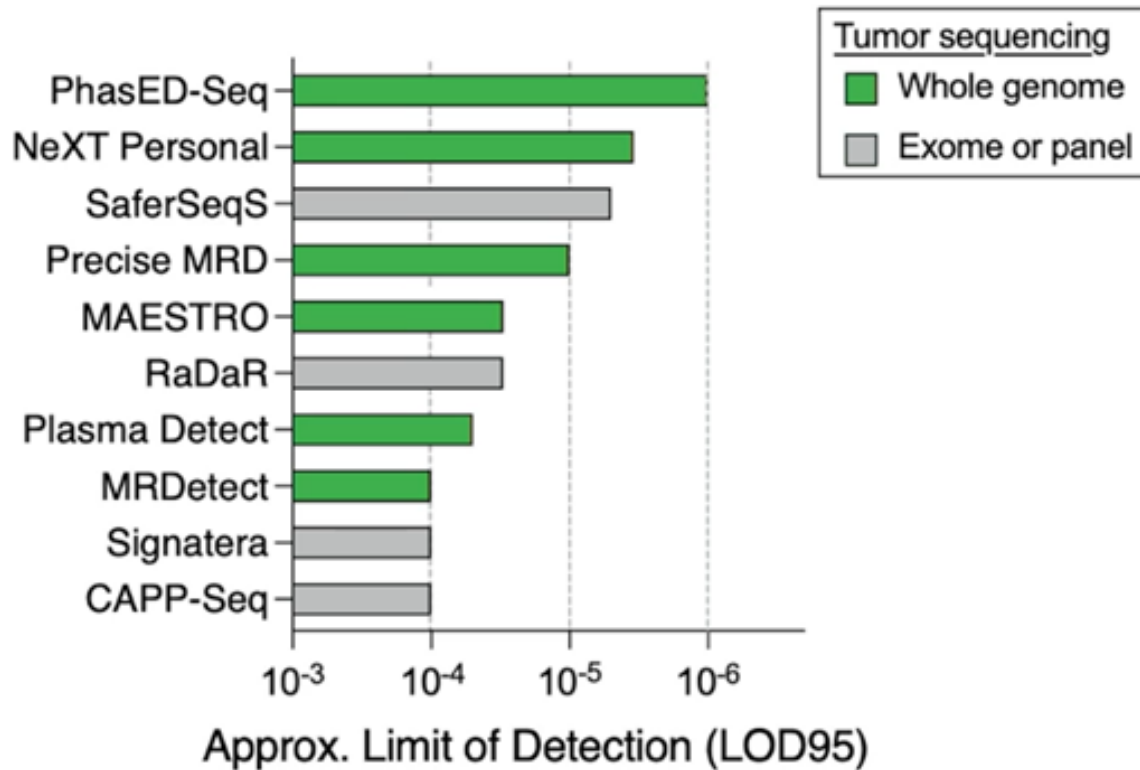
The MRD assay should have high sensitivity and negative predictive value (NPV) for supporting de-escalation of treatment and high specificity and positive predictive value (PPV) for supporting escalation of treatment.

- MRD detection with 1st generation assays has a high positive predictive value.
- Sensitivity remains a challenge

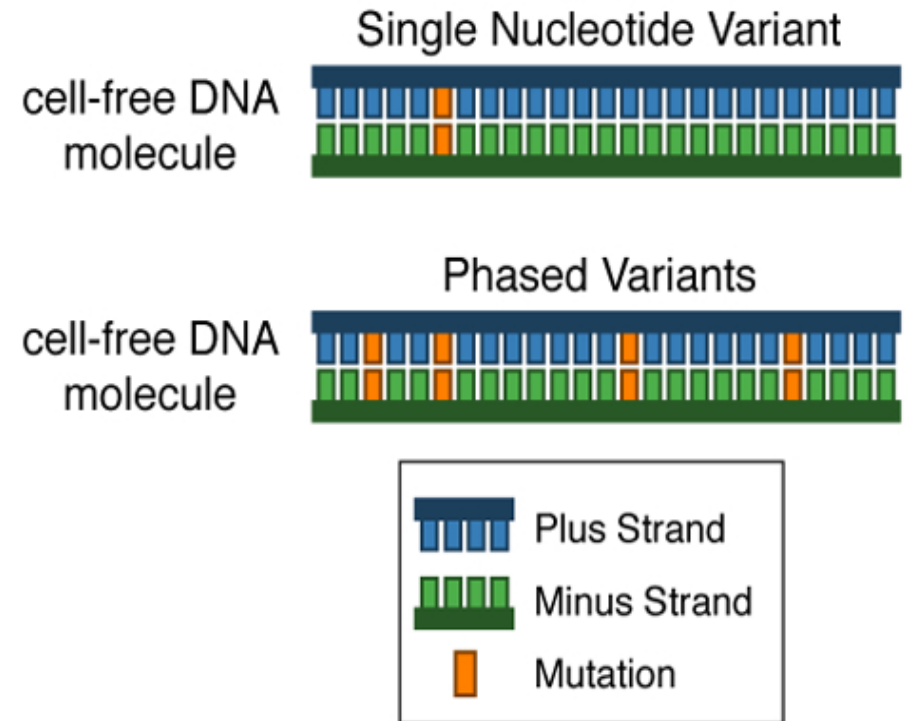


# APPROACHES TO IMPROVE LOD

- ✓ Sequence more genome
- ✓ Track more samples
- ✓ Decrease error rate



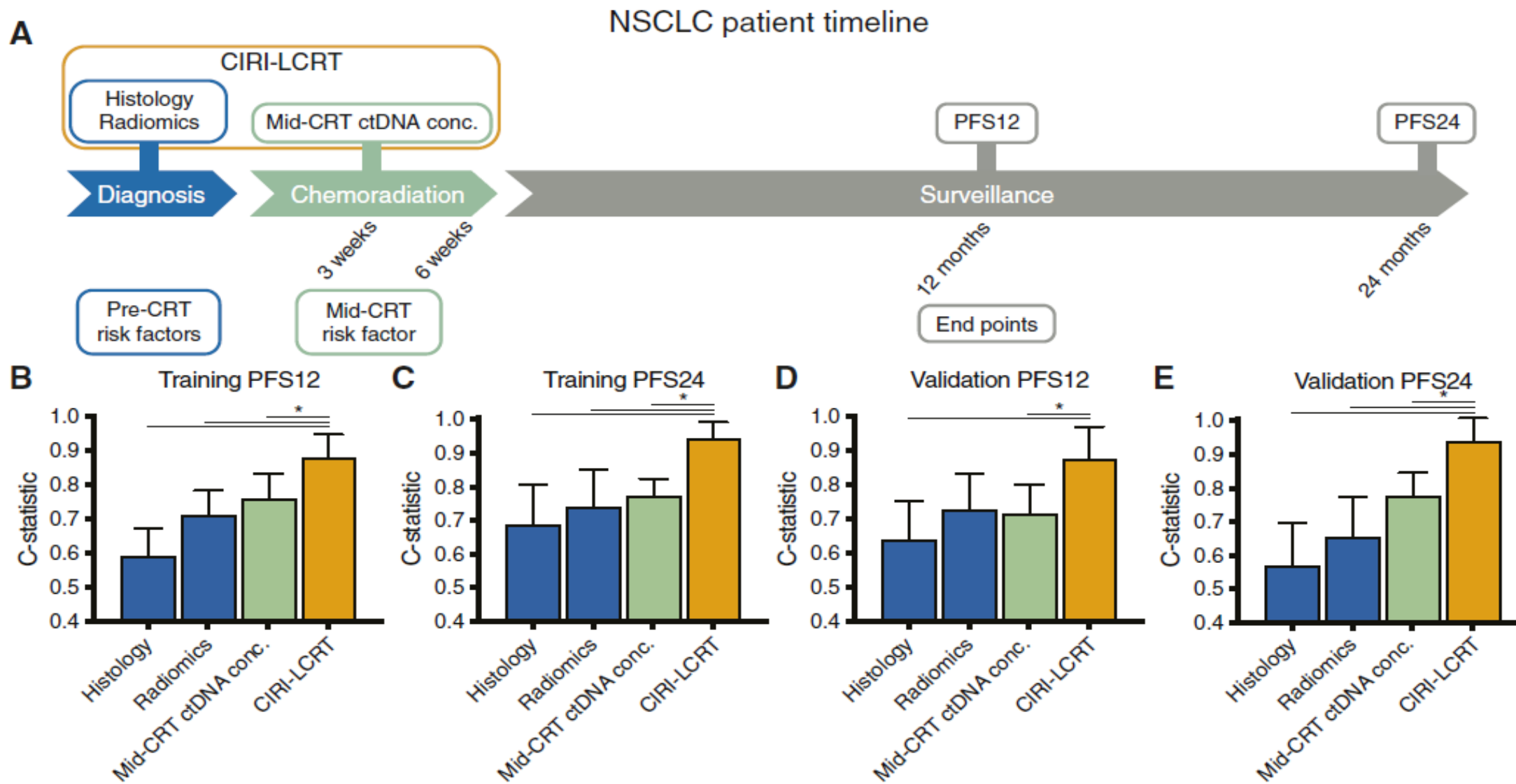
## Phased variants in ctDNA







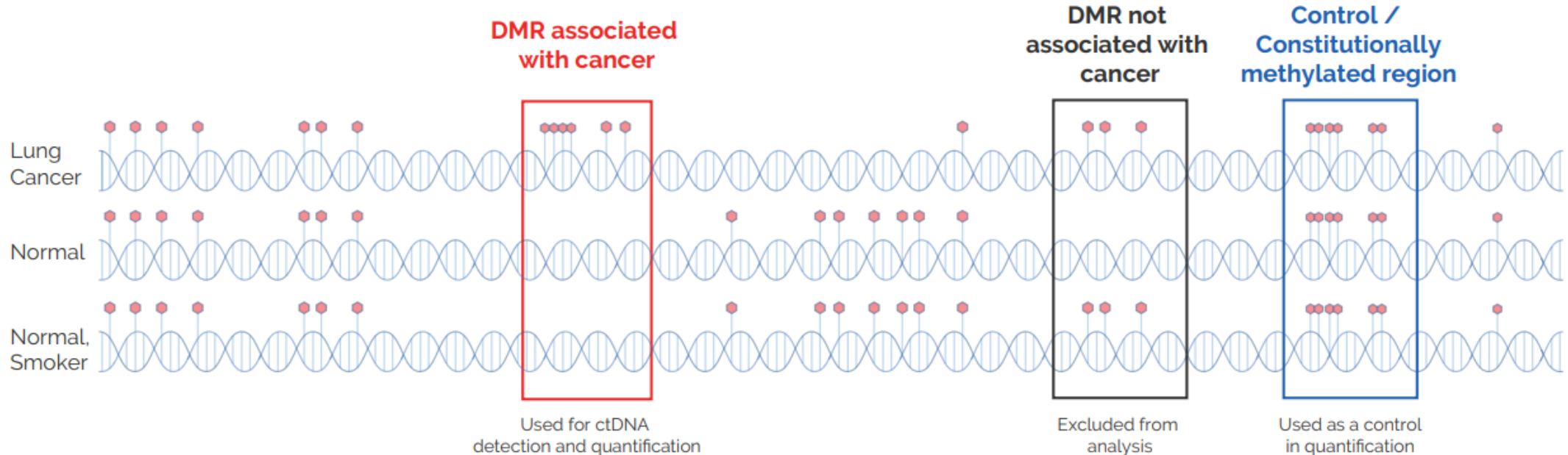
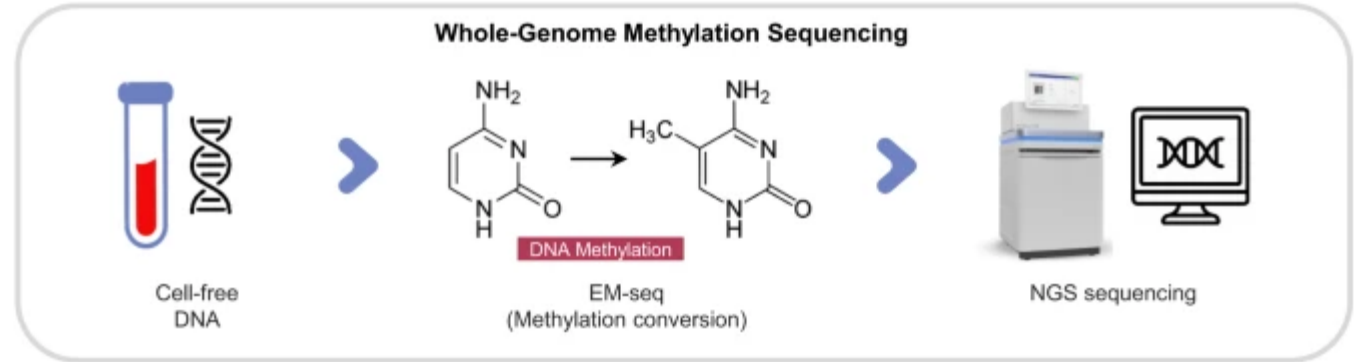
# APPROACHES TO IMPROVE LOD





# APPROACHES TO IMPROVE LOD: TUMOR FRACTION. METILATION

- ✓ Sequence more genome
- ✓ Track more samples
- ✓ Decrease error rate
- ✓ Enrichment of minor alleles
- ✓ Integrate radiomics and other biomarkers
- ✓ Combine variant detection with non-genetic features





# APPROACHES TO IMPROVE LOD: FRAGMENTOMICS

Ivanov et al. *BMC Genomics* 2015, **16**(Suppl 13):S1  
<http://www.biomedcentral.com/1471-2164/16/S13/S1>



RESEARCH

Open Access

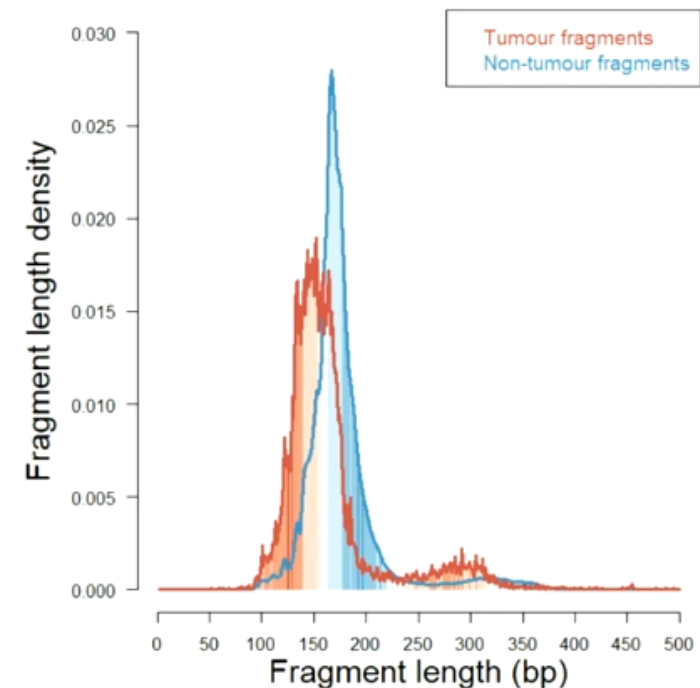
## Non-random fragmentation patterns in circulating cell-free DNA reflect epigenetic regulation

Maxim Ivanov<sup>1,2,3\*</sup>, Ancha Baranova<sup>1,2,3,4,5</sup>, Timothy Butler<sup>6,7</sup>, Paul Spellman<sup>6,7</sup>, Vladislav Mileyko<sup>1,2</sup>

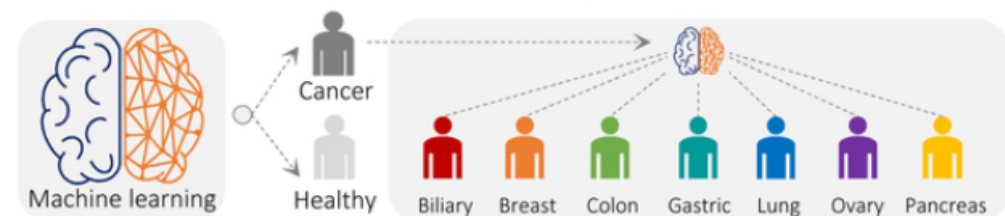
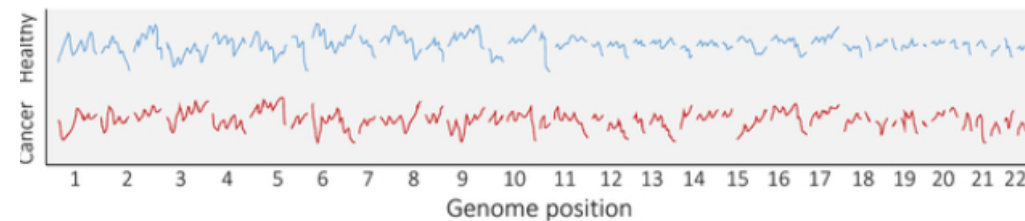
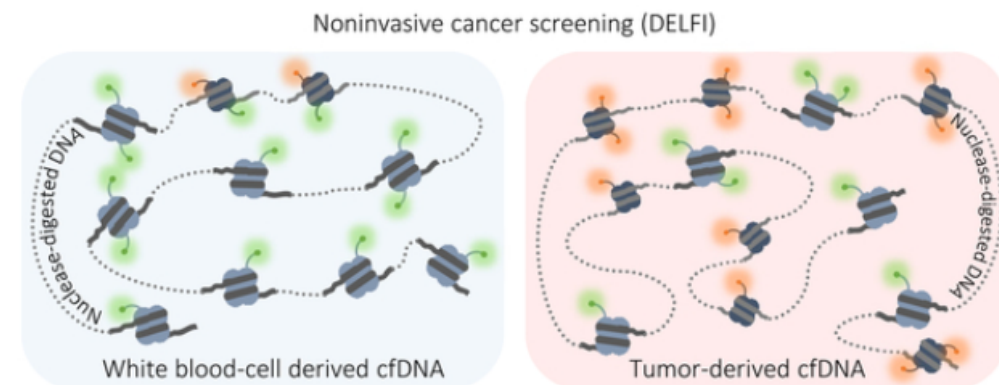
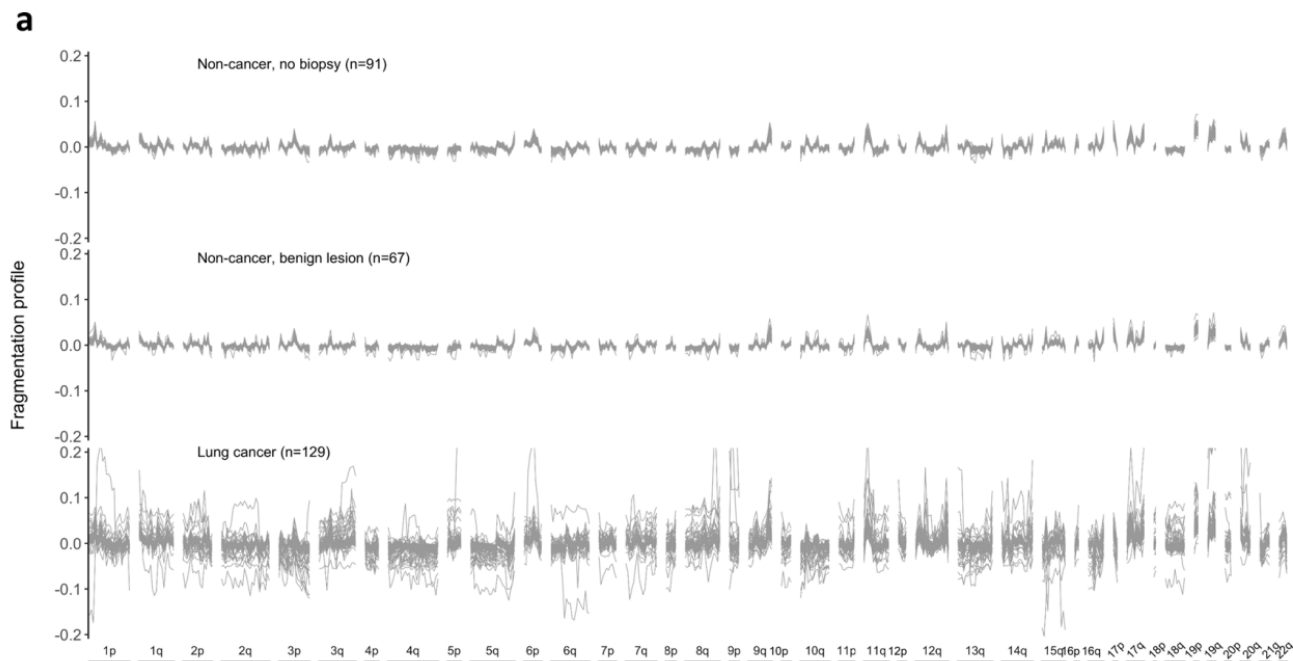
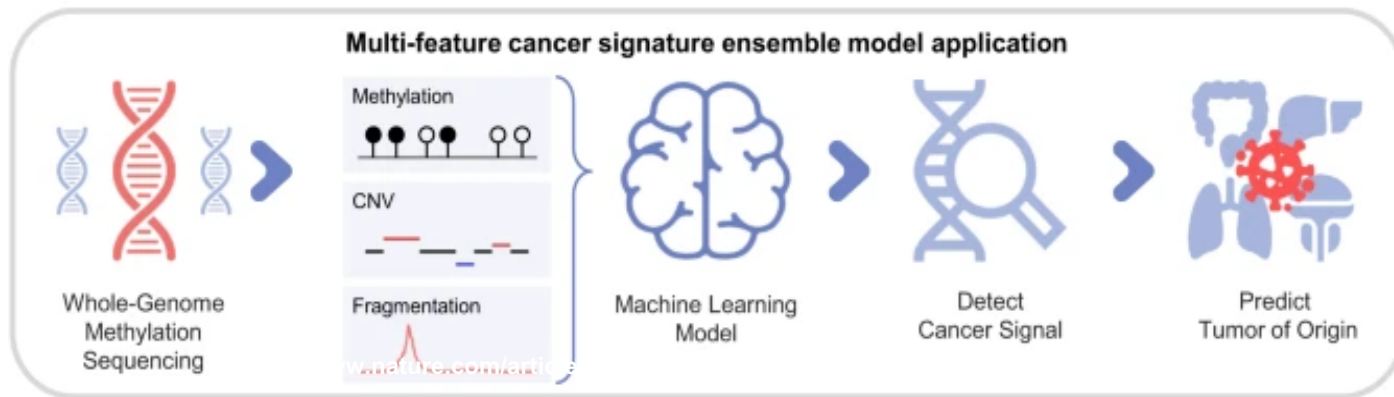
From The 7th International Young Scientists School  
Novosibirsk, Russia. 22-25 June 2015

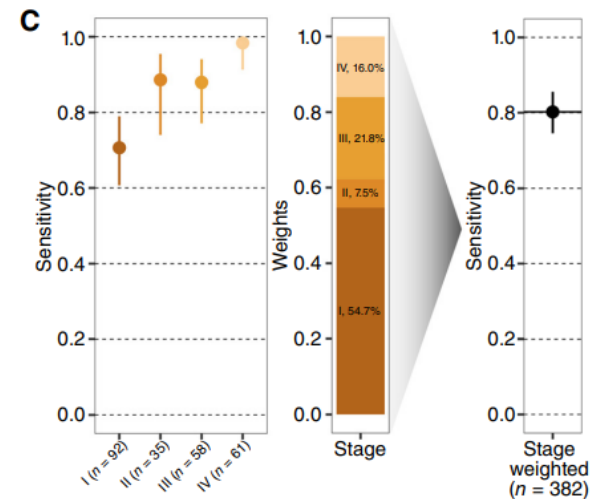
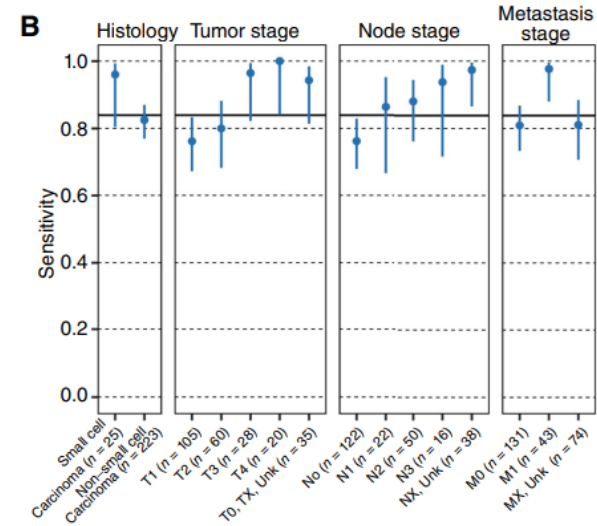
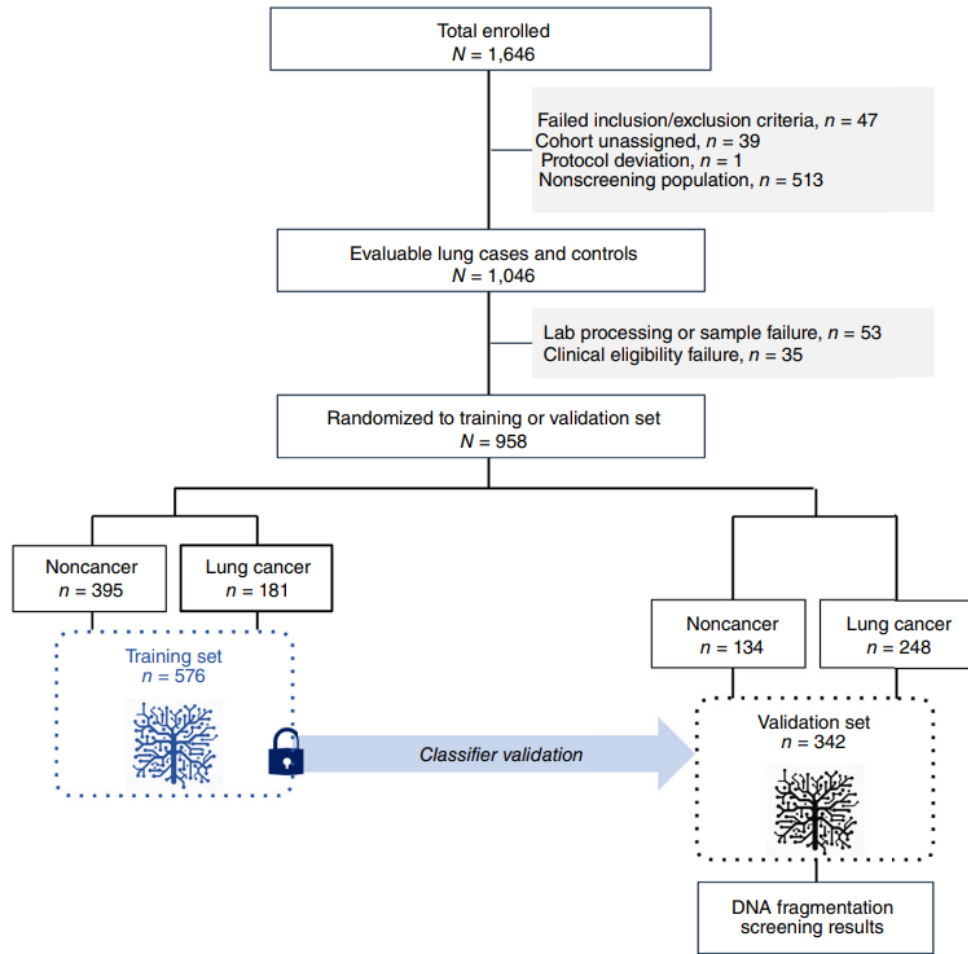
*“...Thus, opening a novel filed in biomarker research that we may tentatively call **“fragmentomics”**....In this paper, we employ high throughput sequencing of human cfDNA to analyze the properties of cfDNA fragmentation patterns.”*

Ivanov M et al. *BMC Genomics* 2015



Vessies et al., *Molecular Oncology* 16:2719–2732 (2022)







# APPROACHES TO IMPROVE LOD: FRAGMENTOMICS

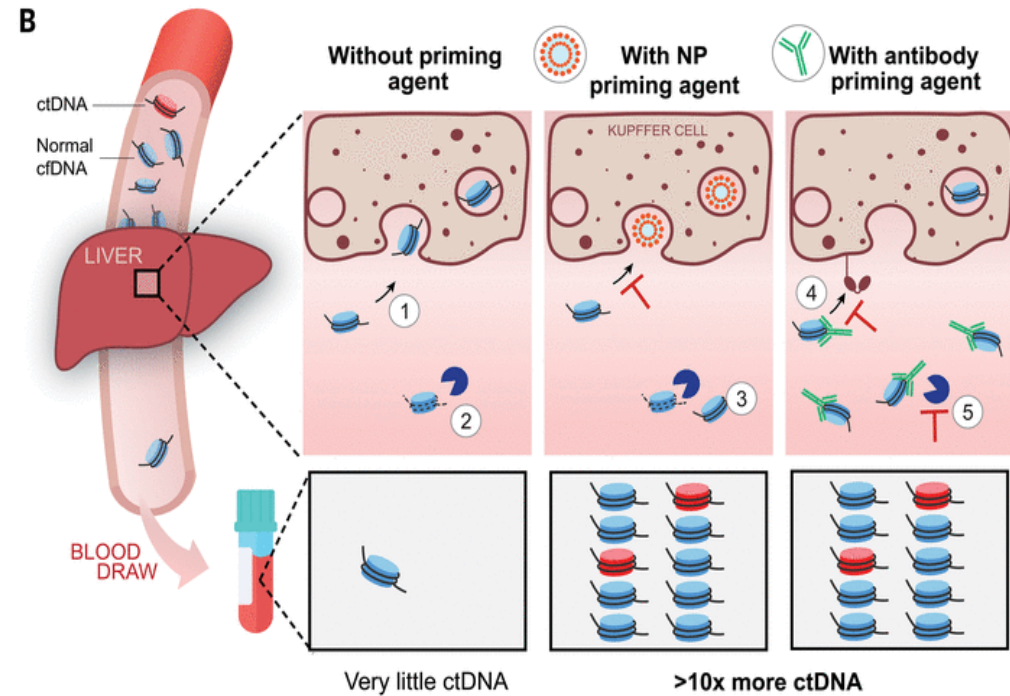
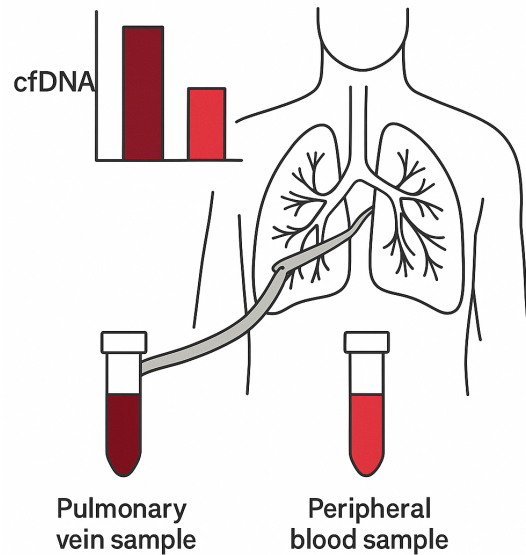
**Table 2. Selected studies utilizing different fragmentomics approaches**

Study	Fragment feature	Application	Technology	Key findings and limitations
Mouliere et al. 2018 <sup>54</sup>	Fragment size difference between cancer and healthy on a global level, 10 bp oscillations on subnucleosomal level	Cancer diagnosis (pan tumor)	Shallow WGS, WES, size selection enrichment	AUC 0.91–0.99 depending on cancer type. Low sequencing depth (0.4x). Sensitive at low MAFs (after size specific enrichment). Only late-stage cancers
Cristiano et al. 2019 <sup>61</sup>	Fragment size difference between cancer and healthy on a regional level	Cancer diagnosis (pan-tumor)	Delfi (WGS)	AUC 0.94 (Sens 57–99%, Spec 98%) supervised model, non-age matched controls
Ulz et al. 2019 <sup>59</sup>	Coverage at TFBS and TSS	Prostate cancer subtyping and early detection	WGS	High tumor fraction required
Snyder et al. 2016 <sup>65</sup>	Fragment endpoints, coverage near TFBS and near TSS	Cell of origin	Windowed protection score – WPS (WGS inc single strand)	Single strand sequencing enriched shorter fragments. Small sample numbers, high sequencing depth required (~100x).
Esfahani et al. 2022 <sup>70</sup>	Fragment length diversity at promoter regions ‘promoter fragment entropy’, coverage at regions near TSS (“nucleosome depleted regions”)	Diagnosis and subtype classification (lung cancer, diffuse large B cell lymphoma)	EPIQ-Seq (Targeted sequencing, also used WGS/WES)	Composite model of PFE/NDR, Lung cancer from healthy AUC 0.91 (training), 0.83 (validation), NSCLC subtype AUC 0.9, DLBCL from healthy AUC 0.92 (training) AUC 0.96 (validation). Requires disease specific panels for EPIC-seq, less sensitive at early stage
De Sarkar et al. 2023 <sup>66</sup>	Coverage at TFBS and TSS, nucleosome phasing (periodicity of nucleosome positions)	Prostate cancer phenotyping	Keraon, ctdPheno (WGS)	AUC 0.96 (90.4% sensitivity, 97.5% specificity) for phenotyping. Lower limit of 8% and 3% tumor fraction required. (ctDPheno/Keraon respectively)
Sun et al. 2019 <sup>57</sup>	Strand orientation	HCC diagnosis and tissue of origin	Orientation-aware cfDNA fragmentation-OCF (WGS)	67% sensitivity, 93.8% specificity for HCC. Lower tissue fraction required than some approaches e.g., ~5%. Based on known open chromatin regions with limited independent validation.
Jiang et al. 2020 <sup>58</sup>	End-motif frequency	Cancer diagnosis (mainly HCC)	WGS, WGBS	AUC 0.86 for HCC. Accurate at 4% tumor fraction. Requires deep sequencing for accuracy. Limited independent validation.
Doebley et al. 2022 <sup>71</sup>	TFBS coverage by fragment midpoint	Cancer detection (pan-tumor), breast cancer subtyping.	Griffin (WGS)	Ultra-low-pass WGS (0.1x). Cancer vs. non-cancer: AUC 0.89 for 0.1x coverage. AUC 0.92 for breast cancer subtyping. Mainly existing cohorts, limited independent validation.

WGS, whole-genome sequencing; WES, whole exome sequencing; WGBS, whole genome bisulfite sequencing; TSS, transcription start site; TFBS, transcription factor binding site; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; VAF, variant allele frequency; PFE, promoter fragment entropy; NDR, nucleosome depleted regions; AUC, area under the curve.

# APPROACHES TO IMPROVE LOD: FRAGMENTOMICS

- ✓ Sequence more genome
- ✓ Track more samples
- ✓ Decrease error rate
- ✓ Enrichment of minor alleles
- ✓ Integrate radiomics and other biomarkers
- ✓ Combine variant detection with non-genetic features
- ✓ Increase the amount of cfDNA





## TAKE HOME MESSAGE

- ctDNA levels at baseline as well as ctDNA clearance are of prognostic significance in the neoadjuvant setting
- MRD monitoring can help identify cancer patients with excellent prognosis who may be candidates for treatment de-escalation.
- MRD assays used for de-escalation should demonstrate high sensitivity and high negative predictive value (NPV).
- 1st generation MRD assays provide high positive predictive value but may be less suitable for guiding treatment de-escalation.
- Novel methods such as Phased-Seq and Minor Allele Enriched Sequencing show promise for improving MRD measurement.
- While cfDNA profiling has traditionally relied on mutation detection, moving beyond mutations—through fragmentation-based profiling—offers the potential for greater sensitivity.
- Approaches to improve DNA yield are under investigation and may improve LOD; EVs also show promise as an alternative source
- Multimodal approaches (e.g., combining radiomics and ctDNA MRD) may enhance the prediction of survival outcomes.

# GRACIAS!

II JORNADA TRASLACIONAL  
DE ONCOLOGÍA DE PRECISIÓN: A TRAVÉS DE LAS VÍAS  
DE SEÑALIZACIÓN  
SEVILLA, 6 Y 7  
DE FEBRERO DE 2025



Plan de  
Recuperación,  
Transformación  
y Resiliencia



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