16th CONGRESS Lung ON CANCER

BARCELONA 27 / 28 NOVEMBER 2025

GECP lung cancer research

Liquid Biopsy:Monitoring response in oncogenic drivers

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27-11-2025

CONFLICTO DE INTERESES

- Consultant or Advisory Role: Novartis, AstraZeneca, Boehringer-Ingelheim, Roche, BMS, Lilly, MSD, Takeda, Phyzer, Kyowa, Sanofi,Jansen
- Research Funding: BMS
- Speaking: Novartis, AstraZeneca, Boehringer-Ingelheim, Roche, BMS, Lilly, MSD, Takeda, Kyowa, Pierre-fabre, Novocure, Sanofi, Jansen, Daychi

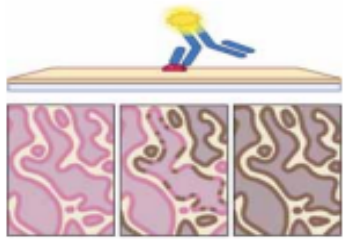
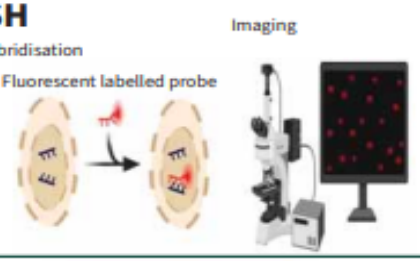
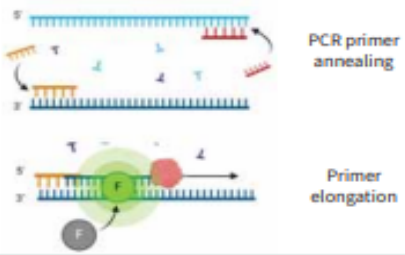
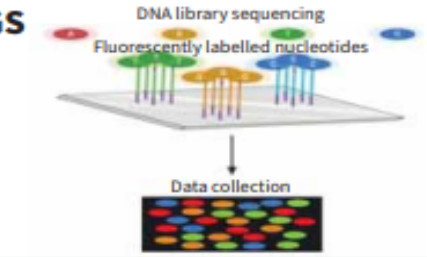
SUMMARY

1.- New technology approach

2.- Monitoring mechanism of resistance

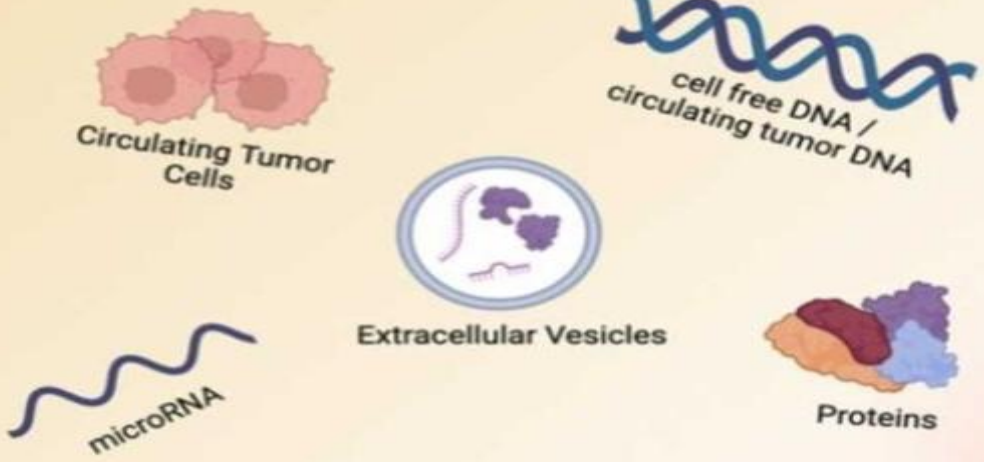
3.- Monitoring response and early relapse

SUMMARY

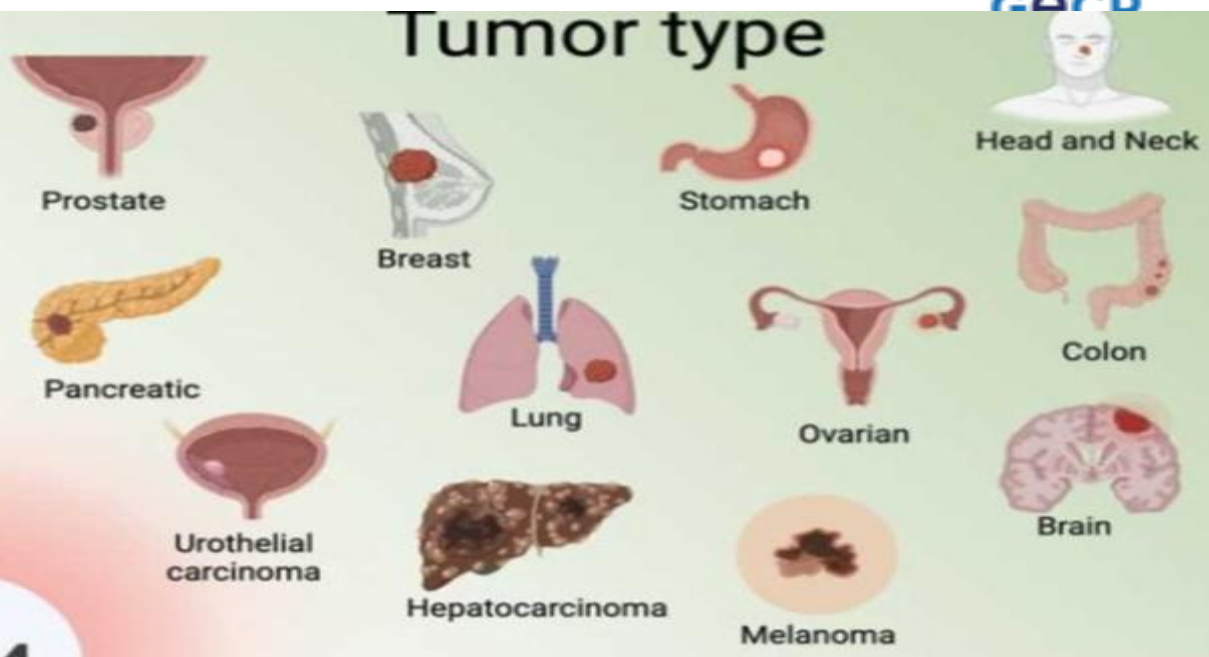
	Principles	Purpose	Strengths	Limitations
IHC 	Uses antibodies to detect specific proteins in tissue sections	Histological classification; detection of protein overexpression (*e.g. EGFR*)	Rapid and widely available; useful for protein-level changes	Cannot detect underlying genetic mutations; limited to known targets
FISH 	Employs fluorescent DNA probes to bind specific gene regions in chromosomes	Detects gene fusions, amplifications, and deletions (*e.g. ALK, ROS1*)	High specificity for structural chromosomal alterations	Labour-intensive; unable to detect small mutations or novel variants
PCR 	Amplifies target DNA regions using primers and DNA polymerase	Identifies specific known point mutations or small indels	High sensitivity; rapid results	Restricted to predefined mutations; cannot detect unknown alterations
NGS 	Simultaneously sequences multiple genes in parallel	Broad genetic profiling including known and unknown variants	Comprehensive; detects various mutation types (SNVs, indels, fusions, CNVs)	Requires high-quality nucleic acids; longer turnaround; more expensive

Toland S,. Liquid biopsy in lung cancer. Breathe (Sheff). 2025 Aug 19;21(3):250051

Analyte



Tumor type



2024

Analysis


Next Generation Sequencing

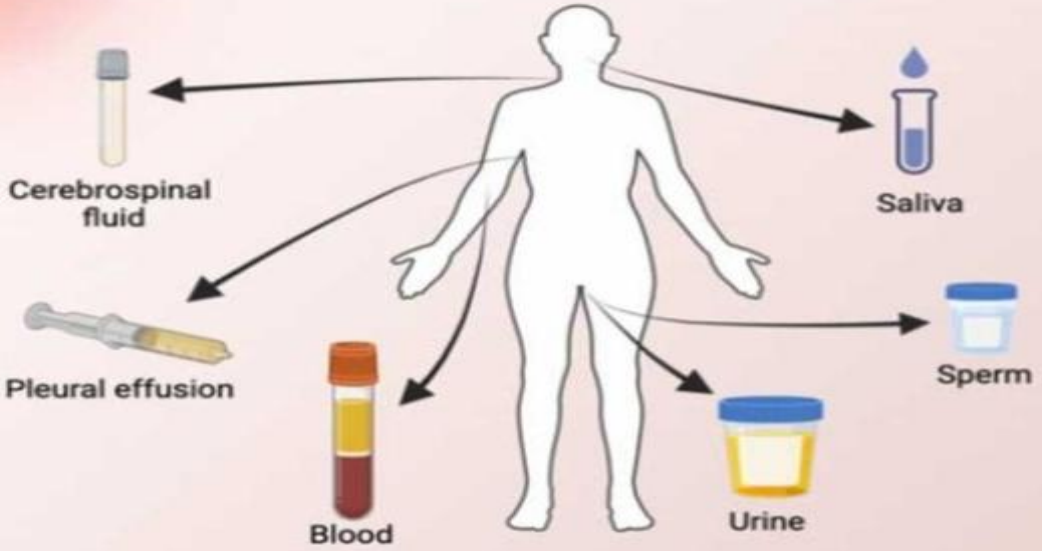

Machine Learning Models


Digital Polymerase Chain Reaction


Flow Cytometry

- CfDNA Integrity
- Characterization (miRNA)
- Comprehensive Genomic Profiling
- Copy Number Variations
- Methylation Profile
- Proteomics
- Quantification (ctDNA, CTCs)
- Tumor Fraction
- Fragmentomics
- Tumor Mutational Burden
- Variant Allele Frequency

Liquid type



Characteristic	Tissue NGS	Liquid biopsy
Procedure invasiveness	Invasive (requires tissue sampling *via* biopsy)	Minimally invasive (typically blood draw or fluid aspiration)
Sample origin	Localised tumour tissue (often from a single site)	Circulating tumour-derived DNA from primary and metastatic sites
Turnaround time	Longer (due to histological processing and logistics)	Shorter (typically within days)
Tumour heterogeneity assessment	Limited (single spatial point)	Broader (captures multi-site and subclonal variations)
Feasibility for serial monitoring	Limited (risk and feasibility of repeated biopsies)	High (repeatable with minimal patient burden)
Sensitivity in early-stage disease	Higher	Lower (due to low ctDNA shedding)
Sensitivity in advanced disease	High	Moderate to high (~70%)
Detection of resistance mutations	Requires repeat invasive biopsy	Often feasible noninvasively through ctDNA profiling
Histology and PD-L1 status	Assessable	Not assessable
Risk of clonal haematopoiesis interference	None	Present (can cause false positives, *e.g.* TP53, KRAS)
Pre-analytical variability	Moderate (affected by tissue quality and fixation methods)	Higher (sensitive to sample handling and cfDNA degradation)

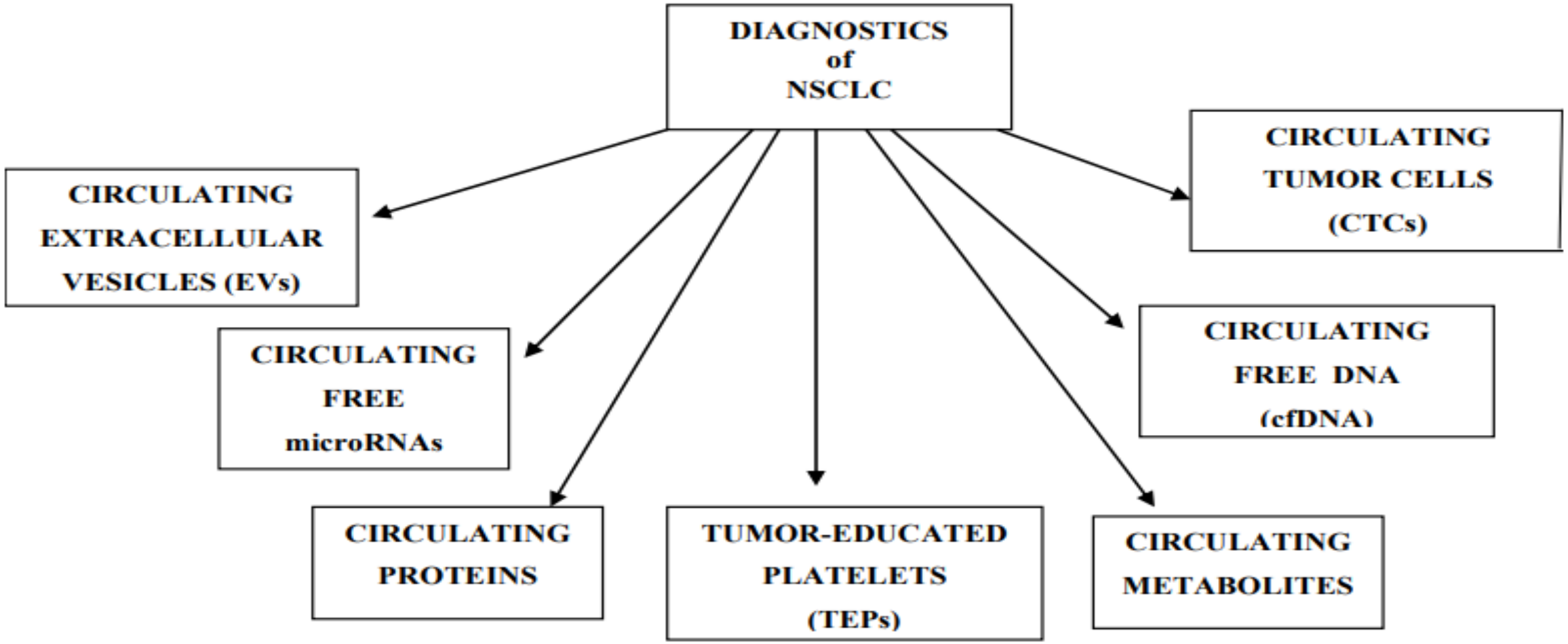
ctDNA: circulating tumour DNA; PD-L1: programmed death-ligand 1; cfDNA: cell-free DNA.

TABLE 3 Clinical indications and limitations of liquid biopsy in lung cancer

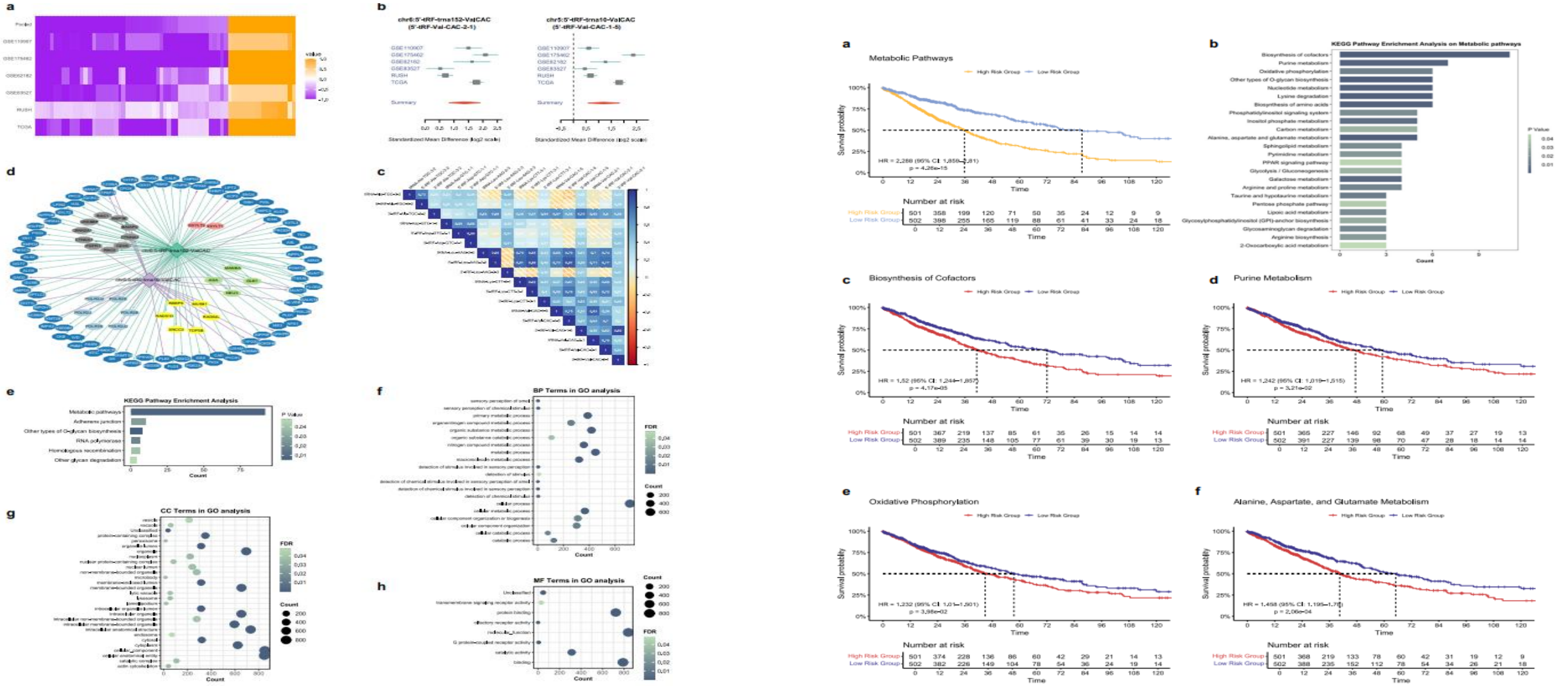
Clinical scenario	Is liquid biopsy appropriate?
Patient is unfit for invasive biopsy due to comorbidities or poor lung function	Yes
Tumour is located adjacent to major vessels or inaccessible by bronchoscopic/computed tomography-guided means	Yes
Tissue biopsy obtained but insufficient for next-generation sequencing or reflex molecular testing	Yes
Disease progression suspected and re-biopsy is high risk or not feasible	Yes
Histological diagnosis not yet established	No
Tumour is readily accessible for biopsy and tissue is sufficient for molecular profiling	No, prefer tissue biopsy
Early-stage disease with low circulating tumour DNA shedding (*e.g.* stage I)	No, currently limited sensitivity

Biomarker type	Source	Analytes	Advantages	Limitations	Clinical applications
Circulating tumour DNA	Tumour-derived DNA fragments in blood	Mutations, methylation, copy number variants	High tumour specificity; dynamic monitoring	Low abundance in early-stage cancers	Mutation detection, minimal residual disease, treatment response
Circulating tumour cells	Intact tumour cells in circulation	DNA, RNA, proteins, morphology	Comprehensive tumour profiling; potential for culturing	Rare and difficult to isolate	Prognosis, therapy selection, metastasis study
Exosomes	Extracellular vesicles secreted by tumours	RNA, DNA, proteins, lipids	Stable, protected cargo; reflects tumour microenvironment	Complex isolation; heterogeneous populations	Biomarker discovery, monitoring
MicroRNA	Small non-coding RNA fragments in blood or other fluids	MicroRNA expression profiles	Stable in circulation; potential for early detection	May lack tumour specificity; expression influenced by non-cancerous conditions	Early detection, prognosis, and therapy response prediction

Group/Markers	Significance
Circulating tumor cells	Diagnosis, prognostic, monitoring response to treatment
Circulating free microRNAs	Early diagnosis, prognostic, metastasis, monitoring, response to treatment
Circulating free DNA	Diagnosis and prognostic
Tumor-educated platelets	Early diagnosis, monitoring, response to treatment
Circulating extracellular vesicles	Diagnosis, metastasis, response to treatment
Metabolomic markers	Early diagnosis, prediction, response to treatment
Proteomics markers	Early diagnosis, prognostic, monitoring

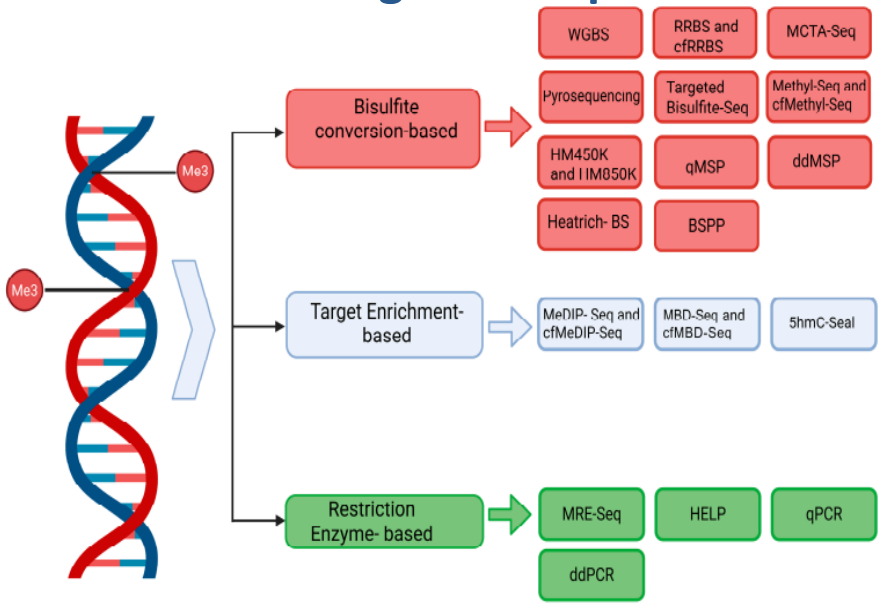


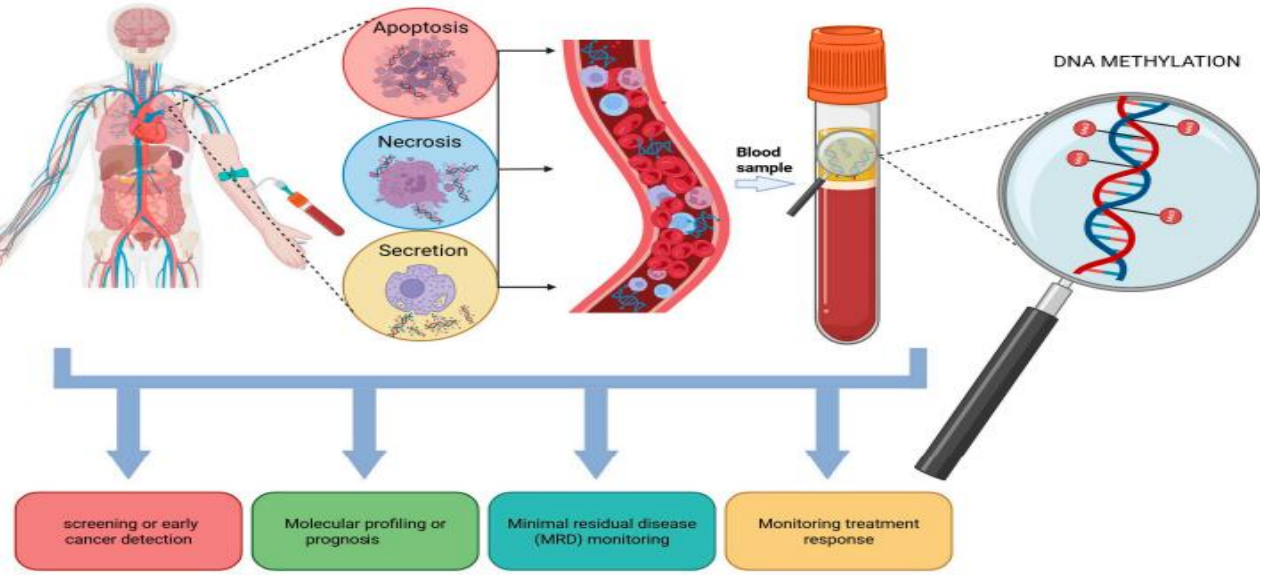
Liquid biopsy diagnostics for non-small cell lung cancer via elucidation of tRNA signatures



Feng Z,. Liquid biopsy diagnostics for non-small cell lung cancer via elucidation of tRNA signatures. Commun Med (Lond). 2025 Aug 21;5(1):364.

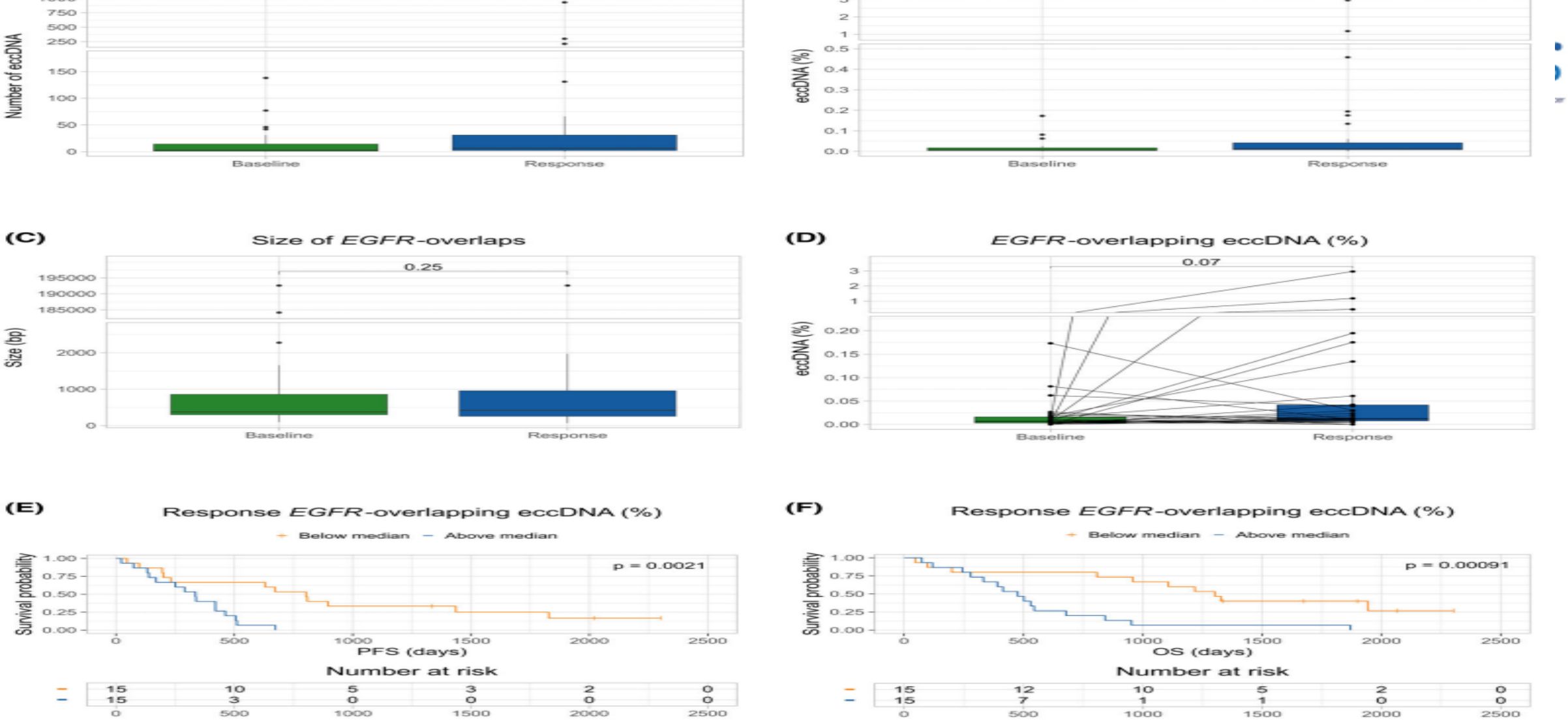
Methylation Analyses in Liquid Biopsy of Lung Cancer Patients: A Novel and Intriguing Approach Against Resistance to Target Therapies and Immunotherapies





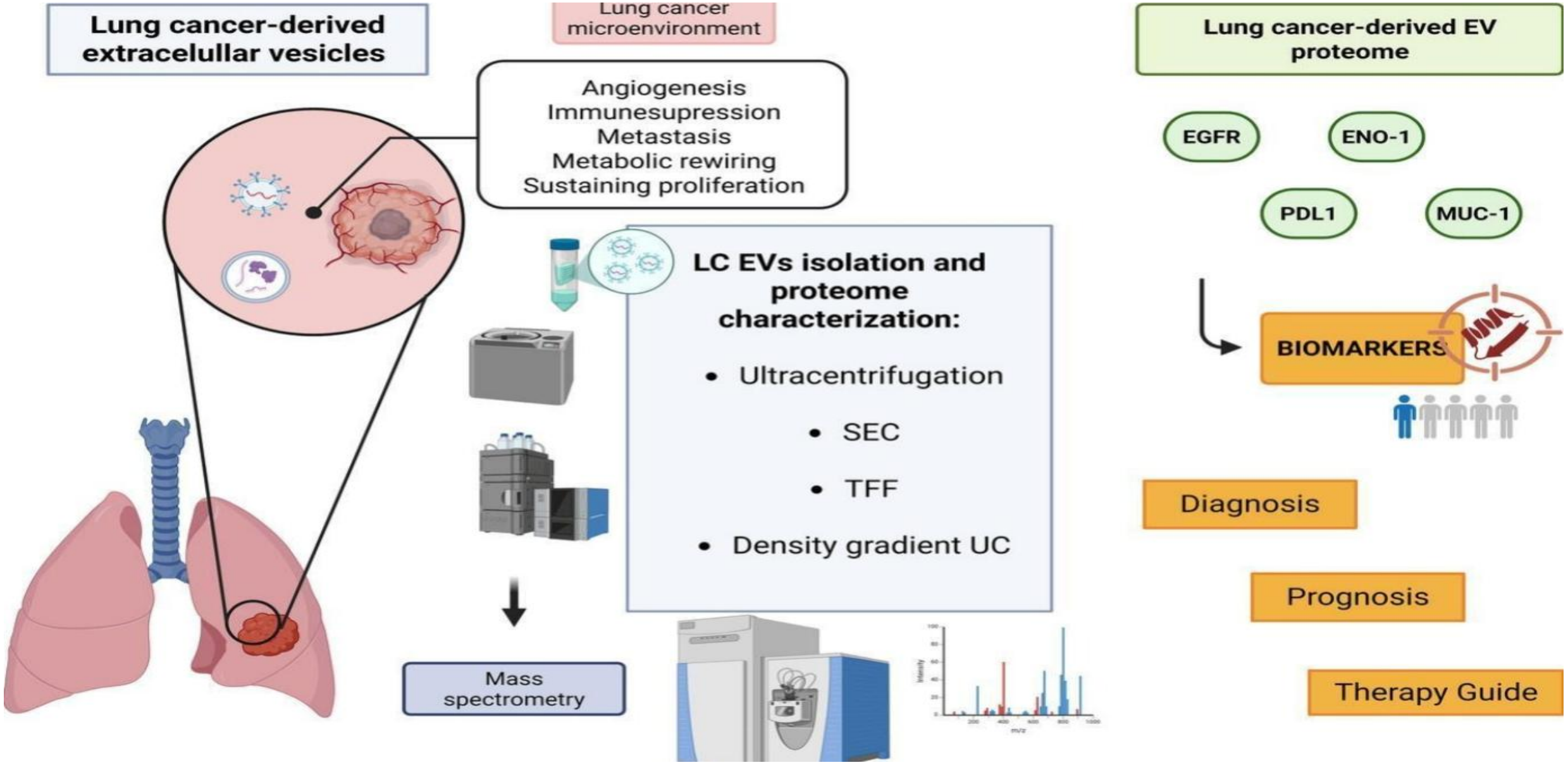
Target	Population and Therapy	Biological Effects
EGFR T790M mutation	8 pts with IV stage LUAD osimertinib (post-1L)	Methylation levels are higher in ctDNA of pts with detectable somatic mutations than in pts without somatic mutations. The decrease in methylation levels and maxAF reflects treatment efficacy and the increase reflects PD.
EGFR mutation	42 pts with IV stage LUAD osimertinib (post-1L)	A significant increase in methylation is found for at least one of the 9 tested genes at PD compared to baseline. Difference trend in PFS is shown between pts who are positive for DNA methylation of at least one gene at PD and those who are negative.
EGFR mutation	27 pts with IV stage LUAD osimertinib (post-1L)	The increase in methylation is found for at least one of the nine tested genes at PD compared with baseline in both plasma cfDNA and paired CTC analysis.
EGFR mutation	Pts with IV stage LUAD gefitinib (1L)	Methylation level of *WIF1* promoter is lower in the cfDNA of pts with a complete or partial response to gefitinib. Pts with hypomethylated WIF1 have better PFS and OS.
EGFR mutation	122 pts with III-IV stage LUAD gefitinib, erlotinib, afatinib	Higher hypomethylation is found in cases with on-target resistances compared with those with off-target mutations. Hipo-methylation and CNA correlate with the duration of response only in *EGFR* amplification cases.
EGFR mutation	103 pts with III-IV stage LUAD afatinib (1L)	cfDNA methylation levels are correlated with PFS are clustered in the cadherin, Wnt and EGFR signalling pathways. Pre-afatinib levels of *CEP170* and *CHCHD6* cfDNA methylation are associated with both PFS and OS. Pre-afatinib and post-afatinib levels of *SLC9A3R2* and *INTS1* cfDNA methylation correlate with bone metastasis.
EGFR mutation	32 pts with IV stage LUAD EGFR-TKI	Histone modifications, DNA methylation, and chromatin accessibility allow discrimination between cfDNA samples from pts with tSCLC and EGFR-mutated LAUD.
ALK-rearranged	21 pts with IV stage LUAD crizotinib, ceritinib, alectinib, brigatinib, lorlatinib	Higher 5-mC scores is associated with shorter OS. 5-mC scores can predict treatment response and PD.

Trombetta D,. Methylation Analyses in Liquid Biopsy of Lung Cancer Patients: A Novel and Intriguing Approach Against Resistance to Target Therapies and Immunotherapies. Cancers (Basel). 2025 Sep 16;17(18):3021.



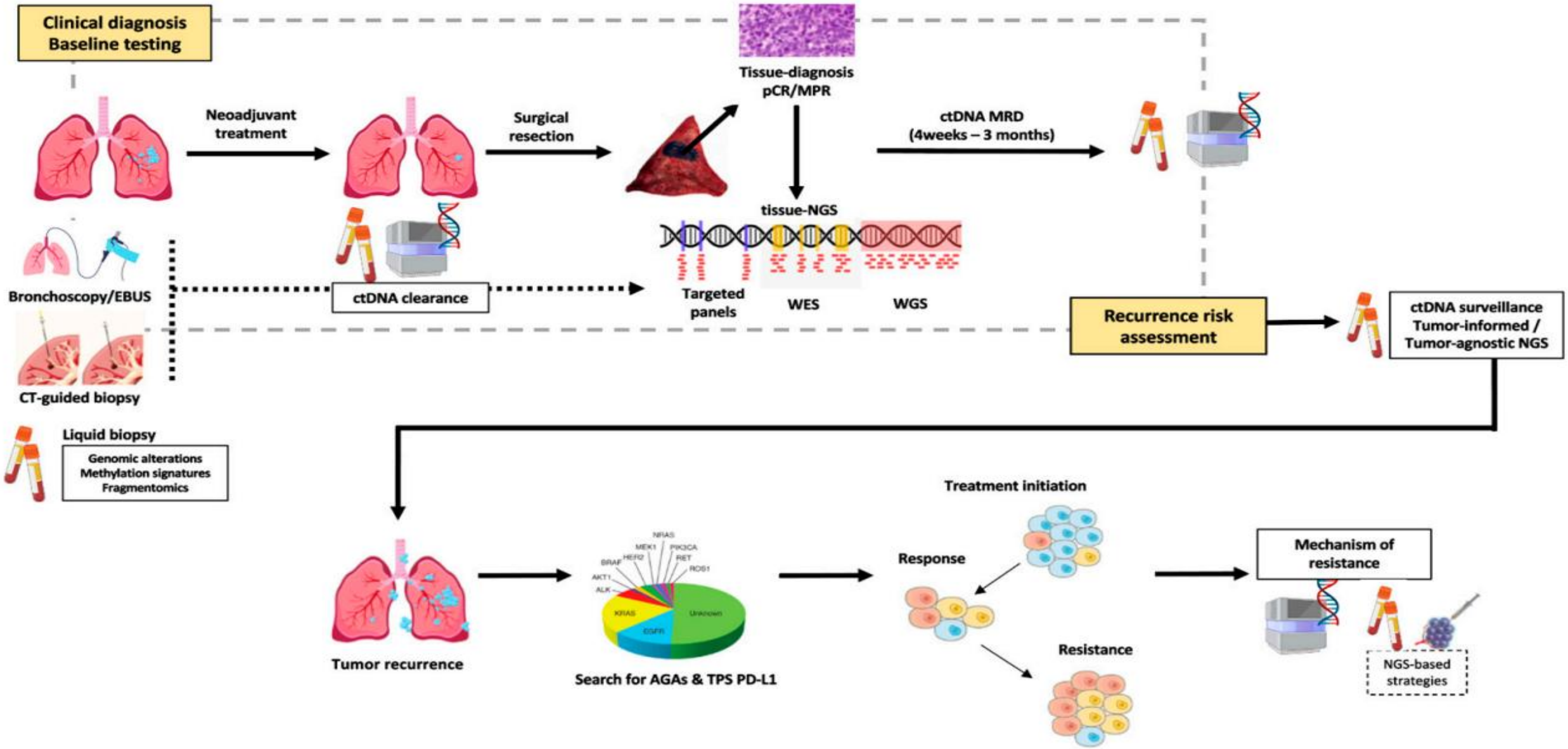
investigated whether the identified eccDNA contained parts of the EGFR gene; these eccDNA are referred to as EGFR-overlapping eccDNA. EGFR-overlapping eccDNA was identified in 29/32 (90.6%) of the baseline samples

Stensgaard S, BS. Plasma extrachromosomal circular DNA as a biomarker in EGFR-targeted therapy of non-small cell lung cancer. Mol Oncol. 2025 Oct 30.



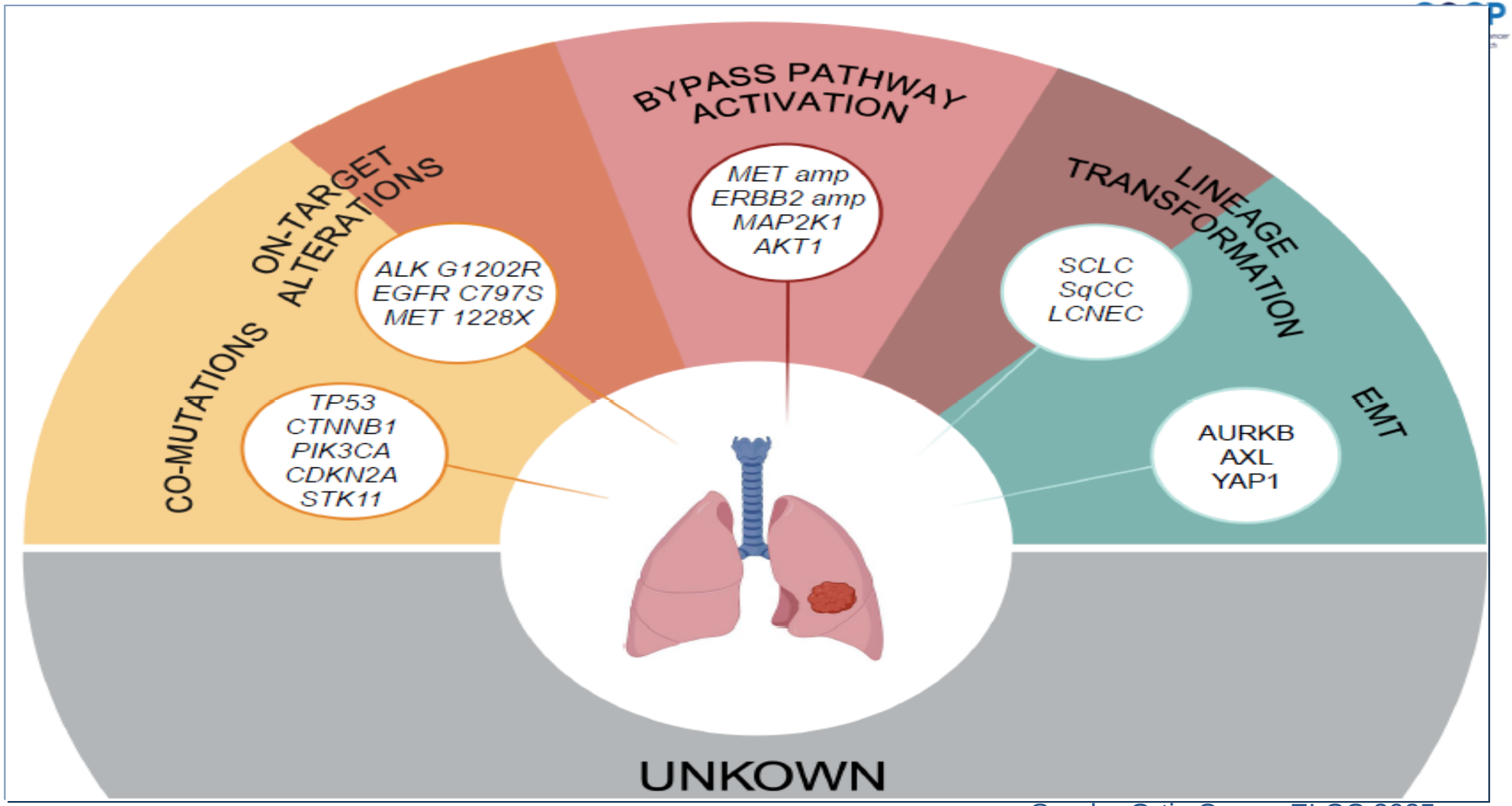
Morales-Tarré O, Navarro XP, Encarnación-Guevara S. The protein cargo of extracellular vesicles. Recent advances in lung cancer research. J Proteomics. 2025 Nov 7;323:105557

SUMMARY

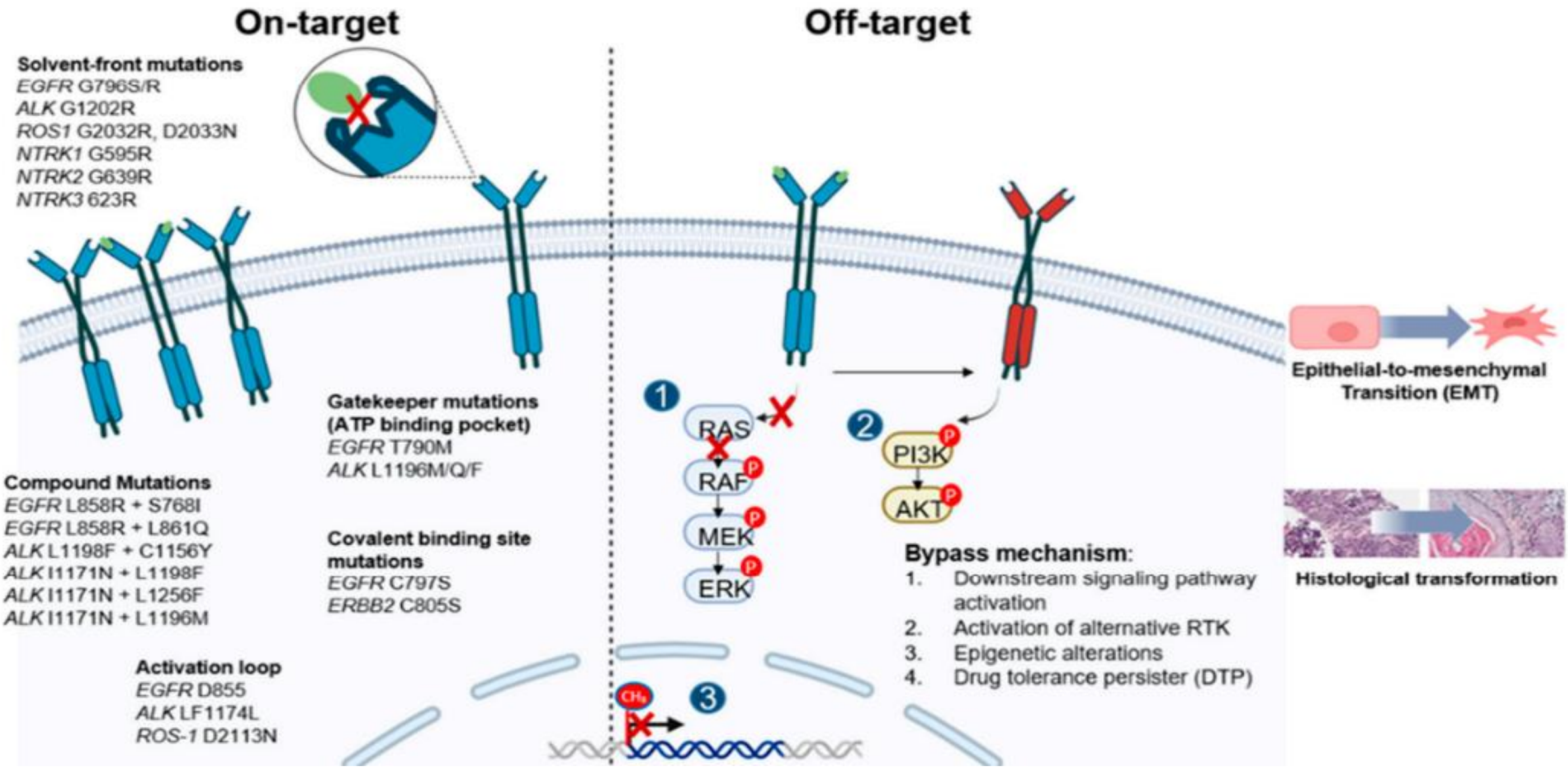


Urtecho SB, Jimenez Munarriz B, Rabey MR, Leighl NB. Liquid Biopsy in Lung Cancer: Tracking Resistance to Targeted Therapies. Cancers (Basel). 2025 Oct 29;17(21):3474.

Resistencia a las terapias dirigidas a Dianas moleculares

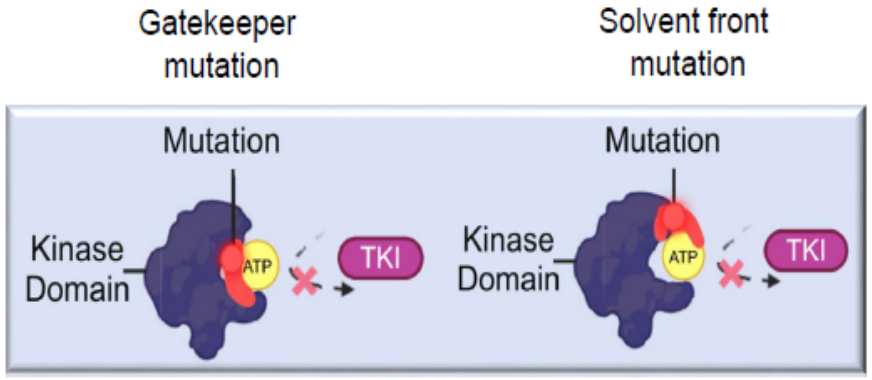


Sandra Ortiz Curan. ELCC 2025



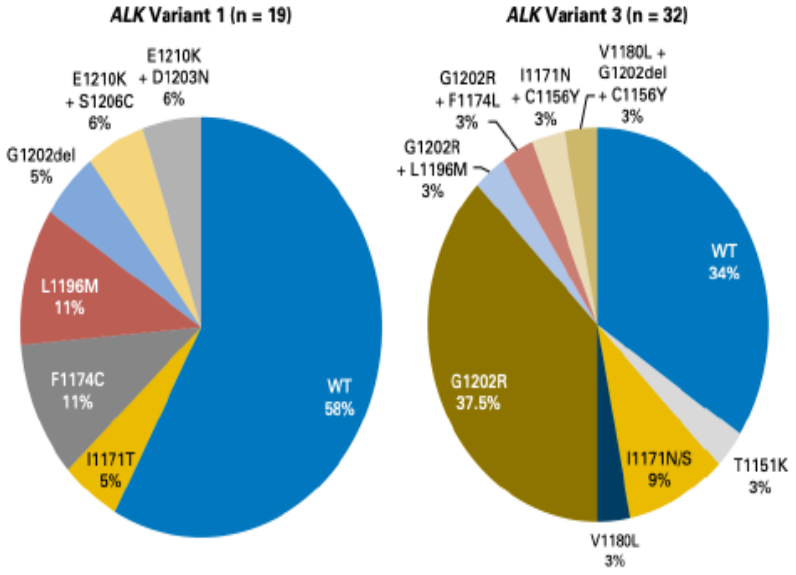
Urtecho SB, Jimenez Munarriz B, Rabey MR, Leighl NB. Liquid Biopsy in Lung Cancer: Tracking Resistance to Targeted Therapies. Cancers (Basel). 2025 Oct 29;17(21):3474.

Resistencia a las terapias diriginas a Dianas moleculares “On target”



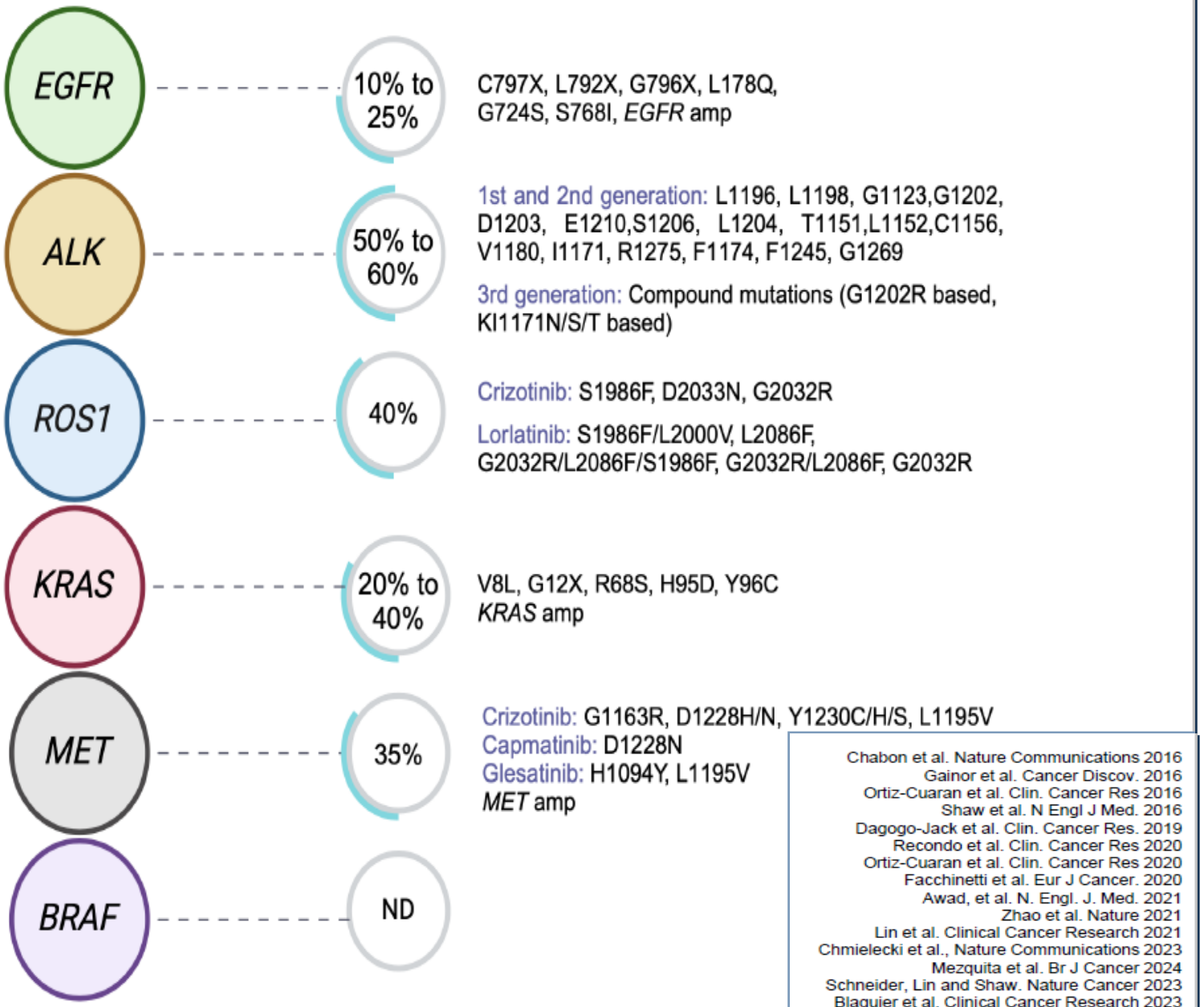
Adapted from Ou et al. MedComm 2024

ALK Resistance Mutations by Variant



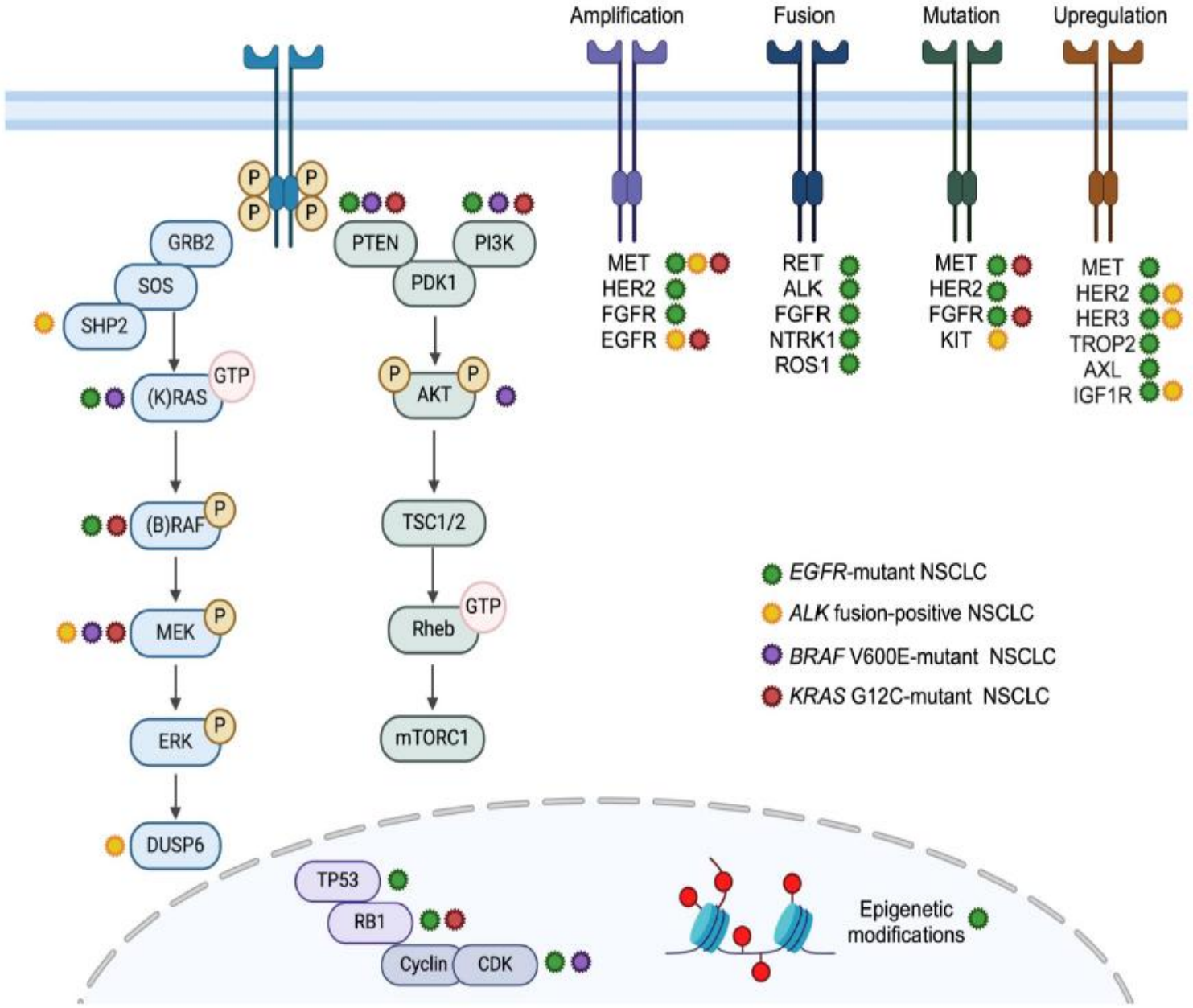
	Variant 1	Variant 3	*P*
% *ALK* mutations	42	66	.145
% G1202R	0	43.5	.001

Lin et al. J Clin Oncol. 2018



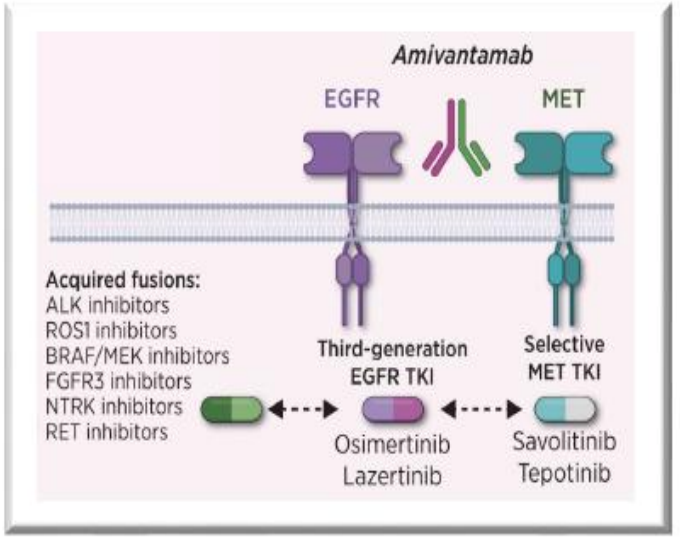
Chabon et al. Nature Communications 2016
Gainor et al. Cancer Discov. 2016
Ortiz-Cuaran et al. Clin. Cancer Res 2016
Shaw et al. N Engl J Med. 2016
Dagogo-Jack et al. Clin. Cancer Res. 2019
Recondo et al. Clin. Cancer Res 2020
Ortiz-Cuaran et al. Clin. Cancer Res 2020
Facchinetti et al. Eur J Cancer. 2020
Awad, et al. N. Engl. J. Med. 2021
Zhao et al. Nature 2021
Lin et al. Clinical Cancer Research 2021
Chmielecki et al., Nature Communications 2023
Mezquita et al. Br J Cancer 2024
Schneider, Lin and Shaw. Nature Cancer 2023
Blaquier et al. Clinical Cancer Research 2023

Activación`por “By Pass” Vías alternativas “Out target”



Targeting bypass pathway activation:

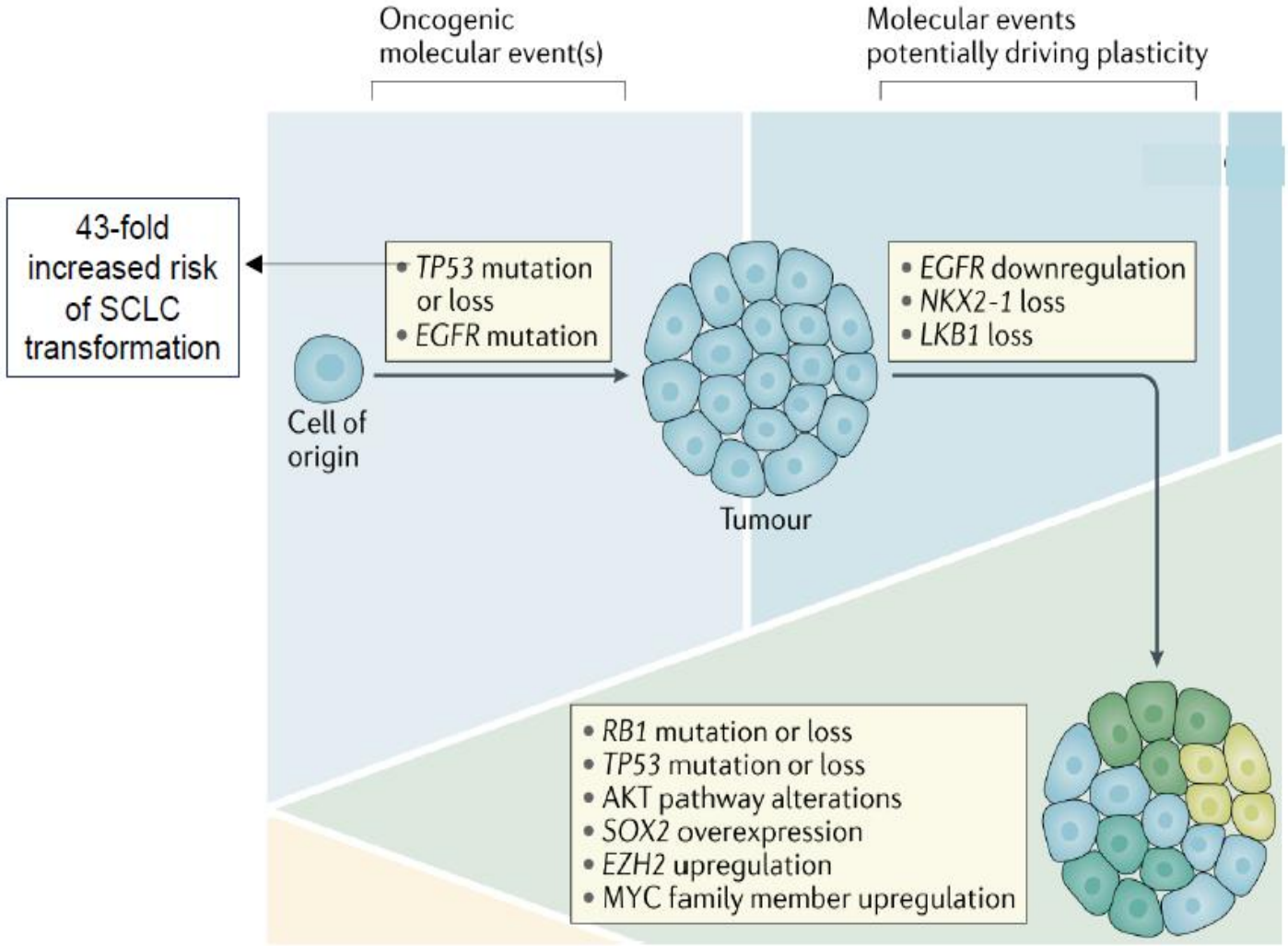
- ADC
- Specific driven TKI
- Combination of mAB plus TKI

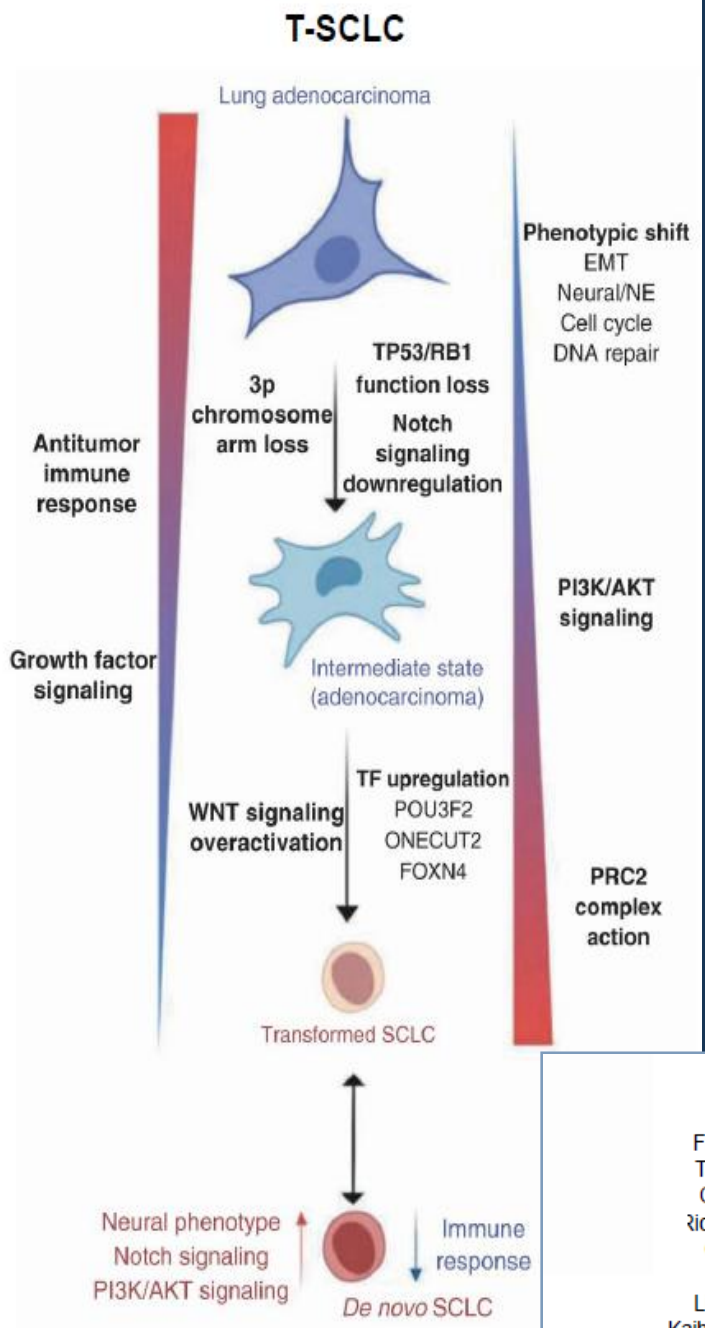


Ortiz-Cuaran et al. Clin. Cancer Res 2016
Ortiz-Cuaran et al. Clin. Cancer Res 2020
Facchinetti et al. Eur J Cancer. 2020
Awad, et al. N. Engl. J. Med. 2021
Zhao et al. Nature 2021
Mezquita et al. Br J Cancer 2024
Schneider, Lin and Shaw. Nature Cancer 2023
Adapted from Passaro et al. Nature Cancer 2021
Adapted from Blaquier et al. Clinical Cancer Research 2023

Lineage transformation to SCLC or SqCC

From adenocarcinoma to squamous or neuroendocrine carcinoma: 3% to 14% of patients with *EGFR-mutated* NSCLC.



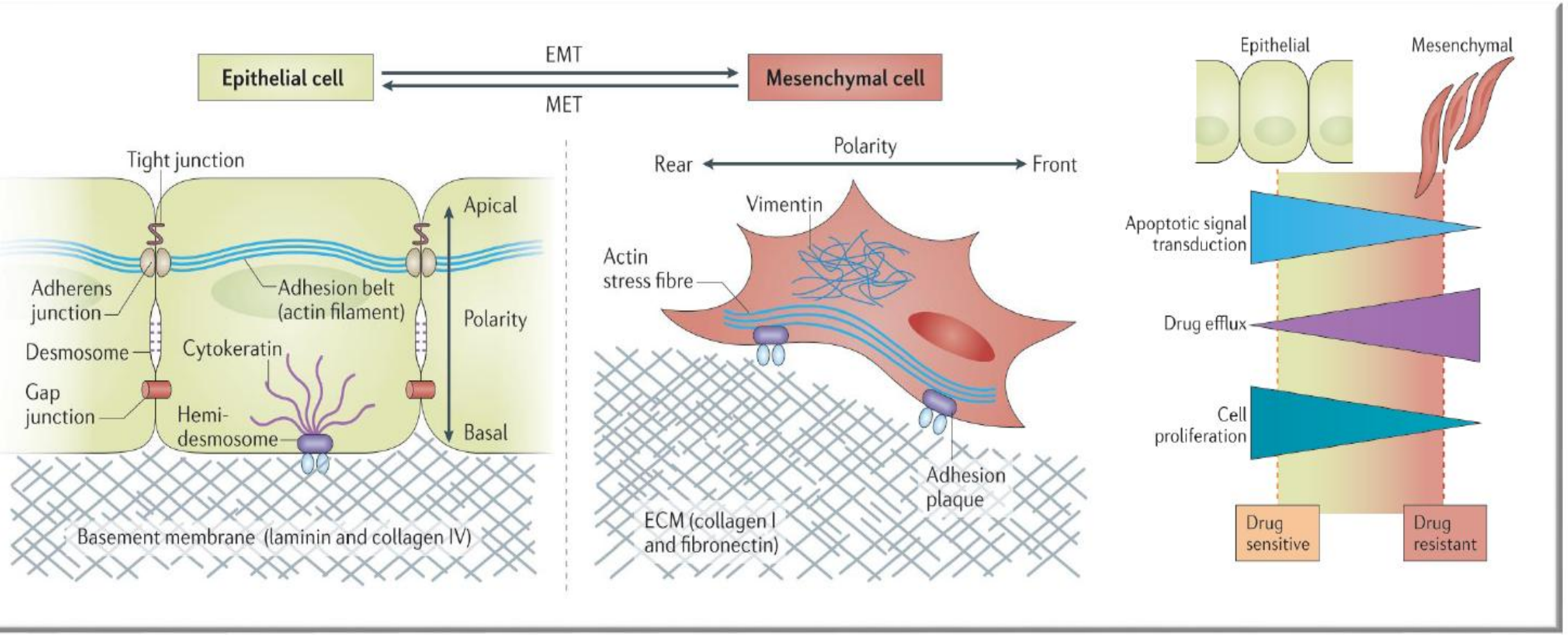


Sequist et al., STM 2011
Niederst et al. Nat Comm 2015
Oser et al. Lancet Oncol. 2015
Fujita et al. J. Thorac.Oncol. 2016
Takegawa et al. Ann. Oncol. 2016
Cha et al. J. Thorac. Oncol. 2016
Ricordel et al. J Thorac Oncol 2017
Okabe et al. J Thorac Onco2017
Lee et al. J. Clin. Oncol. 2017
Lin et al. NPJ Precis. Oncol. 2020
Kaiho et al. Onco Targets Ther. 2020
Schoenfeld et al. Clin Cancer Res 2020
Quintanal-Villalonga et al. Cancer Discovery 2021
Quintanal-Villalonga et al. J Hematol Oncol 2021

Sandra Ortiz-Cuaran

SqCC differentiation occurrs in vivo and in vitro → does not require stromal cells.

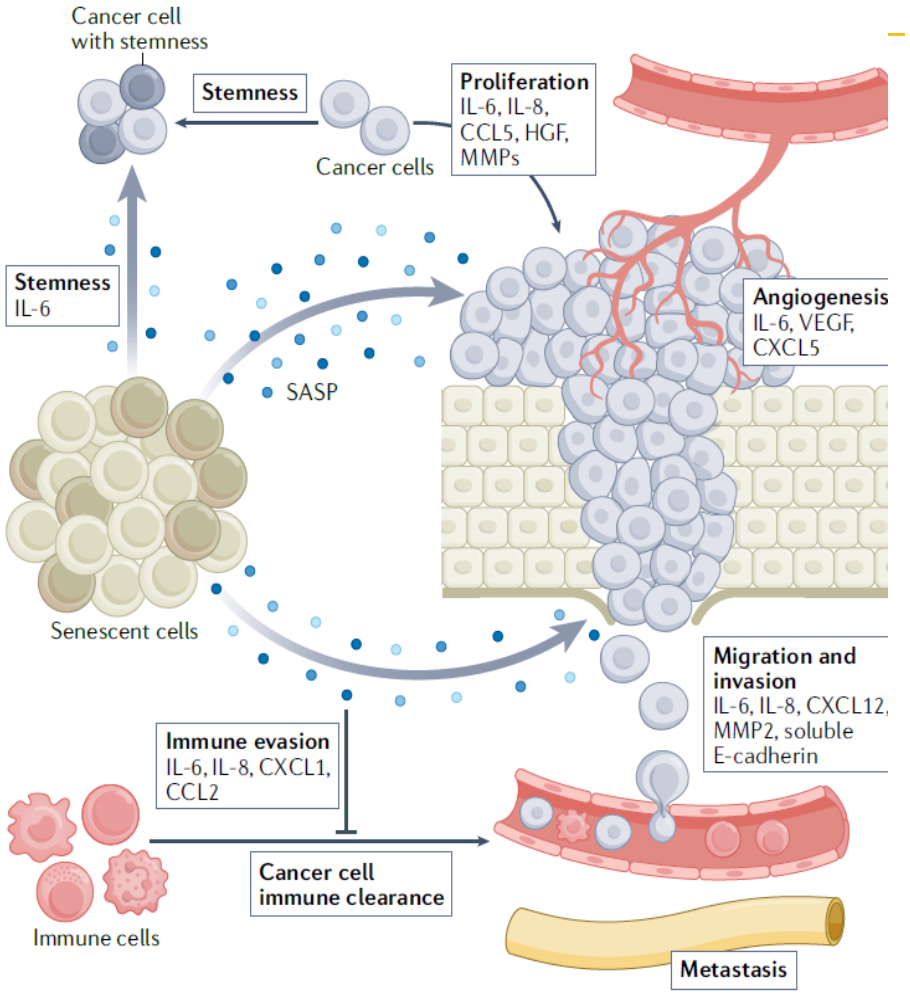
Phenotypic switching via epithelial-to-mesenchymal transition

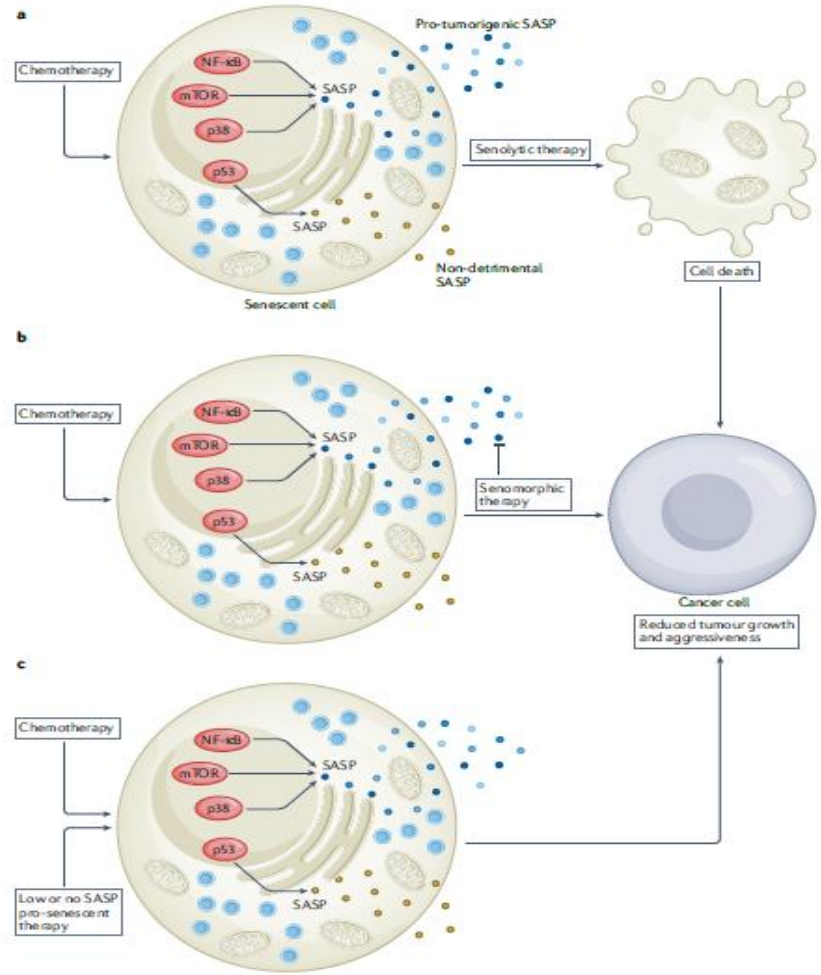


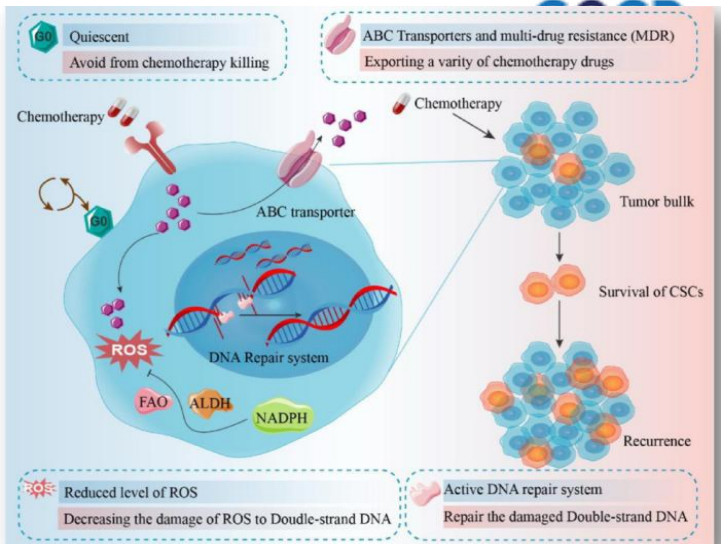
Shibue and Weinberg. Nat Rev Clin Oncol. 2017
Zhang et al., Nat Commun. 2016
Larsen et al., J Clin Invest. 2016

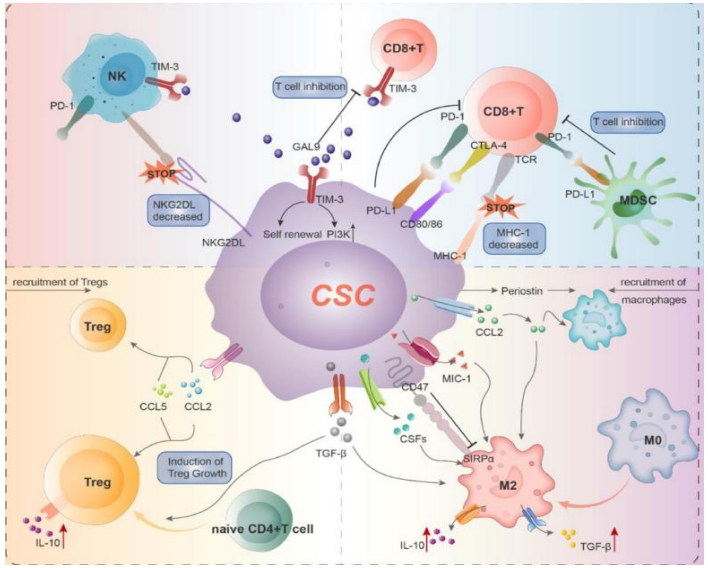
Induction of senescence by Target drugs induces the STEM phenotype:

senolitic or senomorfic therapy









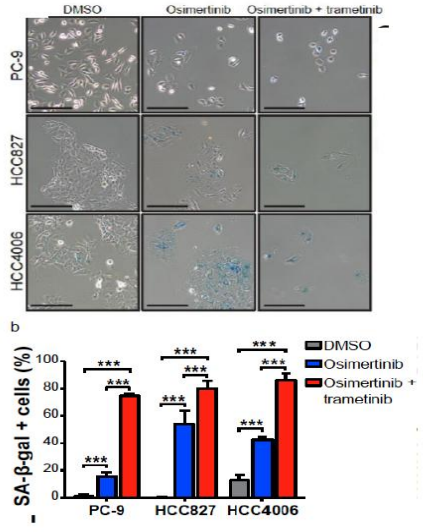
Roles of cellular senescence in tumour promotion. Following induction of senescence by intrinsic or therapeutic stresses, cancer cells can secrete senescence- associated factors that mediate secondary effects on tumour progression and contribute to **cancer stemness**, proliferation, migration, invasion and metastasis, thus enhancing the malignant potential of the cancer cell population.

Without senolitic or senomorfic therapy,
Target drugs induced senescence
And so: STEMNESS phenotype

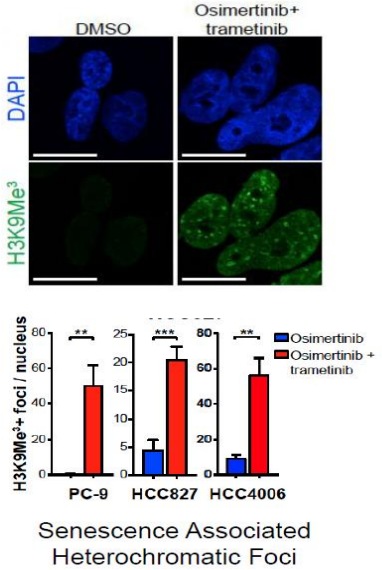
STEM pnenotype induced immunoresistence
And resistance to target drugs

Clemens A. Schmitt. Nature Review Clin Oncol 2022
Wenwen Guo. Sem in cancer Biol 2022

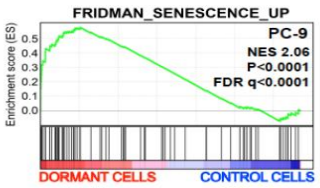
DTP cells exhibit features of cellular senescence



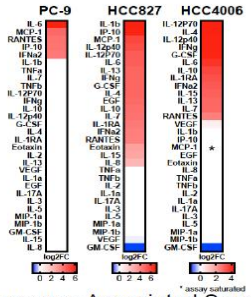
β-galactosidase activity



Senescence Associated Heterochromatic Foci



Senescence Associated Gene Signature



Senescence Associated Secretory Phenotype

Kari Kurppla, Heidi Haikala, Tran Thai, David Barbie

cancers

MDPI

Review

Senolytics for Cancer Therapy: Is All that Glitters Really Gold?

Valerie J. Carpenter [1,2], Tareq Saleh [3] and David A. Gewirtz [1,2,*]

FEBS Journal

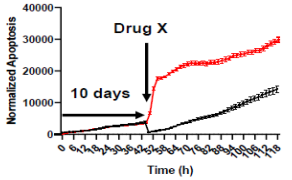
FEBS PRESS

DISCOVERY-IN-CONTEXT REVIEW

Discovery, development, and future application of senolytics: theories and predictions

Erin O. Wissler Gerdes, Yi Zhu, Tamar Tchkonia and James L. Kirkland

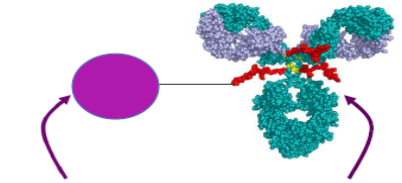
Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, USA



DRUG	TARGET	Apoptosis induction in DTPS ?
NAVITOCLAX	Bcl-2/Bcl-xL/Bcl-w	+++
A-1155463	Bcl-xL	+++
AZD8055	mTOR	-
QUERCETIN	Bcl-2 family	-
SERTRALINE	mTOR/BIM induction	-
DIGOXIN	Na/K ATPase	-
PANOBINOSTAT	HDAC	-
17-DMAG	HSP-90	-

Kari Kurppa and Simon Baldacci

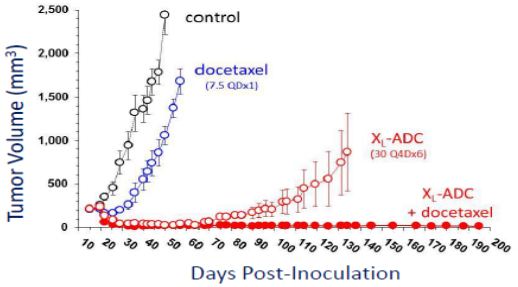
Targeted delivery of a BCL-XL inhibitor using antibody drug conjugate technology



BCL-XL Inhibitor Targeting Antibody

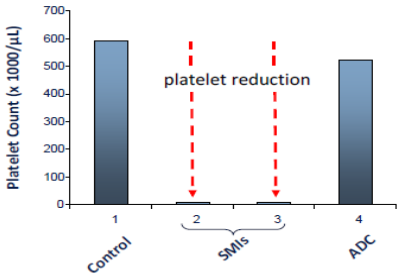
- Targeted delivery to tumor tissues
- Potentially avoidance of systemic toxicity (thrombocytopenia)
- ? Wider therapeutic index

NSCLC Xenograft model (NCI-H1650, EGFR^{3+})



Maximum Platelet Reduction in Mouse

- Bcl-X_Li reduce platelets >90% by 6 hr
- Bcl-X_Li-ADC shows negligible platelet ↓



Andrew Souers - AbbVie

Molecular vulnerabilities of EMT in TKI resistant NSCLC

Different types of bispecifics (bsAb) in development

bsAbs for dual receptor inhibition
- amivantamab
- zenocutuzumab
- biparatopic bsABs

bsAbs for ligand-receptor inhibition
- ivonescimab

bsAbs for receptor activation

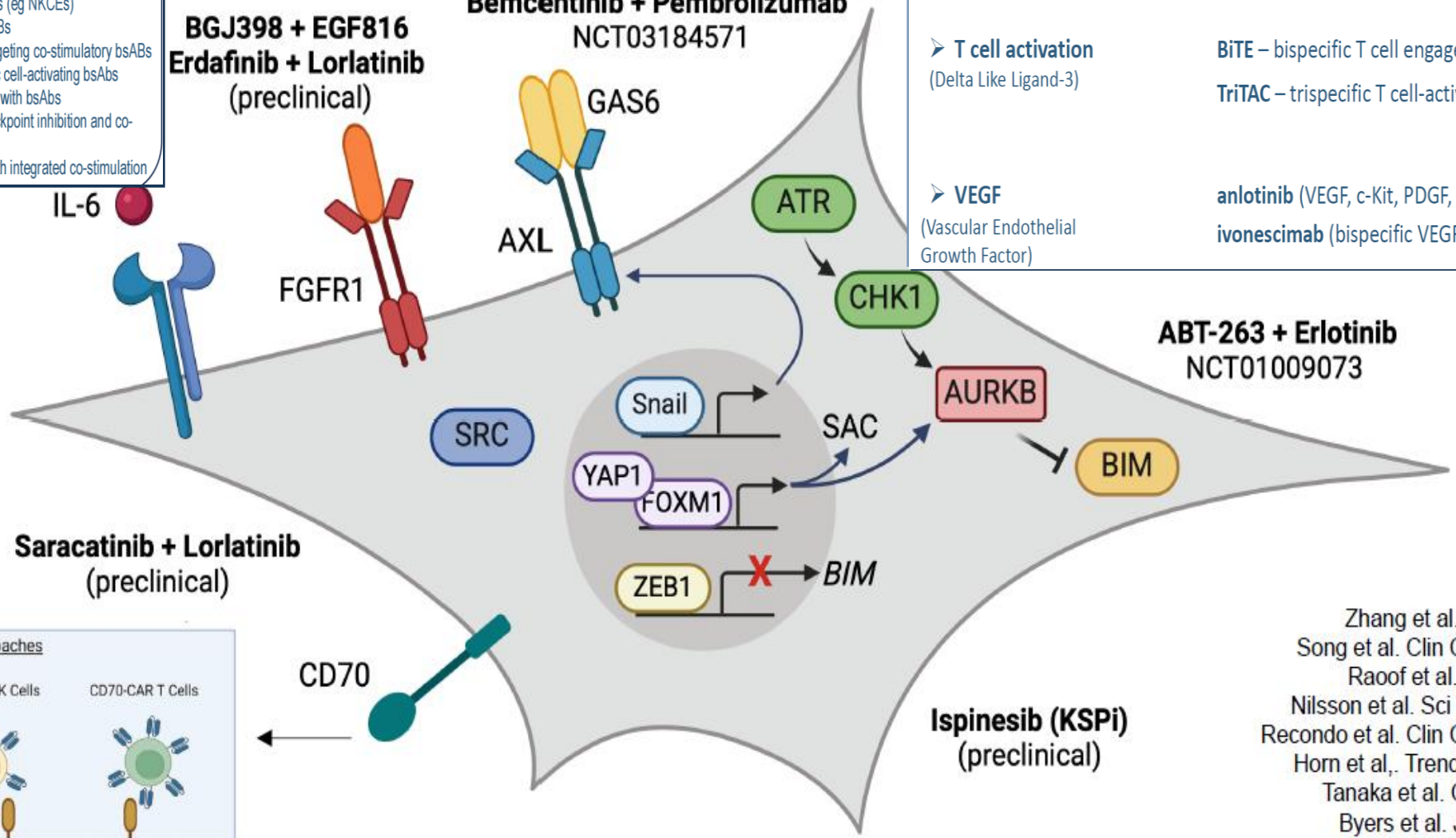
bsAbs for targeted payload delivery
- E.G. bsADCs

Dual CPIs
- PD1 x CTLA4 or PD1 x LAG3

Effector cell engagers
- T-cell engagers (CD3 engaging)
- Innate cell engagers (eg NKCEs)
- Co-stimulatory bsABs
- Tumour antigen targeting co-stimulatory bsABs
- MDCs and dendritic cell-activating bsAbs
- Dual co-stimulation with bsAbs
- bsAbs for dual checkpoint inhibition and co-stimulation
- Trispecific TCEs with integrated co-stimulation

Bemcentinib + Erlotinib
NCT02424617 (I/II) - 1 PR and 9 SD
Bemcentinib + Pembrolizumab
NCT03184571

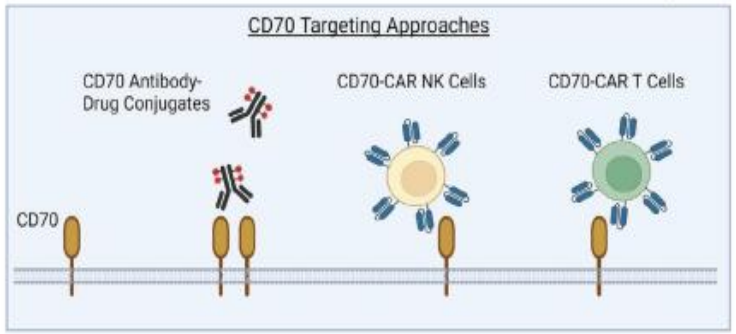
BGJ398 + EGF816
Erdafinib + Lorlatinib
(preclinical)



ABT-263 + Erlotinib
NCT01009073

Saracatinib + Lorlatinib
(preclinical)

Ispinesib (KSPi)
(preclinical)



- **ADC** (Antibody Drug Conjugate)
  - **TROP-2** (Trophoblast cell surface antigen 2)
  - **B7-H3** (immune checkpoint protein CD276)
  - **SEZ6** (Seizure-related 6 homolog)
- **T cell activation** (Delta Like Ligand-3)
  - **BiTE** – bispecific T cell engager
  - **TriTAC** – trispecific T cell-activating construct
- **VEGF** (Vascular Endothelial Growth Factor)
  - **anlotinib** (VEGF, c-Kit, PDGF, FGF inhibitor)
  - **ivonescimab** (bispecific VEGF and PD-1 inhibitor)

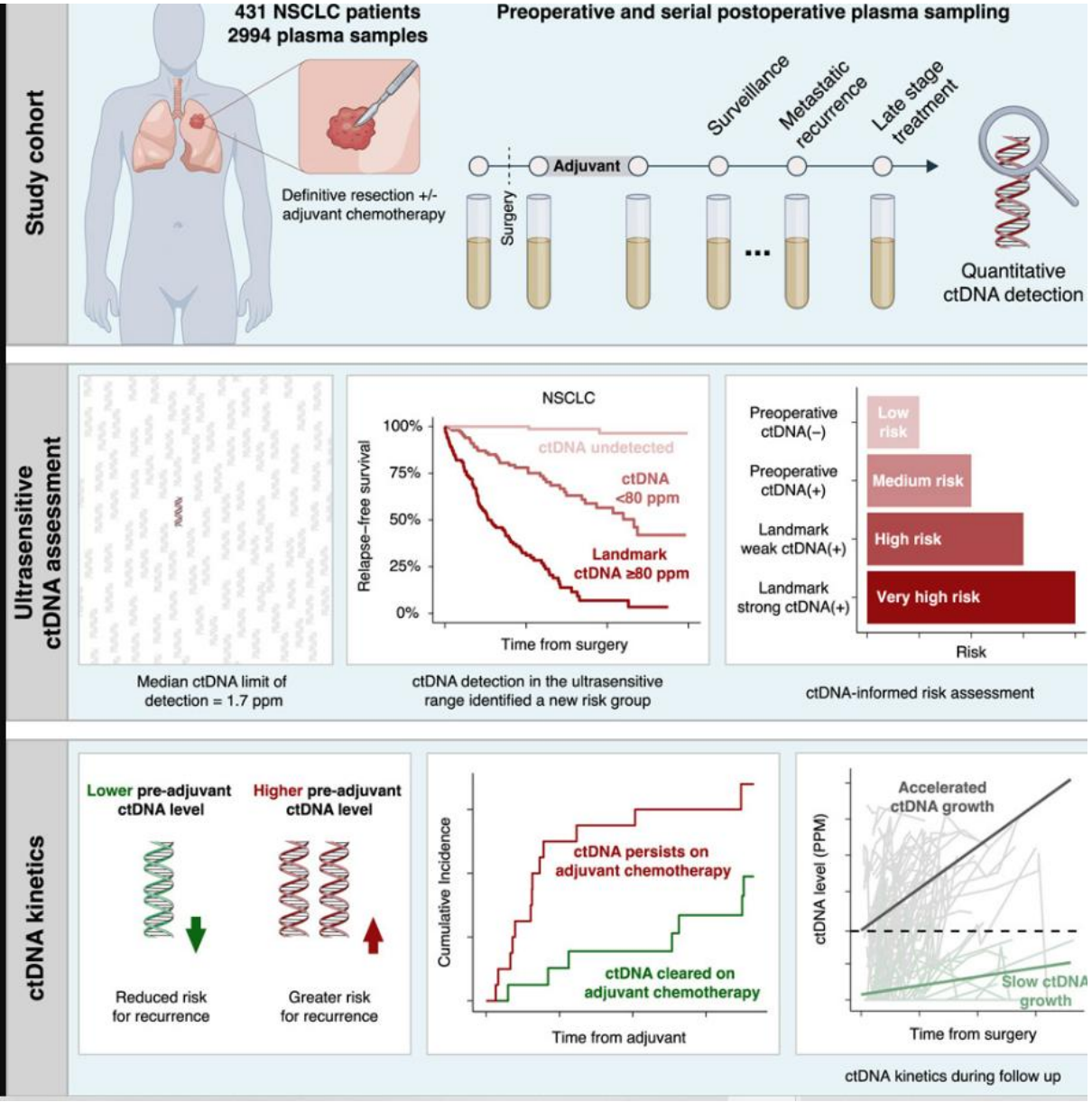
Zhang et al. Nat Genet. 2012
Song et al. Clin Cancer Res. 2018
Raoof et al., Oncogene 2019
Nilsson et al. Sci Transl Med. 2020
Recondo et al. Clin Cancer Res. 2020
Horn et al,. Trends in Cancer 2020
Tanaka et al. Cancer Cell. 2021
Byers et al. J Clin Oncol 2021
Nilsson et al. Cancer Cell 2023
Patel et al., Clin Cancer Res 2023

SUMMARY

1.- New technology approach

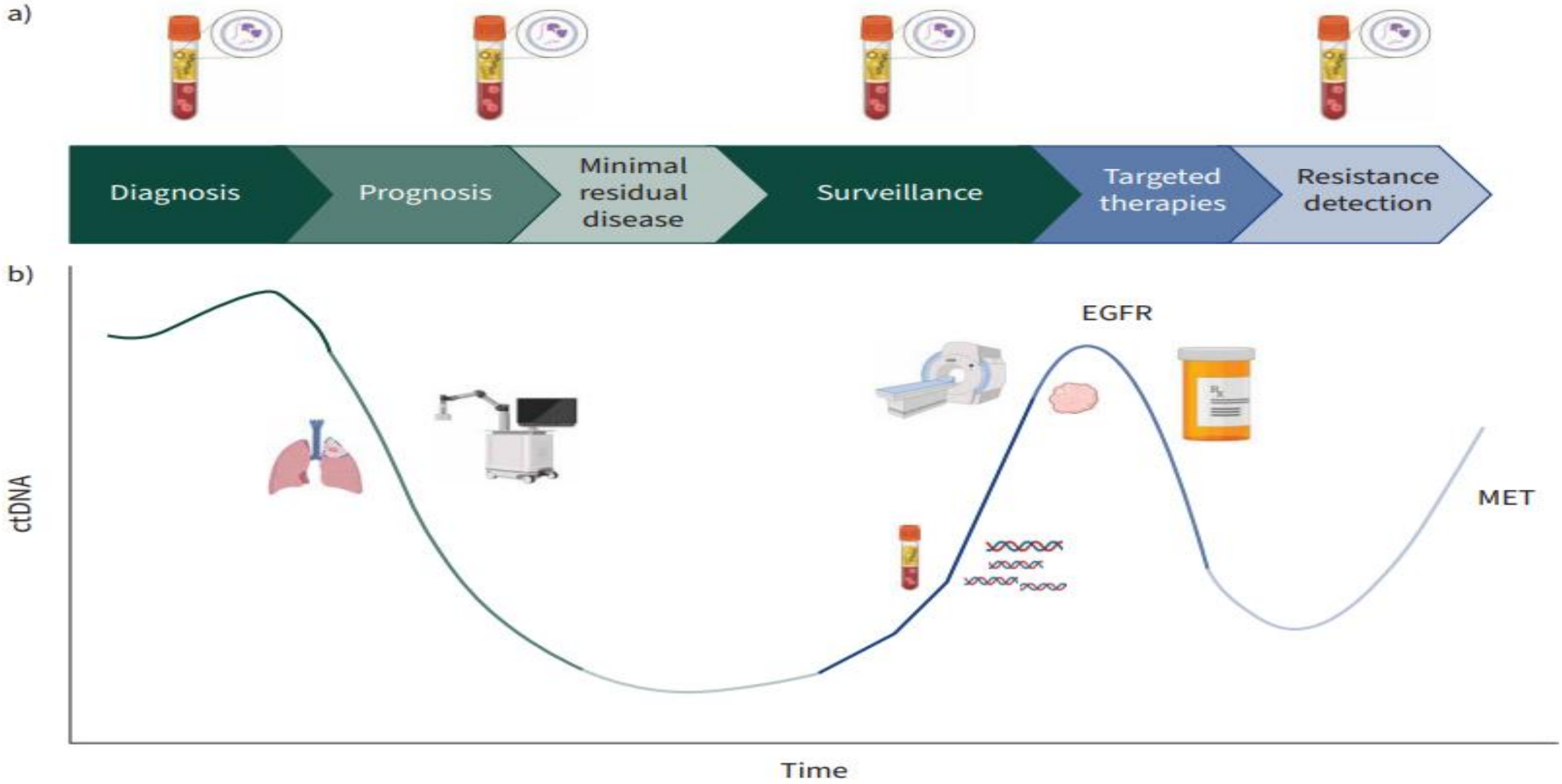
2.- Monitoring mechanism of resistance

3.- Monitoring response and early relapse



Preoperative ctDNA status	Landmark ctDNA status	ctDNA risk	Potential clinical recommendations
Preoperative ctDNA(-)		Low	• Infrequent (e.g. annual) intrathoracic radiographic surveillance Stage I: de-escalation of surveillance acceptable • Systemic screening could be omitted in this group • Consider clinical trials for de-escalation of adjuvant therapy
Preoperative ctDNA(+)	Landmark ctDNA(-)	Medium	• Infrequent (e.g 6 monthly) intra and extrathoracic radiographic surveillance Stage I: de-escalation of surveillance acceptable • Give adjuvant therapy where appropriate (clinical trials of de-escalation of adjuvant therapy could be considered) • High-cadence longitudinal ctDNA monitoring to detect impending relapse
	Landmark weak ctDNA(+)	High	• More frequent scanning (e.g. intra and extrathoracic radiographic surveillance 3-6 monthly) • Consider adjuvant therapy even in early-stage 1 disease • High-cadence longitudinal ctDNA monitoring through adjuvant therapy for clearance and thereafter
	Landmark strong ctDNA(+)	Very high	• More frequent scanning (e.g. intra and extrathoracic radiographic surveillance 3 monthly) • Consider adjuvant therapy even in early-stage 1 disease • Consider clinical trials for escalation of adjuvant therapy • High-cadence longitudinal ctDNA monitoring for treatment response

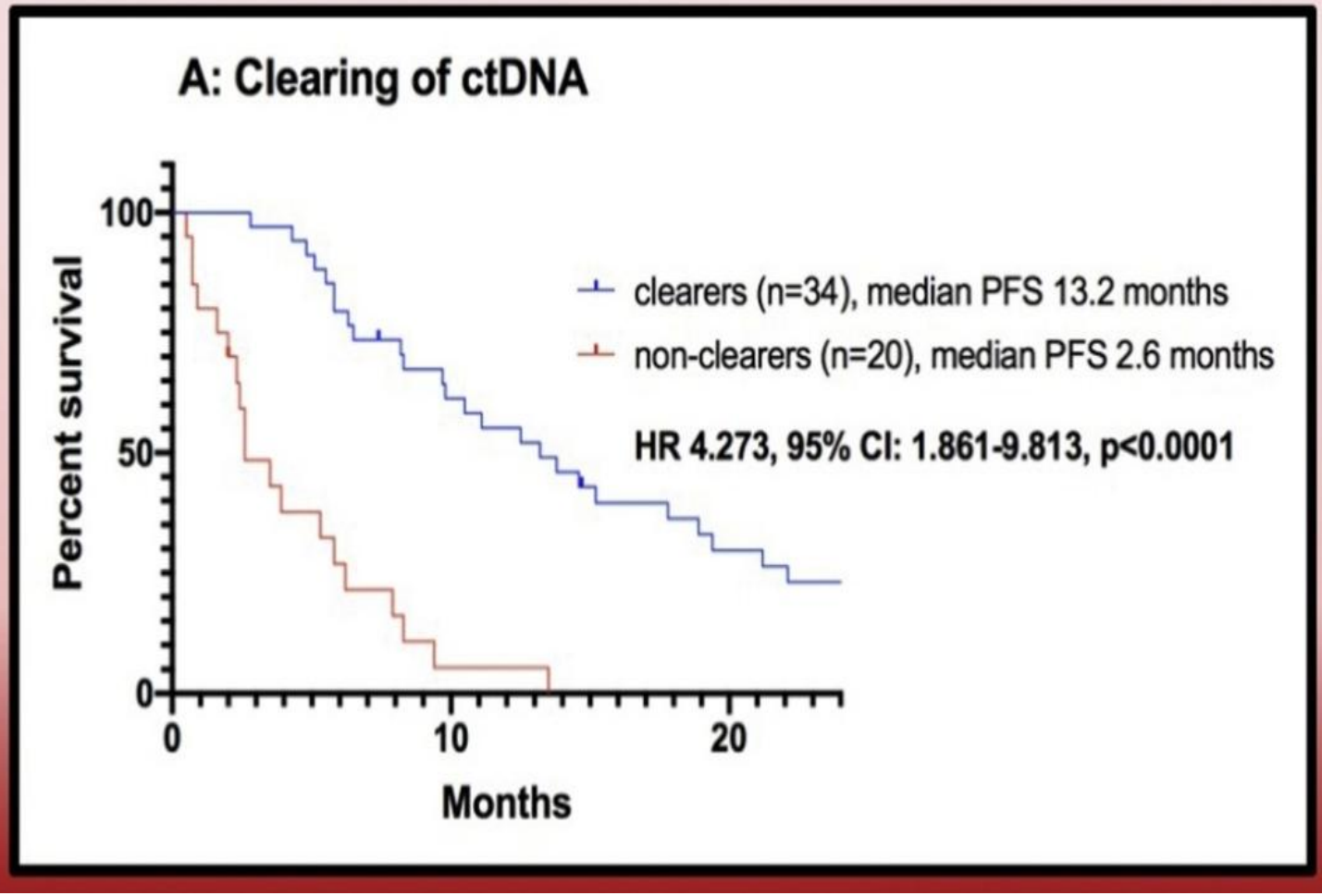
Black JRM,. Longitudinal ultrasensitive ctDNA monitoring for high-resolution lung cancer risk prediction. Cell. 2025 Nov 7:S0092-8674(25)01192-4


a)
Diagnosis
Prognosis
Minimal residual disease
Surveillance
Targeted therapies
Resistance detection
b)
ctDNA
EGFR
MET
Time

EGFR mutations in ctDNA at PD to 1st line systemic therapy

Osimertinib as 2nd line systemic therapy

ctDNA: clearers vs. non-clearers



Boysen Fynboe. Clearing of circulating tumour DNA predicts clinical response to osimertinib in EGFR mutated lung cancer patients. Lung Cancer. 2020 May;143:67-72

Predictive significance of circulating tumor DNA against patients with T790M-positive EGFR-mutant NSCLC receiving osimertinib.

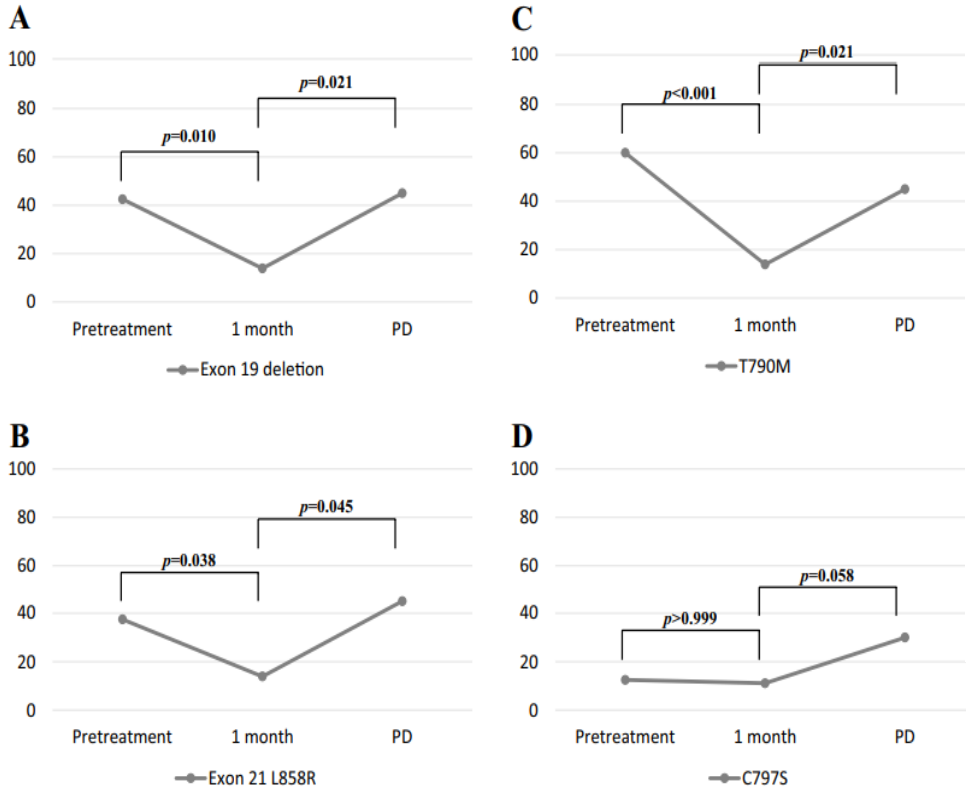


Figure 2. Comparison of positive rate of copy numbers in exon 19 deletion (**A**), L858R (**B**), T790M (**C**), and C797S (**D**), according to pretreatment, 1 month, and at progressive disease after osimertinib initiation.

Different variables		N = 40 (%)
Age (year)	Median (range)	69 (33–85)
Gender	Male/Female	12/28 (30.0/70.0)
Clinical stage	III/IV/Ope rec.	4/30/6 (10.0/75.0/15.0)
Smoking history	Yes/No	9/31 (22.4/77.6)
ECOG PS	0–1/2	35/5 (87.5/12.5)
Histology	AC/other	40/0 (100/0)
EGFR mutation status	Exon 19/L858R/L861Q	24/15/1 (60.0/37.5/2.5)
Numbers of prior treatment	1/2 or more lines	29/11 (72.5/27.5)
Prior EGFR-TKIs regimens	Gefitinib/Erlotinib/Afatinib	11/12/17 (27.5/30.0/42.5)
Brain metastases	Yes/No	17/23 (42.5/57.5)
PD-L1 expression	≥ 50%/< 50%/unknown	1/17/22 (2.5/42.5/55.0)

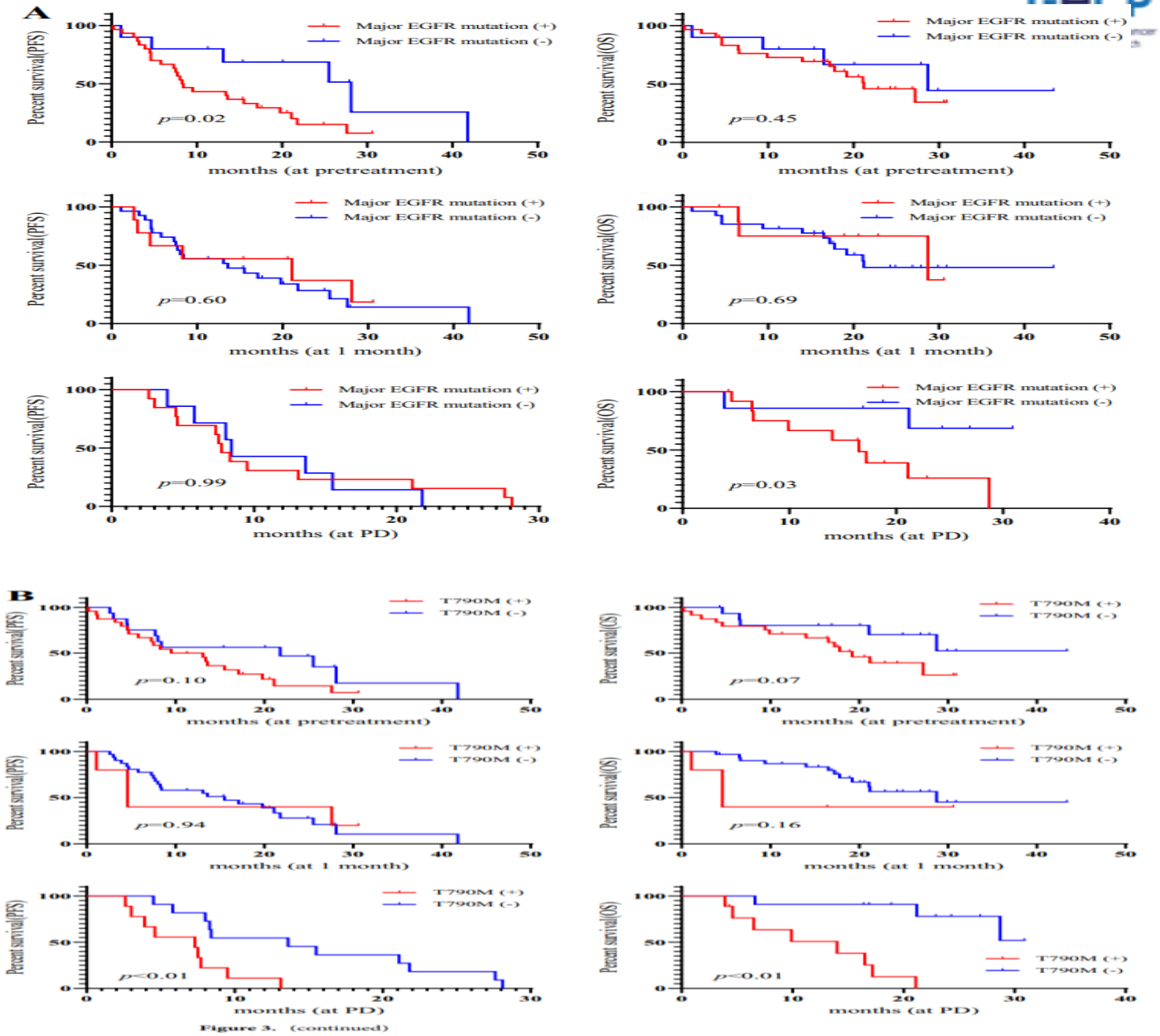
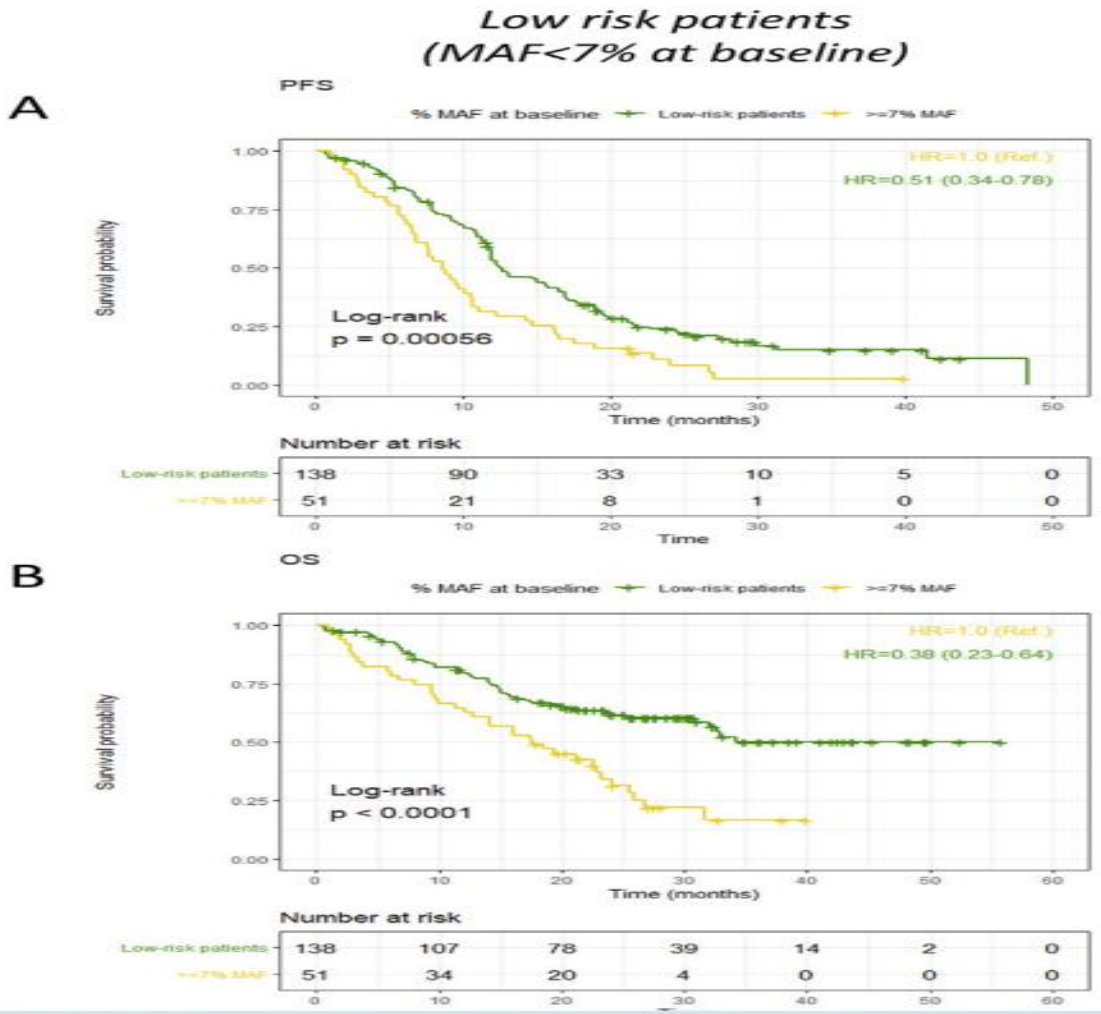
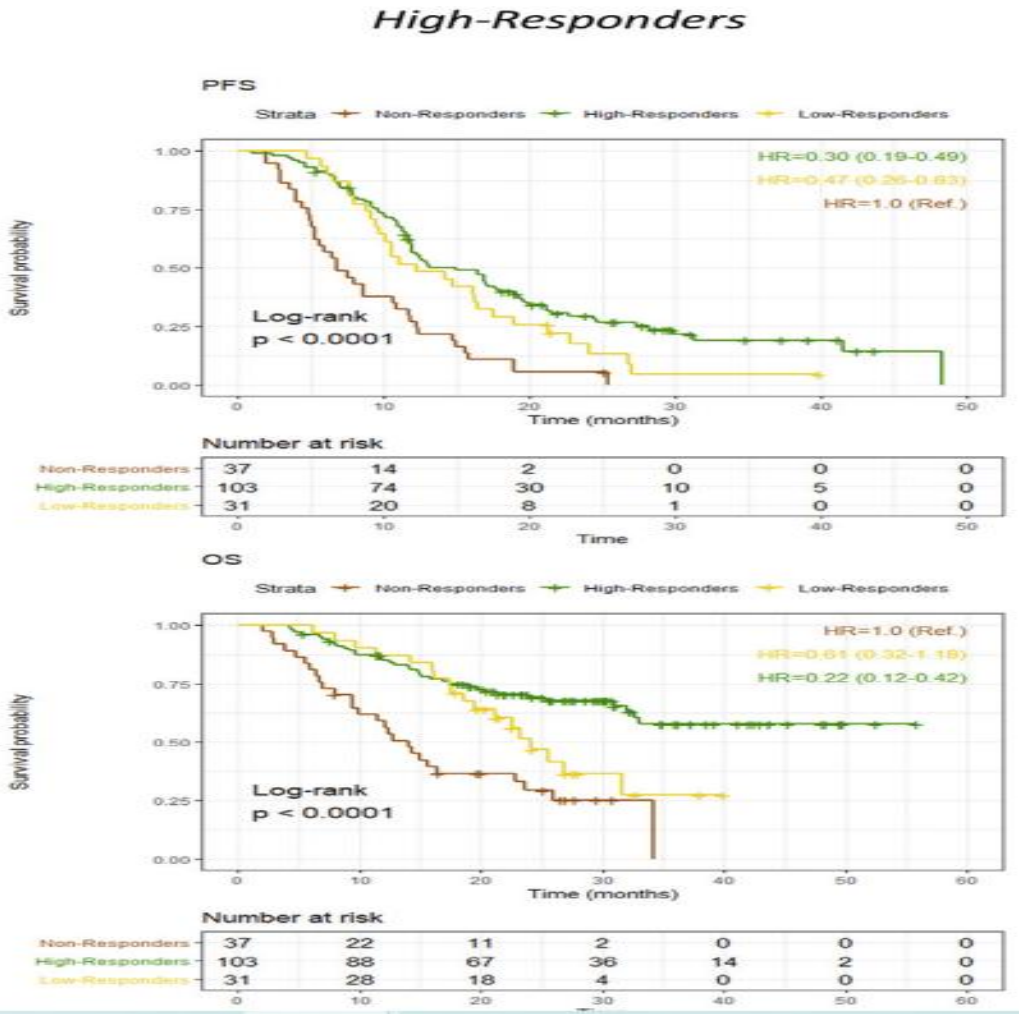


Figure 3. (continued)

Yamaguchi O, Predictive significance of circulating tumor DNA against patients with T790M-positive EGFR-mutant NSCLC receiving osimertinib. Sci Rep. 2023 Nov 27;13(1):20848.

Analysis of circulating tumour DNA to identify patients with epidermal growth factor receptor-positive non-small cell lung cancer who might benefit from sequential tyrosine kinase inhibitor treatment





Curves for high responders (patients with an MAF at baseline and undetectable ctDNA at 3 or 6 months) are coloured in green.

Provencio M, Serna-Blasco R, Franco F, Calvo V, Royuela A, Auglytė M, Sánchez-Hernández A, de Julián Campayo M, García-Girón C, Dómine M, Blasco A, Sánchez JM, Oramas J, Bosch-Barrera J, Sala MÁ, Sereno M, Ortega AL, Chara L, Hernández B, Padilla A, Coves J, Blanco R, Balsalobre J, Mielgo X, Bueno C, Jantus-Lewintre E, Molina-Vila MÁ, Romero A. Analysis of circulating tumour DNA to identify patients with epidermal growth factor receptor-positive non-small cell lung cancer who might benefit from sequential tyrosine kinase inhibitor treatment. Eur J Cancer. 2021 May;149:61-72.

IASLC 2025 World Conference on Lung Cancer

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wclc.iaslc.org #WCLC25

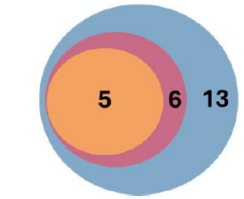
Comparative Analysis of Circulating Tumor DNA Monitoring Strategies in Advanced NSCLC With MET Exon 14 Skipping Treated With Ensartinib: A Biomarker Study Embedded in the EMBRACE Trial

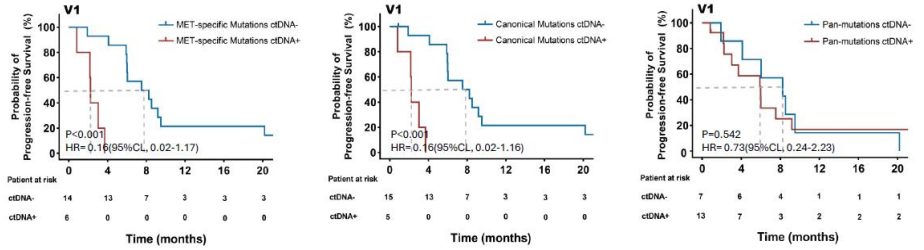
Yang Xia, Mo Zhou, Jing Zhao, Xuqi Sun, Haifeng Shen, Xiuning Le

Associate Professor
Vice Chair, Department of Pulmonary Medicine,
Second Affiliated Hospital,

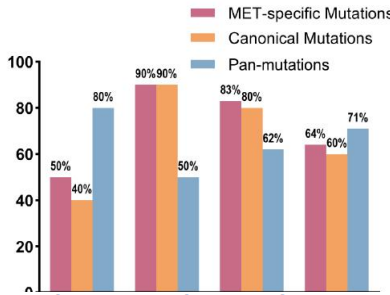
IASLC 2025 World Conference on Lung Cancer SEPTEMBER 6-9, 2025 | BARCELONA, SPAIN #WCLC25

The association between ctDNA after 4-week ensartinib and treatment outcomes





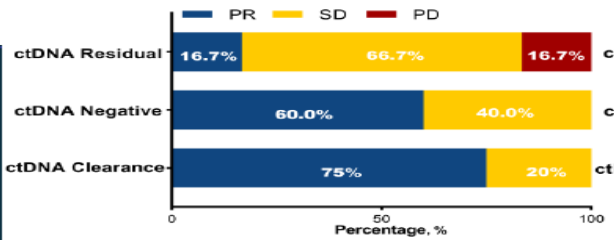
	ctDNA –	ctDNA+
MET-specific Mutations		
ORR	64.3%	16.7%
mPFS	7.9m	2.2m
Canonical Mutations		
ORR	60.0%	20.0%
mPFS	7.9m	2.2m
Pan-mutations		
ORR	71.4%	38.5%

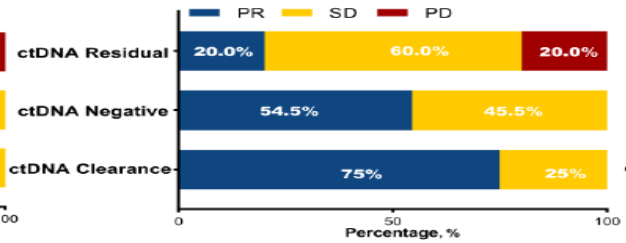


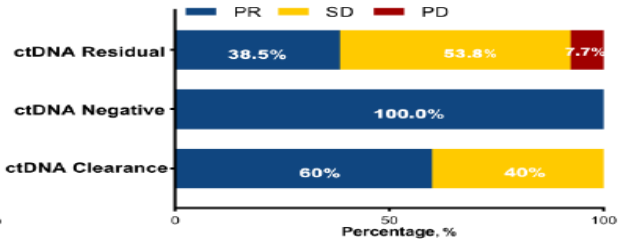
- By V1 at week 4, detection rates fell: MET-specific 30% (6/20), canonical 25% (5/20), pan 65% (13/20).
- MET-specific negativity at week 4 associated with clearly superior ORR and longer PFS; Canonical showed a similar trend; By contrast, pan-mutation failed to separate PFS difference.
- Pan-mutations offered the highest sensitivity (~80%) but low specificity; MET-specific achieved a balanced profile with 50% sensitivity, 90% specificity, and 83% PPV, more decision-ready for ruling in true non-responders.

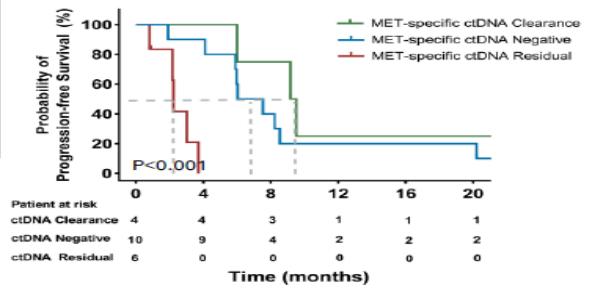
The association between dynamic changes of ctDNA and treatment outcomes

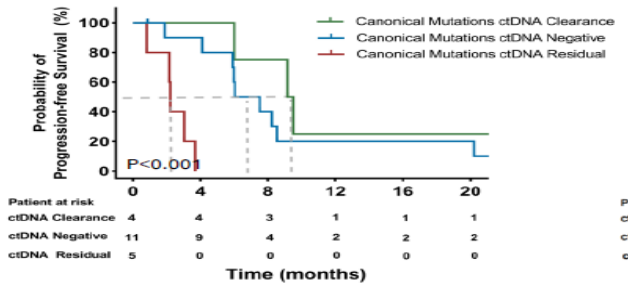
	ctDNA Residual	ctDNA Negative	ctDNA Clearance
MET-specific Mutations			
ORR	16.7%	60%	75.0%
mPFS	2.2m	6.8m	9.3m
Canonical Mutations			
ORR	20%	54.5%	75.0%
mPFS	2.2m	6.8m	9.3m
Pan-mutations			
ORR	38.5%	100%	60.0%
mPFS	5.9m	7.1m	8.5m

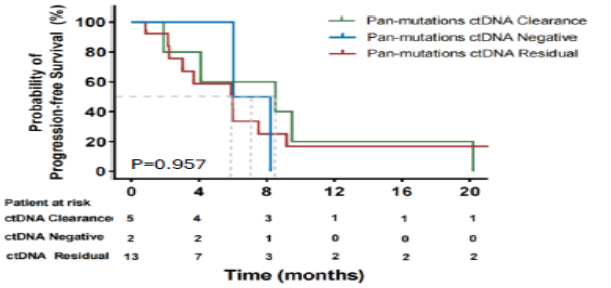












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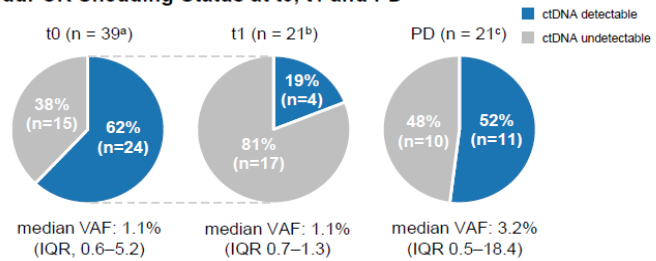
Liquid biopsy in BRAF V600E NSCLC treated with dabrafenib plus trametinib: final analysis of LiBRA study (GOIRC-03-2020)

Alessandro Leonetti, MD, PhD

Results – ddPCR Analysis

ddPCR Shedding Status at t0, t1 and PD

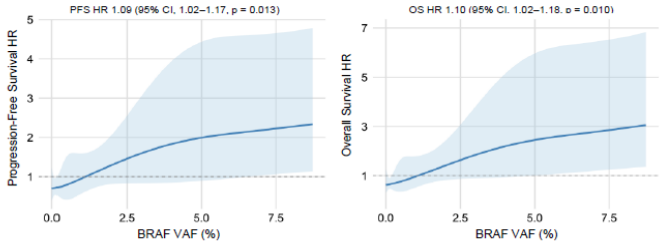


median VAF: 1.1% (IQR, 0.6–5.2)

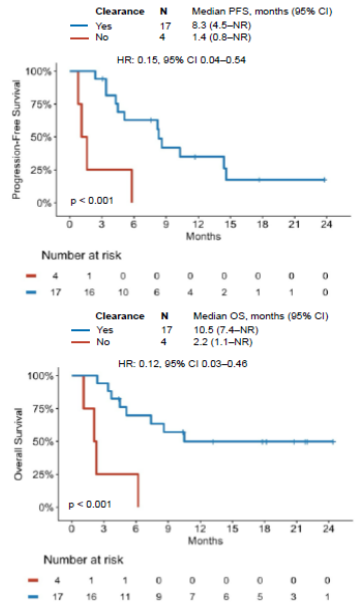
median VAF: 1.1% (IQR 0.7–1.3)

median VAF: 3.2% (IQR 0.5–18.4)

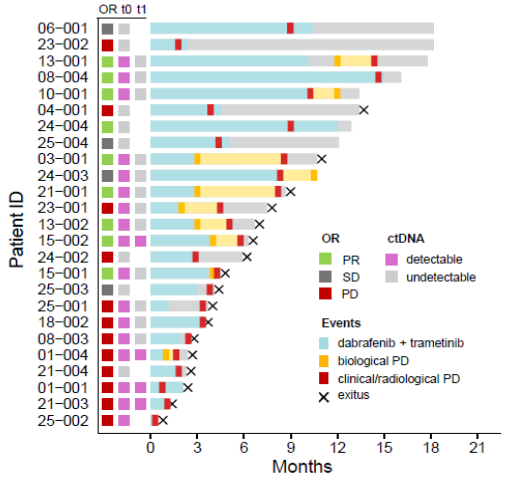
Association between BRAF VAF at t0 and PFS/OS



KM Curves for Clearance at t1



Timeline of Key Events in Patients with PD

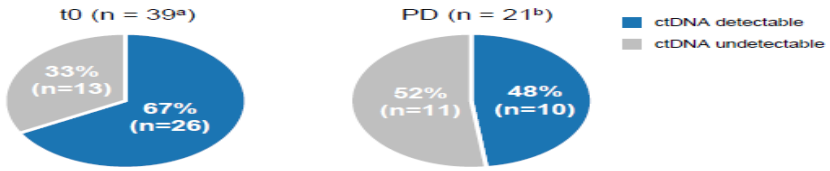


- Among patients with radiological/clinical PD (n = 25), 10 (40%) experienced biological PD
- Median interval from biological PD to radiological/clinical PD: 4.9 weeks (IQR 1.4–9.8)

Abbreviations: CI, Confidence Interval; ctDNA, circulating tumor DNA; ddPCR, Digital Droplet Polymerase Chain Reaction; HR, Hazard Ratio; IQR, Interquartil Range, KM, Kaplan Meier; n, number; NR, Not Reached; OR, Overall Response; OS, Overall Survival; PD, Progression of Disease; PFS, Progression-Free Survival; VAF, Variant Allele Frequency; ª1 patient missed t0 sample for compliance; ᵇ3 basal shedder patients missed t1 sample for compliance; ᶜ4 patients missed PD sample for compliance

Results – NGS Analysis

NGS Shedding Status at t0 and PD

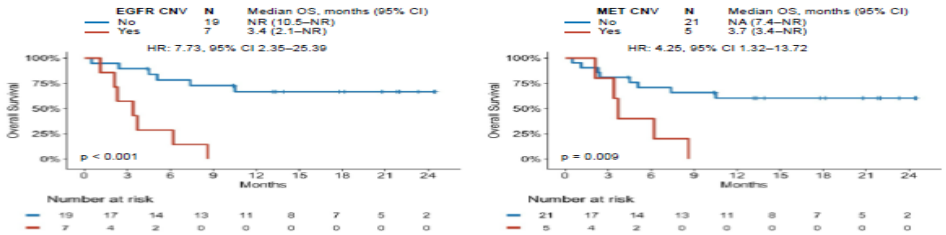


median VAF: 0.8% (IQR, 0.4–5.0)

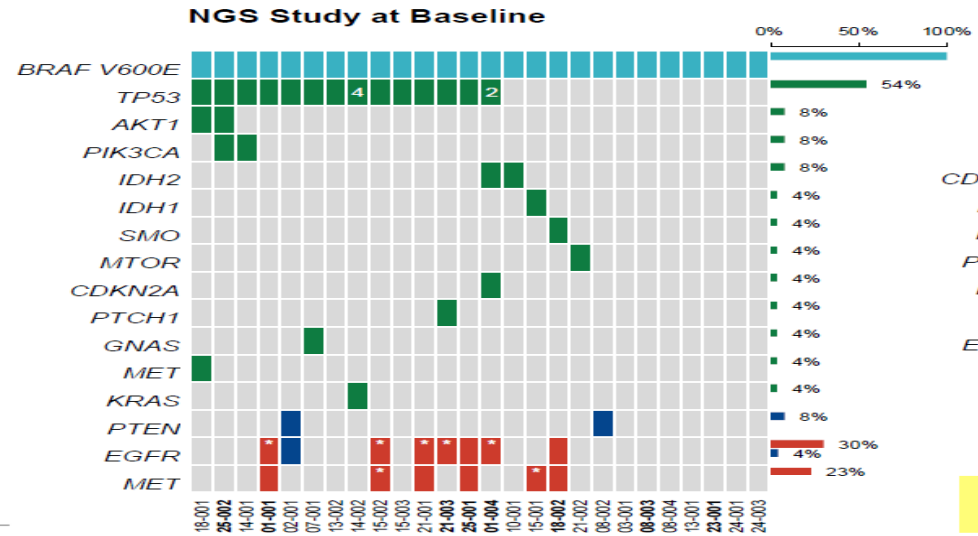
median VAF: 2.9% (IQR 0.8–19.8)

- Concordance between ddPCR and NGS: 95%

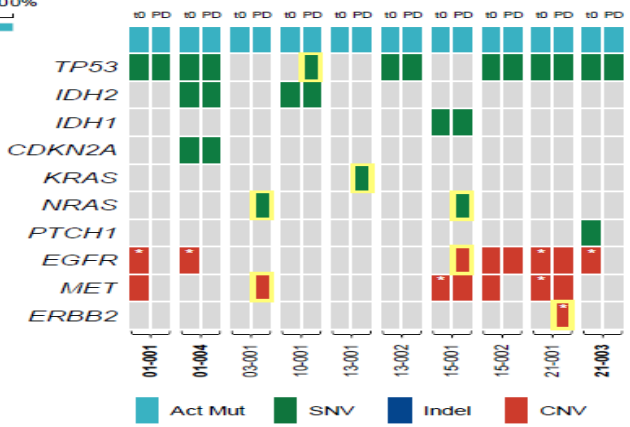
KM Curves for Basal EGFR/MET CNVs



NGS Study at Baseline



Matched NGS (PD *vs* Baseline)



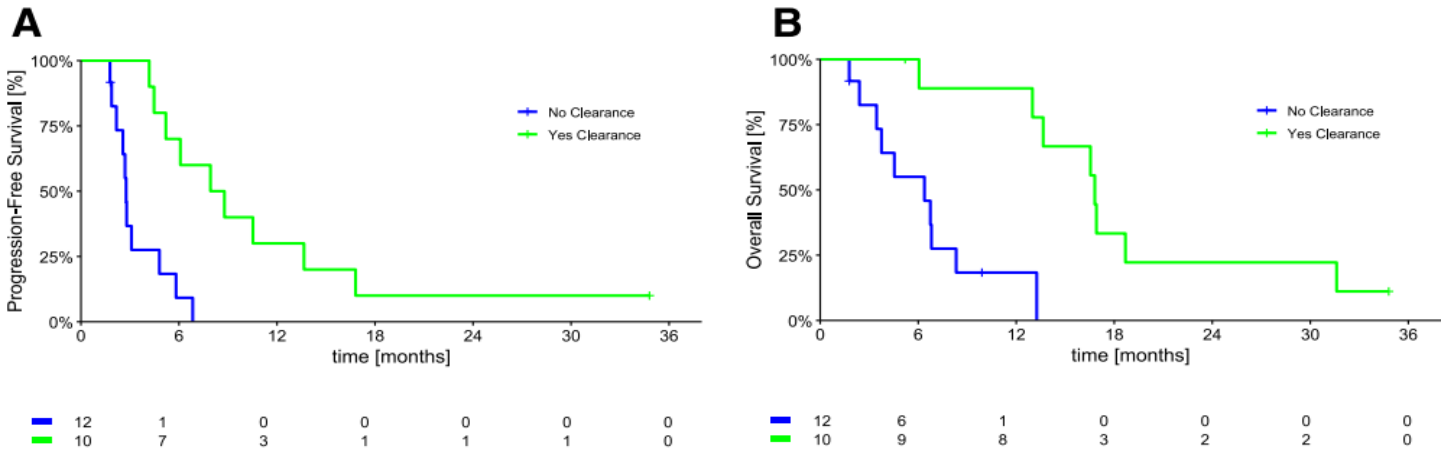
At resistance = NRAS, KRAS, TP53 SNV
EGFR, MET, HER2 CNV

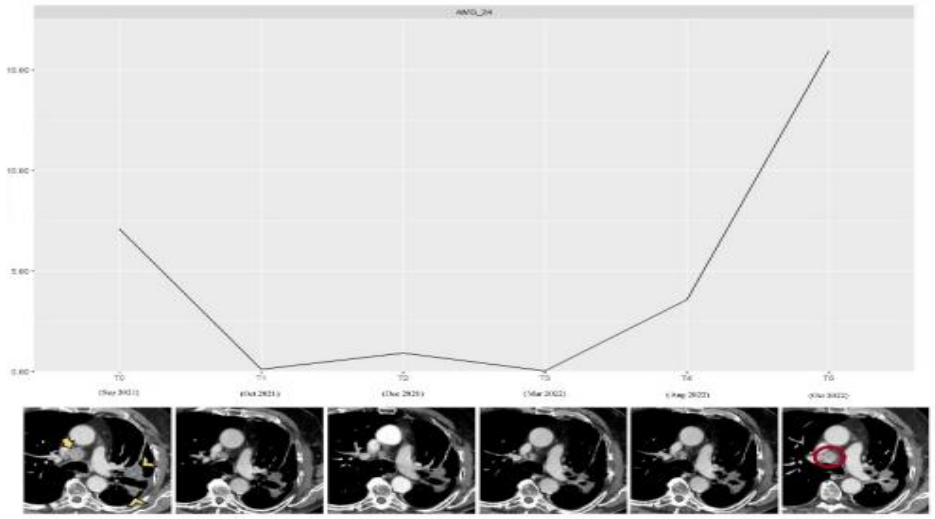
- Co-mutations at t0 in 19/26 (73%) shedders

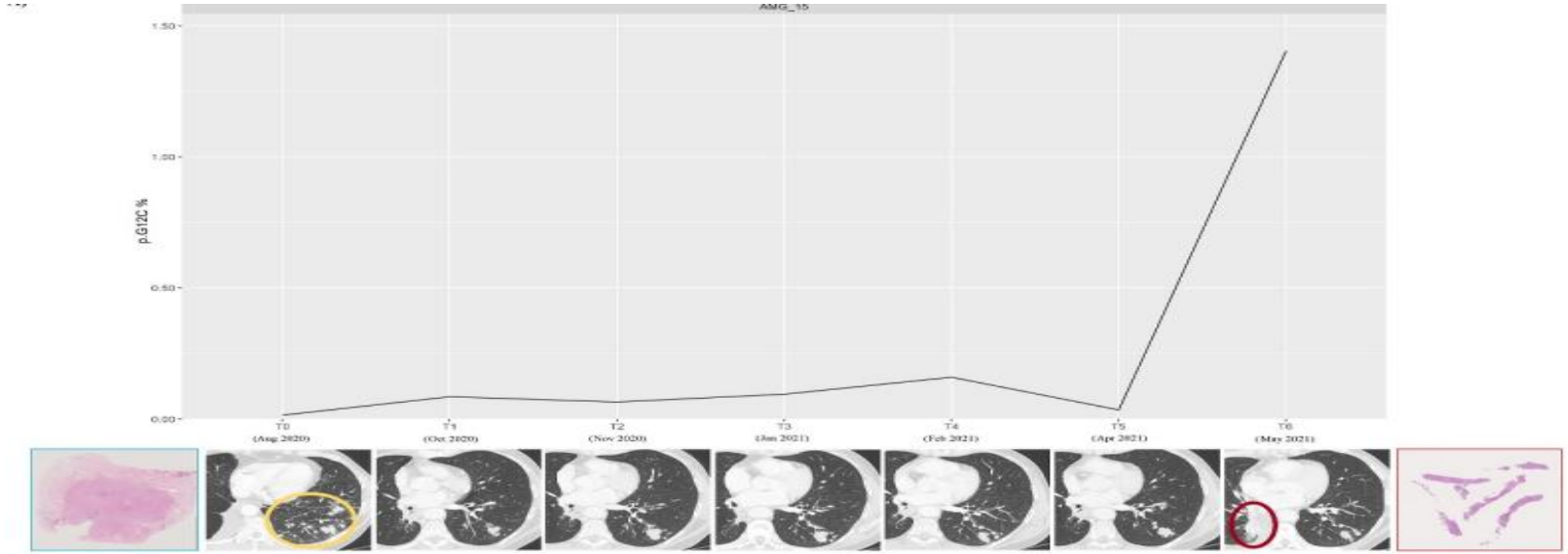
*The value has a score between 0–5; Patient IDs shown in **bold** indicate primary resistance

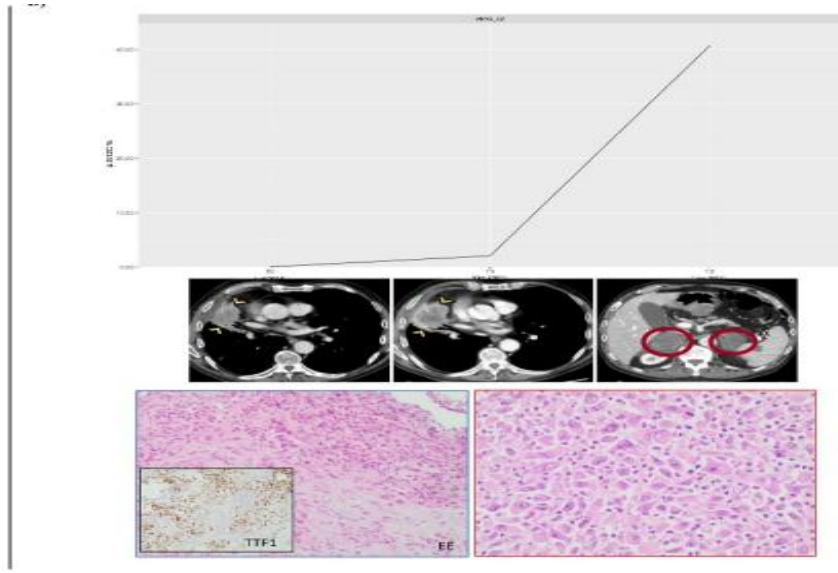
Abbreviations: ActMut, Activating Mutation; amp, amplification; CI, Confidence Interval; CNV; Copy Number Variation; HR, Hazard Ratio; Indel, Insertion-Deletion; KM, Kaplan Meier; n, number; NGS, Next-Generation Sequencing; NR, Not Reached; PD, Progression of Disease; SNV, Single Nucleotide Variant; VAF, Variant Allele Frequency; ª1 patient missed t0 sample for compliance; ᵇ4 patients missed PD sample for compliance

Circulating tumor DNA dynamic variation predicts sotorasib efficacy in KRASp.G12C-mutated advanced non-small cell lung cancer



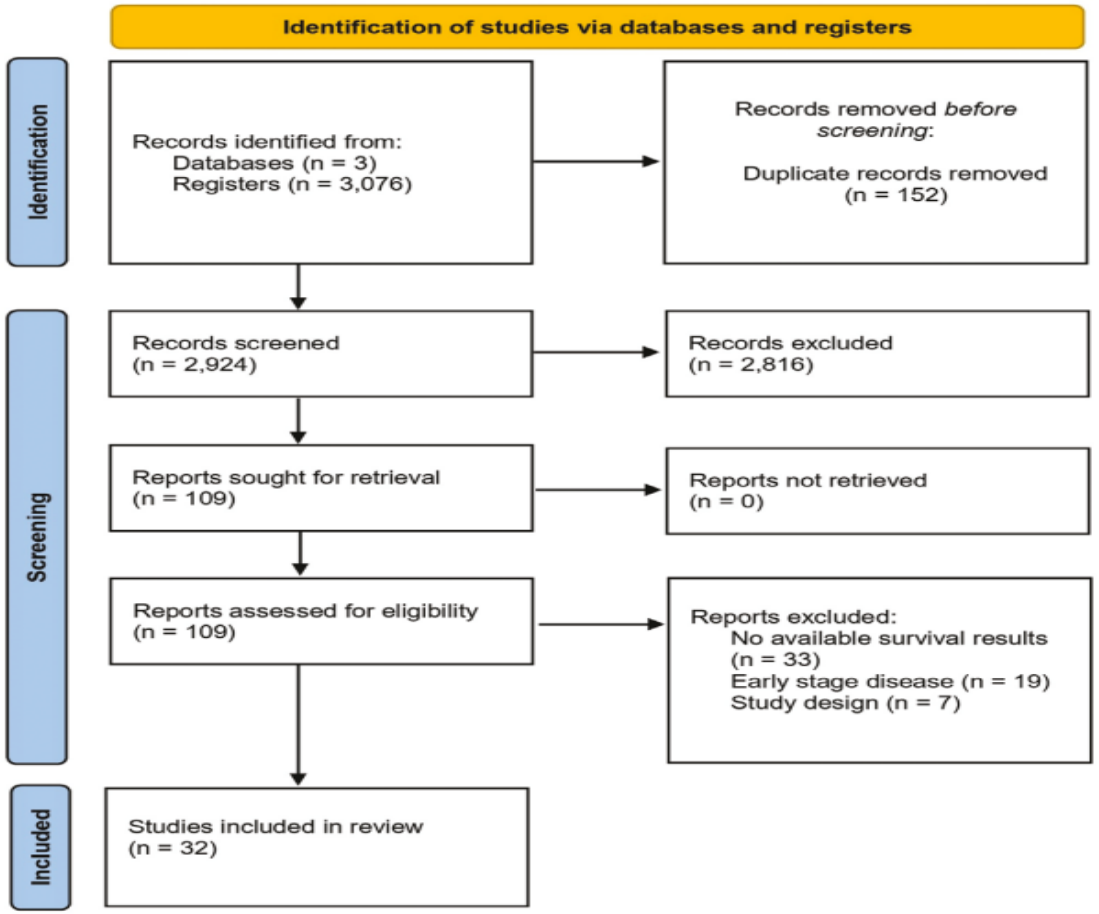






Passiglia F,. Circulating tumor DNA dynamic variation predicts sotorasib efficacy in KRASp.G12C-mutated advanced non-small cell lung cancer. Cancer. 2025 Jun 1;131(11):e35917

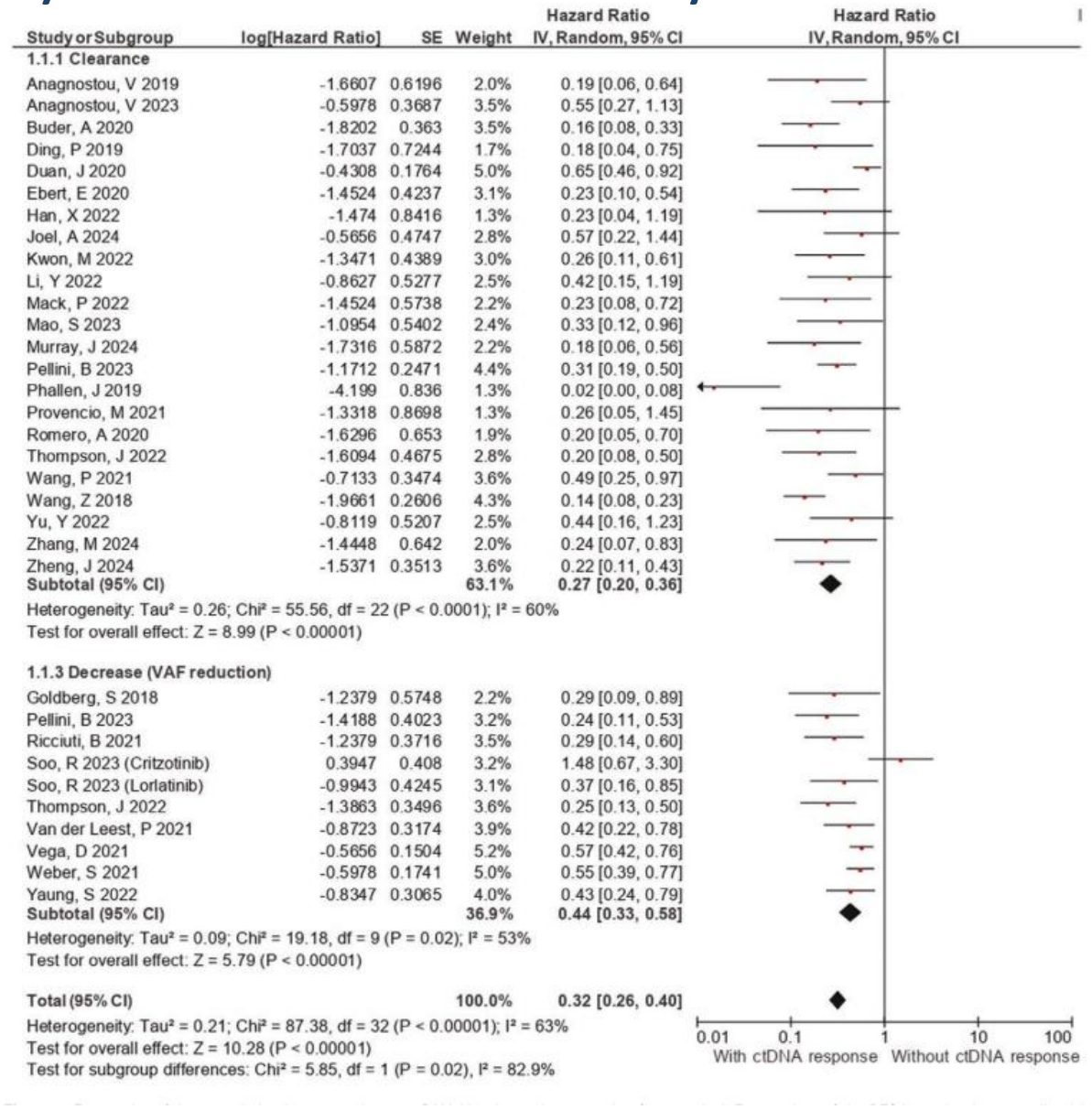
Plasma ctDNA kinetics as a predictor of systemic therapy response for advanced non-small cell lung cancer: a systematic review and meta-analysis

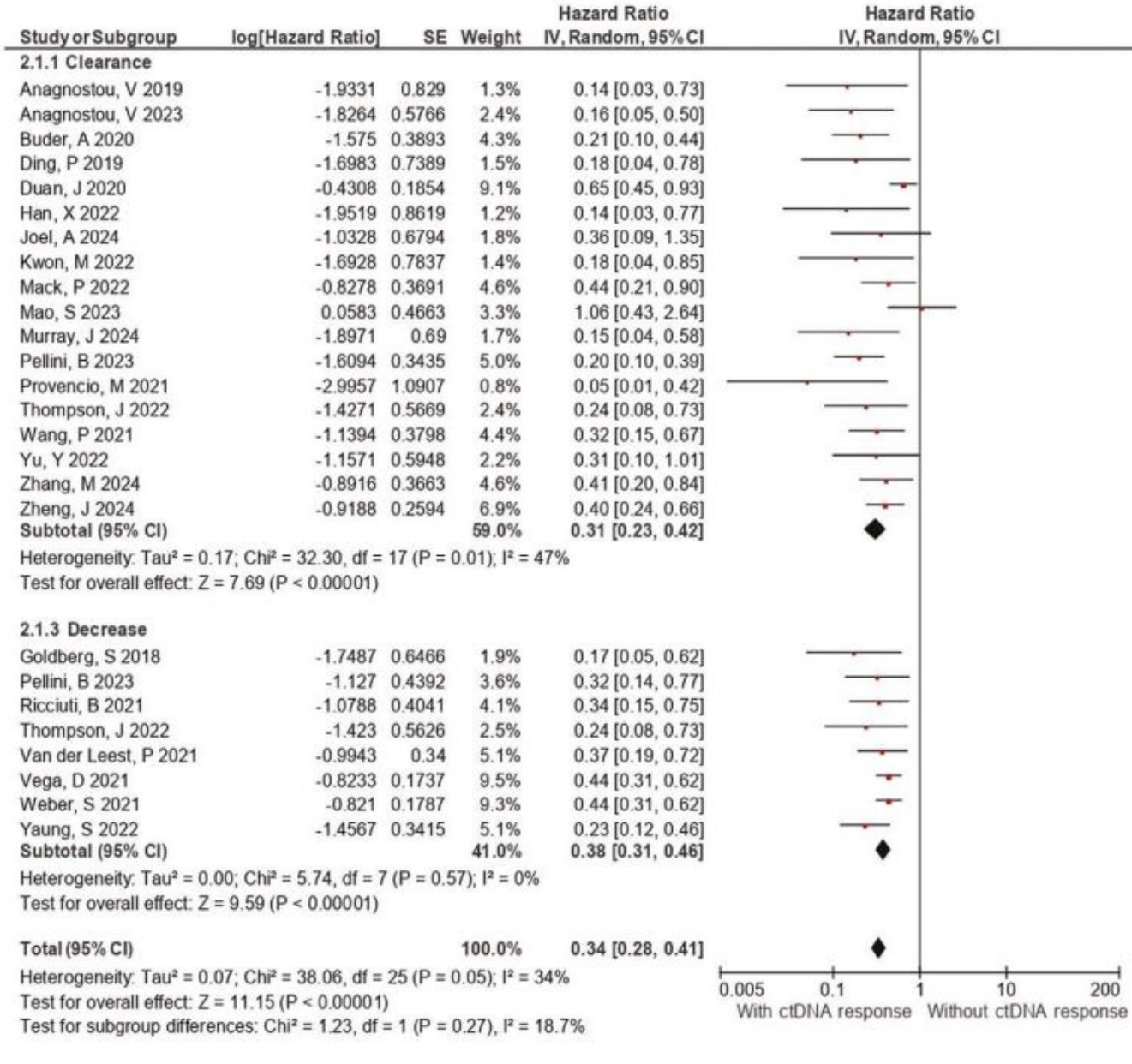


First Author/year	Study design	NSCLC Driver mutation	Number of patients	Treatment	ctDNA detection method
Anagnostou, 2019	Prospective cohort	n/a	24	ICB	NGS, tumor informed
Anagnostou, 2023	Clinical trial	n/a	50	ICB	NGS, tumor agnostic
Buder, 2020	Retrospective cohort	*EGFR*	141	*EGFR TKI*	PCR, tumor informed
Ding, 2019	Clinical trial	*EGFR*	28	*EGFR TKI*	PCR, tumor informed
Duan, 2020	Clinical trial	*EGFR*	180	*EGFR TKI*	NGS, tumor informed
Ebert, 2020	Prospective cohort	*EGFR*	82	*EGFR TKI*	PCR, tumor informed
Goldberg, 2018	Retrospective cohort	n/a	28	ICB	NGS, tumor informed
Han, 2022	Clinical trial	n/a	33	Chemoimmunotherapy	NGS, tumor agnostic
Joel, 2024	Prospective cohort	*EGFR*	66	*EGFR TKI*	PCR, tumor informed
Kwon, 2022	Prospective cohort	*ALK*	92	*EGFR TKIs*	NGS, tumor informed
Li, 2022	Retrospective cohort	*EGFR*	20	*EGFR TKIs*	NGS, tumor agnostic
Mack, 2022	Clinical trial	*EGFR*	106	*EGFR TKIs*	NGS, tumor informed
Mao, 2023	Retrospective cohort	*HER2*	50	HER2 TKIs	NGS, tumor agnostic
Murray, 2024	Prospective cohort	n/a	30	Chemoimmunotherapy or immunotherapy	NGS, tumor informed
Phallen, 2019	Retrospective cohort	n/a	28	*EGFR TKIs*	NGS, tumor agnostic
Provencio, 2021	Clinical trial	n/a	15	Chemoradiotherapy	PCR, tumor informed
Ricciuti, 2021	Prospective cohort	n/a	62	ICB	NGS, tumor informed
Romero, 2020	Prospective cohort	*EGFR*	22	*EGFR TKI*	NGS, tumor agnostic
Song, 2020	Clinical trial	n/a	248	Diverse	NGS, tumor agnostic
Soo, 2023	Clinical trial	*ALK*	291	*EGFR TKI*	NGS, tumor agnostic
Thompson, 2022	Prospective cohort	n/a	67	ICB	NGS, tumor agnostic
van der Leest, 2021	Prospective cohort	n/a	100	ICB	PCR, tumor informed
Vega, 2021	Clinical trial	n/a	200	ICB	NGS, tumor agnostic
Wang, 2018	Prospective cohort	*EGFR*	183	*EGFR TKI*	PCR, tumor informed
Wang, 2021	Clinical trial	*EGFR*	106	*EGFR TKI*	NGS, tumor agnostic
Weber, 2021	Prospective cohort	n/a	152	ICB	NGS, tumor agnostic
Yaung, 2022	Prospective cohort	n/a	92	Chemotherapy	NGS, tumor agnostic
Yu, 2022	Clinical trial	*METex14*	66	*MET TKI*	NGS, tumor agnostic
Zhang, 2024	Clinical trial	n/a	22	Chemoimmunotherapy	NGS, tumor agnostic
Pellini, 2023	Clinical trial	n/a	221	*EGFR* TKI and chemotherapy	PCR, tumor informed
Zheng, 2022	Retrospective cohort	*EGFR*	51	*EGFR TKI*	NGS, tumor agnostic
Zheng, 2024	Clinical trial	*ALK*	180	ALK TKI	NGS

Leite da Silva LF,. Plasma ctDNA kinetics as a predictor of systemic therapy response for advanced non-small cell lung cancer: a systematic review and meta-analysis. Oncologist. 2025 Feb 6;30(2):oyae344.

Plasma ctDNA kinetics as a predictor of systemic therapy response for advanced non-small cell lung cancer: a systematic review and meta-analysis





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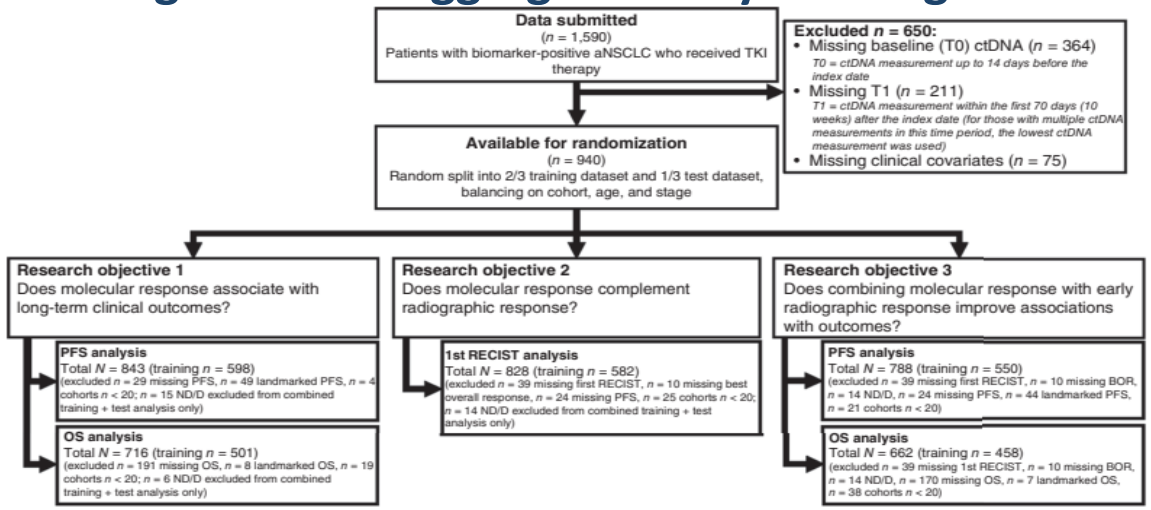
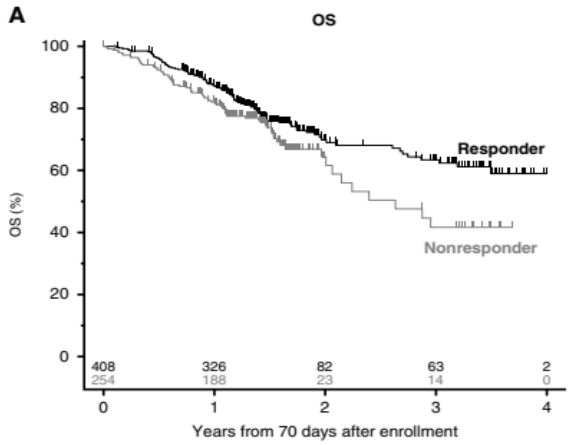
Plasma ctDNA kinetics as a predictor of systemic therapy response for advanced non-small cell lung cancer: a systematic review and meta-analysis

Subgroups	Progression-free survival				Overall survival			
	HR (95% CI)	*P*	I2	Test for subgroup difference	HR (95% CI)	*P*	I2	Test for subgroup difference
Treatment class				*P* = .93				*P* = .27
ICB	0.33 [0.24, 0.46]	<.01	68%		0.32 [0.25, 0.41]	<.01	41%	
Targeted therapies	0.34 [0.24, 0.46]	<.01	61%		0.41 [0.28, 0.58]	<.01	0%	
Assessment time-point				*P* = .76				*P* = .07
Timepoint < =4 weeks after baseline	0.29 [0.16, 0.53]	<.01	72%		0.32 [0.15, 0.36]	<.01	0%	
Timepoint > 4 weeks after baseline	0.33[0.25, 0.42]	<.01	63%		0.37 [0.29, 0.47	<.01	42%	
Study design				*P* = .11				*P* = .22
Clinical trial	0.40 [0.28, 0.57]	.01	68%		0.36 [0.29, 0.43]	<.01	43%	
Observational	0.28 [0.31, 0.36]	<.01	53%		0.32 [0.26, 0.40]	<.01	6%	
Assay type				*P* = .53				*P* = .17
Tumor agnostic	0.37 [0.27, 0.50]	<.01	65%		0.36 [0.29, 0.46]	<.01	25%	
Tumor informed	0.32 [0.25, 0.42]	<.01	43%		0.27 [0.19, 0.38]	<.01	55%	

Leite da Silva LF,. Plasma ctDNA kinetics as a predictor of systemic therapy response for advanced non-small cell lung cancer: a systematic review and meta-analysis. Oncologist. 2025 Feb 6;30(2):oyae344.

ctDNA Clearance as an Early Indicator of Improved Clinical Outcomes in Advanced NSCLC Treated with TKI: Findings from an Aggregate Analysis of Eight Clinical Trials

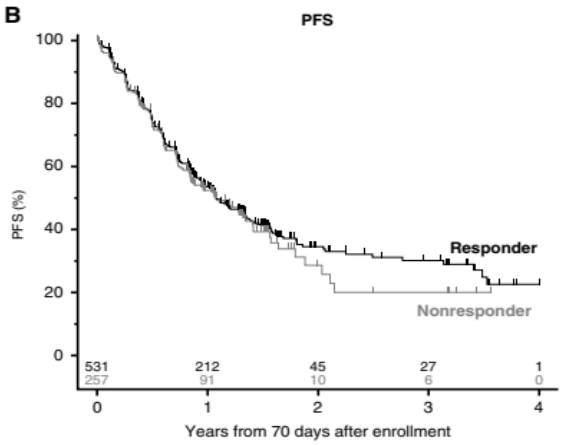




	Deaths/N	Median (Years)	1-Year estimate
Responder	103/408	NR	87% (84–91)
Nonresponder	75/254	2.6 (2.1–NR)	82% (77–87)

Multivariable associations

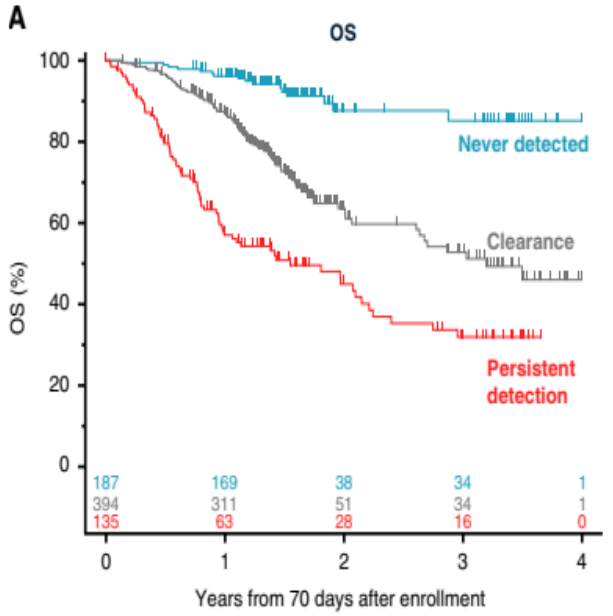
Comparison	HR (95% CI), *P* value
Responder vs. Nonresponder	1.21 (0.87–1.67), 0.254



	Events/N	Median (Years)	1-Year estimate
Responder	281/531	1.1 (1.0–1.3)	54% (49–58)
Nonresponder	131/257	1.1 (0.8–1.4)	52% (46–59)

Multivariable associations

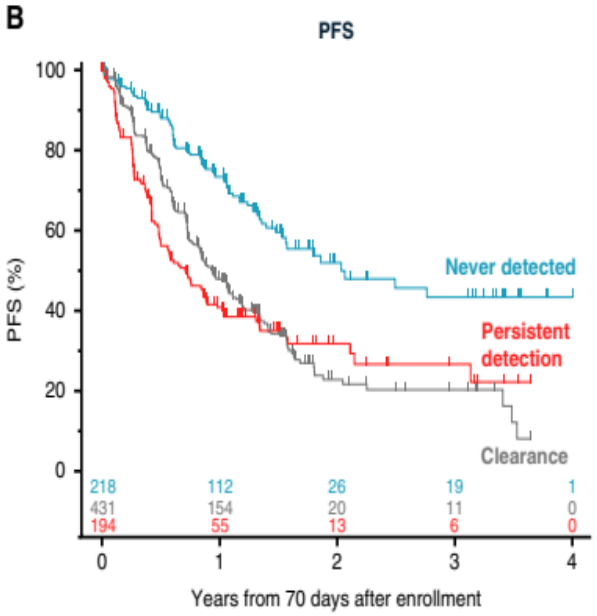
Comparison	HR (95% CI), *P* value
Responder vs. Nonresponder	0.96 (0.77–1.20), 0.728



	Deaths/N	Median (Years)	1-Year estimate
Never detected	16/187	NR	96% (93–99)
Clearance	114/394	3.2 (2.6–NR)	87% (84–91)
Persistent detection	72/135	1.5 (1–2.2)	57% (48–66)

Multivariable associations

Comparison	HR (95% CI), *P* value
Clearance vs. never detected	2.95 (1.58–5.48), <0.001
Persistent detection vs. never detected	6.25 (3.10–12.62), <0.001
Persistent detection vs. clearance	2.12 (1.50–3.01), <0.001



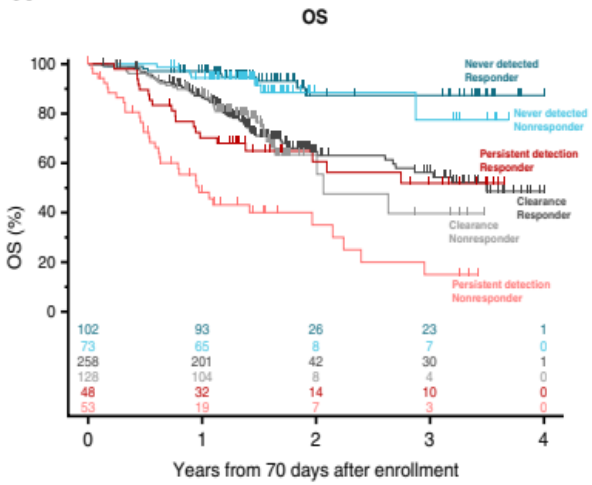
	Events/N	Median (Years)	1-Year estimate
Never detected	77/218	2 (1.6–NR)	73% (67–80)
Clearance	253/431	0.9 (0.8–1.1)	48% (43–53)
Persistent detection	114/194	0.7 (0.5–0.9)	41% (33–48)

Multivariable associations

Comparison	HR (95% CI), *P* value
Clearance vs. never detected	2.11 (1.54–2.91), <0.001
Persistent detection vs. never detected	3.21 (2.17–4.76), <0.001
Persistent detection vs. clearance	1.52 (1.17–1.98), 0.002

Andrews HS,. ctDNA Clearance as an Early Indicator of Improved Clinical Outcomes in Advanced NSCLC Treated with TKI: Findings from an Aggregate Analysis of Eight Clinical Trials. Clin Cancer Res. 2025 Jun 3;31(11):2162-2172.

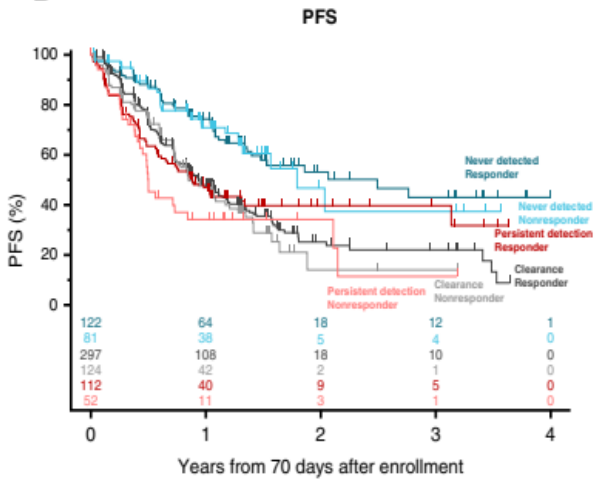
ctDNA Clearance as an Early Indicator of Improved Clinical Outcomes in Advanced NSCLC Treated with TKI: Findings from an Aggregate Analysis of Eight Clinical Trials



Category	Deaths/N	Median (Years)	1-Year estimate
Never detected Responder	8/102	NR	97% (94–100)
Never detected Nonresponder	7/73	NR	94% (89–100)
Clearance Responder	76/258	3.5 (2.7–NR)	87% (82–91)
Clearance Nonresponder	35/128	2.1 (2.0–NR)	88% (82–94)
Persistent detection Responder	19/48	NR	70% (57–83)
Persistent detection Nonresponder	33/53	1.0 (0.6–2.1)	48% (34–63)

Multivariable associations

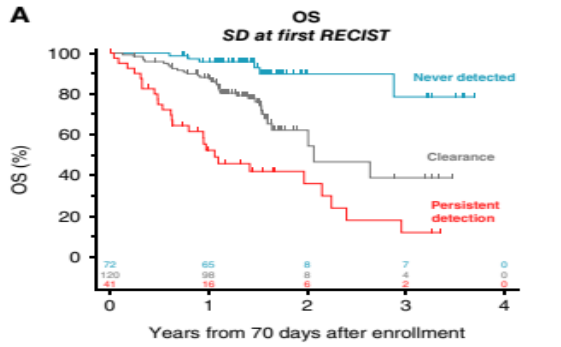
Comparison	HR (95% CI), *P* value
Never detected Nonresponder vs. Responder	0.95 (0.34–2.64), 0.917
Clearance Nonresponder vs. Responder	0.91 (0.60–1.39), 0.675
Persistent detection Nonresponder vs. Responder	1.93 (1.04–3.58), 0.037



Category	Events/N	Median (Years)	1-Year estimate
Never detected Responder	45/122	2.5 (1.4–NR)	74% (66–83)
Never detected Nonresponder	27/81	1.8 (1.3–NR)	71% (60–82)
Clearance Responder	175/297	0.9 (0.8–1.1)	48% (42–54)
Clearance Nonresponder	73/124	0.8 (0.7–1.2)	48% (38–57)
Persistent detection Responder	61/112	0.9 (0.6–1.3)	47% (37–57)
Persistent detection Nonresponder	31/52	0.5 (0.4–0.8)	34% (19–49)

Multivariable associations

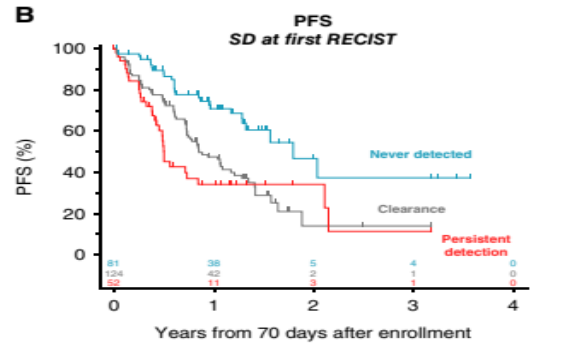
Comparison	HR (95% CI), *P* value
Never detected Nonresponder vs. Responder	0.80 (0.49–1.30), 0.373
Clearance Nonresponder vs. Responder	0.94 (0.71–1.25), 0.670
Persistent detection Nonresponder vs. Responder	1.29 (0.80–2.07), 0.296



	Deaths/N	Median (Years)	1-Year estimate
Never detected	6/72	NR	96% (91–100)
Clearance	34/120	2.1 (1.6–NR)	88% (82–94)
Persistent detection	26/41	1.1 (0.6–2.1)	52% (36–69)

Multivariable associations

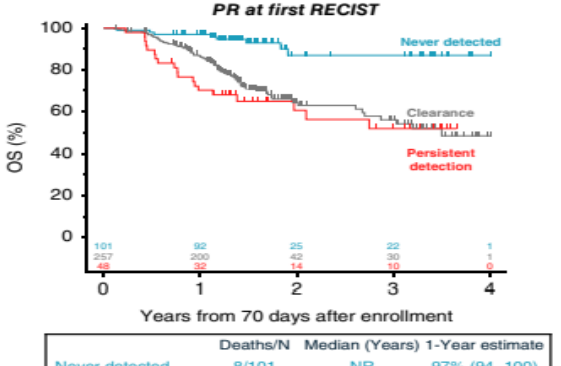
Comparison	HR (95% CI), *P* value
Clearance vs. never detected	4.80 (1.78–12.92), 0.002
Persistent detection vs. never detected	19.93 (5.89–67.44), <0.001
Persistent detection vs. clearance	4.15 (2.07–8.33), <0.001



	Deaths/N	Median (Years)	1-Year estimate
Never detected	27/81	1.8 (1.3–NR)	71% (60–82)
Clearance	73/124	0.8 (0.7–1.2)	48% (38–57)
Persistent detection	31/52	0.5 (0.4–0.8)	34% (19–49)

Multivariable associations

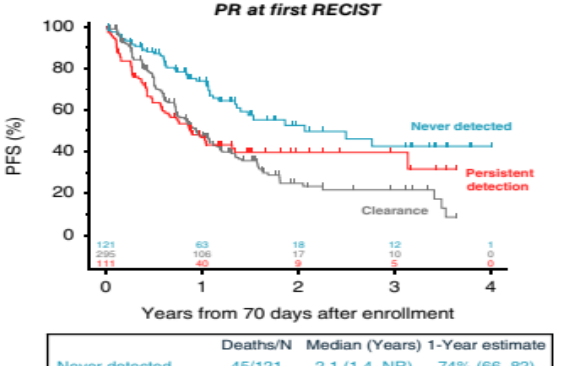
Comparison	HR (95% CI), *P* value
Clearance vs. never detected	3.11 (1.82–5.32), <0.001
Persistent detection vs. never detected	7.92 (3.77–16.65), <0.001
Persistent detection vs. clearance	2.55 (1.50–4.31), <0.001



	Deaths/N	Median (Years)	1-Year estimate
Never detected	8/101	NR	97% (94–100)
Clearance	76/257	3.5 (2.7–NR)	87% (82–91)
Persistent detection	19/48	NR	70% (57–83)

Multivariable associations

Comparison	HR (95% CI), *P* value
Clearance vs. never detected	2.05 (0.82–5.13), 0.124
Persistent detection vs. never detected	**3.32 (1.18–9.33), 0.023**
Persistent detection vs. clearance	1.62 (0.95–2.75), 0.077

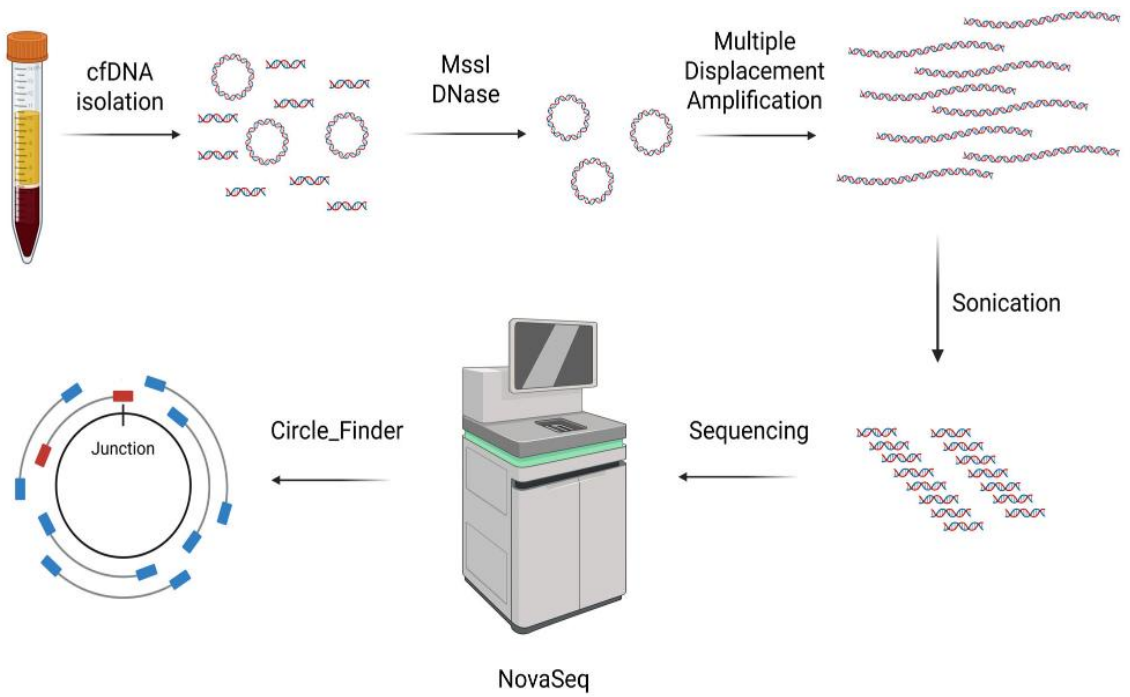


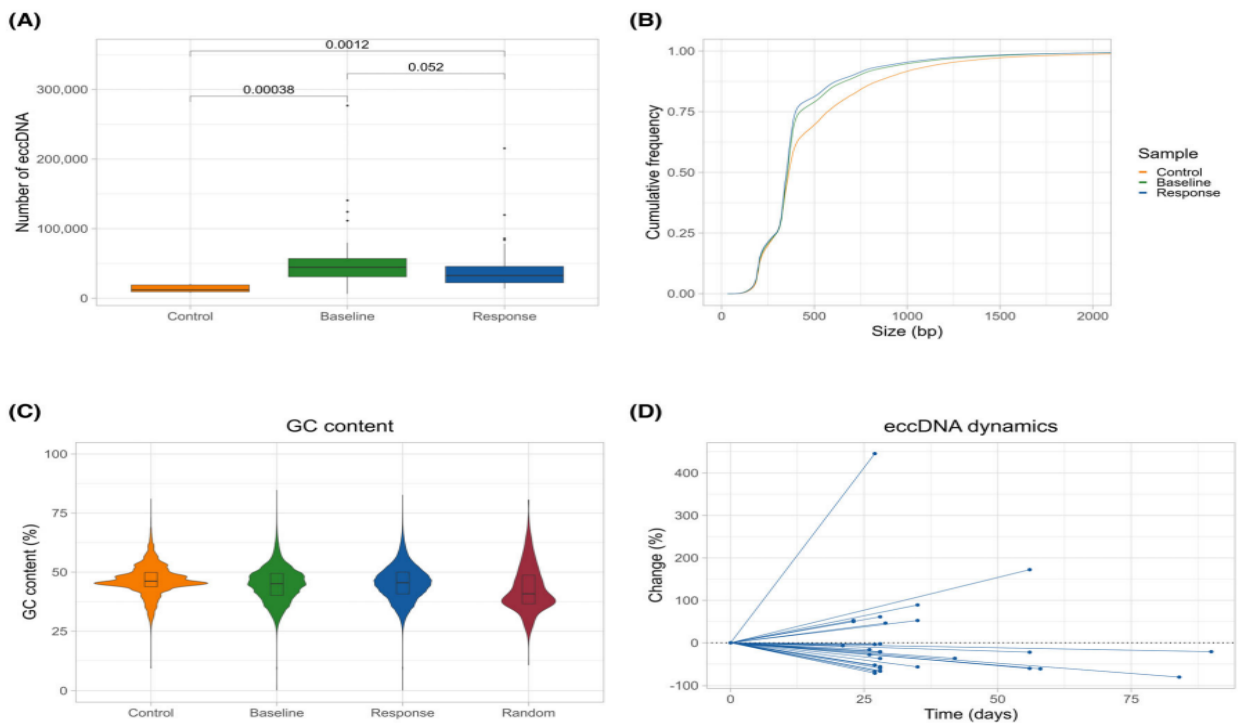
	Deaths/N	Median (Years)	1-Year estimate
Never detected	45/121	2.1 (1.4–NR)	74% (66–82)
Clearance	174/295	0.9 (0.8–1.1)	47% (41–54)
Persistent detection	61/111	0.9 (0.6–1.3)	47% (37–57)

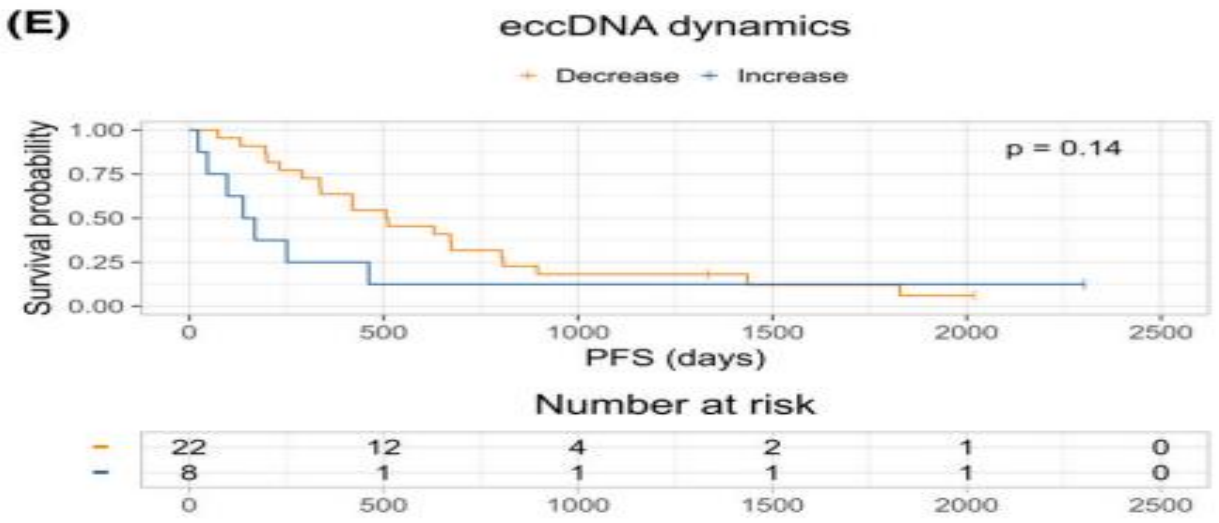
Multivariable associations

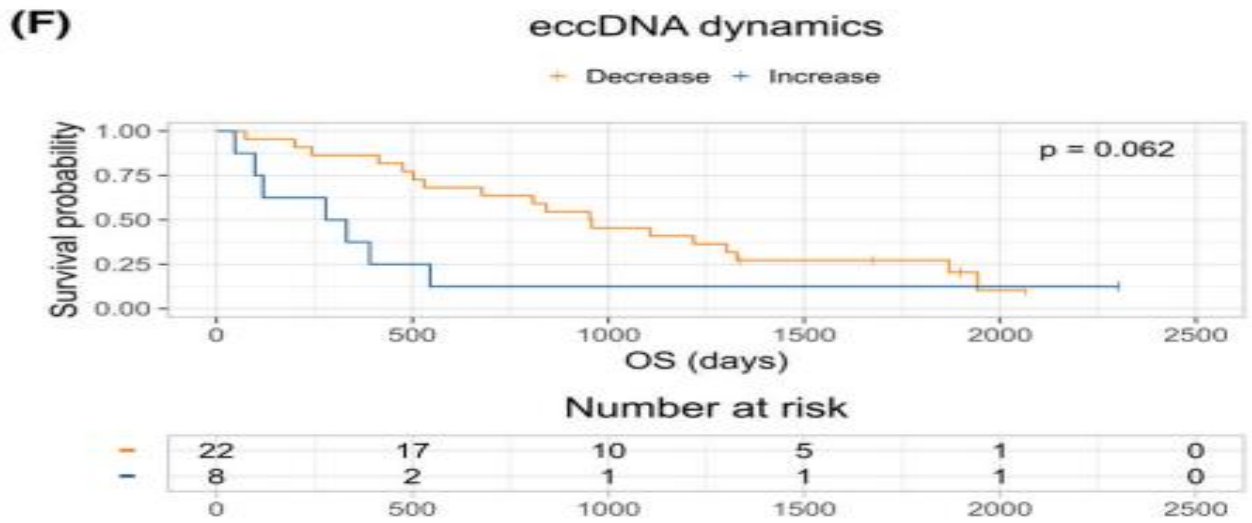
Comparison	HR (95% CI), *P* value
Clearance vs. never detected	**1.64 (1.08–2.51), 0.021**
Persistent detection vs. never detected	**2.28 (1.39–3.75), 0.001**
Persistent detection vs. clearance	1.39 (0.99–1.94), 0.054

Andrews HS,. ctDNA Clearance as an Early Indicator of Improved Clinical Outcomes in Advanced NSCLC Treated with TKI: Findings from an Aggregate Analysis of Eight Clinical Trials. Clin Cancer Res. 2025 Jun 3;31(11):2162-2172.



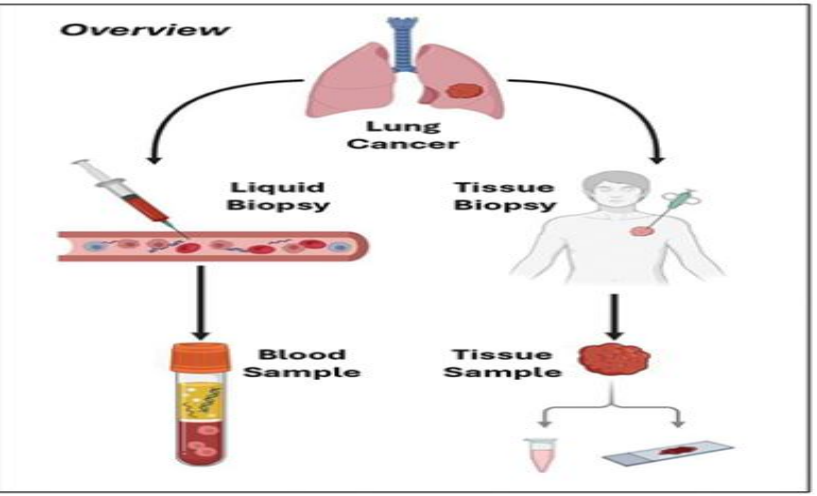


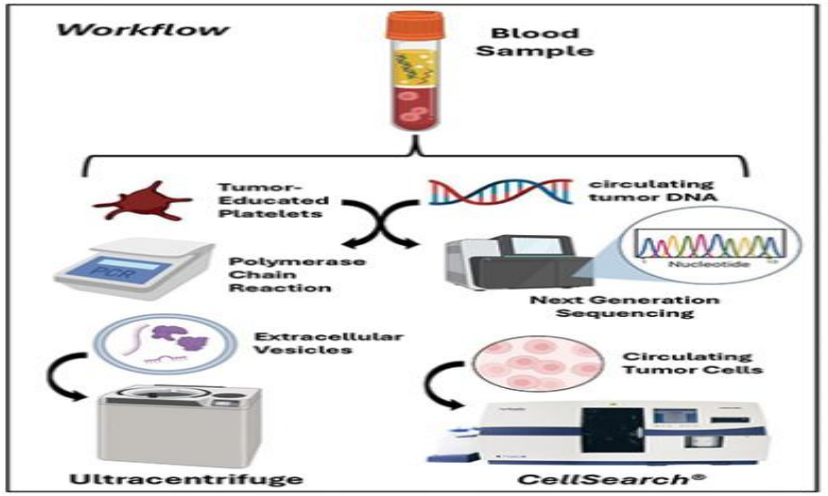


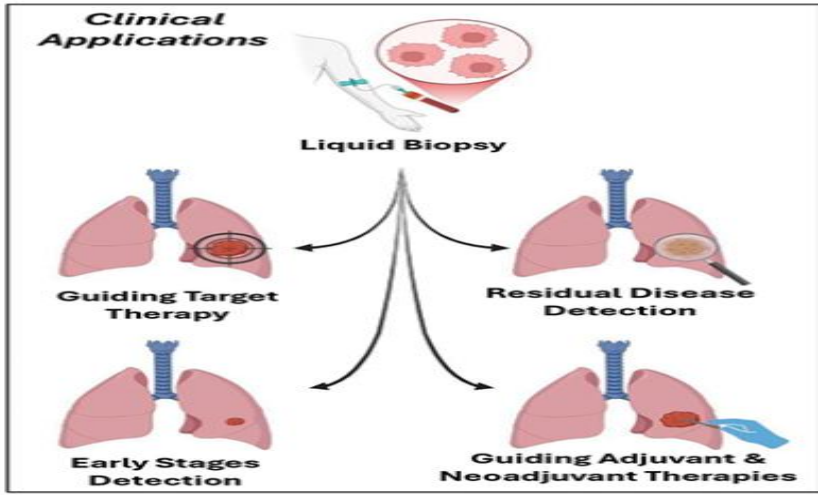


Stensgaard S, BS. Plasma extrachromosomal circular DNA as a biomarker in EGFR-targeted therapy of non-small cell lung cancer. Mol Oncol. 2025 Oct 30.

SUMMARY. CONCLUSION

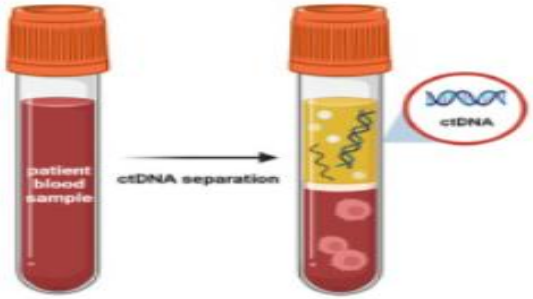




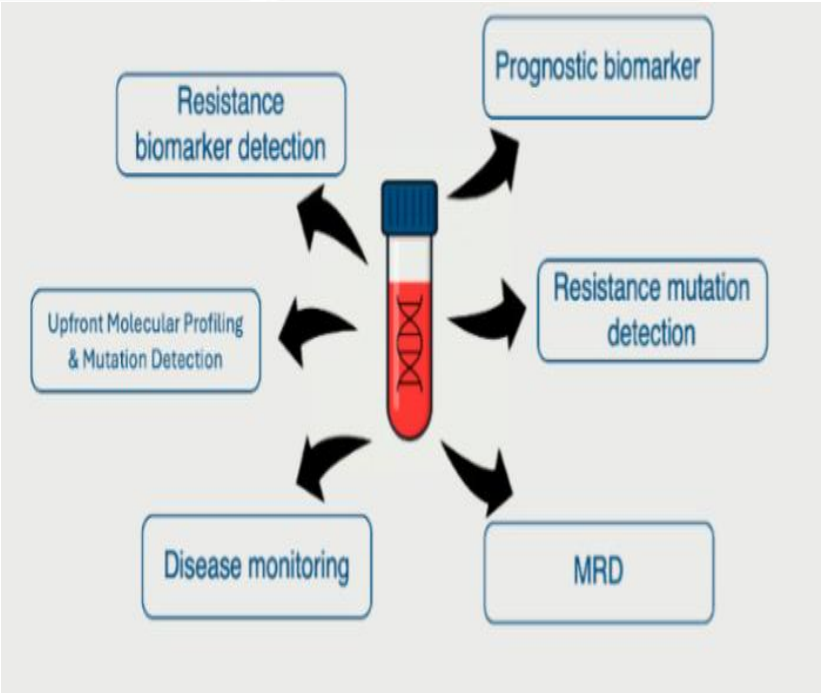


Next Steps

- Integrating into clinical practice
- Enhance through new technologies
- Clarify cost-effectiveness
- Resolving regulatory and reimbursement issues

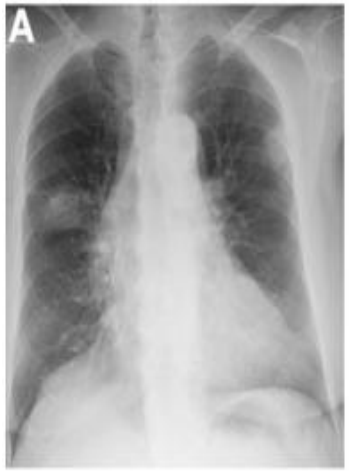


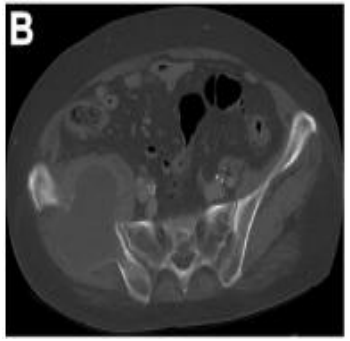
Analyte	Method	Purpose
	NGS-BASED ASSAYS	• Genotyping for actionable targets detection • Minimum residual disease monitoring
	FRAGMENTOMIC ANALYSIS	• Assessing fragment size to identify tumor-derived mutations in cfDNA (e.g. Lung-CLiP)
	MASS SPECTROMETRY	
	PCR BASED ASSAYS	• Epigenetic assays for detection of treatment targets through reprogramming • Novel diagnostic assays
	MICROARRAY	

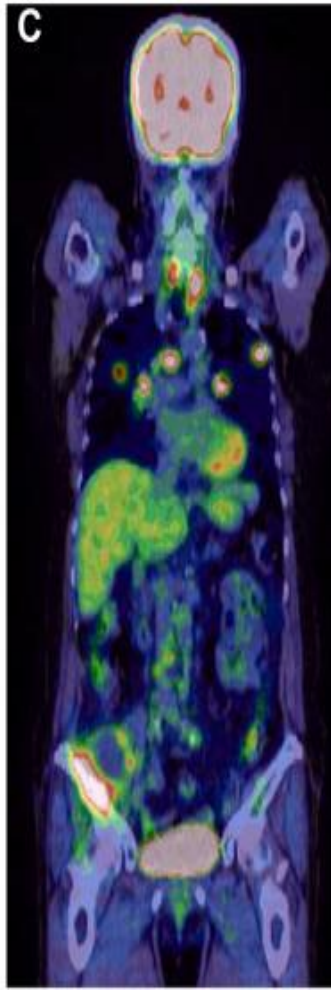


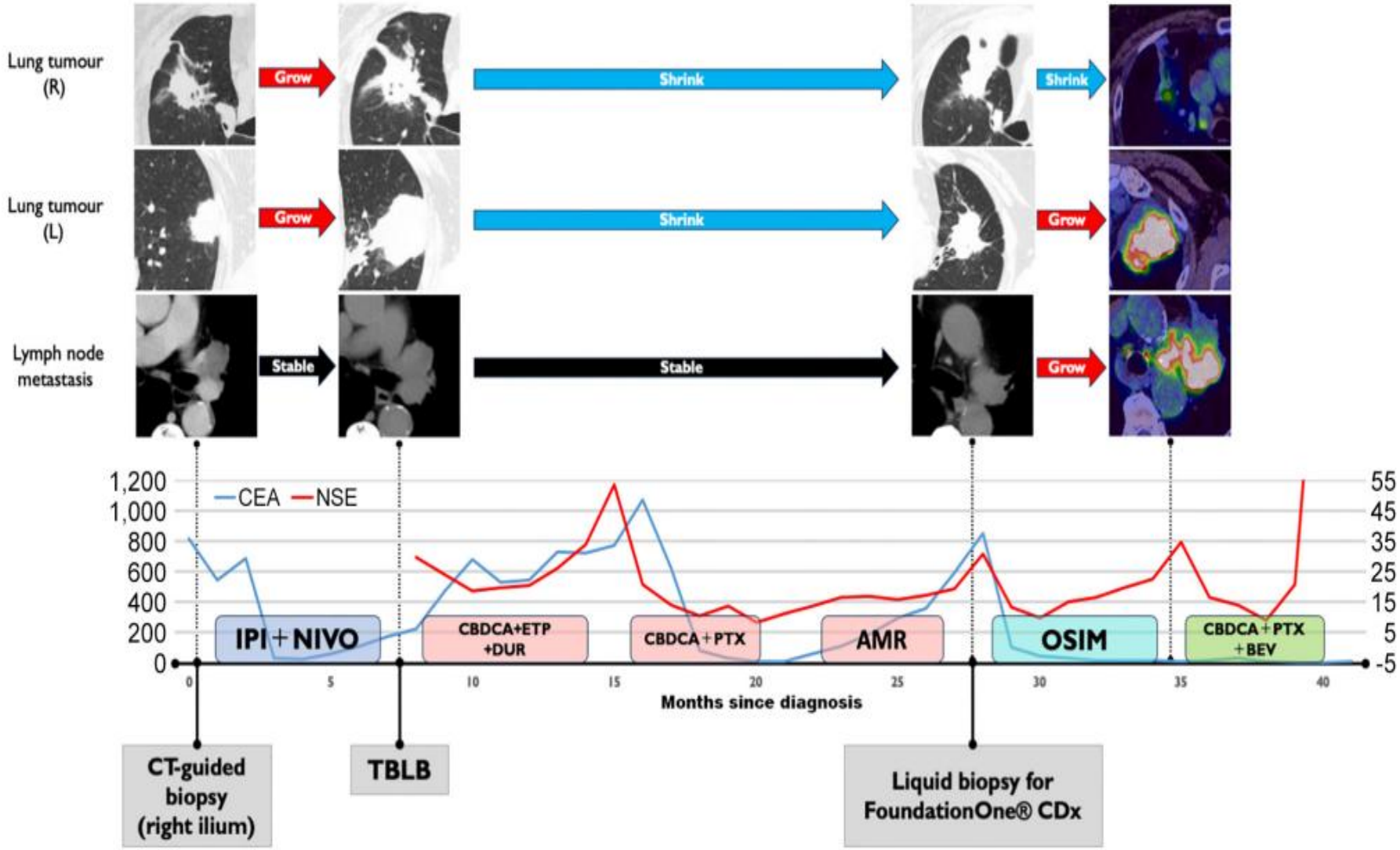
Kontic M, Stjepanovic M, Markovic F. Beyond the Tissue: Unlocking NSCLC Treatment Potential Through Liquid Biopsy. Genes (Basel). 2025 Aug 13;16(8):954.

Case of Combined Small-cell Carcinoma and Adenocarcinoma of the Lung With *EGFR* Exon 19 Deletion Identified *via* Liquid Genomic Profiling









ukuda Y, A Case of Combined Small-cell Carcinoma and Adenocarcinoma of the Lung With *EGFR* Exon 19 Deletion Identified *via* Liquid Genomic Profiling. In Vivo. 2025 Nov-Dec;39(6):3652-3655

La biopsia liquida nos ayudara a generar un nuevo mantra en la oncologia

- No dejes que tu mente se adapte siempre a un protocolo estructurado… A un devenir previsible..

… Que sólo sirve para restarnos parcialmente la ansiedad ante la incertidumbre

- No hay nada previsible cuando cada tumor en cada persona es una huella digital genética diferente, y por tanto.. “singularidad” en si misma y una “singularidad” metacrónica a lo largo de su propio tiempo evolutivo

- La biopsia liquida nos ayudará a percibir Una de las formas mas sencillas de sabiduria..

…. Es ESPERAR LO INESPERABLE

Por que los que nos diferenciarà de la IA … no es la inteligencia… es la sabiduria

16th CONGRESS Lung ON CANCER

BARCELONA 27/28 NOVEMBER 2025

THANK YOU

GECP lung cancer research