Minimal Residual Disease (MRD) as Detected by Liquid Biopsy:

Where are We Going?

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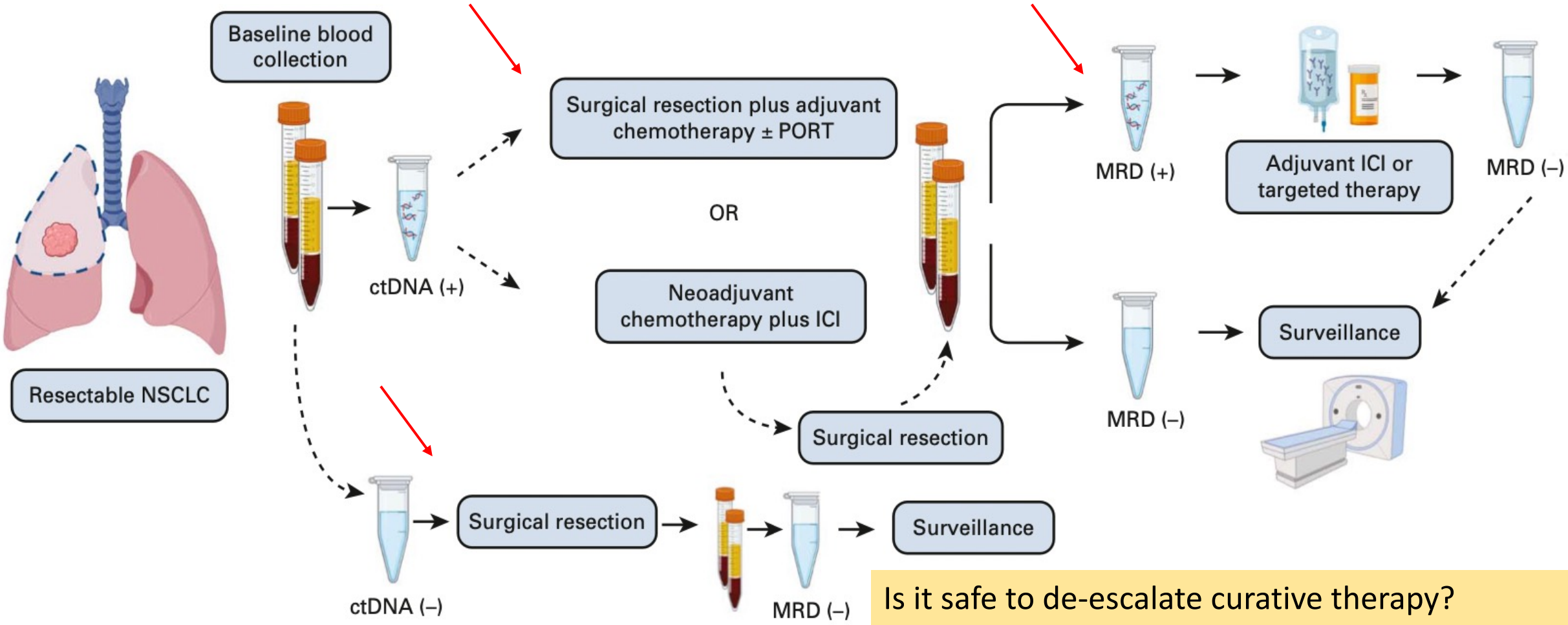
Plasma ctDNA/MRD for treatment selection– not ready for prime time…

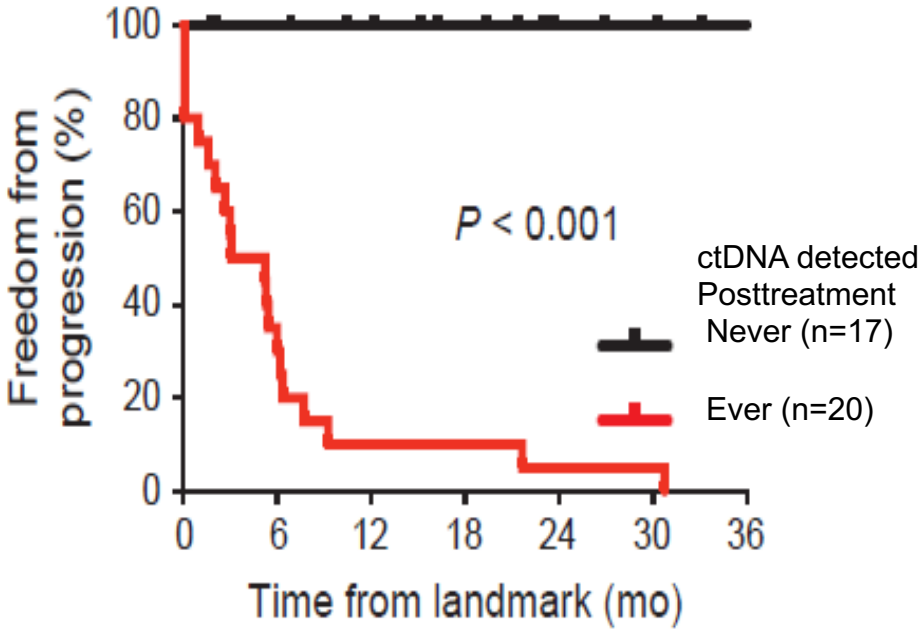


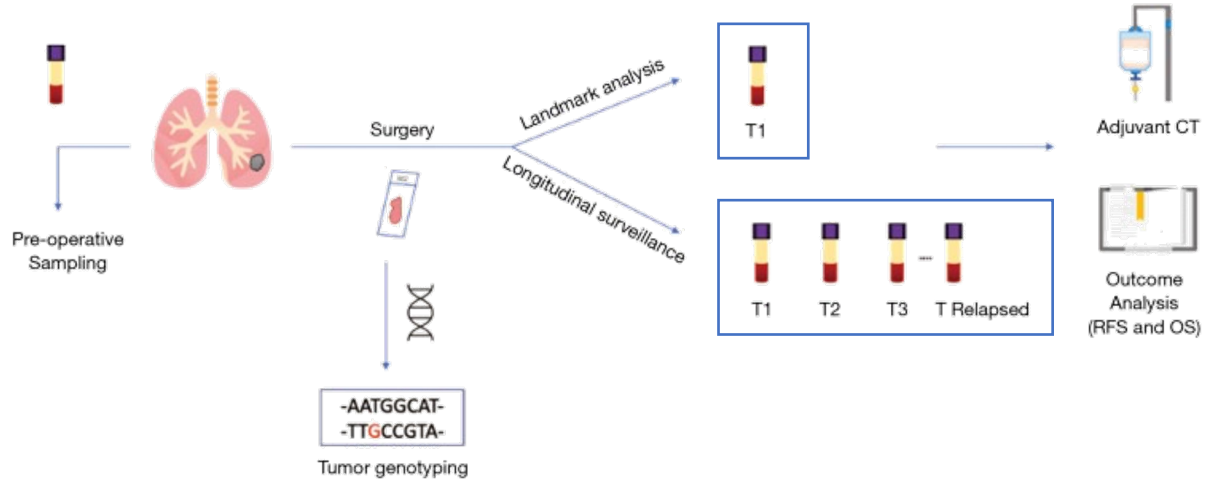
Is it safe to de-escalate curative therapy?
Do we have good options for escalating therapy?
Do patients really benefit?

Pellini B, Chaudhuri AA. J Clin Oncol. 2022 Feb 20;40(6):567-575.

MRD in early-stage NSCLC across studies[1,2]

Post-treatment ctDNA levels in patients with stage I-III NSCLC (N=40)



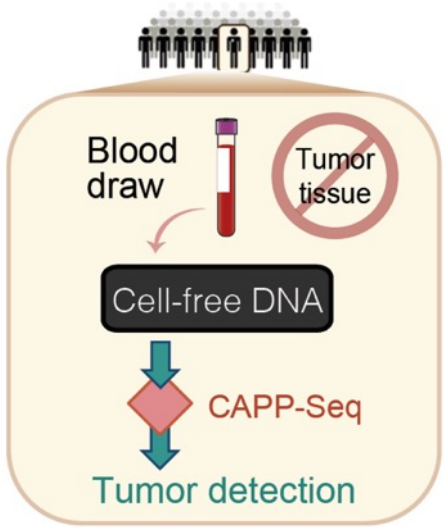


- MRD is a powerful prognostic indicator, with data on >1000 early stage NSCLC patients
- Using a single post-operative timepoint, MRD predicts clinical relapse with 36–100% sensitivity[2]
- Using serial timepoints post-treatment, this increases to 82–100%[2]
- MRD detection precedes clinical recurrence by 5.5 months (mean)[1]
- MRD+ patients derive greater RFS benefit from adjuvant chemotherapy[1]

1. Verzè M, et al. Transl Lung Cancer Res. 2022;11:2588–600;
2. Pellini and Chaudhuri. J Clin Oncol 2022; 40:567-575

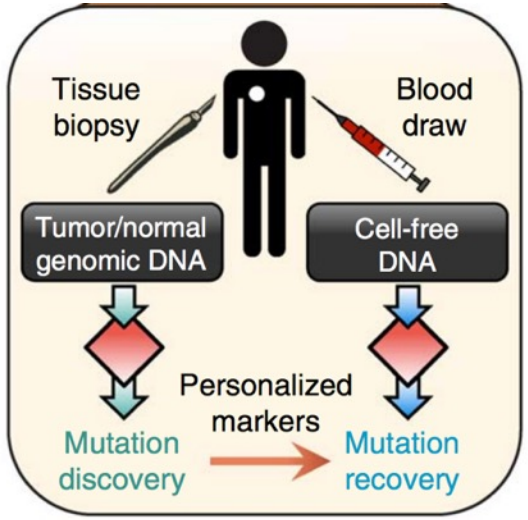
Different types of ctDNA MRD assays

Tumour-naive[1]



- Genotyping with **no knowledge of tumour mutations** (“off the shelf”)[3]
- Faster, less expensive[3]
- Limit of detection ~0.1%[4,5]

Tumour-informed[2]



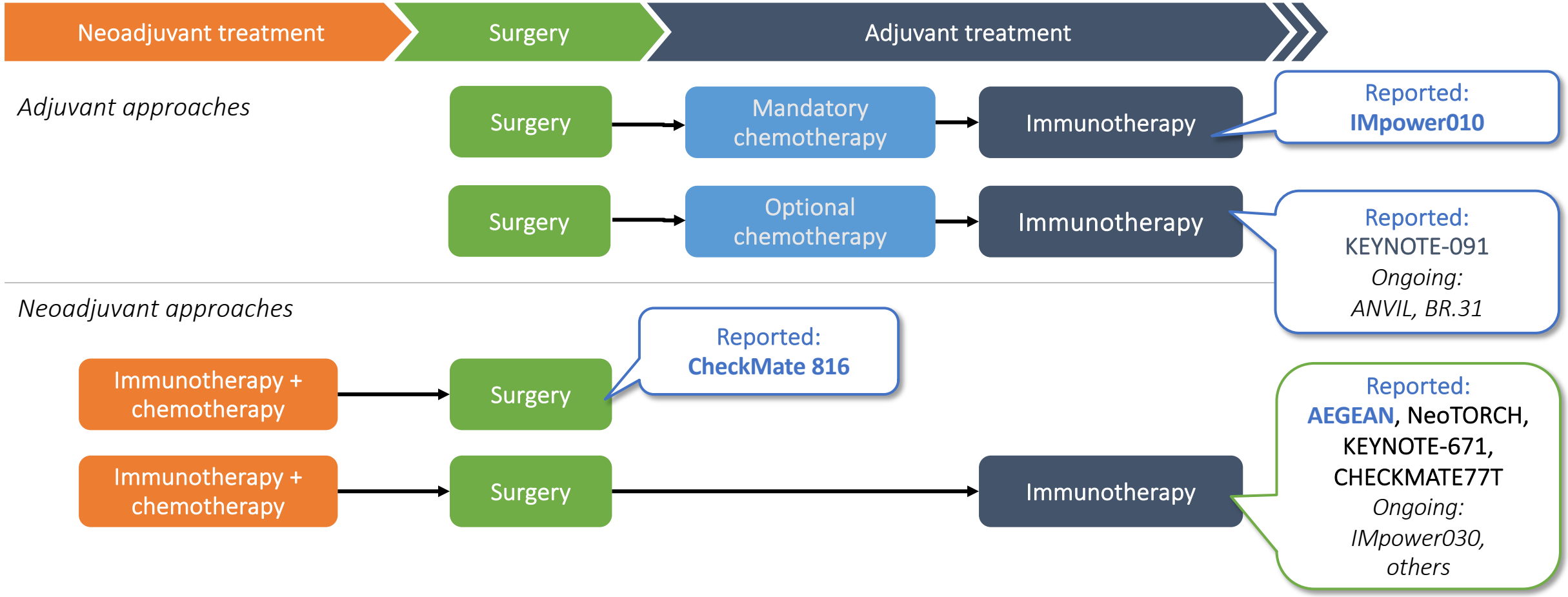
- Tracking **multiple known** mutations (bespoke or personalised)[3]
- Requires tumour tissue, time, $$[3]
- Limit of detection ~0.01%[5]

Slide courtesy of Dr. Max Diehn

CAPP-Seq, cancer personalised profiling by deep sequencing; ctDNA, circulating tumour DNA; MRD, minimal residual disease

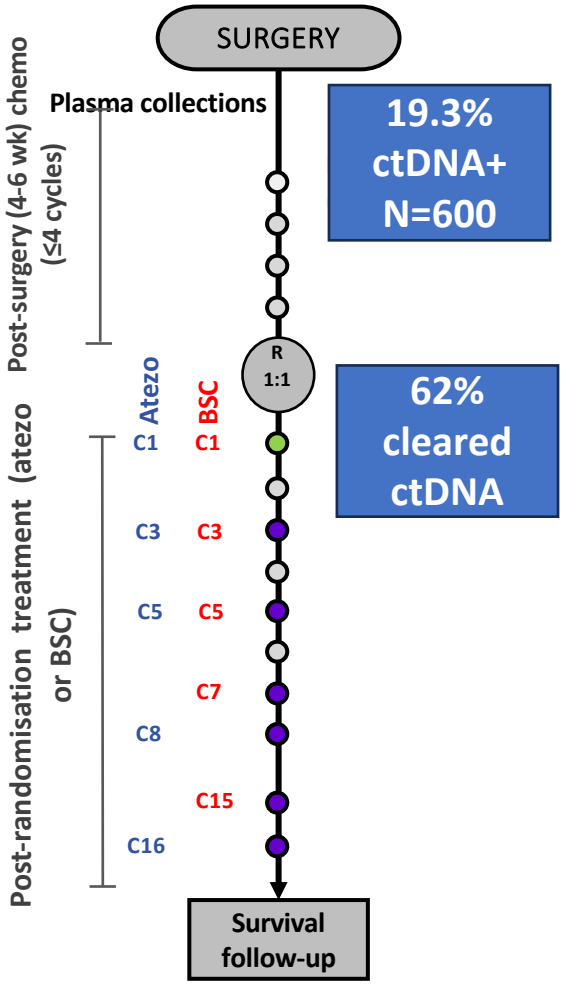
1. Newman A. Nat Biotechnol 2016;34:547–55; 2. Newman AM, et al. Nat Med 2014;20:548–54; 3. Chen H, et al. Oncol Rep 2023;49:106; 4. Chan HT, et al. Front Oncol 2022;12:1055968; 5. Moding E, et al. Cancer Discov 2022;11:2968–2986

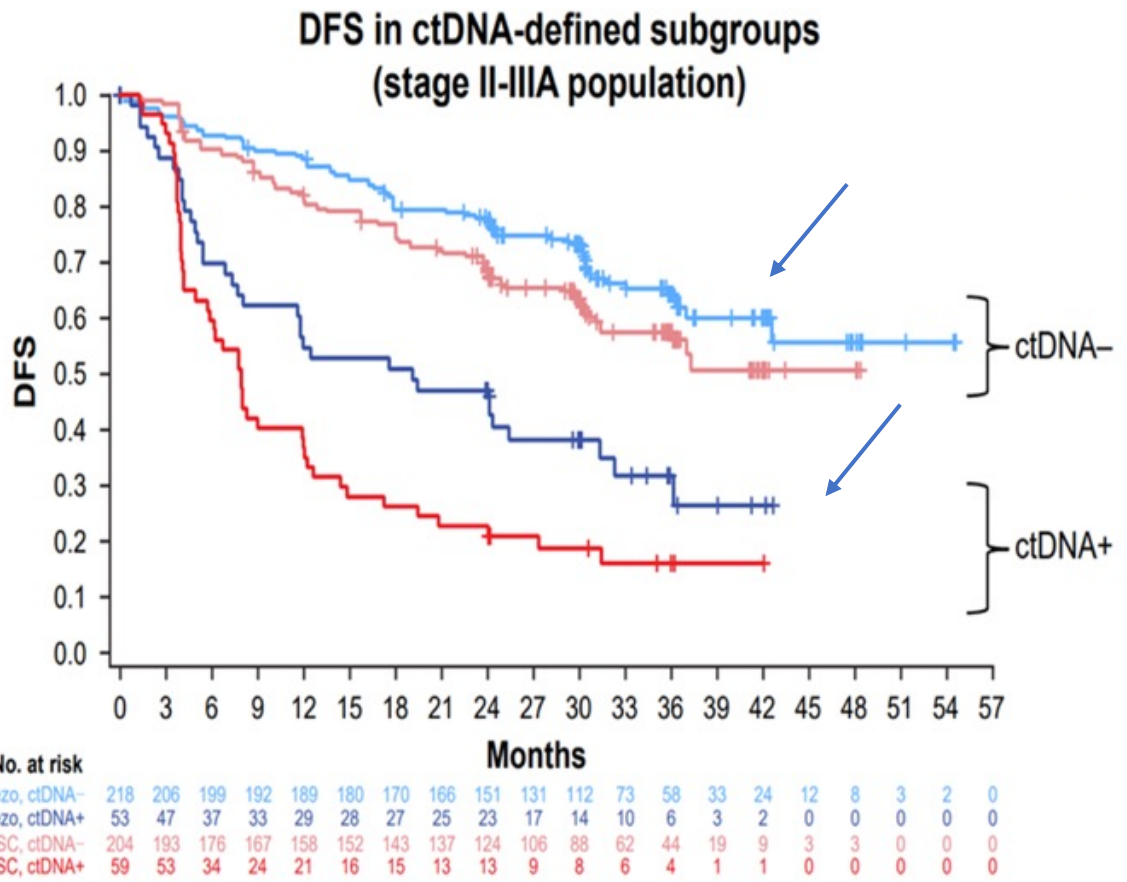
Phase III studies in resectable NSCLC



Modified from Dr. S. Peters, EPICS2022

IMpower-010: post-operative ctDNA (tumor informed assay) prognostic in early-stage NSCLC, but does not help select adjuvant therapy





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

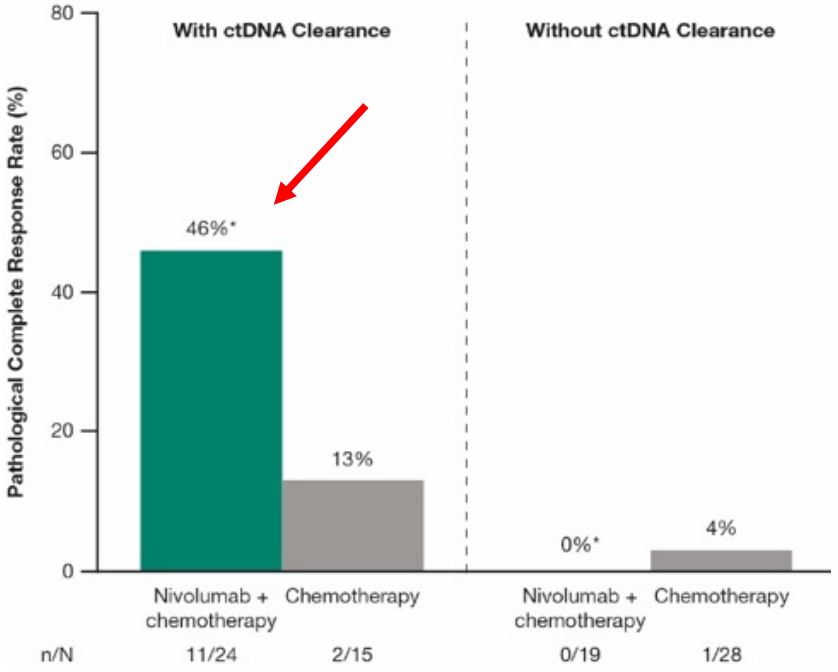
ctDNA+	Atezo (n=53)	BSC (n=59)
mDFS, mo	19.1	7.9
HR (95% CI)	0.61 (0.39, 0.94)	

Tumor informed assay

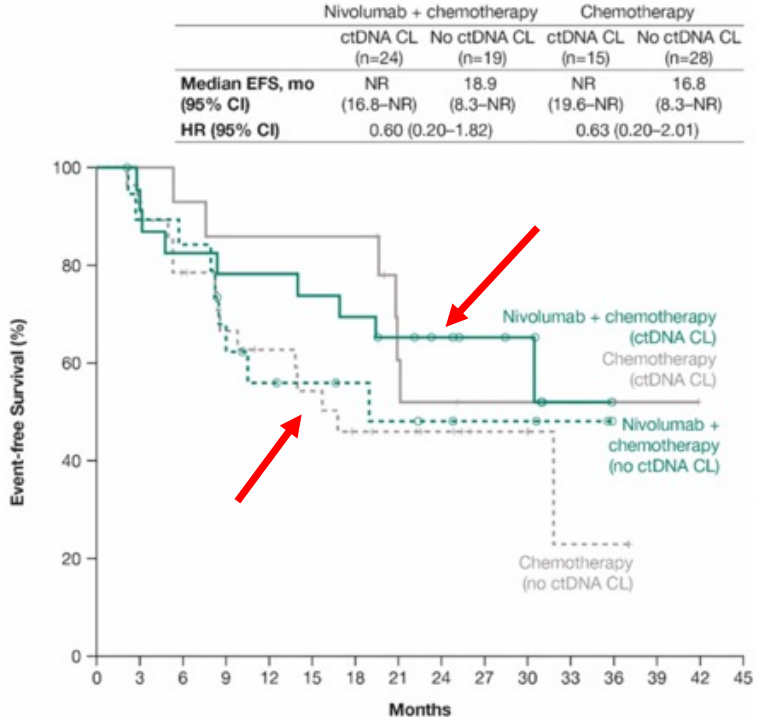
More sensitive assays are needed!

Atezo, atezolizumab; BSC, best supportive care; CI, confidence interval; ctDNA, circulating tumour DNA: DFS, disease-free survival; HR, hazard ratio; mDFS, median DFS; NR, not reached NSCLC, non-small cell lung cancer. Zhou C, et al. Oral presentation presented at ESMO IO 2021. Felip E, et al. Oral presentation presented at ESMO 2022.

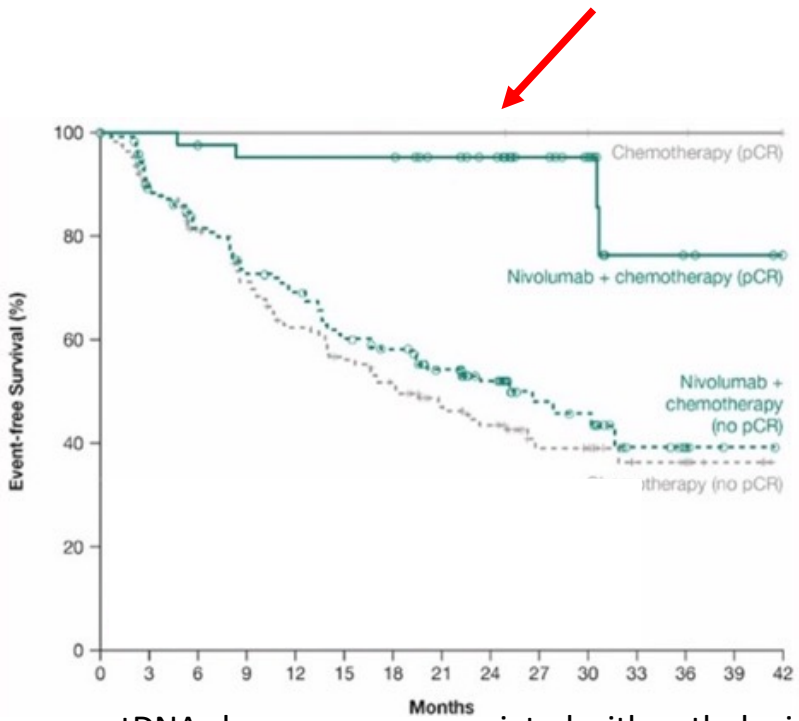
CheckMate 816: Preoperative ctDNA clearance associates with pathologic complete response and event-free survival *(but how does that help in clinic?)*



EFS by ctDNA clearance



EFS by pCR status

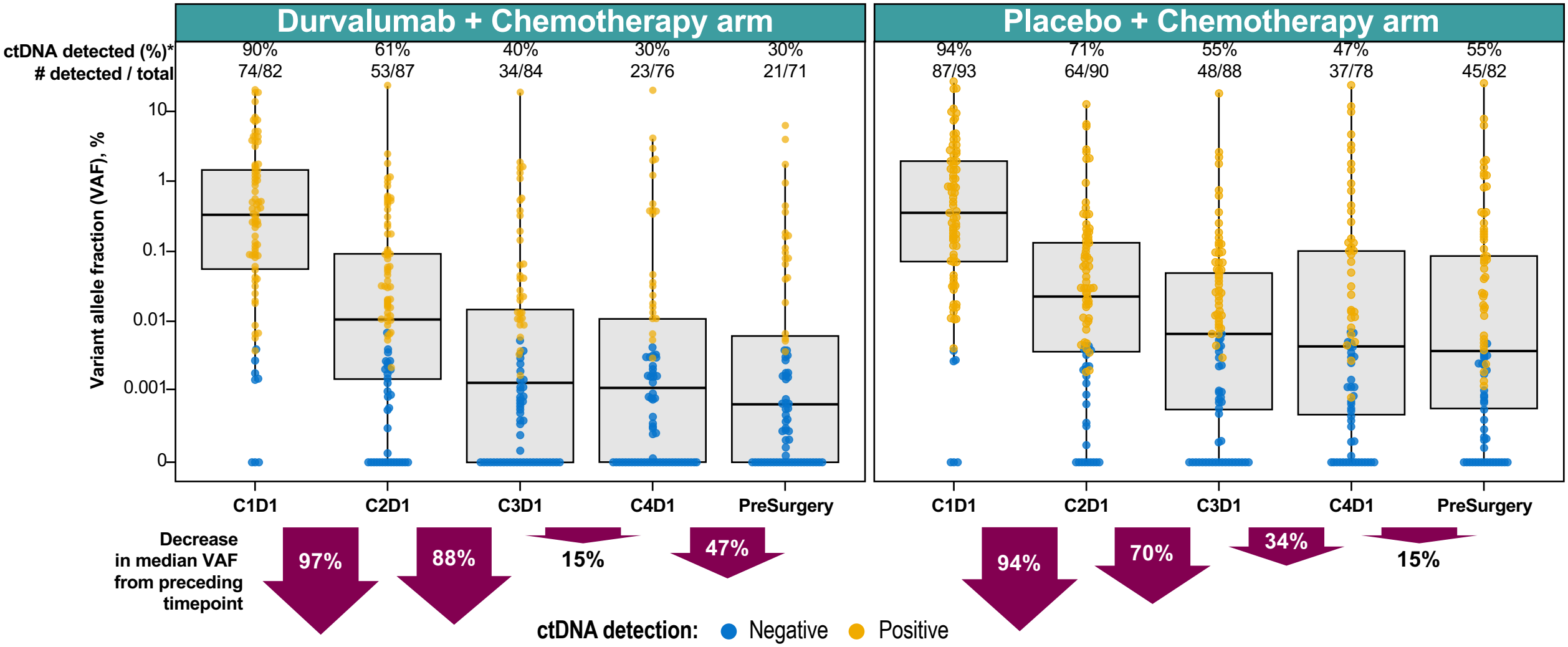


The use of ctDNA complements other surrogate endpoints such as pCR

ctDNA clearance was associated with pathologic CR and longer EFS

CI, confidence interval; CL, clearance; CR, complete response; ctDNA, circulating tumour DNA; EFS, event-free survival; HR, hazard ratio; NR, not reported; NSCLC, non-small cell lung cancer pCR, pathological CR. Forde PM, et al. N Engl J Med 2022;386:1973–85 (incl. suppl.)

AEGEAN: Preoperative ctDNA falls with neoadjuvant treatment (greatest in cycle 1)

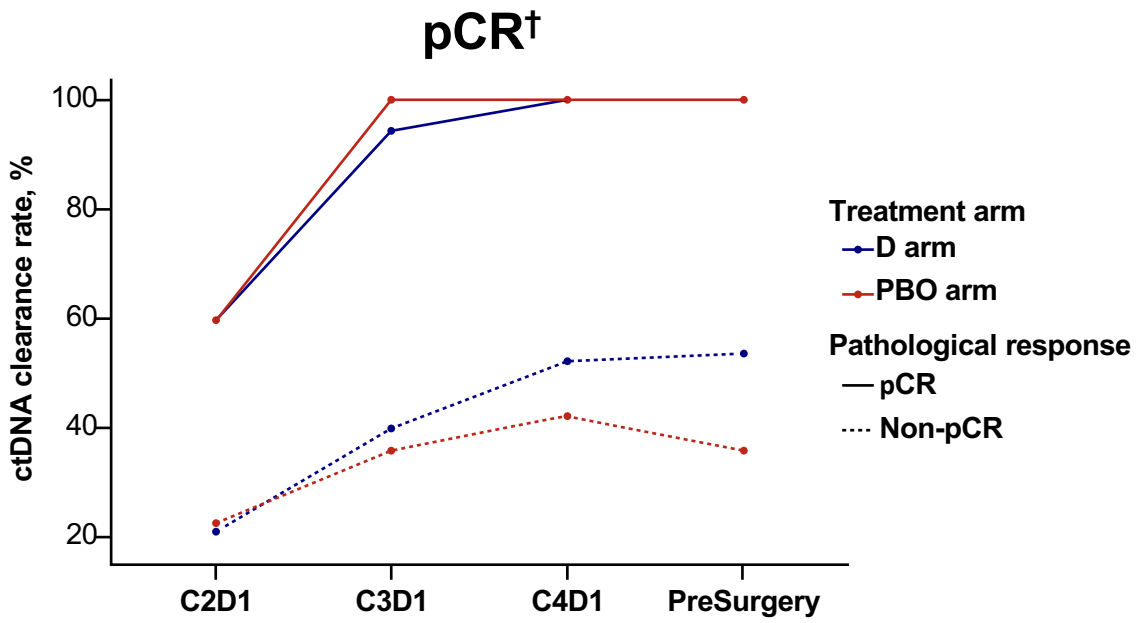


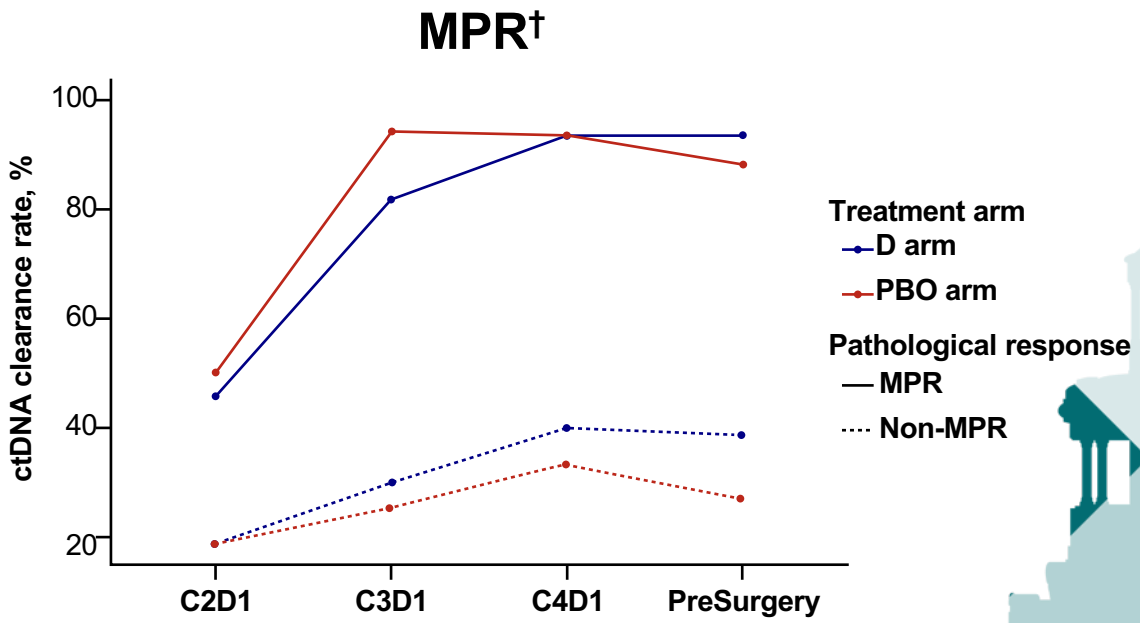
Reck M, et al. ESMO 2023 Congress LBA59

*ctDNA was considered detected if the signal to noise exceeded a threshold of P<0.01.
There is a variable number of patients analysed per timepoint, as indicated.
†There was no difference in median VAF levels at baseline between the two treatment arms.

Association of ctDNA Clearance with pCR/MPR and Its Predictive Utility

- Among patients who were ctDNA-positive at baseline (C1D1), all patients achieving pCR and >90% of all patients achieving MPR had ctDNA clearance at C4D1*





- Patients without ctDNA clearance were unlikely to achieve pCR (NPV > 84.0% at C2D1 in both arms)
- Patients who achieved ctDNA clearance in the D arm vs the PBO arm were more likely to achieve pCR (PPV = 50.0% vs 14.3% at C2D1)

Predictive value of ctDNA clearance at different timepoints for pCR

D arm	pCR	
	PPV	NPV
C2D1	**50.0%**	84.9%
C3D1	43.6%	97.1%
C4D1	40.5%	100.0%
PreSurgery	41.5%	**100.0%**

PBO arm	pCR	
	PPV	NPV
C2D1	14.3%	96.9%
C3D1	18.2%	100.0%
C4D1	18.2%	100.0%
PreSurgery	19.4%	**100.0%**

*In the BEP, pCR (25.6% vs 6.3%) and MPR (44.4% vs 18.8%) rates were higher in the D arm vs the PBO arm.
†The plots include all evaluable patients at each timepoint.
NPV, negative predictive value; PPV, positive predictive value.

Reck M, et al. ESMO 2023 Congress LBA59

Ongoing or pending trials in early stage lung cancer

Number	Prior tx	Stage	N	ctDNA-positive intervention	ctDNA-negative intervention	Phase	Primary endpoint	Site(s)
NCT04585477	Surgery or RT +/- chemo	I–III	80	Durvalumab	None	II	ctDNA change	Stanford
NCT04585490	chemoRT + several cycles durvalumab	III	48	Durvalumab + platinum doublet chemotherapy	Durvalumab	III	ctDNA change	Stanford
NCT04966663	Surgery	I	66	Nivolumab + chemotherapy vs observation	None	II RCT	RFS	Toronto
NCT05536505	Surgery	I–III EGFR mt	180	Icotinib	None	II	DFS	Guangdong
TBD	Surgery	II EGFR wt	1204	Adjuvant vs observation	Adjuvant vs observation	III	DFS	Gustave Roussy + EU + Toronto
TBD	None	I	TBD	Preoperative datopotamab deruxtecan	None		TBD	

ctDNA, circulating tumour DNA

ClinicalTrials.gov. NCT04585477. Available at: https://classic.clinicaltrials.gov/ct2/show/NCT04585477 (accessed September 2023);
ClinicalTrials.gov. NCT04585490. Available at: https://classic.clinicaltrials.gov/ct2/show/NCT04585490 (accessed September 2023);
ClinicalTrials.gov. NCT04966663. Available at: https://classic.clinicaltrials.gov/ct2/show/ NCT04966663 (accessed September 2023);
ClinicalTrials.gov. NCT05536505. Available at: https://classic.clinicaltrials.gov/ct2/show/ NCT05536505 (accessed September 2023)

ctDNA Lung DETECT study

Clinical stage I NSCLC

n=360 patients with operable (**T1-2 <4cm and T3-4 multifocal**) NSCLC

Complete (R0) Resection

Real-time ctDNA assessment

ctDNA positive (est ~20%) n=72

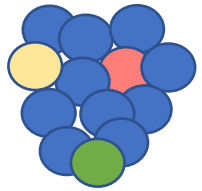
ctDNA negative n=288

Adjuvant chemo-immunotherapy

ctDNA RCT

Observation (Standard)

Pre-op n=360



Resection sample n=360

3-6 weeks Post-op n=360

At relapse or 12 month follow-up n=360

<u>ctDNA Detect:</u>

ctDNA association with RFS, OS across timepoints

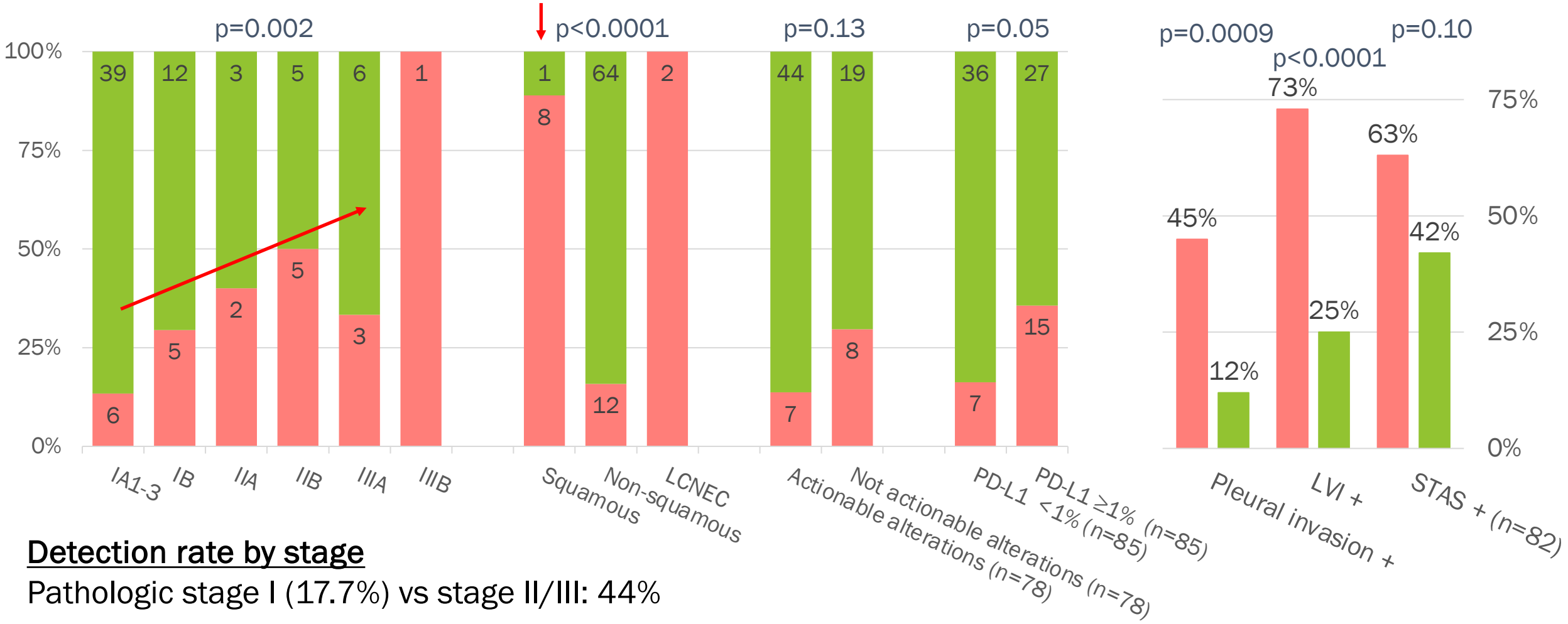
<u>ctDNA RCT:</u>

2 year RFS with adjuvant therapy

NCT05254782; NCT04966663

Pre-operative positivity in 25% of samples (n=87)

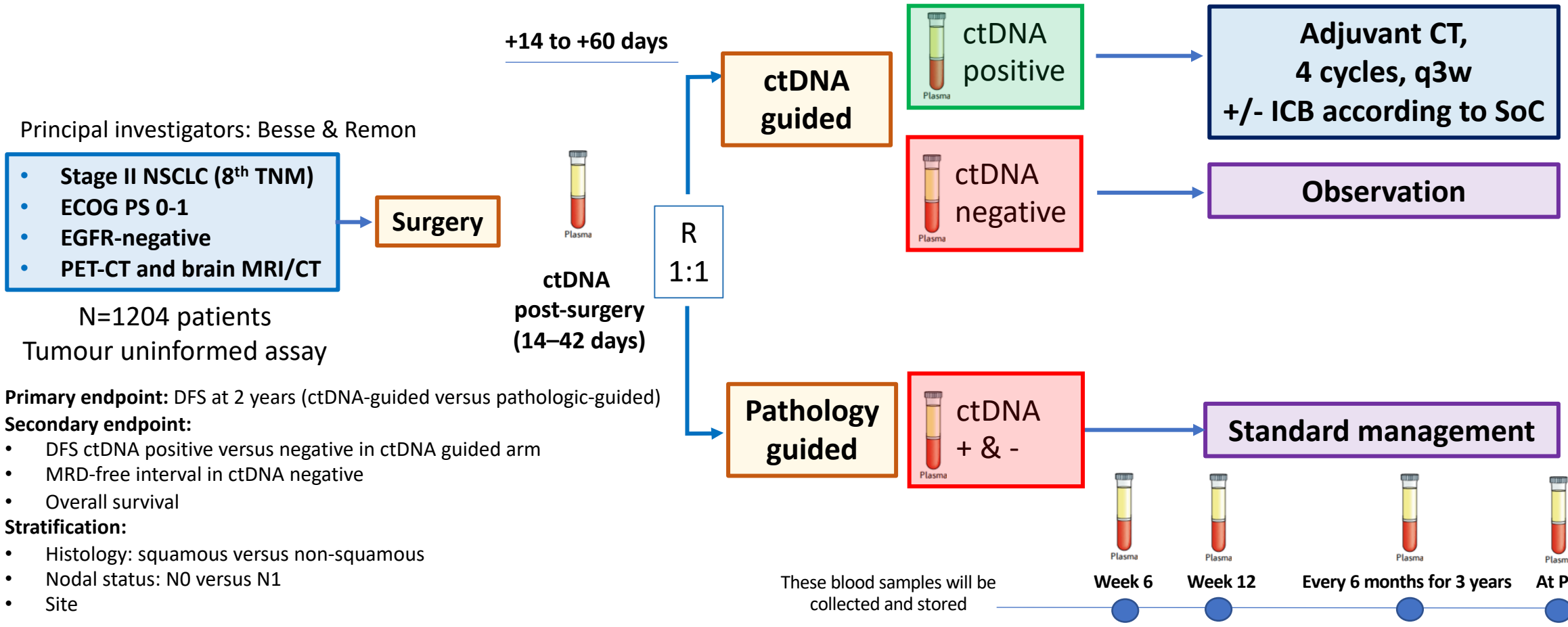
ctDNA positive tumors larger: **25 vs 19mm**, p=0.007



Detection rate by stage

Pathologic stage I (17.7%) vs stage II/III: 44%

ADMIRO trial – Can we de-escalate?



Primary endpoint: DFS at 2 years (ctDNA-guided versus pathologic-guided)

Secondary endpoint:

- DFS ctDNA positive versus negative in ctDNA guided arm
- MRD-free interval in ctDNA negative
- Overall survival

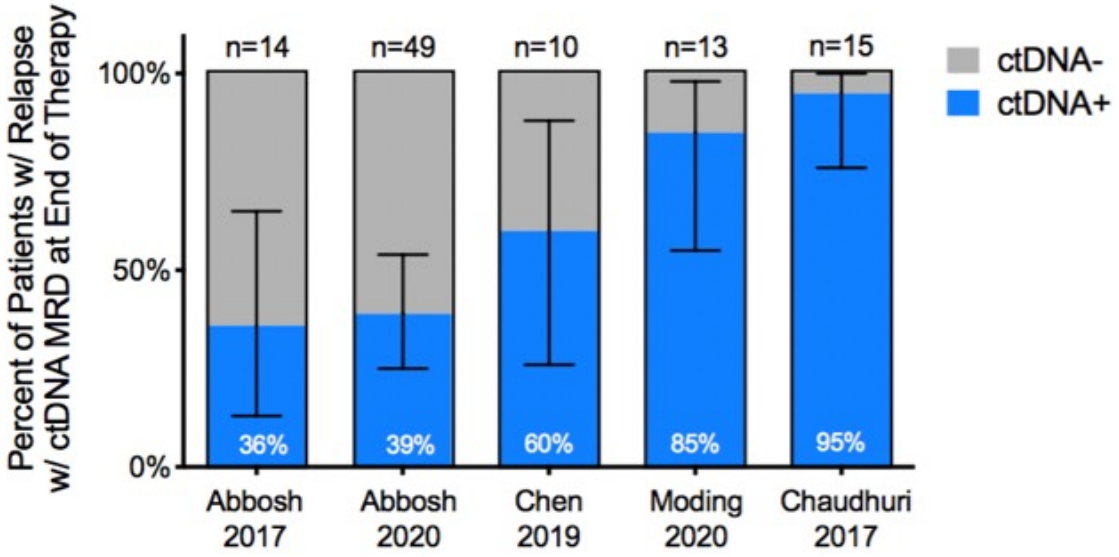
Stratification:

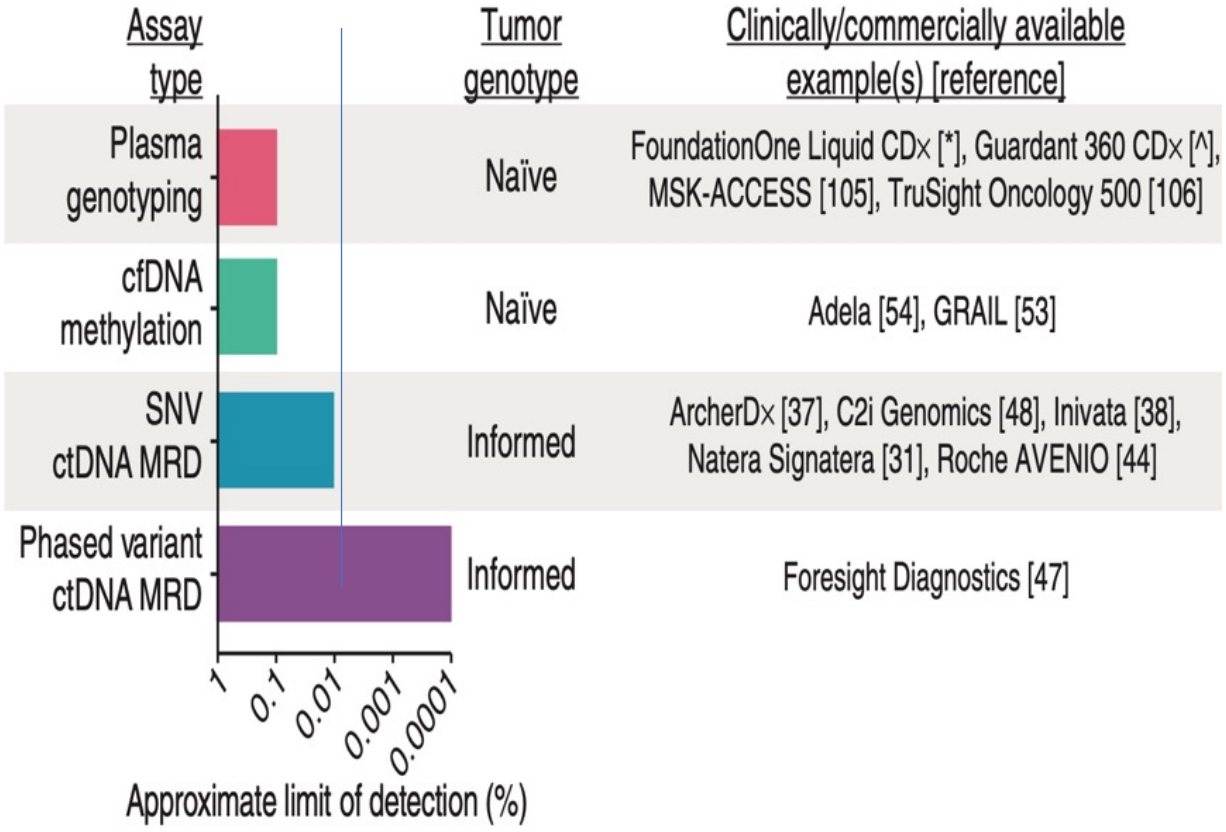
- Histology: squamous versus non-squamous
- Nodal status: N0 versus N1
- Site

CT, chemotherapy; ctDNA, circulating tumour DNA; DFS, disease-free survival; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; ICB, immune checkpoint blockade; MRD, minimal residual disease; MRI, magnetic resonance imaging; NSCLC, non-small cell lung cancer; R, randomisation; PET-CT, Positron emission tomography–computed tomography; PD, progressive disease; q3w, every 3 weeks; SoC, standard of care; TNM, tumour, node and metastasis. Adapted Courtesy of: Dr Jordi Remon

#1 challenge – sensitivity of MRD assay

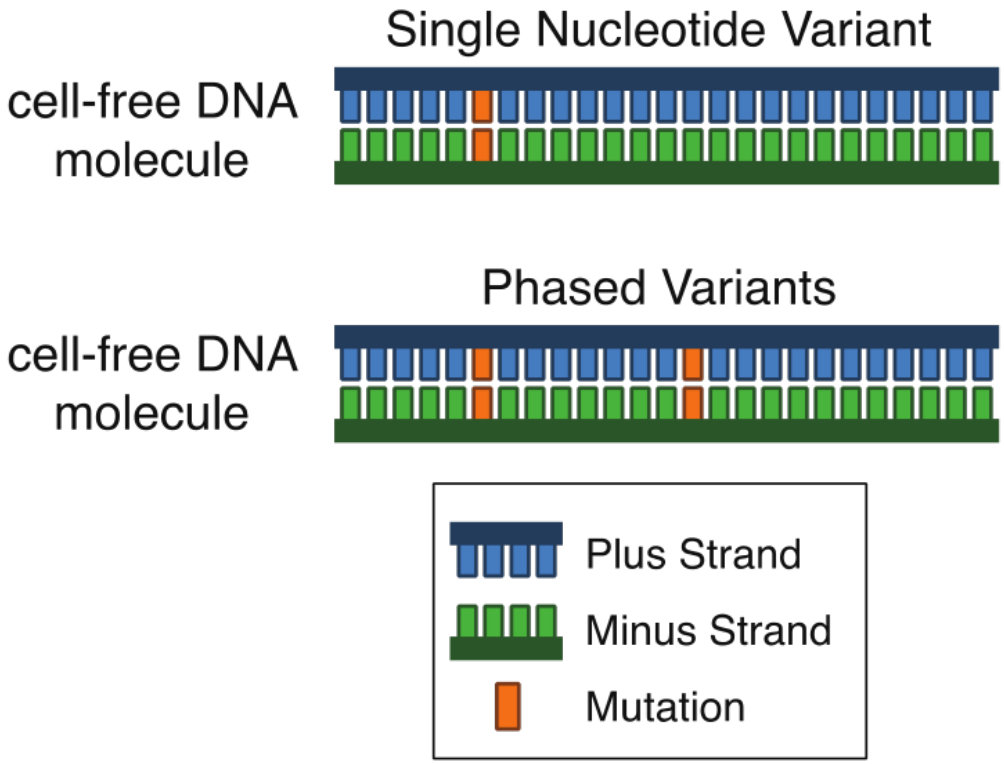
- False negatives





Source: Dr. Max Diehn, ESMO Applications of Liquid Biopsy Series – Lung Cancer, October 2021
Chin et al Mol Diagn Ther; Moding et al Cancer Discovery 2021

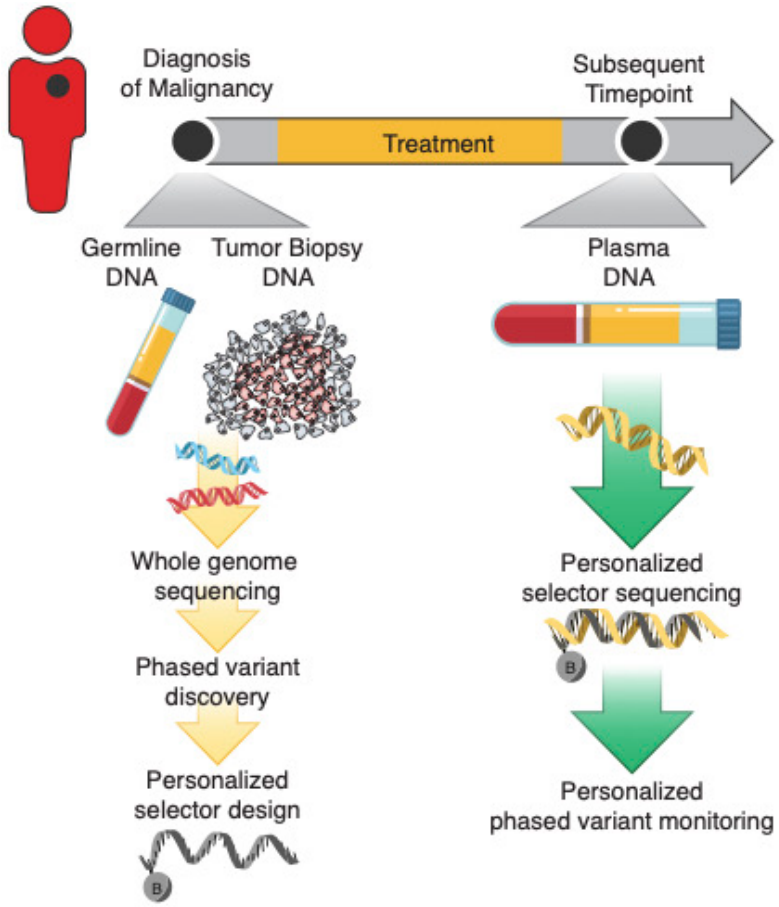
Novel ways to improve LOD: Phased Variants



Limit of Detection

~0.01%

0.01% * 0.01% ≤ 1e-6

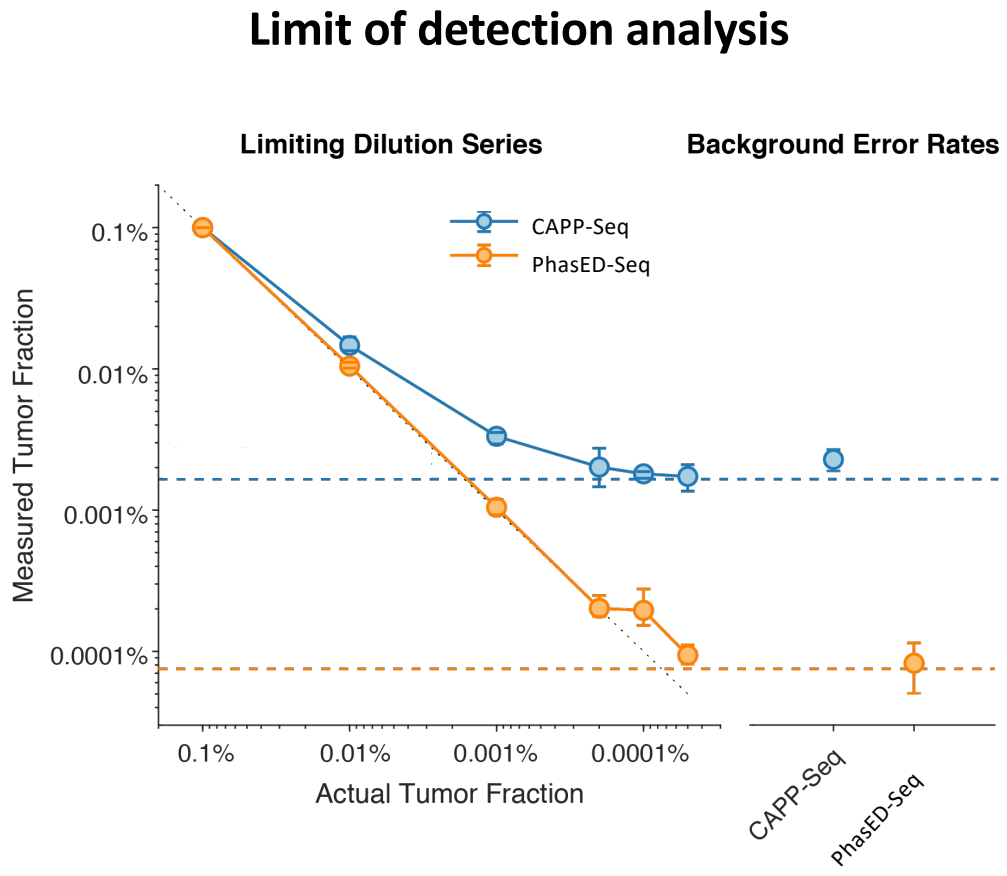


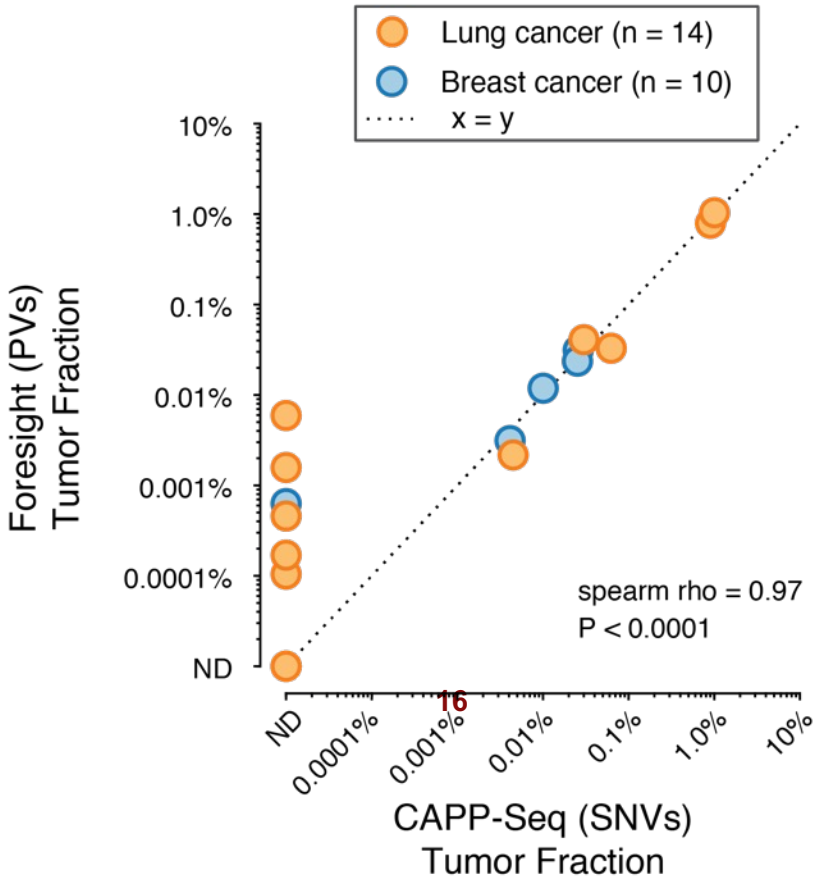
Median of ~1,000 PVs per NSCLC

Kurtz et al. *Nature Biotechnology* 2021; courtesy Dr. Max Diehn

More sensitive ctDNA Detection in Lung and Breast Cancers

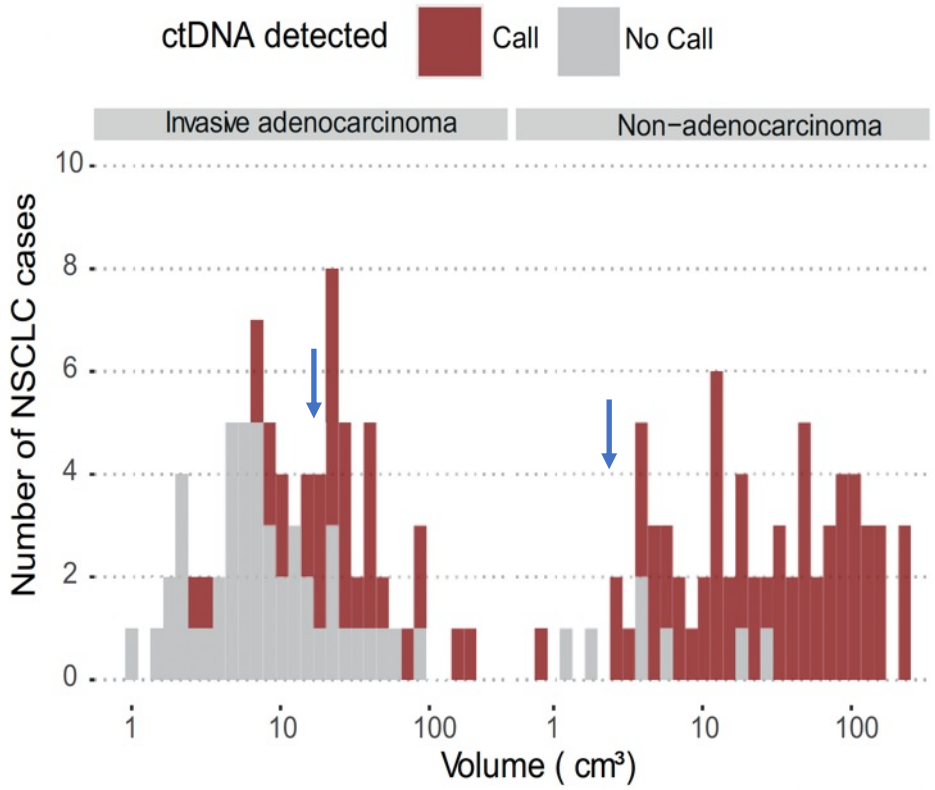
Minimize risk of false negative results – potential to de-escalate therapy?

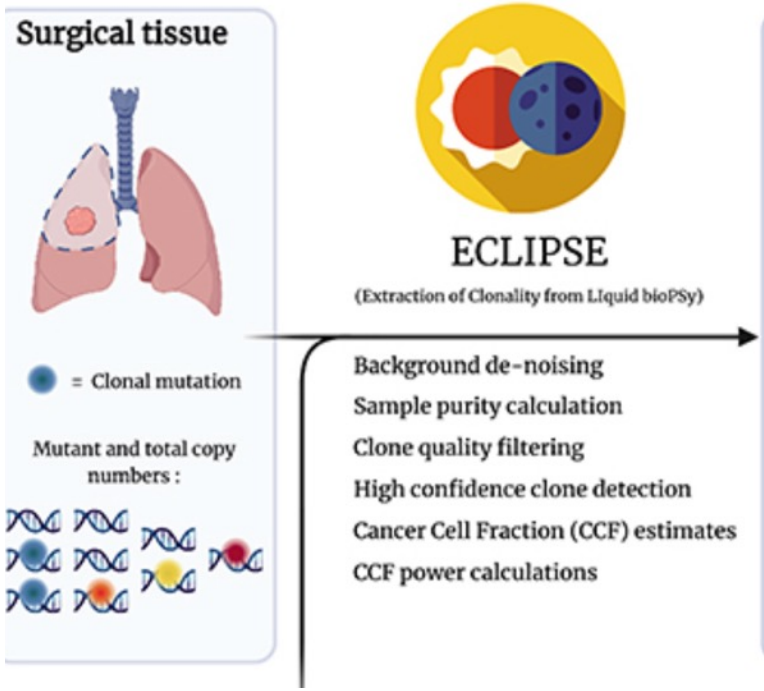


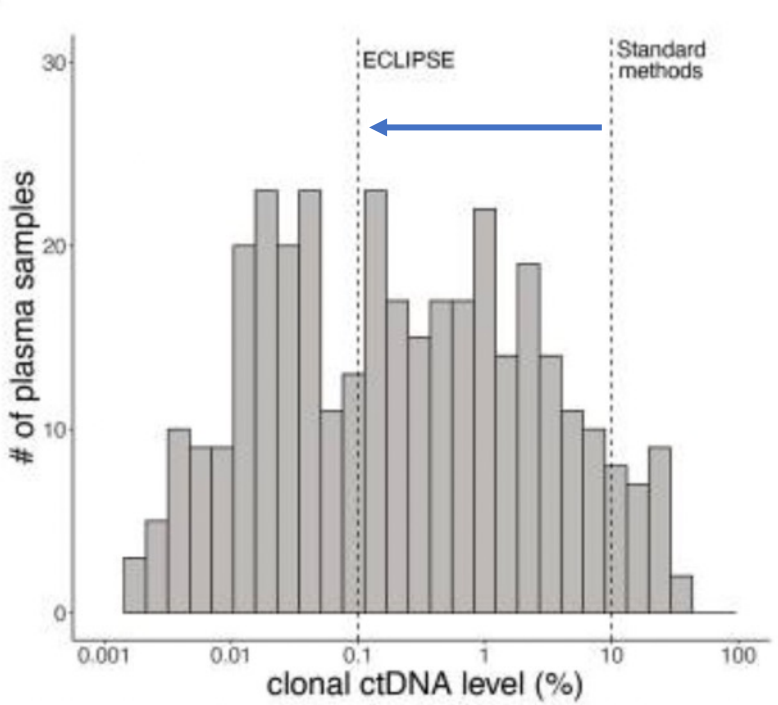


Kurtz et al. *Nature Biotechnology* 2021

TRACERx: Detecting MRD using subclonal populations and AI







Abbosh C, et al. Nature 2023;616:553–562

Clinical utility of liquid biopsy monitoring still under investigation

APPLE phase II trial: study design

- Advanced NSCLC.
- Del19, L858R *EGFR* mut.
- EGFR TKI naïve.
- ECOG PS 0-2.
- Stable BM allowed w/o steroids.

R 1:1:1

A N=53 → Osimertinib 80 mg daily (QD) until RECIST 1.1 PD

B N=52 → Gefitinib 250 mg QD until central ct-DNA T790M + or RECIST 1.1 PD → Osimertinib 80 mg QD until RECIST 1.1 PD

C N=51 → Gefitinib 250 mg QD until RECIST 1.1 PD → Osimertinib 80 mg QD until RECIST 1.1 PD

Stratification criteria:

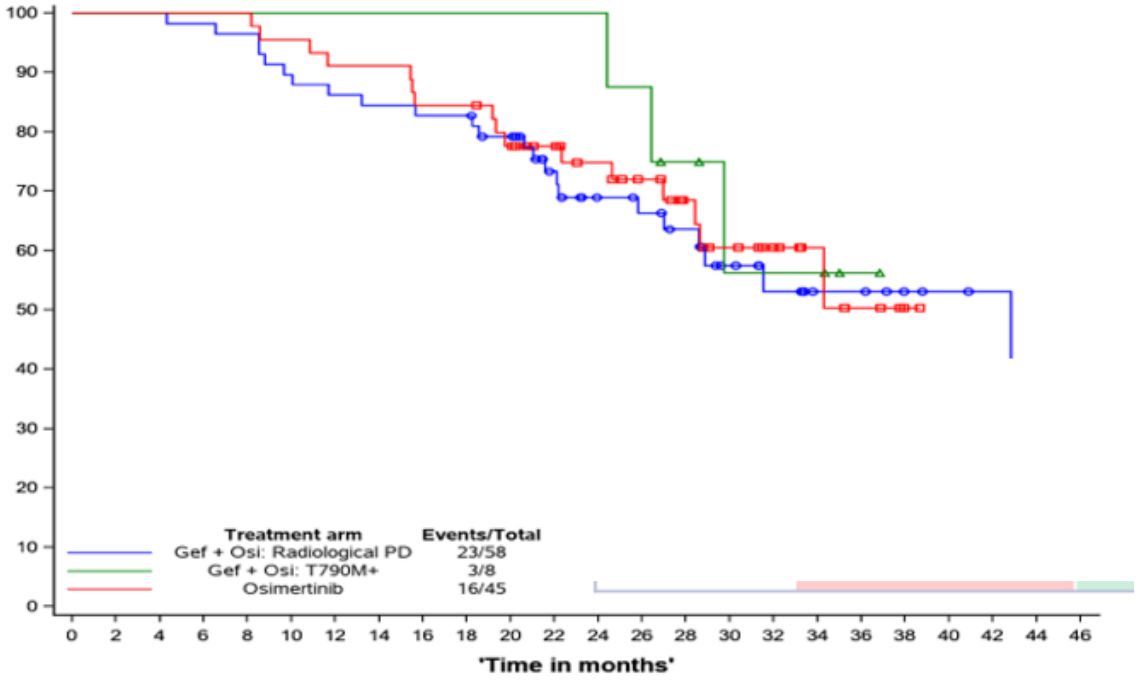
- *EGFR* mutation subtype (Del19 *vs.* L858R)
- Brain metastasis (present *vs.* absent)
- T790M at baseline (positive *vs.* negative)

CENTRAL ct-DNA cobas *EGFR* Mutation Test v2, performed Q4W.
Only applied as a predictive for making treatment decisions in arm B

RECIST 1.1 assessment with thorax, abdomen and brain CT-scans Q8W.

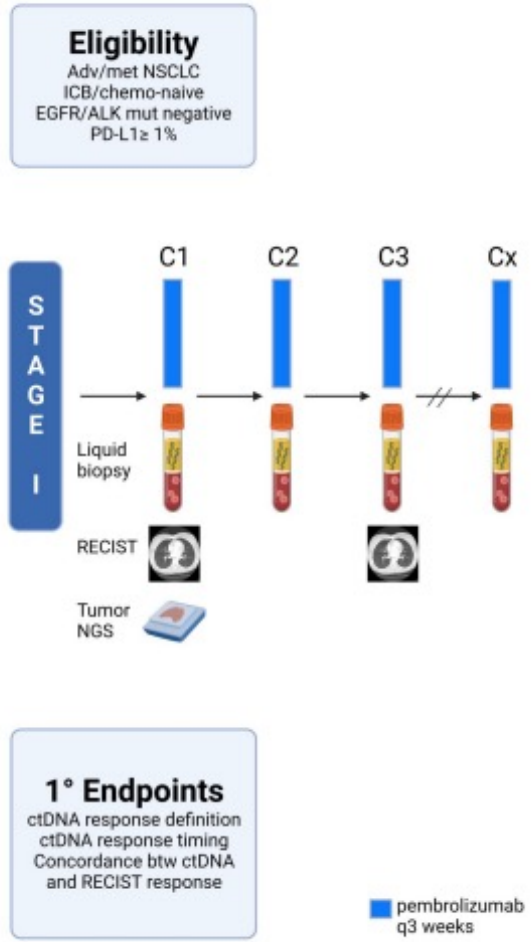
17% switched early (molecular PD)
Better PFS from starting 2L osimertinib (?lead time bias)

No difference in survival between arms A, B or C
Osimertinib upfront similar PFS, OS but better CNS PFS

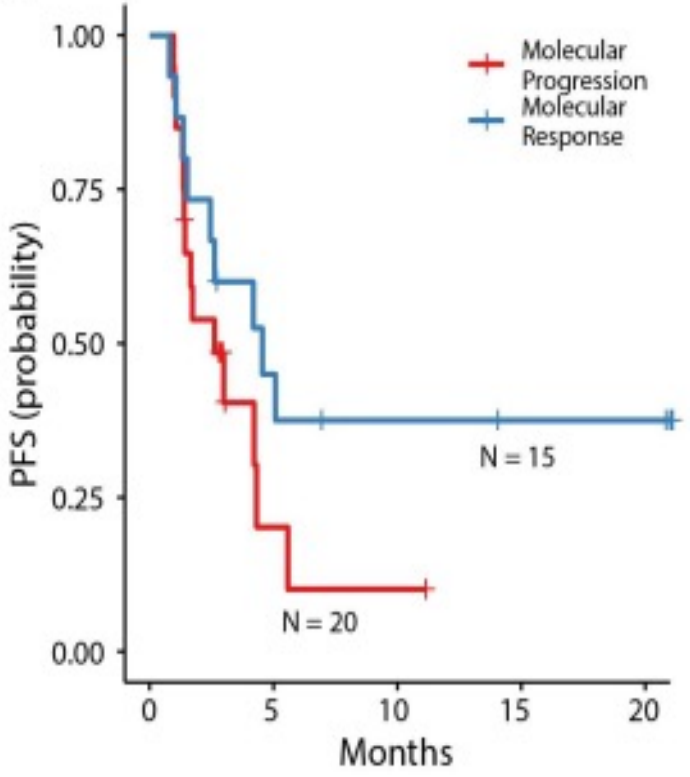


Remon et al ESMO 2022; Remon et al ELCC 2023; Remon et al Ann Oncol 2023

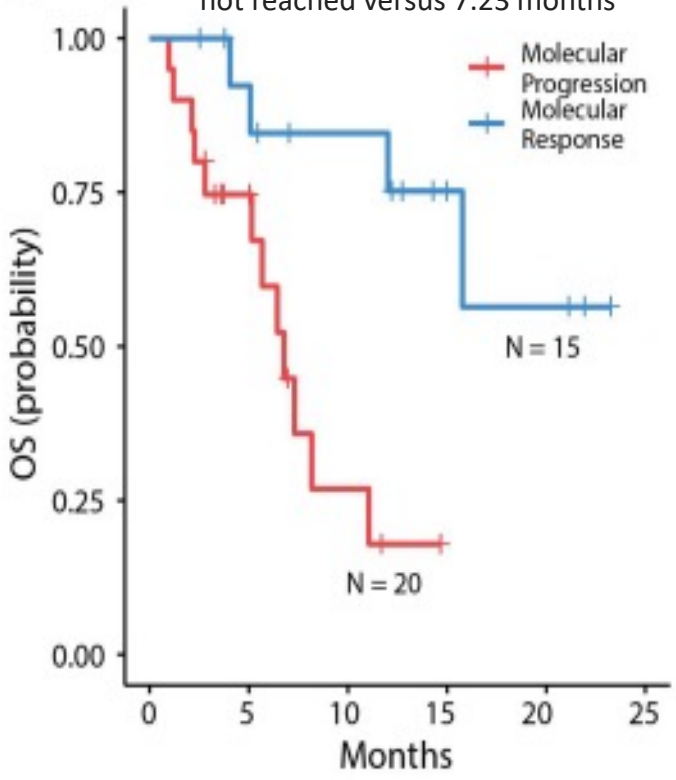
CCTG BR.36: ctDNA response 82% concordant with RECIST molecular response associated with PFS and OS (even in SD)



Median PFS in molecular response
5.0 versus 2.6 months



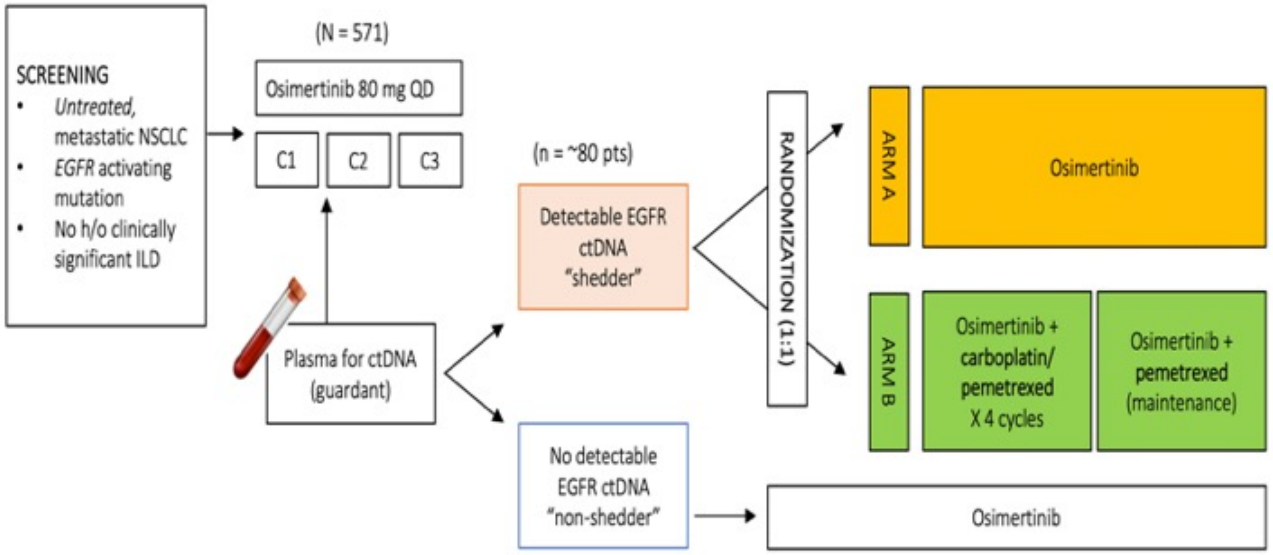
Median OS in molecular response
not reached versus 7.23 months



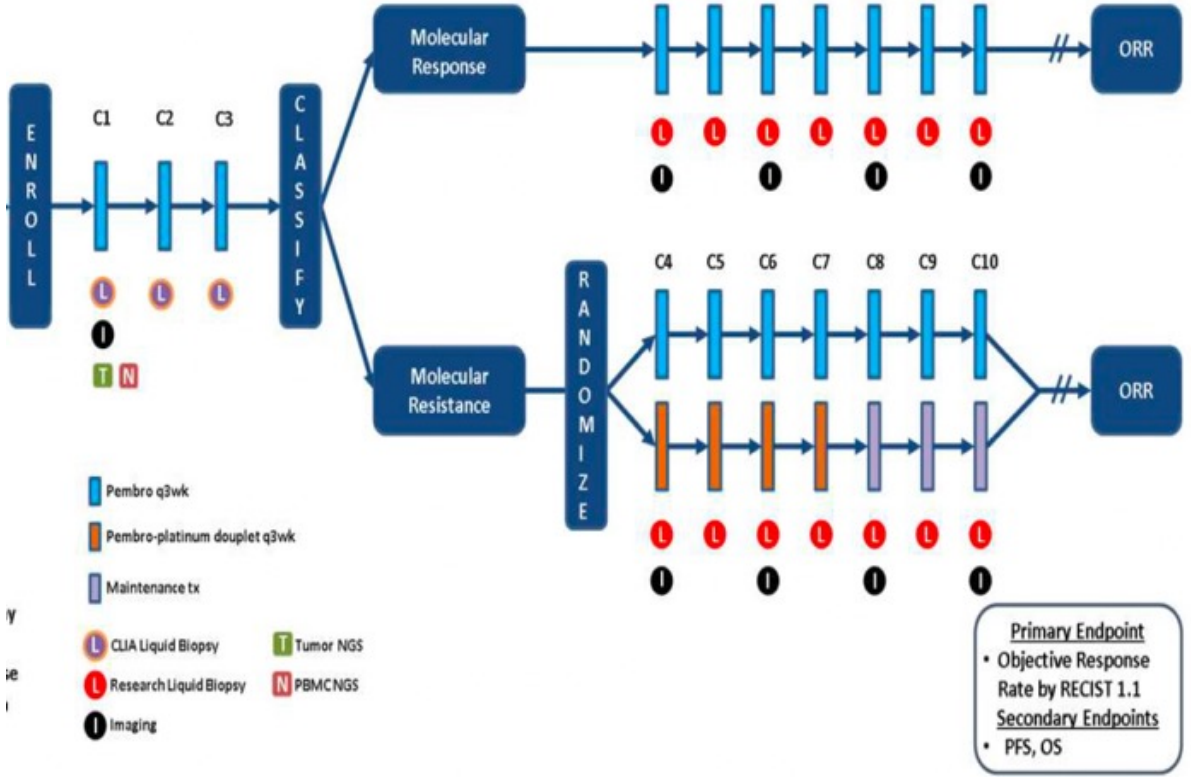
ALK, anaplastic lymphoma kinase; C, cycle; ctDNA, circulating tumour DNA; ICB, immune checkpoint blockade; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; OS, overall survival
PD, progressive disease; PD-L1, programmed cell death ligand-1; PFS, progression-free survival; q6/9/12w, every 6/9/12 weeks; RECIST, Response Evaluation Criteria in Solid Tumours; SD, stable disease; W, weeks 1. Anagnostou V, et al. Poster presented at AACR 2023 (Poster CT212); 2. ClinicalTrail.gov. NCT04093167. Available at: https://classic.clinicaltrials.gov/ct2/show/NCT04093167 (accessed September 2023)

Some exciting ctDNA guided trials in this space

Osimertinib +/- Chemotherapy
PI: Dr. Helena Yu, MSKCC



Pembrolizumab +/- Chemotherapy
PIs: Dr. Cheryl Ho (BC), Dr. Elsa Anagnastou (JHU)



ClinicalTrial.gov. NCT04410796. Available at: https://classic.clinicaltrials.gov/ct2/show/NCT04410796 (accessed September 2023)
ClinicalTrial.gov. NCT04093167. Available at: https://classic.clinicaltrials.gov/ct2/show/NCT04093167 (accessed September 2023)

Key Take Aways

- MRD and ctDNA monitoring not yet ready for prime time in NSCLC but this may change soon…

- MRD is a rapidly emerging biomarker in early and late stage disease

    - ctDNA strongly prognostic at all timepoints
    - ctDNA clearance with treatment prognostic
    - More trials are needed to prospectively test interventions based on ctDNA results
    - Next generation assays needed to improve sensitivity to decrease false negative rate

The future….