Assessment of MRD

Dr. Margarita Majem

Hospital de la Santa Creu i Sant Pau

Barcelona

COI Disclosure

- Honoraria: Amgen, AstraZeneca, Roche, Bristol Myers Squibb, Merck Sharp & Dohme, Pfizer, Boehringer Ingelheim, Novartis, Immedica, Helsinn Therapeutics, Takeda, Sanofi, Johnson & Johnson, Pierre Fabre, Beigene, Lilly.

- Research Funding: Bristol-Myers Squibb (Inst), AstraZeneca (Inst), Roche (Inst)

- Travel, Accommodations: AstraZeneca, Roche, Pfizer, MSD, Johnson & Johnson.

Background

- **Circulating tumor DNA (ctDNA)**
  - ctDNA is a fraction of cell-free DNA (cfDNA) usually isolated from plasma.
  - Fragments of DNA shed from tumor cells into the bloodstream.
  - Detected and quantified using sensitive molecular techniques (NGS).
  - Non-invasive biomarker for tumor genotyping, monitoring treatment response, and identifying emerging resistance mechanisms.

- **Molecular Residual Disease (MRD) in NSCLC**
  - **Clinical state** in which a small number of cancer cells remain in the body **after curative-intent treatment,** undetectable by conventional imaging or pathology.
  - **MRD is detected by the presence of ctDNA in plasma after treatment** → strongly predictive of disease recurrence, often preceding radiologic relapse by several months.

ctDNA in early stage

- Greater risk of disease recurrence when detected before and/or after **surgery or CT-RT.**
  - Longitudinal monitoring after surgery increase the prognostic value → detection recurrence earlier than clinical or radiological progression.

- To date, limitations of studies do not support the routine clinical use of ctDNA monitoring:
  - Limited number of patients enrolled
  - Different technologies used for ctDNA testing

- Urgent need for interventional studies to provide evidence for implementing ctDNA testing in this setting.

Why MRD /ctDNA matters in NSCLC?

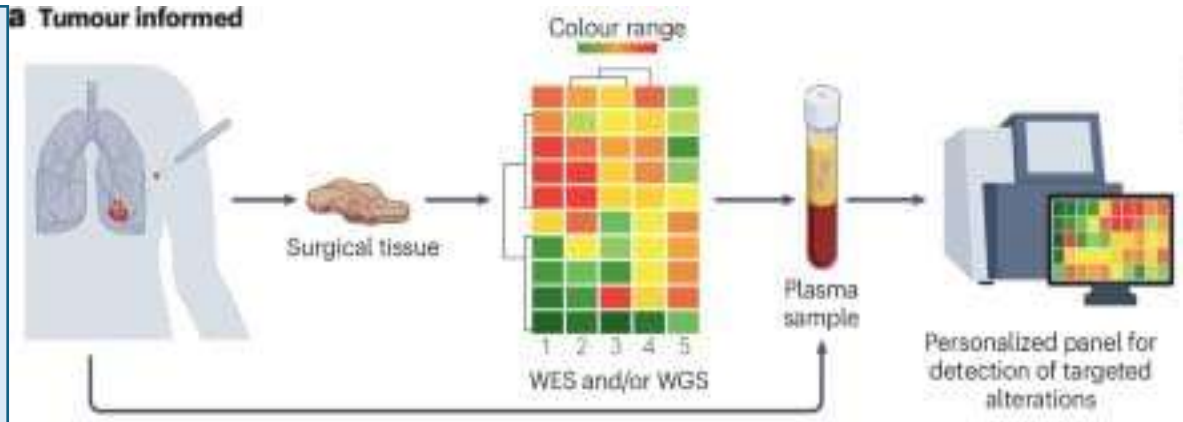
- Despite improvements in the **management of early stages**, 30%-50% of NSCLC patients will experience recurrence.
  - TNM staging is the main prognostic factor for treatment decisions.
  - Patients with early-stage lung cancer might be treated sub-optimally.
  - Toxic treatment is often administered to patients who do not need it, while those who could benefit to prevent relapse do not receive it.

- **Non-invasive biomarkers are urgently needed to help us to predict disease relapse.**
  - ctDNA detects recurrence months before it is visible.
  - It may help for accurate risk stratification.

Rajaram R. Chest 2024

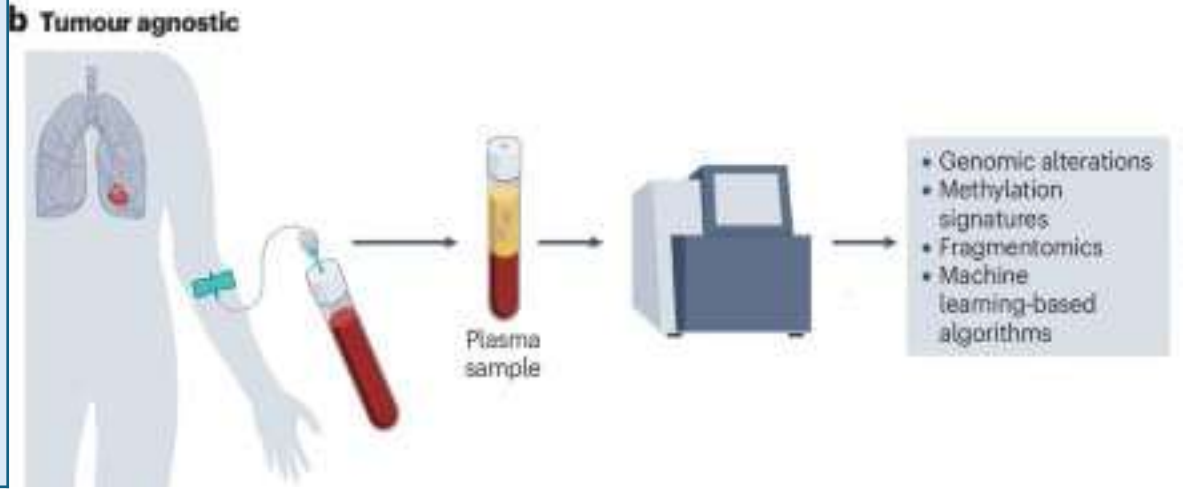
Testing strategies for the detection of ctDNA in early-stage NSCLC

Tumor Informed



- Tumor sequencing → identify mutations.
- Germline sequencing (to rule out CHIP).
- Create personalized panel
- Detection in blood before/ after surgery and follow-up.

Tumor Agnostic



- No tumor required.
- Detects tumor signal without a custom panel.
- Analyzes mutational ctDNA, methylation patterns
- Bioinformatic algorithms

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Testing strategies for the detection of ctDNA in early-stage NSCLC

Tumor Informed	Personalized panel with patient-specific mutations	• High specificity / sensibility • Higher success rate • Higher LOD	• Requires tumor tissue (No tumour tissue if pCR or near pCR) • Longer TAT • Higher cost • WES or WGS affected by quality and amount of tissue samples (esp. for early biopsy) • Does not detect temporal or spatial heterogeneity • Number of targeted variants affects LOD
Tumor Agnostic	Predefined cancer gene panel.	• Rapid TAT • No need for tissue sample • Lower cost • Representative of temporal or spatial heterogeneity	• False-negative (limited coverage of tumour mutations) • False-positive: Interference of clonal hematopoiesis (CHIP) • In study, new generation of assays and the use of methylation and fragmentomic profiles as highly sensitive biomarkers

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Assays to Detect Minimal Residual Disease

Assay	Method	Tumor Genotype Informed	Variants Assessed	Reported LOD	Published Validation Studies in NSCLC (n)
Signatera[19]	Multiplex PCR based NGS	Yes	Top 16 somatic SNVs and Indels	0.01% VAF	Abbosh et al (n = 100)[18]
RaDaR[20]	Multiplex PCR based NGS	Yes	SNVs, indels and CNAs	0.001% VAF	Gale (n = 88)[21]
CAPP-Seq[22]	Hybridization capture based NGS	Yes	SNVs	0.003% VAF	Chaudhuri et al (n = 37)[22] Moding et al (n = 65)[23] Jun et al (n = 39)[24] [Abstract only]
AVENIO Surveillance kit[27]	Hybridization capture based NGS	Yes	SNVs, indels, fusions and CNAs	0.1% VAF	Nil to date
PhasED-seq[28]	Hybridization capture based NGS	Yes	SNVs and Phased Variants	0.000094% VAF	Kurtz et al (n = 5)[28]
MRDetect[67]	WGS-based	Yes	SNVs and CNAs	0.001% VAF	Zviran et al (n = 22)[30] Tan et al (n = 52)[68] [Abstract only]
Guardant Reveal[33,69]	Hybrid capture based NGS and methylation	No	SNVs, indels and methylation	0.01% VAF	Nil to date
DELFI[36]	Fragmentomics	No	Fragment size	NA	Cristiano et al (n = 12)[36] Mathios et al (n = 46)[37]

Abbreviations: LOD, limit of detection; VAF, variant allele frequency; NGS, next generation sequencing; PCR, polymerase chain reaction; WGS, whole genome sequencing; CNAs, copy-number aberrations; indel, insertion or deletion; SNVs, single-nucleotide variants; CAPP-Seq, cancer personalized profiling by deep sequencing; DELFI, DNA evaluation of fragments for early interception; NA, not applicable.

O'Sullivan OncoTargets and Therapy 2023

ctDNA as a biomarker in early-stage NSCLC

1. Preoperative ctDNA testing
2. ctDNA testing during neoadjuvant therapy
3. Detection of MRD after NSCLC resection
4. ctDNA as a biomarker in locally advanced unresectable NSCLC

Preoperative ctDNA testing

Preoperative ctDNA testing

- **ctDNA + before** surgery or CTRT might correlate with progression and inform on the response to neoadjuvant therapy
- **ctDNA testing after** surgery or CTRT can enable **detection of MRD** or identify recurrence earlier during follow-up.
- **Preoperative ctDNA+ shorter RFS** (heterogeneous studies, not confirmed in multivariate analyses)
- ctDNA rate: Stage I 13% -stage III 88%

Table 1 | Clinical studies testing upfront ctDNA in patients with NSCLC

Study	Patients (*n*); disease stage	Time point	ctDNA assay (*n* of genes)	LOD	HR for RFS in patients with ctDNA positive vs negative status	ctDNA detection rate (overall; per stage)	Comments
Studies using tumour-informed and tumour-agnostic strategies							
Chabon et al. (2020)[61]	85; IA (15%), IB (39%), IIA (11%), IIB (14%), IIIA (14%) and IIIB (5%)	Before surgery or radiotherapy	CAPP-Seq (255)	≥0.01%	3.39 (95% CI 1.23–9.38; *P*=0.026) overall; 3.69 (95% CI 1.04–13.19; *P*=0.046) for stage I disease	57%; 42%, 67% and 88% for stages I, II and III, respectively	Small cohort size; ctDNA was detected with tumour-agnostic (*n*=68) and tumour-informed (*n*=17) strategies
Studies using tumour-informed strategies							
Gale et al. (2022)[62]	88; I (49%), II (28%) and III (23%)	Before surgery or chemoradiotherapy	RaDaR (up to 48)	NR	3.14 (95% CI 1.49–6.60; *P*=0.003)	51%; 24%, 77% and 87% for stages I, II and III, respectively	Small cohort size, with ctDNA results available for 78 patients
Abbosh et al. (2023)[45]	187; I–III[a]	Before surgery	Anchored Multiplex PCR (200[b])	0.008%	NR	66%; 42% in adenocarcinomas vs 92% in non-adenocarcinomas	NA
Tan et al. (2024)[63]	57; IA (54%), IB (14%), IIA (9%), IIB (7%), IIIA (14%) and IIIB (2%)	Before surgery	Signatera (up to 16)	NR	3.54 (95% CI 1.00–12.62; *P*=0.009)	26%; 23%, 13%, 0%, 50%, 63% and 100% in stages IA, IB, IIA, IIB, IIIA and IIIB, respectively	Small cohort size
Studies using tumour-agnostic strategies							
Peng et al. (2020)[58]	77; I (53%), II (23%), III (21%) and IV (3%)	1–7 days before surgery	cSMART (127)	NR	3.65 (95% CI 1.84–7.22; *P*=0.0005)	60%; 44%, 72%, 81% and 100% for stages I, II, III and IV, respectively	Small cohort size; higher detection rate in male patients, never smokers, patients with lung squamous histology and/or visceral pleural invasion; the preoperative time point for each patient (1–7 days) was not specified
Kris et al. (2021)[64]	104; IB–IIIB[a]	Before surgery	AVENIO Surveillance (197)	NR	NR	72%	Patients without tumour variants on panel excluded from analyses
Li et al. (2022)[60]	119; IA (22%), IB (43%), IIA (8%), IIB (13%) and IIIA (15%)	7 days before surgery	Geneseeq (425)	NR	2.42 (95% CI 1.11–5.27; *P*=0.022)	25%; 17%, 12%, 44%, 33% and 56% for stages IA, IB, IIA, IIB and IIIA, respectively	Preoperative ctDNA results available for 117 patients
Xia et al. (2022)[59]	330; I (67%), II (18%) and III (15%)	Before surgery	Genecast (769)	NR	4.2 (95% CI 2.6–6.7; *P*< 0.001); 2.6 (95% CI 1.3–5.1; *P*=0.005) for preoperative ctDNA positivity on multivariate analysis	21%	Correlation between disease stages and ctDNA detection: HR for stage II–III vs I 3.8 (95% CI 2.3–6.1; *P*< 0.001) and 2.4 (95% CI 1.1–5.1; *P*=0.026) on multivariate analysis

ctDNA, circulating tumour DNA; LOD, limit of detection; NA, not applicable; NR, not reported; NS[illegible]er; RFS, recurrence-free survival. [a]Percentage not available. [b]Median value.

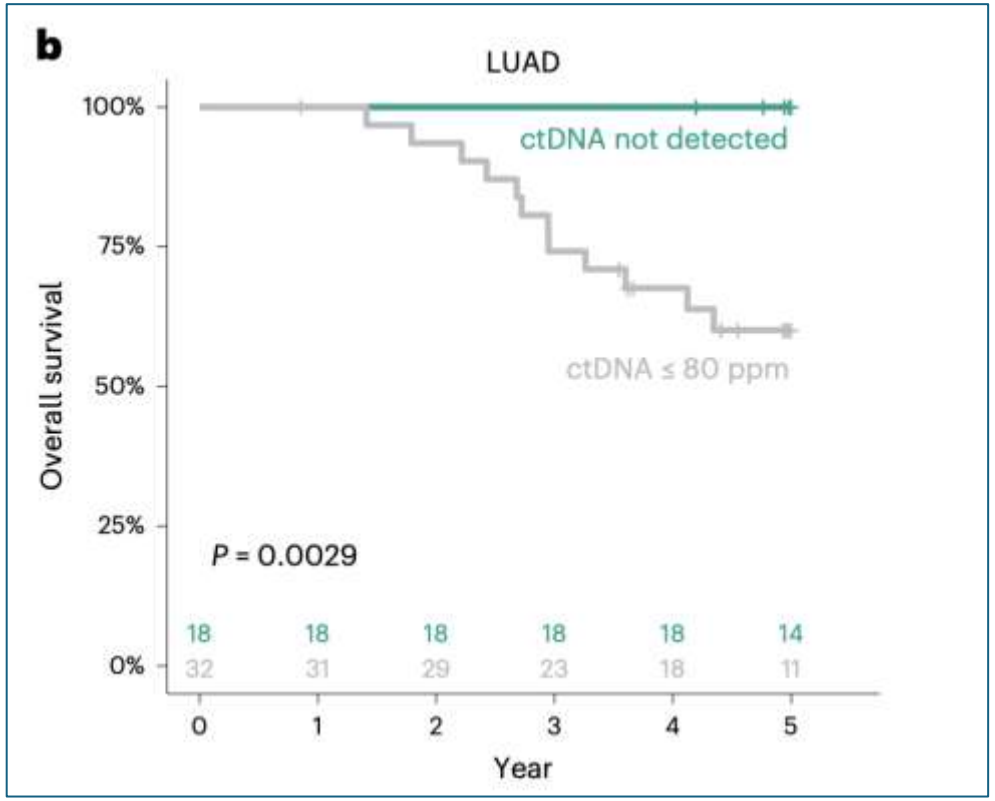
Normanno N. Nature Review Clin Oncol 2025

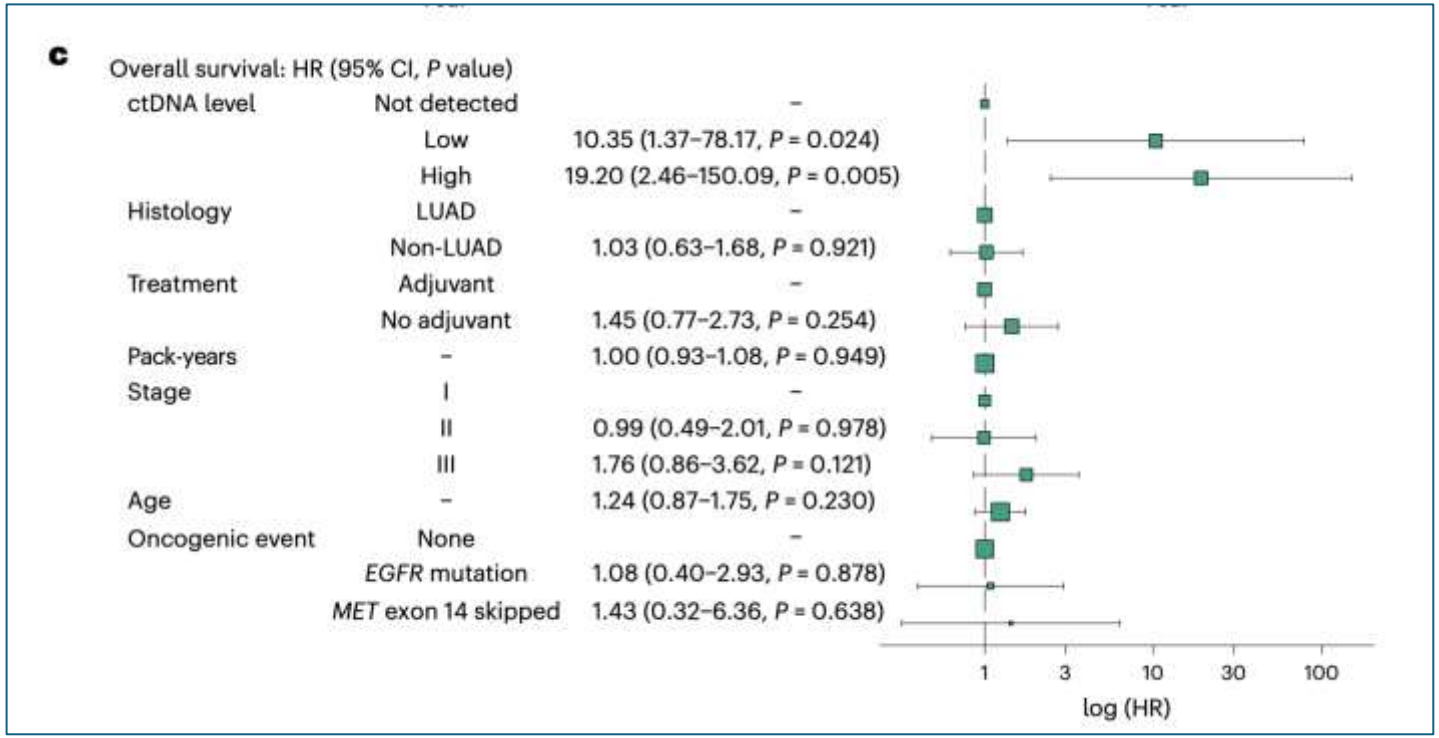
Brief Communication https://doi.org/10.1038/s41591-024-03216-y

Ultrasensitive ctDNA detection for preoperative disease stratification in early-stage lung adenocarcinoma

TRACERx study

- **Baseline ctDNA level is prognostic of OS**
- NeXT Personal → **ultrasensitive**, tumor-informed liquid-biopsy platform
- Identification of ultra-low-risk population with very low ctDNA levels within the adenocarcinoma





Black Natures Medicine 2025

ctDNA testing during neoadjuvant therapy

ctDNA testing during neoadjuvant therapy

- **ctDNA dynamics during NA** therapy can provide relevant information on treatment efficacy in patients with early stage NSCLC

- **NADIM TRIAL**
  - ctDNA <1% before and after NA had better PFS and OS.
  - **ctDNA clearance better PFS and OS.**
    - No association with pathological response.
    - Not association with PFS nor OS in patients with a pCR.
  - ctDNA dynamics retained prognostic value in patients without a pCR.

Table 2 | Clinical studies testing ctDNA in patients with NSCLC receiving neoadjuvant treatment

Study	Patients (*n*); disease stage	Time point	Neoadjuvant therapy	ctDNA assay (*n* of genes)	LOD	ctDNA detection rate	ctDNA clearance before surgery	Outcomes	Comments
Studies using tumour-informed strategies									
Forde et al. (2022), Deutsch et al. (2024)	358; IB–II (35%) and IIIA (64%)	Before cycles 1 and 3	Platinum-based chemotherapy ± nivolumab	ArcherDX Personalized Cancer Monitoring	NR	Before cycle 1: 93% and 95% with and without nivolumab, respectively; before cycle 3: 42% and 62%	56% and 35% with and without nivolumab	pCR in patients with ctDNA clearance: 46% and 13% with and without nivolumab, respectively; pCR in patients with no ctDNA clearance: 0% and 4%; longer EFS in patients with ctDNA clearance (HR 0.60, 95% CI 0.20–1.82 and HR 0.63, 95% CI 0.20–2.01 with and without nivolumab, respectively)	Small cohort size, with ctDNA data available only for 86 patients
Reck et al. (2024)	283; II (31%), IIIA (45%) and IIIB (24%)	Before each one of 4 neoadjuvant cycles, and before and after surgery	Platinum-based chemotherapy+ durvalumab or placebo	Invitae Personalized Cancer Monitoring (16–50 variants)	Up to 0.008%	90% at baseline	65% and 42% with or without durvalumab, respectively	ctDNA clearance at cycle 4: 100% in patients with pCR and >93% in patients with MPR; longer EFS for ctDNA clearance before surgery vs no clearance (HR 0.26, 95% CI 0.13–0.54 and HR 0.47, 95% CI 0.26–0.84 with durvalumab and placebo, respectively)	ctDNA evaluated in all enrolled patients before each one of 4 neoadjuvant cycles, and before and after surgery
Studies using tumour-agnostic strategies									
Kris et al. (2021)	104; IB–IIIB[a]	Before and after neoadjuvant treatment, and after surgery	Atezolizumab	AVENIO Surveillance (197)	NR	72%, 56% and 12% before and after neoadjuvant treatment, and after surgery,	32% of ctDNA-positive patients at baseline	≥twofold decrease in ctDNA levels correlated with MPR ($P < 0.001$); ctDNA negativity after atezolizumab correlated with improved 2-year DFS (HR 0.49,	Patients with negative findings on tumour samples excluded from study
Provencio et al. (2022, 2024)	46; IIIA (100%)	Before and after neoadjuvant treatment	Paclitaxel+ carboplatin+ nivolumab	Oncomine Pan-Cancer Cell-Free Assay (52)	0.1%	70% both before and after therapy	68%	No increase in ctDNA levels in 92% of patients with pCR and in 86% with MPR, and increase in 33% with incomplete pathological responses; longer PFS (HR 0.26, 95% CI 0.07–0.93; $P = 0.038$) and OS (HR 0.004, 95% CI 0.00–0.55; $P = 0.015$) in those with undetectable ctDNA; baseline ctDNA MAF levels <1% associated with improved PFS (HR 0.20, 95% CI 0.06–0.73; $P = 0.006$) and OS (HR 0.07, 95% CI 0.01–0.39; $P > 0.002$) on multivariate analysis; 5-year PFS and OS for patients with MAF ≥1% of 48.6% and 56.3%, respectively, compared with 83.8% and 86.2% for patients with MAF <1%	Very small cohort size

ctDNA, circulating tumour DNA; DFS, disease-free survival; EFS, event-free survival; LOD, limit of detection; MAF, mutant allele fraction; MPR, major pathological response; NR, not reported; NSCLC, non-small cell lung cancer; OS, overall survival; pCR, pathological complete response; PFS, progression-free survival.

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ctDNA testing during neoadjuvant therapy

- **CHECKMATE 816**
  - Tumor informed panel.
  - N: 86/358 patients.
  - **ctDNA clearance as a potential early predictor of favourable outcomes.**
    - CT-Nivolumab greater rate of ctDNA clearance.
    - **ctDNA clearance longer EFS and greater pCR in both arms.**

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Rizk et al. (2024)	283; II (31%), IIIA (45%) and IIIB (24%)	Before each one of 4 neoadjuvant cycles, and before and after surgery	Platinum-based chemotherapy+ durvalumab or placebo	Invitae Personalized Cancer Monitoring (16–50 variants)	Up to 0.008%	90% at baseline	65% and 42% with or without durvalumab, respectively	ctDNA clearance at cycle 4: 100% in patients with pCR and >93% in patients with MPR; longer EFS for ctDNA clearance before surgery vs no clearance (HR 0.26, 95% CI 0.13–0.54 and HR 0.47, 95% CI 0.26–0.84 with durvalumab and placebo, respectively)	ctDNA evaluated in all enrolled patients before each one of 4 neoadjuvant cycles, and before and after surgery
Studies using tumour-agnostic strategies									
Kris et al. (2021)	104; IB–IIIB[a]	Before and after neoadjuvant treatment, and after surgery	Atezolizumab	AVENIO Surveillance (197)	NR	72%, 56% and 12% before and after neoadjuvant treatment, and after surgery, respectively	32% of ctDNA-positive patients at baseline	≥twofold decrease in ctDNA levels correlated with MPR ($P < 0.001$); ctDNA negativity after atezolizumab correlated with improved 2-year DFS (HR 0.49, 95% CI 0.20–1.19; $P = 0.12$)	Patients with negative findings on tumour samples excluded from study
Provencio et al. (2022, 2024)	46; IIIA (100%)	Before and after neoadjuvant treatment	Paclitaxel+ carboplatin+ nivolumab	Oncomine Pan-Cancer Cell-Free Assay (52)	0.1%	70% both before and after therapy	68%	No increase in ctDNA levels in 92% of patients with pCR and in 86% with MPR, and increase in 33% with incomplete pathological responses; longer PFS (HR 0.26, 95% CI 0.07–0.93; $P = 0.038$) and OS (HR 0.004, 95% CI 0.00–0.55; $P = 0.015$) in those with undetectable ctDNA; baseline ctDNA MAF levels <1% associated with improved PFS (HR 0.20, 95% CI 0.06–0.73; $P = 0.006$) and OS (HR 0.07, 95% CI 0.01–0.39; $P = 0.002$) on multivariate analysis; 5-year PFS and OS for patients with MAF ≥1% of 48.6% and 56.3%, respectively, compared with 83.8% and 86.2% for patients with MAF <1%	Very small cohort size

ctDNA, circulating tumour DNA; DFS, disease-free survival; EFS, event-free survival; LOD, limit of detection; MAF, mutant allele fraction; MPR, major pathological response; NR, not reported; NSCLC, non-small-cell lung cancer; OS, overall survival; pCR, pathological complete response; PFS, progression-free survival.

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ctDNA testing during neoadjuvant therapy

- **AEGEAN**
  - Tumour-informed panel
  - **ctDNA clearance before surgery strong prognostic factor.**
  - Correlation between ctDNA clearance and pCR.

Table 2 | Clinical studies testing ctDNA in patients with NSCLC receiving neoadjuvant treatment

Study	Patients (*n*); disease stage	Time point	Neoadjuvant therapy	ctDNA assay (*n* of genes)	LOD	ctDNA detection rate	ctDNA clearance before surgery	Outcomes	Comments
Studies using tumour-informed strategies									
Forde et al. (2022)[87], Deutsch et al. (2024)[88]	358; IB–II (35%) and IIIA (64%)	Before cycles 1 and 3	Platinum-based chemotherapy ± nivolumab	ArcherDX Personalized Cancer Monitoring	NR	Before cycle 1: 93% and 95% with and without nivolumab, respectively; before cycle 3: 42% and 62%	56% and 35% with and without nivolumab	pCR in patients with ctDNA clearance: 46% and 13% with and without nivolumab, respectively; pCR in patients with no ctDNA clearance: 0% and 4%; longer EFS in patients with ctDNA clearance (HR 0.60, 95% CI 0.20–1.82 and HR 0.63, 95% CI 0.20–2.01 with and without	Small cohort size, with ctDNA data available only for 86 patients
Reck et al. (2024)[89]	283; II (31%), IIIA (45%) and IIIB (24%)	Before each one of 4 neoadjuvant cycles, and before and after surgery	Platinum-based chemotherapy+ durvalumab or placebo	Invitae Personalized Cancer Monitoring (16–50 variants)	Up to 0.008%	90% at baseline	65% and 42% with or without durvalumab, respectively	ctDNA clearance at cycle 4: 100% in patients with pCR and >93% in patients with MPR; longer EFS for ctDNA clearance before surgery vs no clearance (HR 0.26, 95% CI 0.13–0.54 and HR 0.47, 95% CI 0.26–0.84 with durvalumab and placebo, respectively)	ctDNA evaluated in all enrolled patients before each one of 4 neoadjuvant cycles, and before and after surgery
Studies using tumour-agnostic strategies									
Kris et al. (2021)[66]	104; IB–IIIB[a]	Before and after neoadjuvant treatment, and after surgery	Atezolizumab	AVENIO Surveillance (197)	NR	72%, 56% and 12% before and after neoadjuvant treatment, and after surgery, respectively	32% of ctDNA-positive patients at baseline	≥twofold decrease in ctDNA levels correlated with MPR ($P < 0.001$); ctDNA negativity after atezolizumab correlated with improved 2-year DFS (HR 0.49, 95% CI 0.20–1.19; $P = 0.12$)	Patients with negative findings on tumour samples excluded from study
Provencio et al. (2022, 2024)[84,85]	46; IIIA (100%)	Before and after neoadjuvant treatment	Paclitaxel+ carboplatin+ nivolumab	Oncomine Pan-Cancer Cell-Free Assay (52)	0.1%	70% both before and after therapy	68%	No increase in ctDNA levels in 92% of patients with pCR and in 86% with MPR, and increase in 33% with incomplete pathological responses; longer PFS (HR 0.26, 95% CI 0.07–0.93; $P = 0.038$) and OS (HR 0.004, 95% CI 0.00–0.55; $P = 0.015$) in those with undetectable ctDNA; baseline ctDNA MAF levels <1% associated with improved PFS (HR 0.20, 95% CI 0.06–0.73; $P = 0.006$) and OS (HR 0.07, 95% CI 0.01–0.39; $P > 0.002$) on multivariate analysis; 5-year PFS and OS for patients with MAF ≥1% of 48.6% and 56.3%, respectively, compared with 83.8% and 86.2% for patients with MAF <1%	Very small cohort size

ctDNA, circulating tumour DNA; DFS, disease-free survival; EFS, event-free survival; LOD, limit of detection; MAF, mutant allele fraction; MPR, major pathological response; NR, not reported; NSCLC, non-small-cell lung cancer; OS, overall survival; pCR, pathological complete response; PFS, progression-free survival.

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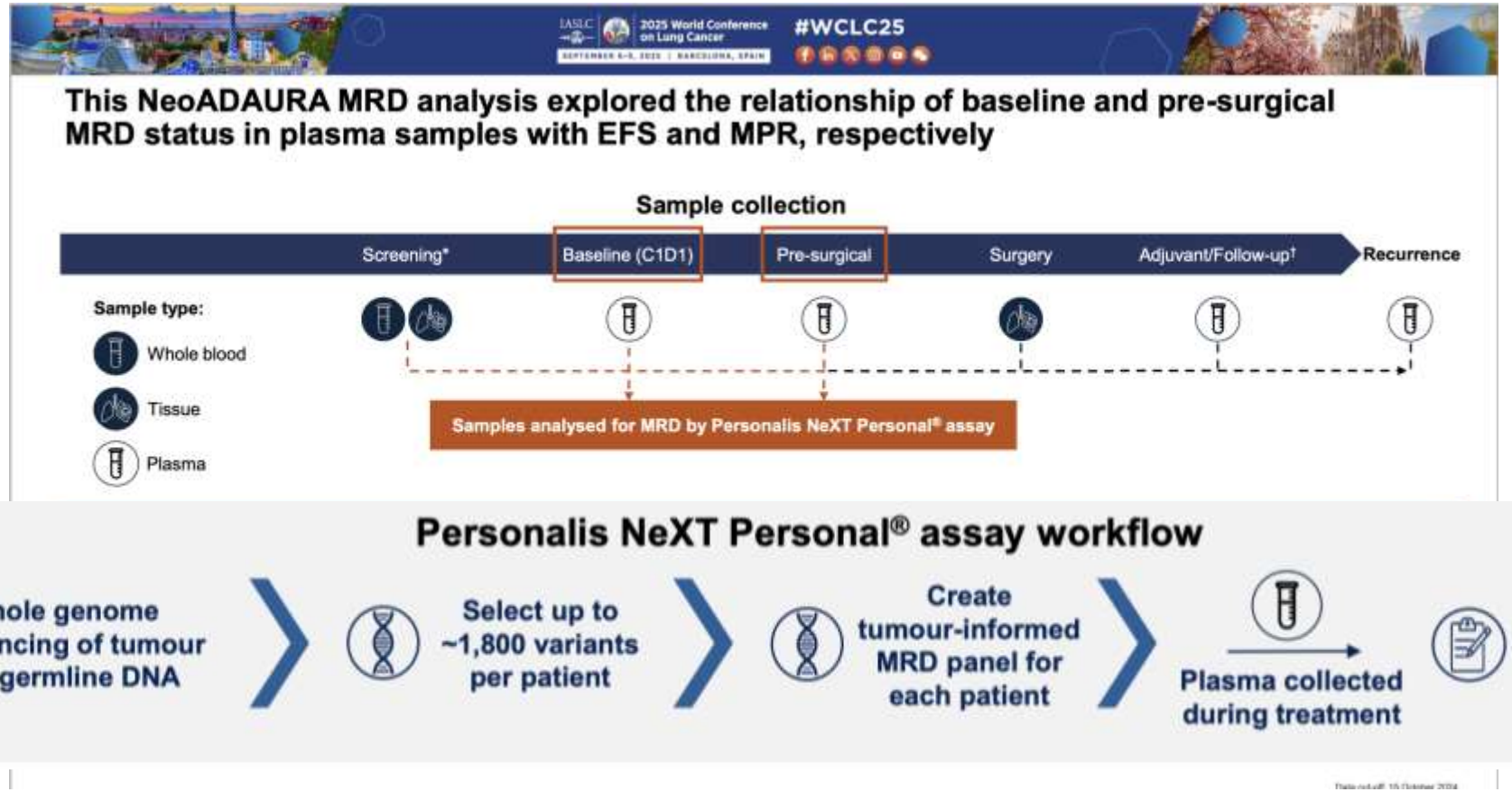
ctDNA testing during neoadjuvant therapy

NeoADAURA trial

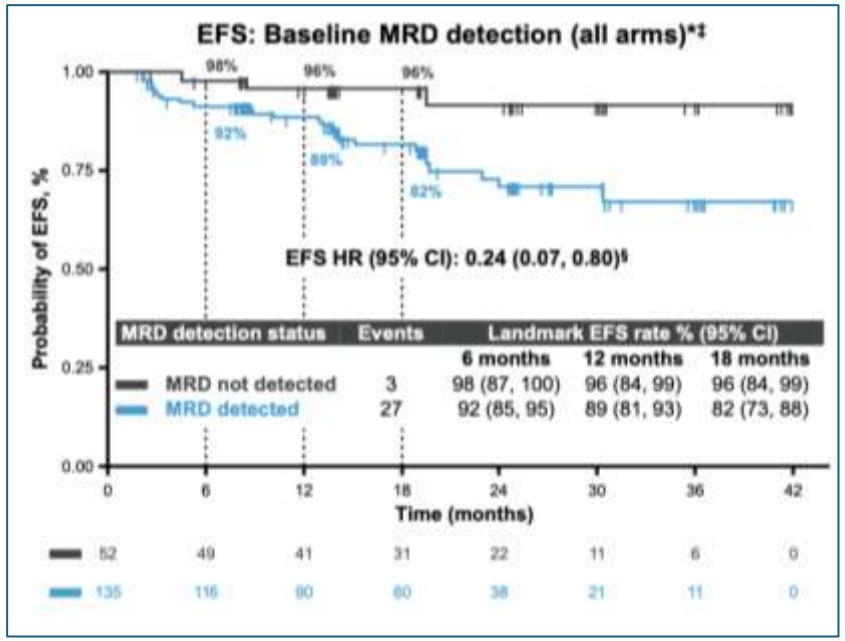


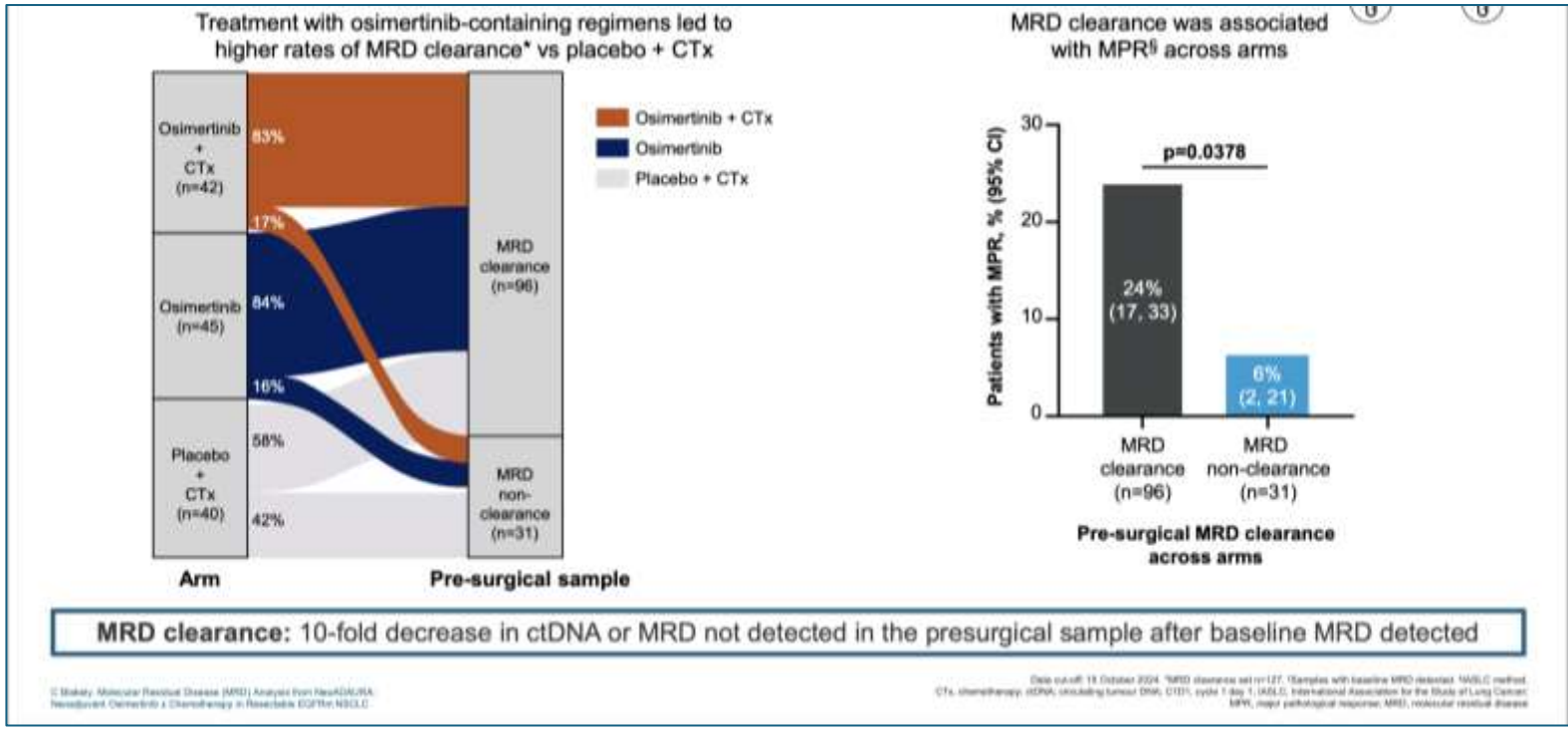
Blakely WCLC 2025

ctDNA testing during neoadjuvant therapy

NeoADAURA trial

- Baseline MRD (ctDNA+) prognostic for EFS.
- Pre-surgical MRD clearance enriched with osimertinib-containing regimens and in pts with MPR.



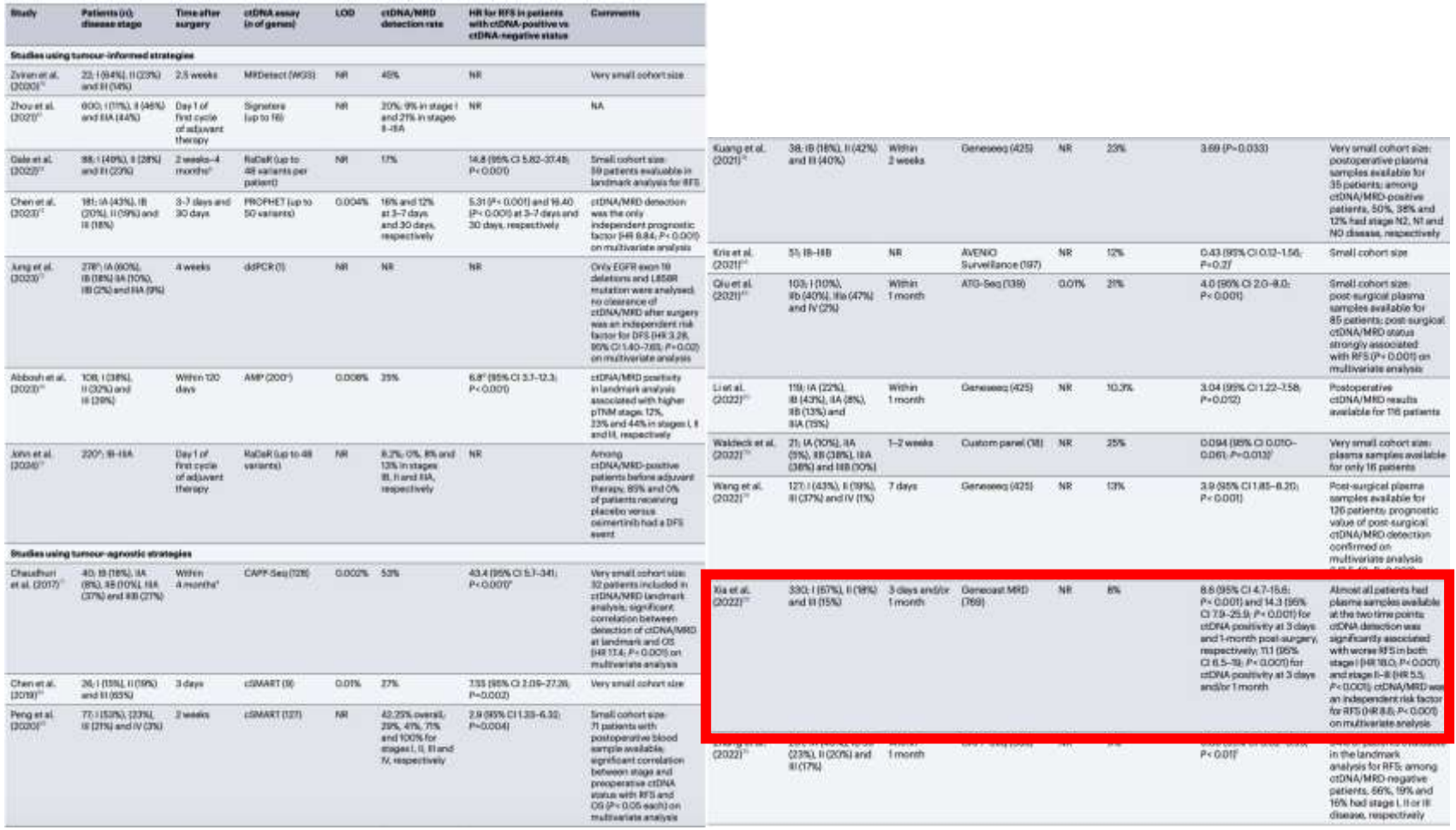


Blakely WCLC 2025

Detection of MRD after NSCLC resection

Detection of MRD after NSCLC resection

- Heterogeneous studies (unselected /EGFR, sample, endpoint, testing,...)
- Relevance of ctDNA reported in few studies.
- **LUNGCA-1:** RFS HR ctDNA + vs ctDNA -:8.6 and 14.3 (P < 0.001) at 3 days and 1 month.
- Best prognostic value of ctDNA **few weeks after surgery** (reduction of DNA shedding).



Study	Patients (n); disease stage	Time after surgery	ctDNA assay (n of genes)	LOD	ctDNA/MRD detection rate	HR for RFS in patients with ctDNA-positive vs ctDNA-negative status	Comments
Studies using tumour-informed strategies							
Zviran et al. (2020)[14]	22; I (64%), II (23%) and III (14%)	2.5 weeks	MRDetect (WGS)	NR	45%	NR	Very small cohort size
Zhou et al. (2021)[15]	600; I (11%), II (46%) and IIIA (44%)	Day 1 of first cycle of adjuvant therapy	Signatera (up to 16)	NR	20%, 9% in stage I and 21% in stages II–IIIA	NR	NA
Gale et al. (2022)[16]	88; I (40%), II (28%) and III (33%)	2 weeks–4 months[a]	RaDaR (up to 48 variants per patient)	NR	17%	14.8 (95% CI 5.82–37.48); P < 0.001	Small cohort size: 59 patients evaluable in landmark analysis for RFS
Chen et al. (2023)[17]	181; IA (43%), IB (20%), II (19%) and III (18%)	3–7 days and 30 days	PROPHET (up to 50 variants)	0.004%	16% and 12% at 3–7 days and 30 days, respectively	5.31 (P < 0.001) and 16.40 (P < 0.001) at 3–7 days and 30 days, respectively	ctDNA/MRD detection was the only independent prognostic factor (HR 8.84, P < 0.001) on multivariate analysis
Jung et al. (2023)[18]	278[b]; IA (60%), IB (18%) IIA (10%), IIB (2%) and IIIA (9%)	4 weeks	ddPCR (1)	NR	NR	NR	Only EGFR exon 19 deletions and L858R mutation were analysed; no clearance of ctDNA/MRD after surgery was an independent risk factor for DFS (HR 3.28, 95% CI 1.40–7.65; P = 0.02) on multivariate analysis
Abbosh et al. (2023)[19]	108; I (38%), II (32%) and III (29%)	Within 120 days	AMP (200[c])	0.008%	35%	6.8[d] (95% CI 3.1–12.3; P < 0.001)	ctDNA/MRD positivity in landmark analysis associated with higher pTNM stage; 12%, 33% and 44% in stages I, II and III, respectively
John et al. (2024)[20]	220[e]; III–IIIA	Day 1 of first cycle of adjuvant therapy	RaDaR (up to 48 variants)	NR	8.2%, 0%, 8% and 13% in stages III, II and IIIA, respectively	NR	Among ctDNA/MRD-positive patients before adjuvant therapy, 85% and 0% of patients receiving placebo versus osimertinib had a DFS event
Studies using tumour-agnostic strategies							
Chaudhuri et al. (2017)[21]	40; IB (18%), IIA (8%), IIB (10%), IIIA (37%) and IIIB (27%)	Within 4 months[f]	CAPP-Seq (128)	0.002%	53%	43.4 (95% CI 5.7–341); P < 0.001[g]	Very small cohort size: 32 patients included in ctDNA/MRD landmark analysis; significant correlation between detection of ctDNA/MRD at landmark and OS (HR 17.4; P = 0.025) on multivariate analysis
Chen et al. (2019)[22]	26; I (15%), II (19%) and III (65%)	3 days	cSMART (9)	0.01%	27%	7.55 (95% CI 2.09–27.28; P = 0.002)	Very small cohort size
Peng et al. (2020)[23]	77; I (53%), (23%), II (21%) and IV (3%)	2 weeks	cSMART (127)	NR	42.25% overall, 28%, 41%, 71% and 100% for stages I, II, III and IV, respectively	2.9 (95% CI 1.33–6.32; P = 0.004)	Small cohort size: 71 patients with postoperative blood sample available; significant correlation between stage and preoperative ctDNA status with RFS and OS (P < 0.05 each) on multivariate analysis
Kuang et al. (2021)[24]	38; IB (18%), II (42%) and III (40%)	Within 2 weeks	Geneseeq (425)	NR	23%	3.69 (P = 0.033)	Very small cohort size: postoperative plasma samples available for 35 patients; among ctDNA/MRD-positive patients, 50%, 38% and 12% had stage N2, N1 and N0 disease, respectively
Kris et al. (2021)[25]	51; IB–IIIB	NR	AVENIO Surveillance (197)	NR	12%	0.43 (95% CI 0.12–1.56; P = 0.2)	Small cohort size
Qiu et al. (2021)[26]	103; I (10%), IIb (40%), IIIa (47%) and IV (2%)	Within 1 month	ATG-Seq (139)	0.01%	21%	4.0 (95% CI 2.0–8.0; P < 0.001)	Small cohort size: post-surgical plasma samples available for 85 patients; post-surgical ctDNA/MRD status strongly associated with RFS (P < 0.001) on multivariate analysis
Li et al. (2022)[27]	119; IA (22%), IB (43%), IIA (8%), IIB (13%) and IIIA (13%)	Within 1 month	Geneseeq (425)	NR	10.3%	3.04 (95% CI 1.22–7.58; P = 0.012)	Postoperative ctDNA/MRD results available for 116 patients
Waldeck et al. (2022)[28]	21; IA (10%), IIA (5%), IIB (38%), IIIA (38%) and IIIB (10%)	1–2 weeks	Custom panel (18)	NR	25%	0.094 (95% CI 0.010–0.861; P = 0.013)[h]	Very small cohort size: plasma samples available for only 16 patients
Wang et al. (2022)[29]	127; I (43%), II (19%), III (37%) and IV (1%)	7 days	Geneseeq (425)	NR	13%	3.9 (95% CI 1.85–8.21; P < 0.001)	Post-surgical plasma samples available for 126 patients; prognostic value of post-surgical ctDNA/MRD detection confirmed on multivariate analysis
Xia et al. (2022)[30]	330; I (67%), II (18%) and III (15%)	3 days and/or 1 month	Genecast MRD (769)	NR	8%	8.6 (95% CI 4.7–15.6; P < 0.001) and 14.3 (95% CI 7.9–25.9; P < 0.001) for ctDNA positivity at 3 days and 1-month post-surgery, respectively; 11.1 (95% CI 6.5–19; P < 0.001) for ctDNA positivity at 3 days and/or 1 month	Almost all patients had plasma samples available at the two time points; ctDNA detection was significantly associated with worse RFS in both stage I (HR 18.0; P < 0.001) and stage II–III (HR 5.5; P < 0.001); ctDNA/MRD was an independent risk factor for RFS (HR 8.8; P < 0.001) on multivariate analysis
[illegible] (2022)[31]	[illegible] (23%), II (20%) and III (17%)	1 month	[illegible]	[illegible]	[illegible]	P < 0.01[i]	[illegible] in the landmark analysis for RFS; among ctDNA/MRD-negative patients, 66%, 19% and 16% had stage I, II or III disease, respectively

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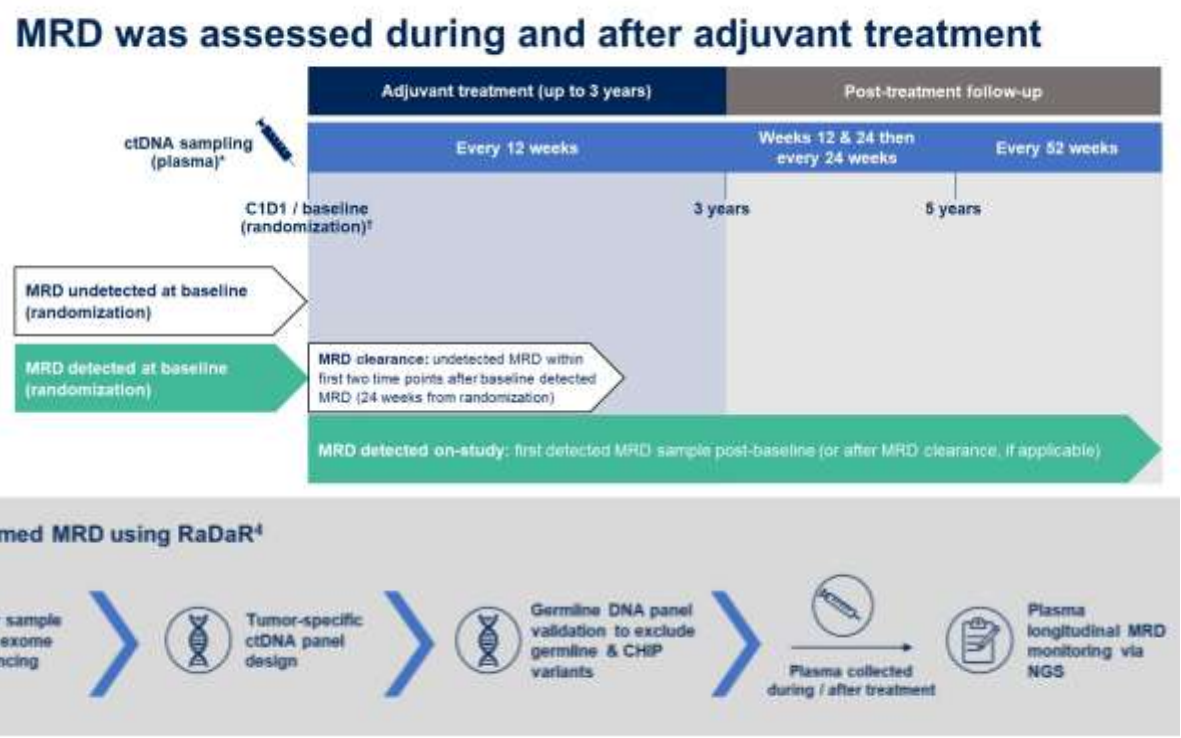
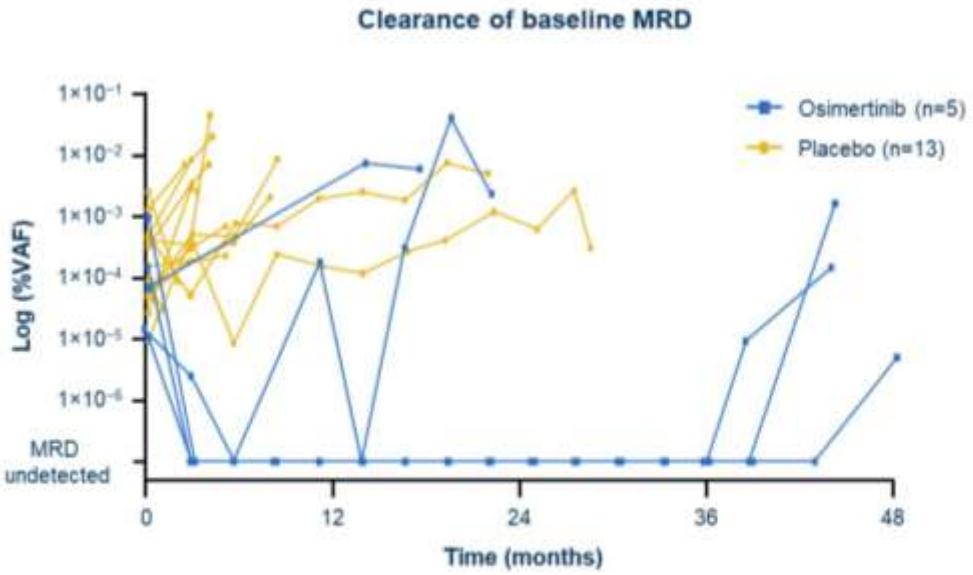
Detection of MRD after NSCLC resection

ADAURA trial





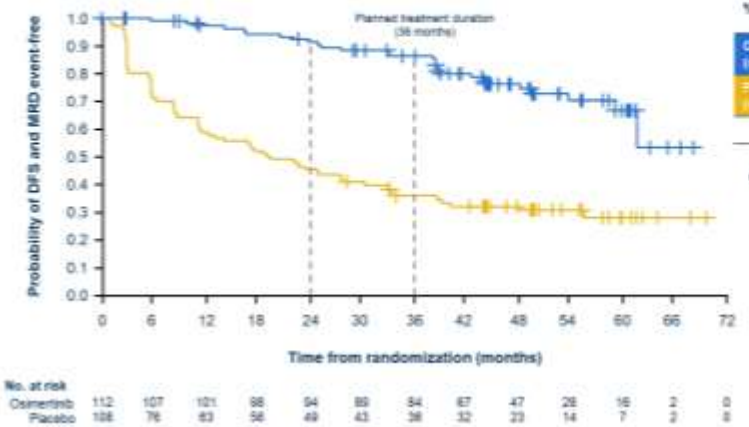
- Of 18 patients with detected MRD at baseline
  - 4 / 5 patients receiving osimertinib cleared MRD
  - 0 / 13 patients receiving placebo cleared MRD

John ASCO 2024

Detection of MRD after NSCLC resection

ADAURA trial

Patients receiving osimertinib were more likely to be DFS and MRD event-free* vs. placebo



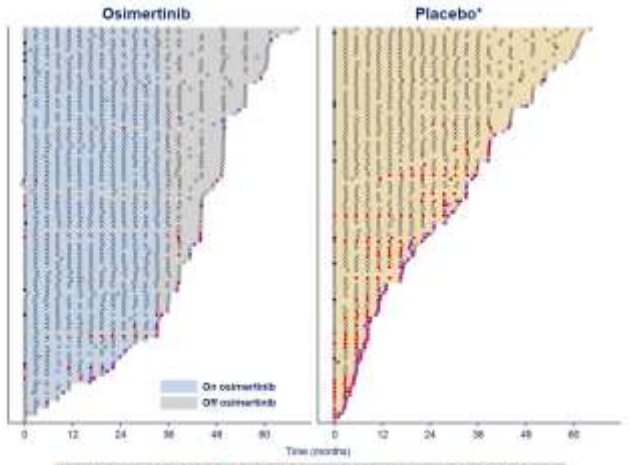
% (95% CI)	DFS and MRD event-free rate	
	24 months	36 months
Osimertinib (n=112)	91 (84, 95)	88 (78, 92)
Placebo (n=108)	48 (38, 58)	36 (27, 45)

HR (95% CI) 0.23 (0.15, 0.36)[†]

Median follow-up time, months (95% CI):
osimertinib 44.2 (42.4, 46.1), placebo 19.1 (11.1, 20.3)

MRD events were detected more frequently with placebo vs. osimertinib

- MRD events were detected in 68 patients:
  - 13% (15 / 112) occurred in osimertinib group
  - 49% (53 / 108) occurred in placebo group



2024 ASCO ANNUAL MEETING #ASCO24

Tumor-informed MRD analysis demonstrated maintenance of DFS and MRD event-free status for most patients during and after osimertinib treatment

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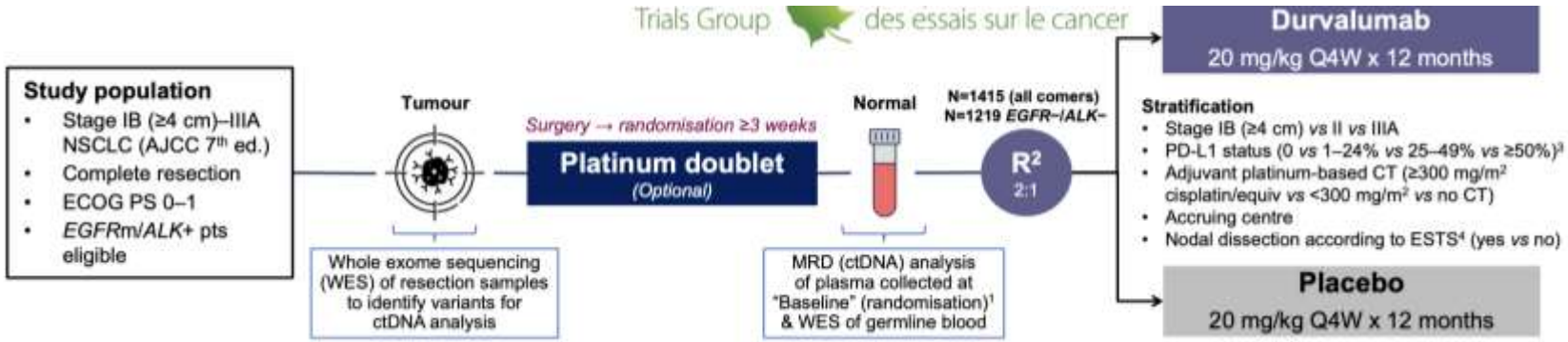
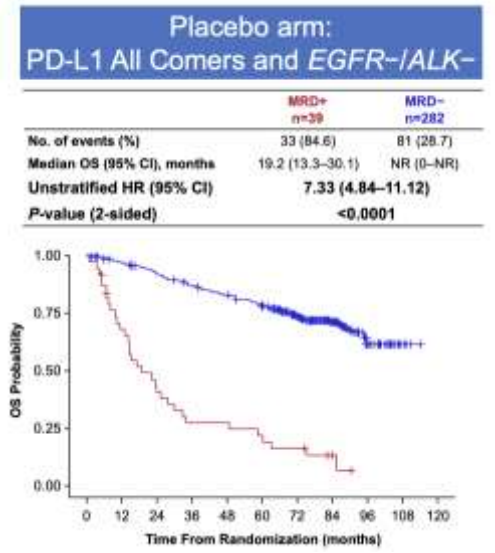
Detection of MRD after NSCLC resection

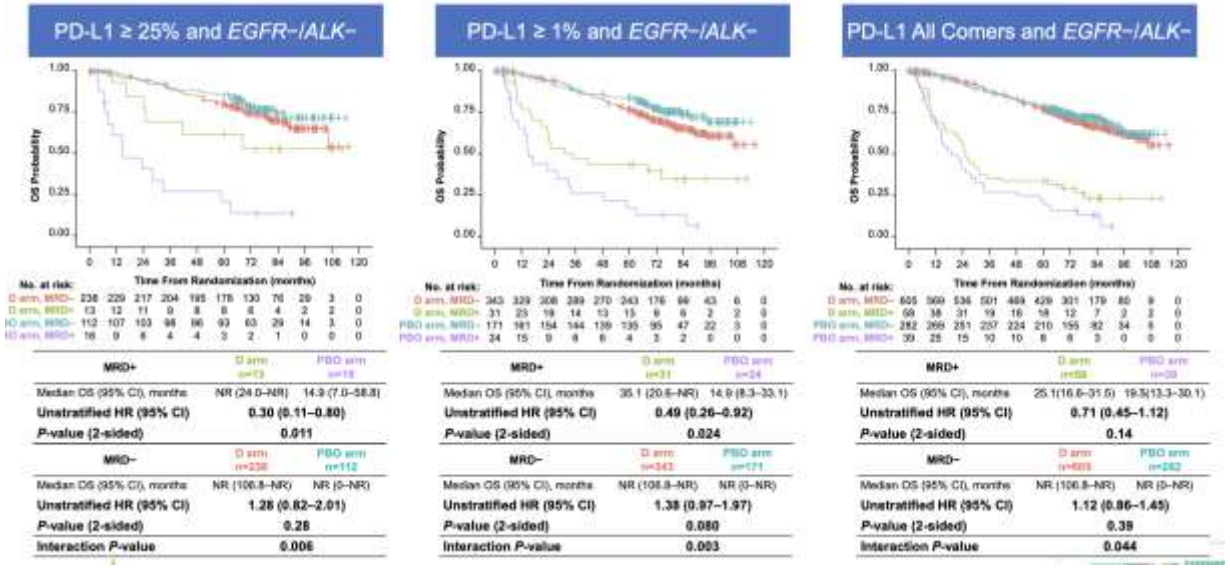
BR.31 trial



MRD + poor prognostic



MRD + is predictive for OS of durvalumab in PD-L1 + subpopulations



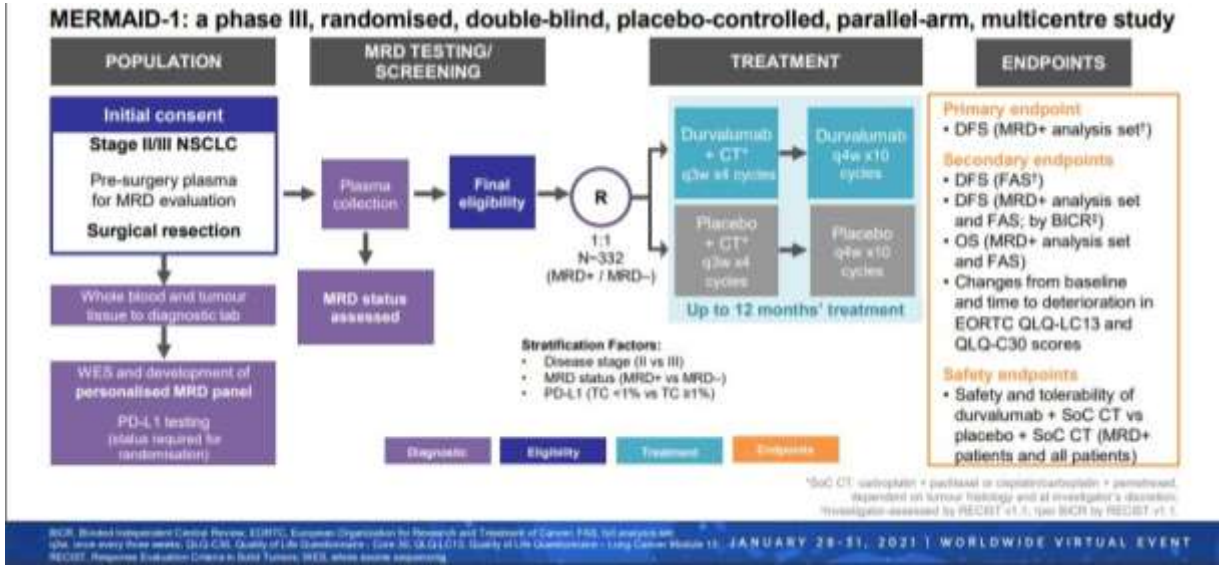
Detection of MRD after NSCLC resection

- **Adjuvant immunotherapy/targeted therapy** may convert **ctDNA +** patients to negative and may **delay progression** in **ctDNA-negative** patients (exploratory data).
- **MRD detection** after surgery identifies patients at **high recurrence risk**; **ctDNA+** shows **high PPV** despite study limitations.
- **Some MRD-negative patients still relapse**, highlighting:
  - the need for **more sensitive assays** to reduce false negatives.
  - the fact that **MRD-negative recurrences** are often **locoregional or intracranial**, where ctDNA shedding is low.
- **Adjuvant therapy decisions** in early-stage NSCLC **cannot rely solely on ctDNA/MRD results**; clinical and pathological factors must also be considered.

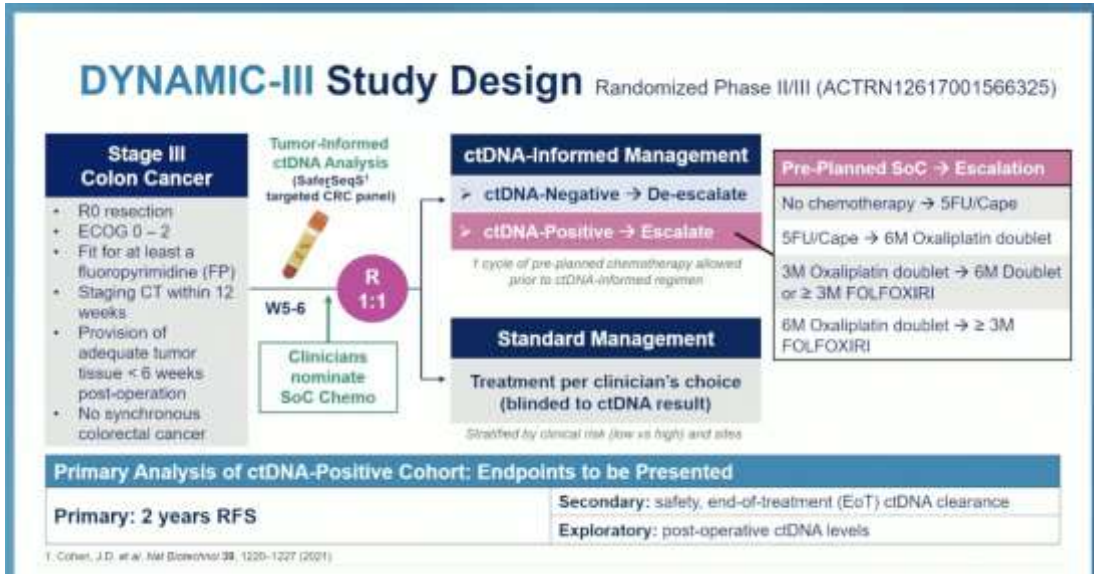
Detection of MRD after NSCLC resection

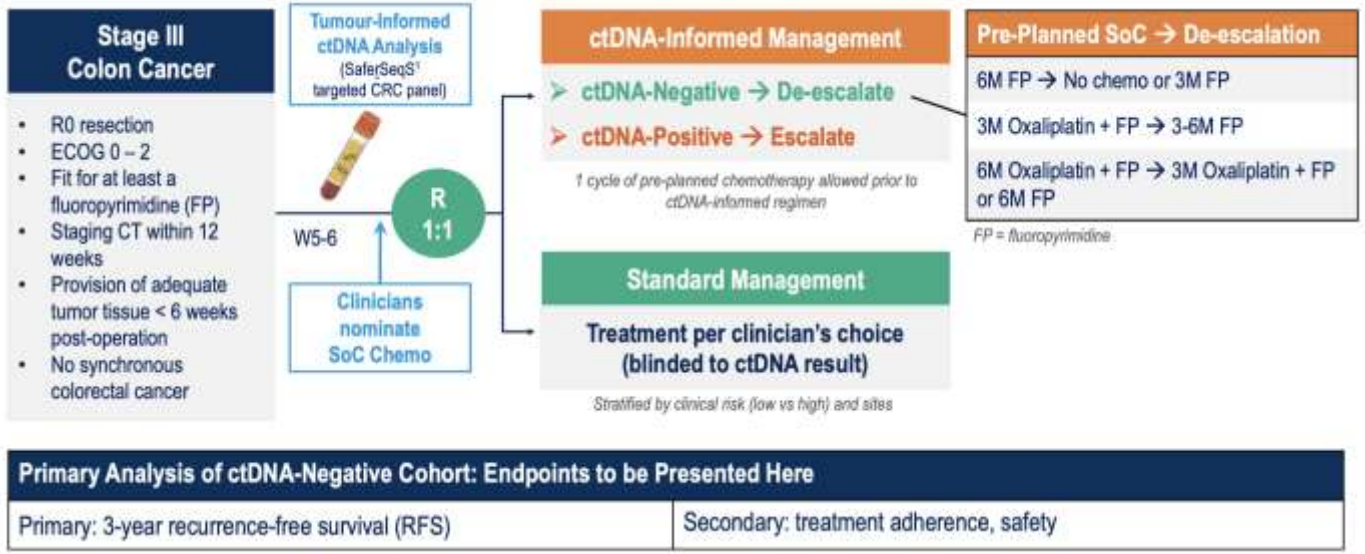
MRD to guide adjuvant therapy (escalation and de-escalation)

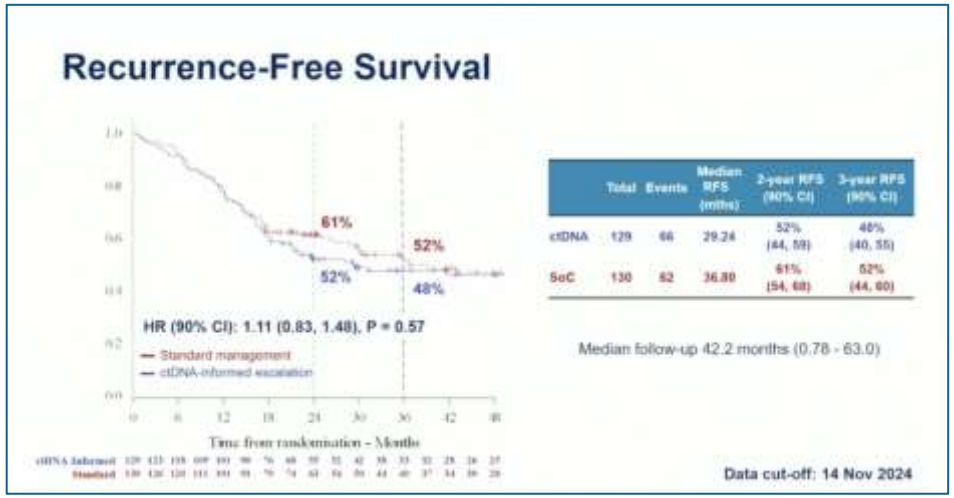
- **Escalation:** intensified adjuvant therapy in MRD-positive patients (targeted therapy or immunotherapy, longer adjuvant treatment),
- **De-escalation:** to avoid overtreatment in MRD-negative patients (no adjuvant treatment or shorter), reduce toxicity and costs.

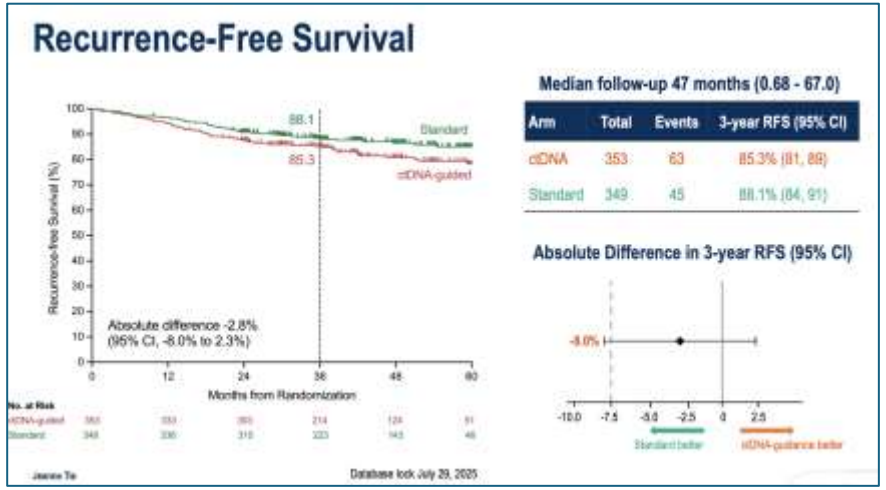


Detection of MRD after COLON resection









Tie ASCO 2025; Goss ESMO 2025

ctDNA as a biomarker in locally advanced unresectable NSCLC

ctDNA as a biomarker in locally advanced unresectable NSCLC

- Prognostic role for ctDNA detection after CTRT.
  - ctDNA + → worse prognosis.
  - ctDNA/MRD +, consolidation ICIs improve PFS.
  - "All-negative" or "early clearance" → best PFS and do not benefit from consolidation ICIs.
  - Consolidation mainly helpful for high-risk patients defined by ctDNA kinetics.

Study	Patients (*n*); disease stage	Time after chemoradiotherapy	ctDNA assay (*n* of genes)[a]	LOD	ctDNA detection rate	HR for PFS in patients with ctDNA-negative vs ctDNA-positive status	Comments
Moding et al. (2020)[96]	65; chemoradiotherapy cohort: IIB (14%), IIIA (54%) and IIIB (32%); ICI cohort: IIB (4%), IIIA (64%) and IIIB (32%)	Within 4 months	CAPP-Seq (139)	NR	Before treatment: 78% and 75% in chemoradiotherapy and ICI cohorts, respectively; after treatment 50% and 41%, respectively	Not reached[b]; 0% and 100% in patients with detectable vs undetectable ctDNA ($P < 0.001$)	Very small cohort size; 13 patients with no variants detected in baseline tumour or plasma were excluded from the analysis
Yang et al. (2022)[97]	55; I (4%), II (9%), IIIA (22%), IIIB (49%) and IIIC (16%)	1 month	Geneseeq (474)	NR	57% and 29% before and after treatment, respectively	0.104 (95% CI 0.036–0.302; $P < 0.0001$)	Very small cohort size: discovery set included only 47 patients; correlation of ctDNA detection with outcome confirmed in a validation set of patients with stage III disease ($n = 20$)
Pan et al. (2023)[98]	139; IIB (4%), IIIA (27%), IIIB (55%) and IIIC (14%)	Last day of radiotherapy	CAPP-Seq (338)	0.02%	73% and 53% before and after treatment, respectively	0.43 (95% CI 0.29–0.65; $P < 0.001$)	Data after chemoradiotherapy available for 96% of patients

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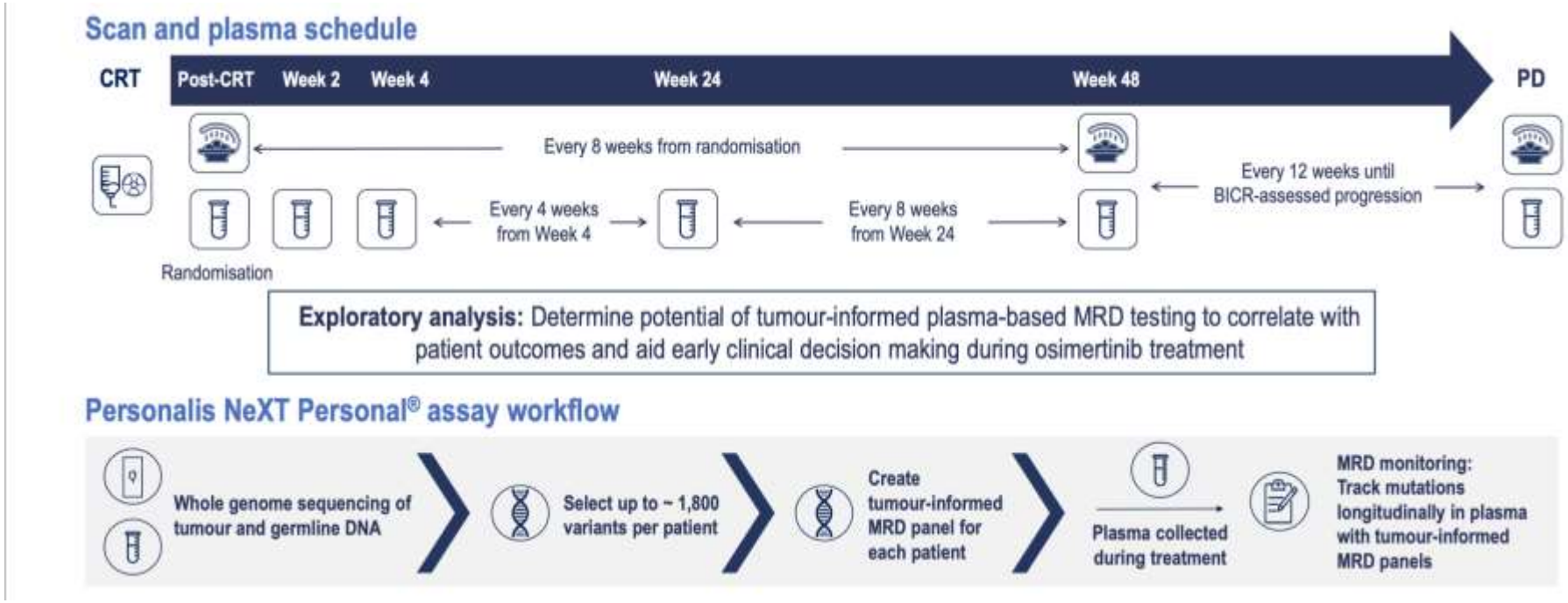
ctDNA as a biomarker in locally advanced unresectable NSCLC

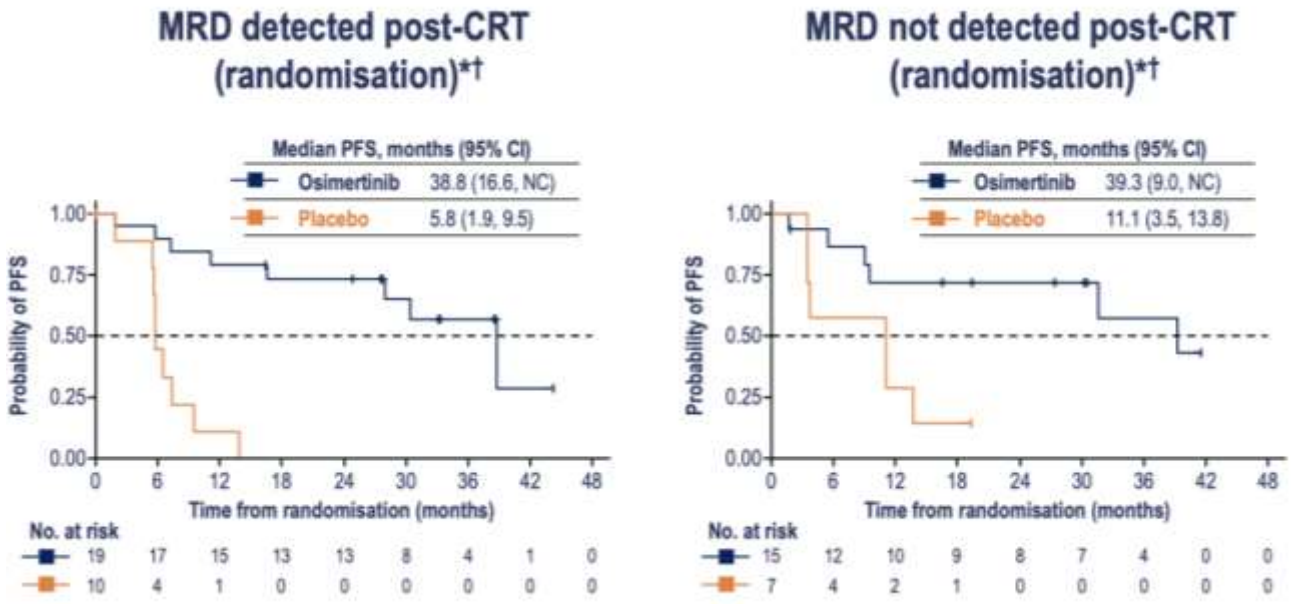
LAURA TRIAL



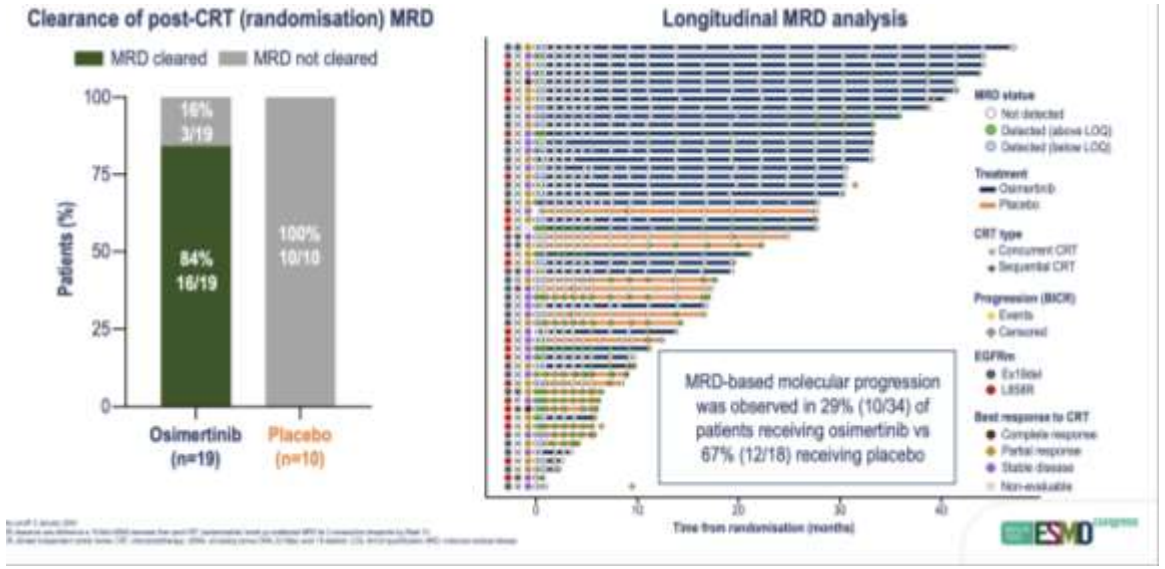
Arriola ESMO 2025

ctDNA as a biomarker in locally advanced unresectable NSCLC

LAURA TRIAL



- Irrespective of post-CRT MRD status, patients benefited from osimertinib treatment vs placebo



- MRD clearance exclusively in osimertinib arm
- Molecular progression less frequently with osimertinib

Arriola ESMO 2025

Longitudinal ctDNA testing in NSCLC

Normanno N. Nature Review Clin Oncol 2025

Longitudinal ctDNA testing in NSCLC

- MRD + correlates with disease recurrence.
  - Detection of ctDNA during follow-up → significantly poorer RFS.
- **MRD + can precede radiological recurrence in 20%-84% of patients, ranging between 2.8 and 12.6 months.**
- More sensitive NGS assays might improve ctDNA detection.
- N: 261 pts stage I–III NSCLC → 97% MRD - free from disease after a median follow-up of 19.7m → potentially cured subpopulation.
  - Clinical trials are need to demonstrate an advantage of starting treatment before clinical or radiological evidence of progression.

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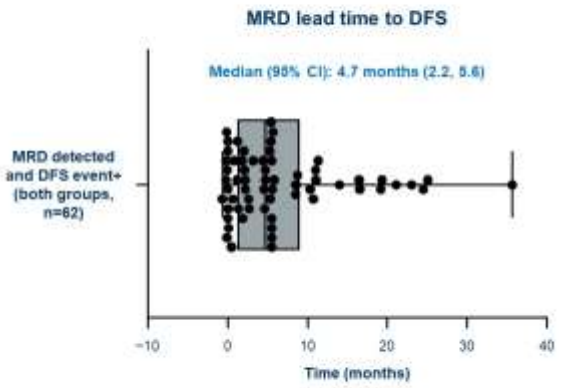
Longitudinal ctDNA testing in NSCLC

ADAURA TRIAL

MRD identified recurrence with a median lead time of 4.7 months

		MRD status,*†n	
		Detected (n=68)	Undetected (n=152)
DFS status,*†n	Event (n=96)	**62**	34
	Censor (n=124)	6	**118**

Concordance of MRD with DFS,‡ % (95% CI)	
PPA (sensitivity)	**65 (55, 74)**
NPA (specificity)	**95 (91, 99)**
Positive predictive value	91 (84, 98)
Negative predictive value	78 (71, 84)
Overall percent agreement	82 (77, 87)

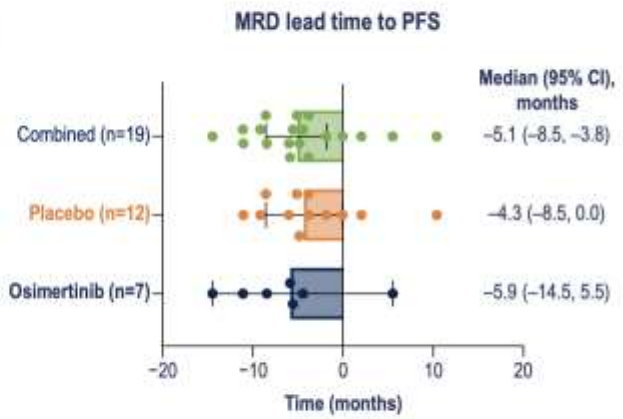


LAURA TRIAL

MRD-based molecular progression had a clinical sensitivity of 63% and a median lead time of –5.1 months across arms

		MRD status, n	
		Molecular progression (n=22)	No molecular progression (n=30)
PFS status, n	Event (n=30)	19	11
	Censored (n=22)	3	19

Concordance of MRD with PFS, % (95% CI)	
PPA (sensitivity)	63 (46, 81)
NPA (specificity)	86 (72, 100)
PPV	86 (72, 100)
NPV	63 (46, 81)

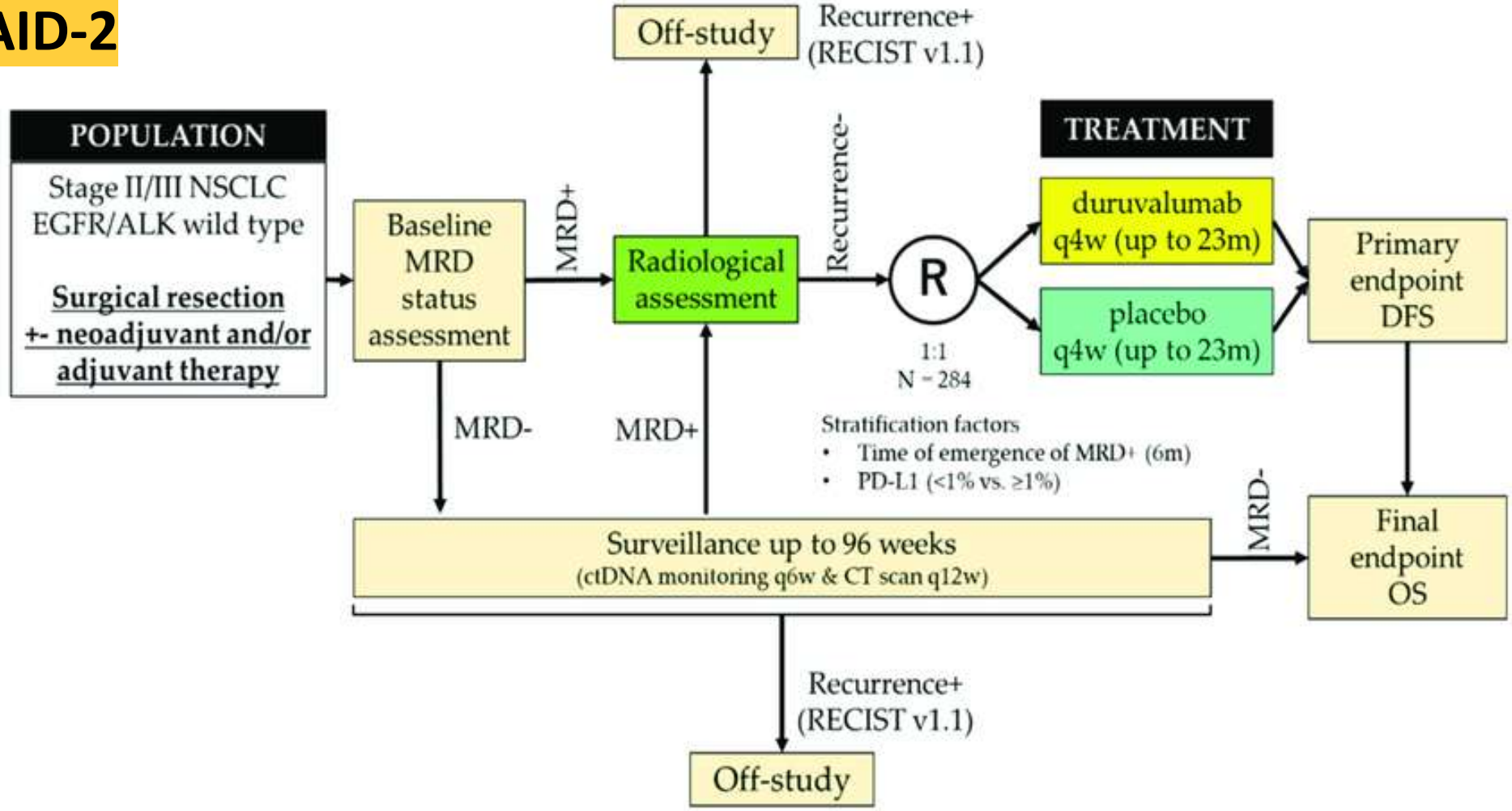


MRD monitoring was able to predict disease progression prior to radiological progression

John ASCO 2024; Arriola ESMO 2025

Longitudinal ctDNA testing in NSCLC

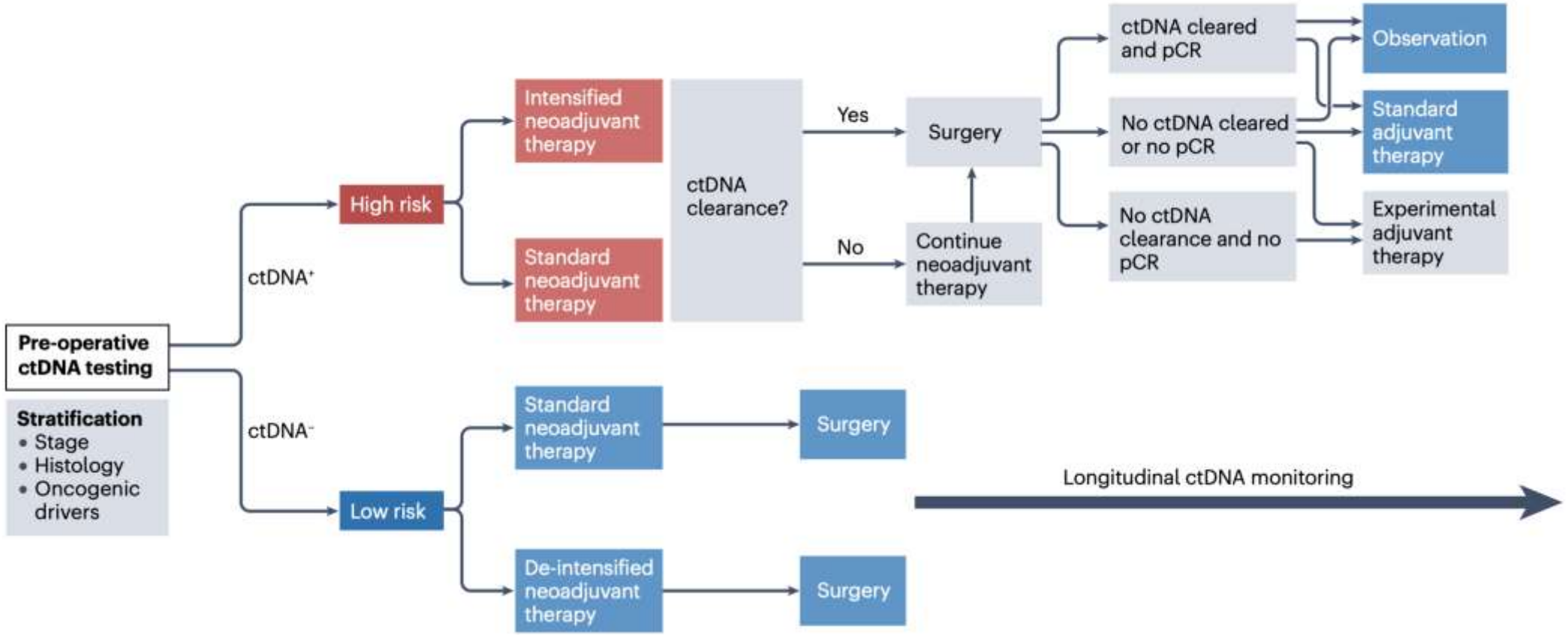
MERMAID-2



Need for prospective clinical trials

Need for prospective clinical trials

ctDNA testing in patients with early-stage NSCLC eligible for neoadjuvant therapy



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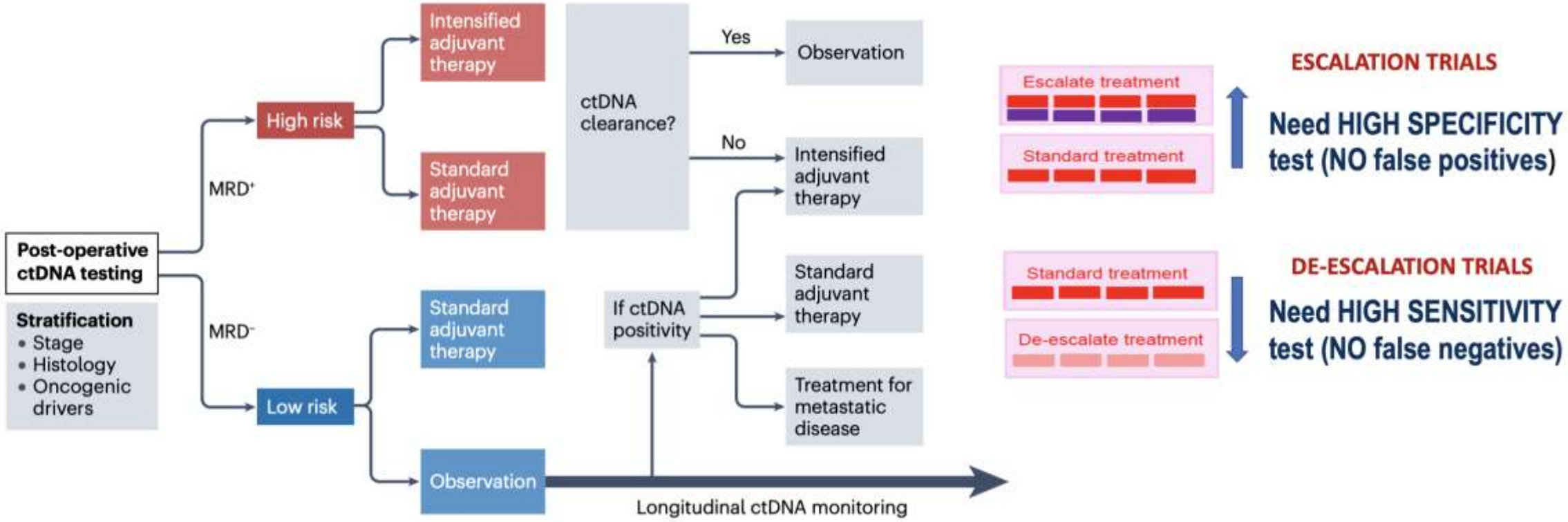
Need for prospective clinical trials

ctDNA/MRD testing in patients with resected early-stage NSCLC



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Ongoing clinical trials in NSCLC

Trial	Phase	MRD Trial Type	Assay	Patient Population	Intervention	Primary Endpoint
ORACLE NCT05059444	NA	Surveillance	Guardant Reveal	Stage II–III NSCLC Undergoing curative intent treatment *Other tumor types also enrolled	CtDNA assessment at the end of radical treatment and during follow up.	Distant Recurrence Free Interval
BTCRC-LUN19-396 NCT04367311	II	Surveillance	CAPP-seq	Resected stage IB, II, IIIA NSCLC	All patients have adjuvant platinum-based doublet CT + 13 cycles of atezolizumab. CtDNA testing every 3 months.	Percentage with undetectable ctDNA at defined time points
NCT04585477	II	Treatment Intensification	AVENIO surveillance kit	Stage I–III NSCLC who have completed surgery or definitive SABR and SOC adjuvant CT if required Patients planned for adjuvant ICI will be excluded	MRD positive patients will receive durvalumab for 12 months. MRD negative patients will receive standard of care surveillance.	Change in ctDNA level after 2 cycles of durvalumab
SCION NCT04944173	II	Treatment Intensification	AVENIO surveillance kit	Stage I NSCLC	SABR and 4 cycles of durvalumab, then evaluated for MRD. MRD negative patients will have no further therapy. MRD positive patients will be randomized to no further therapy or 8 further cycles of durvalumab.	Overall Risk of Relapse
MERMAID-1 NCT04385368	III	Treatment Intensification	ArcherDx	Resectable stage II–III NSCLC	MRD-positive patients post operatively are randomized to adjuvant durvalumab plus platinum-based doublet CT or placebo plus platinum-based doublet CT (SOC).	DFS in MRD positive patients
MERMAID-2 NCT04642469	III	Treatment Intensification	ArcherDx	Resectable stage II–III NSCLC	Patients who become MRD positive during a 96-week surveillance period will be randomized to durvalumab or placebo.	DFS in the PD-L1 TC≥1% analysis set
NCT04585490	III	Treatment Intensification	AVENIO surveillance kit	Unresectable stage III NSCLC that have completed definitive CRT	MRD positive patients will receive 4 cycles of platinum-based doublet CT plus durvalumab. MRD negative patients will receive durvalumab (SOC).	Change in ctDNA level following CT
NCT05286957	II	Treatment Intensification	Not specified	Resected stage IIA, IIB, IIIA NSCLC who have completed adjuvant CT	MRD positive patients will receive tislelizumab.	Percentage of patients changed from MRD positive to MRD negative post 8 cycles of Tislelizumab
NCT05457049	NA	Treatment De-escalation	Not specified	Stage IB-IIIA NSCLC patients who have a complete resection and undetectable landmark MRD.	Patients will have MRD assessed at two time points post operatively. If MRD negative, they will not have adjuvant CT and undergo routine MRD assessment instead.	Two-years DFS rates for patients with longitudinal undetectable MRD

Abbreviations: SABR, stereotactic radiotherapy; MRD, minimal residual disease; DFS, disease free survival; CT, chemotherapy; vs, versus; SOC, standard of care; ICI, immune check point inhibitors; NA, not applicable; CRT, chemoradiotherapy; PD-L1 TC, programmed death-ligand 1 tumor cells.

O'Sullivan OncoTargets and Therapy 2023

Limitations of clinical implementation of ctDNA testing

- **Lack of standardization**, leading to high variability across platforms and laboratories.
- **Limited prospective evidence**; most data come from small, exploratory studies.
- **Limited access** to ctDNA/MRD assays in many healthcare settings.
- **Need for harmonized assay performance and standardized operating procedures**.
- **Requirement for ultra-sensitive assays** (very low LOD) to improve risk stratification and reduce false negatives.
- **Short turnaround times** are essential to inform timely clinical decisions.
- **Adequate tumor tissue** is not always available for tumor-informed approaches.
- **Optimal timing of blood collection** (post-surgery and during follow-up) remains undefined.
- **High cost** of testing limits widespread implementation.

Conclusions

- ctDNA/MRD is a promising tool for managing advanced NSCLC, allowing non-invasive disease monitoring and treatment guidance.
- In early-stage and locally advanced NSCLC, ctDNA/MRD serves as a potential **prognostic** marker for recurrence risk and a **predictive** marker of response to neo/adjuvant therapy.
- Well-designed, adequately powered clinical trials are required to validate its clinical utility and support routine adoption.
- Overcoming current technical, logistical, and standardization challenges is essential for effective clinical implementation.


Thank You
For Your
Attention !!