ctDNA-based MRD testing and monitoring in gynecologic cancers

Robert W. Holloway, MD, DHc, FACOG, FACS
Director Gynecologic Oncology
AdventHealth Cancer Institute
Orlando, FL

Thomas J. Rutherford, PhD, MD
Director, College of Medicine Obstetrics & Gynecology, UCF
Medical Director, Tampa General Hospital Cancer Institute
Tampa, FL

Disclosures-Holloway

- Advisory Boards:

  Caris, Natera, GSK, Genelux

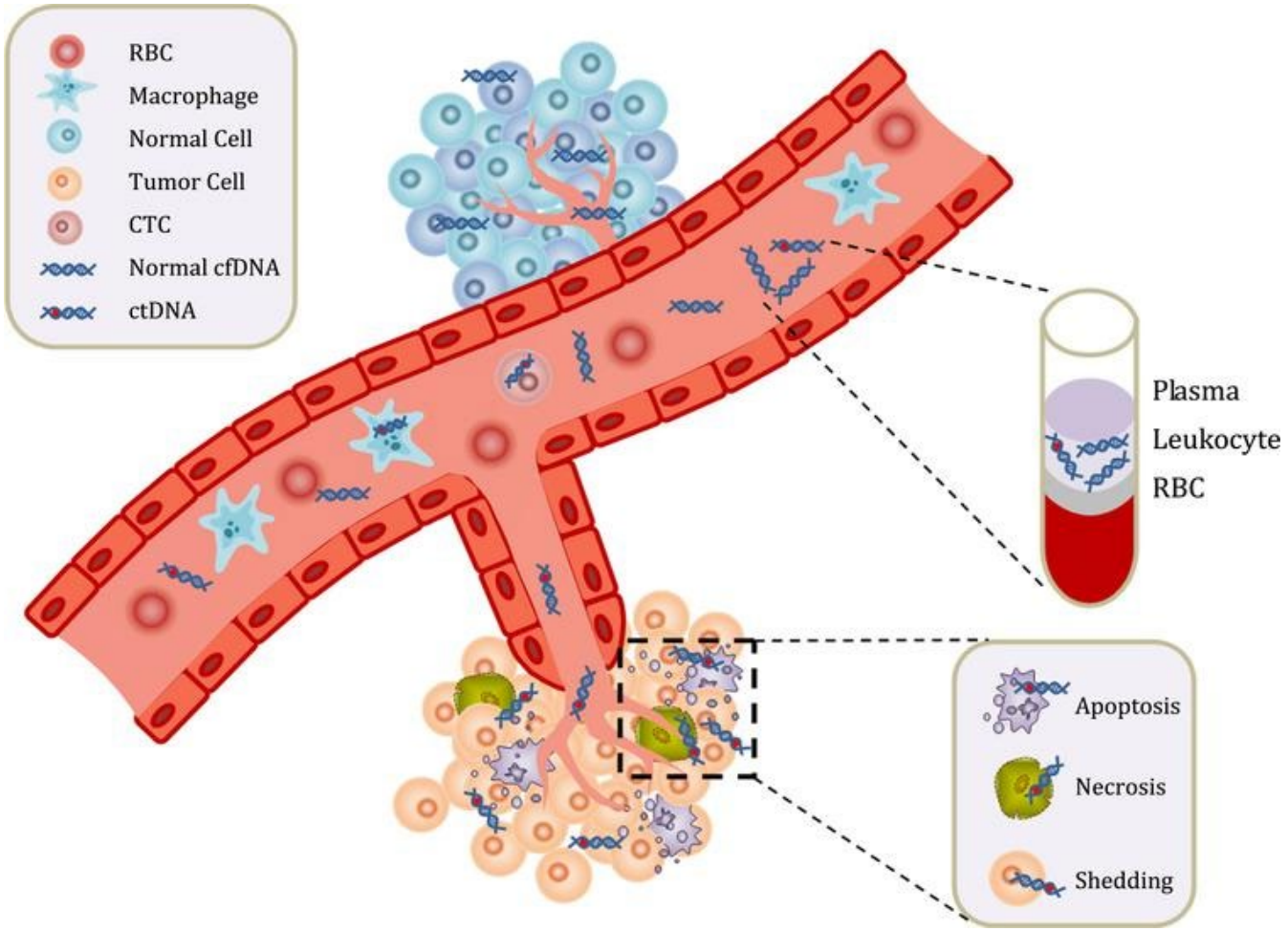
- Speaker Honoraria:

  Caris, Natera, GSK, Merck, AstraZeneca, Abbvie

ctDNA provides a non-invasive real time assessment of tumor biology

- Applications
  - mutation profiling
  - early detection
  - minimal residual disease
  - treatment monitoring
  - guiding therapeutic decisions

ctDNA as a new tool



- Circulating tumor DNA (ctDNA) refers to small fragments of DNA released from tumor cells into the bloodstream
- ctDNA is a non-invasive, highly specific and dynamic blood-based cancer biomarker
- ctDNA dynamics can be used to assess disease burden in real-time with a short half-life (~2hrs)

Hahn et al. *Kidney Cancer.* 2019;3:7–13.

Definitions

- ***Liquid Biopsy***:

  Tumor biomarker information that is detectable through blood samples

- ***Cell Free DNA (cfDNA):***

  Extracellular DNA detectable in the plasma (mostly from normal cells)

- ***Circulating Tumor DNA (ctDNA):***

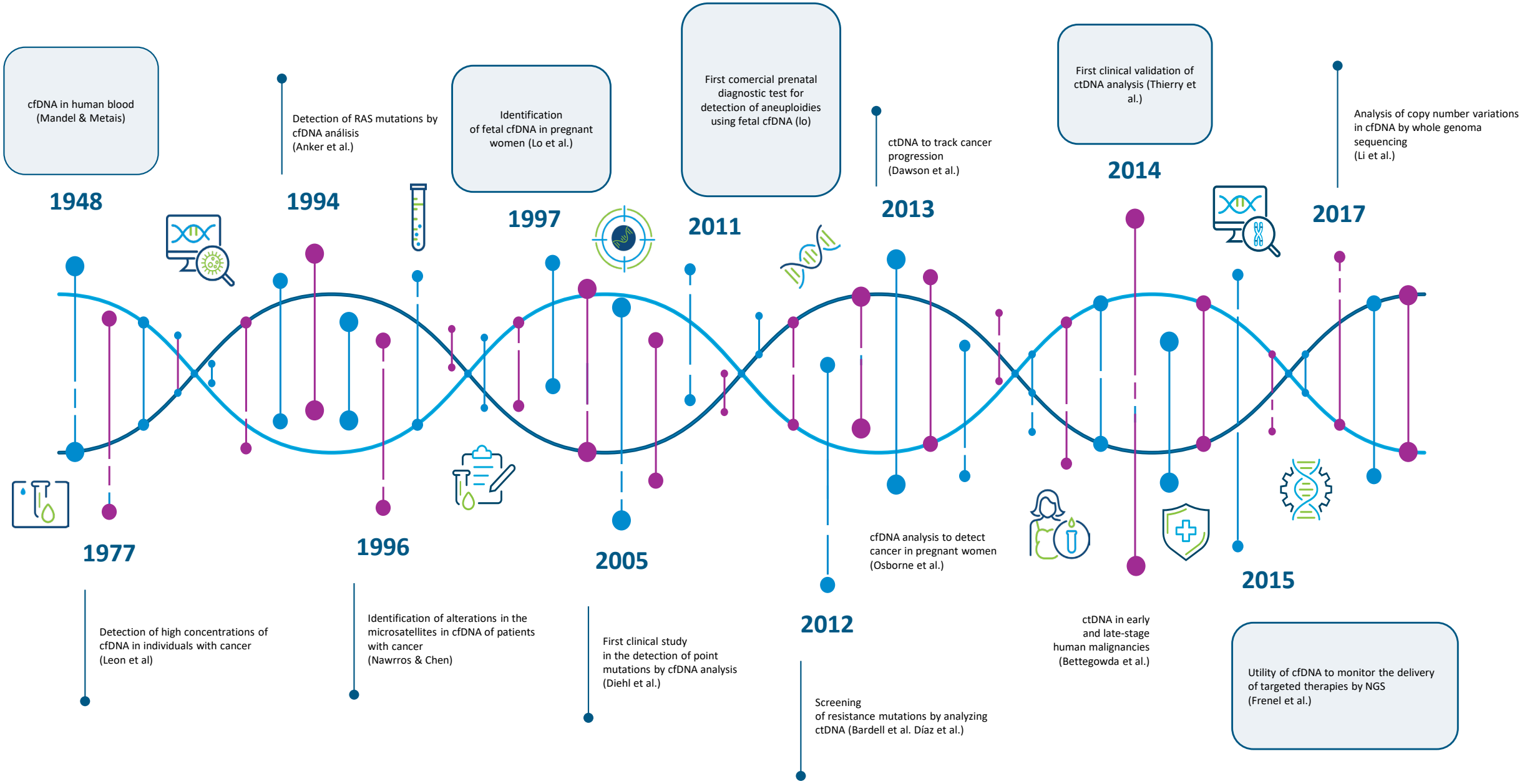
  A minor fraction of cfDNA consisting of DNA from tumor cells

- ***Minimal/Molecular Residual Disease (MRD):***

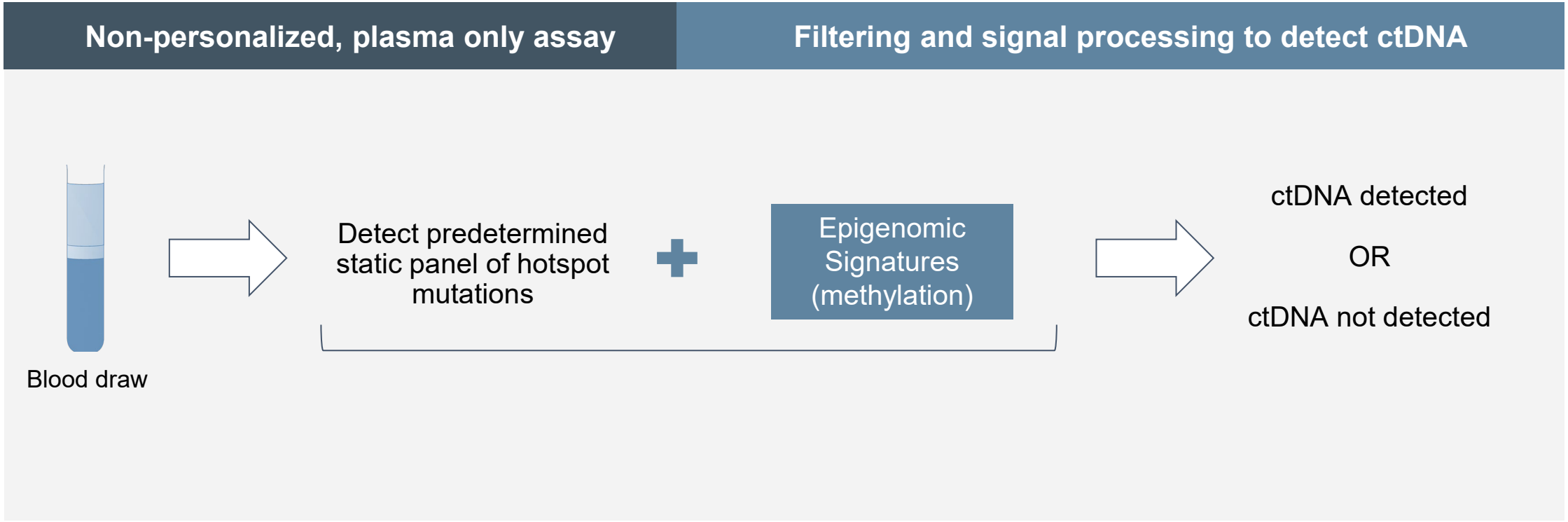
  Tumor burden below the limit of detection of usual clinical/radiological tests

Jantus-Lewintre E et al. *J Liq Biopsy.* 2026;12:100462.

Circulating cfDNA Benchmarks



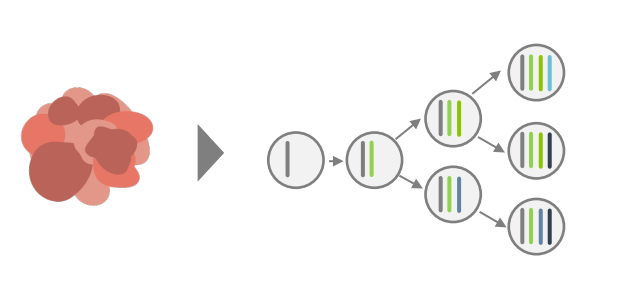
Tumor-naive MRD assay



Tumor-informed MRD assay

Personalized custom designed assay

Tracks mutational signatures derived from the primary tumor to create a customized assay

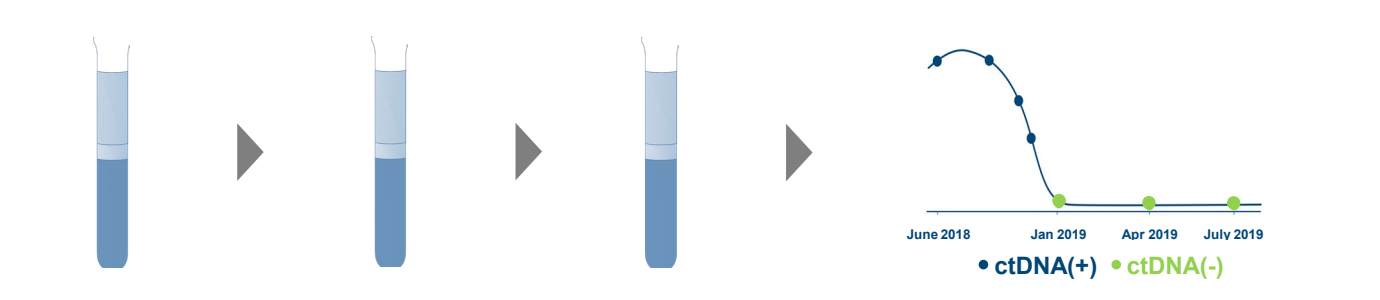


Test design: 3-4 weeks

Serial blood tests to detect ctDNA

Personalized assay to test patient's blood serially

Track ctDNA dynamics over time



Turnaround time: ~7-10 days/test

Tumor-informed MRD can provide detection down to single digit PPM, with lowering limits of detection/increasing sensitivity seen with incorporation of broader tumor sequencing and/or other types of variants such as phased variants.

MRD utility in the current landscape

Other Tumor Guidelines (2025)

National Comprehensive Cancer Network – U.S.

- **Colon & Rectal:** prognostic factor in the adjuvant setting[1,2]
- **Merkel Cell**: recommended for assessing disease burden[3]
- **Diffuse Large B-Cell Lymphoma (DLBCL):** recommended for evaluating PET-positive results at the end of first-line therapy[4]

Japan Society of Clinical Oncology

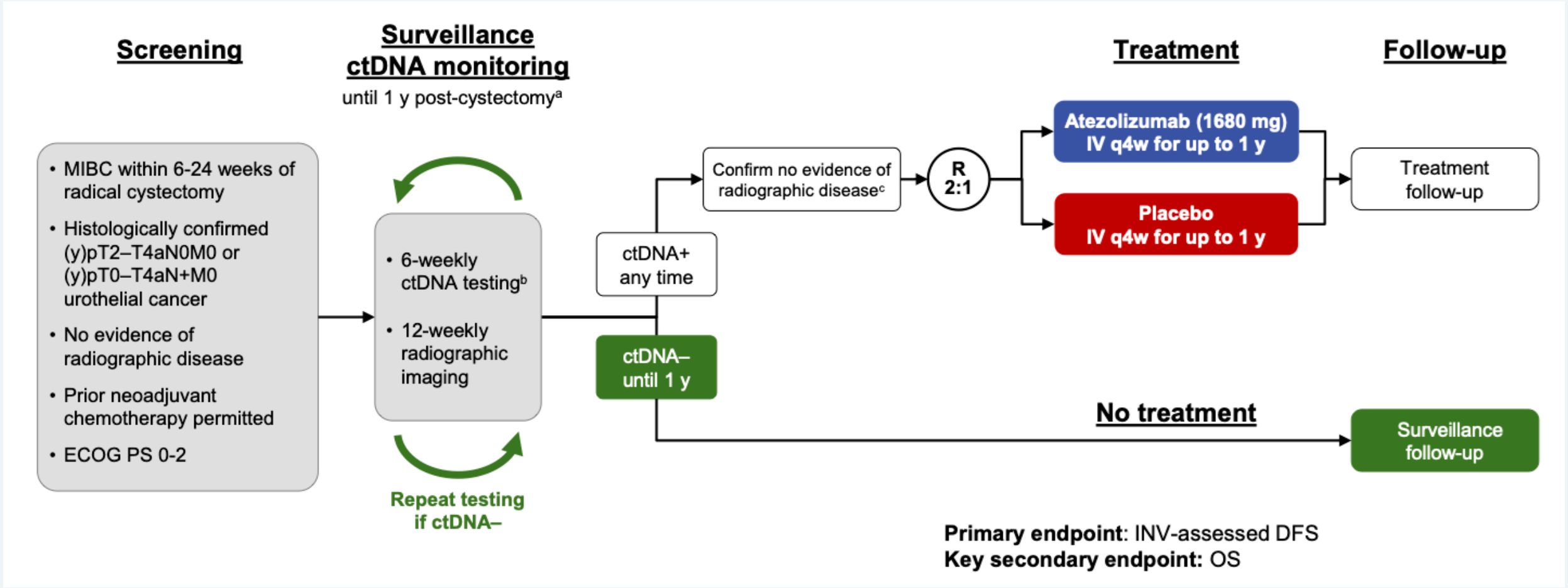
- **Colorectal:** MRD testing is recommended[5]

MRD in GYN

- MRD use in GYN cancers is increasing
- SGO guidelines recognize that MRD may be useful or considered in select cases[6]
- **Actionability is often the key question for clinicians**

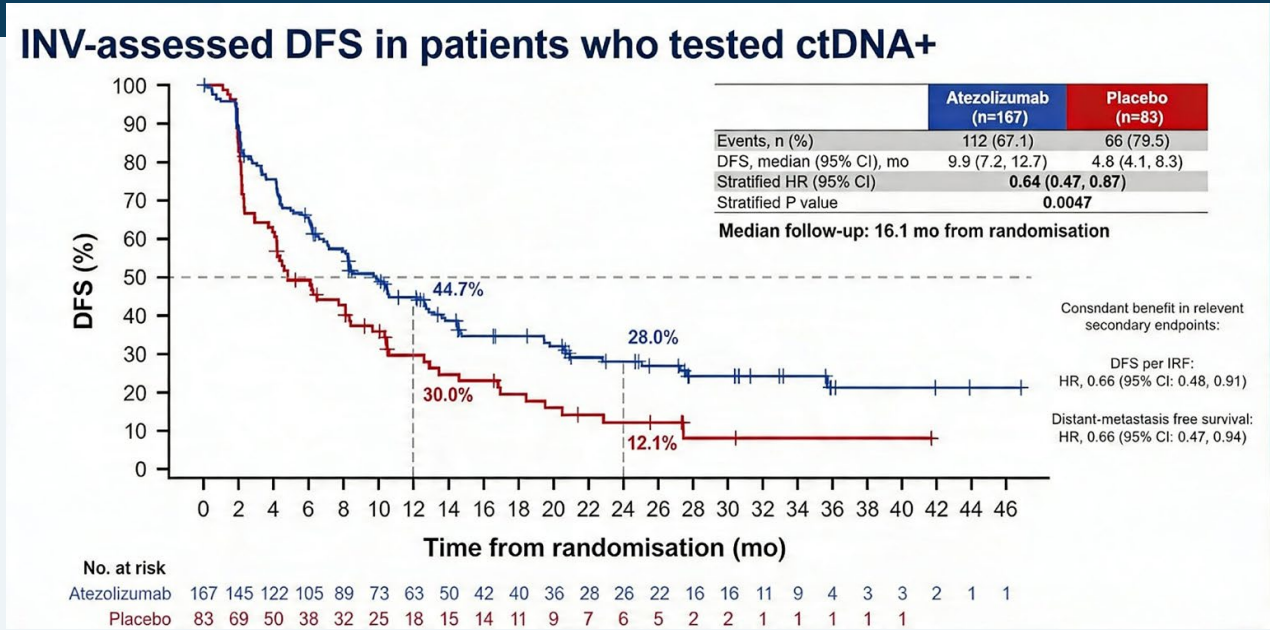
1. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Colon Cancer Version 1.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed February 7, 2025.
2. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Rectal Cancer Version 1.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed February 7, 2025.
3. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Merkel Cell Carcinoma Version 1.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed January 17, 2025.
4. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: B-Cell Lymphomas. Version 1.2025. Published December 20, 2024. Accessed March 20, 2025.
5. Japanese Society of Medical Oncology. Clinical Guidelines: Molecular Testing for Colorectal Cancer Treatment. 5th ed. Published November 21, 2023. https://www.jsmo.or.jp/about/doc/guideline_20231121.pdf
6. Salani R et al. *Gynecol Oncol.* 2026;204:109-17.

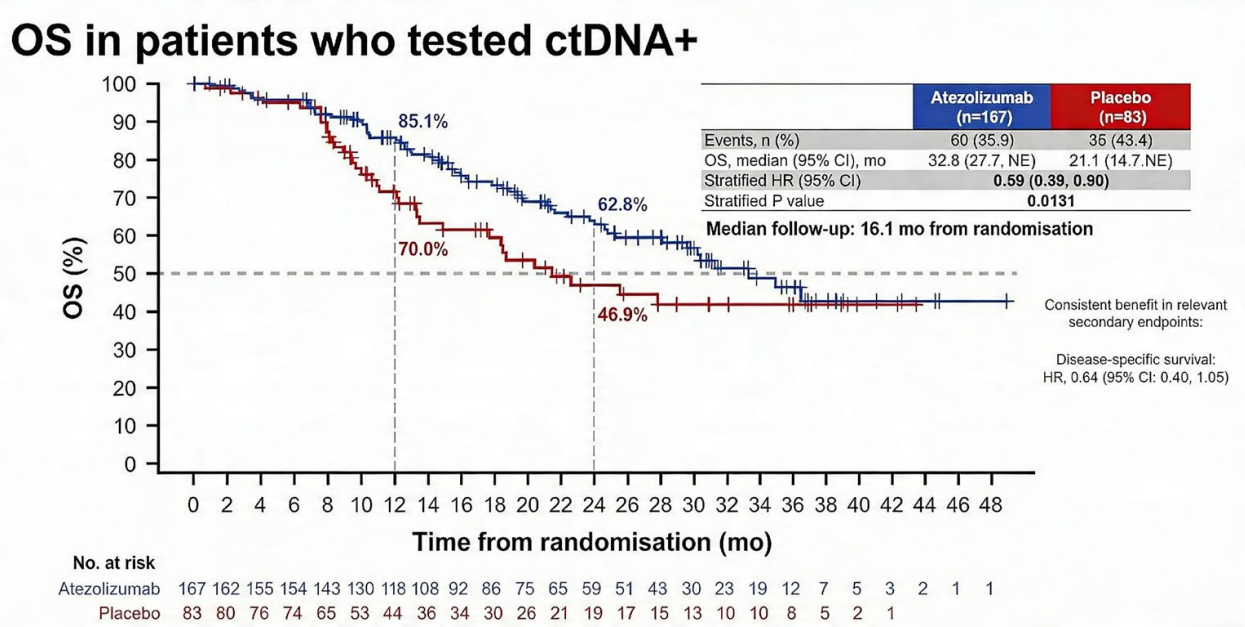
Bladder cancer- IMvigor 011 study



Powles T et al. *N Engl J Med.* 2025;393(24).

Bladder cancer- IMvigor 011 study





Patients in the atezolizumab arm had statistically significant improvements in disease free survival (HR 0.64, 95% CI 0.47, 0.87; p = 0.0047) versus the placebo arm

Patients in the atezolizumab arm had statistically significant improvements in overall survival (HR 0.59, 95% CI 0.39, 0.90; p = 0.0131) versus the placebo arm

Powles T et al. *N Engl J Med.* 2025;393(24):2395-2408.

Practical Uses in the Real World

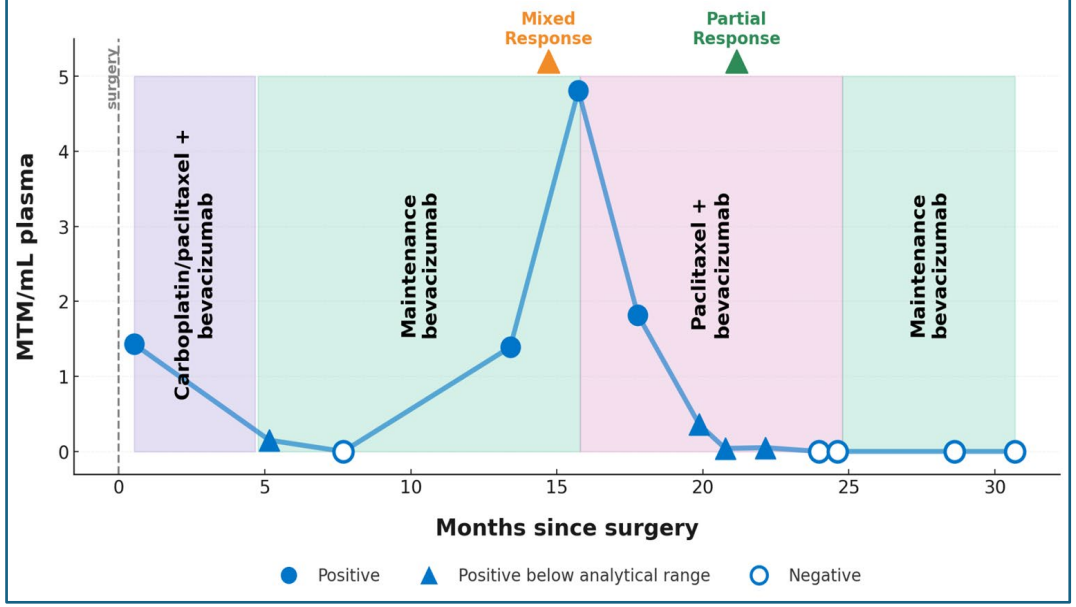
Treating an MRD-detected recurrence on maintenance therapy

Case Study

65yo with Stage IIIC high grade serous carcinoma of the fallopian tube (pT3cN1b)

- Exploratory laparotomy with radical tumor debulking
- HRD neg, *CCNE1* amp, *TP53* mut, PDL1+, BRCA neg, ERBB2 neg, ER/PR neg, FOLR1 neg
- ctDNA positive post-surgery; decreased with chemotherapy
  - Switched to maintenance bevacizumab
- ctDNA started rising, prompting a PET scan that showed mixed response
  - Patient was switched to paclitaxel + bev and ctDNA cleared
- Offered continue maintenance bev vs observation and chose maintenance therapy, where she has sustained ctDNA clearance

Historical ctDNA MRD Results



Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

natera™

ctDNA/MRD in Ovarian Cancer

- Many patients in clinical remission have some level of MRD.

  42% MRD+ on SLL

- ctDNA positivity often detects disease relapse earlier than CA-125 or imaging.

  Median lead time of 6-10 months

- ctDNA negativity does not exclude residual disease.

  50% of patients with surgical MRD+ were ctDNA negative

- ctDNA is highly prognostic.

  ctDNA positivity correlates with worse outcomes

  PFS 6.4 vs 28.1 months

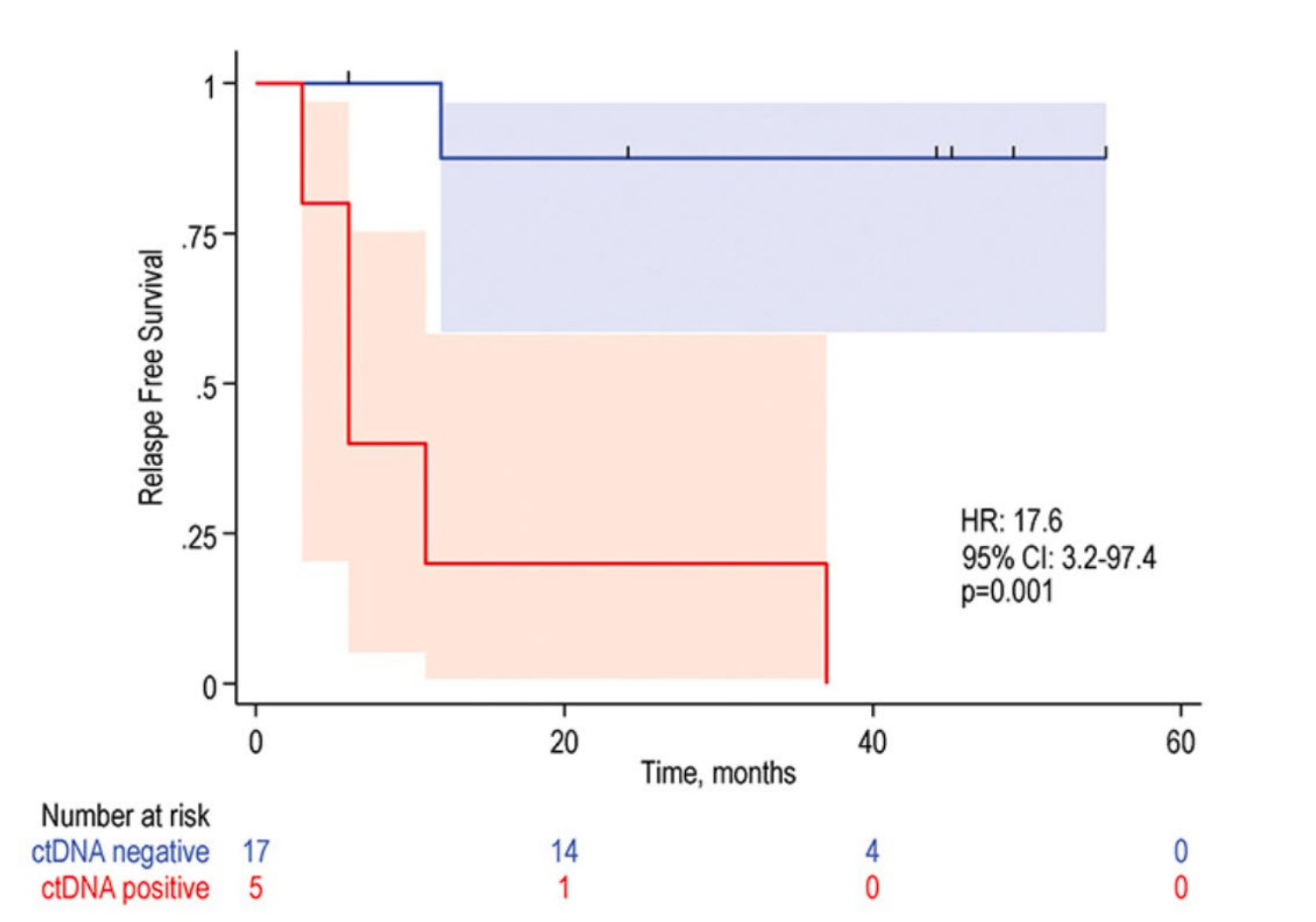
Knisely A et al. *Clin Cancer Res* 2025;31(19):4122-35. Kallio HML et al. *Life Sci Alliance* 2024;7(6).
Hou JY et al. *Gynecol Oncol* 2022;167(2):334-41. Zhang Y et al. *BMC Cancer* 2024;24(1):1565.

ctDNA monitoring for early recurrence

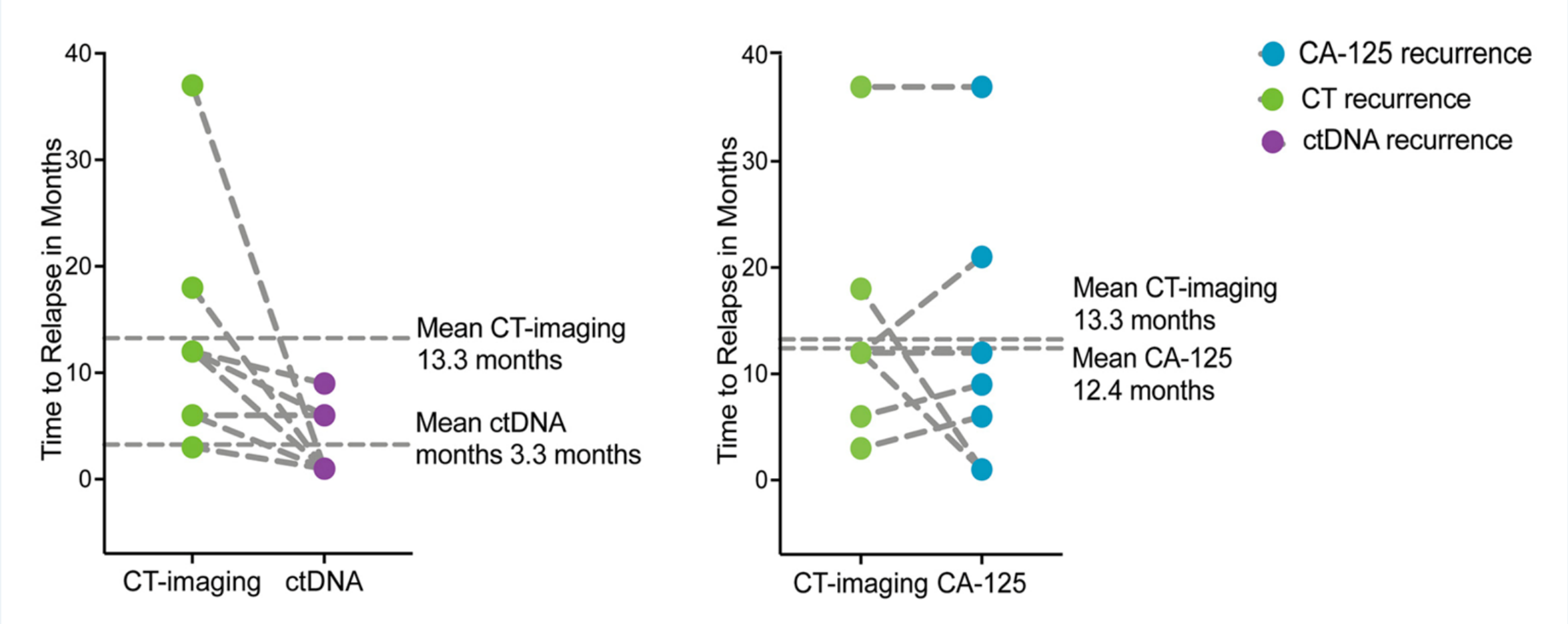
- Study enrolled stage I-IV ovarian cancer patients
  - cohort A: pre-surgical samples (N = 44)
  - cohort B: serially collected, post-surgical samples (N = 12)
  - cohort C: during surveillance (N = 13)
- In cohort B and C, ctDNA was only detected in patients who relapsed (100% sensitivity and specificity)
- ctDNA preceded radiological findings by an average of 10 months.
- The presence of ctDNA was a strong predictor of relapse (HR:17.6, p = 0.001)
- These results suggest that monitoring ctDNA could be beneficial in clinical decision-making for EOC patients.

Hou JY et al. *Gynecol Oncol* 2022;167(2):334-41.

Single post-definitive treatment ctDNA timepoint in OC



Hou JY et al. *Gynecol Oncol* 2022;167(2):334-41.



ctDNA was detectable on average 10 months prior to clinical progression

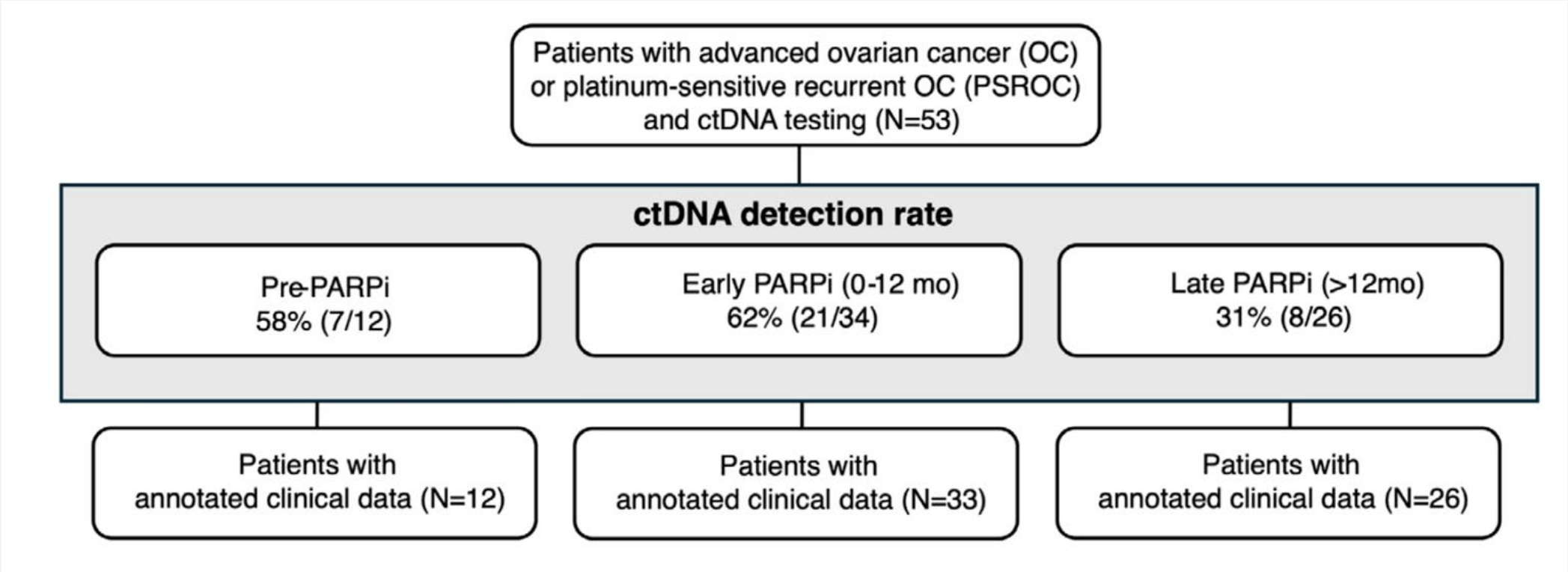
CA-125 had a mean lead time of only ~1 month

Hou JY et al. *Gynecol Oncol* 2022;167(2):334-41.

ctDNA for PARPi maintenance therapy

- A multi-center retrospective cohort study enrolled 53 patients with newly diagnosed Stage III/IV OC or PSROC receiving PARPi maintenance
- Personalized, tumor-informed, exome-based NGS assay
- Samples collected:
  - i) prior to starting PARPi (pre-PARPi; N = 12)
  - ii) during the first 12 months on PARPi (early; N = 34)
  - iii) beyond 12 months of PARPi therapy (late; N = 26)

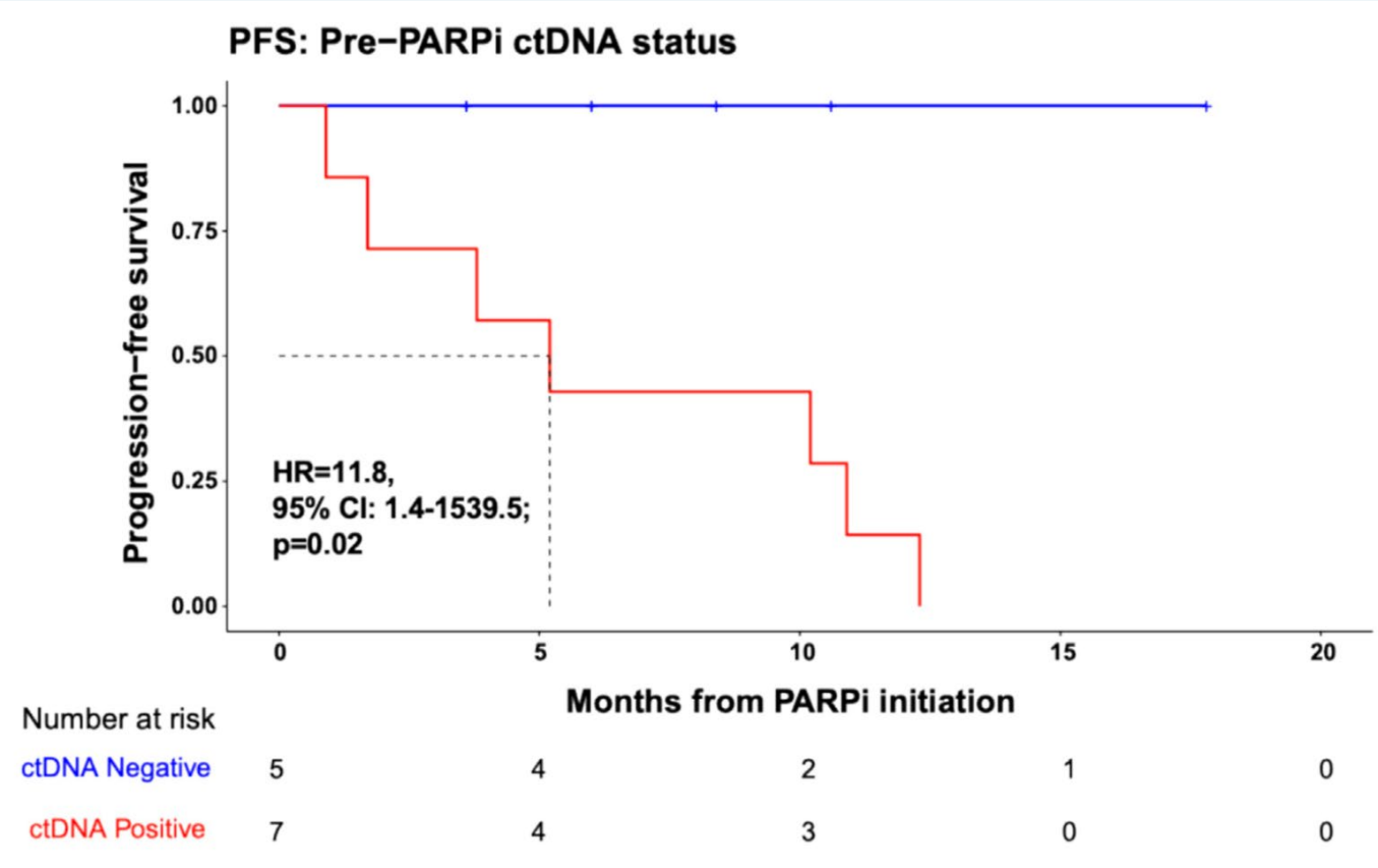
Toboni MD et al. *Gynecol Oncol.* 2026;204:37-43.



Study flowchart detailing the number of patients with OC receiving PARPi therapy, stratified by treatment time points. ctDNA detection rates are shown for three separate windows

Toboni MD et al. *Gynecol Oncol.* 2026;204:37-43.

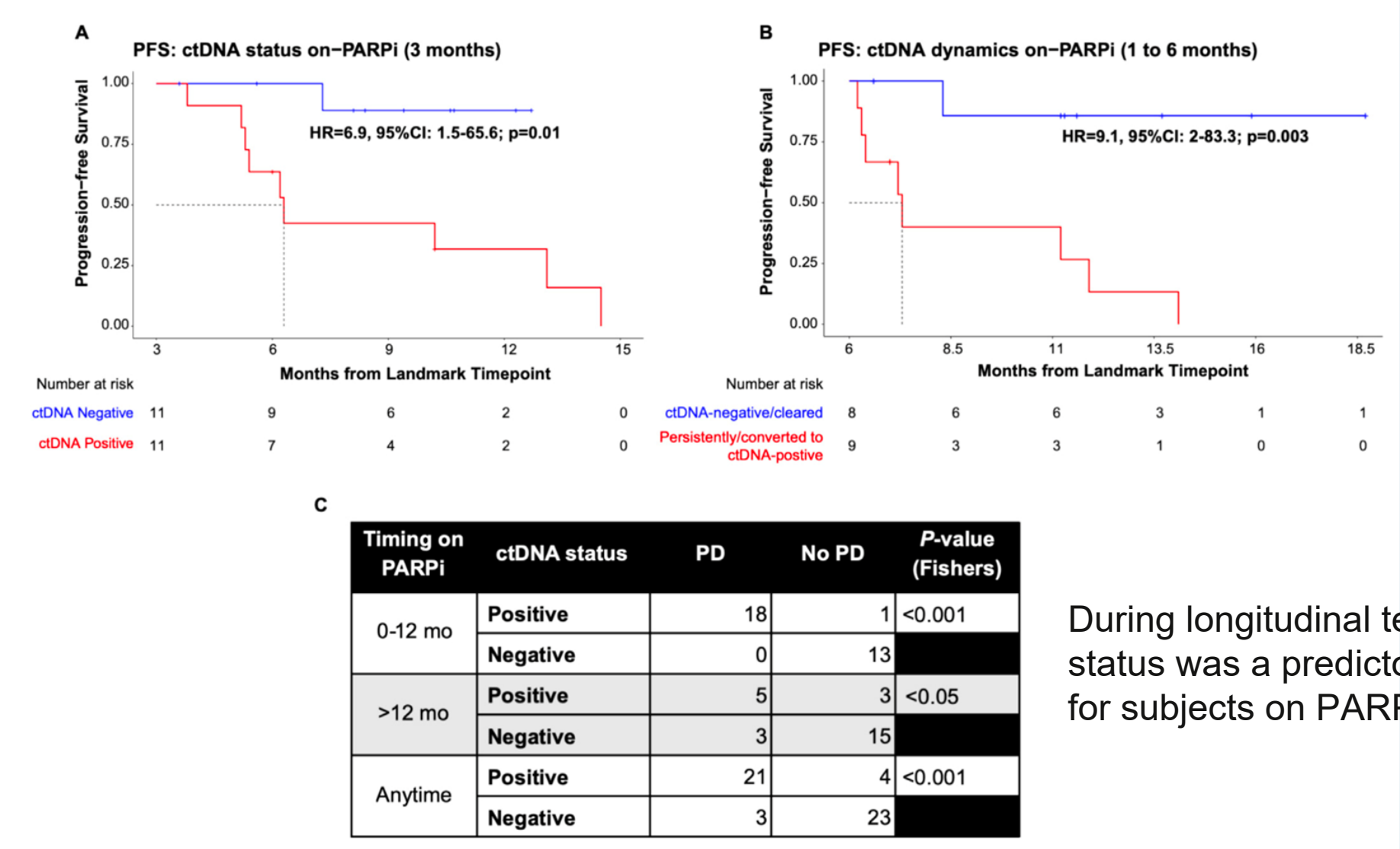
Pre-PARPi ctDNA positivity was associated with inferior PFS



- The median PFS for ctDNA-positive patients was 5.2 (range: 0.9–12.3) months from the initiation of PARPi therapy.
- None of the ctDNA-negative patients recurred at the last known follow-up

Toboni MD et al. *Gynecol Oncol.* 2026;204:37-43.

ctDNA positivity on treatment was associated with inferior PFS



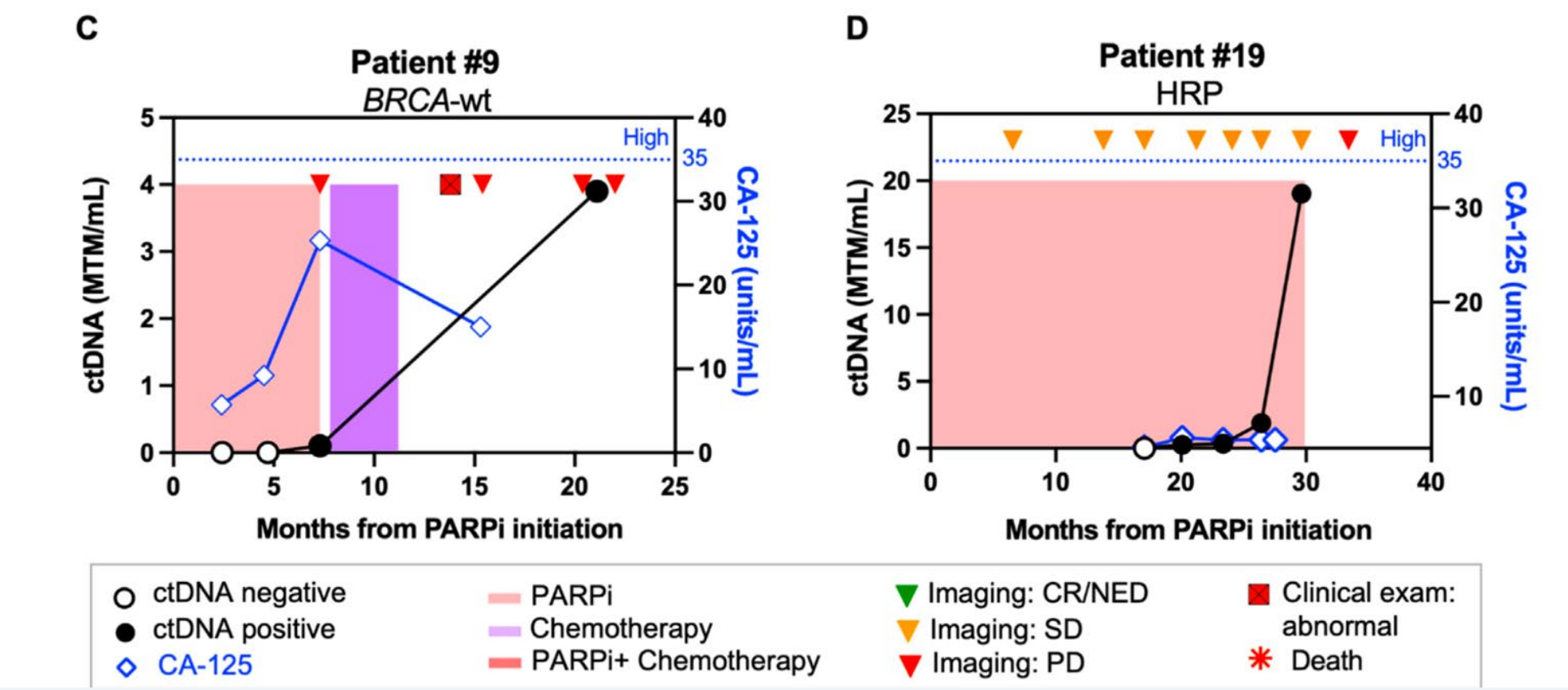
C

Timing on PARPi	ctDNA status	PD	No PD	*P*-value (Fishers)
0-12 mo	**Positive**	18	1	<0.001
	Negative	0	13	
>12 mo	**Positive**	5	3	<0.05
	Negative	3	15	
Anytime	**Positive**	21	4	<0.001
	Negative	3	23	

During longitudinal testing, ctDNA status was a predictor of PFS for subjects on PARPi therapy

Toboni MD et al. *Gynecol Oncol.* 2026;204:37-43.

ctDNA dynamics and radiologic findings relative to treatment in patients with OC



Both converted from ctDNA-negative on PARPi therapy to ctDNA-positive with corresponding PD on imaging, while CA125 remained within normal limits.

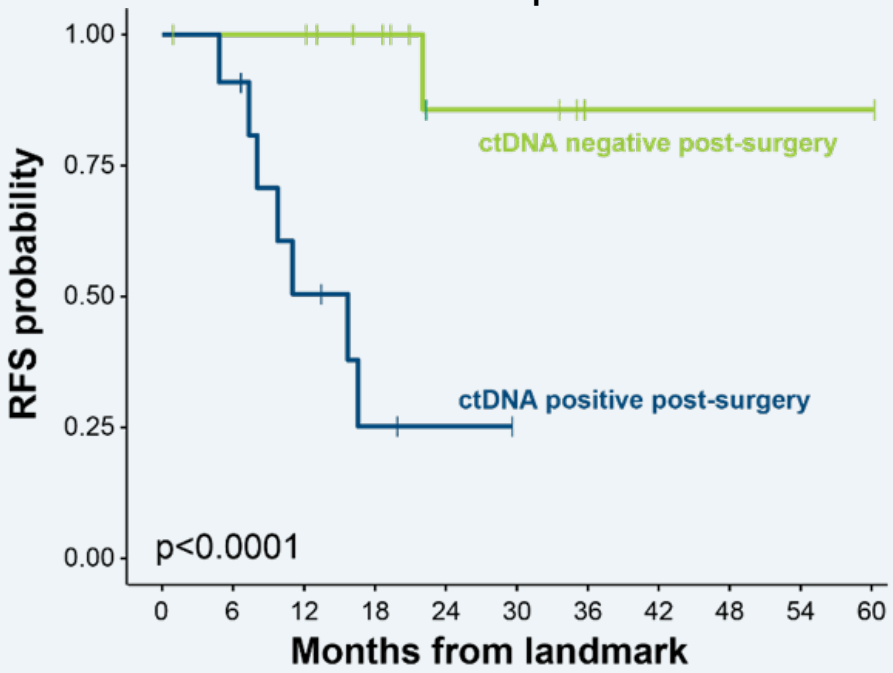
Toboni MD et al. *Gynecol Oncol.* 2026;204:37-43.

Post-Treatment Whole Genome-based ctDNA Monitoring in OC

**Presented by Sophie Blakey-Cheung of AdventHealth Orlando*

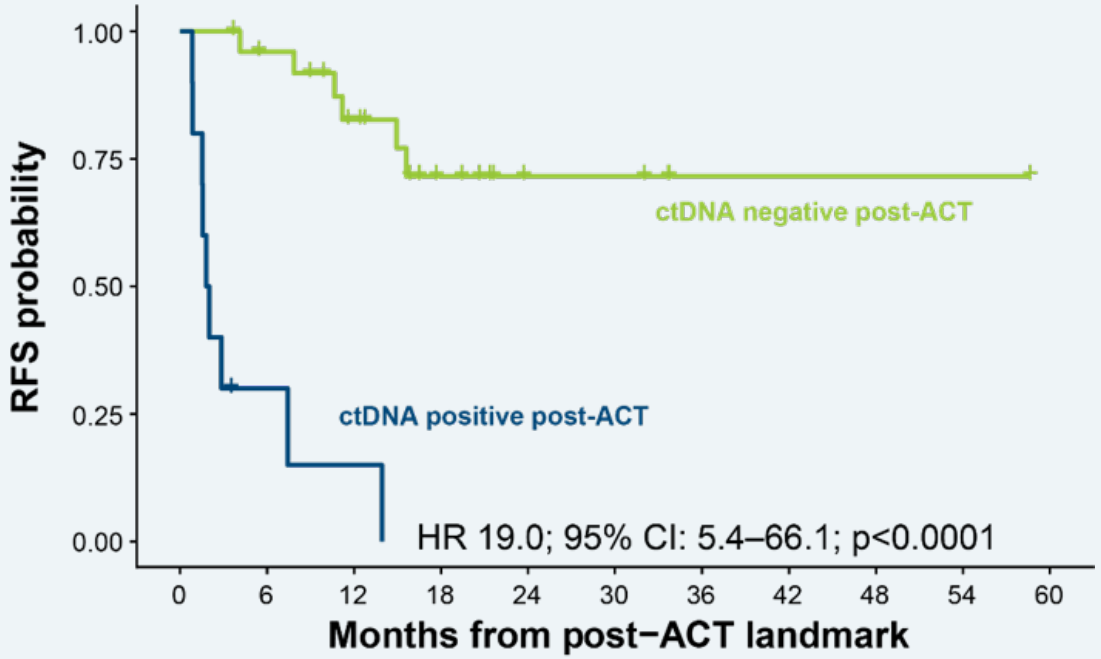
N=120

Landmark prior to ACT



Number at risk											
ctDNA negative	13	12	12	9	4	4	1	1	1	1	1
ctDNA positive	11	10	5	2	1	0	0	0	0	0	0

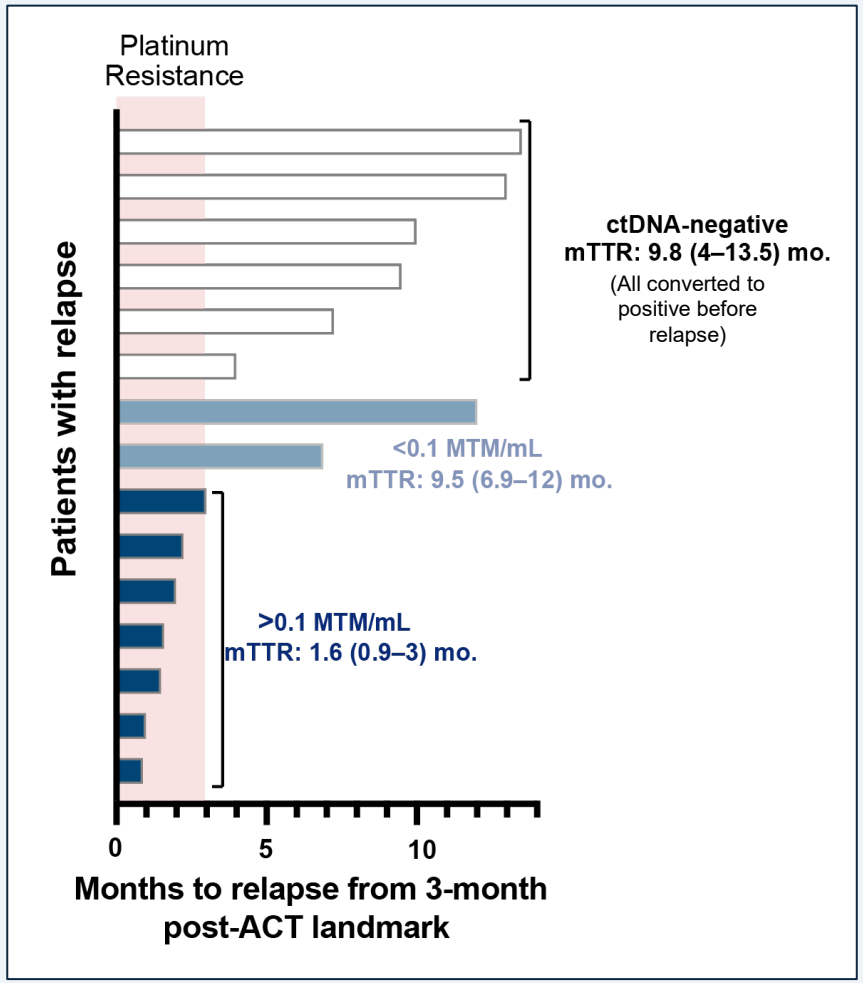
Landmark within 3 months of ACT



Number at risk											
ctDNA negative	26	23	17	9	4	4	1	1	1	1	0
ctDNA positive	10	2	1	0	0	0	0	0	0	0	0

Blakey-Cheung S et al. ASCO 2026: Abstract 5592

ctDNA levels and post-treatment relapse in OC



<u>Among patients who relapsed</u> (N=15)

- **All patients with ctDNA > 0.1MTM/mL post Rx relapsed platinum resistant.**
- **All patients with initial post Rx ctDNA < 0.1MTM/mL relapsed platinum sensitive**
  - **Med TTR 9.5 mo (6.9-12 mo)**
- **All ctDNA-negative patients post Rx were platinum sensitive**
  - **Med TTR 9.8 mo (4-13.5 mo)**
  - **All converted to positive before relapse**

Blakey-Cheung S et al. ASCO 2026: Abstract 5592

ctDNA/MRD in Endometrial Cancer

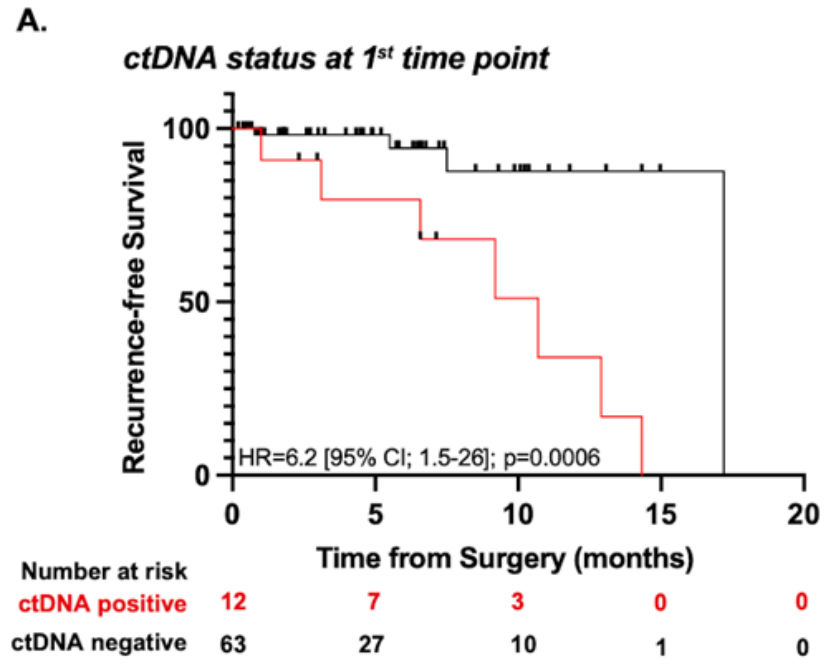
- Endometrial cancer is the most common gynecologic malignancy in the US
- Blood-based biomarkers in EC such as CA-125 and HE4 are of limited clinical utility and not routinely recommended
- Post-surgical ctDNA detection has been associated with shortened RFS in patients with endometrial cancer.

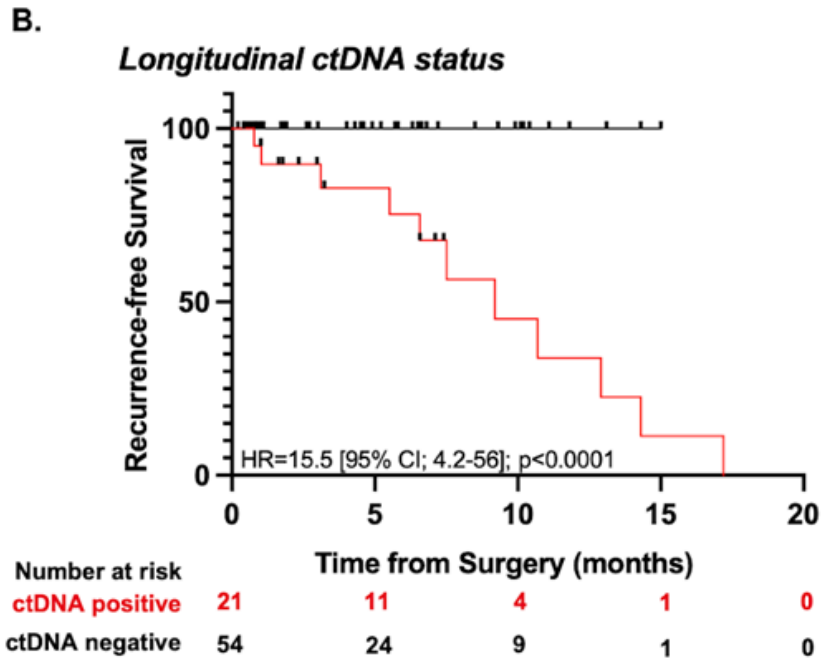
Siegel RL et al. *CA Cancer J Clin.* 2025;75(1):10-45. Recio F et al. *Gynecol Oncol*. 2024;182:63-9. Behrouzi R et al. *Cancers (Basel).* 2021;13(19):4764. Quan Q et al. *Sci. Rep.* 2021;11(1):21694. Ahmed A et al. *Gynecol Oncol.* 2026;205:1-8.

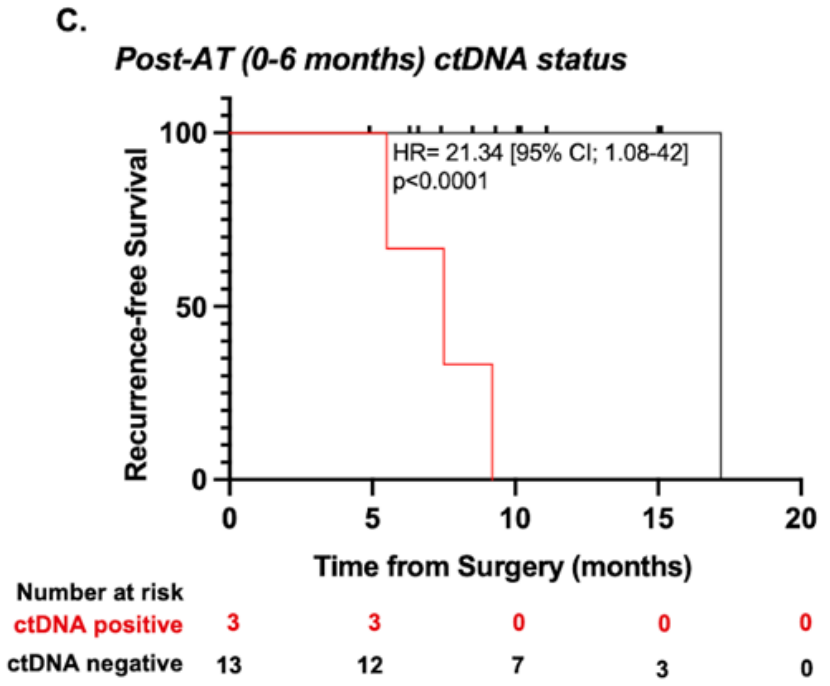
Real-world observational data

- 101 patients with stage I EC were enrolled from multiple institutions
- 267 plasma samples were tested using a personalized, tumor-informed, exome-based ctDNA assay.
- Patients were followed post-surgically and monitored with ctDNA testing
- Based on the GOG 249 criteria, patients were stratified into low (15/ 101; 15%), H-IR (22/101; 22%), high (52/101; 51%), sarcoma (9/101; 9%), and unknown (3/101; 3%) risk groups

Recio F et al. *Gynecol Oncol*. 2024;182:63-9.







ctDNA status at 1st time point

RFS: HR = 6.2; p = 0.0006
Recurrence rate: 58% vs. 6%

Longitudinal ctDNA status

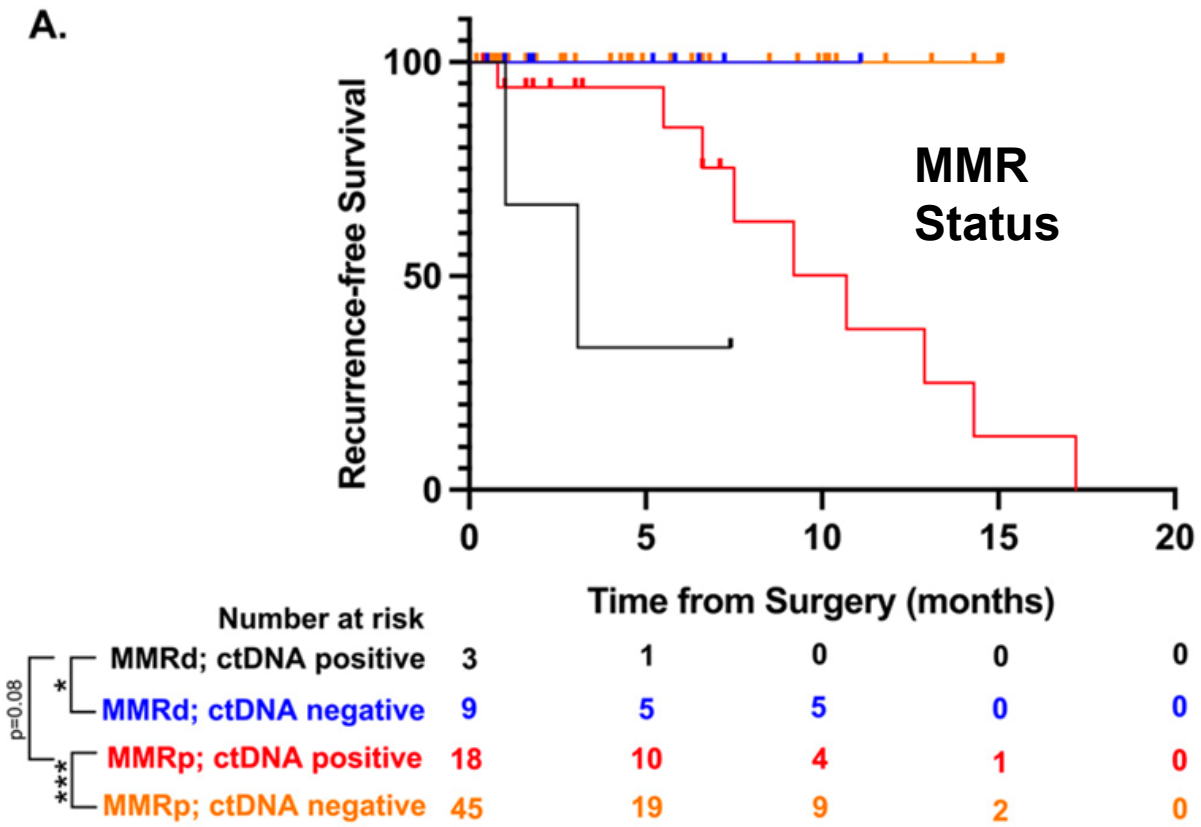
RFS: HR = 15.5; p < 0.0001
Recurrence rate: 52% vs. 0%

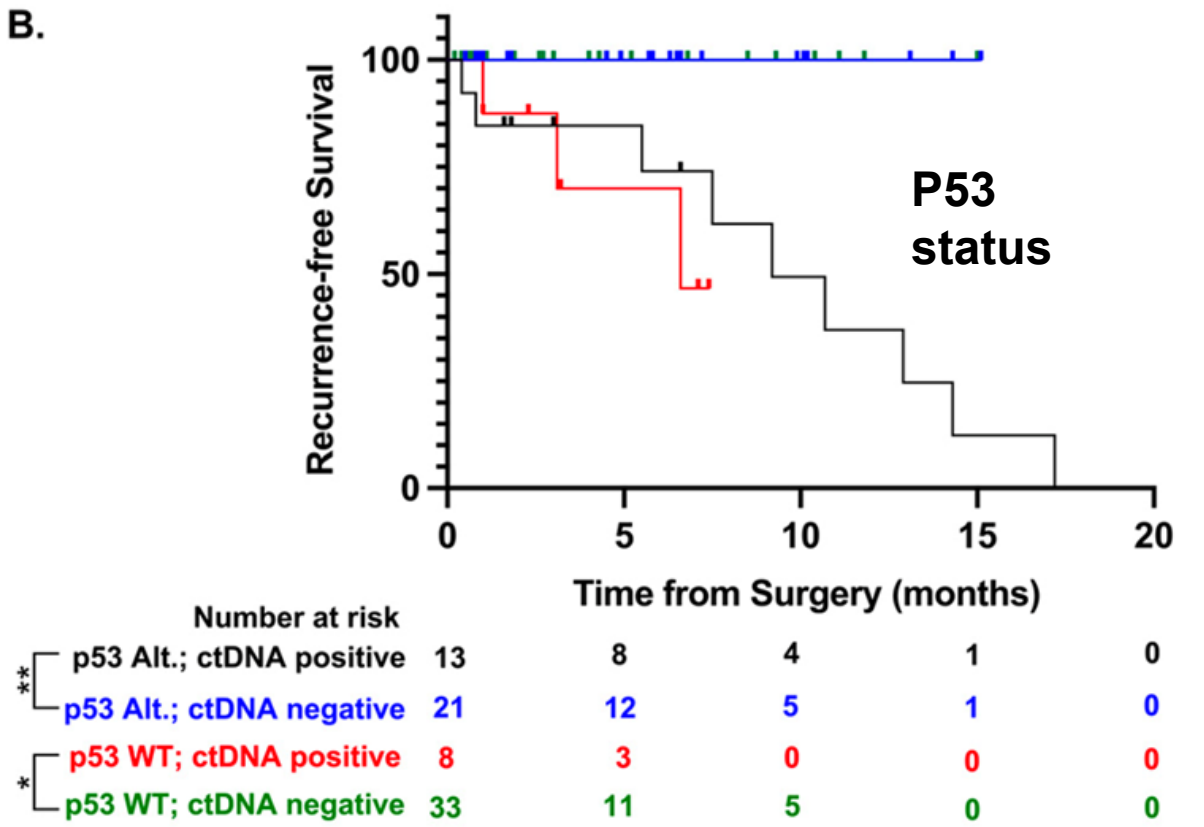
Post-AT (0-6 months) ctDNA status

RFS: HR = 21.34, p < 0.0001)
Recurrence rate: 100% vs. 8%

75 patients had imaging or physical exam (in absence of imaging) results available

Recio F et al. *Gynecol Oncol*. 2024;182:63-9.

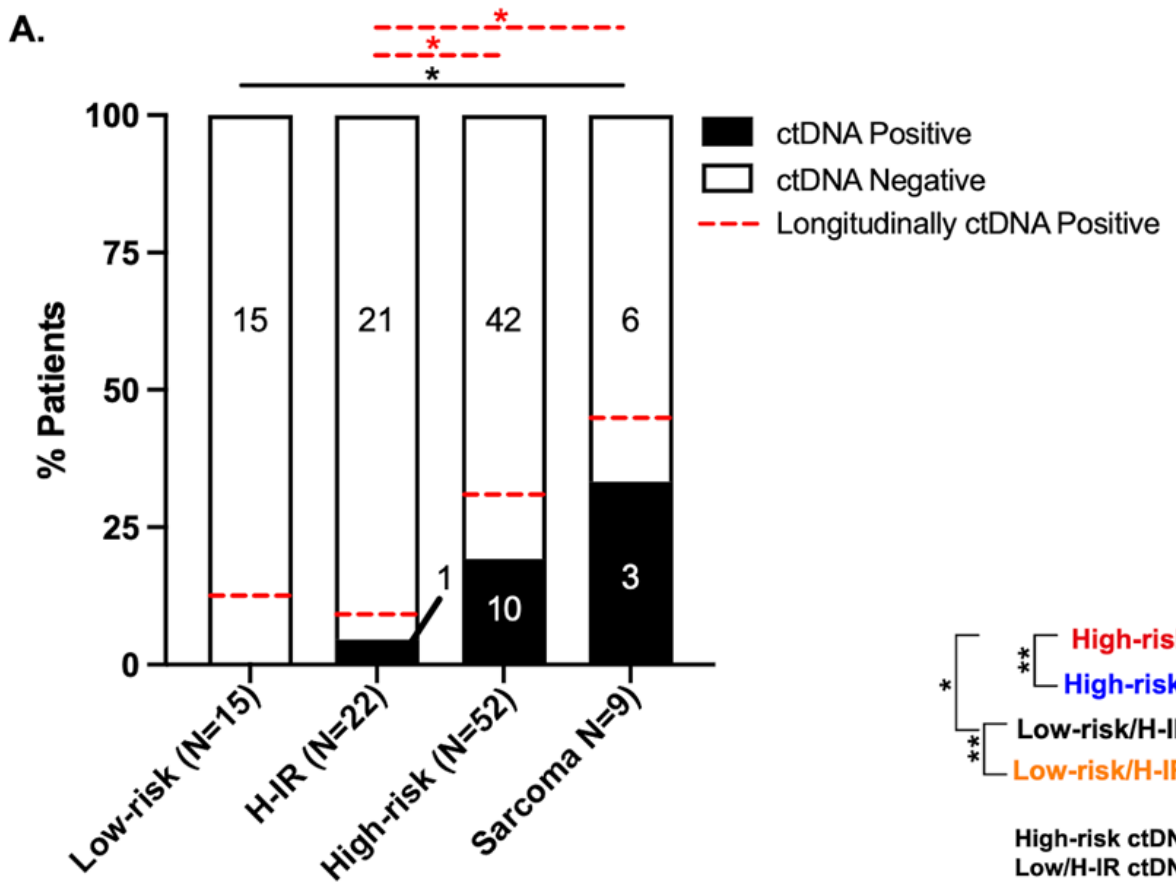


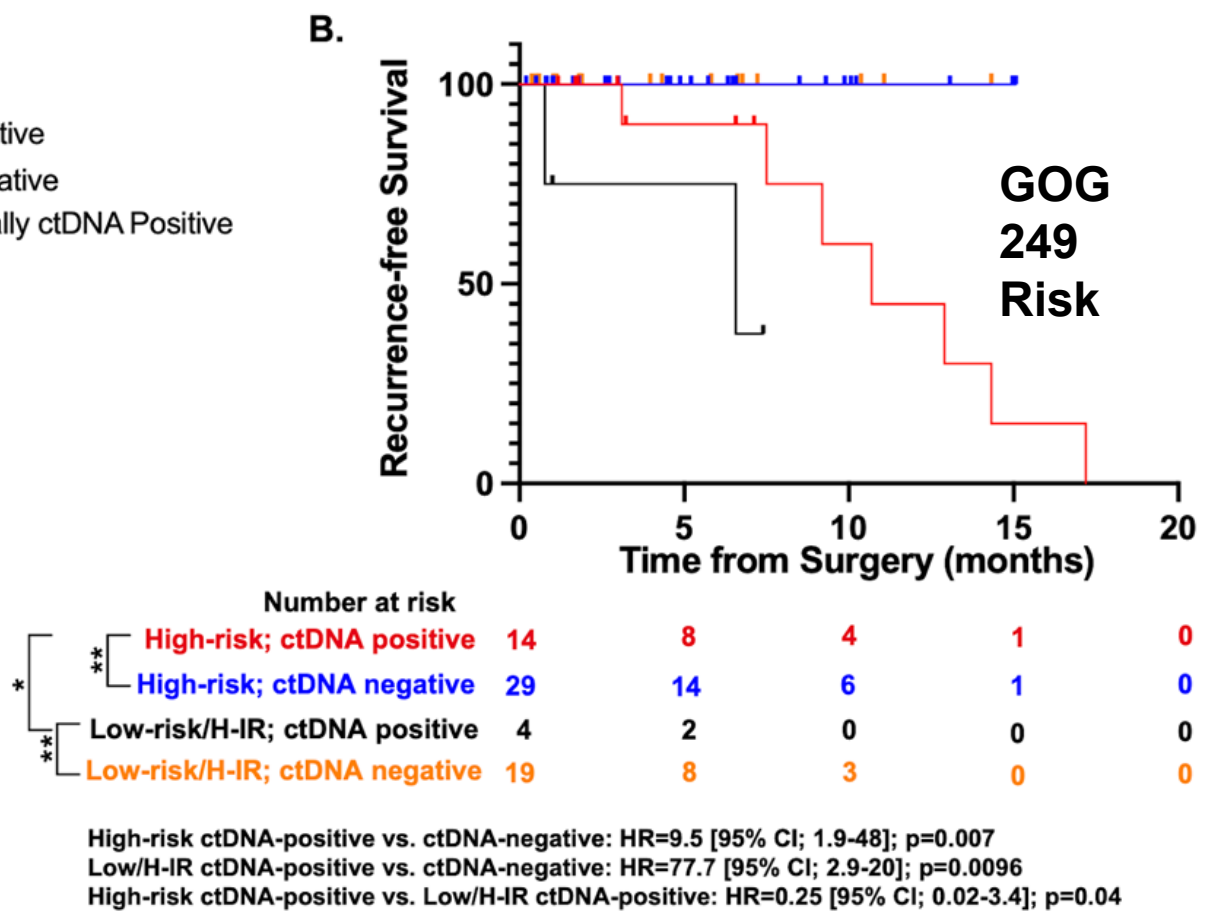


- MMRd ctDNA-positive vs. ctDNA-negative: HR=12.08 [95% CI: 0.293-498]; p=0.03
- MMRp ctDNA-positive vs. ctDNA-negative: HR=29.9 [95% CI: 1.438-621.658]; p=0.0002
- MMRp ctDNA-positive vs. MMRd ctDNA-positive: HR=4.462 [95% CI: 0.733-27.156]; p=0.08

- p53ALT ctDNA-positive vs. ctDNA-negative: HR=9.1 [95% CI: 2.237]; p=0.002
- p53WT ctDNA-positive vs. ctDNA-negative: HR=9.8 [95% CI: 1-99]; p=0.01

Recio F et al. *Gynecol Oncol*. 2024;182:63-9.





A. Percentage of patients with exome-based ctDNA detection at the first post-surgical time point (black bars) and longitudinally (dotted red line)

B. Association of ctDNA and risk groups with RFS

High-risk ctDNA-positive vs. ctDNA-negative: HR=9.5 [95% CI; 1.9-48]; p=0.007
Low/H-IR ctDNA-positive vs. ctDNA-negative: HR=77.7 [95% CI; 2.9-20]; p=0.0096
High-risk ctDNA-positive vs. Low/H-IR ctDNA-positive: HR=0.25 [95% CI; 0.02-3.4]; p=0.04

Recio F et al. *Gynecol Oncol*. 2024;182:63-9.

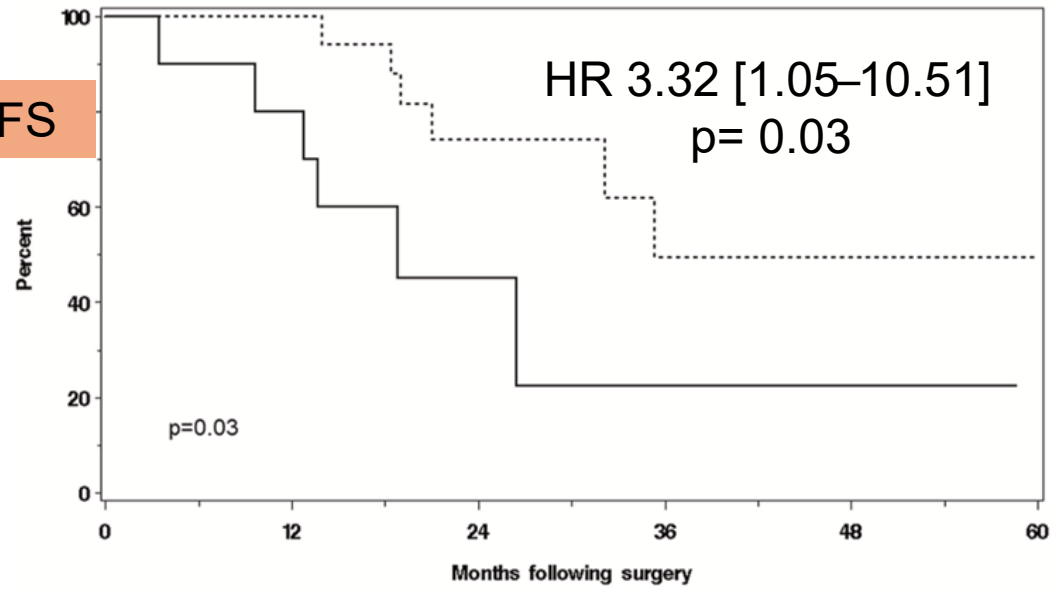
A prospective observational study

- Endometrial cancer
- Blood samples were collected before and 10 weeks after surgery
- Personalized, tumor-informed NGS assay
- Thirty-six patients were included: 6 (16.7 %) intermediate risk, 1 (2.8 %) high-intermediate risk, 28 (77.8 %) high risk, and 1 (2.8 %) advanced metastatic.
- Post-surgical ctDNA was associated with increased risk of recurrence (HR 3.32 [1.05–10.51]) and death (HR 5.97 [1.11–36.08]).

De Vitis LA et al. *Eur J Surg Oncol.* 2026;52(3):111388.

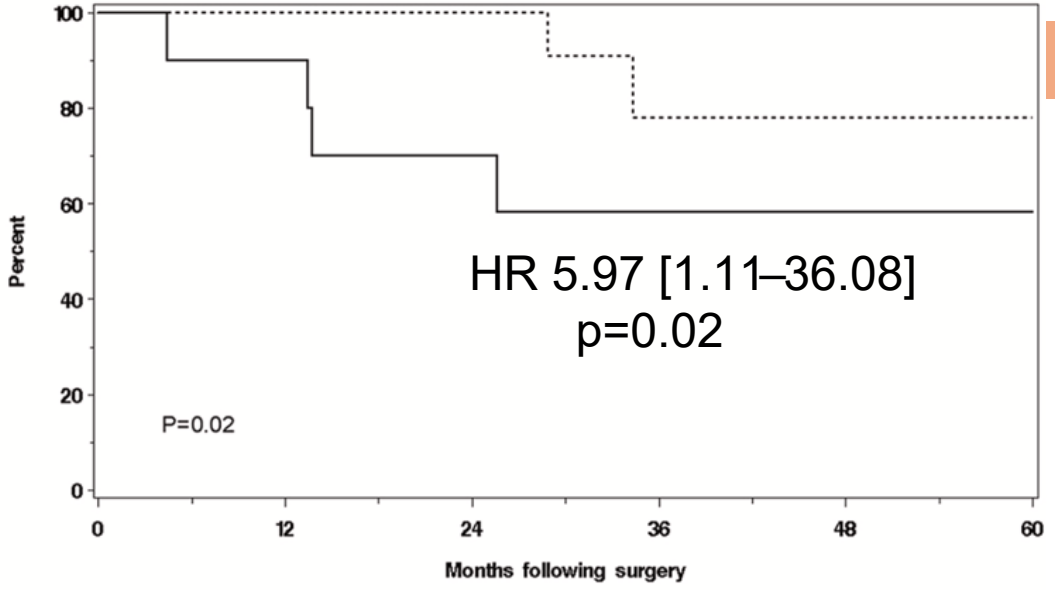
A prospective observational study

DFS



Number at risk	0 mo	12 mo	24 mo	36 mo	48 mo	60 mo
ctDNA detected	10	8	3	1	1	0
ctDNA undetected	18	18	10	4	4	2

OS

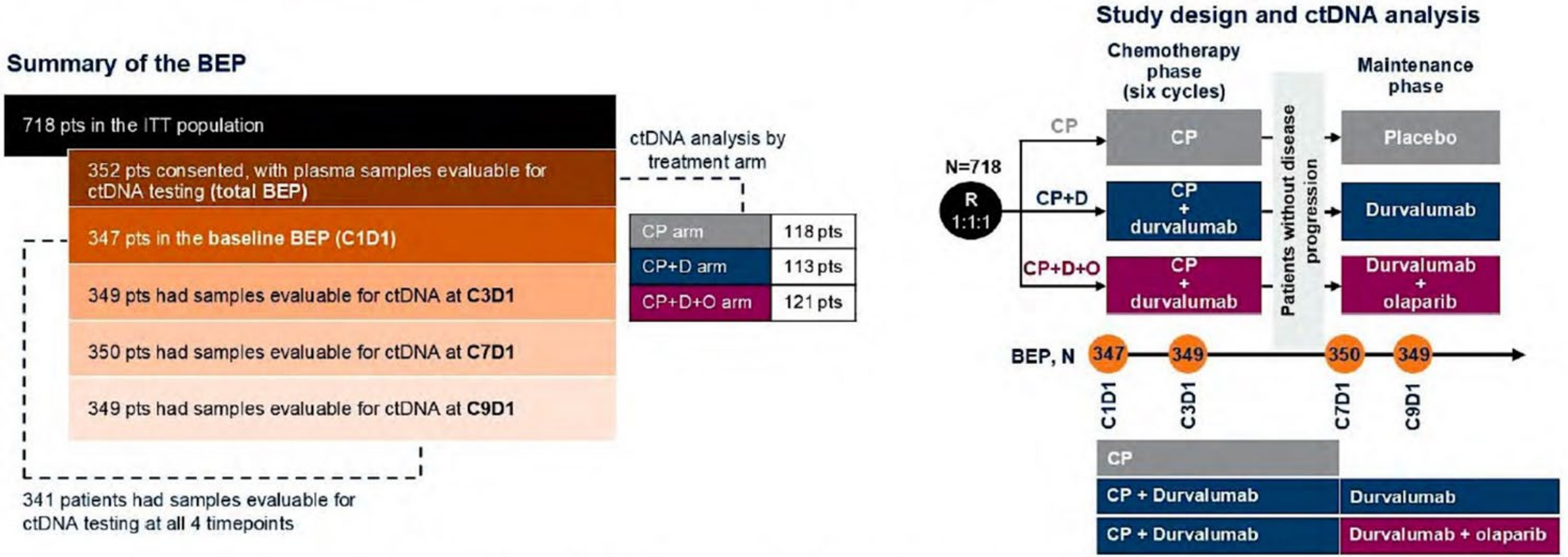


Number at risk	0 mo	12 mo	24 mo	36 mo	48 mo	60 mo
ctDNA detected	10	9	6	2	2	2
ctDNA undetected	18	18	13	6	6	4

Post-surgical ctDNA was associated with increased risk of recurrence and death

De Vitis LA et al. *Eur J Surg Oncol.* 2026;52(3):111388.

DUO-E post hoc ctDNA analysis



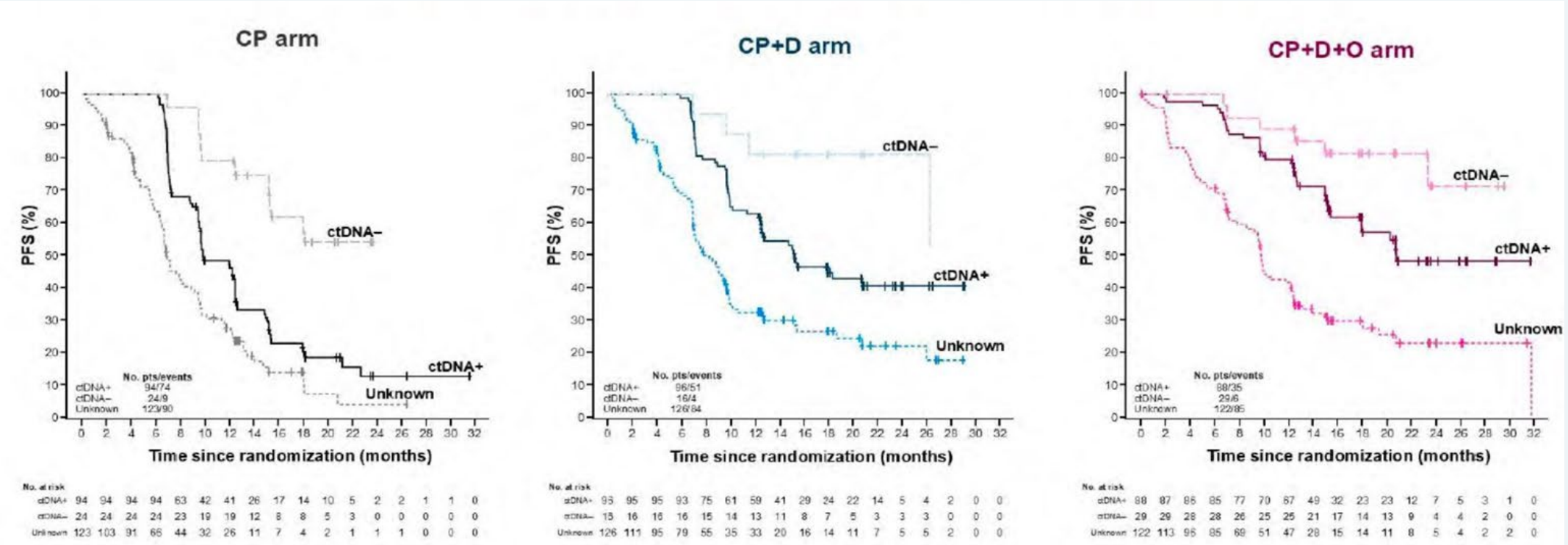
- Samples were collected at baseline (C1D1), during the chemotherapy phase (C3D1), prior to maintenance initiation (C7D1), and during the maintenance phase (C9D1)
- ctDNA was analyzed using the methylation-based Guardant Infinity™ assay (Guardant Health, Palo Alto, CA)

C, cycle; D, day; pts, patients

BEP = biomarker-evaluable population; ITT = intent-to-treat

Westin SN et al. *J Clin Oncol.* 43;16:5512. (ASCO 2025: abstract 5512).

DUO-E: PFS by ctDNA status in ITT



- ctDNA was detectable in 80% (278/347) of C1D1 samples
- Presence of BL ctDNA was associated with shorter PFS across treatment arms

Westin SN et al. *J Clin Oncol.* 43;16:5512. (ASCO 2025: abstract 5512).

Practical Uses in the Real World

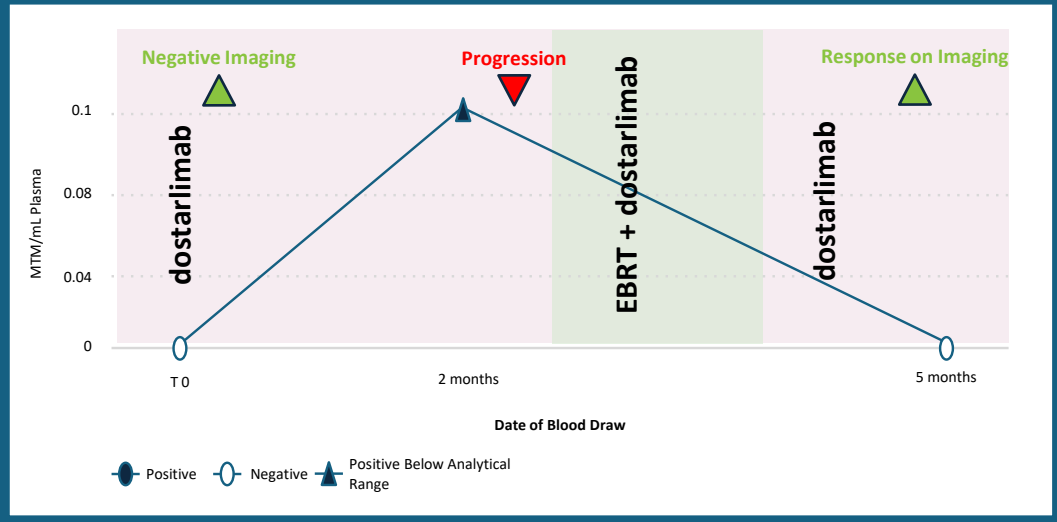
Treating an MRD-detected recurrence on ICI maintenance therapy

Case Study

64 year old with advanced FIGO grade 3 endometrioid endometrial cancer underwent debulking surgery with TAH/BSO

- Following surgery, patient completed 6 cycles of taxol/carboplatin/dostarlimab and was transitioned to dostarlimab
- CT C/A/P obtained with new pulmonary findings
  - IR biopsy suggestive of histoplasmosis
  - Follow-up imaging showed stable lung findings
- ctDNA ordered for IO maintenance monitoring
- Positive ctDNA prompted imaging that confirmed recurrence
- Recurrence successfully treated with radiation

Historical ctDNA MRD Results



Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

natera™

ctDNA/MRD in Cervical Cancer

Locally advanced CC (LACC): stages IB3–IVA, high risk for recurrence and mortality

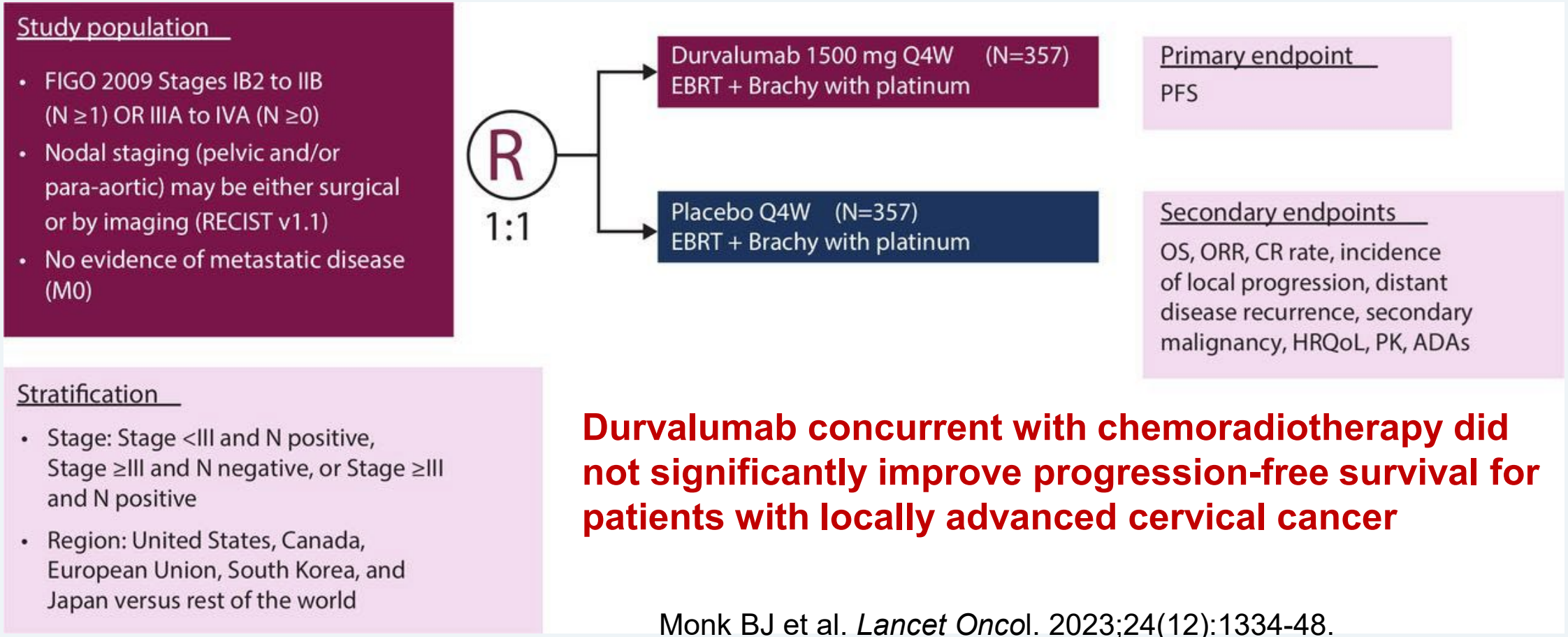
- 30–50% of cases recur within two years of definitive treatment end
- Recurrence typically identified based on symptoms and imaging
- No single biomarker has been universally established for the sensitive detection of cervical cancer recurrence

Are there sensitive biomarkers for monitoring or early detection of cervical cancer recurrence？

Monk BJ et al. *Lancet Onco*l. 2023;24(12):1334-48. (CALLA)
Takekuma M et al. *J Gynecol Oncol.* 2025;36(6):e116. (BEATcc)
Lorusso D et al. *Lancet.* 2024;403(10434):1341-50. (KN A18)

The CALLA Trial

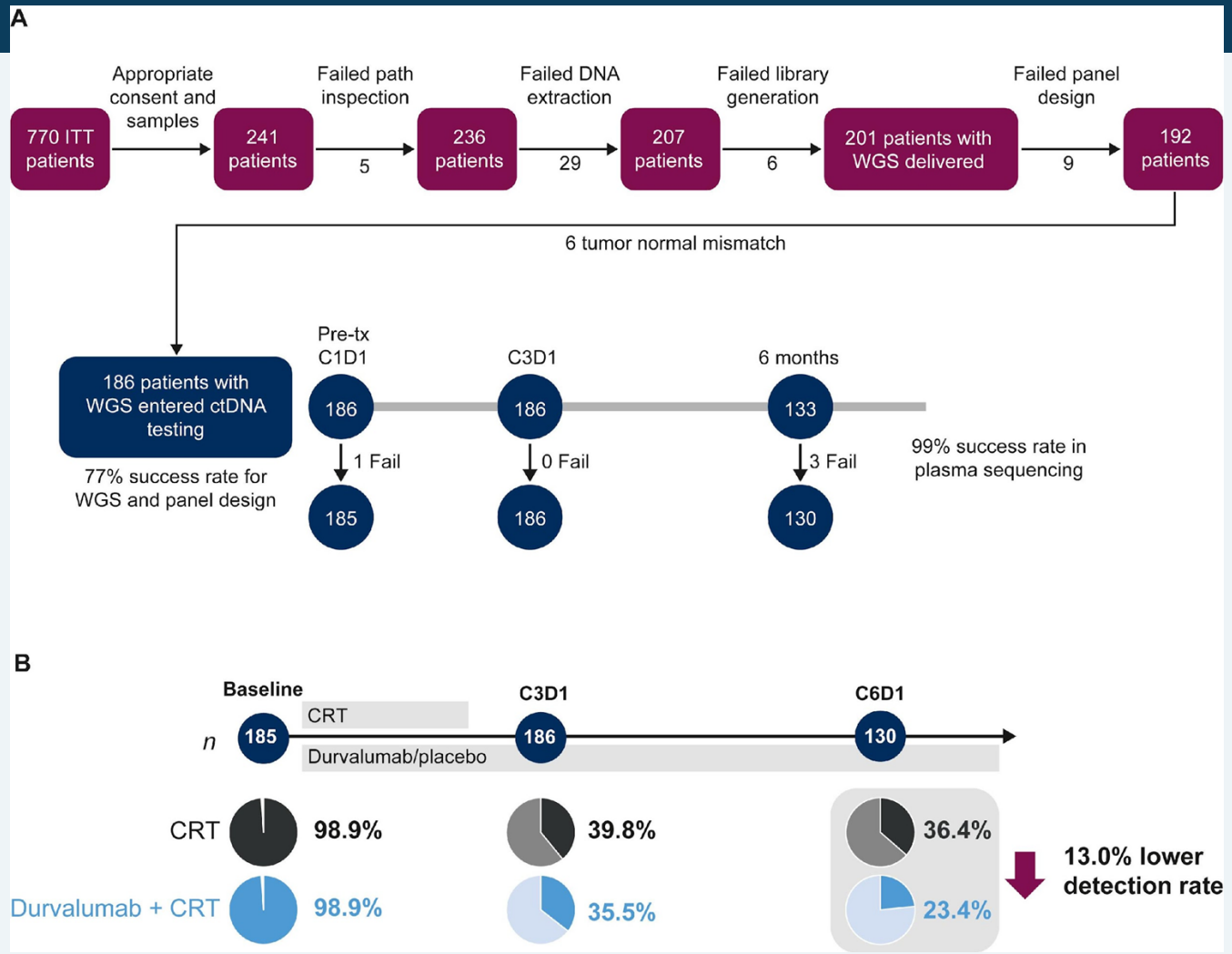
A phase III, randomized, double- blind, placebo-controlled trial, was designed to determine whether durvalumab combined with and following concurrent CRT could improve outcomes compared with CRT alone.



Durvalumab concurrent with chemoradiotherapy did not significantly improve progression-free survival for patients with locally advanced cervical cancer

Monk BJ et al. *Lancet Oncol*. 2023;24(12):1334-48.

The CALLA Trial- an exploratory analysis



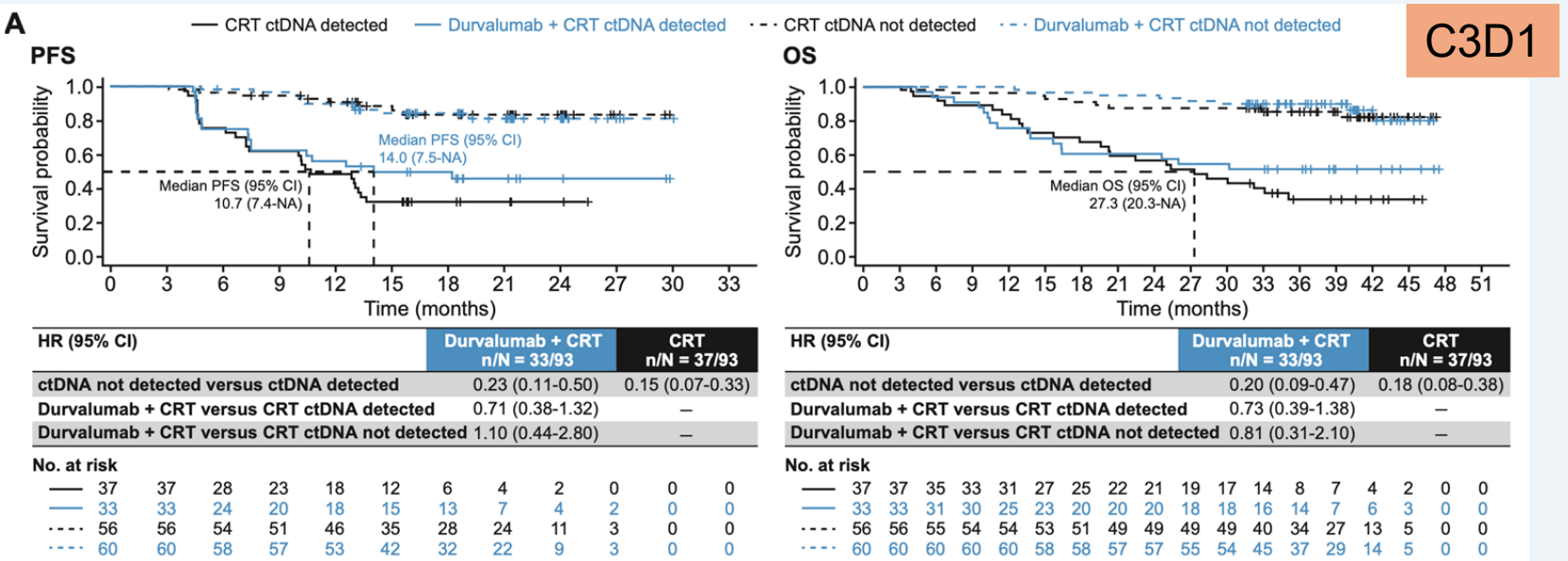
- ctDNA collected at
  C1D1 (baseline before CRT)
  C3D1 (after CRT)
  C6D1 (3 months after CRT)
- ctDNA test:personalized WGS-based tumor-informed assay
- cHPV DNA :primers specific to the 23 most common HPV variants.

- ctDNA detection rates
  at baseline was 98.9% (183/185)
  at C3D1 and C6D1, were 35.5% and 23.4% for the durvalumab＋CRT arm and 39.8% and 36.4% for the CRT arm

Mayadev J et al. *Ann Oncol*. 2025;36(9):1047-57

The CALLA Trial- an exploratory analysis

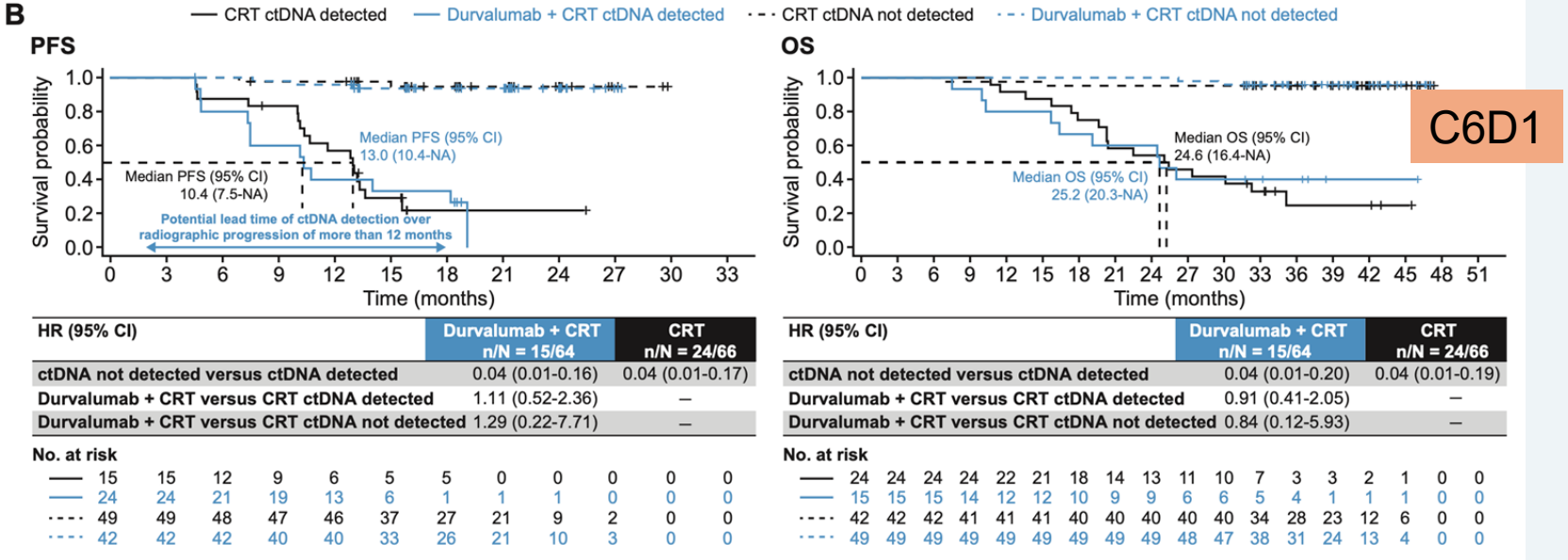
- Detection levels of ctDNA were predictive of disease progression and survival at baseline: in the ctDNA less than median versus ctDNA greater than median subgroups
  - Durvalumab+CRT: PFS 0.61 (0.28-1.35) and OS 0.55 (0.23-1.35)
  - CRT: PFS 0.49 (0.26-0.95) and OS 0.65 (0.33-1.28)
- ctDNA detection at C3D1 occurred a median of 164 days before clinical progression.
- The PPV and NPV of C3D1 ctDNA detection were 61% and 82% with respect to subsequent progression.
- The PPV and NPV for C6D1 ctDNA detection with respect to subsequent progression were 74% and 94%.



C3D1

HR (95% CI)	Durvalumab + CRT n/N = 33/93	CRT n/N = 37/93
ctDNA not detected versus ctDNA detected	0.23 (0.11-0.50)	0.15 (0.07-0.33)
Durvalumab + CRT versus CRT ctDNA detected	0.71 (0.38-1.32)	–
Durvalumab + CRT versus CRT ctDNA not detected	1.10 (0.44-2.80)	–

HR (95% CI)	Durvalumab + CRT n/N = 33/93	CRT n/N = 37/93
ctDNA not detected versus ctDNA detected	0.20 (0.09-0.47)	0.18 (0.08-0.38)
Durvalumab + CRT versus CRT ctDNA detected	0.73 (0.39-1.38)	–
Durvalumab + CRT versus CRT ctDNA not detected	0.81 (0.31-2.10)	–



C6D1

HR (95% CI)	Durvalumab + CRT n/N = 15/64	CRT n/N = 24/66
ctDNA not detected versus ctDNA detected	0.04 (0.01-0.16)	0.04 (0.01-0.17)
Durvalumab + CRT versus CRT ctDNA detected	1.11 (0.52-2.36)	–
Durvalumab + CRT versus CRT ctDNA not detected	1.29 (0.22-7.71)	–

HR (95% CI)	Durvalumab + CRT n/N = 15/64	CRT n/N = 24/66
ctDNA not detected versus ctDNA detected	0.04 (0.01-0.20)	0.04 (0.01-0.19)
Durvalumab + CRT versus CRT ctDNA detected	0.91 (0.41-2.05)	–
Durvalumab + CRT versus CRT ctDNA not detected	0.84 (0.12-5.93)	–

The HRs comparing PFS and OS, in the ctDNA negative versus positive subgroups

- Durvalumab+CRT arm:
  0.23 (0.11-0.50) and 0.20 (0.09-0.47)
- CRT arm:
  0.15 (0.07-0.33) and 0.18 (0.08- 0.38)

The HRs comparing PFS and OS, in the ctDNA negative versus positive subgroups

- Durvalumab+CRT arm:
  0.04 (0.01-0.16) and 0.04 (0.01-0.20)
- CRT arm:
  0.04 (0.01-0.17) and 0.04 (0.01-0.19)

Association of PFS and OS with on-treatment ctDNA and cHPV DNA

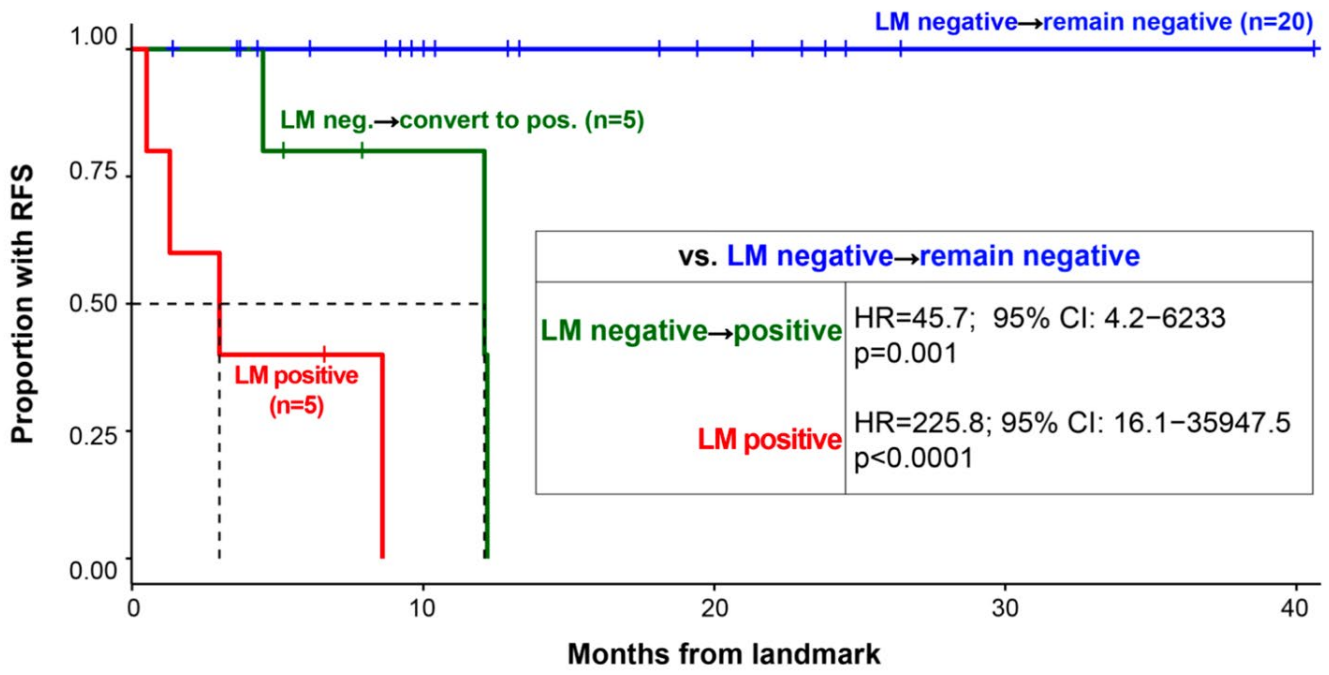
Circulating tumor DNA in locally advanced cervical cancer: A real-world cohort study

- A retrospective real-word cohort study included 58 locally advanced cervical cancer patients
  - 78% [45/58] had squamous cell carcinoma
  - 72% [42/58] LACC (IB3-IVA)
- ctDNA testing on plasma samples using personalized, tumor-informed exome-based assay
- Objective: Explore longitudinal circulating tumor DNA (ctDNA) testing as a potential biomarker to predict cancer recurrence in LACC patients following definitive treatment

Kuhn T et al. *Gynecol Oncol.* 2026;208:S50-S51 (SGO 2026)

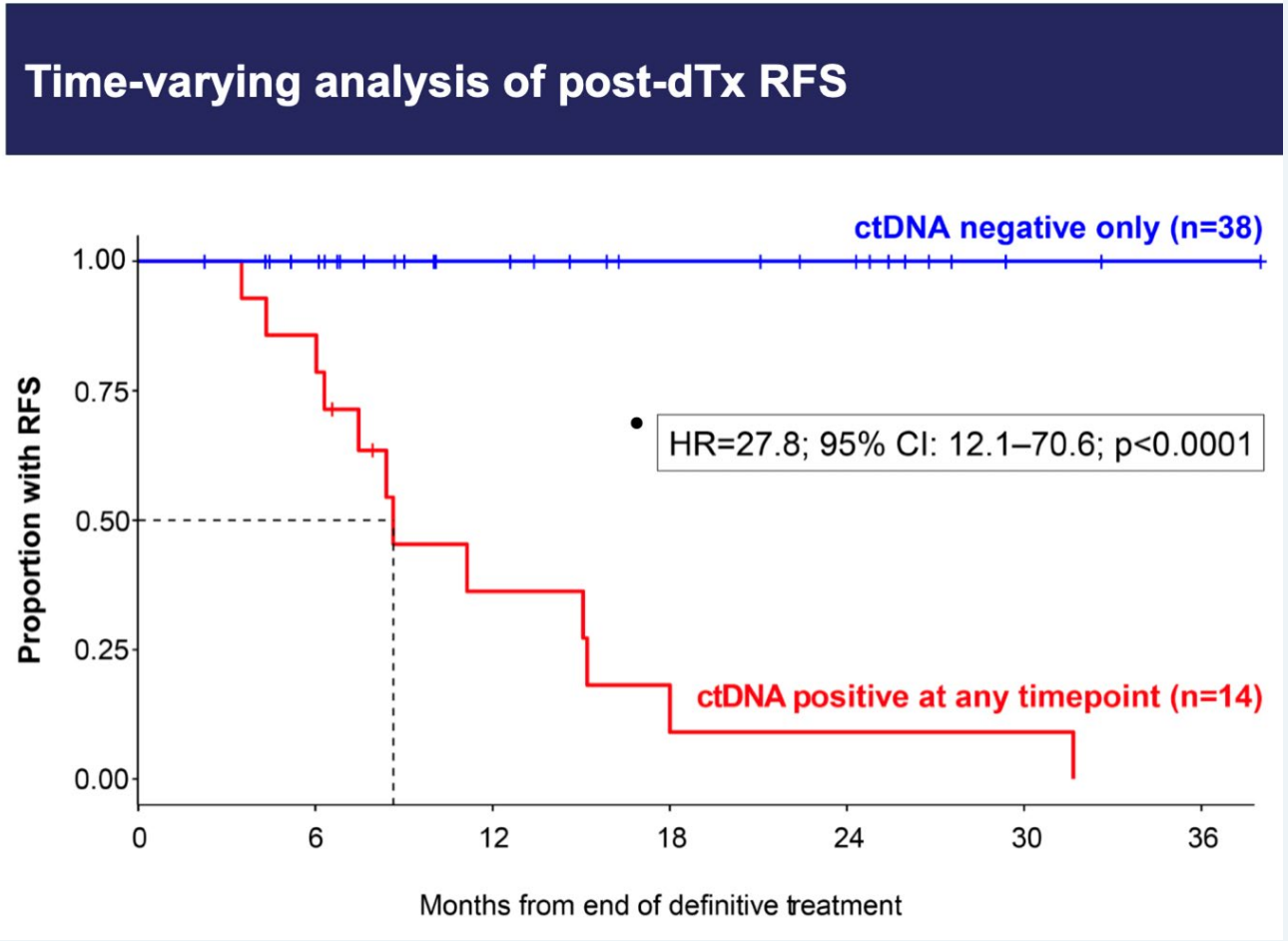
ctDNA status after definitive treatment predicts relapse

Landmark (LM) analysis of relapse-free survival (RFS)
LM = 0–3 months after dTx



- ctDNA-positivity at LM or conversion from LM negative to positive had worse RFS compared patients who were LM negative and remained negative
- Median RFS was 3.0 months for LM positive patients
- Median (range) follow-up of 9.4 (0.5–40.6) months

Longitudinal ctDNA testing predicts relapse



- No recurrences were seen among ctDNA negative-only patients

- 14 of 16 ctDNA-positive patients recurred;
  - 2 patients without recurrence had a median f/u of 7.3 mo. post-dTx)

- All 14 recurrences were ctDNA-positive prior to or at the time of recurrence
  - 9/14 were positive at first ctDNA timept
  - 5/14 converted from ctDNA neg. to positive at a median of 6.5 (5–12) months from initial ctDNA-negative test

Summary

- ctDNA-based MRD testing is a rapidly emerging prognostic and predictive tool across gynecologic cancers.
- Accumulating prospective and retrospective evidence consistently demonstrates that post-treatment ctDNA positivity is a powerful predictor of recurrence and inferior survival, often outperforming conventional biomarkers such as CA-125 and imaging.
- ctDNA-based MRD testing in gynecologic cancers remains investigational and is not yet incorporated into standard-of-care guidelines.
- Interventional clinical studies are required to investigate the ability of longitudinal ctDNA to modify treatment and improve survival in specific disease settings. This includes maintenance escalation or de-escalation.

“There is no absolute knowledge. And those who claim it, whether they are scientists or dogmatists, open the door to tragedy. All information is imperfect. We have to treat it with humility.”

Jacob Bronowski, scientist, broadcaster, The Ascent of Man, 1973