An Industry Supported Symposium at the IGCS 2025 Annual Global Meeting

Beyond the image: New opportunities in gynecologic cancer testing

This session is not included in the main event CME/CPD credit.

Cape Town, South Africa

Friday, November 7, 2025

07:45 - 08:45 GMT+2

This educational activity is supported by an independent medical education grant from Natera

Welcome, introductions and review of learning objectives

Thomas J. Herzog, MD

University of Cincinnati

Cincinnati, Ohio, USA

Learning Objectives

1. Describe the principles of MRD testing using circulating tumor DNA (ctDNA) and its application in gynecologic cancers.
2. Compare ctDNA-based MRD monitoring with traditional methods such as imaging and serum biomarkers.
3. Evaluate the evidence for ctDNA use in detecting recurrence and guiding treatment decisions in endometrial, ovarian, and cervical cancers.
4. Discuss limitations, alternative approaches, and considerations for integrating MRD testing into clinical practice.

Agenda

07:45 – 07:50: **Welcome, introductions and review of learning objectives**
Thomas J. Herzog, MD, University of Cincinnati, Cincinnati, Ohio, USA

07:50 – 08:05: **Understanding the Science and Technology of ctDNA Testing**
Stephanie Lheureux, MD, Princess Margaret Cancer Centre, Toronto, Ontario, Canada

08:05 – 08:20: **Clinical Applications in Gynecologic Cancers**
Kathleen Moore, MD, University of Oklahoma, Health Sciences Center, Stephenson Cancer Center, Oklahoma City, Oklahoma, USA

08:20 – 08:40: **Interactive Cases and Understanding Challenges and Considerations**
All Faculty

08:40 – 08:45: **Key takeaways and Q&A**
Thomas J. Herzog, MD, University of Cincinnati, Cincinnati, Ohio, USA

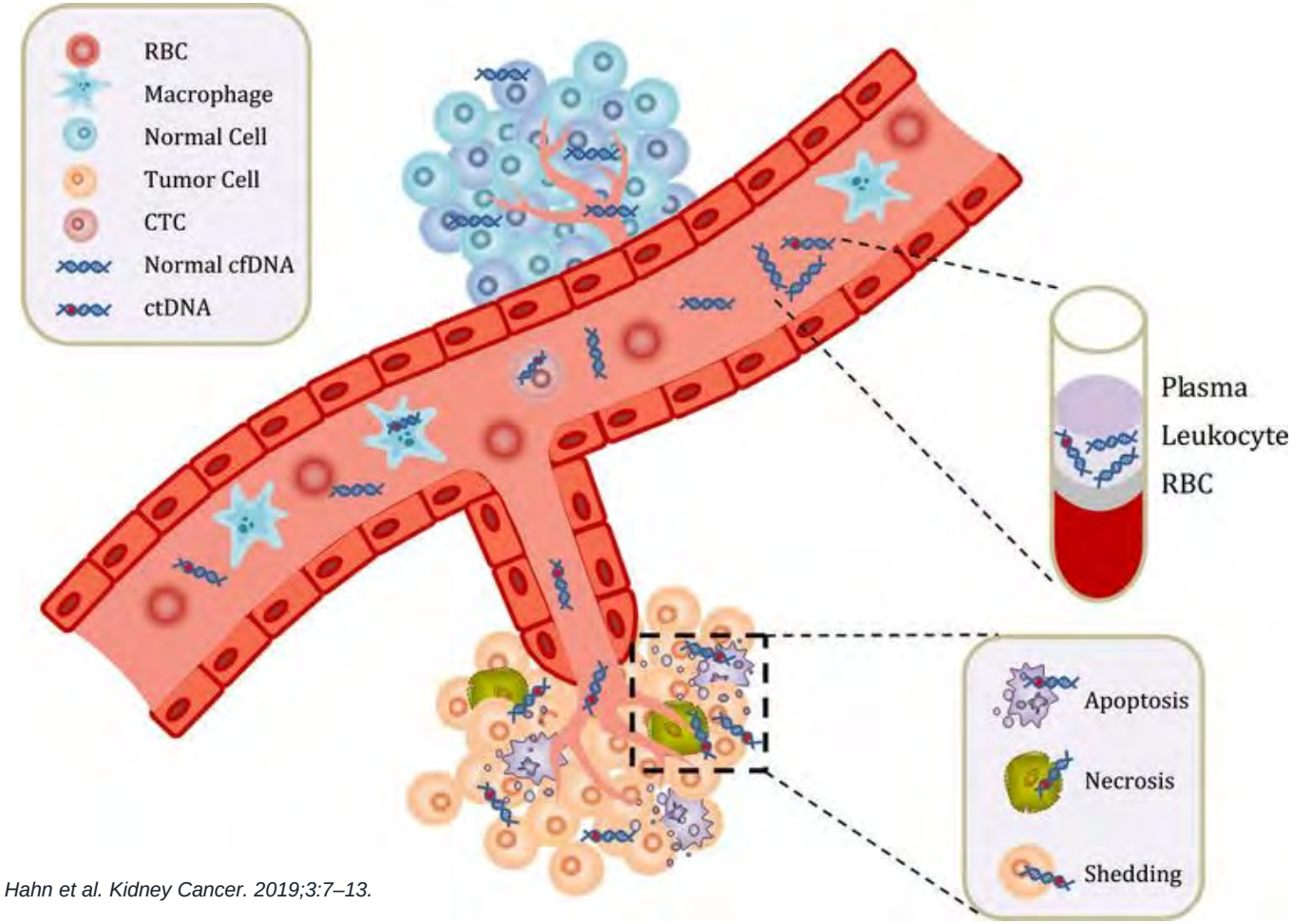
Understanding the science and technology of ctDNA testing

Stephanie Lheureux, MD, PhD

Princess Margaret Cancer Centre

Toronto, Ontario, Canada

ctDNA as a new tool



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

- 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)

✓ **ctDNA for Molecular Residual Disease:** May detect cancer recurrence and monitor treatment response, across multiple cancers and treatment modalities

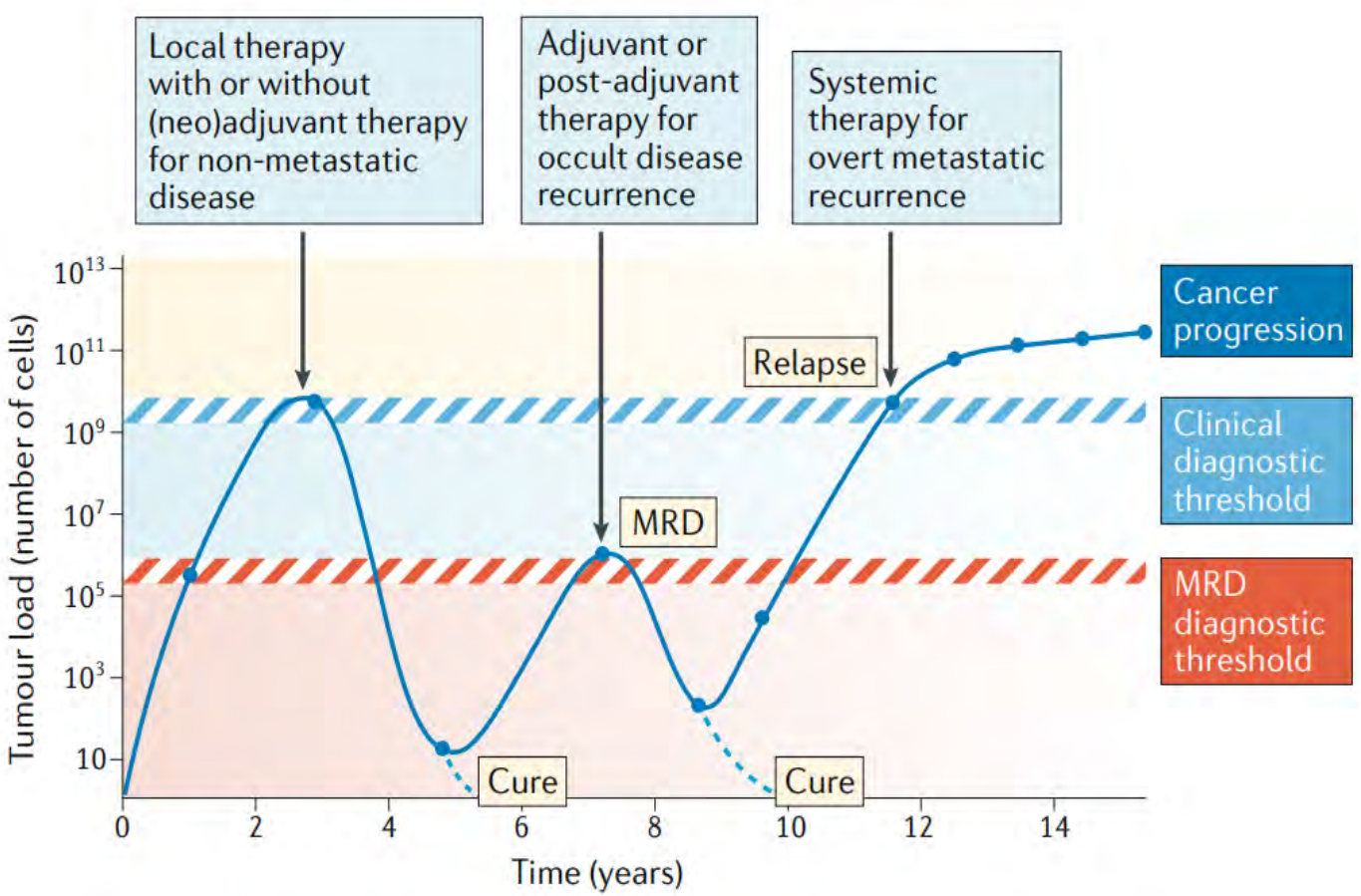
✓ **ctDNA for Liquid Biopsy:** Reflects tumor heterogeneity and may allow for real-time tracking of tumor evolution and resistance

ctDNA Applications

	Unknown if cancer is present	Known cancer present
Definition	**Molecular Residual Disease Testing**	**Liquid Biopsy**
Purpose	**Detection of persistent or recurrent disease following therapy or on surveillance.** • Risk stratification following conclusion of therapy or after surgery • Track treatment response • Identify emergence or persistence of disease	**Identify genetic alterations in patients known to have disease present** • Inform biomarker-based therapeutic decisions • Better understand cancer evolution • Identify drivers of drug resistance

1. *Ketch, et al. Utilizing Circulating Tumor DNA (ctDNA)-based Molecular Residual Disease Detection for Postoperative Monitoring in Early-Stage Uterine Cancer. JCO Precis Oncol 9, e2500286(2025*
2. *Han, K., Zou, J., Zhao, Z., Baskurt, Z., Zheng, Y., Barnes, E., ... & Leung, E. (2024). Clinical validation of human papilloma virus circulating tumor DNA for early detection of residual disease after chemoradiation in cervical cancer. Journal of Clinical Oncology, 42(4), 431-440.*
3. *Williams, M. J., Vázquez-García, I., Tam, G., Wu, M., Varice, N., Havasov, E., ... & Shah, S. P. (2024). Tracking clonal evolution of drug resistance in ovarian cancer patients by exploiting structural variants in cfDNA. Biorxiv.*
4. *Soberanis Pina, P., Clemens, K., Bubie, A., Grant, B., Haynes, G., Zhang, N., ... & Lheureux, S. (2024). Genomic landscape of ctDNA and real-world outcomes in advanced endometrial cancer. Clinical Cancer Research, 30(24), 5657-5665.*

What is molecular residual disease (MRD)?



- Minimal/molecular residual disease (MRD) detection is widely used in hematologic malignancies
- MRD is cancer persisting after treatment that cannot be detected with current imaging tools (occult metastatic disease)
- MRD may be used in solid malignancies to more accurately monitor tumor burden levels

1. Pantel K, Alix-Panabieres C. Liquid biopsy and minimal residual disease - latest advances and implications for cure. Nat Rev Clin Oncol. 2019;16(7):409-424.

MRD assays can be tumor informed or tumor naive

Non-personalized, plasma only assay

Detect predetermined static panel of hotspot mutations

+

Epigenomic Signatures (methylation)

ctDNA detected

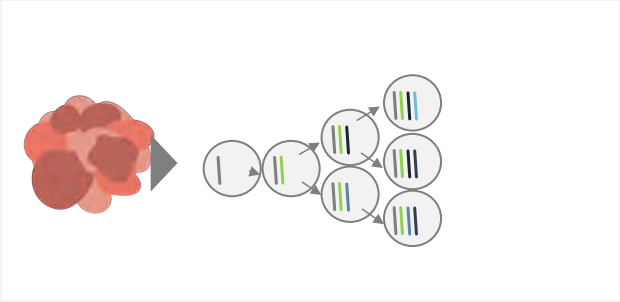
OR

ctDNA not detected

Turnaround time: ~7-10 days/test

Personalized, tumor informed assay

Blood draw



Test design: 3-4 weeks

Serial blood tests to detect ctDNA

Turnaround time: ~7-10 days/test

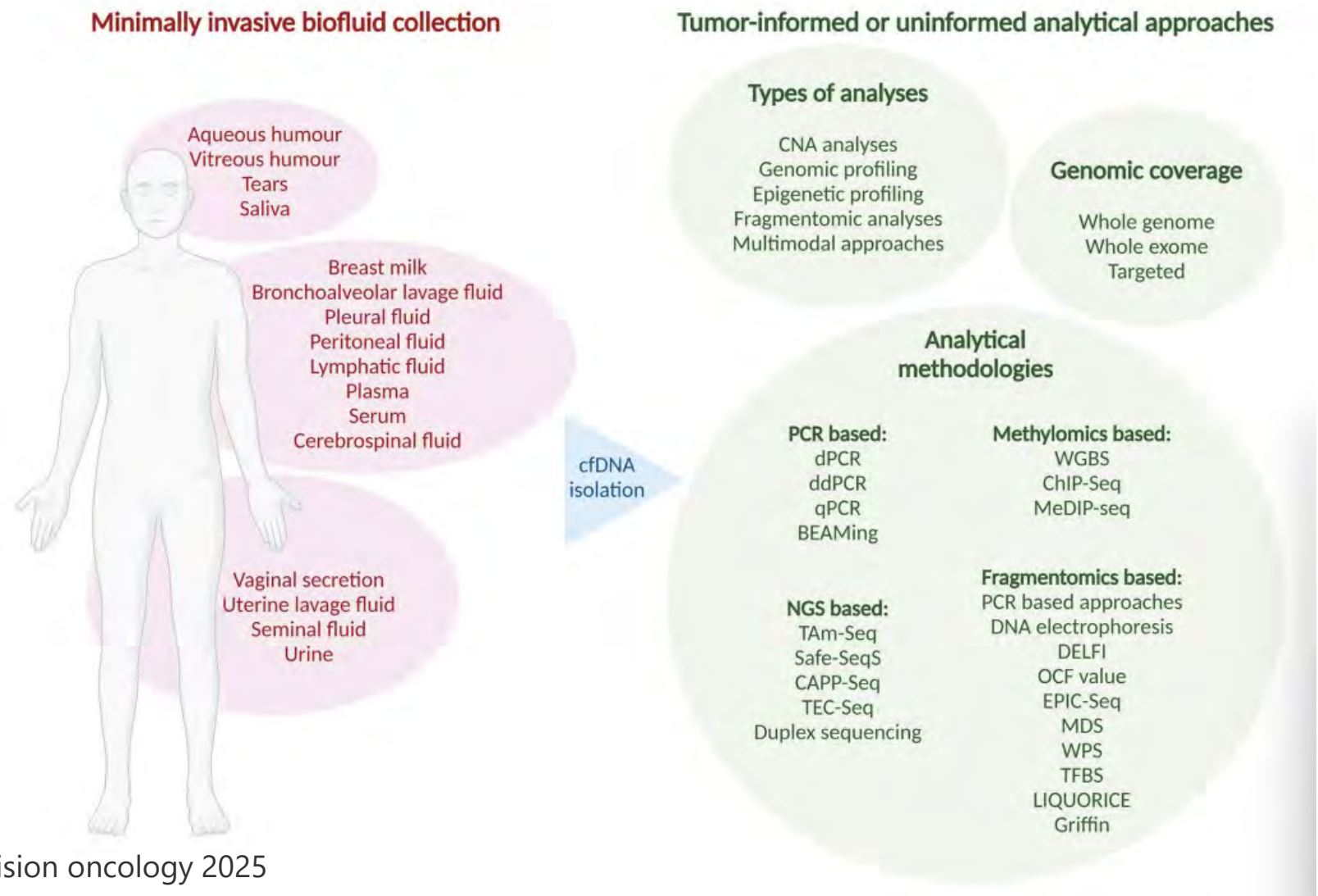
MRD Modalities

	Tumor-Informed Assay	Tumor-Agnostic Assay
Exome Sequencing Analysis of coding region of DNA	2025 Data in GYN Serial, personalized MRD testing identified 100% of uterine cancer recurrences ahead of conventional methods[1]	
Genome Sequencing Analysis of coding and non-coding regions	2025 Data in GYN As part of the CALLA trial, MRD positivity Post-CRT/C3D1 was prognostic for PFS and OS with a PPV of 61%[2]	
Epigenetic Analysis Epigenetic features, such as methylation patterns, are analyzed		2025 Data in GYN As part of the DUO-E study, MRD positivity at baseline correlated with lower PFS in all treatment arms compared to MRD negativity at baseline[3]

Note: There have been no head-to-head comparisons of MRD modalities.

1. Ketch, et al. Utilizing Circulating Tumor DNA (ctDNA)-based Molecular Residual Disease Detection for Postoperative Monitoring in Early-Stage Uterine Cancer. JCO Precis Oncol 9, e2500286(2025
2. Mayadev, J., Vázquez Limón, J. C., Ramírez Godinez, F. J., Leiva, M., Cetina-Pérez, L. D. C., Varga, S., ... & Monk, B. J. (2025). Ultrasensitive detection and tracking of circulating tumor DNA (ctDNA) and association with relapse and survival in locally advanced cervical cancer (LACC): Phase 3 CALLA trial analyses.
3. Westin, S. N., Moore, K. N., Guy, M., Jordan, S., McHale, M., Miller, E., ... & Van Nieuwenhuysen, E. (2025). Durvalumab plus carboplatin/paclitaxel followed by durvalumab with or without olaparib as first-line treatment for endometrial cancer: Longitudinal changes in circulating tumor DNA.

Types of biofluids, analyses, genomic regions, and methodologies that can be used for ctDNA monitoring in cancer patients.



Bartolomucci et al - npj | precision oncology 2025

Considerations when assessing MRD testing performance

- **ctDNA shed** – Some tumor types shed less ctDNA than others (GYN cancers tend to shed ctDNA well)
- **Tissue availability** - For some tumor types (i.e. lung cancers) tissue availability can be a challenge (in GYN, this does not tend to be the case as often)
- **Accounting for clonal hematopoiesis/CHIP mutations –** Selected assay should have a method to account for CHIP mutations via filtering and/or germline analysis
- **Balancing sensitivity and specificity** - Assays need to maximize sensitivity but not at the expense of specificity/PPV. Clinical outcomes is the ultimate metric of sens/spec.
- **Performance in clinical cohorts** - Selected assay should have performance validated in clinical cohorts. Analytical datasets from cell lines may not correlate to clinical performance.
- **Therapy-induced ctDNA suppression** - Therapies may induce suppression or even clearances of ctDNA – Serial MRD testing throughout the treatment journey is needed
- **Best practices/standardization** - MRD assay reporting is evolving (e.g. MTM, ppm, tumor fraction, VAF)

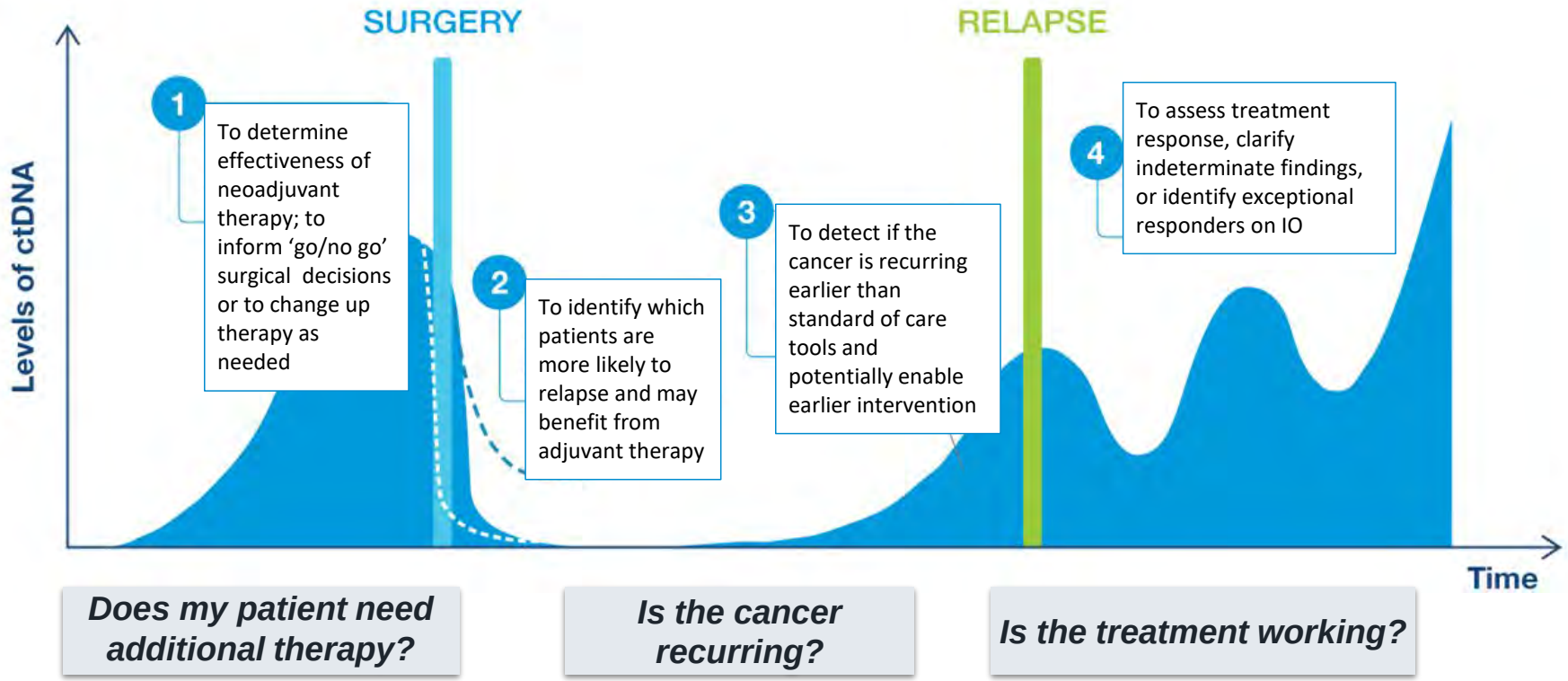
Clinical applications in gynecologic cancers

Kathleen Moore, MD

University of Oklahoma, Health Sciences Center
Stephenson Cancer Center

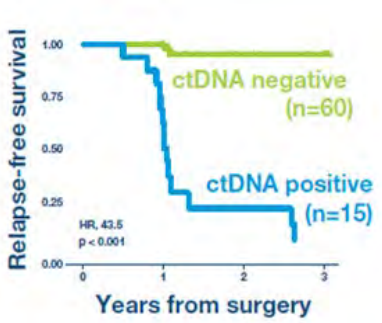
Oklahoma City, Oklahoma, USA

Clinical Applications for MRD along the continuum of care



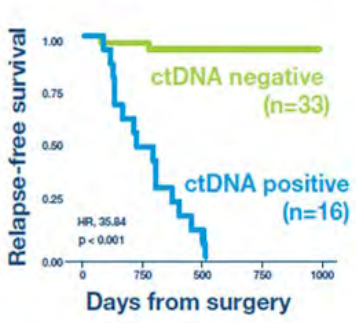
Prognostic of disease recurrence across multiple tumor types*

Colon[1,7]
JAMA Oncology



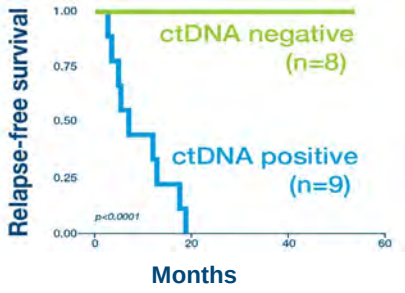
88%-93% sensitivity
98% specificity

Breast[2, 3]
Clinical Cancer Research



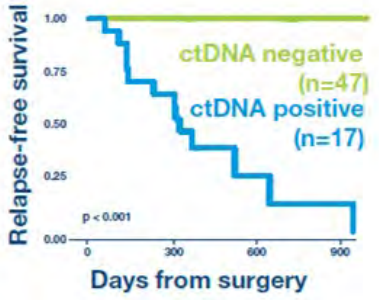
88-89% sensitivity
95-99% specificity

Lung[4,5]
Frontiers in Oncology



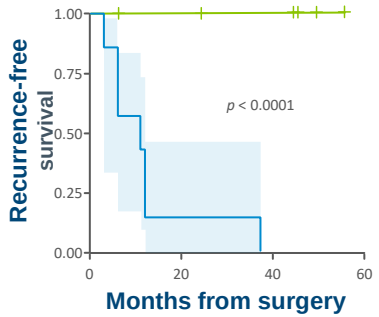
80%-99% sensitivity
96%-99% specificity

Bladder[6]
Jrnl of Clin Oncology



99% sensitivity
98% specificity

Ovarian[8]
Gynecologic Oncology



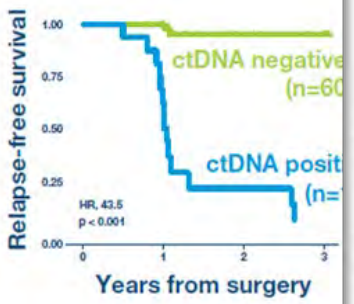
99% sensitivity
99% specificity

*1. Reinert T, Henriksen TV, Christensen E, et al. Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer. JAMA Oncol. 2019. 2. Coombes RC, Page K, Salari R, et al. Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence. Clin Cancer Res. 2019;25(14):4255-4263 3. Shaw et al., Serial Postoperative Circulating Tumor DNA Assessment Has Strong Prognostic Value During Long-Term Follow-Up in Patients With Breast Cancer. JCO Precis Oncol 8, e2300456(2024). DOI:10.1200/PO.23.00456. 4. Lebow, E. et al. ctDNA-based detection of molecular residual disease in stage I-III non-small cell lung cancer patients treated with definitive radiotherapy. Front. Oncol. 2023,13:1253629. 5 Martin T, Dinerman A, Sudhaman S, et al. Early real-world experience monitoring circulating tumor DNA in resected early-stage non-small cell lung cancer. J Thorac Cardiovasc Surg. 2024. 6. Christensen E, Birkenkamp-Demtroder K, Sethi H, et al. Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma. J Clin Oncol. 2019;37(18):1547-1557. 7 Kotani D. et al., Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer, Nature Medicine v29 Issue 1 Jan 2023. 8. . Hou JY, et al. Gynecologic Oncology. 2022; 167: 334-341. *Longitudinal sensitivity and specificity*

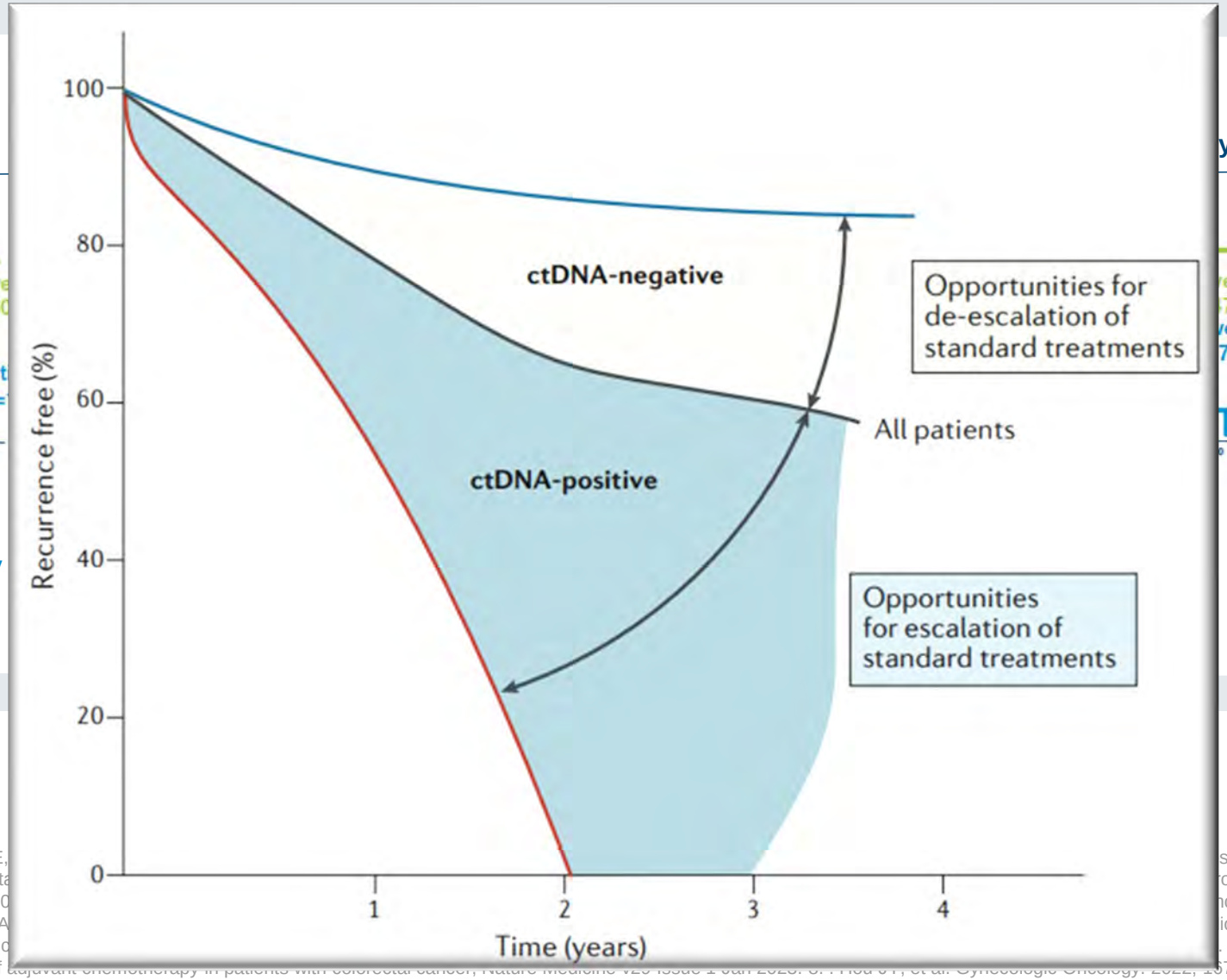
MRD allows for tailoring therapy to each patient

Colon[1,7]

JAMA Oncology

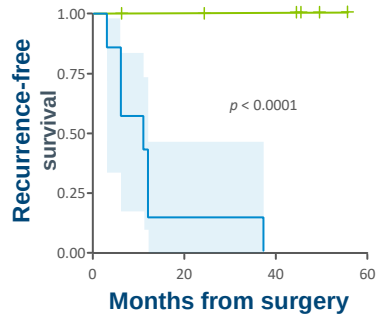


88%-93% sensitivity
98% specificity



Ovarian[8]

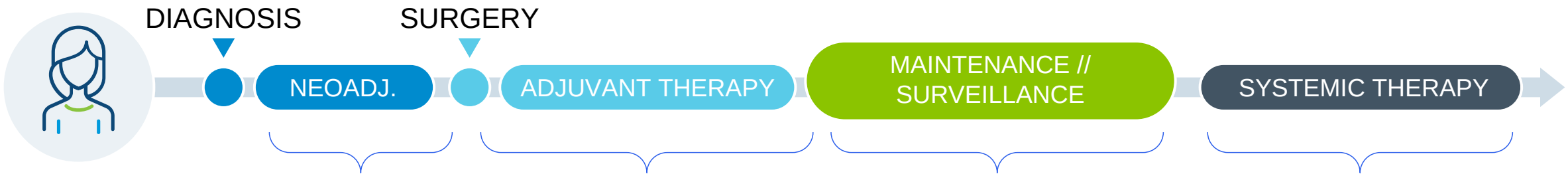
Gynecologic Oncology



99% sensitivity
99% specificity

1. Reinert T, Henriksen TV, Christensen E, ... s RC, Page K, Salari R, et al. Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Meta... ognostic Value During Long-Term Follow-Up in Patients With Breast Cancer. JCO Precis Oncol 8, e2300... ncer patients treated with definitive radiotherapy. Front. Oncol. 2023,13:1253629. 5 Martin T, Dinerman A... iovasc Surg. 2024. 6. Christensen E, Birkenkamp-Demtroder K, Sethi H, et al. Early Detection of Metastatic... J Clin Oncol. 2019;37(18):1547-1557. 7 Kotani D. et al., Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer, Nature Medicine v29 Issue 1 Jan 2023. 8. Hou Y, et al. Gynecologic Oncology. 2022, 167: 334-341. *Longitudinal sensitivity and specificity

Opportunities to improve care with ctDNA MRD



Assess early response

Optimize adjuvant treatment

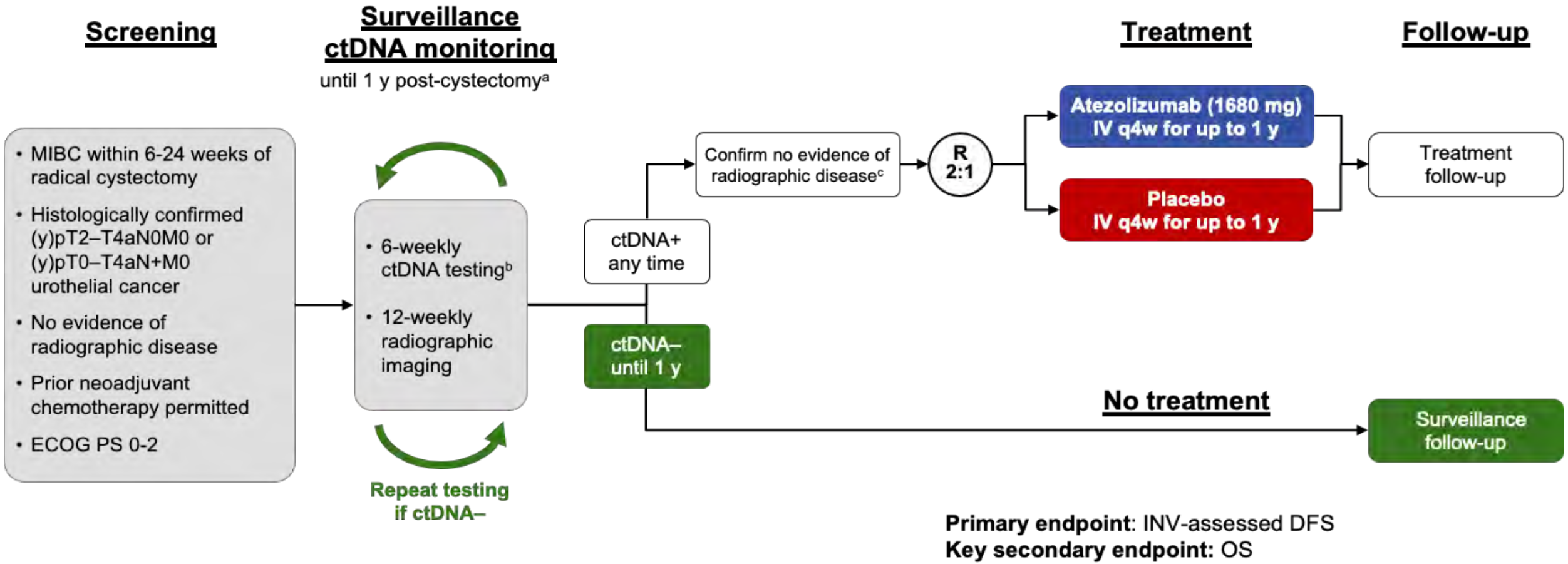
Detect molecular relapse to guide surveillance and maintenance

Monitor treatment response to optimize clinical management

MRD to Direct Adjuvant Therapy

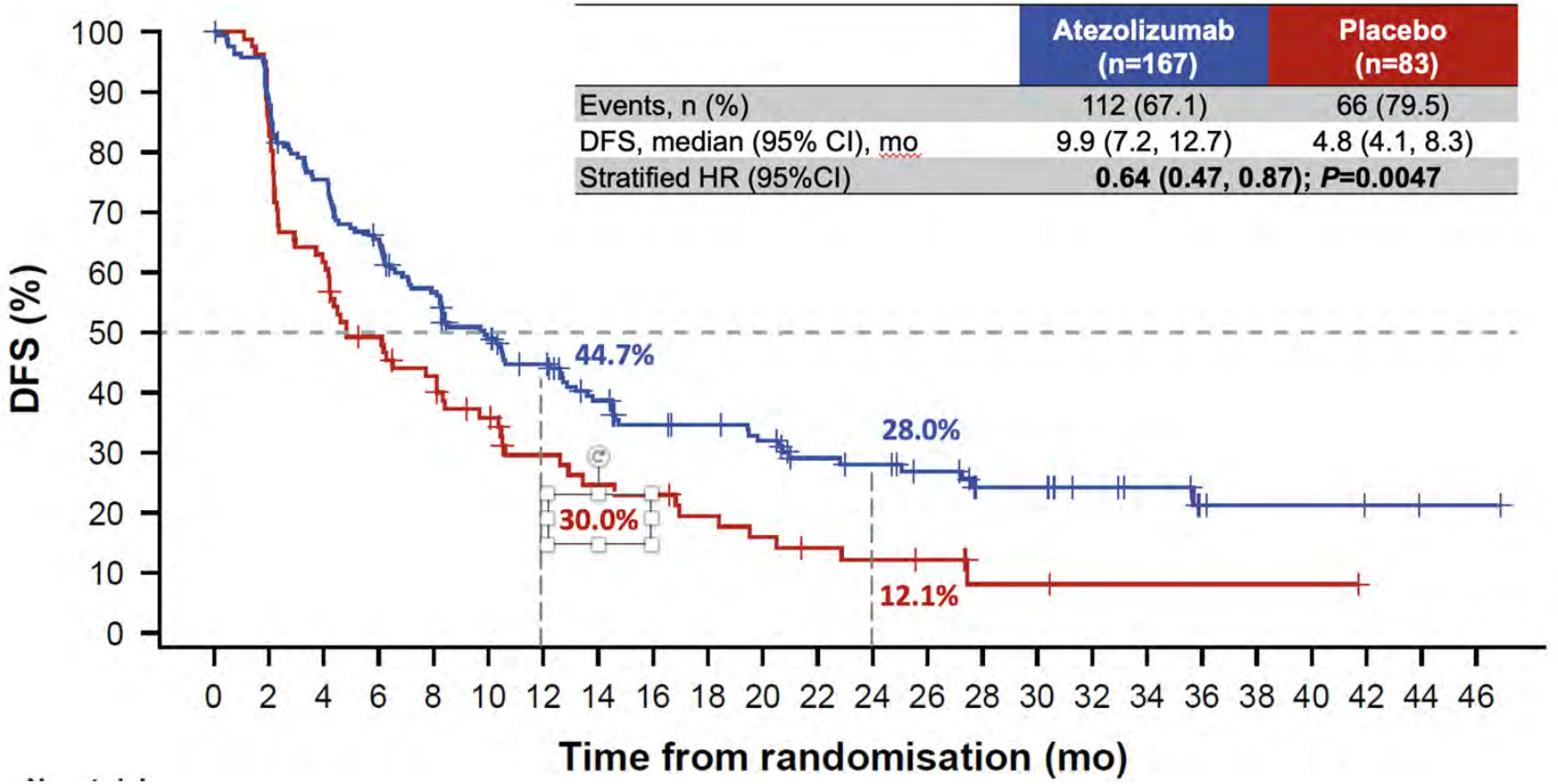
Recent Data from ESMO (IMvigor011 & Checkmate 274)

IMvigor011: First personalized, tumor-informed MRD guided treatment phase III trial based on molecular status in MIBC patients after radical cystectomy



Powles, ESMO 2025 (Pending final reference). Adapted from Powles T, et al. ESMO 2021 (Abstract 3716) with permission.

MRD guided treatment improves DFS for high-risk patients



	Atezolizumab (n=167)	Placebo (n=83)
Events, n (%)	112 (67.1)	66 (79.5)
DFS, median (95% CI), mo	9.9 (7.2, 12.7)	4.8 (4.1, 8.3)
Stratified HR (95%CI)	**0.64 (0.47, 0.87); *P*=0.0047**	

Consistent benefit in relevant secondary endpoints:

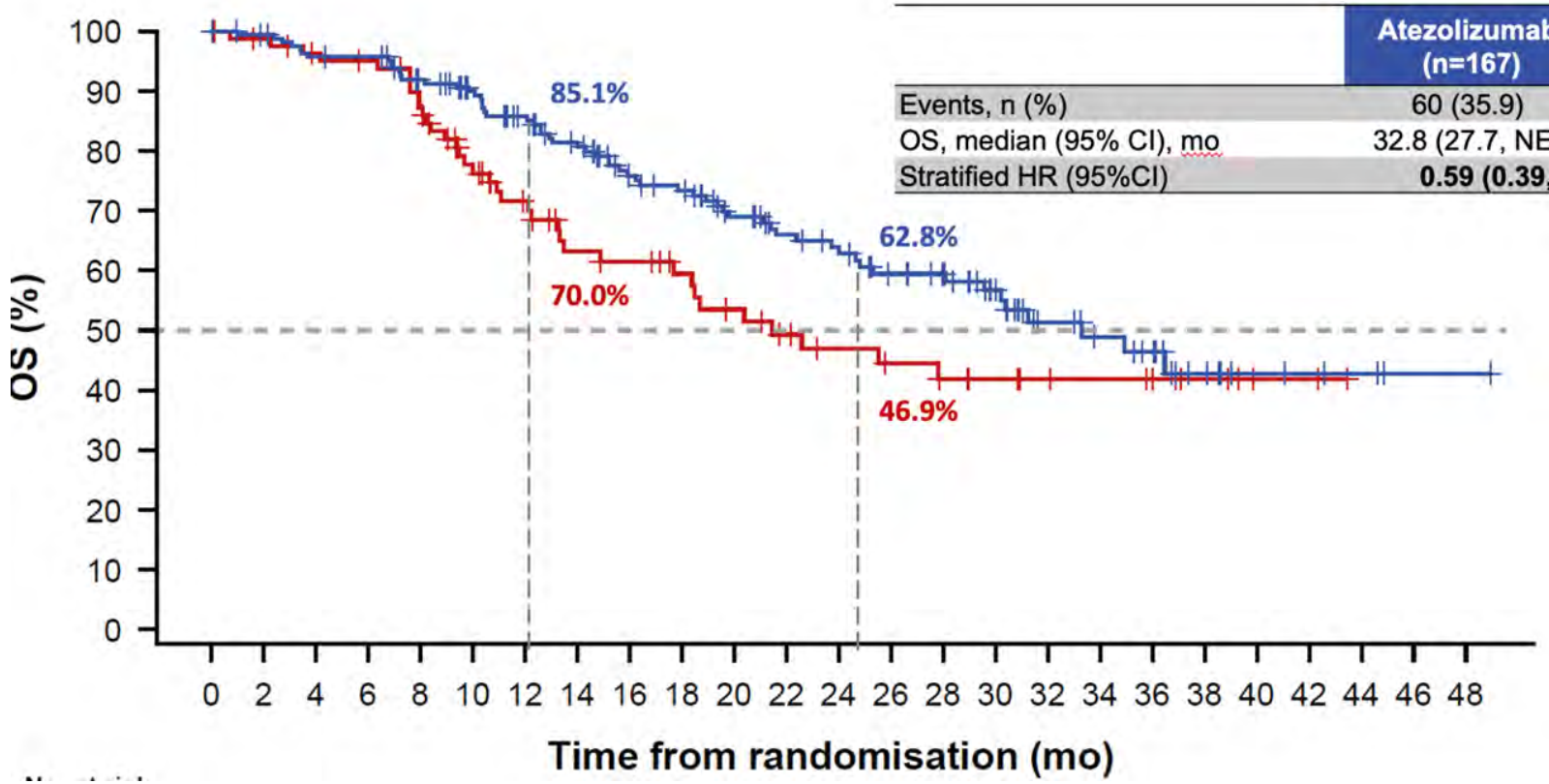
DFS per IRF:
HR, 0.66 (95% CI: 0.48, 0.91)

Distant-metastasis free survival:
HR, 0.66 (95% CI: 0.47, 0.94)

MRD-guided treatment more than doubled DFS in ctDNA-positive patients

Powles, ESMO 2025 (Pending final reference)

MRD guided treatment improves OS for high-risk patients



	Atezolizumab (n=167)	Placebo (n=83)
Events, n (%)	60 (35.9)	36 (43.4)
OS, median (95% CI), mo	32.8 (27.7, NE)	21.1 (14.7,NE)
Stratified HR (95%CI)	**0.59 (0.39, 0.90); *P*=0.0131**	

Consistent benefit in relevant secondary endpoints:

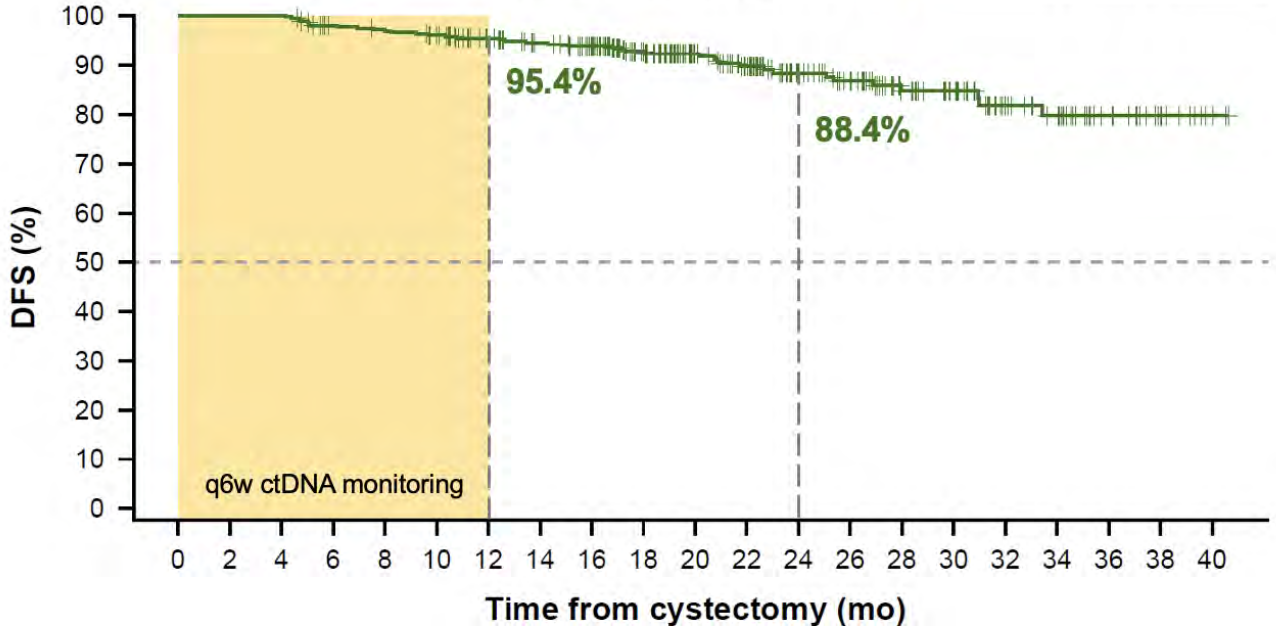
Disease-specific survival:
HR, 0.64 (95% CI: 0.40, 1.05)

MRD-guided treatment decreased the risk of death by 41% in ctDNA-positive patients.

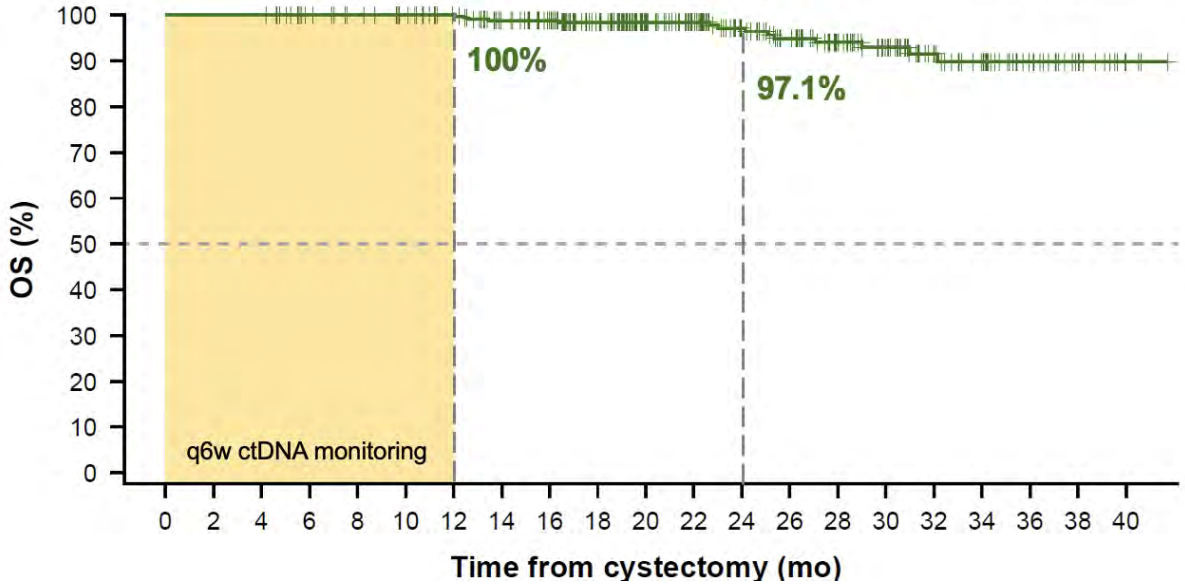
Powles, ESMO 2025 (Pending final reference)

Persistently ctDNA-negative patients had excellent survival without adjuvant therapy

	No treatment (n=357)
Events, n (%)	39 (10.9)
DFS, median (95% CI), mo	NE (NE)



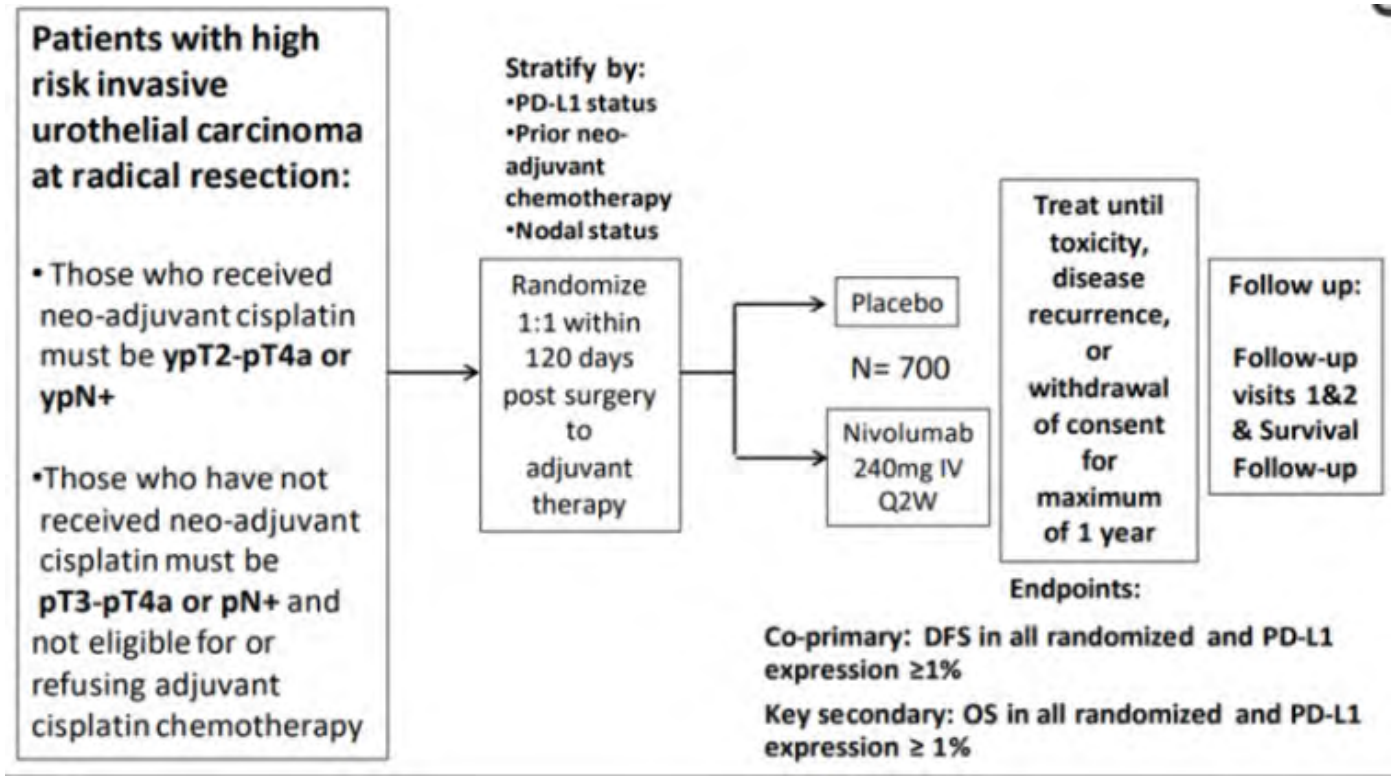
	No treatment (n=357)
Events, n (%)	14 (3.9)
OS, median (95% CI), mo	NE (NE)

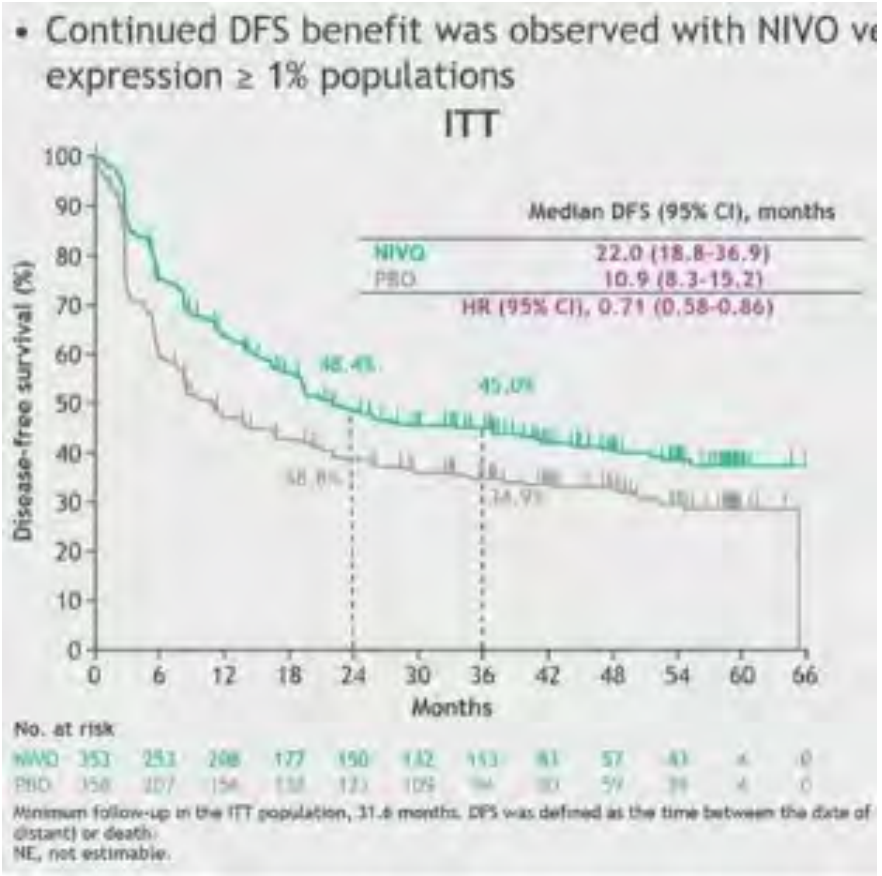


Serial MRD monitoring can identify persistently ctDNA negative patients who can be spared from unnecessary treatment

Powles, ESMO 2025 (Pending final reference)

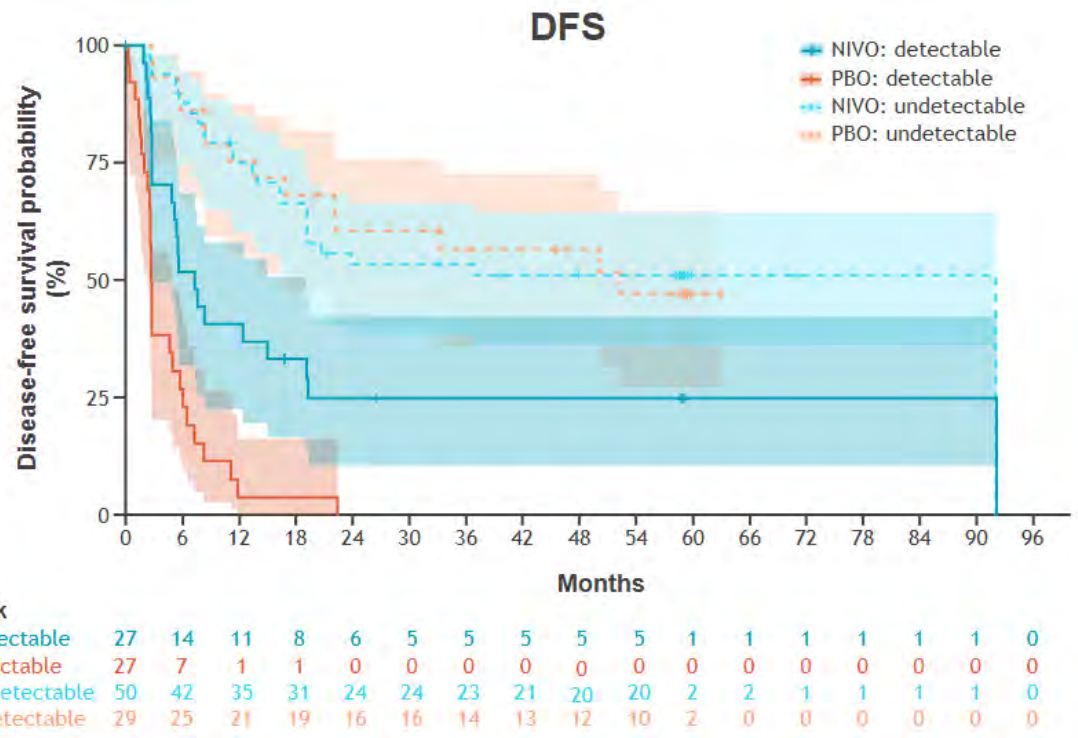
CheckMate 274: Study Design and DFS

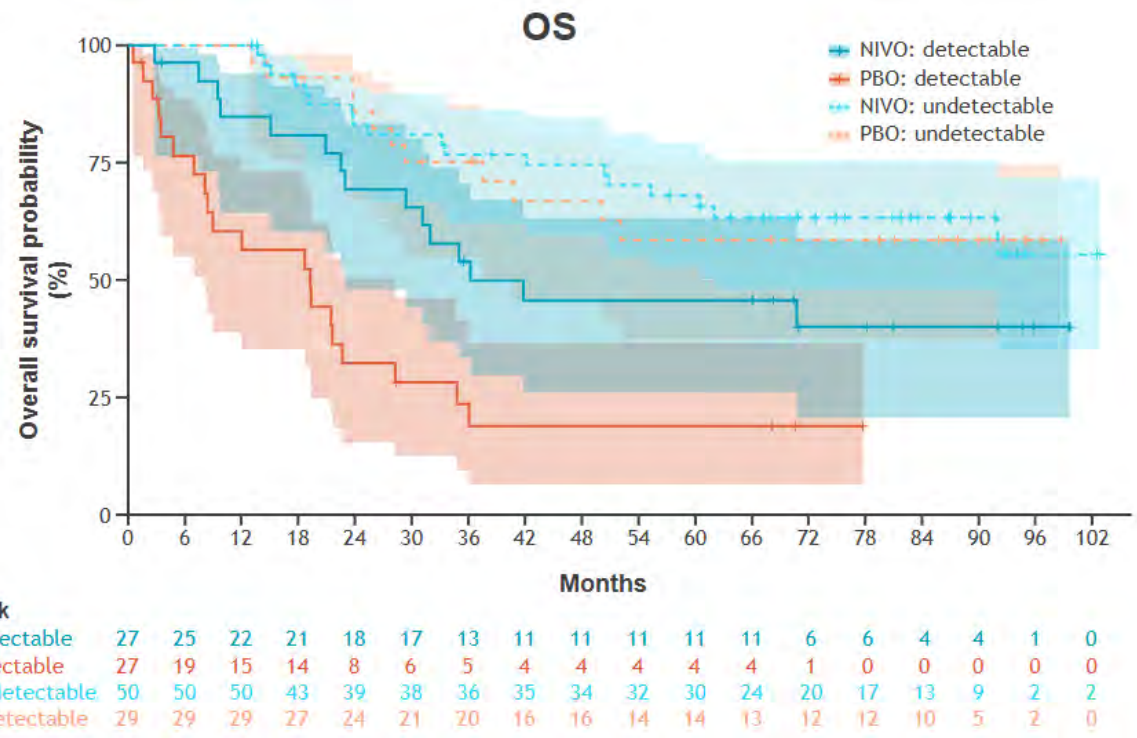




Galsky M, et al. J Clin Oncol 2025 Jan;43(1):15-21. doi: 10.1200/JCO.24.00340. Epub 2024 Oct 11.

CheckMate 274: Correlation of ctDNA and treatment with DFS and OS





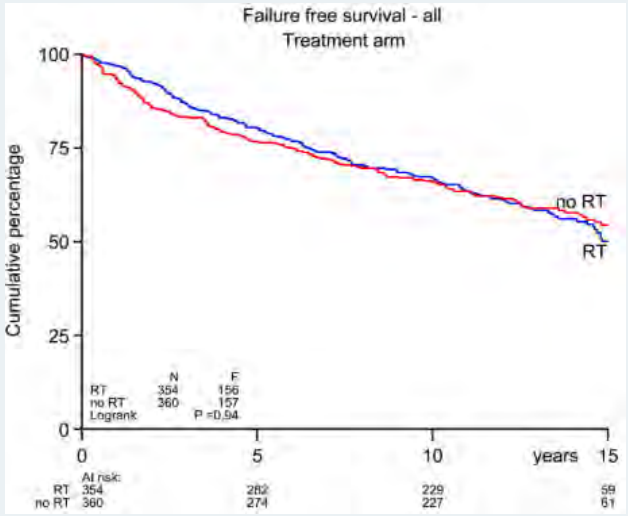
ctDNA detectable	NIVO (n = 27)	PBO (n = 27)
Median DFS (95% CI), months	7.4 (2.8-19.2)	2.8 (2.4-5.0)
DFS HR (95% CI)	0.35 (0.18-0.66)	
ctDNA undetectable	**NIVO (n = 50)**	**PBO (n = 29)**
Median DFS (95% CI), months	91.9 (19.2-NE)	52.2 (16.9-NE)
DFS HR (95% CI)	0.99 (0.51-1.93)	

ctDNA detectable	NIVO (n = 27)	PBO (n = 27)
Median OS (95% CI), months	36.2 (23.0-NE)	19.3 (8.1-28.2)
OS HR (95% CI)	0.41 (0.20-0.83)	
ctDNA undetectable	**NIVO (n = 50)**	**PBO (n = 29)**
Median OS (95% CI), months	NR (62.0-NE)	NR (40.7-NE)
OS HR (95% CI)	0.87 (0.41-1.84)	

Galsky M, et al. J Clin Oncol 2025 Jan;43(1):15-21. doi: 10.1200/JCO.24.00340. Epub 2024 Oct 11.

Where are there needs to identify patients at high risk of recurrence in GYN?

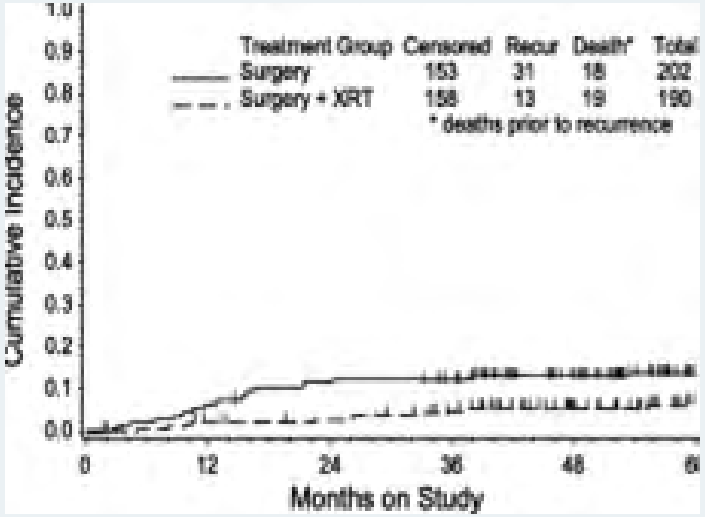
PORTEC-1[1]



Obs vs. WPRT in H-IR EC patients

While a decrease in RFS was observed, there was no difference seen in OS.

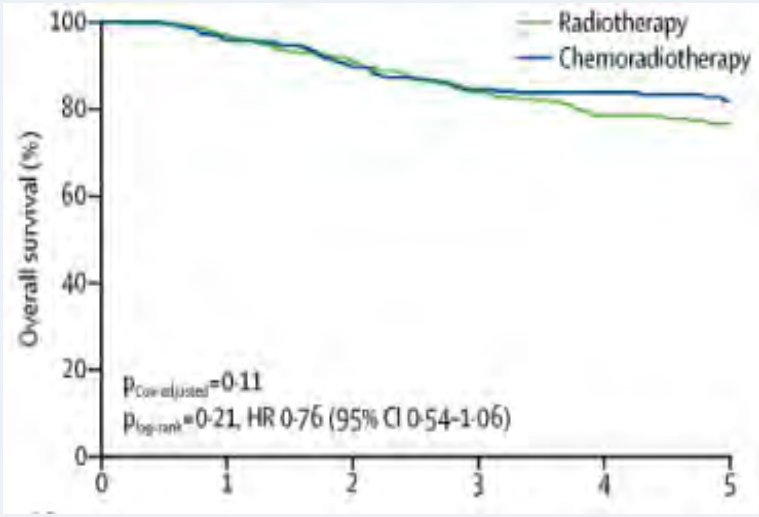
GOG-99[2]



Obs vs. WPRT in H-IR EC patients

While a decrease in RFS was observed, there was no difference seen in OS.

PORTEC-3[3]



Addition of chemo to XRT in stg I-III EC patients

Lack of benefit in OS with the addition of chemotherapy to radiotherapy.

There is an evident need for an additional molecular biomarker, such as ctDNA, in early-stage EC to optimize risk assessment.

1. Nout RA, et al. Long-term outcome and quality of life of patients with endometrial carcinoma treated with or without pelvic radiotherapy in the post operative radiation therapy in endometrial carcinoma 1 (PORTEC-1) trial. J Clin Oncol. 2011 May 1;29(13):1692-700.
2. Keys HM, et al; Gynecologic Oncology Group. A phase III trial of surgery with or without adjunctive external pelvic radiation therapy in intermediate risk endometrial adenocarcinoma. Gynecol Oncol. 2004 Mar;92(3):744-51.
3. Adjuvant chemoradiotherapy versus radiotherapy alone for women with high-risk endometrial cancer (PORTEC-3): final results of an international, open-label, multicentre, randomised, phase 3 trial S de Boer, M McCormack, et al. The Lancet Oncology, Volume 19, Issue 3, 295 - 309

Utilizing Circulating Tumor DNA (ctDNA)-based Molecular Residual Disease Detection for Postoperative Monitoring in Early-Stage Uterine Cancer

Peter W. Ketch[1], Carly Bess Scalise[2], Fernando Recio[3], Tara Berman[4], Nicole Hook[2], Paul Loar[5], Rebecca Arend[1], Punashi Dutta[2], Zach Gentry[1], Ekaterina Kalashnikova[2], Meenakshi Malhotra[2], Minetta C. Liu[2], Luis Vaccarello[6], Adam C. ElNaggar[2], Robert Holloway[3], Michael D. Toboni[1*]

[1]*The University of Alabama at Birmingham, School of Medicine, Birmingham, AL*

[2]*Natera, Inc, Austin, TX, USA*

[3]*AdventHealth Cancer Institute, Orlando, FL, USA*

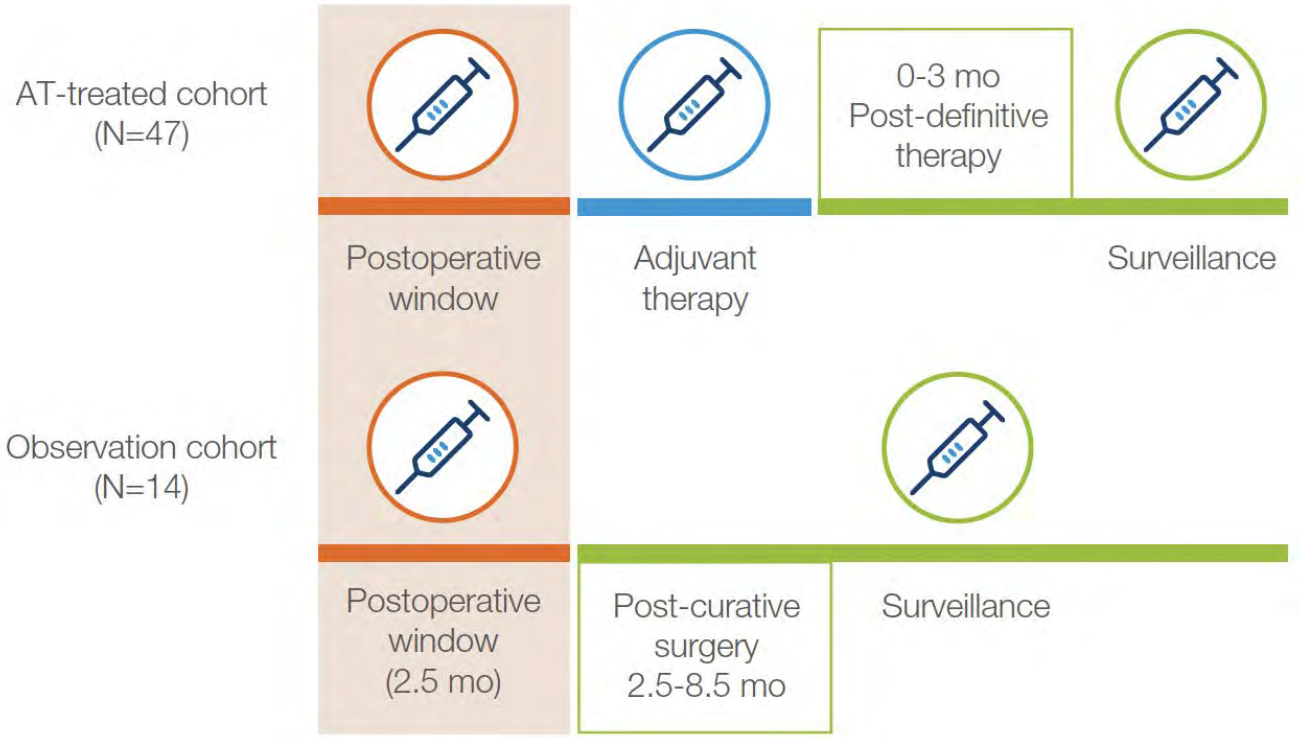
[4] *Inova Schar Cancer Institute, Fairfax, VA*

[5] *Texas Oncology, Austin, TX, USA*

[6]*Zangmeister Cancer Center, Columbus, OH, USA*

Study Cohort

- Overall ctDNA Cohort: N=61 patients, 233 timepoints
- Clinical follow-up available up to ~29 months (median: ~11 months)



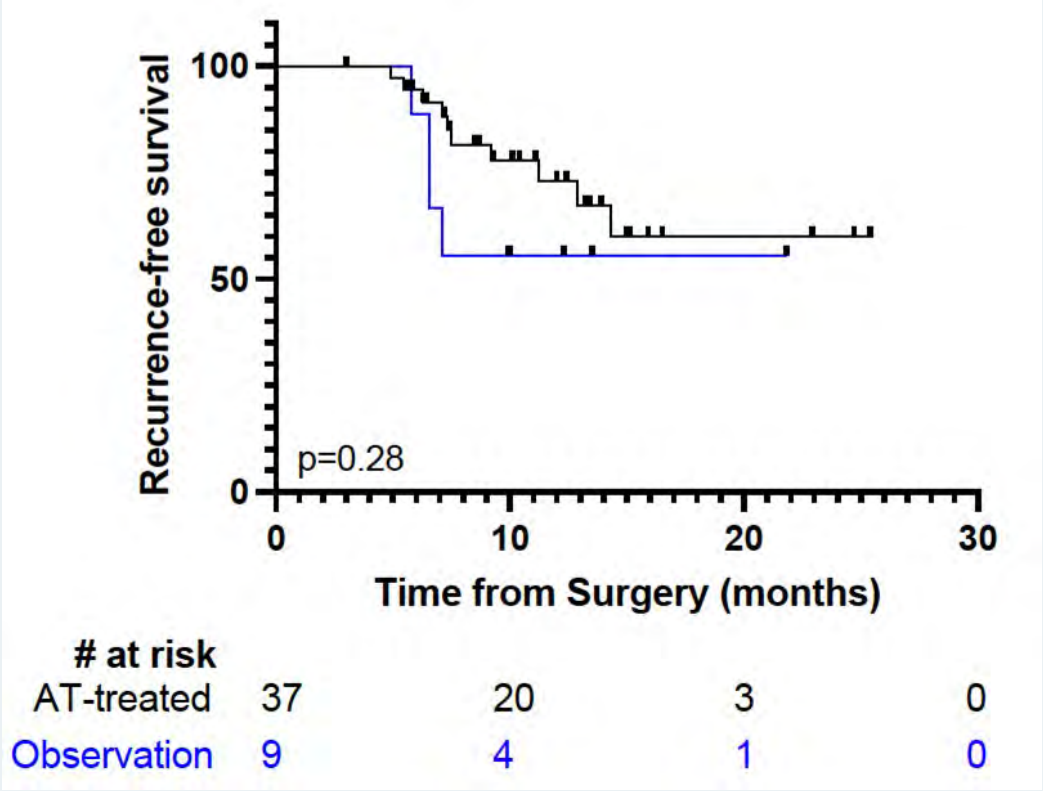
Cohort Characteristics	# Patients (%) (Total N=61)
Risk Groups (per GOG-249)	
Low	4 (6.5%)
High-Intermediate Risk (H-IR)	23 (38%)
High	30 (49%)
Sarcoma	4 (6.5%)
MMR Status	
Deficient (MMRd)	13 (21%)
Proficient (MMRp)	48 (79%)
p53 Status	
Altered (Alt.)	25 (41%)
Wild-type (WT)	36 (59%)

Ketch, et al. Utilizing Circulating Tumor DNA (ctDNA)-based Molecular Residual Disease Detection for Postoperative Monitoring in Early-Stage Uterine Cancer. JCO Precis Oncol 9, e2500286(2025

Ketch et al
JCO PO 2025

Current risk classifications are limited in predicting who will benefit from adjuvant therapy in stage I EC

AT-treated vs. Observation



There was no significant difference in recurrence between patients who received adjuvant treatment and those who were observed, across all risk groups

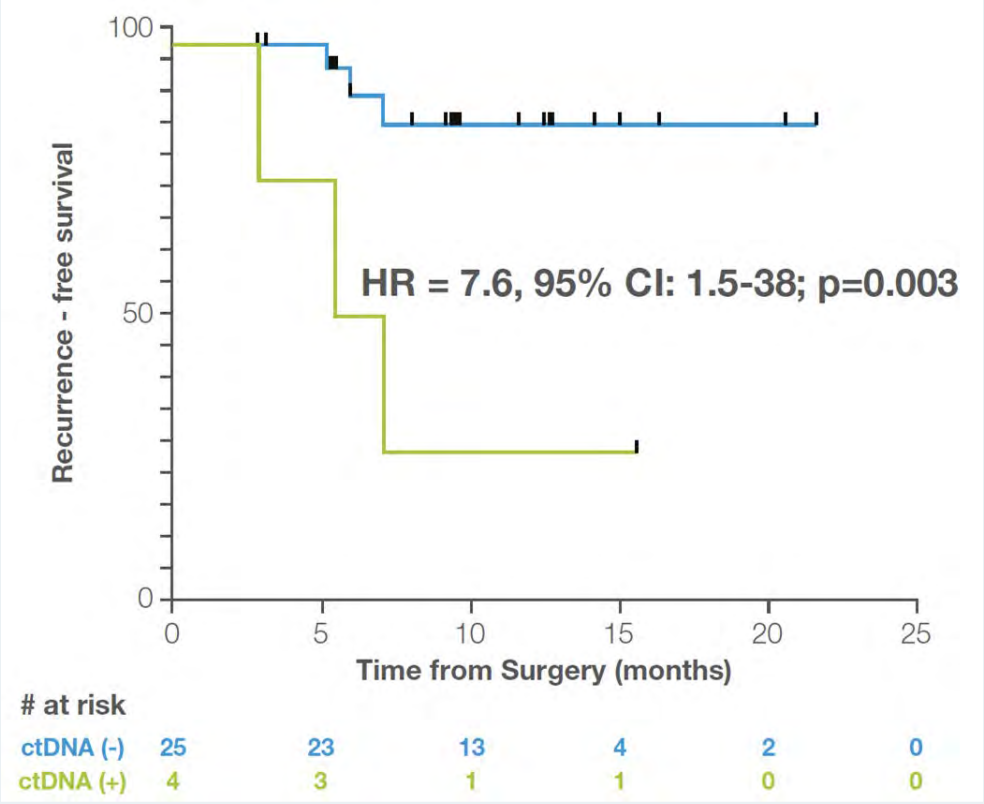
Ketch, et al. Utilizing Circulating Tumor DNA (ctDNA)-based Molecular Residual Disease Detection for Postoperative Monitoring in Early-Stage Uterine Cancer. JCO Precis Oncol 9, e2500286(2025

ctDNA-positivity post-surgery was associated with reduced RFS

Post-surgical window up to 2.5 months post-op

Ketch et al
JCO PO 2025

Post-operative ctDNA status

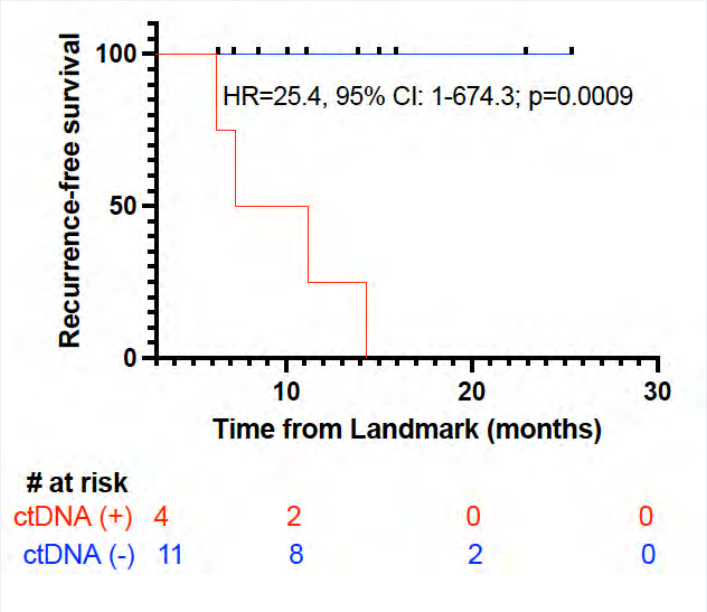


Patients who were ctDNA positive within 2.5 months of surgery were 7.6x more likely to experience recurrence than ctDNA negative patients

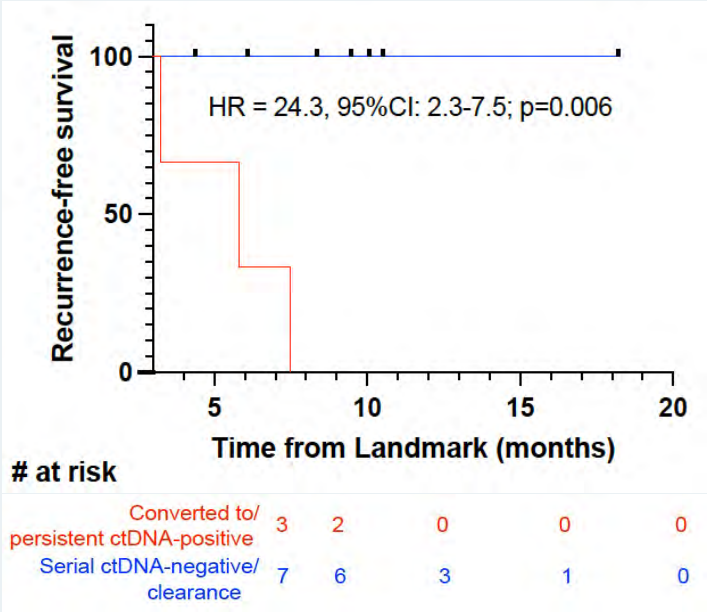
Ketch, et al. Utilizing Circulating Tumor DNA (ctDNA)-based Molecular Residual Disease Detection for Postoperative Monitoring in Early-Stage Uterine Cancer. JCO Precis Oncol 9, e2500286(2025

ctDNA positivity post-definitive therapy conferred a 25x higher risk of recurrence compared to serially ctDNA-negativity

Post-definitive therapy ctDNA status



RFS and ctDNA dynamics from pre to post AT

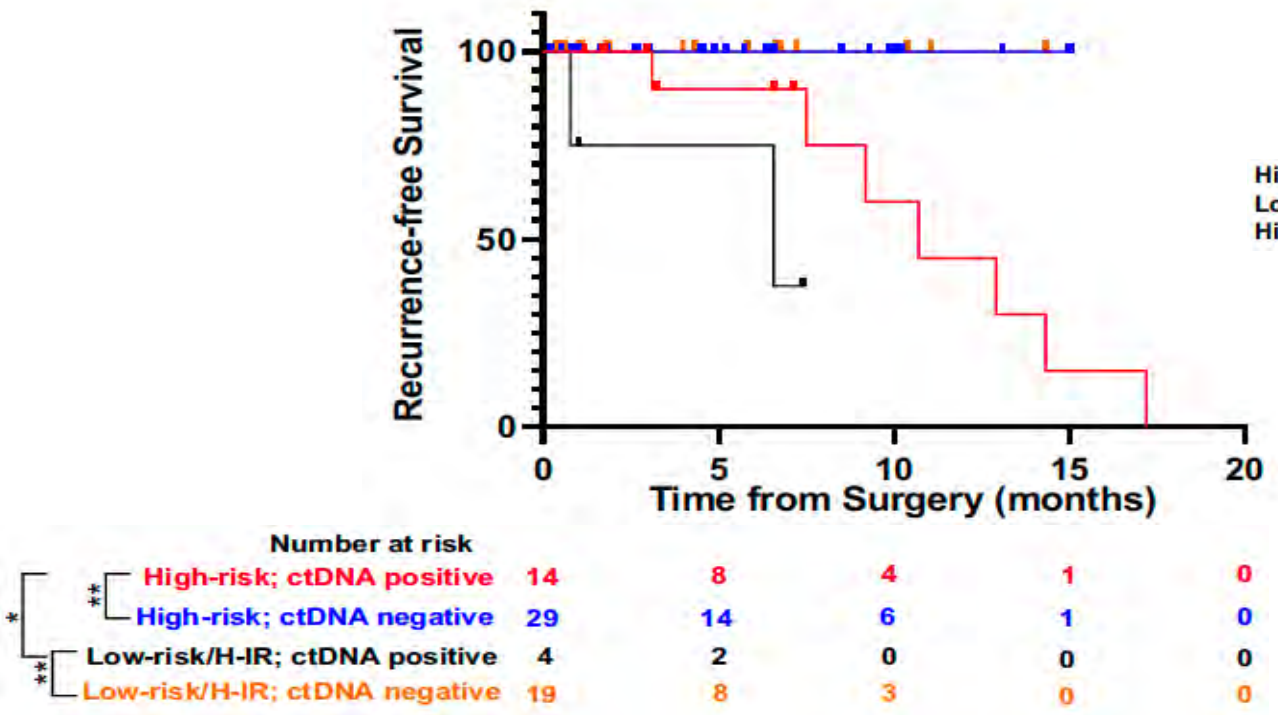


100% of relapse patients were ctDNA positive before or within 6 weeks of recurrence
100% of serially ctDNA-negative patients remained recurrence free

Ketch, et al. Utilizing Circulating Tumor DNA (ctDNA)-based Molecular Residual Disease Detection for Postoperative Monitoring in Early-Stage Uterine Cancer. JCO Precis Oncol 9, e2500286(2025

Recio et al
Gynecologic Oncology
2024

Longitudinal ctDNA Detection by Risk Group



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

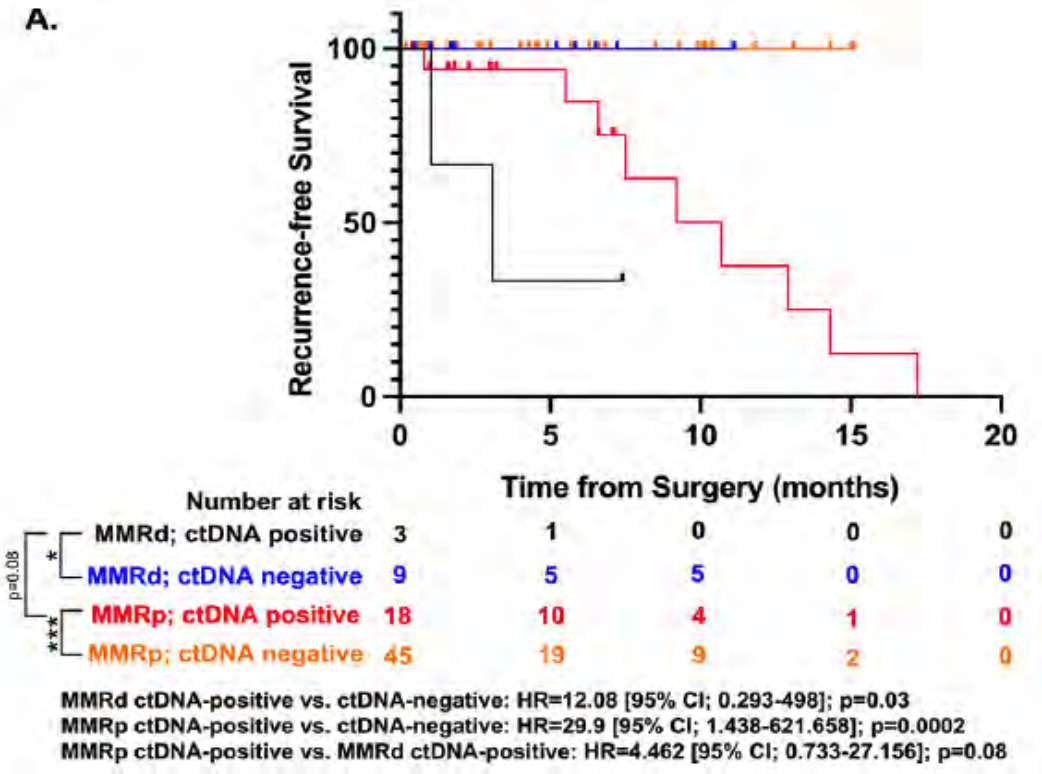
ctDNA positivity was associated with poor RFS, regardless of risk group, but was most significant for low-risk/H-IR patients[1]

None of ctDNA-negative patients experienced disease recurrence, regardless of risk-group[1]

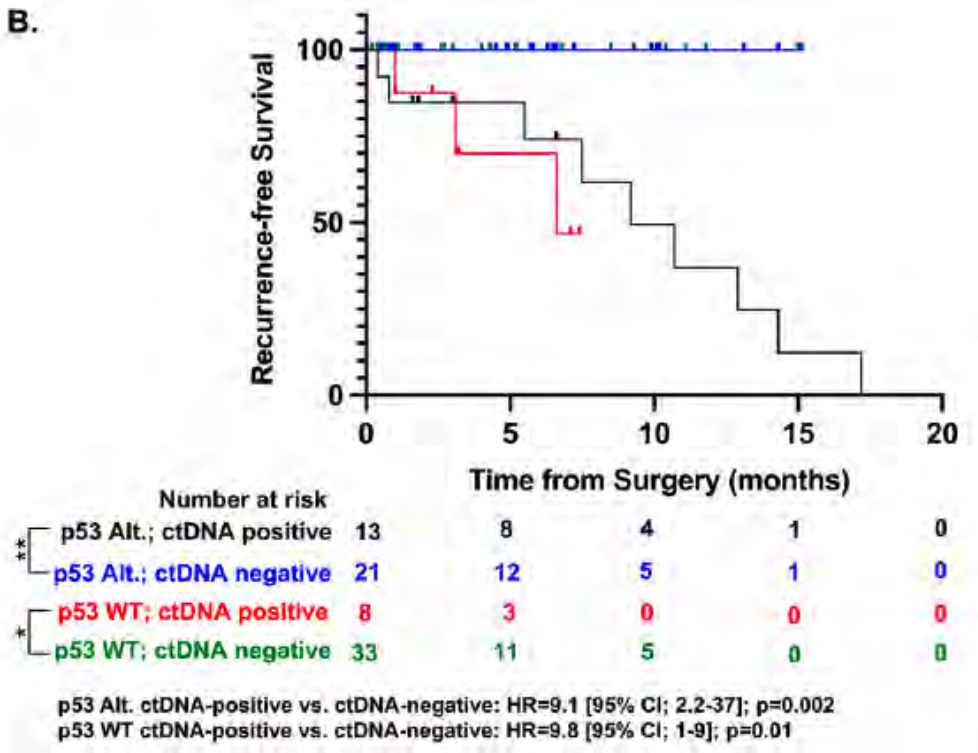
1. *Recio, Scalise, Loar, et al. Post-surgical ctDNA-based molecular residual disease detection in patients with stage I uterine malignancies. Gynecologic Oncology, 2024-03-01, Volume 182, Pages 63-69.*
2. *Ketch, et al. Utilizing Circulating Tumor DNA (ctDNA)-based Molecular Residual Disease Detection for Postoperative Monitoring in Early-Stage Uterine Cancer. JCO Precis Oncol 9, e2500286(2025*

Longitudinal ctDNA Detection by MMR and p53 Status

Longitudinal ctDNA Detection by MMR Status



Longitudinal ctDNA Detection by p53 Status



Any patient (regardless of MMR and p53 status) who was ctDNA positive post-surgery had a reduced RFS

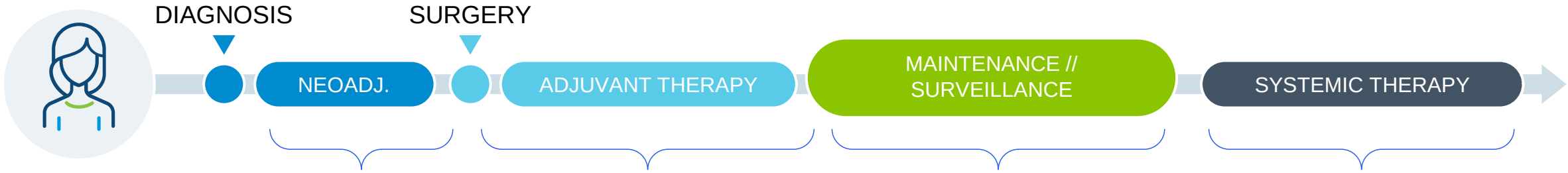
Recio, Scalise, Loar, et al. Post-surgical ctDNA-based molecular residual disease detection in patients with stage I uterine malignancies. Gynecologic Oncology, 2024-03-01, Volume 182, Pages 63-69.

Answering critical questions in ovarian cancer care

How has my patient responded to to (neo)adjuvant chemotherapy?

Is maintenance therapy effective? Is it needed? When to image? Duration?

Is your patient's cancer recurring or progressing?



Assess early response
Timing of surgery
Opportunity to tailor treatment

Optimize adjuvant treatment

Recurrence monitoring and treatment response monitoring

Monitor treatment response to optimize clinical management

Circulating tumor DNA monitoring for early recurrence detection in epithelial ovarian cancer

GYNECOLOGIC ONCOLOGY
OFFICIAL PUBLICATION OF THE SOCIETY OF GYNECOLOGIC ONCOLOGY

June Y. Hou [a,*,1], Jocelyn S. Chapman [b,1], Ekaterina Kalashnikova [c], William Pierson [b], Karen Smith-McCune [b], Geovanni Pineda [b], Reena Marie Vattakalam [a], Alexandra Ross [d], Meredith Mills [d], Carlos J. Suarez [d], Tracy Davis [e], Robert Edwards [e], Michelle Boisen [e], Sarah Sawyer [c], Hsin-Ta Wu [c], Scott Dashner [c], Vasily N. Aushev [c], Giby V. George [c], Meenakshi Malhotra [c], Bernhard Zimmermann [c], Himanshu Sethi [c], Adam C. ElNaggar [c], Alexey Aleshin [c], James M. Ford [d,*]

[a] *Columbia University Irving Medical Center, New York City, NY, United States of America*
[b] *University of California, San Francisco, CA, United States of America*
[c] *Natera, Inc., Austin, TX, United States of America*
[d] *Stanford University, Stanford, CA, United States of America*
[e] *University of Pittsburgh School of Medicine, Pittsburgh, PA, United States of America*

Clinical validation study in ovarian cancer

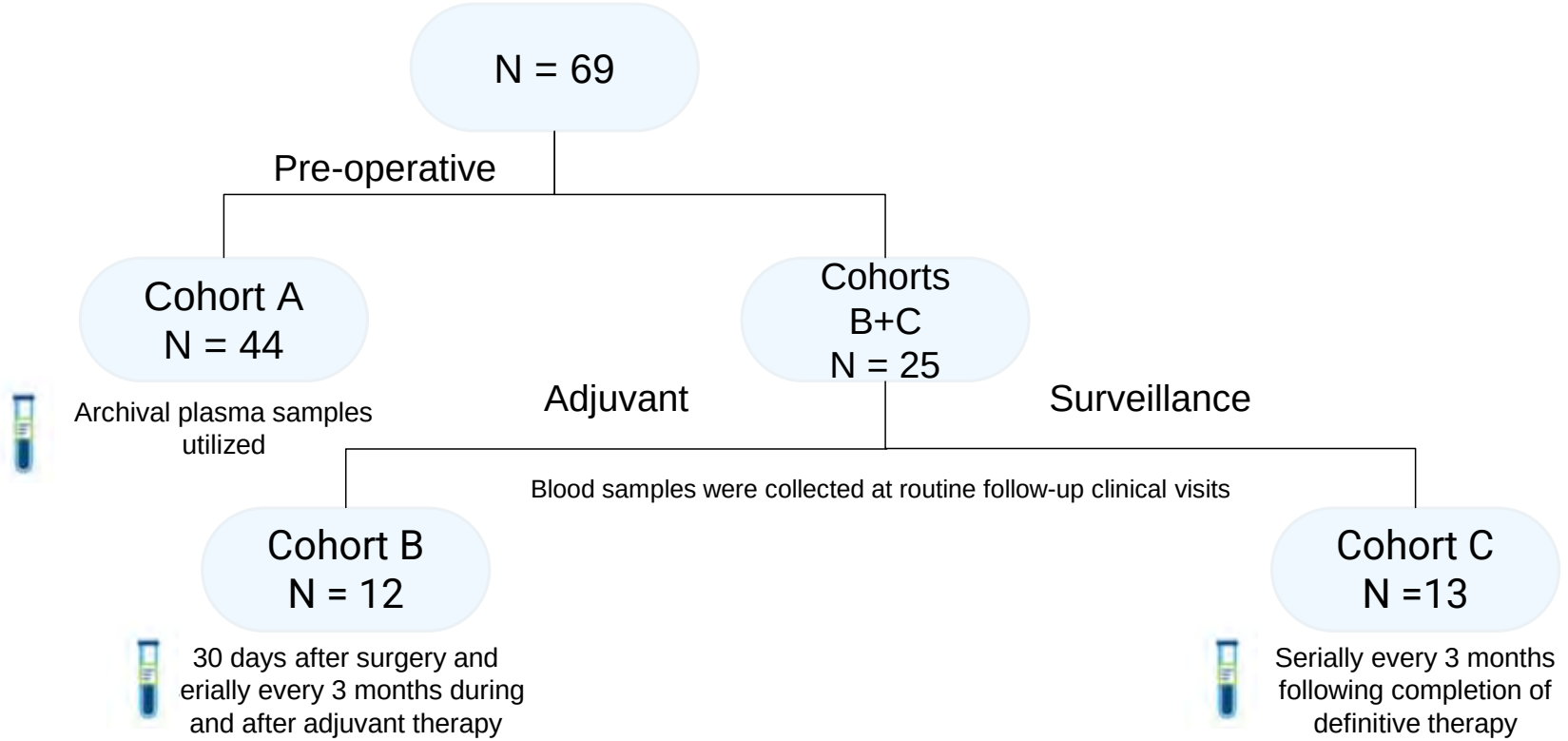
Patient characteristics

Patient characteristics	All patients $n = 69$, (%)
Median Age (at diagnosis)	Median: 55.5 years (range 29–82 years)
Subtype	
Serous	37 (54%)
Clear Cell	9 (13%)
Endometrioid	9 (13%)
Other	14 (20%)
Stage	
I	17 (25%)
II	12 (17%)
III	26 (38%)
IV	3 (4%)
Unstaged	11 (16%)

Hou JY, Chapman JS, Kalashnikova E, et al. Circulating tumor DNA monitoring for early recurrence detection in epithelial ovarian cancer. Gynecologic Oncology. 2022; 167: 334-341. doi: 10.1016/j.ygyno.2022.09.004

Clinical validation study in ovarian cancer

Examine the utility of ctDNA as a biomarker for EOC by assessing its relationship with patient outcomes and CA-125 when measured pre-surgically and during patient monitoring.



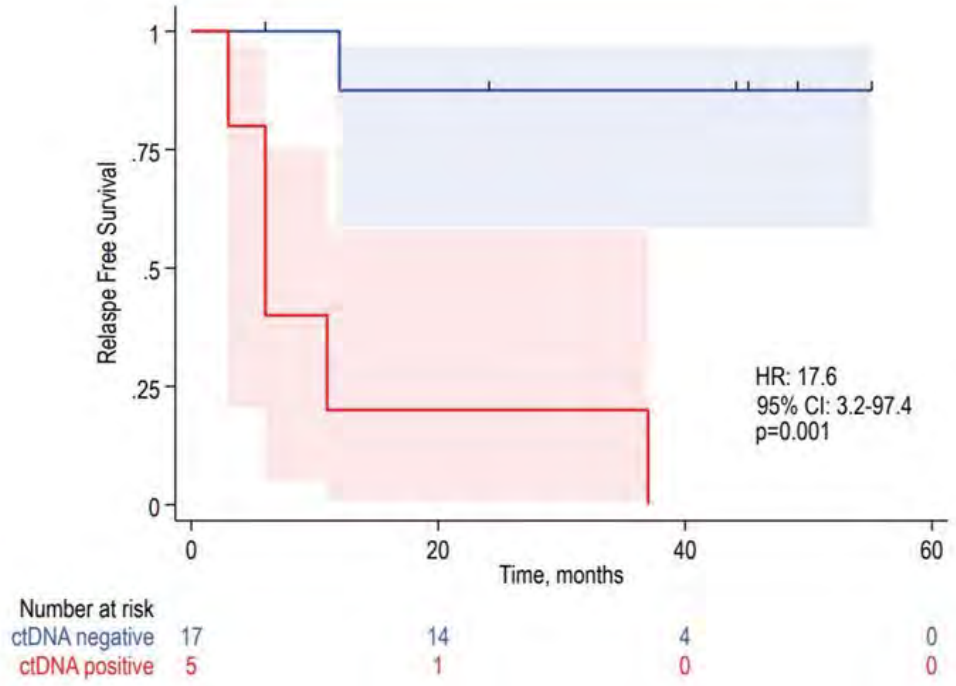
163 plasma samples analyzed with median follow up of 2.7 years (range: 0.08–16.7) for Cohort A and 2 years (range 0.3-4.6) for Cohorts B and C

J.Y. Hou, J.S. Chapman, E. Kalashnikova, et al., Circulating tumor DNA monitoring for early recurrence detection in epithelial ovarian cancer, Gynecologic Oncology, https://doi.org/10/1016/j.ygyno.2022.09.004

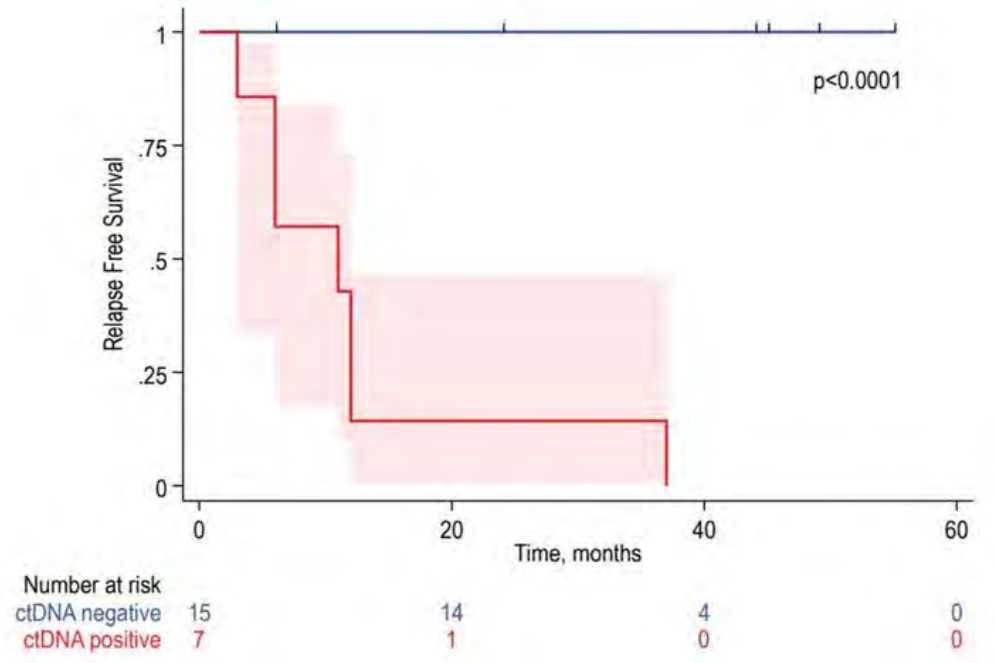
Longitudinal ctDNA monitoring identified relapse with 100% sensitivity and specificity

Cohort B + C, n = 22

RFS at a single time point after completion of definitive treatment



RFS with longitudinal monitoring after completion of definitive treatment

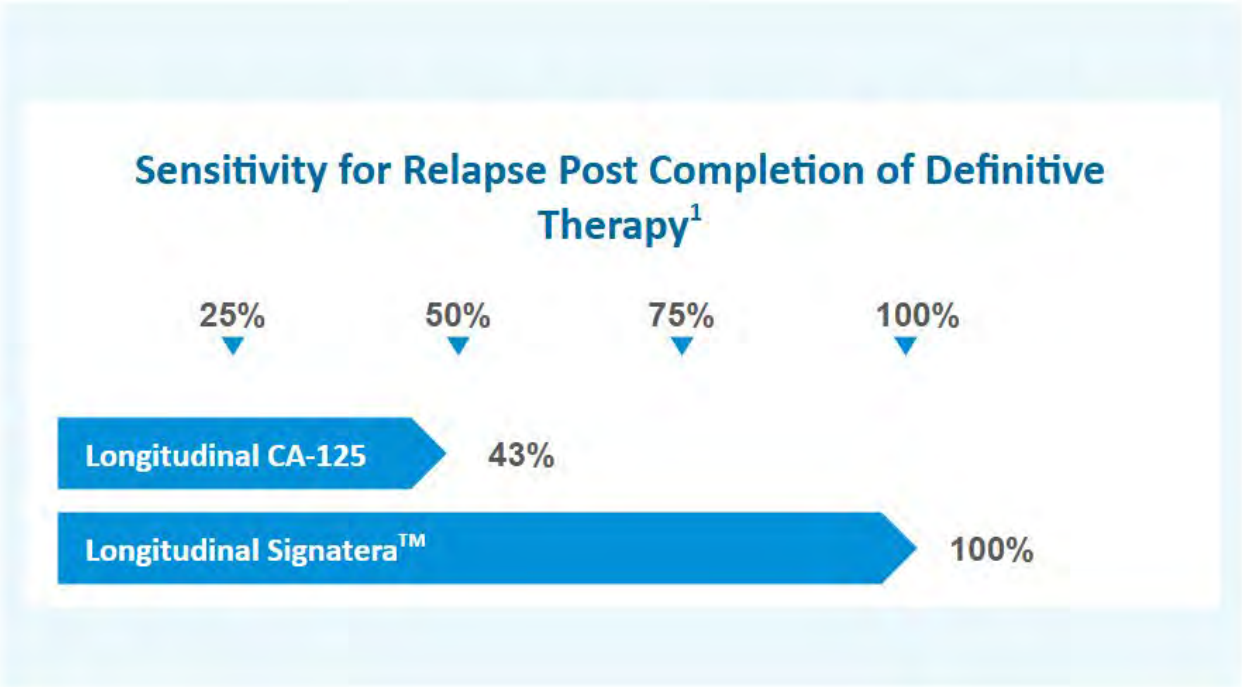


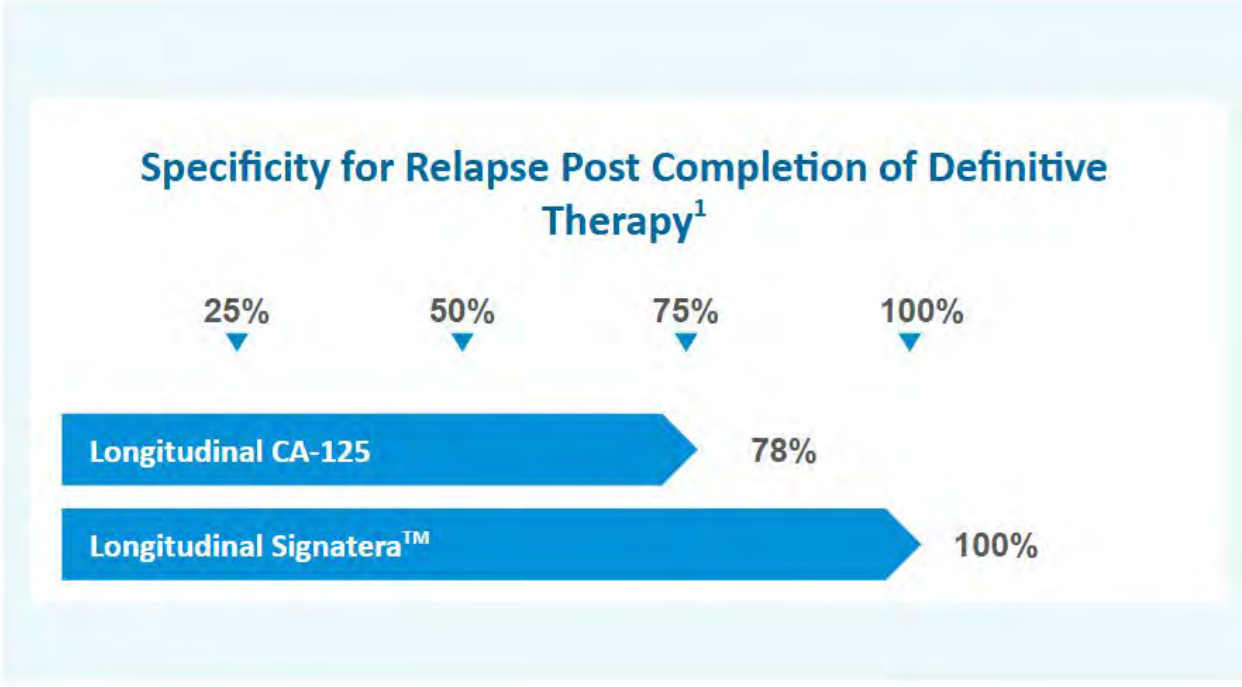
Median follow-up for Cohorts B and C: 2 years

Hou JY, Chapman JS, Kalashnikova E, et al. Circulating tumor DNA monitoring for early recurrence detection in epithelial ovarian cancer. Gynecologic Oncology. 2022; 167: 334-341. doi: 10.1016/j.ygyno.2022.09.004

Hou et al
Gynecologic Oncology 2022

ctDNA was shown to be a more sensitive biomarker for relapse than CA-125



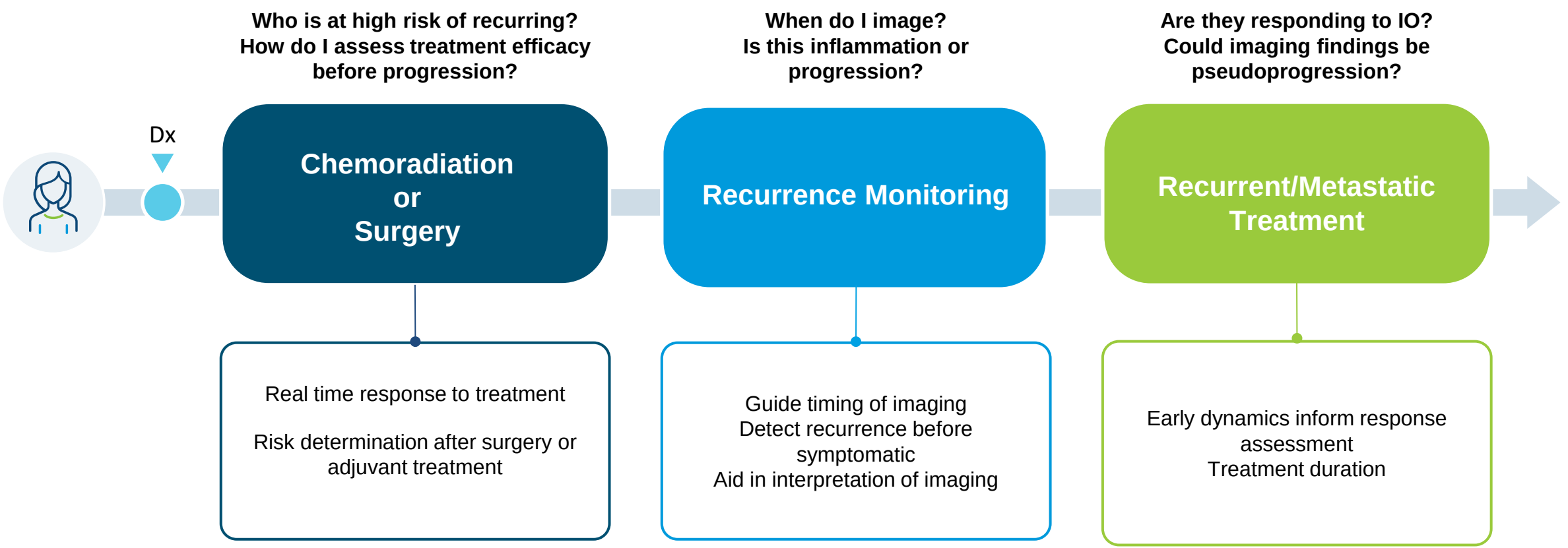


Signatera™ detected relapse an average of 9 months before CA-125[2]

Hou JY, Chapman JS, Kalashnikova E, et al. Circulating tumor DNA monitoring for early recurrence detection in epithelial ovarian cancer. Gynecologic Oncology. 2022; 167: 334-341. doi: 10.1016/j.ygyno.2022.09.004

Answering critical questions in cervical cancer care

CERVICAL CANCER



References: *1. National Comprehensive Cancer Network Guidelines. Cervical Cancer Version 4.2025.2. Venchiarutti Moniz et al., JCO 2024 3. Rotman et al. International Journal of Radiation Oncology Biol. Phys. 2006 4. Kuhn et al. SGO Winter 2025 5. Recio et al., SGO 2023*

The Utility of Circulating Tumor (ct) DNA for Surveillance Monitoring in Cervical Cancer Patients

SGO 2025 WINTER MEETING

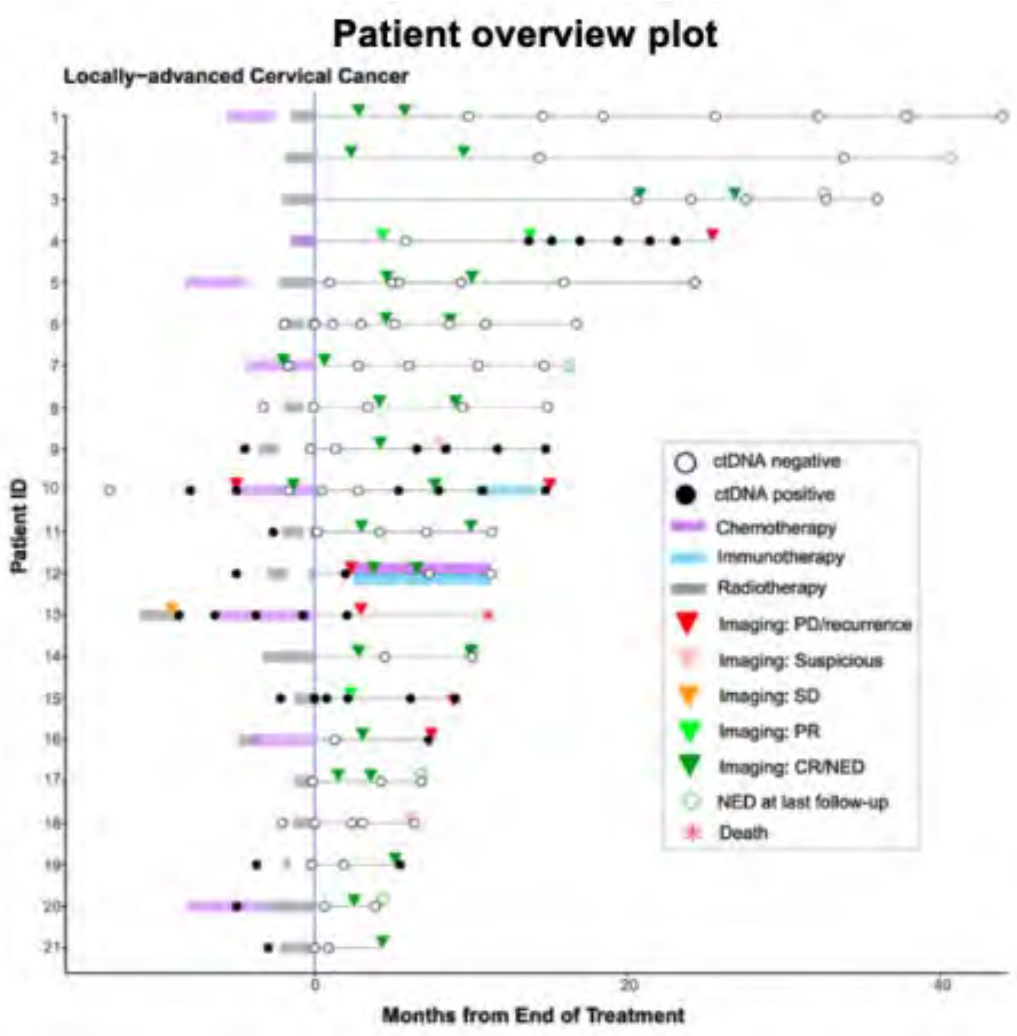
Theresa Kuhn[a], Gabriella Wernicke[b] Peter Ketch[c], Michael Toboni[c], Carly Bess Scalise[d], Nicole Hook[d], Hannah Calkins[d], Faraz Salmasi[d], Adam C. ElNaggar[d], Minetta C. Liu[d], Robert W. Holloway[a]

a AdventHealth Orlando Gynecologic Oncology, Orlando, FL, USA
b Northwell Health Cancer Institute, Hempstead NY
c University of Alabama at Birmingham, Biringham, AL
d Natera, Inc, Austin, TX, USA

Real-world experience locally advanced cervical cancer cohort

Patient Demographics	N=21
Age	Median = 58 (range: 34-85)
Histology	
Adenocarcinoma	1 (5%)
Small Cell Neuroendocrine	2 (10%)
Squamous Cell Carcinoma	17 (80%)
Poorly Differentiated	1 (5%)
Stage	
I	5 (24%)
II	2 (10%)
III	8 (38%)
IV	6 (28%)

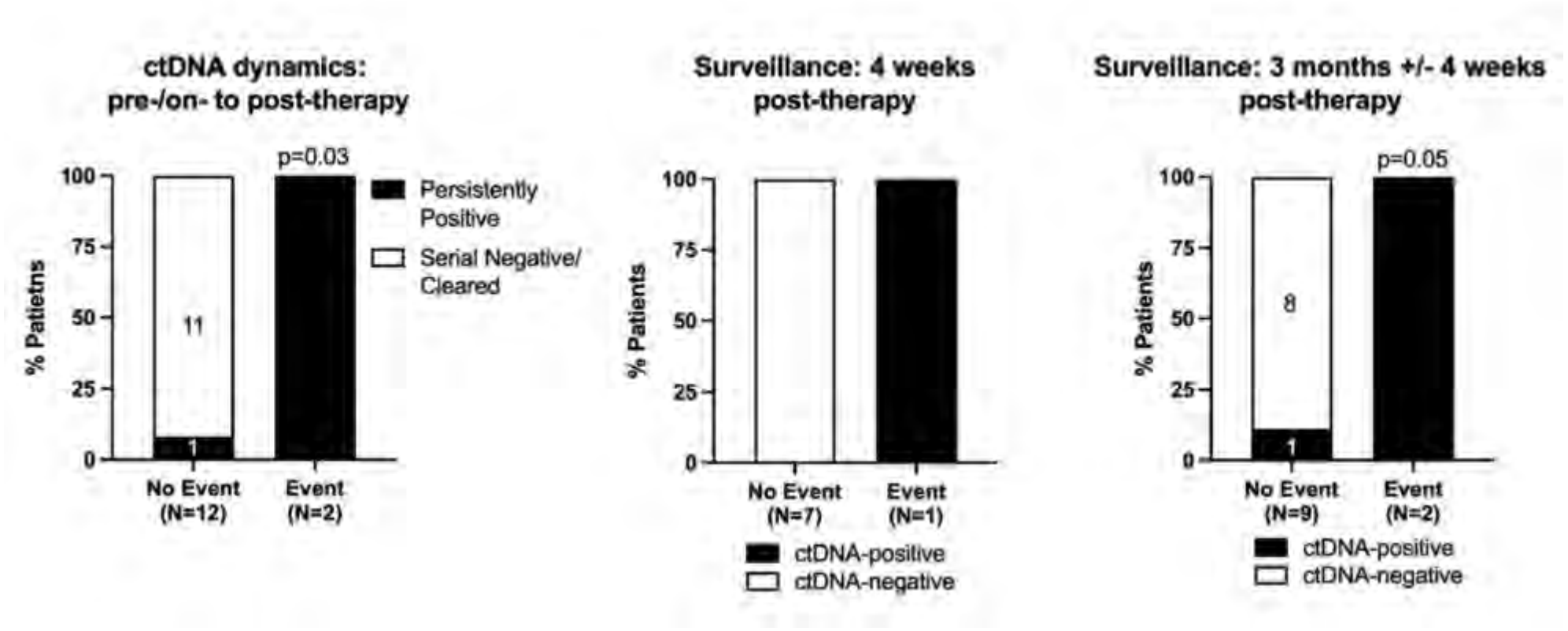
Median f/u for the cohort was 9 (range 4.3-40.6) months with an average of 5 ctDNA tests (range 2-9) per patient



Kuhn et al. The Utility of Circulating Tumor (ct) DNA for Surveillance Monitoring in Cervical Cancer Patients. SGO Winter, Whistler, Canada. January 30 to February 1, 2025.

100% of patients with PD/recurrence had a positive ctDNA test at the time of or prior to clinical recurrence

ctDNA identified PD/recurrence a median of 3 (range 0-11.7) mns prior to radiographic evidence



Persistent ctDNA positivity was significantly associated with poor outcomes (p=0.03)

Kuhn et al. The Utility of Circulating Tumor (ct) DNA for Surveillance Monitoring in Cervical Cancer Patients. SGO Winter, Whistler, Canada. January 30 to February 1, 2025.

Monitoring Treatment Response

ctDNA, Imaging, CA-125

Limitations of CA-125 for treatment response monitoring

Poor Concordance Between CA-125 and RECIST Progression

Platinum-sensitive recurrent ovarian cancer PARPi maintenance trials

- Study 19, SOLO2, ARIEL3, and NOVA
- Almost half with RECIST PD did not have CA-125 PD

Discordance between GCIG CA-125 progression and RECIST progression

CALYPSO trial of patients with platinum-sensitive recurrent ovarian cancer.

- Approximately 2 in 3 women with PSROC have RECIST PD but not CA-125 PD by GCIG criteria

Tjokrowidjaja, et al. J Clin Oncol. 2024 Apr 10;42(11):1301-1310.
Zebic, et al. Br J Cancer 130, 425–433 (2024).

PARPi response monitoring using personalized circulating tumor DNA testing in patients with ovarian cancer

THE POWER OF SHARED PURPOSE:
Transforming Gynecologic Cancer Care

SGO ANNUAL MEETING ON WOMEN'S CANCER
San Diego, CA • 2024

Elizabeth T. Evans[1], Michael Toboni[1], Carly Bess Scalise[2], Melissa Hardesty[3], Tara Berman[4], Nicole Hook[2], Jenifer Ferguson[2], Punashi Dutta[2], Jennah Moore[1], Bailee Dover[1], Minetta C. Liu[2], Adam C. ElNaggar[2], and Rebecca C. Arend[1]

[1]The University of Alabama at Birmingham, School of Medicine, Birmingham, AL; [2]Natera, Inc., Austin, TX; [3]Alaska Women's Cancer Care, Anchorage, AK, USA; [4]Inova Schar Cancer Institute, Fairfax, VA

Real-world study evaluated patients receiving PARPi therapy

- 45 patients with 156 longitudinal plasma samples
  - Pre-PARPi (n = 7)
  - During PARPi (n = 41)
  - Post-PARPi (n = 23)
- Median follow-up 16.8 mns (3.6 - 66.6 mns)

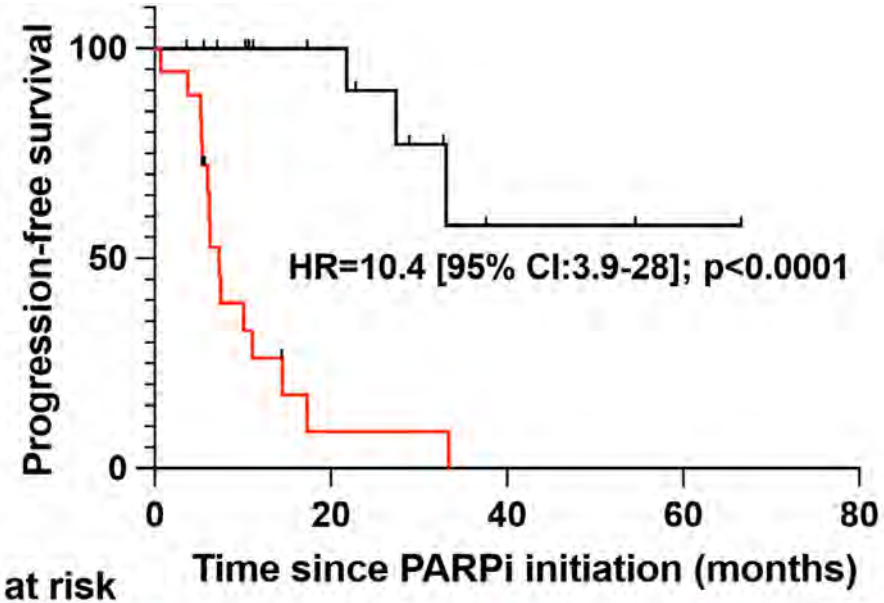
Table 1. Patient and tumor characteristics (N=45)

Parameter		# Patients (%)
Stage		
	I	2 (4.4%)
	II	9 (20%)
	III	21 (46.7%)
	IV	10 (22.2%)
	unknown	3 (6.7%)
Histology		
	Serous	35 (77.8%)
	Clear cell	3 (6.7%)
	Mixed	1 (2.2%)
	Poorly differentiated	1 (2.2%)
	unknown	5 (11.1%)
BRCA Status		
	Negative	21 (46.7%)
	Positive	11 (24.4%)
	unknown	13 (28.9%)
HRD Status		
	Deficient	16 (35.5%)
	Proficient	12 (26.7%)
	unknown	17 (37.8%)

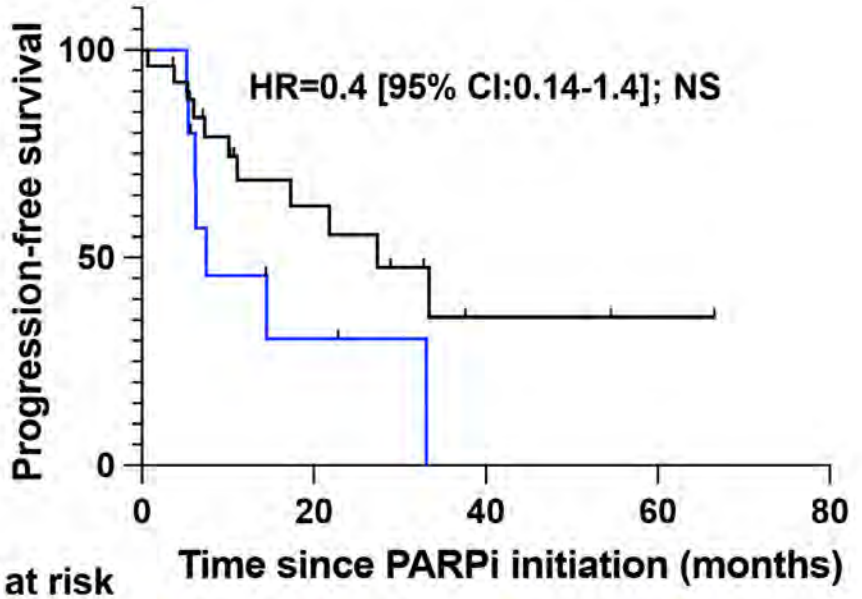
Evans, E, Toboni, M, Scalise, CB, et al. PARPi response monitoring using personalized circulating tumor DNA testing in patients with ovarian cancer. SGO, San Diego, CA. March 16-18, 2024

ctDNA positivity but not elevated CA-125 was significantly associated with inferior PFS

Evans et al SGO 2024



Number at risk					
ctDNA-positive	18	1	0	0	0
ctDNA-negative	18	10	2	1	0



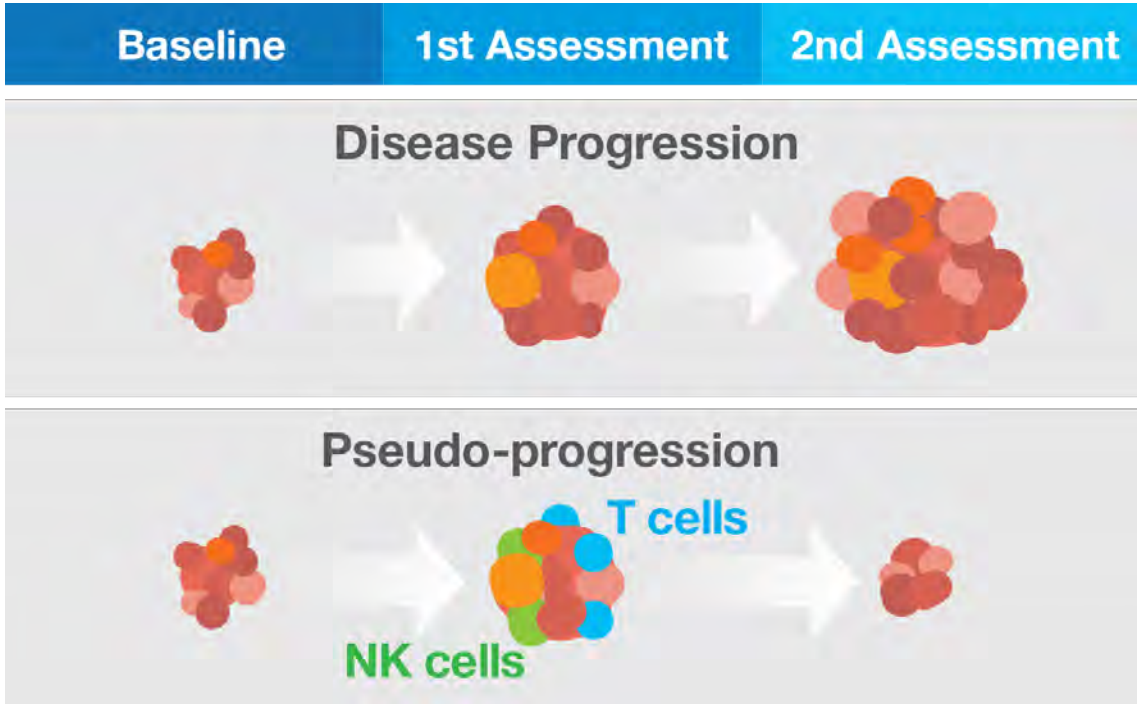
Number at risk					
CA-125 elevated	10	2	0	0	0
CA-125 normal	26	10	2	1	0

ctDNA outperformed CA-125 in identification of patients at high risk of progression while on PARPi

Evans, E, Toboni, M, Scalise, CB, et al. PARPi response monitoring using personalized circulating tumor DNA testing in patients with ovarian cancer. SGO, San Diego, CA. March 16-18, 2024

Difficulty tracking immunotherapy response via imaging

Radiological imaging lacks sensitivity and specificity to distinguish pseudo-progression from true disease progression

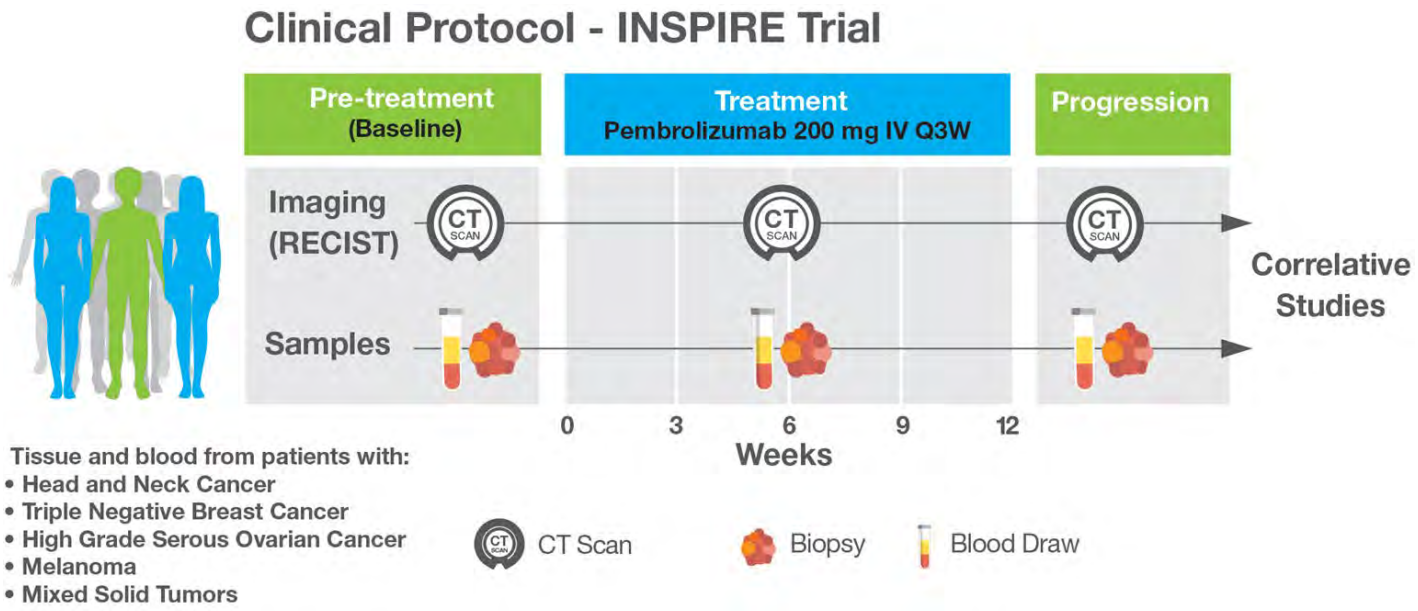


- Pseudo-progression frequency varies by primary site and therapy agent[1]
    - Average 10% rate of pseudo-progression for ICI patients[2]
- Immune related response criteria (irRC)[3]
    - TWO consecutive images done at least FOUR weeks apart is recommended, per

1. *Jia W, Gao Q, Han A, Zhu H, Yu J. The potential mechanism, recognition and clinical significance of tumor pseudoprogression after immunotherapy. Cancer Biol Med. 2019; 16:655-70.*
2. *Patel V. Progress on pseudoprogression. ddPCR for ctDNA could distinguish pseudoprogression from true disease progression. The pathologist. Published May 11, 2018*
3. *Wolchok, J. D. et al. Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria. Clin. Cancer Res. 15, 7412–7420 (2009).*

INSPIRE trial

Study design



94 patients with various solid tumors in the Phase II INSPIRE trial treated with single agent pembrolizumab (200 mg IV q3W)

Patient characteristics

Patients with advanced solid malignancies who have failed standard therapy, for which no standard therapy exists, or who are ineligible for standard therapy
n = 106

Patients analyzed by Signatera
n = 94

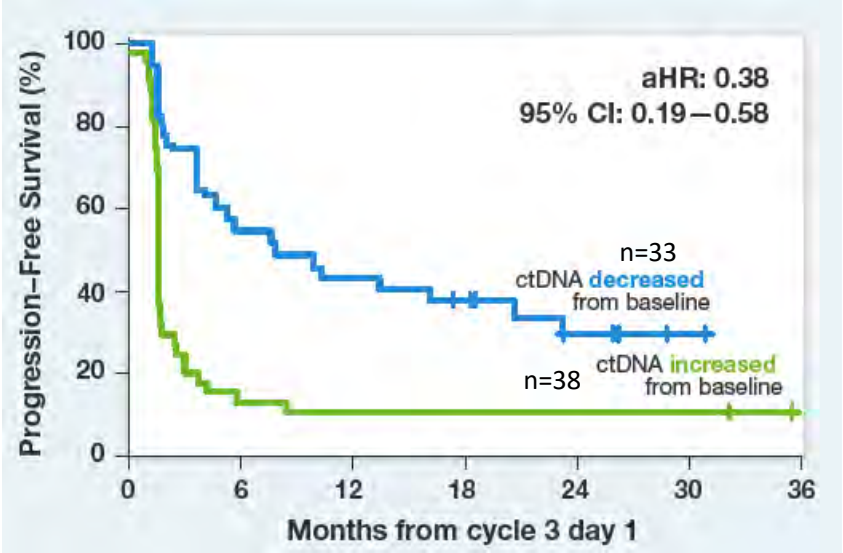
H&N (SCC) n=16	TNBC n=18	Ovarian n=18	Melanoma n=12	Other n=30

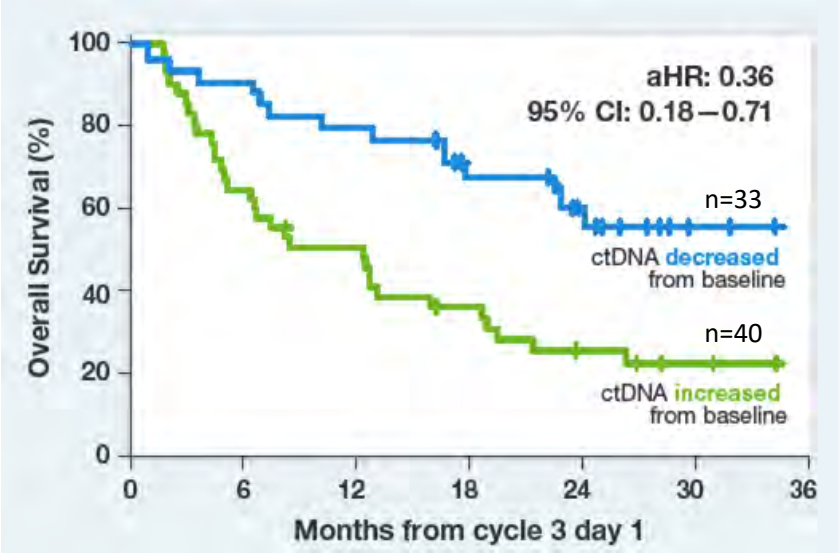
Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nature Cancer. 2020 (Accepted)

Bratman et al. 2020
Nature Cancer

Predict immunotherapy benefit as early as week 6 with ctDNA dynamics

PFS and OS among patients with ctDNA values at both baseline and week 6 (cycle 3 day 1)





of patients had detectable baseline ctDNA

of patients with a ctDNA increase at week 6 did not have an objective response by imaging

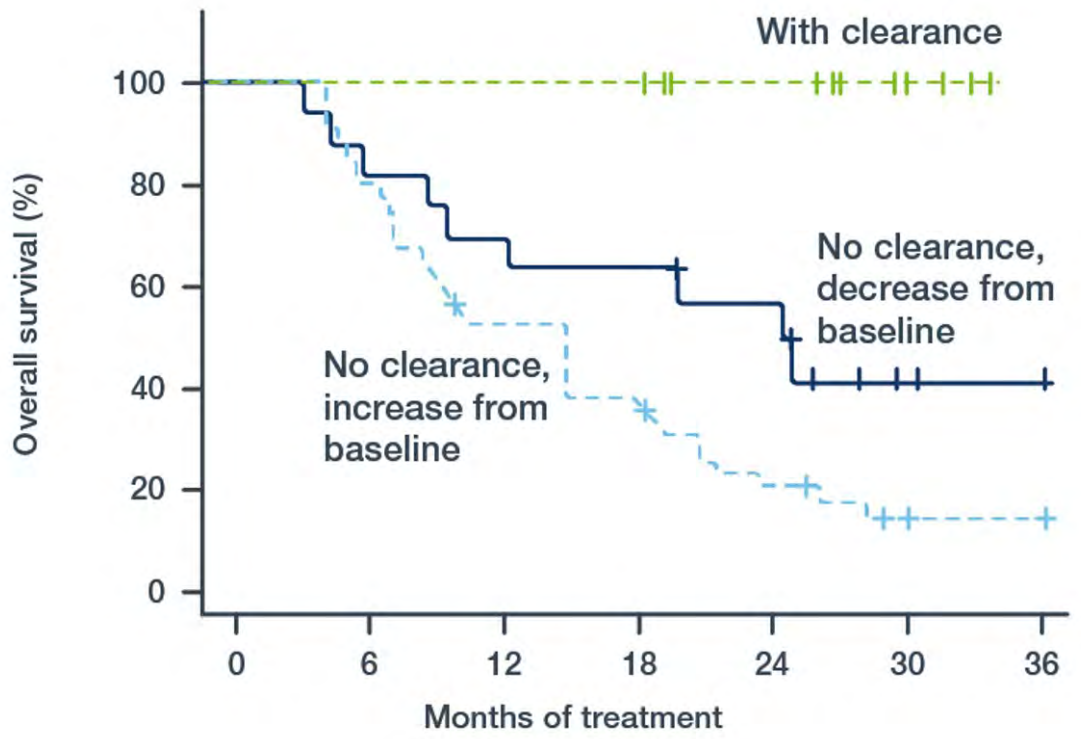
None of the patients (n=30) with an increase in both ctDNA level and tumor size at week 6 achieved objective response at any time during the study

Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881. doi:10.1038/s43018-020-0096-5.

Medicare approved for IO treatment

Response to immunotherapy with ctDNA dynamics

Identify nonresponders and exceptional responders



100% OS*

for patients who achieved ctDNA clearance during treatment

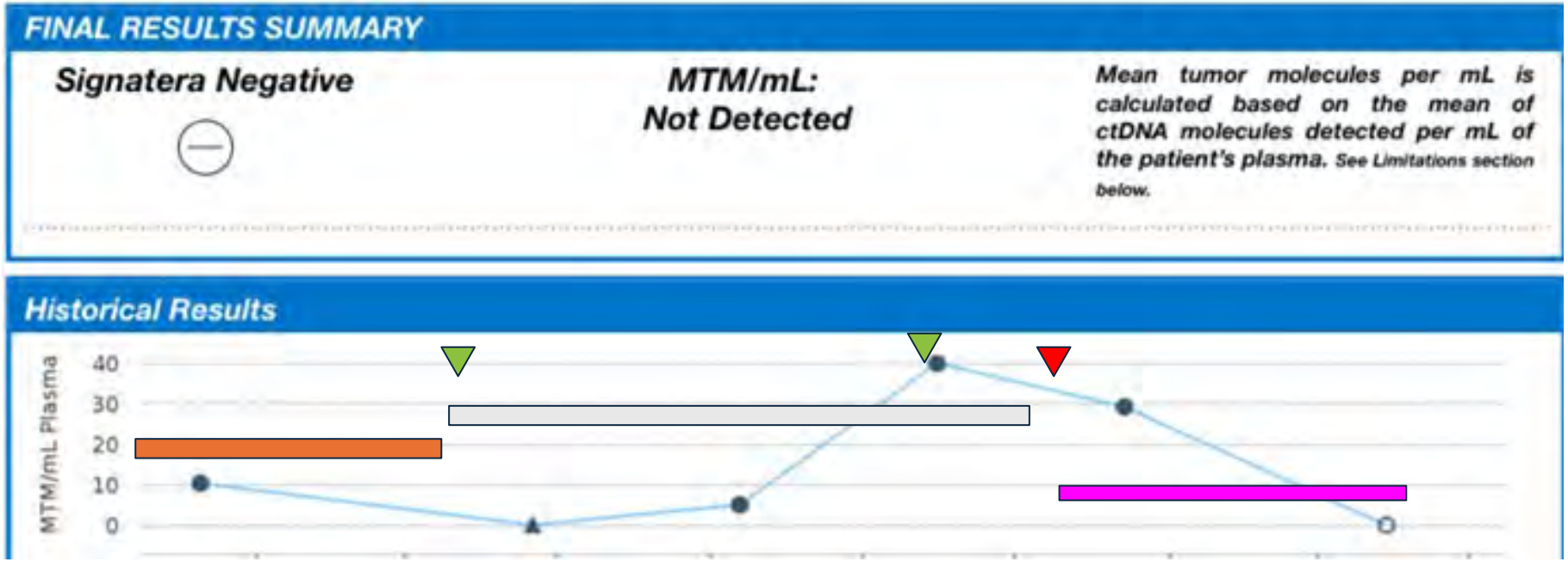
*Median of 25.4 months of follow-up beyond first clearance
ctDNA clearance defined as ctDNA undetectable for at least one on-treatment time point

Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nature Cancer. 2020;1(9):873-881.

Interactive cases and understanding challenges and considerations

All Faculty

MRD case study



72 y/o F with Stage IIIC high grade, serous ovarian cancer

HRD (+), FOLR-1 (+)

Underwent primary debulking surgery to no gross residual disease

Postoperatively, began carboplatin, paclitaxel, and bevacizumab x 6 cycles

Received maintenance bevacizumab + olaparib

▼ Initial PET/CT: NED

▼ CT CAP: 15 x 16 mm retrocaval LN

Began mirvetuximab

Timepoint	Reported MTM/mL
Timepoint 6	0.00
Timepoint 5	29.27
Timepoint 4	40.04
Timepoint 3	5.06
Timepoint 2	0.08
Timepoint 1	10.39

FINAL RESULTS SUMMARY

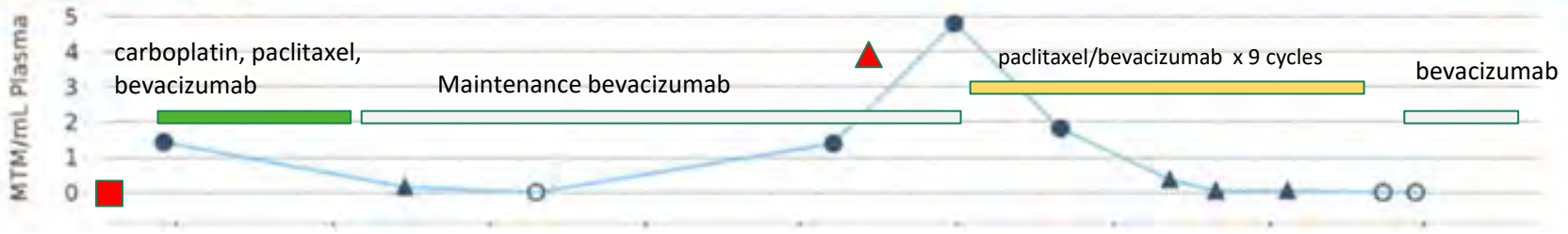
Signatera Negative

MTM/mL: Not Detected

Mean tumor molecules per mL is calculated based on the mean of ctDNA molecules detected per mL of the patient's plasma. ***See Limitations section below.***

Higher than expected cell-free DNA (cfDNA) levels were found in this plasma specimen. Excessive cfDNA may decrease clinical sensitivity.

Historical Results



Timepoint	Reported MTM/mL
Timepoint 11	0.00
Timepoint 10	0.00
Timepoint 9	0.05
Timepoint 8	0.04
Timepoint 7	0.36
Timepoint 6	1.82
Timepoint 5	4.81
Timepoint 4	1.39
TImepoint 3	0.00
Timepoint 2	0.15
Timepoint 1	1.43

MRD case study

67 y/o female:
Stage IIIC, high-grade serous left fallopian tube cancer

■ Primary debulking to no gross residual

Completed 6 cycles of carboplatin, paclitaxel, and bevacizumab

Continued on maintenance bevacizumab

- **Initial response & ctDNA clearance**

- Converted to positive with subsequent rise

▲

- PET: Mixed response, new hypermetabolic left supraclavicular and retroperitoneal LN

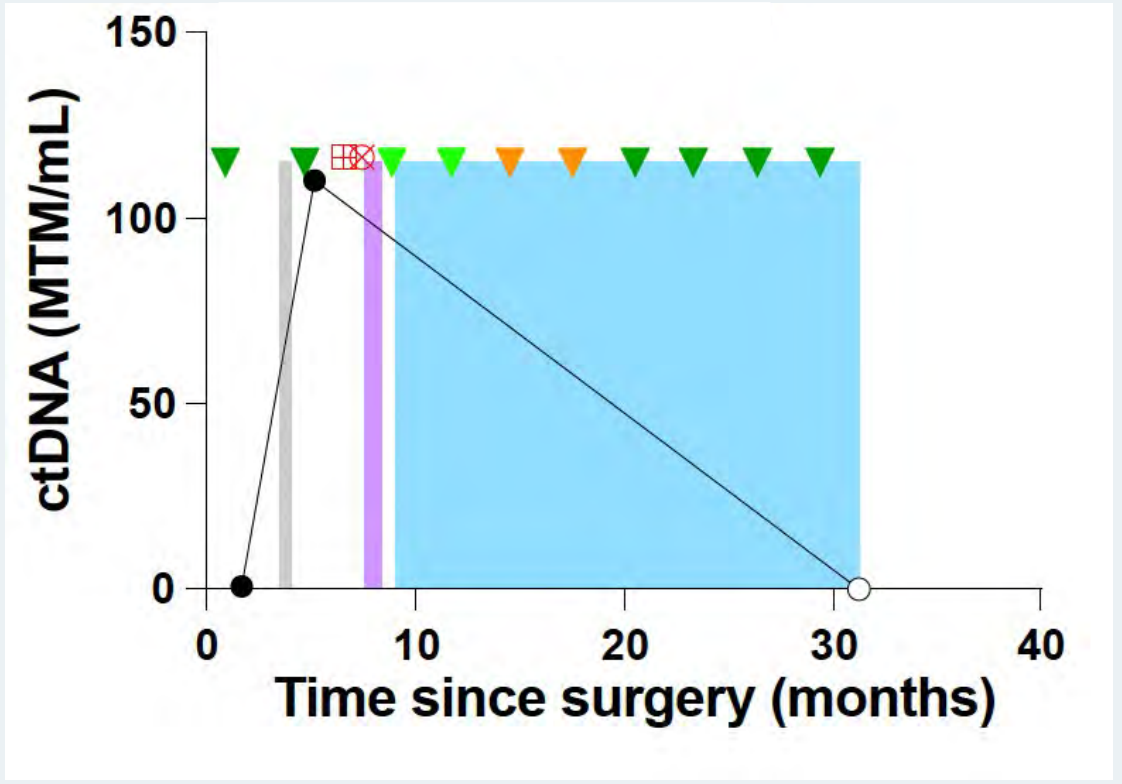
Paclitaxel and bevacizumab x 9 cycles

- ctDNA decline and ultimate clearance
- Concordant with CT CAP

Case Study: MRD predicted relapse and correlated with treatment response in a stg I HR EC patient

57-year-old woman with stg I HR EC

- Patient underwent surgery and adjuvant radiation therapy with NED on imaging post-op and post-AT.
- Signatera post-operatively was positive and an increase in MTM/mL was observed post-AT.
- Shortly after, an abnormal clinical exam was noted and disease recurrence was confirmed on subsequent imaging.
- Immunotherapy was initiated and the patient experienced an objective response with ctDNA clearance.

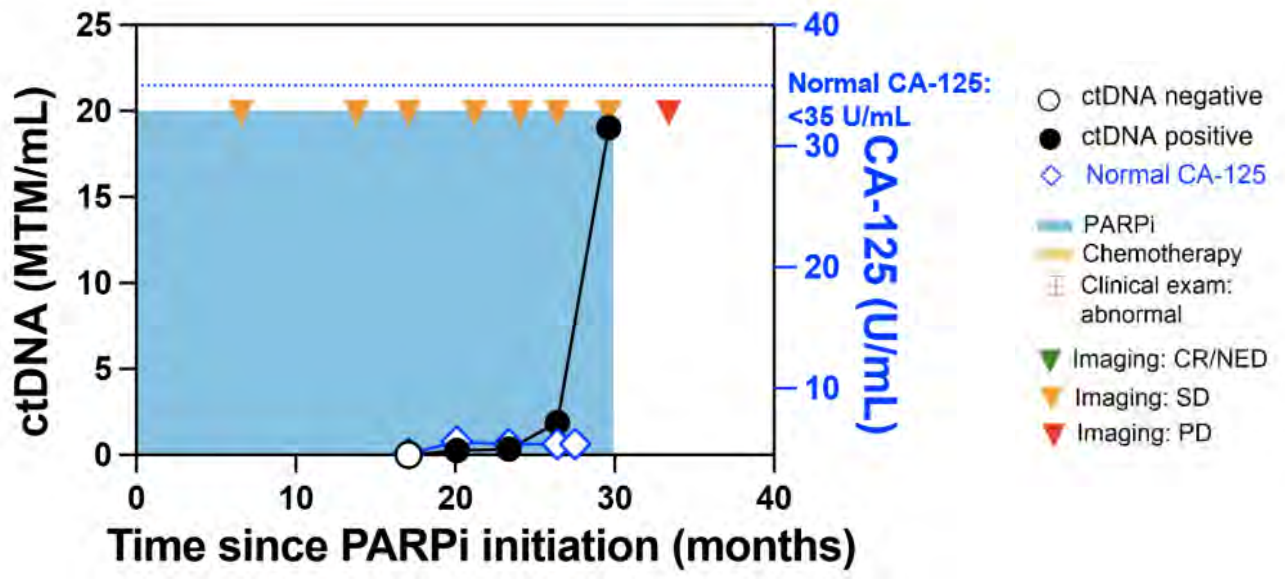


Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

Case Study: MRD positivity on PARPi therapy correlated with progression on imaging in patient with normal CA-125

71-year-old woman with epithelial ovarian cancer

- Patient on PARP maintenance underwent ctDNA testing with intial negative timepoint
- 2 months later ctDNA became positive, increasing over 10 months to 19 MTM/mL, while disease remained stable on imaging
- One year after ctDNA became positive, progressive disease was observed on imaging
- CA-125 remained negative and did not detect disease progression

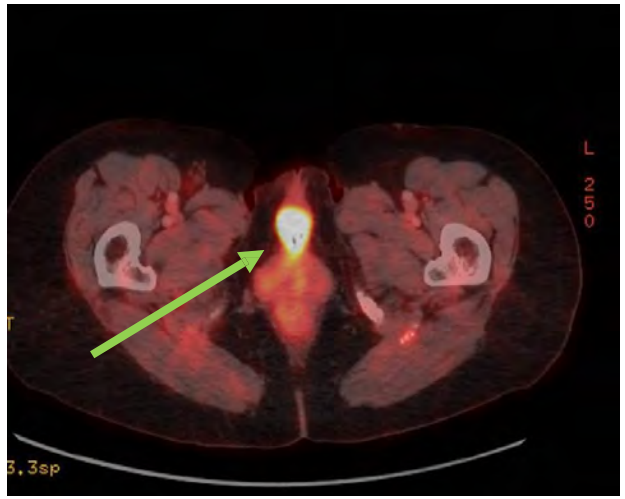


Case features modified to protect patient confidentiality. No treatment recommendations are made or should be implied.

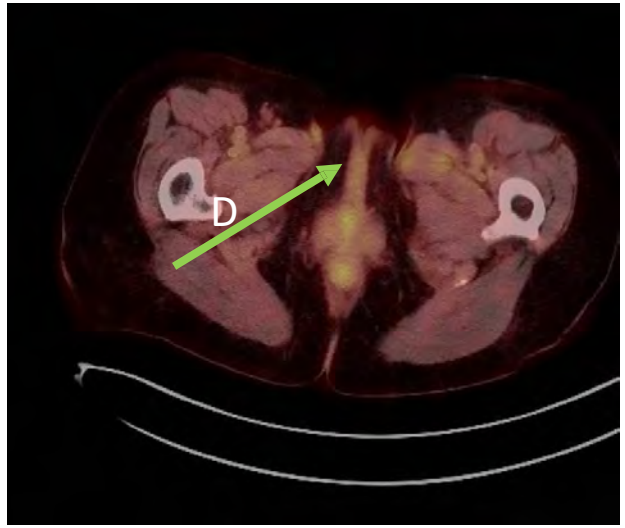
Case Study: MRD correlated with radiation response on imaging

Vulvar Carcinoma

Pre-Tx PET-CT



3-month post-Tx



- Patient with stage III vulvar carcinoma receiving chemoradiation.
- Pre-treatment PET-CT demonstrates a 3.6 x 2.1 cm enhancing mass centered in the clitoris
- 3 month PET-CT demonstrates resolution of measurable mass and SUV max 3.1
- Early clearance on treatment concordant with imaging.

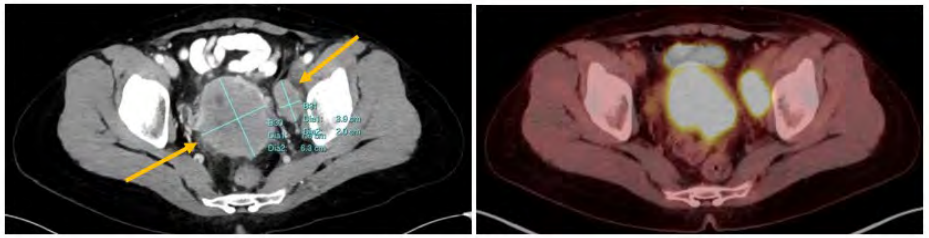
Timepoint	Reported MTM/mL
Timepoint 4	0.00
TImepoint 3	0.00
Timepoint 2	0.00
Timepoint 1	3.17

Wernicke G, Shalamov M, Ottensoser L. Utility of ctDNA as an Early Predictive Biomarker of Response to RT/CRT in Gynecologic Malignancies. Oral presentation presented at SGO, San Diego, CA. March 16-18, 2024.

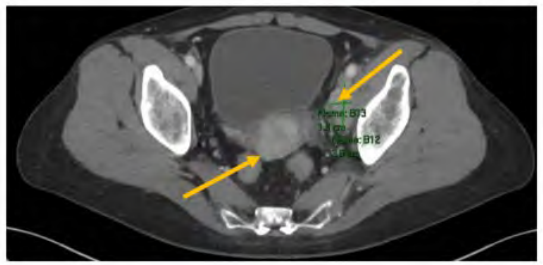
Case Study: MRD correlated with radiation response on imaging

Neuroendocrine carcinoma of the cervix

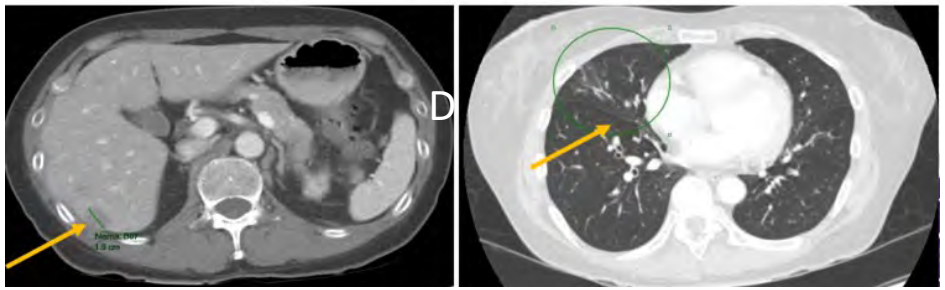
Pre-Tx
PET-CT



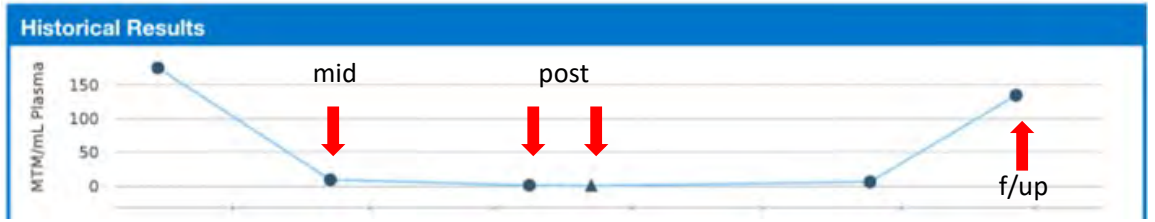
Mid-Tx



3-month
Post-Tx



- Patient underwent systemic therapy and CT/RT
- Mid-treatment showed ctDNA decrease and partial response on imaging
- Post-treatment ctDNA levels were low, but did not clear on therapy
- 3 month later, ctDNA again increased, correlating with metastases in the liver and lung



Timepoint	Reported MTM/mL
Timepoint 6	134.61
Timepoint 5	6.05
Timepoint 4	0.33
TImepoint 3	1.05
Timepoint 2	9.13
Timepoint 1	175.92

Wernicke G, Shalamov M, Ottensoser L. Utility of ctDNA as an Early Predictive Biomarker of Response to RT/CRT in Gynecologic Malignancies. Oral presentation presented at SGO, San Diego, CA. March 16-18, 2024.

Key takeaways and Q&A

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