

# Ultrasensitive ctDNA : Current Evidence and Future Directions in the Genitourinary Malignancies

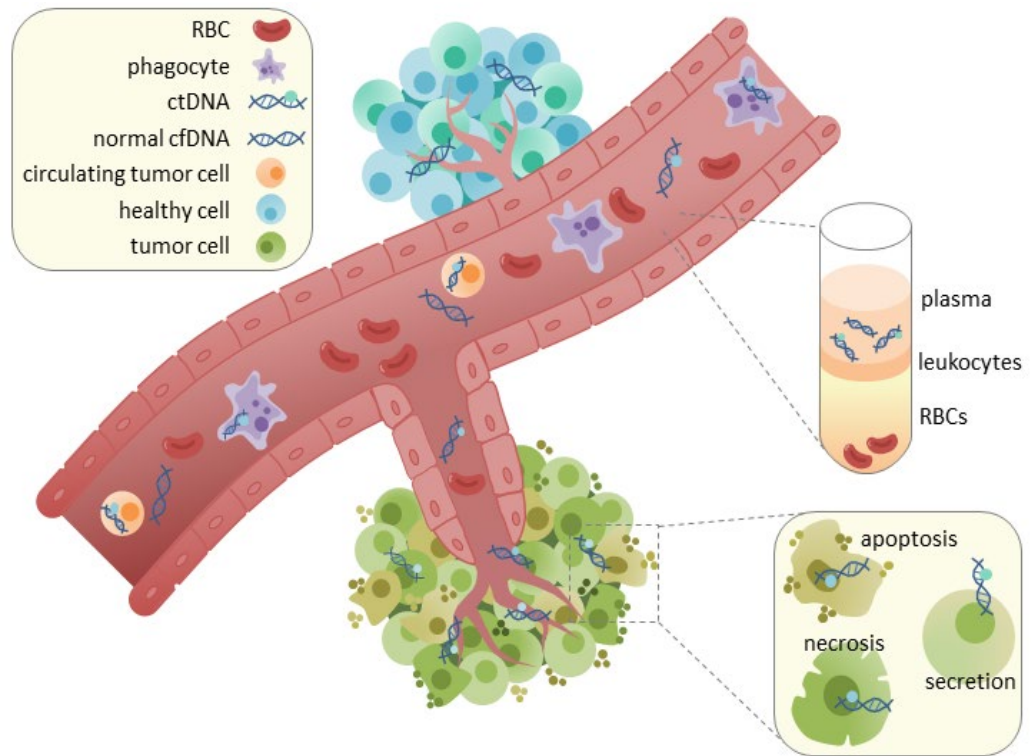
Arnab Basu, MD, MPH  
Site Lead, GU Oncology  
Mayo Clinic, Jacksonville , Florida

# **DISCLOSURE OF RELEVANT FINANCIAL RELATIONSHIP(S) WITH INELIGIBLE COMPANIES**

- Aveo Oncology (Advisory, ISR Funding) , Daiichi Sankyo (Steering Committee) Eisai (Advisory), EMD Serono (Advisory), Arcus Biosciences (Advisory/Research), Pfizer (Advisory/Research) , Natera (Advisory/Research/SC) ,BMS (Research) , Kura Oncology (Research), , Grail (Stock), Exelixis (Advisory/ Research) , Summit Therapeutics (Research), Amgen (Advisory),

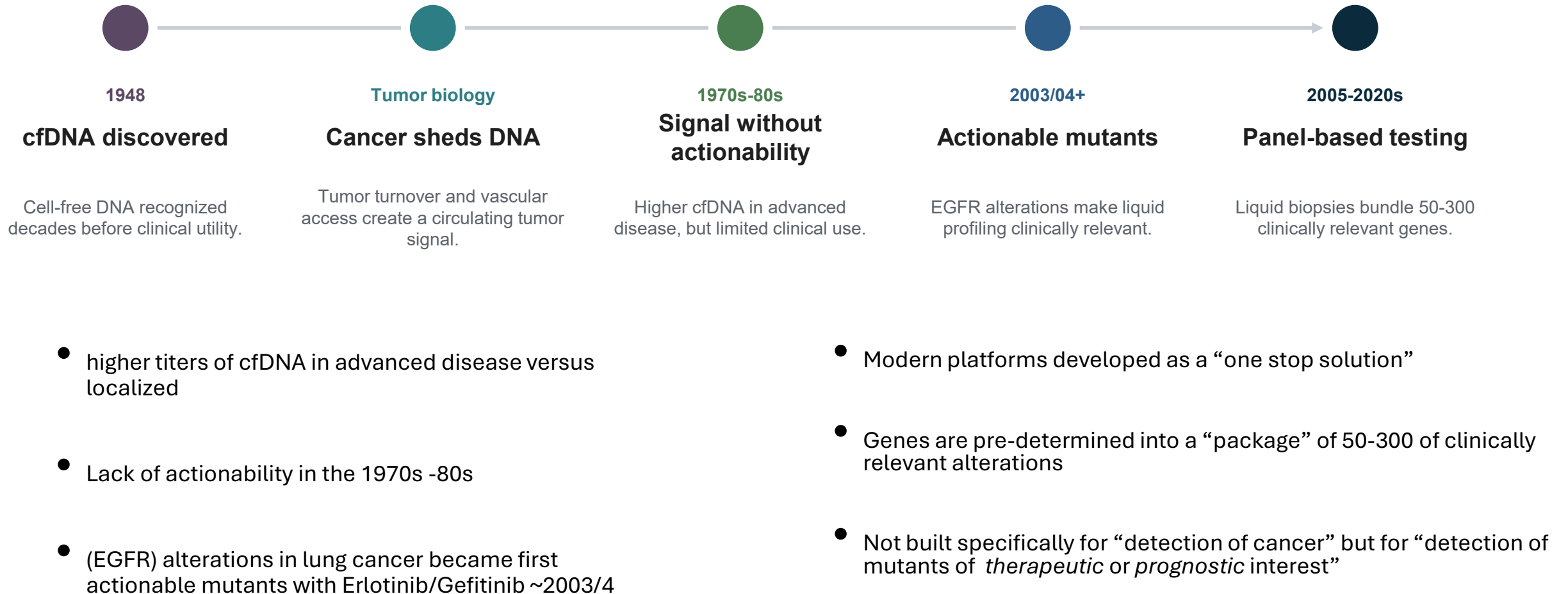
# ctDNA in cancer

- cell free DNA (cfDNA) first described 1948 - dsDNA molecules, usually 1-2 kilobases
- 1 gram tumor has approximately 1,000,000,000 cells
- All connected to circulation
- DNA turnover high in cancers ( apoptosis, necrosis, division, secretion)
- Short half life (  $t_{1/2}$  ~2 hours)



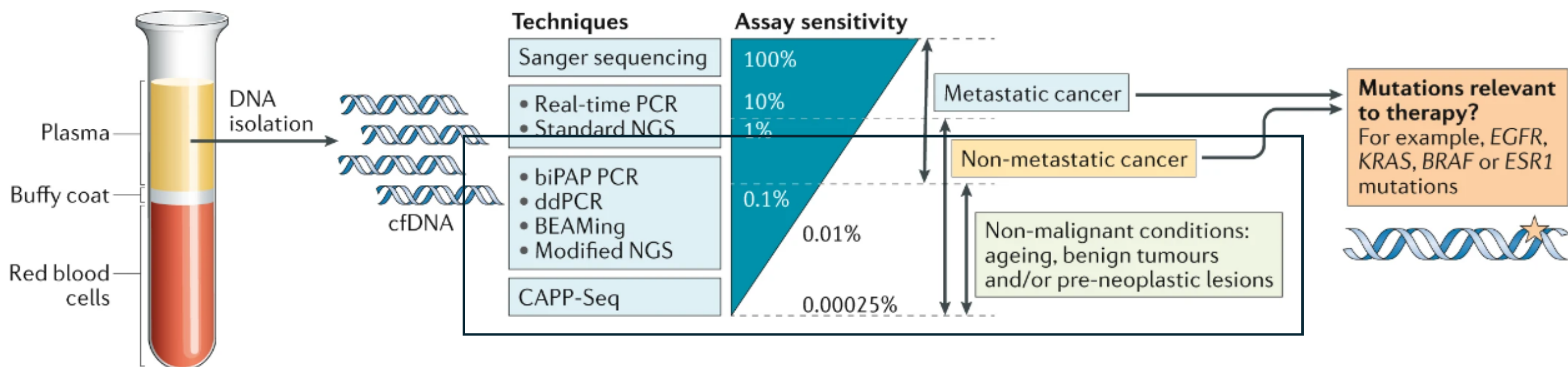
# Progression of ctDNA testing & applications

A shift from biologic observation to clinical decision support



# Ultrasensitive ctDNA tests

From: [Liquid biopsy and minimal residual disease – latest advances and implications for cure](#)

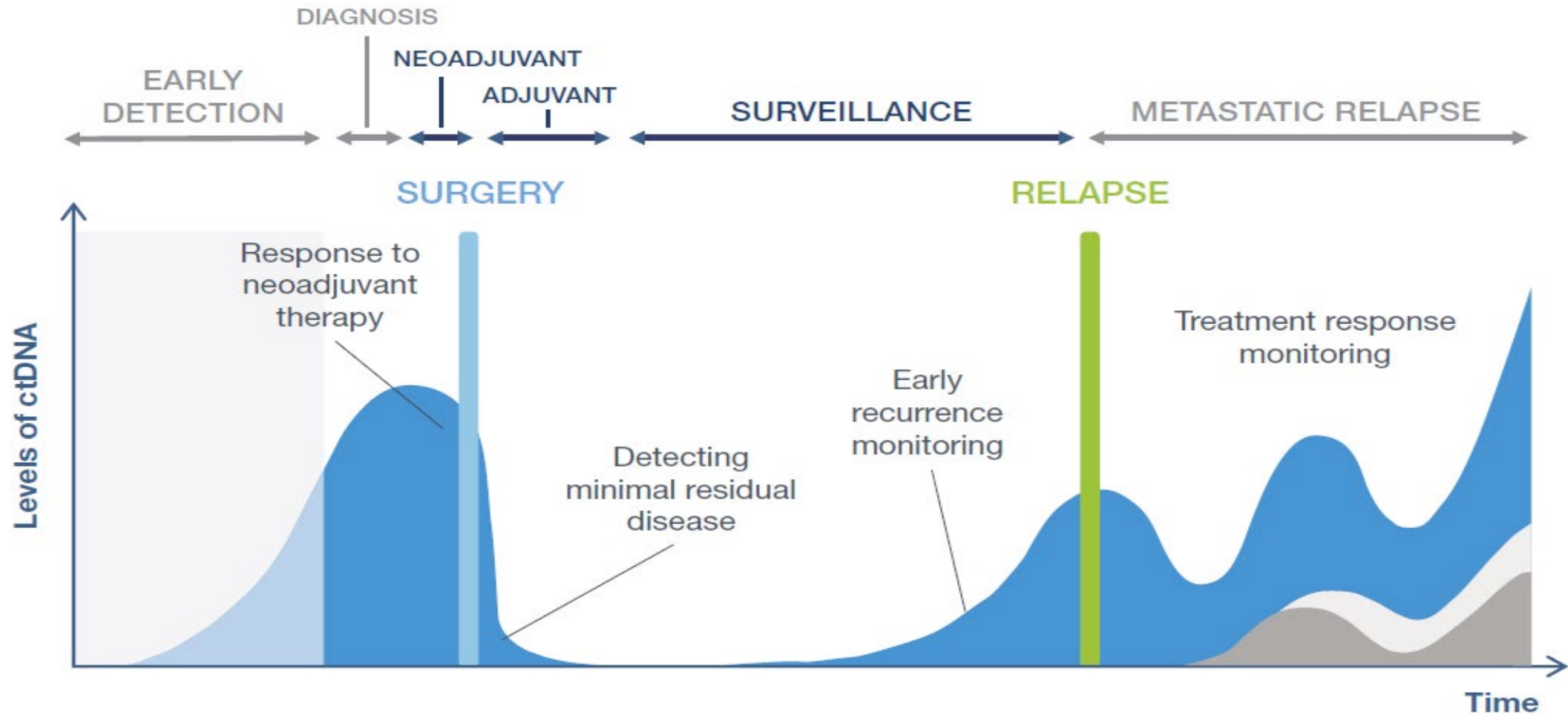


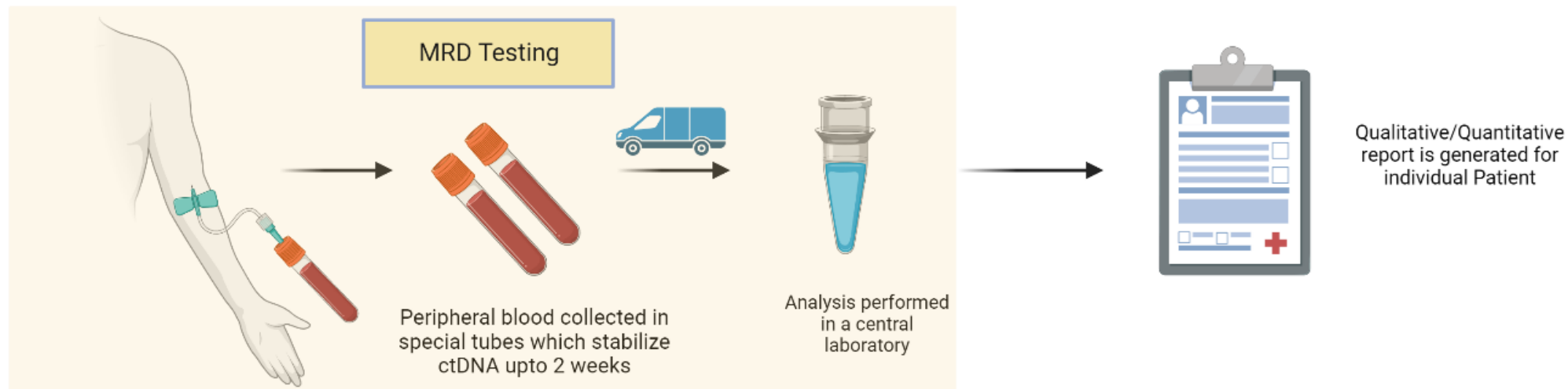
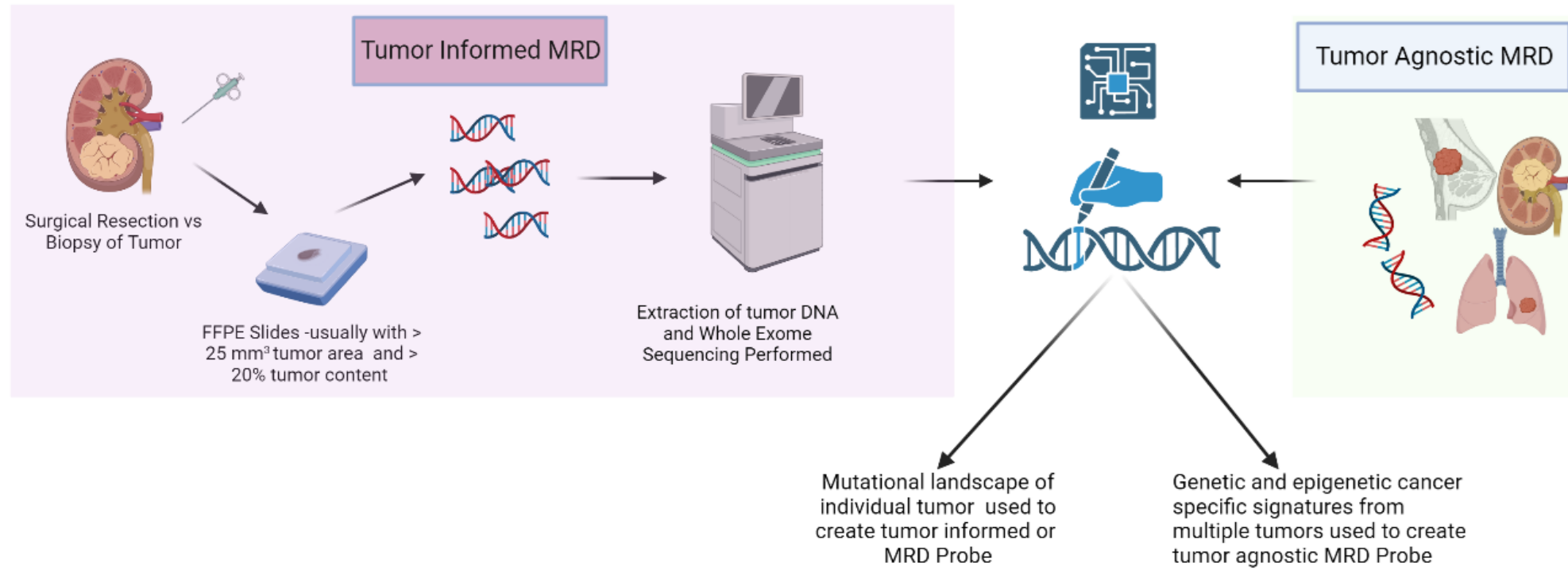
**Liquid biopsy and minimal residual disease – latest advances and implications for cure**

Klaus Pantel & Catherine Alix-Panabières

*Nature Reviews Clinical Oncology* **16**, 409–424 (2019) | [Cite this article](#)

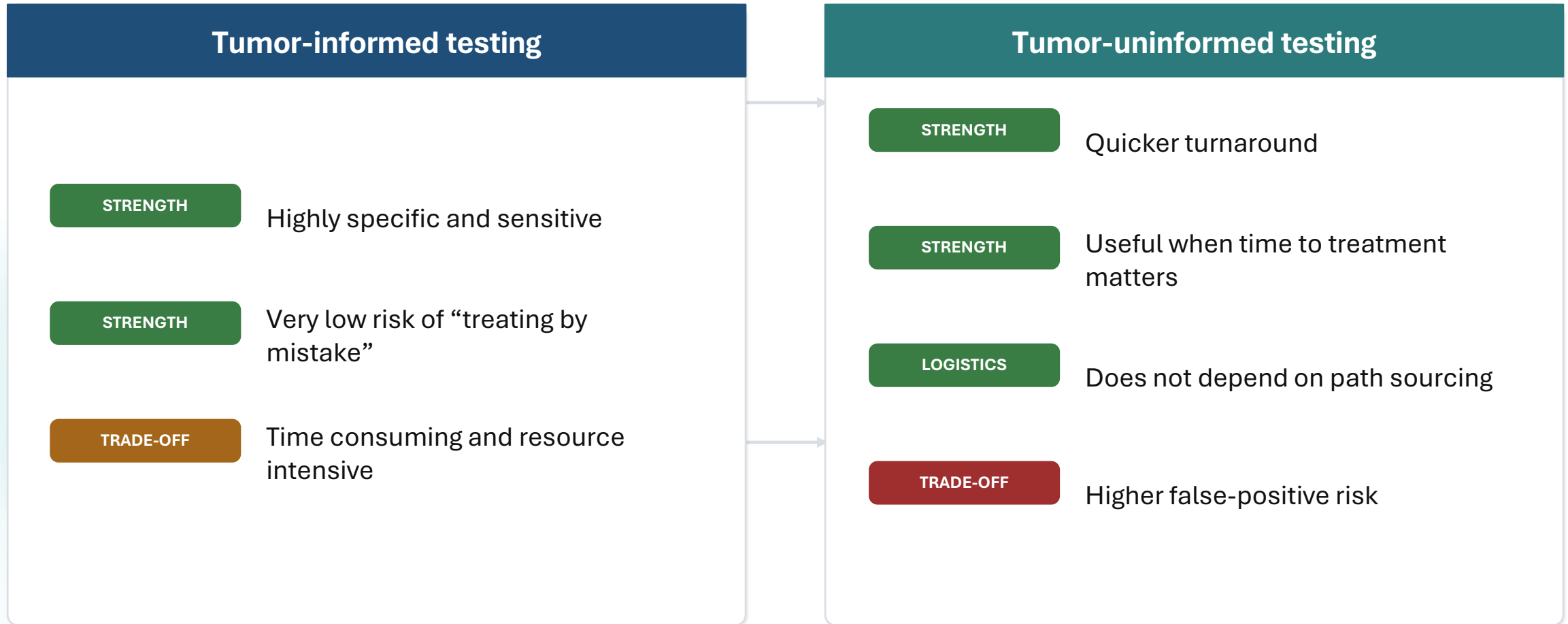
# Clinical applications of ultrasensitive ctDNA





# Practical considerations on MRD testing

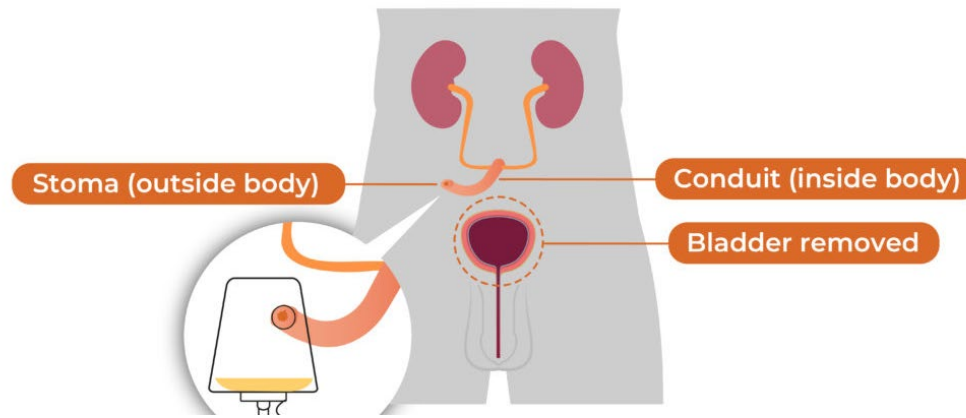
Tumor-informed vs tumor-uninformed approaches



**Key decision:** prioritize specificity and tissue-linked confidence, or speed and tissue-independent access.

# Management of MIBC

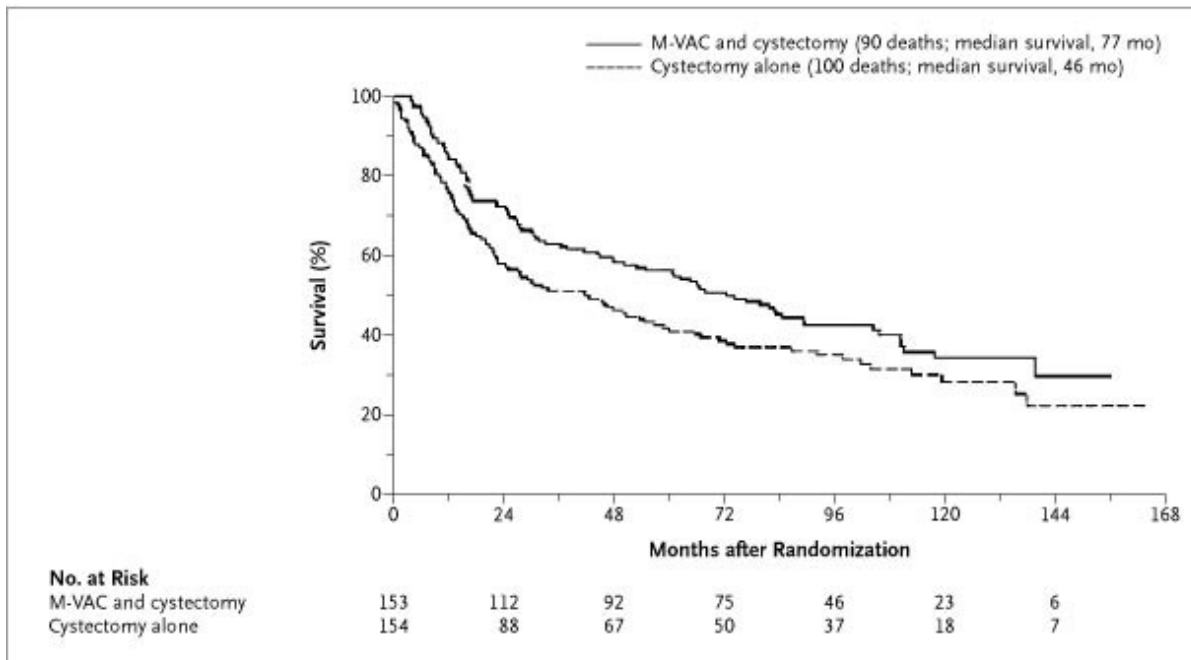
# Neoadjuvant Micro metastasis and Primary directed therapy



# Radical Cystectomy

## Adjuvant – Micro metastasis directed therapy

# Molecular Residual Disease Testing



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ORIGINAL ARTICLE



# Neoadjuvant Chemotherapy plus Cystectomy Compared with Cystectomy Alone for Locally Advanced Bladder Cancer

**i** This article has been corrected. [VIEW THE CORRECTION](#)

**Authors:** H. Barton Grossman, M.D., Ronald B. Natale, M.D., Catherine M. Tangen, Dr.P.H., V.O. Speights, D.O., Nicholas J. Vogelzang, M.D., Donald L. Trump, M.D., Ralph W. deVere White, M.D., Michael F. Sarosdy, M.D., David P. Wood, Jr., M.D., Derek Raghavan, M.D., Ph.D., and E. David Crawford, M.D. [Author Info & Affiliations](#)

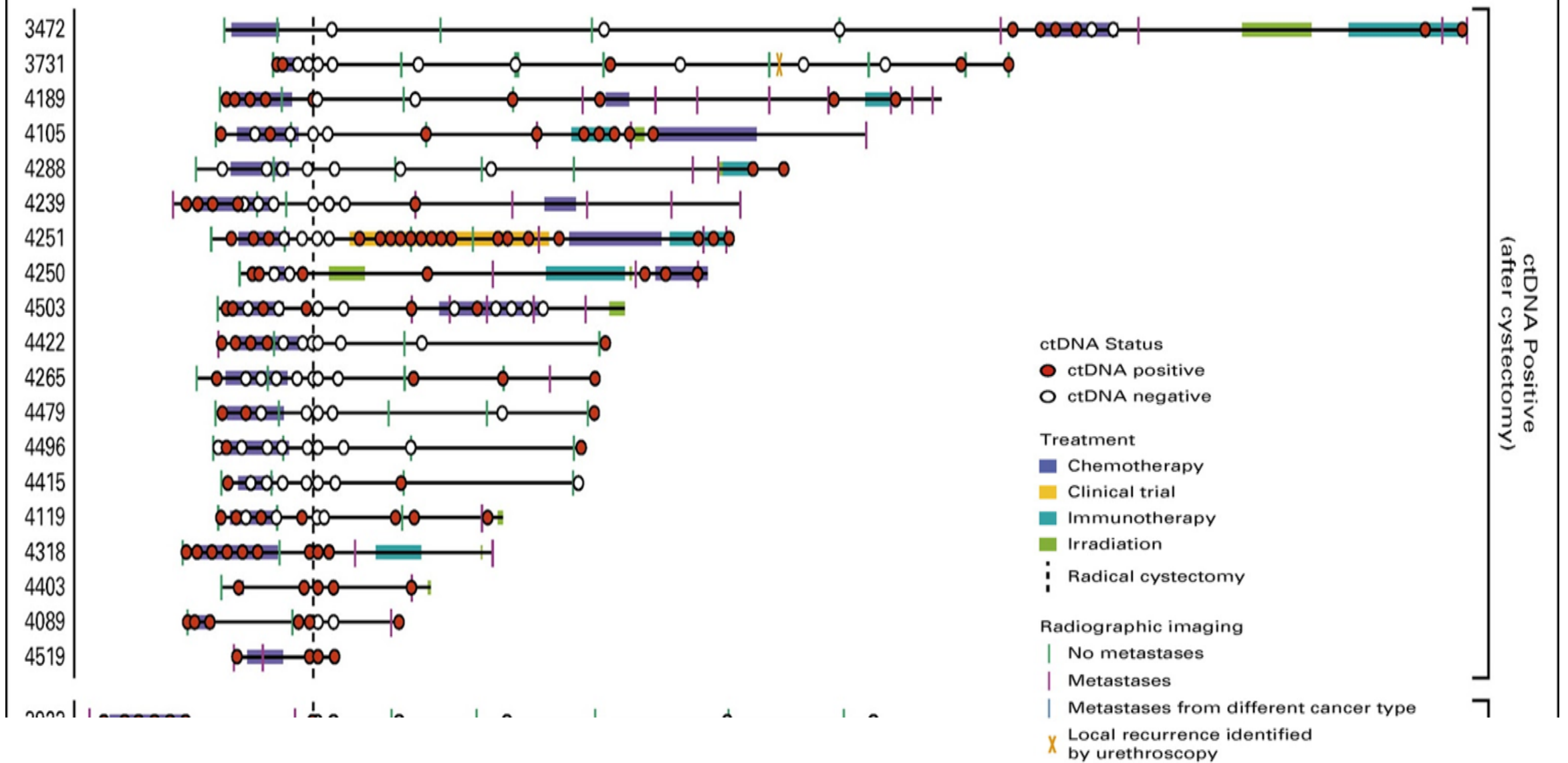
Published August 28, 2003 | N Engl J Med 2003;349:859-866 | DOI: 10.1056/NEJMoa022148 | [VOL. 349 NO. 9](#)

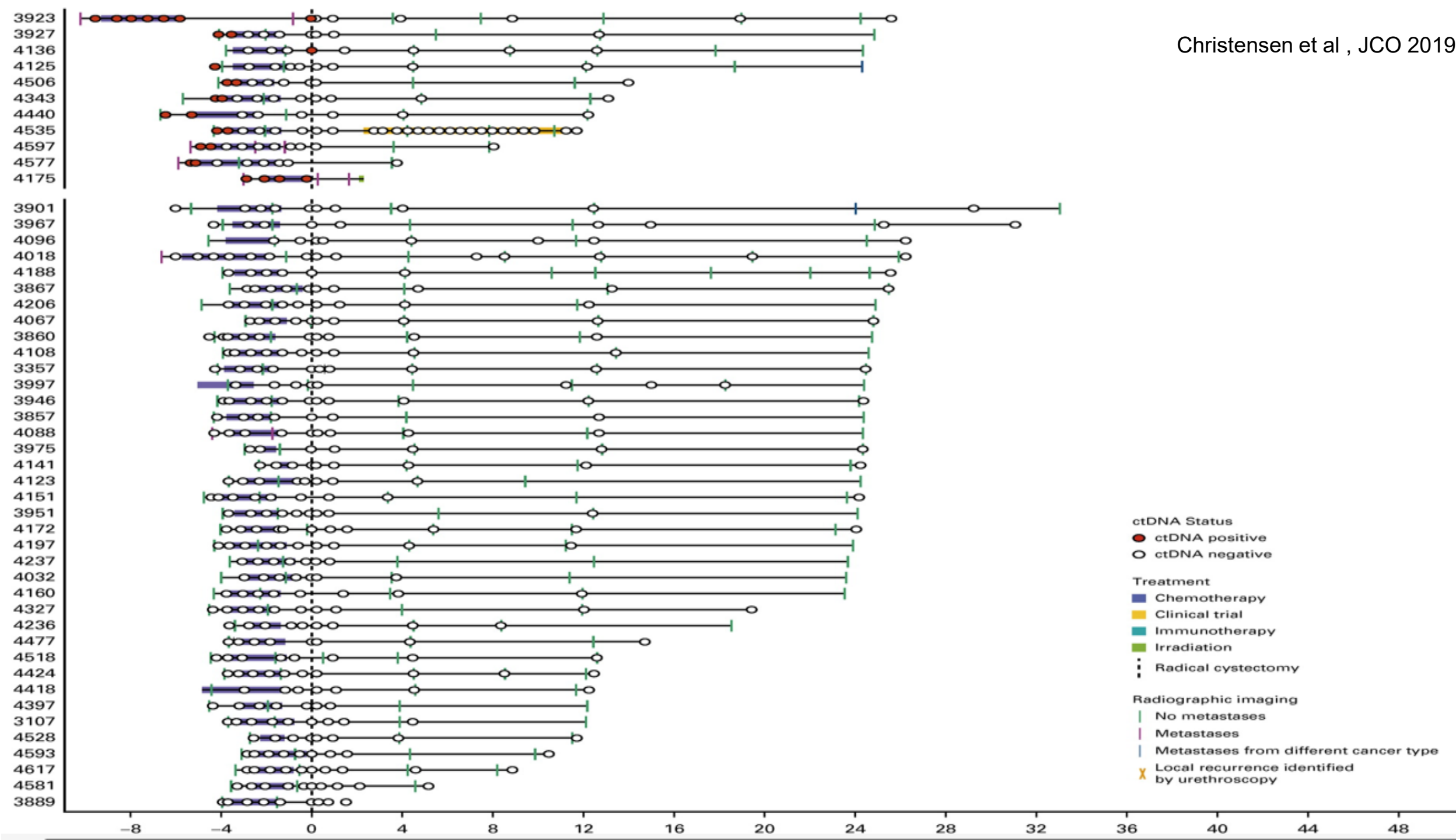
Neoadjuvant chemotherapy demonstrating the benefit of targeting micro-metastatic disease

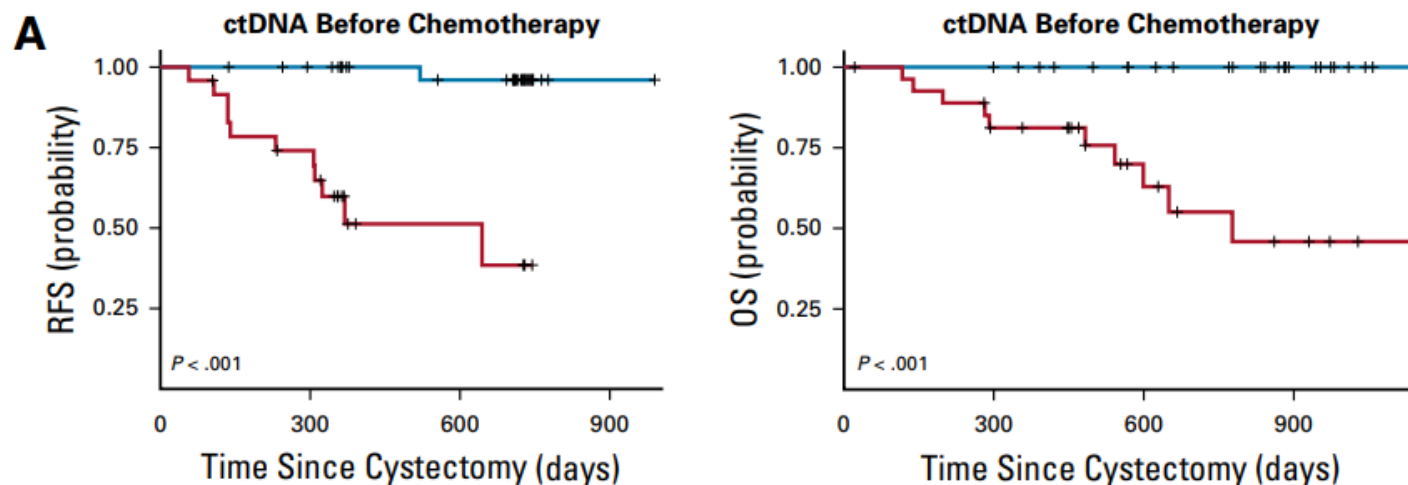
# Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma

Emil Christensen, PhD<sup>1</sup>; Karin Birkenkamp-Demtröder, PhD<sup>1</sup>; Himanshu Sethi, MPH<sup>2</sup>; Svetlana Shchegrova, PhD<sup>2</sup>; Raheleh Salari, PhD<sup>2</sup>; Iver Nordentoft, PhD<sup>1</sup>; Hsin-Ta Wu, PhD<sup>2</sup>; Michael Knudsen, PhD<sup>1</sup>; Philippe Lamy, PhD<sup>1</sup>; Sia Viborg Lindskrog, BS<sup>1</sup>; Ann Taber, MD<sup>1</sup>; Mustafa Balcioglu, PhD<sup>2</sup>; Søren Vang, PhD<sup>1</sup>; Zoe Assaf, PhD<sup>2</sup>; Shruti Sharma, PhD<sup>2</sup>; Antony S. Tin, PhD<sup>2</sup>; Ramya Srinivasan, MS<sup>2</sup>; Dina Hafez, PhD<sup>2</sup>; Thomas Reinert, PhD<sup>1</sup>; Samantha Navarro, BS<sup>2</sup>; Alexander Olson, BS<sup>2</sup>; Rosalyn Ram, PhD<sup>2</sup>; Scott Dashner, BS<sup>2</sup>; Matthew Rabinowitz, PhD<sup>2</sup>; Paul Billings, MD, PhD<sup>2</sup>; Styrmir Sigurjonsson, PhD<sup>2</sup>; Claus Lindbjerg Andersen, PhD<sup>1</sup>; Ryan Swenerton, PhD<sup>2</sup>; Alexey Aleshin, MD<sup>2</sup>; Bernhard Zimmermann, PhD<sup>2</sup>; Mads Agerbæk, MD<sup>1</sup>; Cheng-Ho Jimmy Lin, MD, PhD, MHS<sup>2</sup>; Jørgen Bjerggaard Jensen, MD, DMSc<sup>1,3</sup>; and Lars Dyrskjød, PhD<sup>1,3</sup>

- 68 patients with MIBC at Aarhus University, followed with a tumor informed MRD panel through NAC, cystectomy and followup
- 100% sensitivity for detection of subsequent relapse, 98% specificity







No. at risk

Negative

35

32

23

1

Positive

24

16

4

0

No. at risk

Negative

36

35

26

16

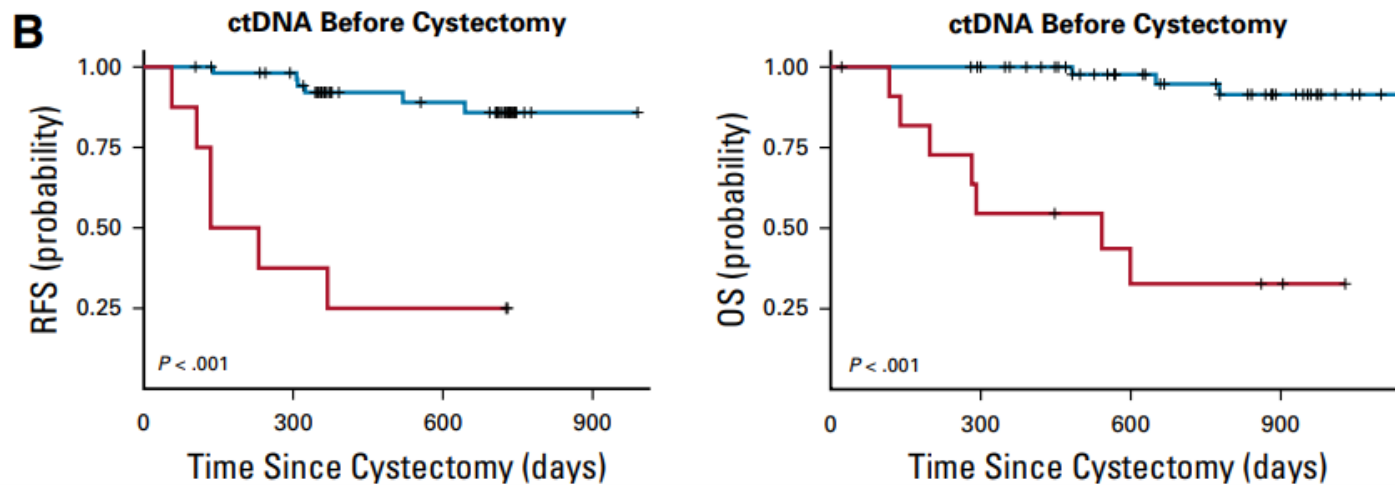
Positive

27

20

9

4

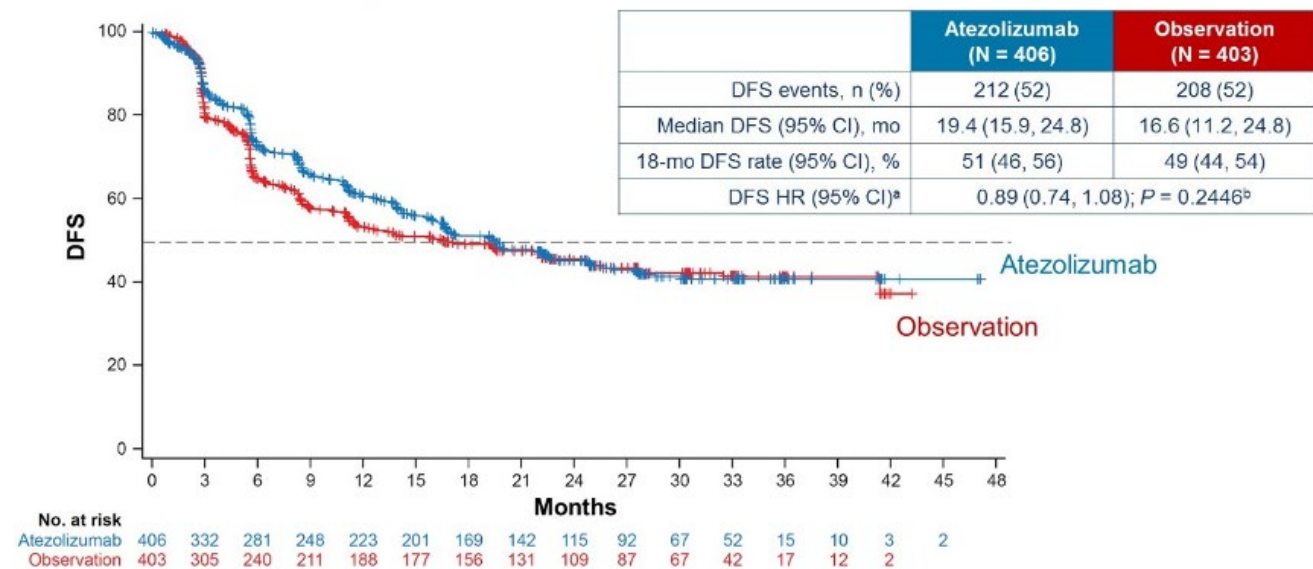


Molecular residual disease clearance is associated with excellent outcomes for NAT

Persistence of ctDNA after NAT is associated with high recurrence risk.

# ctDNA : adjuvant UC

## DFS in ITT Population



Data cutoff: November 30, 2019. Median follow-up: 21.9 mo. <sup>a</sup> Stratified by post-resection tumor stage, nodal status and PD-L1 status. <sup>b</sup> 2-sided.

PRESENTED AT: 2020 ASCO  
ANNUAL MEETING

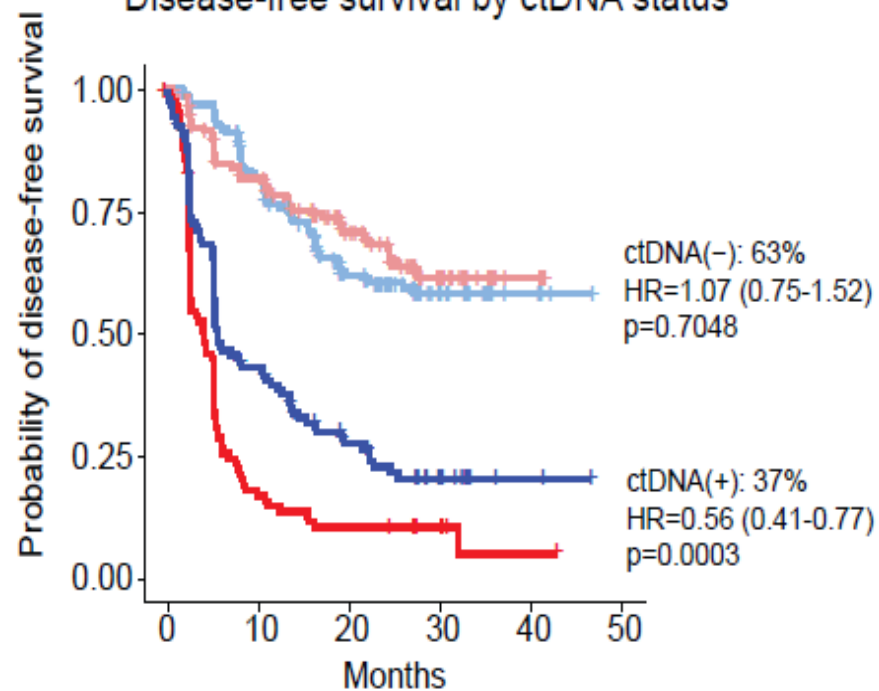
#ASCO20  
Slides are the property of the author.  
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PRESENTED BY: Hussain M. IMVigor010 primary analysis [abs 5000]

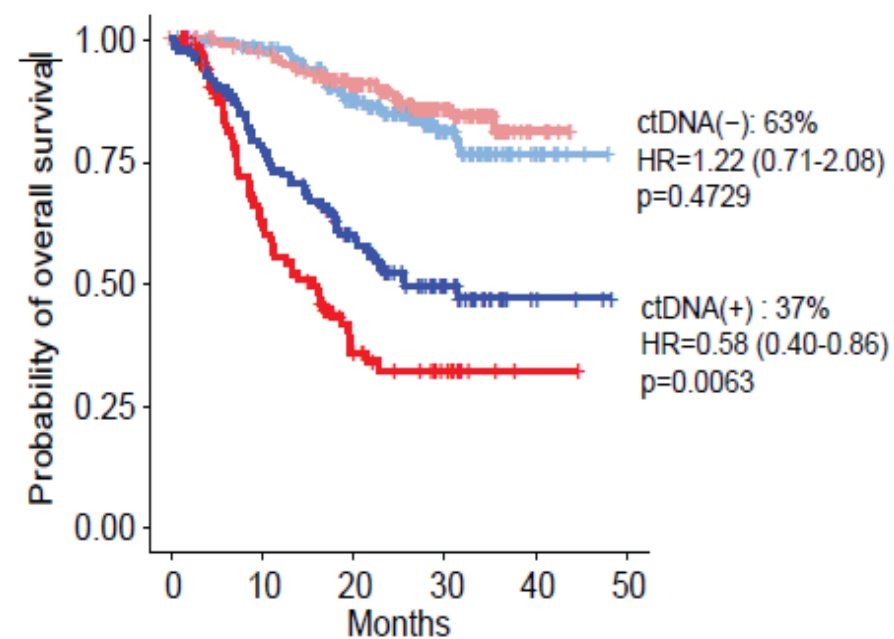
<https://bit.ly/2SKSAD3>



IMVigor010 - No benefit noted of adjuvant atezolizumab in high risk resected MIBC [ ypt2+ or pt3+]

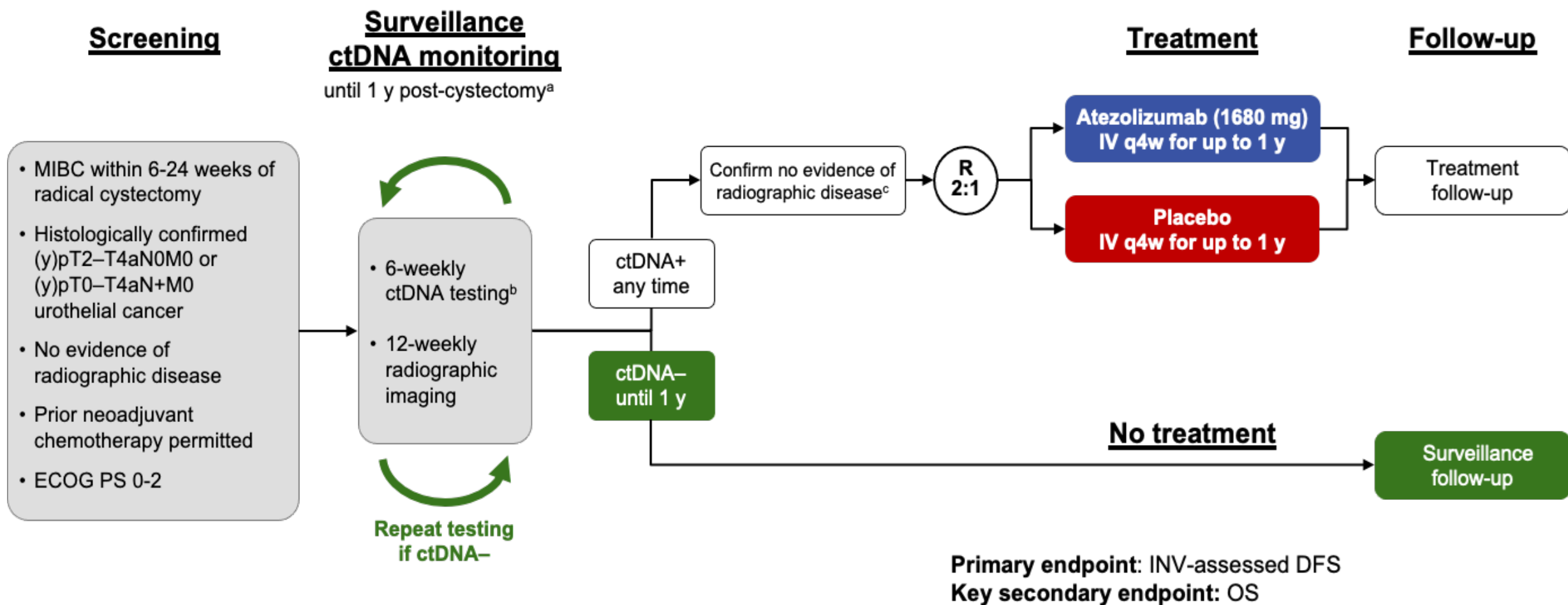
**a****Disease-free survival by ctDNA status**

| No. at risk    |          |     |     |    |    |   |   |
|----------------|----------|-----|-----|----|----|---|---|
| — Atezolizumab | ctDNA(-) | 184 | 144 | 85 | 44 | 5 | 0 |
|                |          | 183 | 140 | 90 | 46 | 6 | 0 |
| — Observation  | ctDNA(+) | 116 | 48  | 25 | 13 | 2 | 0 |
|                |          | 98  | 17  | 10 | 5  | 1 | 0 |

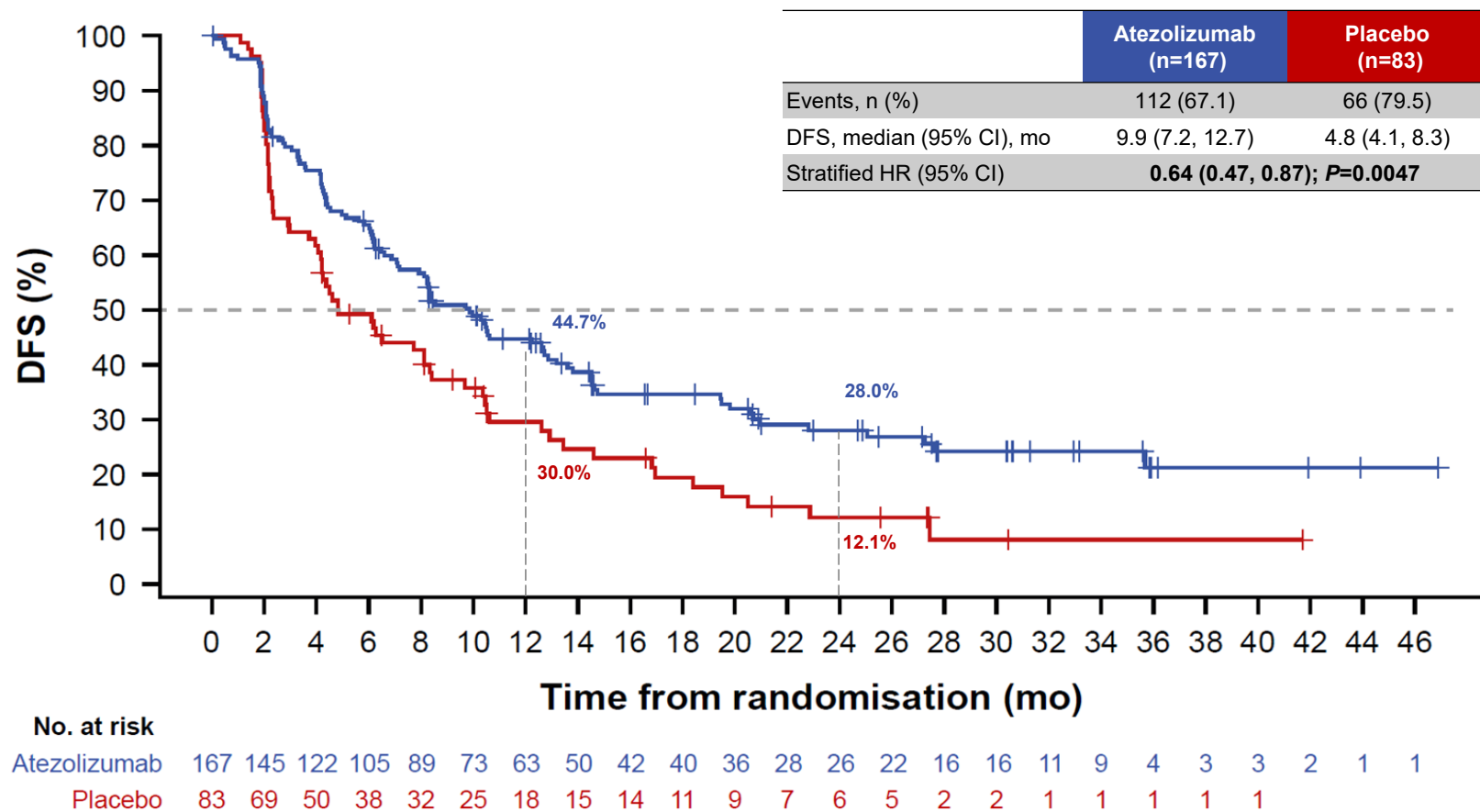
**b****Overall survival by ctDNA status**

| No. at risk    |          |     |     |     |    |    |   |
|----------------|----------|-----|-----|-----|----|----|---|
| — Atezolizumab | ctDNA(-) | 184 | 174 | 129 | 57 | 10 | 0 |
|                |          | 183 | 170 | 130 | 65 | 7  | 0 |
| — Observation  | ctDNA(+) | 116 | 88  | 55  | 25 | 4  | 0 |
|                |          | 98  | 54  | 24  | 11 | 1  | 0 |

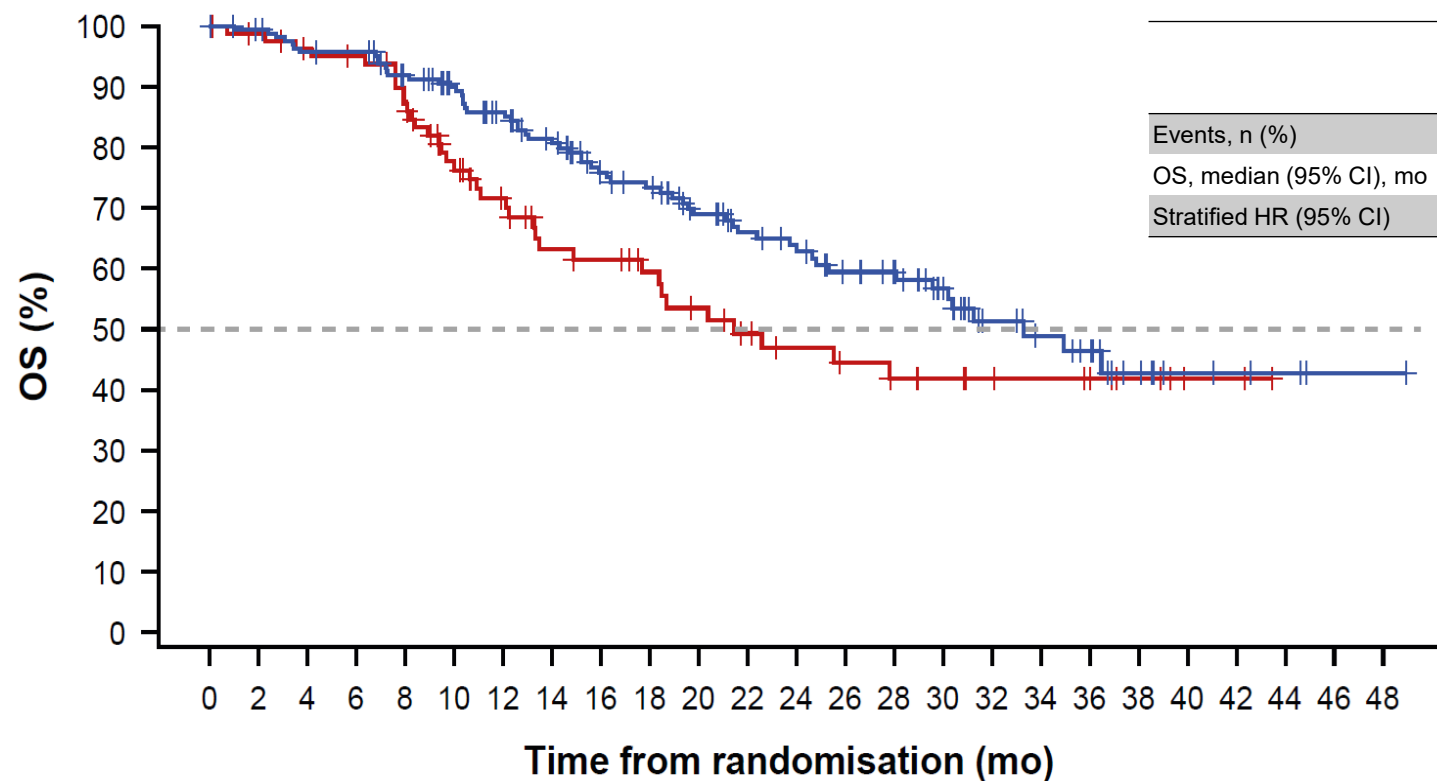
# IMvigor011: Study Design



# Imvigor011 – DFS



# IMvigor011 – Overall Survival



No. at risk

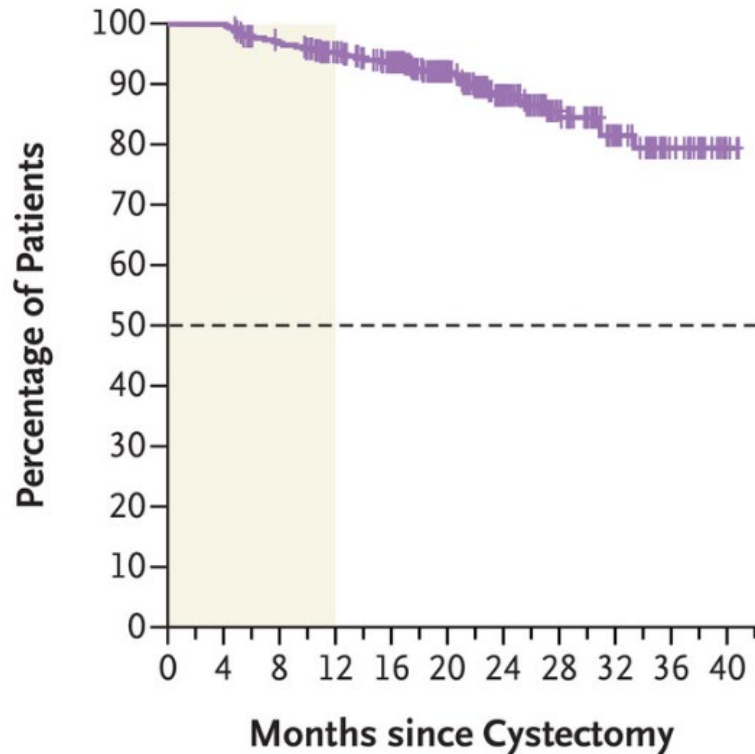
|              |     |     |     |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |   |   |   |   |   |   |
|--------------|-----|-----|-----|-----|-----|-----|-----|-----|----|----|----|----|----|----|----|----|----|----|----|---|---|---|---|---|---|
| Atezolizumab | 167 | 162 | 155 | 154 | 143 | 130 | 118 | 108 | 92 | 86 | 75 | 65 | 59 | 51 | 43 | 30 | 23 | 19 | 12 | 7 | 5 | 3 | 2 | 1 | 1 |
| Placebo      | 83  | 80  | 76  | 74  | 65  | 53  | 44  | 36  | 34 | 30 | 26 | 21 | 19 | 17 | 15 | 13 | 10 | 10 | 8  | 5 | 2 | 1 |   |   |   |

|                         | Atezolizumab<br>(n=167)            | Placebo<br>(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)  | <b>0.59 (0.39, 0.90); P=0.0131</b> |                   |

42% improvement in overall survival in the MRD+ population

# Can We observe patients who don't have MRD?

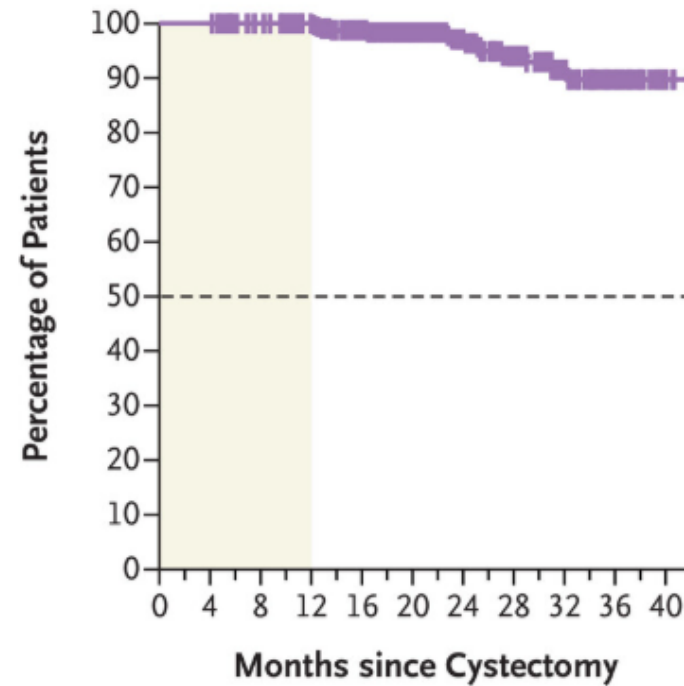
**C** Disease-free Survival among Patients with Persistent ctDNA-Negative Status



**No. at Risk**

No atezolizumab or placebo 357 357 337 303 275 185 116 78 46 20 3

**F** Overall Survival among Patients with Persistent ctDNA-Negative Status



**No. at Risk**

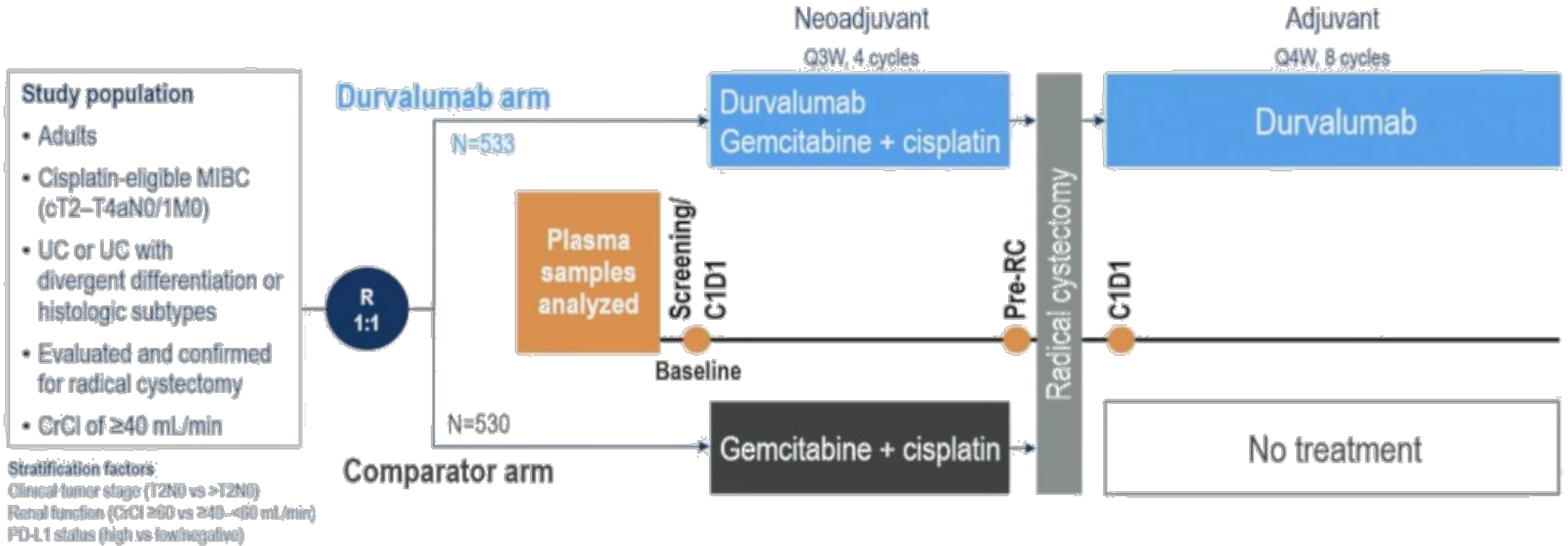
No atezolizumab or placebo 357 357 340 319 288 198 138 96 55 26 4

MRD- patients have very low recurrence risk (31/357; 8.6%) and death

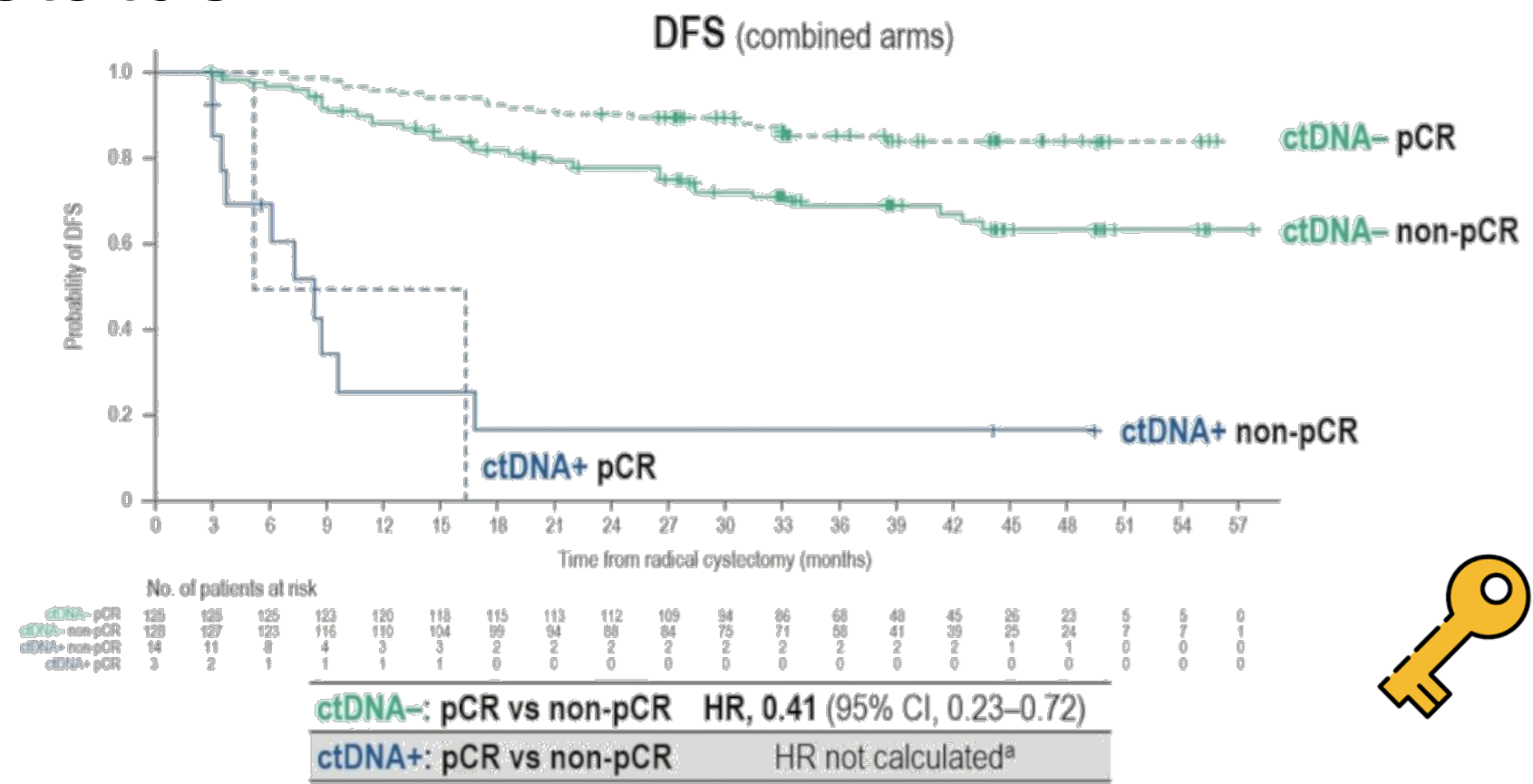
**(OS 100% and 97.1% at year 1 and 2 respectively).**

Powles et al , ESMO 2025

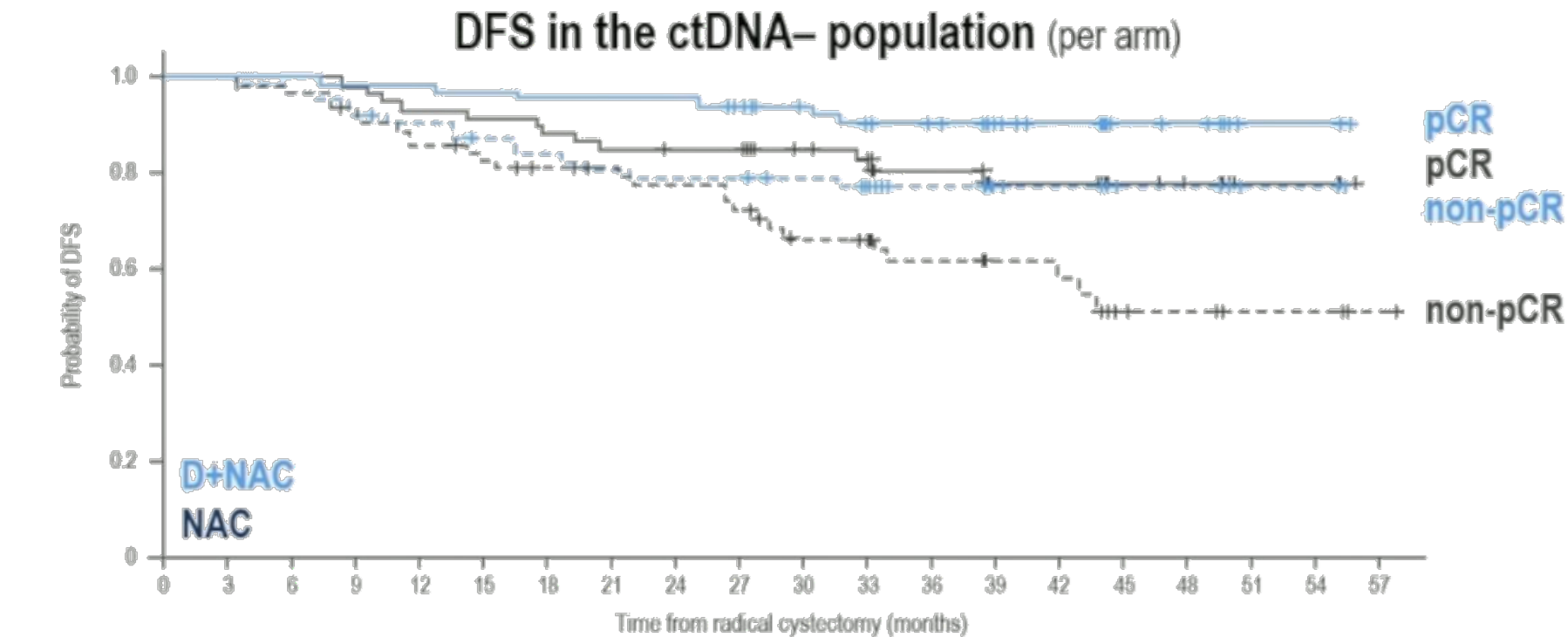
# NIAGARA – ctDNA Analysis



# NIAGARA : Disease Free Survival pCR/ctDNA status



40% of ctDNA –ves at this timepoint turn +ve in Imvigor011



|               | No. of patients at risk |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |   |
|---------------|-------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|
| pCR D+NAC     | 66                      | 66 | 66 | 65 | 65 | 64 | 63 | 63 | 63 | 60 | 53 | 48 | 38 | 26 | 23 | 13 | 12 | 2 |
| pCR NAC       | 59                      | 59 | 59 | 58 | 55 | 54 | 52 | 50 | 49 | 49 | 41 | 38 | 30 | 22 | 22 | 13 | 11 | 3 |
| non-pCR D+NAC | 62                      | 62 | 60 | 57 | 55 | 52 | 50 | 47 | 45 | 45 | 42 | 39 | 33 | 23 | 22 | 15 | 15 | 4 |
| non-pCR NAC   | 66                      | 65 | 63 | 59 | 55 | 52 | 49 | 47 | 43 | 39 | 33 | 32 | 25 | 18 | 17 | 10 | 9  | 3 |

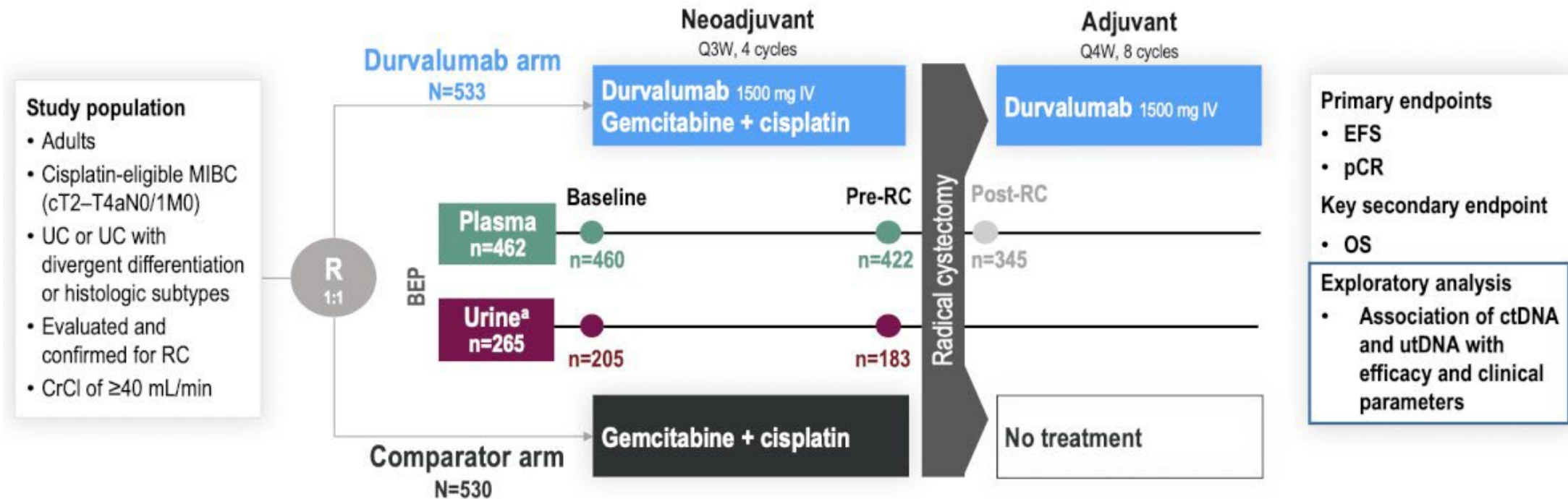
pCR: **D+NAC** vs NAC HR, 0.42 (95% CI, 0.16–1.12)

Non-pCR: **D+NAC** vs NAC HR, 0.54 (95% CI, 0.28–1.03)

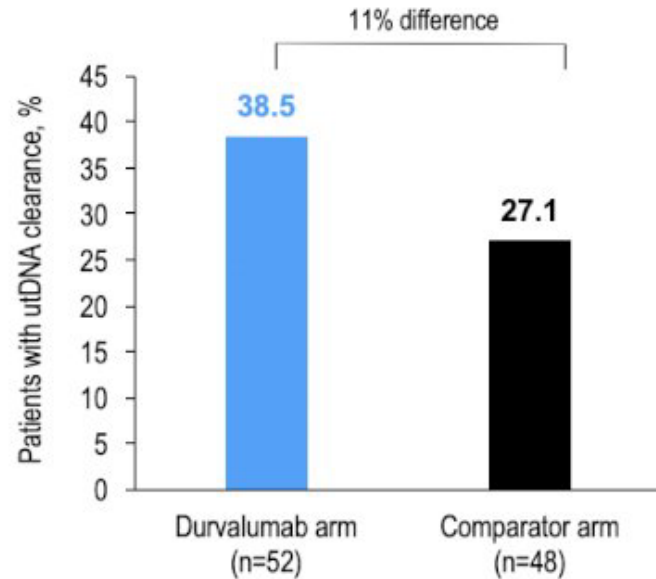
Durvalumab arm = D+NAC; Comparator arm = NAC

Recall, ctDNA –ve here refers to C1D1 population only

# utdna in MIBC – the new kid on the block

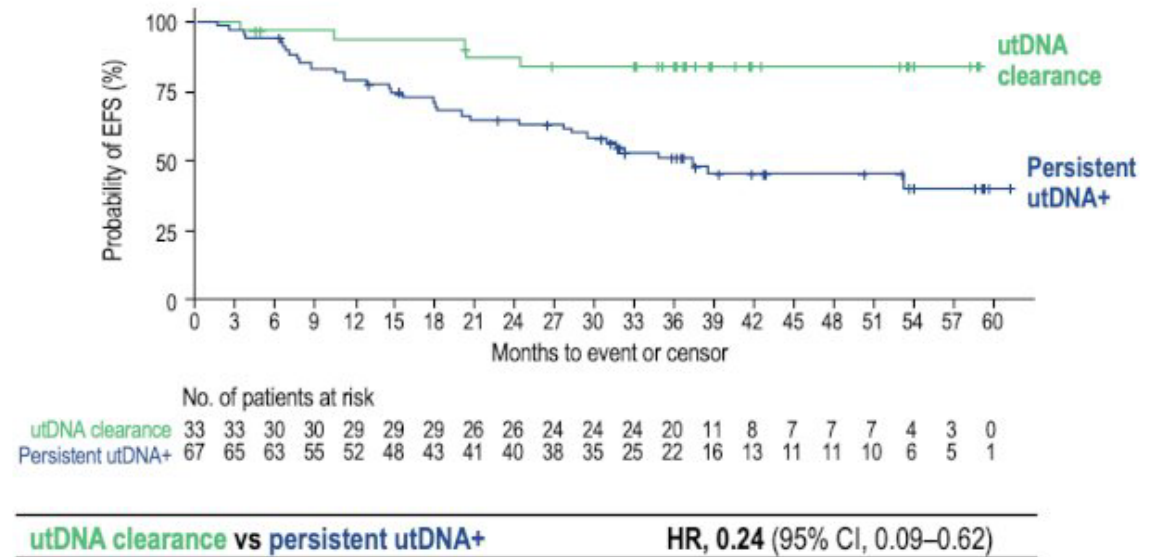


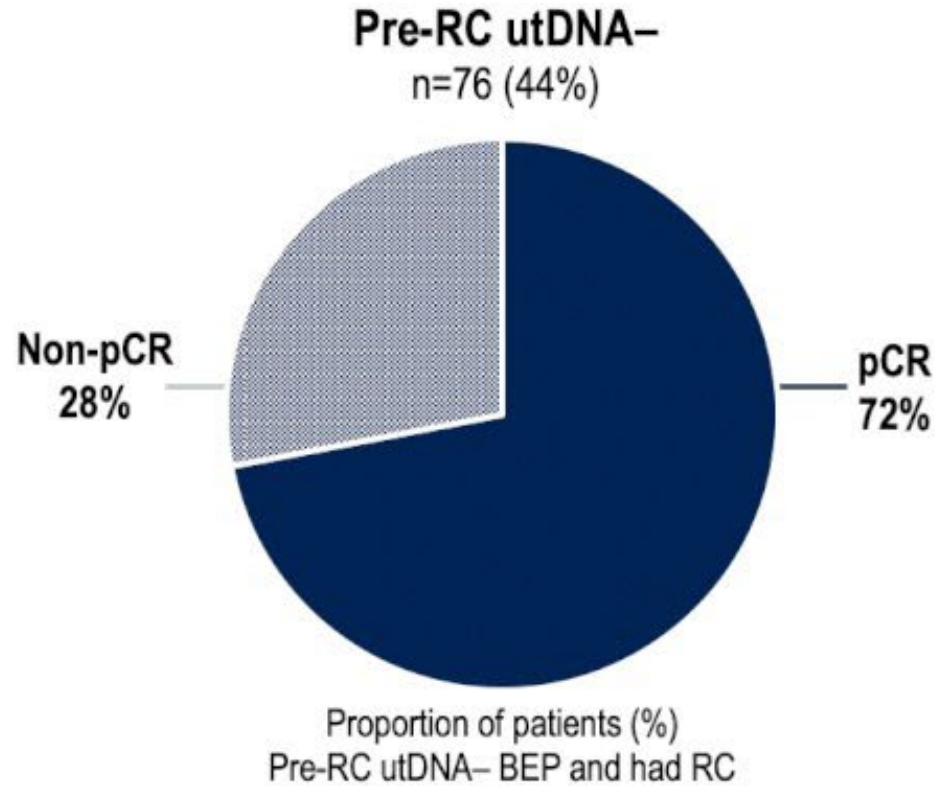
### utDNA clearance from baseline to pre-RC



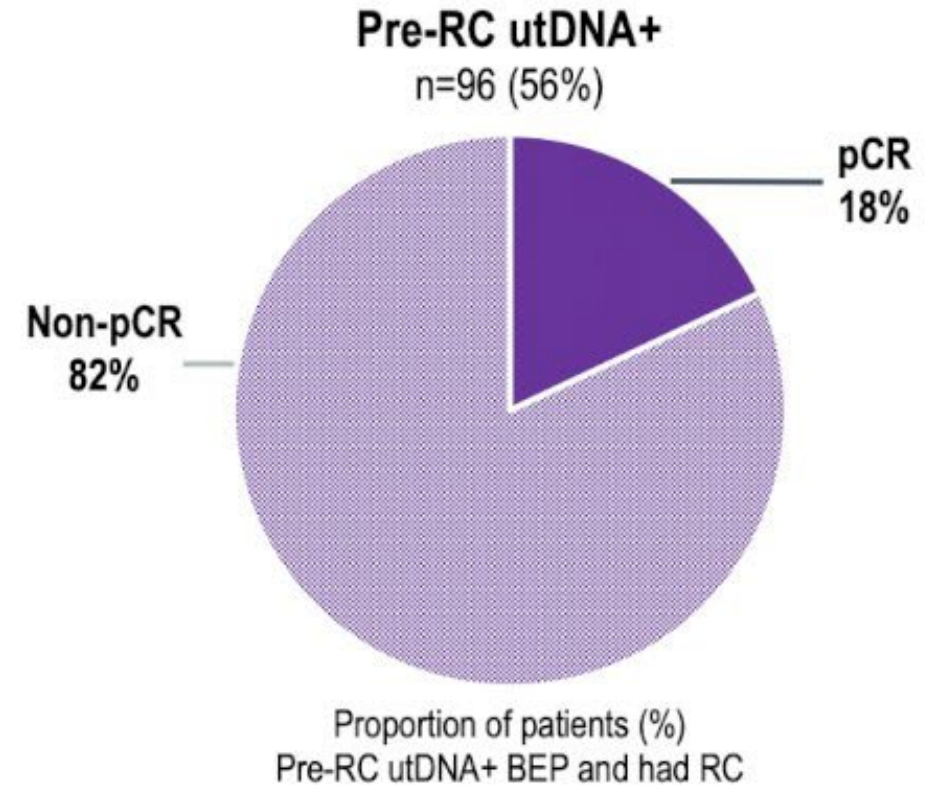
Includes all patients who were utDNA+ at baseline and had pre-RC utDNA samples

### EFS in the baseline utDNA+ population with or without utDNA clearance pre-RC (combined arms)



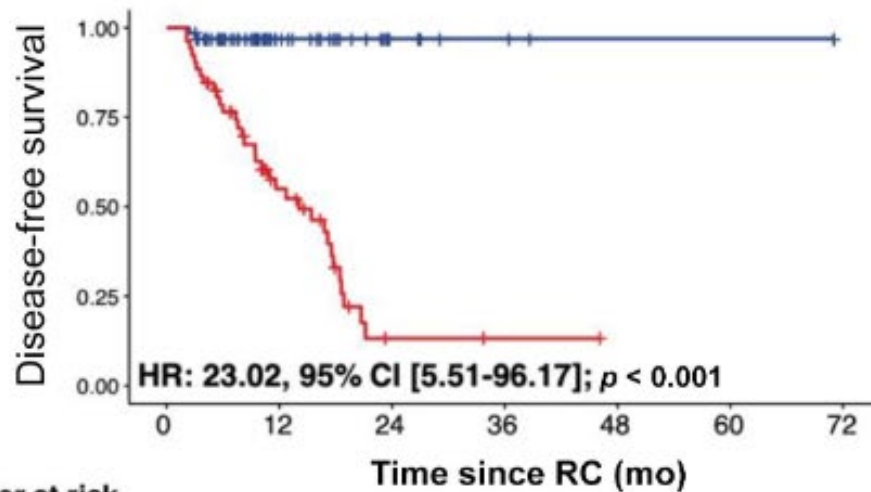


**utDNA– status at pre-RC is associated with higher likelihood of pCR**



**utDNA+ status at pre-RC is associated with higher likelihood of non-pCR**

A

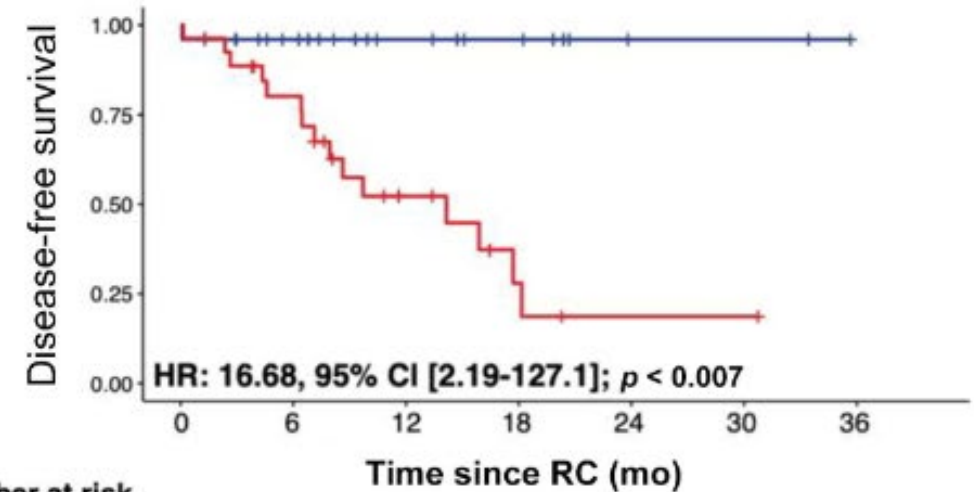


| Number at risk     |    |                    |    |    |    |    |    |    |
|--------------------|----|--------------------|----|----|----|----|----|----|
| Status             |    | Time since RC (mo) |    |    |    |    |    |    |
|                    |    | 0                  | 12 | 24 | 36 | 48 | 60 | 72 |
| ctDNA undetectable | 65 | 24                 | 6  | 3  | 1  | 1  | 0  |    |
| ctDNA detectable   | 52 | 20                 | 2  | 1  | 0  | 0  | 0  |    |

| Status      | ctDNA undetectable | ctDNA detectable |
|-------------|--------------------|------------------|
| Events %    | 3.1 (2/65)         | 63.5 (33/52)     |
| 24-mo DFS % | 96.9 (88.2-99.2)   | 13.2 (3.8-28.6)  |

B



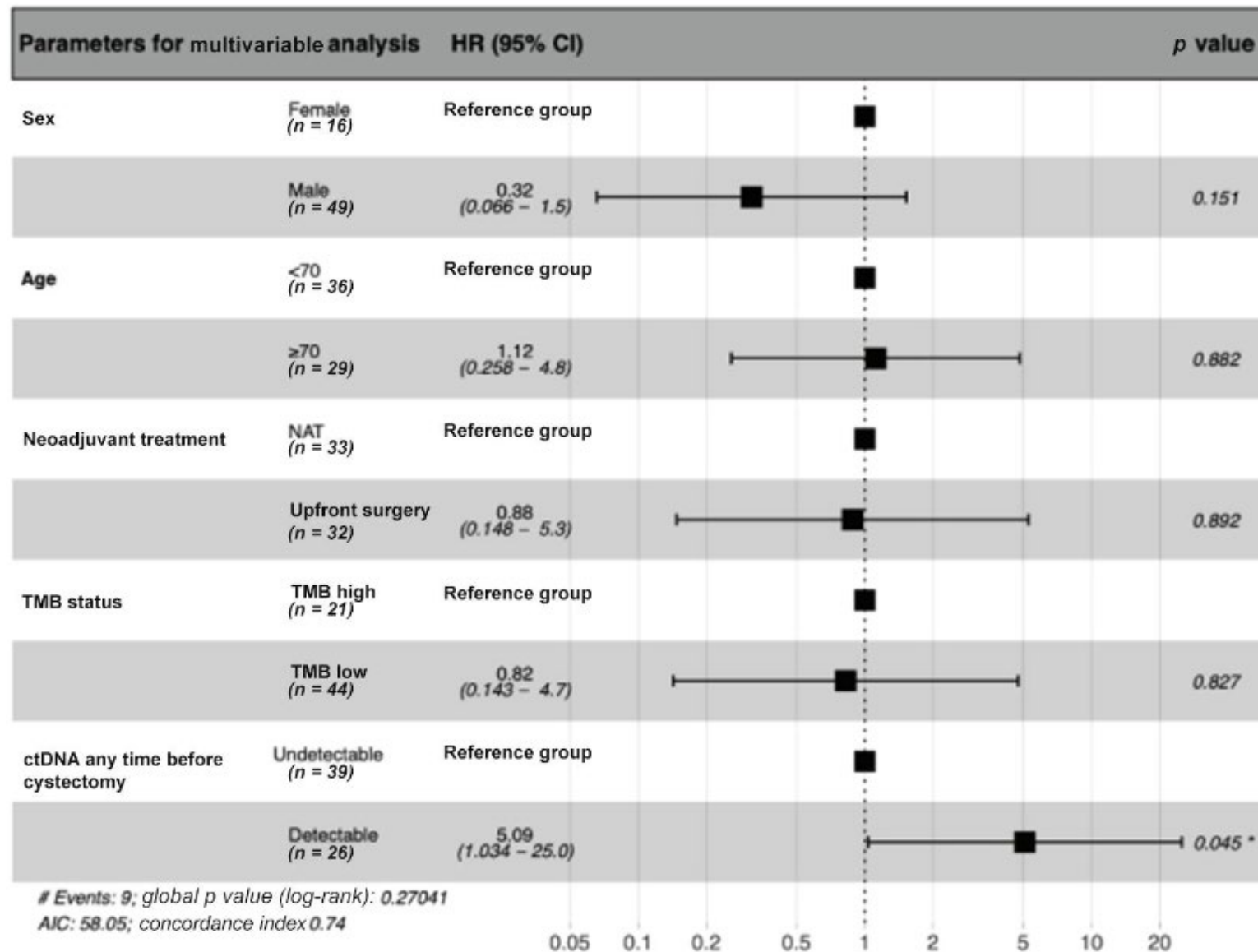
| Number at risk     |    |                    |    |    |    |    |    |    |
|--------------------|----|--------------------|----|----|----|----|----|----|
| Status             |    | Time since RC (mo) |    |    |    |    |    |    |
|                    |    | 0                  | 6  | 12 | 18 | 24 | 30 | 36 |
| ctDNA undetectable | 25 | 18                 | 10 | 7  | 2  | 2  | 0  |    |
| ctDNA detectable   | 27 | 19                 | 8  | 3  | 1  | 1  | 0  |    |

| Status      | ctDNA undetectable | ctDNA detectable |
|-------------|--------------------|------------------|
| Events %    | 4 (1/25)           | 55.6 (15/27)     |
| 24-mo DFS % | 96 (74.8-99.4)     | 18.7 (3.5-43.1)  |

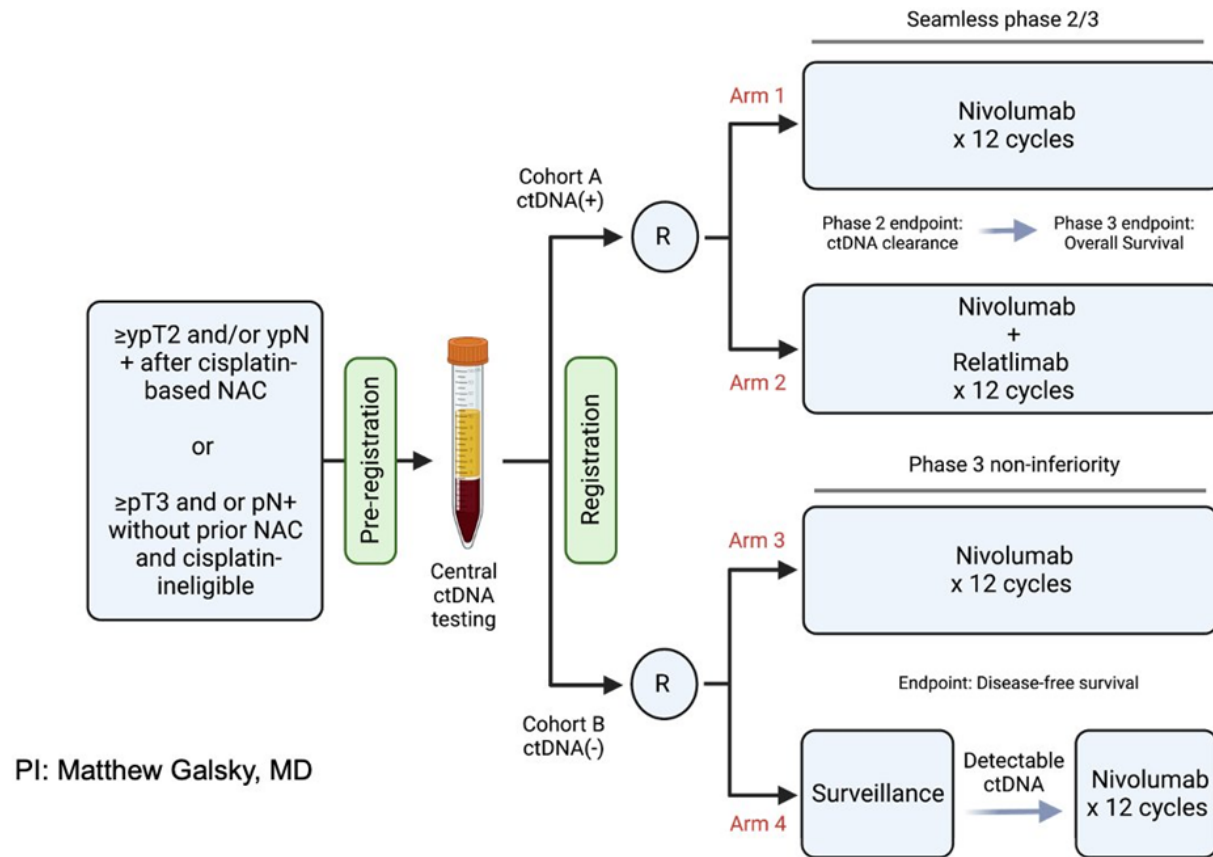
167 patients in RW setting undergoing NAC -> RC and surveillance vs adjuvant therapy

ctDNA (MRD window: hazard ratio 16.68;  $p < 0.001$ ; surveillance window: hazard ratio 23.02;  $p < 0.001$ )



# ctDNA : the future - MODERN

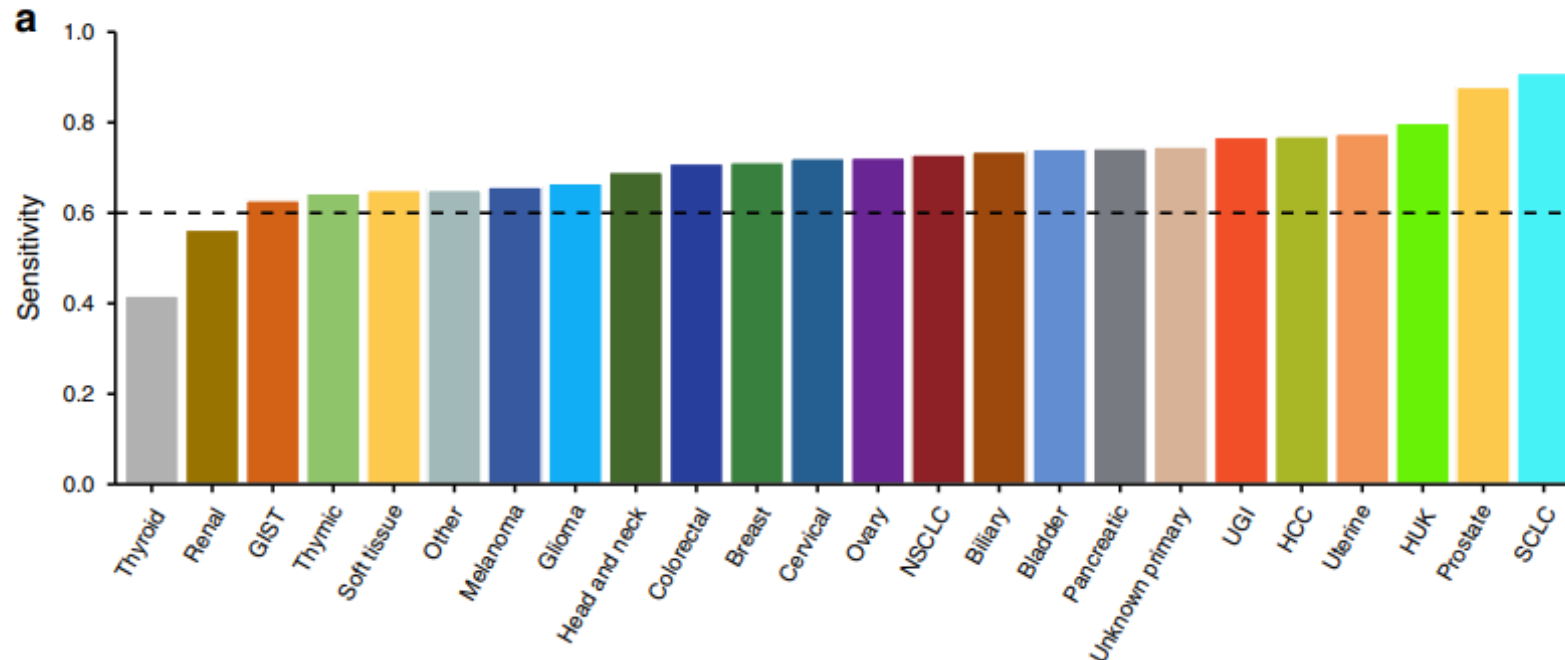
A032103 (MODERN): An integrated Phase 2/3 and Phase 3 Trial of MRD-based Optimization of adjuvant therapy in urothelial cancer



# Next steps in UC

- Validate de-escalation in MRD negative patients on EV Pembro
- Validate escalation in MRD positive patients post effective NAT
- Define optimal IO duration , timepoint of de-escalation ( IITs from OU Health, FCCC)
- Use of hsctDNA as potential Tumor Marker in advanced disease

# ctDNA in RCC



14,972 peripheral blood samples from 12,337 patients

Detection at 56.4%, (95% CI 52.1–60.7% , improved to 63.2% (95% CI 57.4–69.0%) in metastatic disease

Confirms comparatively lower sensitivity

# Association of circulating tumor DNA with patient prognosis in surgically resected renal cell carcinoma



Andres F Correa, Ekaterina Kalashnikova, Hsin-Ta Wu, Ryan M Winters, [Mustafa Balcioglu](#), Sumedha Sudhaman, Denise C Connolly, Yulan Gong, Robert G Uzzo, Himanshu Sethi ...  
[Show more](#)

The Oncologist, Volume 29, Issue 10, October 2024, Pages 887–893,

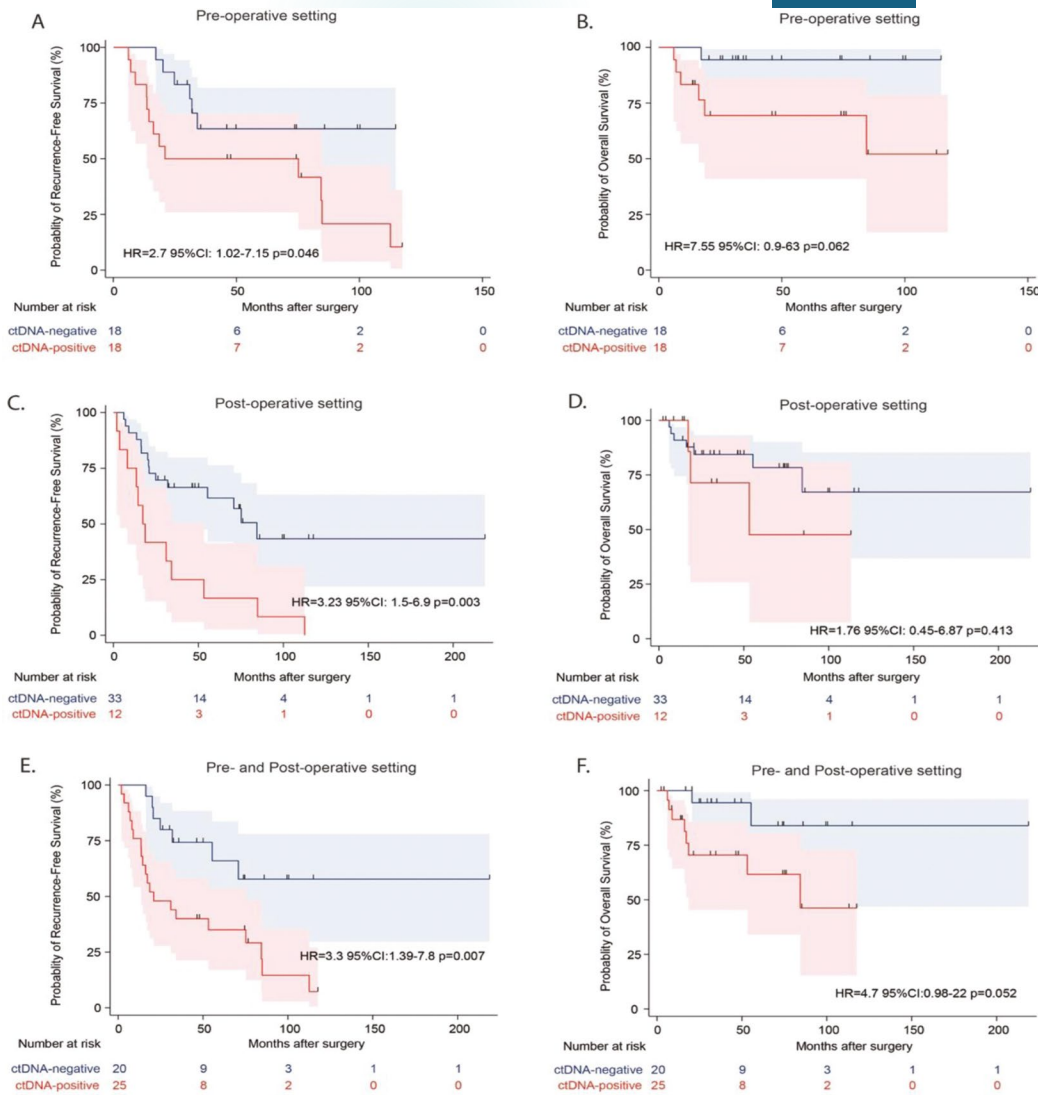
Biorepository plasma sample analysis

N=45 , s/p Nephrectomy (2001-2017).

67% Stage I-II

Pre-operative MRD detection 50%

100% PPV for recurrence.



Correa AF, et al. *Oncologist*. 2024

Figure 3. Association of ctDNA status with RFS within the MRD window

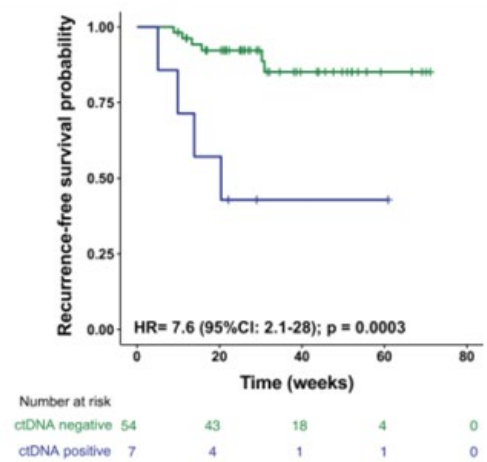


Table 1. Patient & tumor characteristics

| Characteristic               | N = 63 <sup>1</sup> | Characteristic                    | N = 63 <sup>1</sup> | Characteristic                               | N = 63 <sup>1</sup> |
|------------------------------|---------------------|-----------------------------------|---------------------|----------------------------------------------|---------------------|
| <b>Median age (years)</b>    | 64 (21 - 87)        | <b>Risk group classification*</b> | 51/63               | <b>Clinical stage</b>                        |                     |
| <b>Gender</b>                |                     | Intermediate-high                 | 42 (65%)            | I                                            | 1 (2%)              |
| Male                         | 46 (73%)            | High                              | 3 (7%)              | II                                           | 4 (6%)              |
| Female                       | 17 (27%)            | M1 NED <sup>‡</sup>               | 3 (7%)              | III                                          | 52 (83%)            |
| <b>Subtype</b>               |                     | Unknown                           | 3 (5%)              | IV                                           | 6 (9%)              |
| Clear cell                   | 51 (81%)            | <b>Adjuvant Treatment</b>         | 23/63 (37%)         | <b>Median follow-up post-surgery (weeks)</b> | 43.9 (22.9-84.1)    |
| Non-clear cell               | 9 (14%)             | Immunotherapy                     | 20 (32%)            |                                              |                     |
| Unclassified                 | 3 (5%)              | TKI                               | 2 (3%)              |                                              |                     |
| <b>Number of recurrences</b> | 12 (19%)            | Immunotherapy/TKI                 | 3 (5%)              |                                              |                     |

<sup>1</sup>Median (Range); n (%)

\*Risk group classification based on Keynote 564 trial<sup>§</sup>

<sup>‡</sup>M1 (Resection of the primary tumor and solid, isolated, soft-tissue metastases) with no evidence of disease (NED)

RW cohort of patients undergoing nephrectomy (including Metastatectomy)

Median lead time 15 weeks ( 4.3-32) in pts on AT-

NPV 92% during MRD window (4mo)→ 95% longitudinal

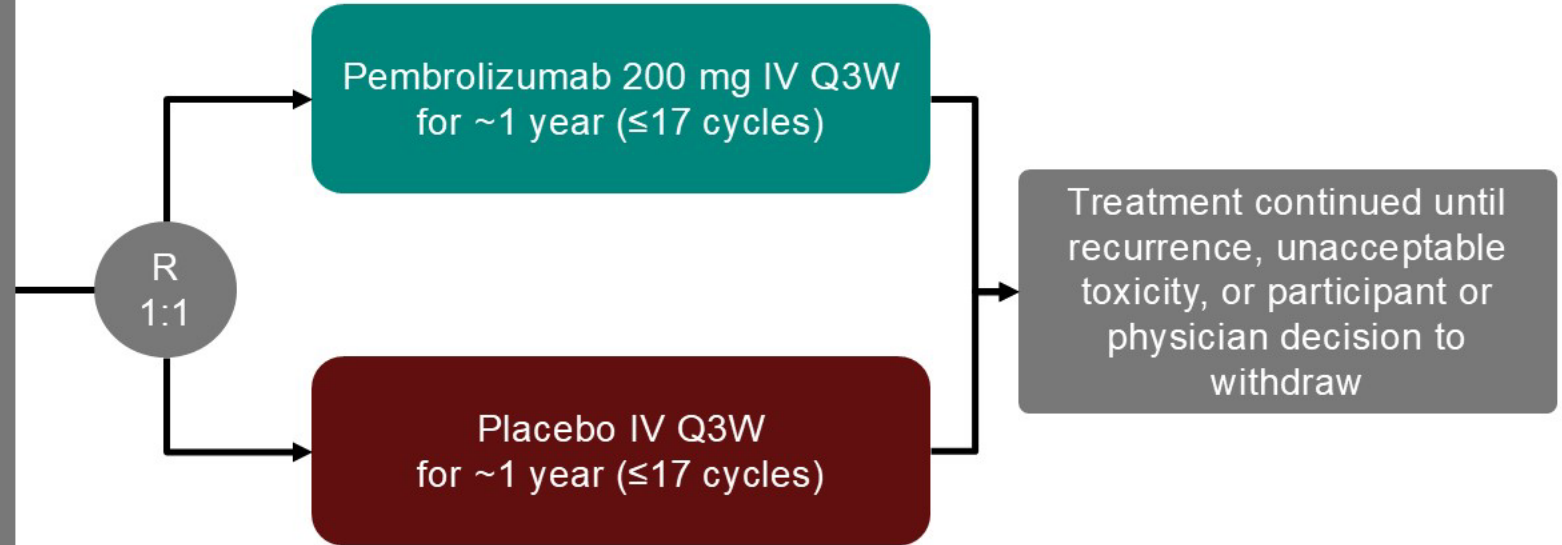
# KEYNOTE-564 Study Design (NCT03142334)

## Key Eligibility Criteria

- Age  $\geq 18$  years
- Histologically confirmed clear cell RCC
- No prior systemic therapy for advanced disease
- Surgery  $\leq 12$  weeks prior to randomization
- Postnephrectomy intermediate-high risk of recurrence (M0):
  - pT2, grade 4 or sarcomatoid, N0
  - pT3, any grade, N0
- Postnephrectomy high risk of recurrence (M0):
  - pT4, any grade, N0
  - Any pT, any grade, N+
- Postnephrectomy and complete resection of metastasis (M1 NED)
- ECOG PS score of 0 or 1

## Stratification Factors

- M stage (M0 vs M1 NED)
- M0 group further stratified:
  - ECOG PS score of 0 vs 1
  - US vs rest of the world



**Primary end point:** DFS by investigator

**Key secondary end point:** OS

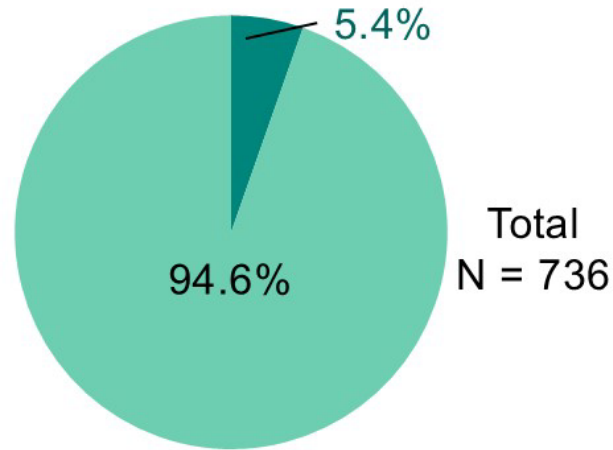
**Other secondary end point:** Safety

**Exploratory end points:** Association of baseline ctDNA metrics and ctDNA change from baseline to cycle 5 day 1 (C5D1) with DFS<sup>a</sup> for pembrolizumab and placebo separately, and ctDNA clearance rate at C5D1

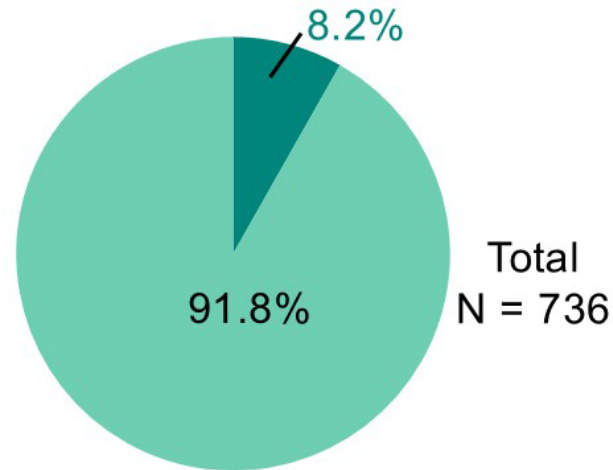
# Exome-based ctDNA Status at Baseline Postsurgery<sup>a</sup>

## Full ctDNA analysis population

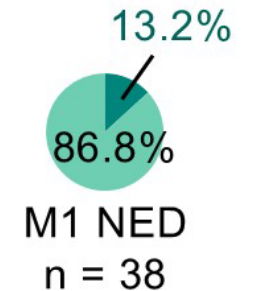
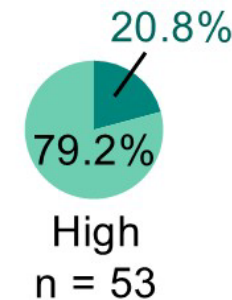
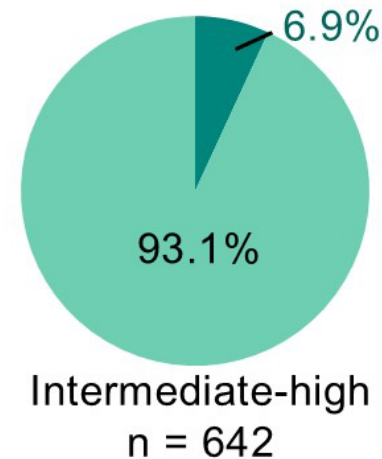
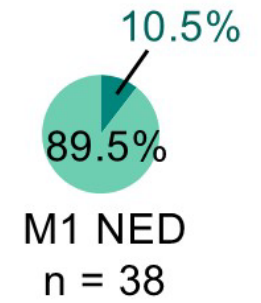
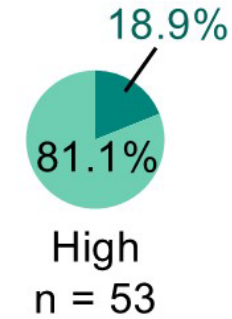
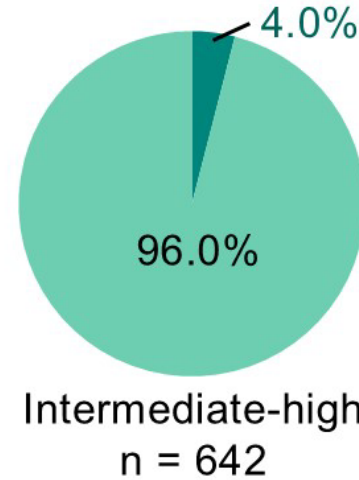
### 16-Plex ctDNA Detection



### 64-Plex ctDNA Detection



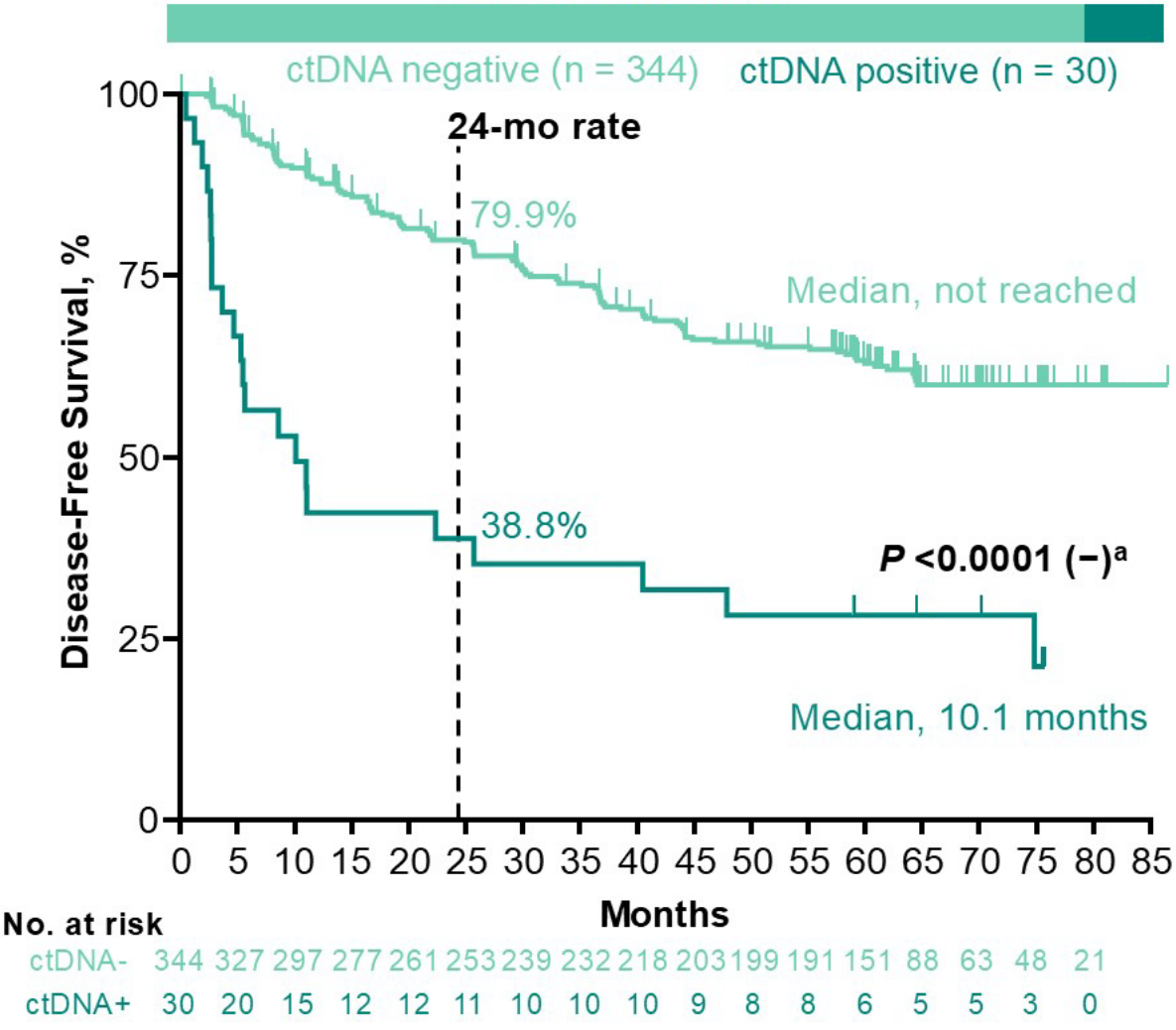
## By risk group



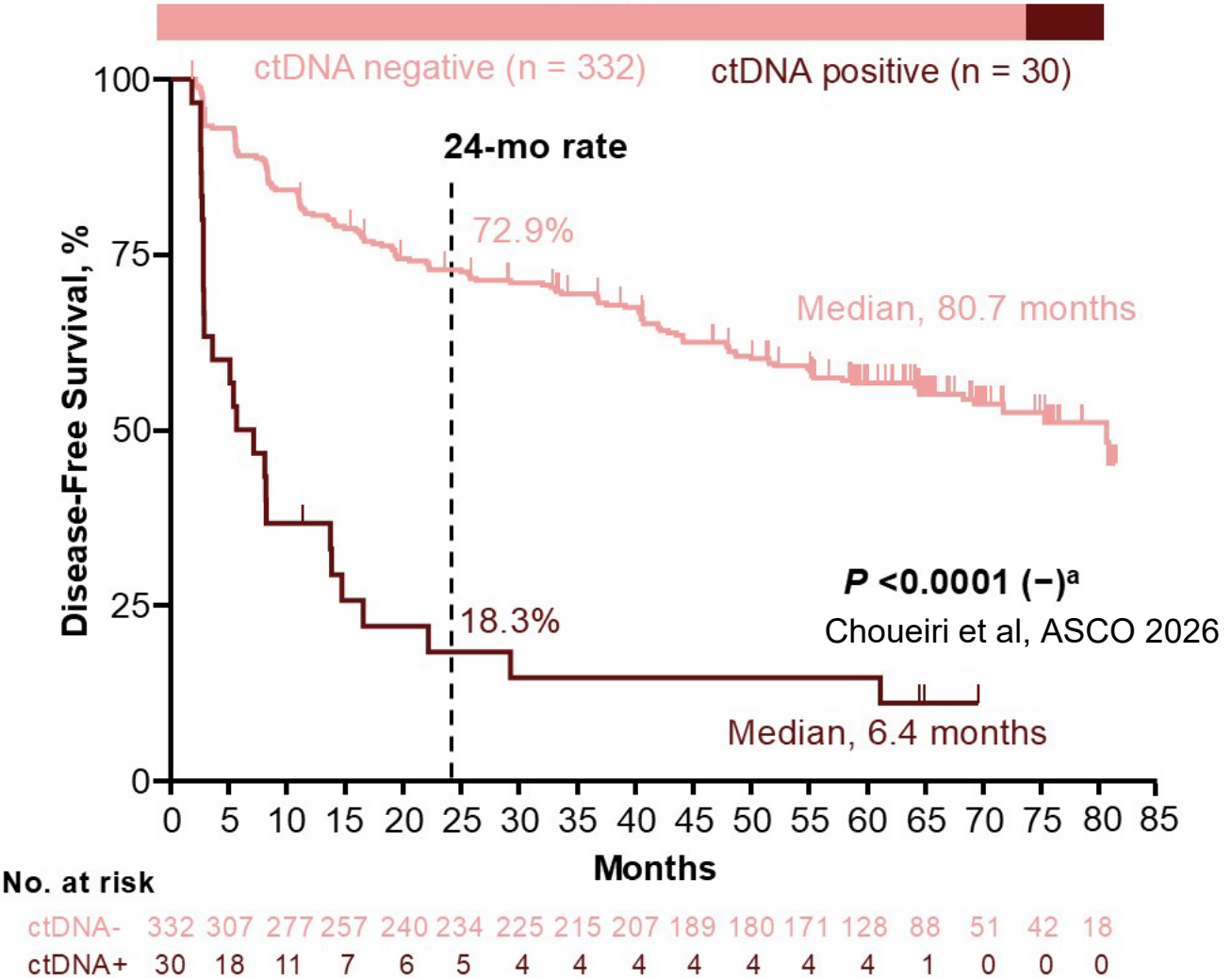
<sup>a</sup>Analysis includes both treatment arms. Clinical data cutoff: September 25, 2024.

# Kaplan-Meier Estimates of DFS by Baseline ctDNA Status Within Each Treatment Arm: Exome-Based 64-Plex Assay

## Pembrolizumab

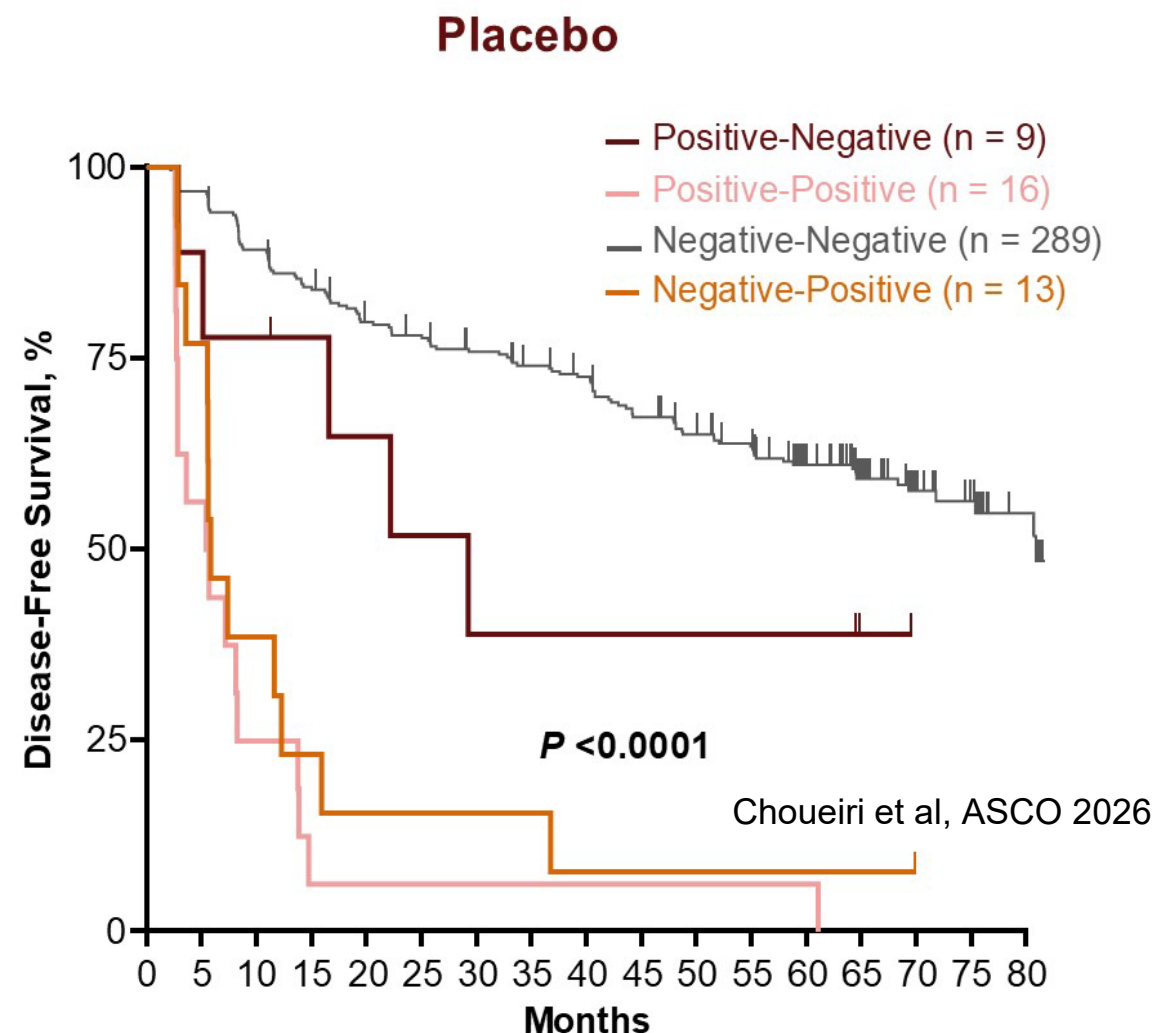
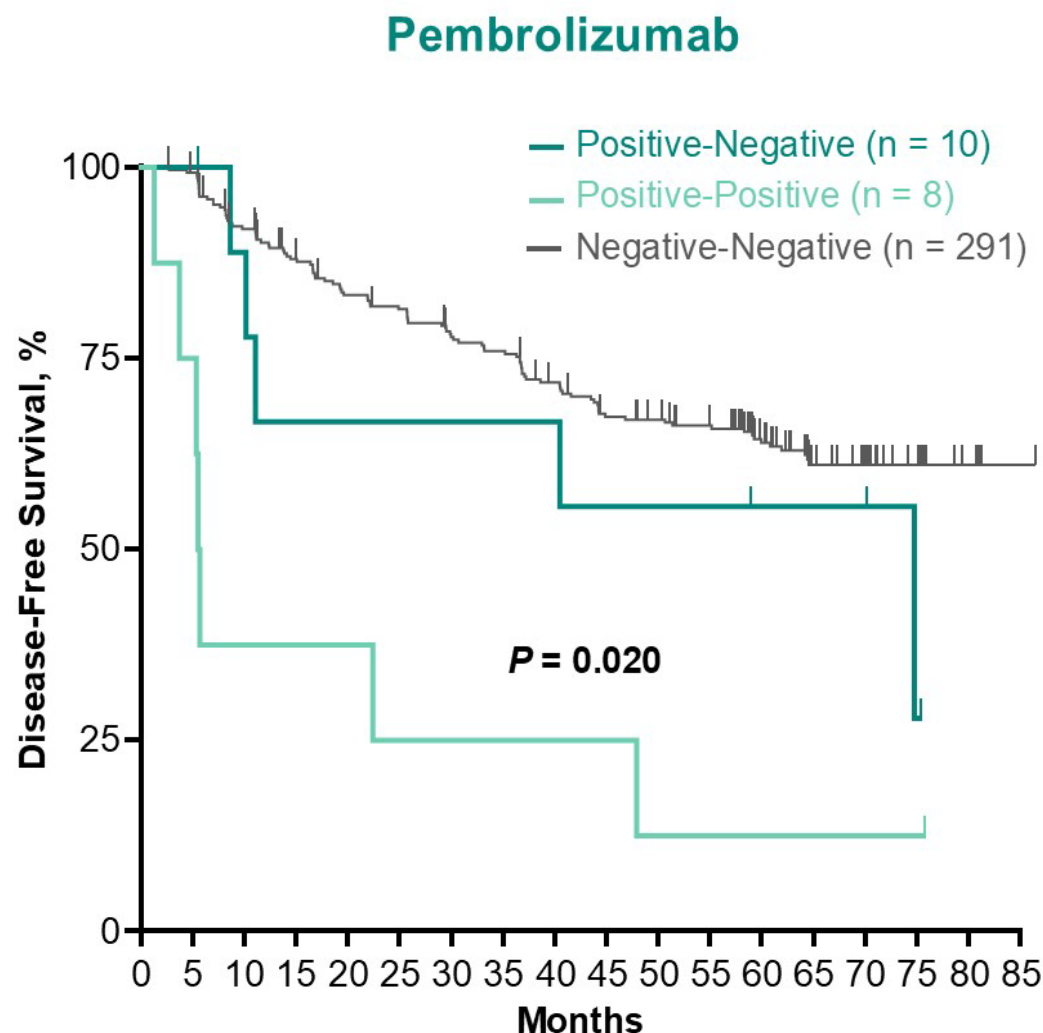


## Placebo



<sup>a</sup>“-” indicates that the observed association is negative. The association between baseline ctDNA metrics and DFS remained significant after adjusting for risk groups and other stratification factors. Statistical significance prespecified at multiplicity-adjusted  $\alpha = 0.05$ . Clinical data cutoff: September 25, 2024.

# Kaplan-Meier Estimates of DFS by ctDNA Change From Baseline at C5D1: Exome-based 64-Plex<sup>a</sup>



Kaplan-Meier curves for subgroups with <5 DFS events are not shown. <sup>a</sup>Kaplan-Meier curves for 16-plex are not shown due to small sample size. For the 16-plex assay, ctDNA change from baseline at C5D1 was significantly negatively associated with DFS for both pembrolizumab and placebo even after adjusting for the baseline ctDNA status. Models were adjusted for baseline ECOG performance status. Likelihood ratio test was performed for each ctDNA change (4-level category) by comparing the full model (with ctDNA change) to the reduced model (without ctDNA change, but with ctDNA baseline). Clinical data cutoff: September 25, 2024.

# Longitudinal Testing of Circulating Tumor DNA in Patients With Metastatic Renal Cell Carcinoma

Arnab Basu, MD, MPH<sup>1</sup>; Cherry Au, MD<sup>2</sup>; Ajitha Kommalapati, MD<sup>1</sup>;  
Hyndavi Kandala, MD<sup>1</sup>; Sumedha Sudhaman, PhD<sup>3</sup>; Tamara Mahmood, MS, PA<sup>3</sup>;  
Carcia Carson, PhD<sup>3</sup>; Natalia Pajak, MSPAS, PA-C<sup>3</sup>; Punashi Dutta, PhD<sup>3</sup>; Mark Calhoun, PhD<sup>3</sup>;  
Meenakshi Malhotra, PhD<sup>3</sup>; Adam C. ElNaggar, MD<sup>3</sup>; Minetta C. Liu, MD<sup>3</sup>;  
James Ferguson III, MD, PhD<sup>1,4</sup>; Charles Peyton, MD<sup>1,4</sup>; Soroush Rais-Bahrami, MD, MBA<sup>1,4,5</sup>;  
and Alan Tan, MD<sup>6</sup>

<sup>1</sup>O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham Heersink School of Medicine, Birmingham, AL;  
<sup>2</sup>Rush University Medical Center, Chicago, IL; <sup>3</sup>Natera, Inc, Austin, TX; <sup>4</sup>Department of Urology, University of Alabama at Birmingham Heersink School of Medicine, Birmingham, AL; <sup>5</sup>Department of Radiology, University of Alabama at Birmingham Heersink School of Medicine, Birmingham, AL; <sup>6</sup>Vanderbilt University Medical Center, Nashville, TN

# Patient characteristics and study design

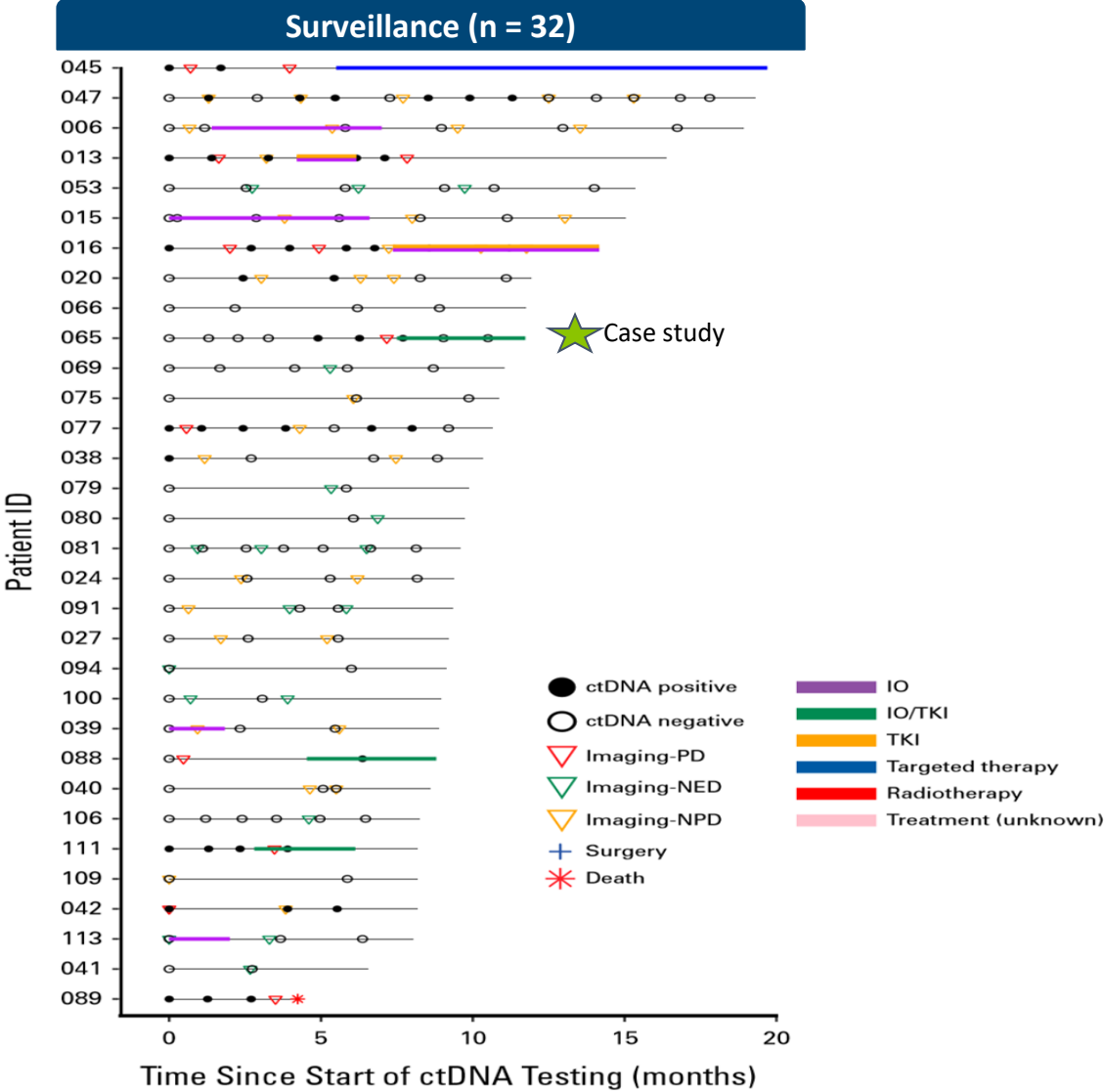
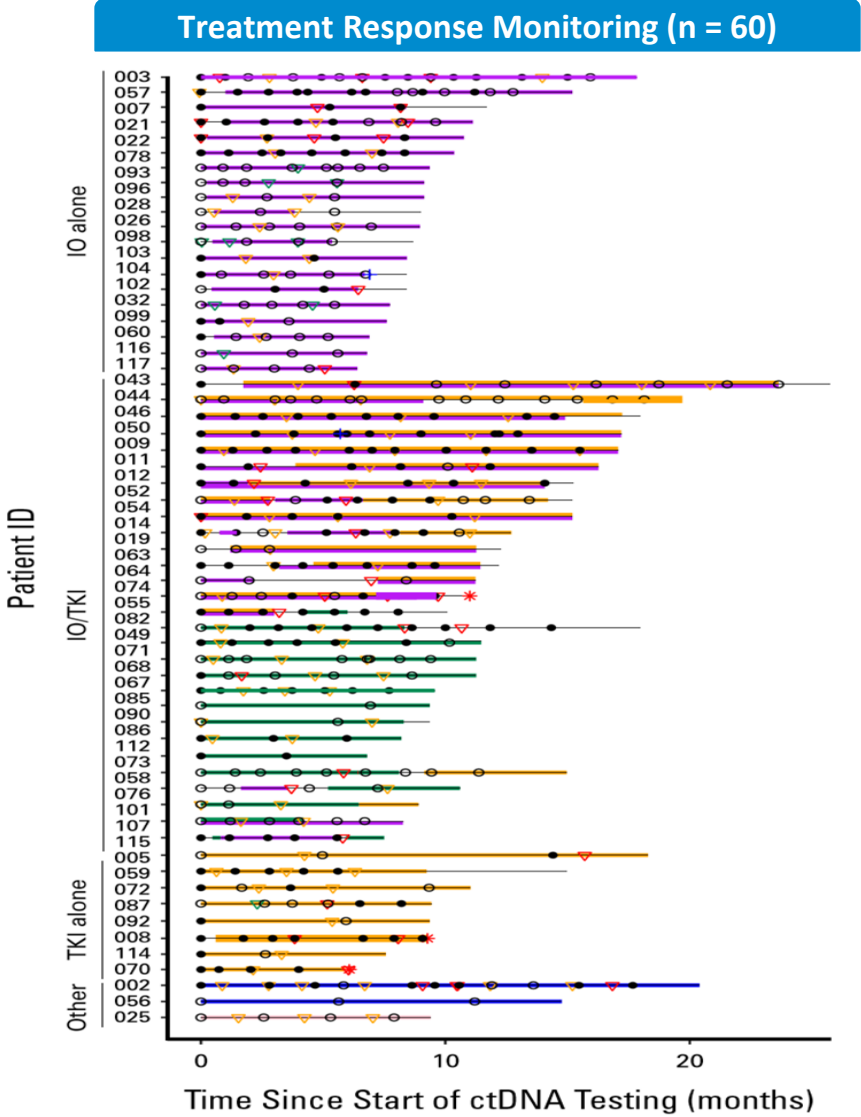
- Real-world cohort of **92 patients** (490 plasma samples) with metastatic RCC
  - 79% clear cell subtype
  - 75% intermediate risk category (IMDC)
  - 49% with lung mets
- Cohort divided by **timing of ctDNA test** (on or off therapy)
  - Treatment response monitoring (n = 60)
  - Surveillance (n = 32)

**TABLE 1.** Demographic Table Highlighting Patient and Tumor Characteristics

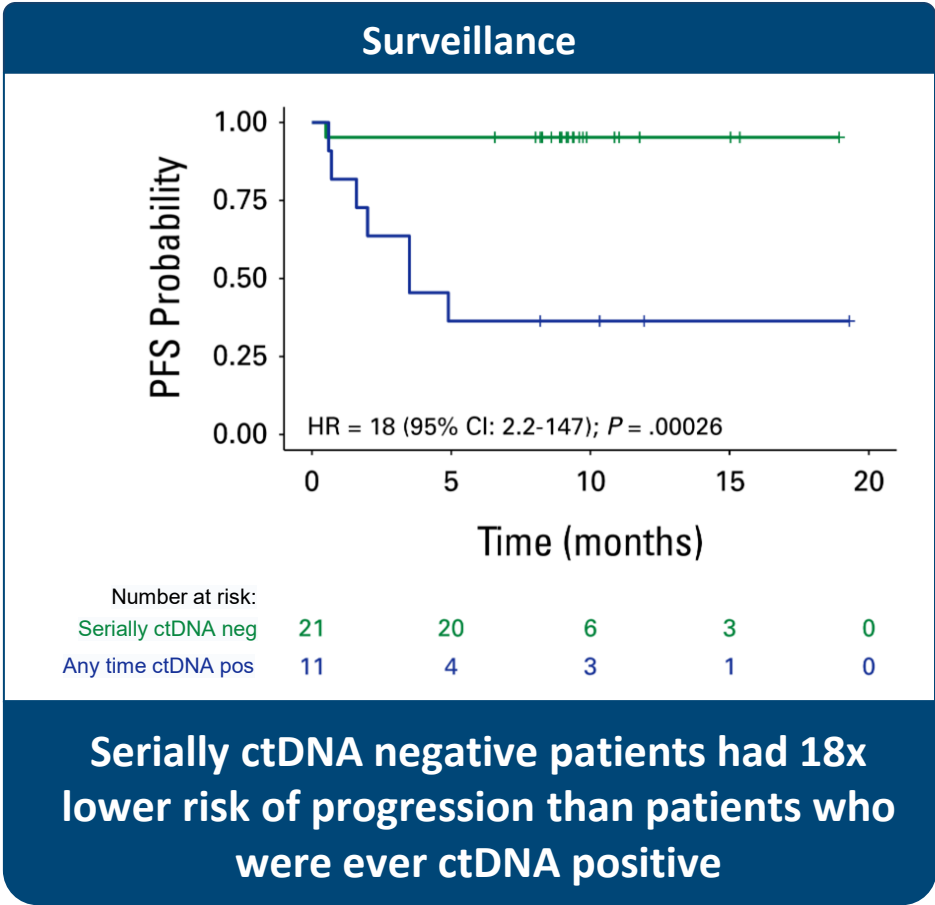
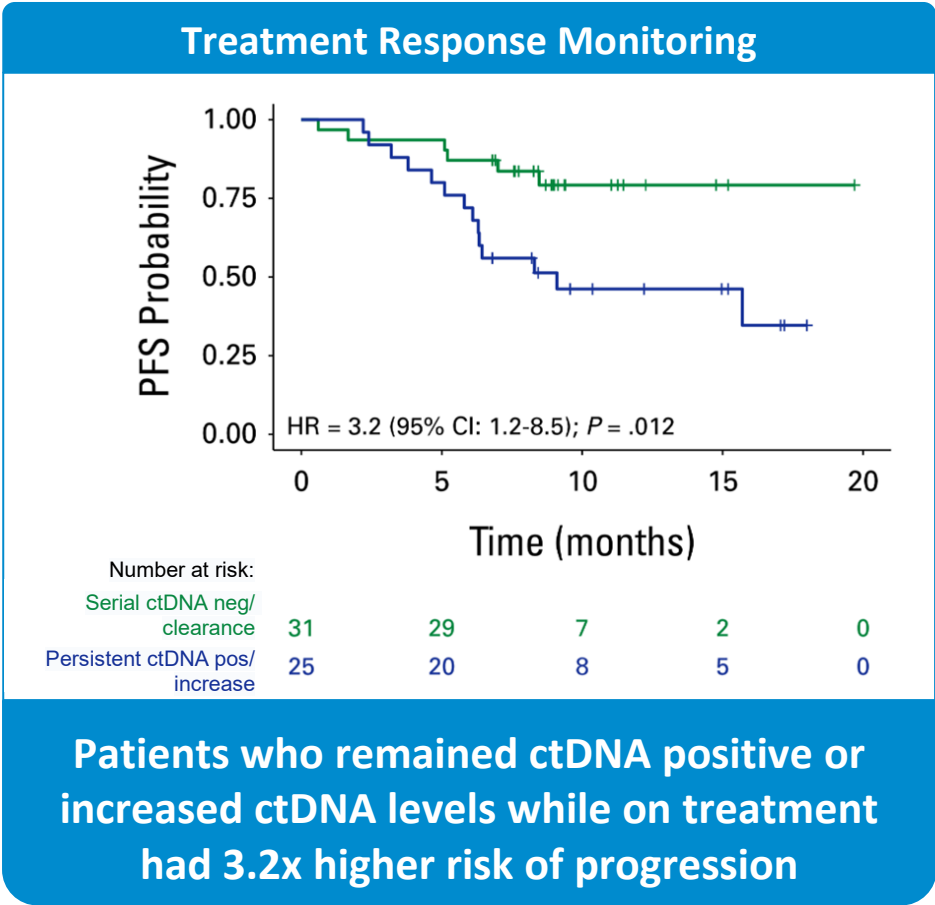
| Patient/Tumor Characteristic          | TRM Cohort (n = 60) | Surveillance Cohort (n = 32) | Overall Cohort (N = 92) |
|---------------------------------------|---------------------|------------------------------|-------------------------|
| Sex, No. (%)                          |                     |                              |                         |
| Male                                  | 44 (73.3)           | 23 (71.9)                    | 67 (72.8)               |
| Female                                | 16 (26.7)           | 9 (28.1)                     | 25 (27.2)               |
| Subtype, No. (%)                      |                     |                              |                         |
| Clear cell                            | 46 (76.7)           | 27 (84.4)                    | 73 (79.3)               |
| Non-clear cell                        | 10 (16.7)           | 3 (9.4)                      | 13 (14.1)               |
| Unclassified                          | 4 (6.7)             | 2 (6.3)                      | 6 (6.5)                 |
| IMDC risk category, No. (%)           |                     |                              |                         |
| Favorable risk (0)                    | 11 (18.3)           | 4 (12.5)                     | 15 (16.3)               |
| Intermediate risk (1-2)               | 43 (71.7)           | 26 (81.3)                    | 69 (75)                 |
| Poor risk (3+)                        | 3 (5.0)             | 1 (3.1)                      | 4 (4.4)                 |
| Unknown                               | 3 (5.0)             | 1 (3.1)                      | 4 (4.4)                 |
| ctDNA status, No. (%)                 |                     |                              |                         |
| Anytime positive                      | 39 (65.0)           | 11 (34.4)                    | 50 (54.3)               |
| Serially negative                     | 21 (35.0)           | 21 (65.6)                    | 42 (45.7)               |
| Age, years, median (range)            | 61 (40-82)          | 64 (35-84)                   | 62 (35-84)              |
| Follow-up, months, median (range)     | 10.5 (6.1-25.8)     | 9.7 (4.2-19.7)               | 10 (4.2-25.8)           |
| Sites of metastasis, No. (%)          |                     |                              |                         |
| Bone                                  | 10 (16.7)           | 5 (15.6)                     | 15 (16.3)               |
| Brain                                 | 5 (8.3)             | 2 (6.3)                      | 7 (7.6)                 |
| Liver                                 | 12 (20.0)           | 1 (3.1)                      | 13 (14.1)               |
| Lung                                  | 32 (53.3)           | 13 (40.6)                    | 45 (48.9)               |
| Lymph node                            | 10 (16.7)           | 3 (9.4)                      | 13 (14.1)               |
| Other                                 | 39 (65.0)           | 11 (34.4)                    | 50 (54.3)               |
| Unknown                               | 5 (8.3)             | 9 (28.1)                     | 14 (15.2)               |
| Number of time points, median (range) | 5 (2-15)            | 3.5 (2-14)                   | 4.5 (2-15)              |

Abbreviations: ctDNA, circulating tumor DNA; IMDC, International Metastatic RCC Database Consortium; TRM, treatment response monitoring.

# Swimmer plots of all patients



# ctDNA status and dynamics were associated with PFS in patients with metastatic RCC



# THE PROGNOSTIC ROLE OF CIRCULATING TUMOR DNA (ctDNA) CLEARANCE AS A BIOMARKER IN LOCALIZED AND METASTATIC RENAL CELL CARCINOMA (RCC): A SINGLE CENTER EXPERIENCE

Adanma Ayanambakkam<sup>1</sup>, Bibi Maryam<sup>1</sup>, Chao Xu<sup>1</sup>, Priyanka Vallabhaneni<sup>1</sup>, Haripriya Andanamala<sup>1</sup>, Vishnu Nagalapuram<sup>1</sup>, Josh Glover<sup>1</sup>, Sanjay Patel<sup>1</sup>, Kelly Stratton<sup>1</sup>, Michael Cookson<sup>1</sup>, Jonathan Heinlen<sup>1</sup>, Andrew McIntosh<sup>1</sup>, Brian Cross<sup>1</sup>, Minh Phan<sup>1</sup>, Ryan Nipp<sup>1</sup>, Rafeh Naqash<sup>1</sup>, Arnab Basu<sup>2</sup> and Rana McKay<sup>3</sup>.  
<sup>1</sup>Stephenson Cancer Center, OK; <sup>2</sup>University of Alabama, AL; <sup>3</sup>University of California San Diego, CA

**Fig 2. (A) LONGITUDINAL ctDNA ANALYSIS IN LOCALIZED RCC**

**Fig 1. ctDNA STATUS AND DYNAMICS ASSOCIATED WITH CLINICAL OUTCOMES**

**Fig 1. (B) LONGITUDINAL ctDNA ANALYSIS IN METASTATIC RCC**

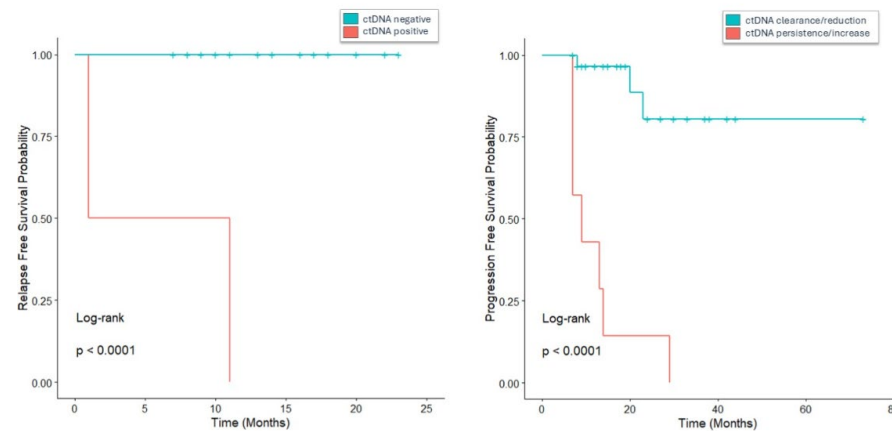
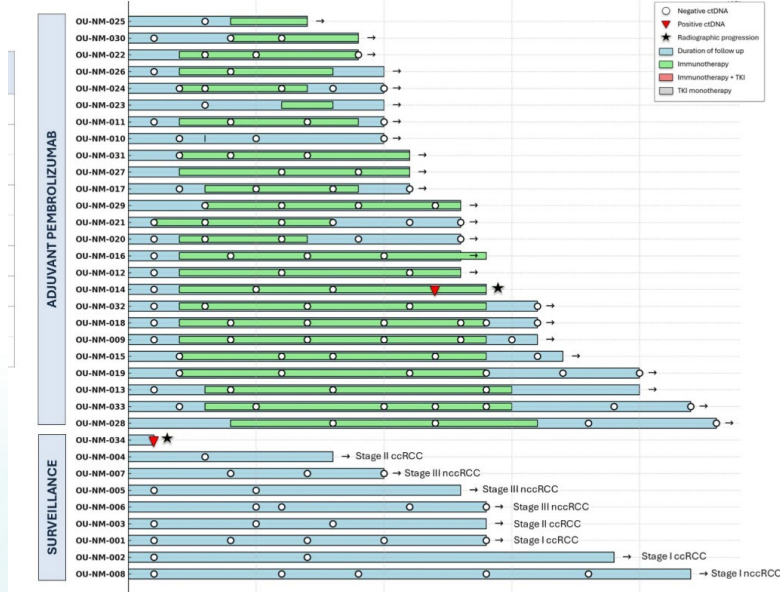


**Figure 2.** Kaplan-Meier estimates for (A) Recurrence Free Survival (RFS) in patients with localized RCC and (B) Progression Free Survival (PFS) in patients with metastatic RCC stratified by ctDNA dynamics or status

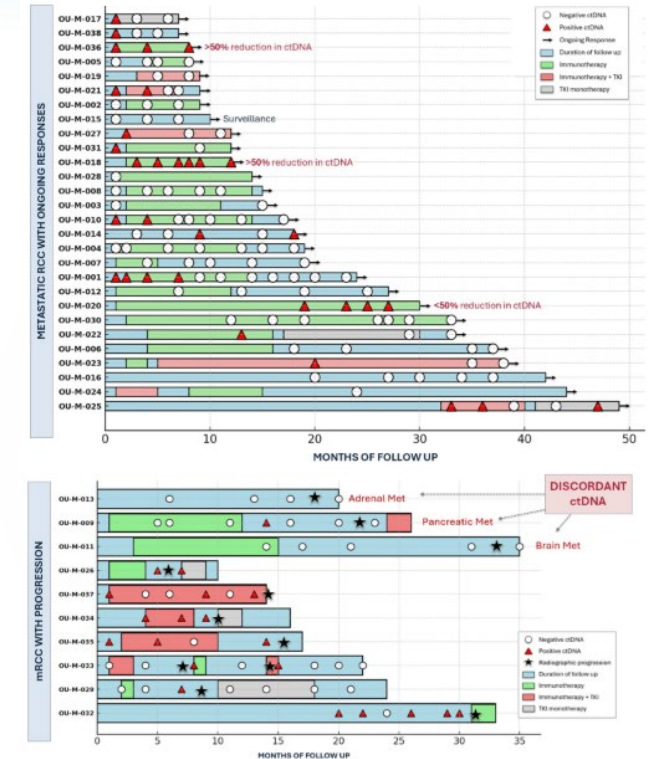


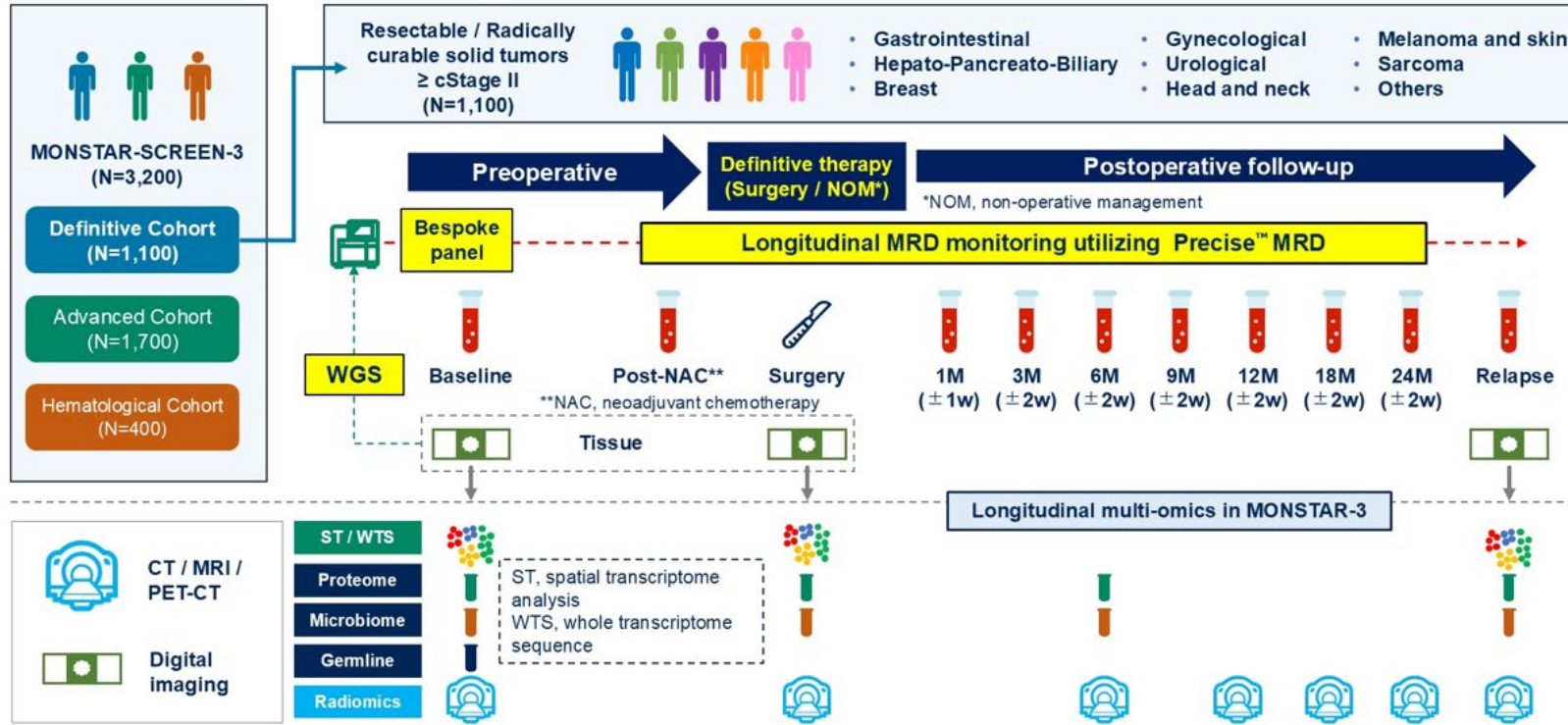
**Figure 1.** Swimmer plot showing clinical outcomes, duration of systemic therapy, and longitudinal ctDNA analysis for three separate sub cohorts of patients with RCC. (A) Patients with non-metastatic RCC (nmRCC), (B) Patients with metastatic RCC (mRCC). Patients are ordered according to descending length of clinical follow up. ctDNA : circulating tumor DNA; TKI : Tyrosine Kinase Inhibitor

34 patients with nmRCC (13 mo f/u) Sensitivity 100% for recurrence

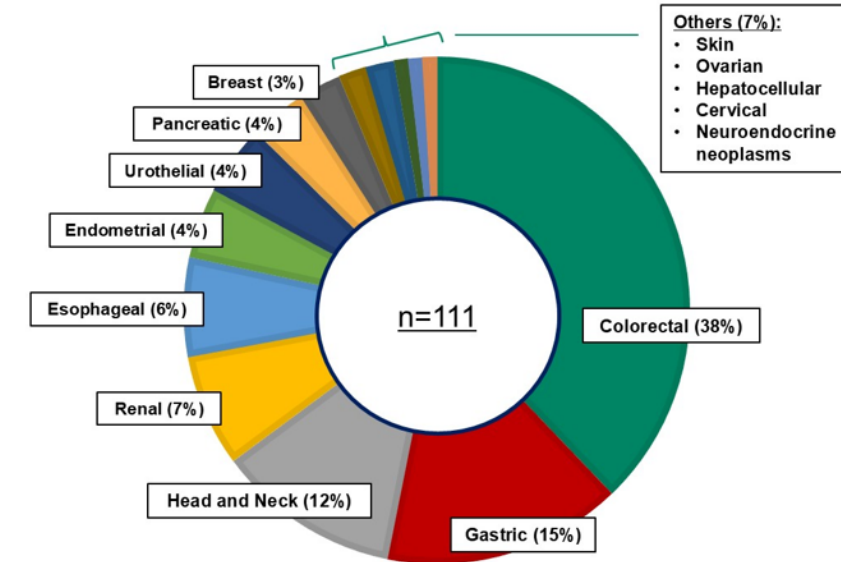
In mRCC clearance favorable prognostic factor

**Arnab Basu/Mayo Clinic**  
**@arnabguonc**

# Study Schema of MONSTAR-SCREEN-3

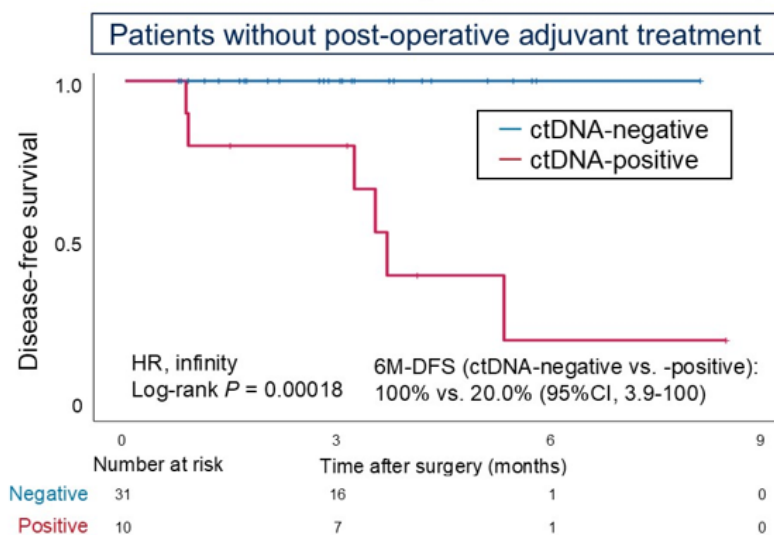
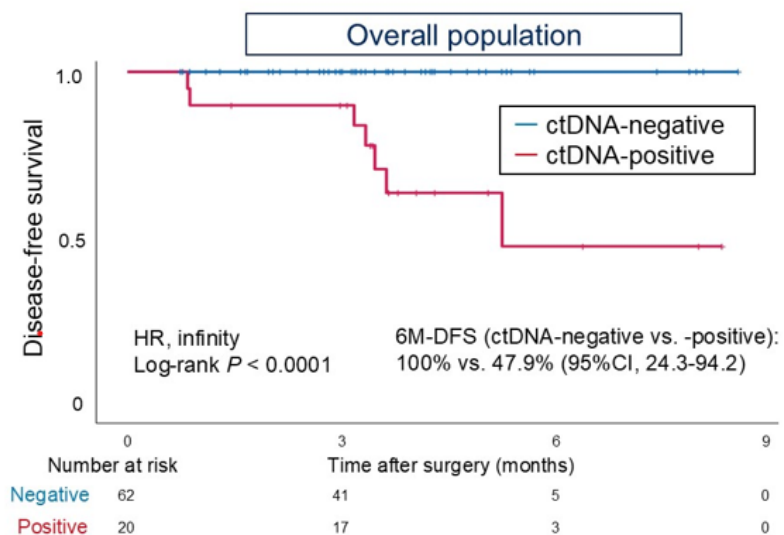


## Patient Characteristics

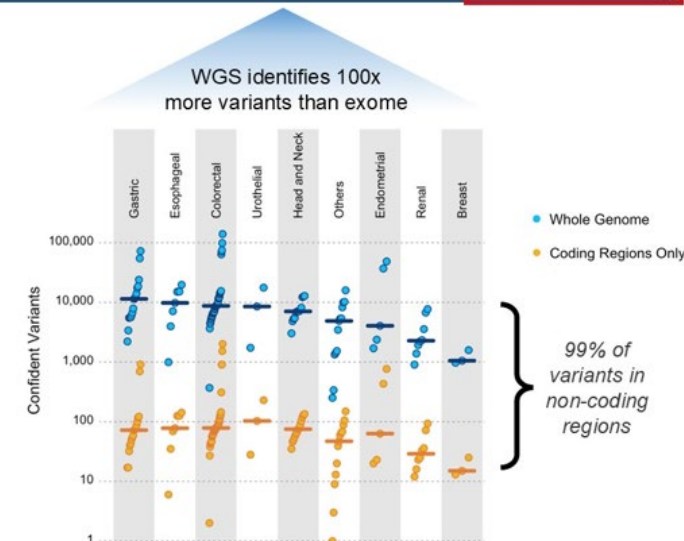
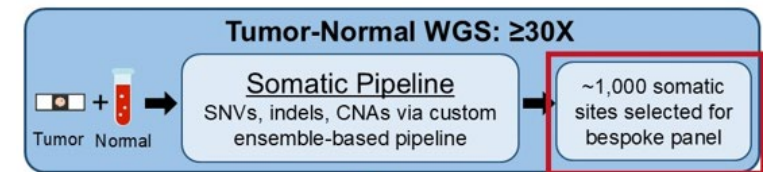


# Disease-free survival by 1M post-surgery ctDNA positivity

Median follow-up period: 3.4 months (range: 0.8–8.7 months)



- Patients who were ctDNA-positive at 1-month post-surgery exhibited significantly worse DFS compared to ctDNA-negative
- 100% of patients with clinical recurrence (7/7) demonstrated ctDNA positivity at 1-month post-surgery



# Clinical performance of Signatera Genome assay in a cohort of patients (pts) with solid tumors

Mridula George<sup>1</sup>, Gary Schwartz<sup>2</sup>, Wassim McHayleh<sup>3</sup>, Vitaly Margulis<sup>4</sup>, Alan Tan<sup>5</sup>, Hideaki Bando<sup>6</sup>, Takayuki Yoshino<sup>6</sup>, Sumedha Sudhaman<sup>7</sup>, Carly Bess Scalise<sup>7</sup>, Bharat Sridhar<sup>7</sup>, Jordan Feeney<sup>7</sup>, Morgan Quail<sup>7</sup>, Sharlene Velichko<sup>7</sup>, Wilson Tan<sup>7</sup>, Alexander Hsieh<sup>7</sup>, Anastasia Butskova<sup>7</sup>, Jose Ortega<sup>7</sup>, Matthew Rabinowitz<sup>7</sup>, Himanshu Sethi<sup>7</sup>, Adam C. ElNaggar<sup>7</sup>, Michael Krainock<sup>7</sup>, Angel A. Rodriguez<sup>7</sup>, Minetta C. Liu<sup>7</sup>, Matthew Galsky<sup>8</sup>

<sup>1</sup>Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey, USA; <sup>2</sup>Baylor University Medical Center, Dallas, TX, USA; <sup>3</sup>Advent Health Cancer Institute, Altamonte Springs, Orlando, FL, USA; <sup>4</sup>UT Southwestern, Dallas, TX, USA; <sup>5</sup>Vanderbilt University Medical Center, Nashville, TN, USA; <sup>6</sup>National Cancer Center Hospital East, Kashiwa, Japan; <sup>7</sup>Natera, Inc. Austin, TX, USA; <sup>8</sup>Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

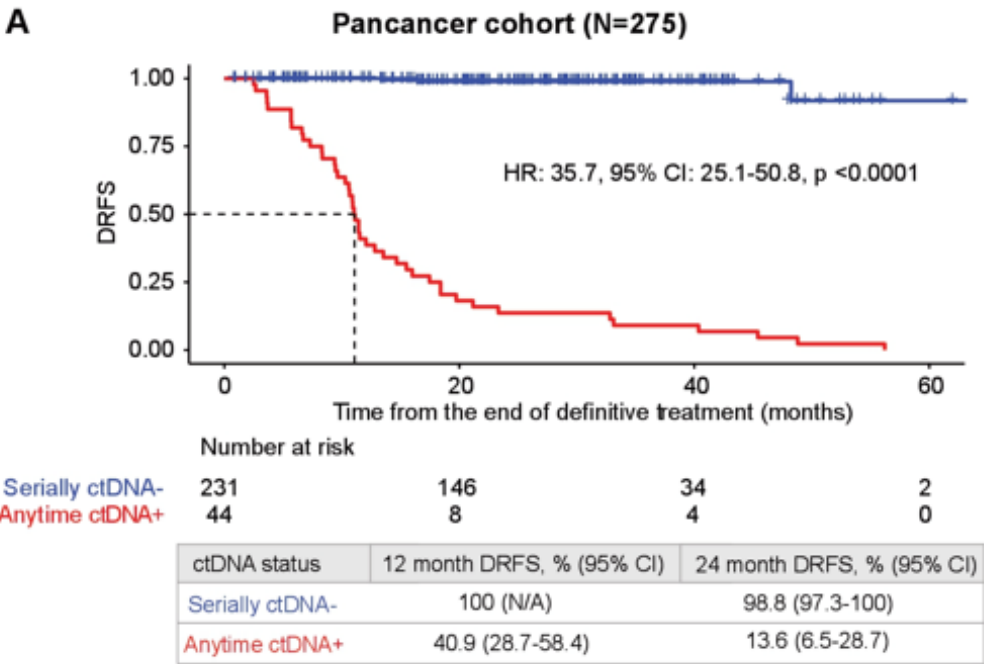
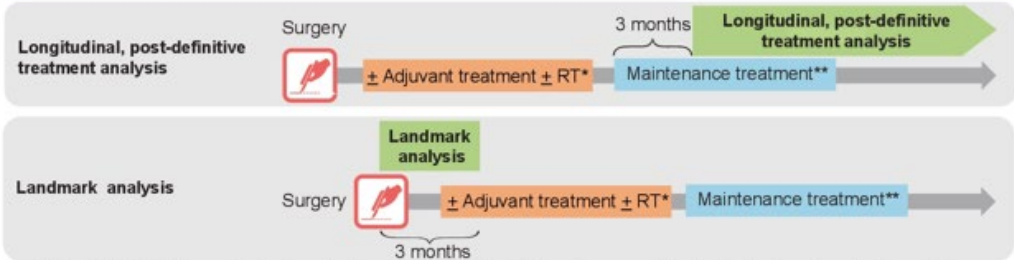


Figure 1. Time points analyzed during landmark and longitudinal post-definitive treatment analyses



\*Includes CDK4/6 inhibitor and other targeted agents for patients with breast cancer. \*\*Endocrine treatment and/or immunotherapy.

Figure 1. The MRD-positive time points had a median observed VAF of 270 ppm (IQR: 42 - 2671.5 ppm), with 39.3% of these samples having VAF in the ultra-sensitive range ( $\leq 100$  ppm), and 37.6% of these detections were at or below 25 ppm. In the surveillance setting, nearly 50% of detected samples were in the ultra-sensitive ( $\leq 100$  ppm) range.

LoD<sub>50</sub> improvement → 17ppm to 5-9ppm (min 1ppm)

Variant Tracking 16 plex → 64 plex

George et al, ASCO Annual Meeting. 2025

# Clinical Performance of a Tumor-Informed Whole Genome-Based (WGS) ctDNA Assay for Recurrence Detection and Treatment Response Monitoring in Localized and Advanced/Metastatic Renal Cell Carcinoma (RCC)

Yash Suri<sup>1</sup> , Sumedha Sudhaman<sup>2</sup> , Bhakti Dwivedi<sup>2</sup> , Rahul K. Kollipara<sup>2</sup> , Arun Singh<sup>2</sup> , Sharlene Velichko<sup>2</sup> , Carcia Carson<sup>2</sup> , Ashley Wray<sup>2</sup> , Punashi Dutta<sup>2</sup> , Meenakshi Malhotra<sup>2</sup> , Adam C. ElNaggar<sup>2</sup> , Gabrielle M. Heilek<sup>2</sup> , Himanshu Sethi<sup>2</sup> , Minetta C. Liu<sup>2</sup> , Alexey Aleshin<sup>2</sup> , Rana R. McKay<sup>3</sup> , Alan Tan<sup>4</sup> , Vitaly Margulis<sup>5</sup> , Arnab Basu<sup>1</sup>

<sup>1</sup> O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham Heersink School of Medicine; <sup>2</sup> Natera, Inc.;

<sup>3</sup>University of California, San Diego; <sup>4</sup> Vanderbilt University Medical Center; <sup>5</sup> Department of Urology, UT Southwestern

**As presented at ESMO Congress 2025**

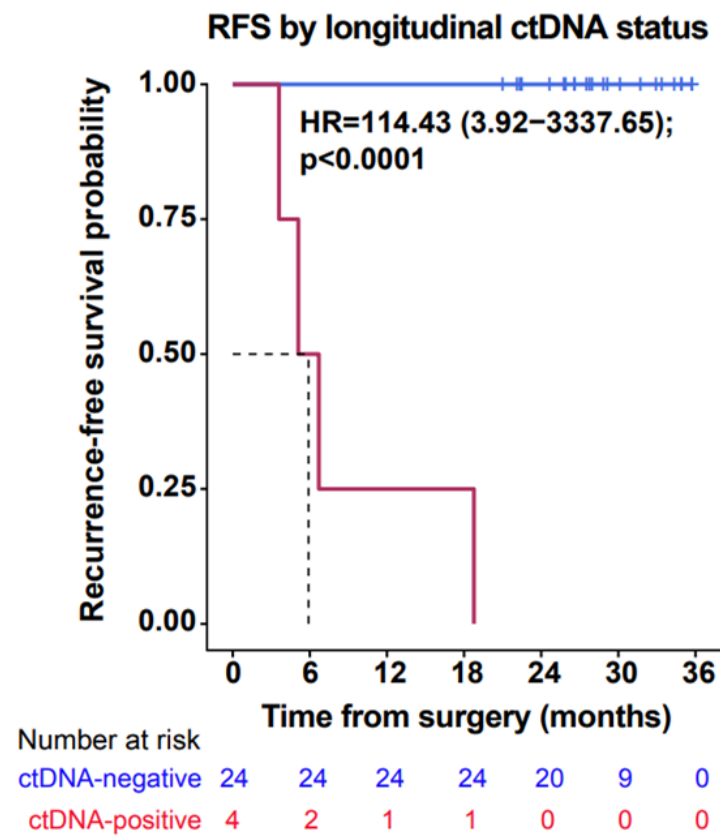
# Patient and Tumor Characteristics

- Retrospective ctDNA analysis of 89 patients with localized and metastatic RCC
- 71% (63/89) presented with stage IV disease
- 81% (72/89) had clear cell histology
- Median number of ctDNA tests across cohorts: 5 (1-23)\*
- Median follow-up:
  - Cohort A: 28.4 (21-35.7) months from surgery
  - Cohort B-TRM: 23.5 (6.03-45.3) months from first ctDNA test
  - Cohort B-Surveillance: 19.3 (3.84-29.6) months from first ctDNA test

| Characteristic               | Overall                             | Cohort A                           | Cohort B                                      |                                               |
|------------------------------|-------------------------------------|------------------------------------|-----------------------------------------------|-----------------------------------------------|
|                              | N = 89 <sup>1</sup>                 | N = 28 <sup>1</sup>                | TRM <sup>1</sup><br>N = 48                    | Surveillance <sup>1</sup><br>N = 13           |
| Median age (years)           | 61.5 (20-78)*                       | 59 (20-78)                         | 61.5 (52.8-67)                                | 67 (63-76)                                    |
| Sex                          |                                     |                                    |                                               |                                               |
| Male                         | 65                                  | 20                                 | 35                                            | 10                                            |
| Female                       | 24                                  | 8                                  | 13                                            | 3                                             |
| Median follow-up (months)    | N/A                                 | 28.4 (21-35.7) months from surgery | 23.5 (6.03-45.3) months from first ctDNA test | 19.3 (3.84-29.6) months from first ctDNA test |
| Median number of ctDNA tests | 5 (1-23)*                           | 4 (1-9)                            | 7 (2-23)                                      | 5 (2-14)                                      |
| Histology                    |                                     |                                    |                                               |                                               |
| Clear cell                   | 72                                  | 22                                 | 39                                            | 11                                            |
| Non-clear cell               | 16                                  | 5                                  | 9                                             | 2                                             |
| Unclassified                 | 1                                   | 1                                  | 0                                             | 0                                             |
| Clinical stage at diagnosis  |                                     |                                    |                                               |                                               |
| I                            | 2                                   | 2                                  | 0                                             | 0                                             |
| II                           | 2                                   | 2                                  | 0                                             | 0                                             |
| III                          | 22                                  | 22                                 | 0                                             | 0                                             |
| IV                           | 63                                  | 2                                  | 48                                            | 13                                            |
| Therapy                      | First-line/systemic therapy (57/89) | First-line therapy (N=9/28)        | Systemic therapy (48/48)                      |                                               |
| IO                           | 23                                  | 8                                  | 15                                            |                                               |
| TX                           | 7                                   | 0                                  | 7                                             | N/A                                           |
| IO+/TX                       | 25                                  | 1                                  | 24                                            |                                               |
| IO+RT                        | 2                                   | 0                                  | 2                                             |                                               |

Suri Y, Sudhaman S, Dwivedi B, et al. Clinical performance of a tumor-informed whole genome-based (WGS) ctDNA assay for recurrence detection and treatment response monitoring in localized and advanced/metastatic renal cell carcinoma (RCC). ESMO Congress, October 17–21, 2025; Berlin, Germany.

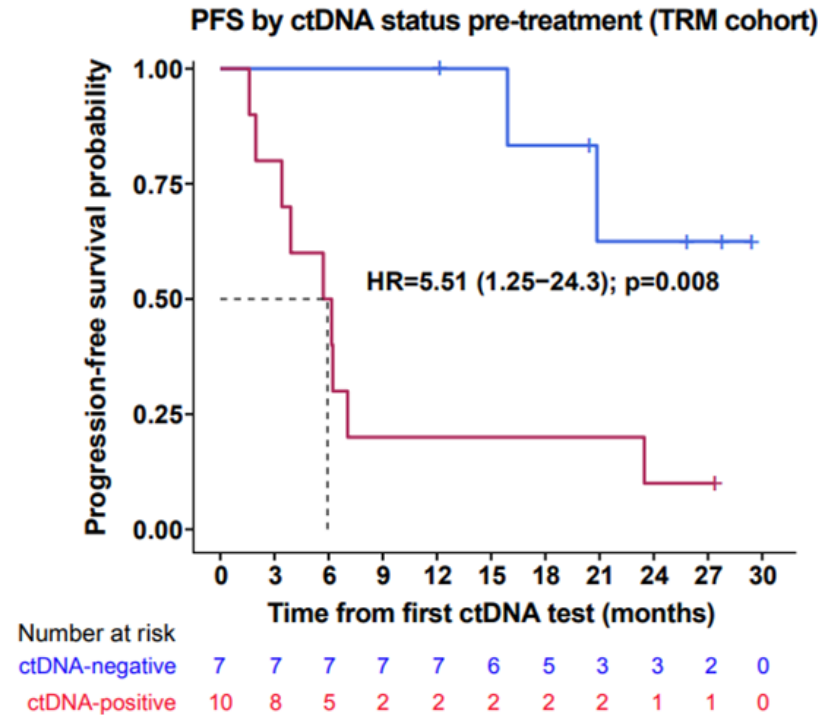
# ctDNA status post-definitive therapy was prognostic for RFS in localized RCC



Longitudinal analysis ctDNA Whole Genome Sequencing assay detected recurrence with 100% sensitivity, specificity, and predictive value in localized RCC

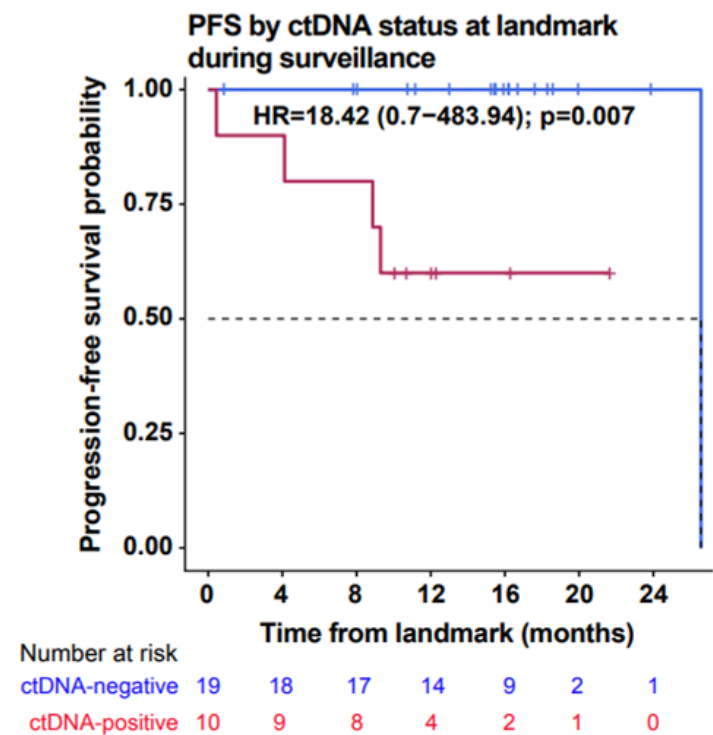
Suri Y, Sudhaman S, Dwivedi B, et al. Clinical performance of a tumor-informed whole genome-based (WGS) ctDNA assay for recurrence detection and treatment response monitoring in localized and advanced/metastatic renal cell carcinoma (RCC). ESMO Congress, October 17–21, 2025; Berlin, Germany.

# Pre-treatment ctDNA status was prognostic for PFS in metastatic RCC



**Patients who were ctDNA positive pre-treatment were 5.5-times more likely to progress than their ctDNA negative counterparts**

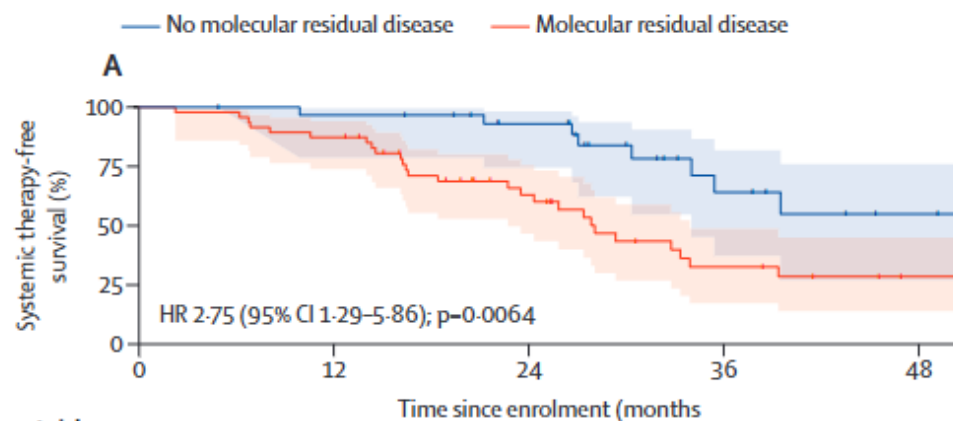
# ctDNA status at landmark during surveillance was prognostic for PFS in metastatic RCC



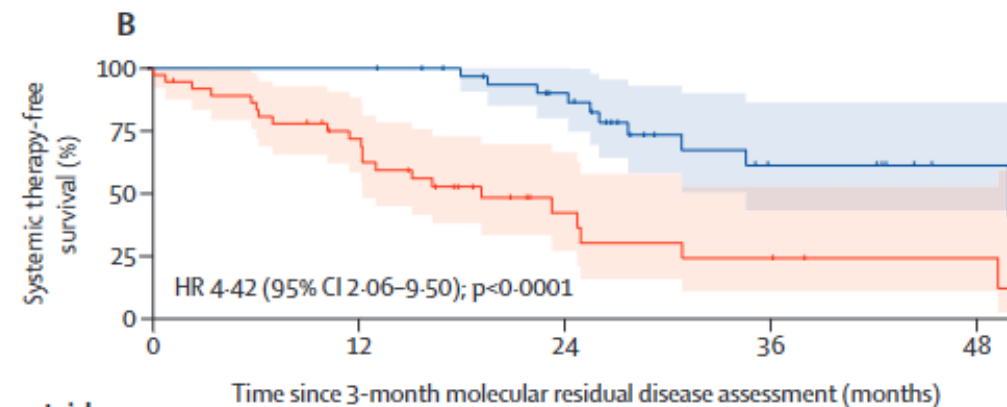
Landmark time point defined as 3 months after the end of treatment or start of surveillance

**Patients who were ctDNA positive at landmark during surveillance were 18.4-times more likely to progress than their ctDNA negative counterparts**

Suri Y, Sudhaman S, Dwivedi B, et al. Clinical performance of a tumor-informed whole genome-based (WGS) ctDNA assay for recurrence detection and treatment response monitoring in localized and advanced/metastatic renal cell carcinoma (RCC). ESMO Congress, October 17–21, 2025; Berlin, Germany.



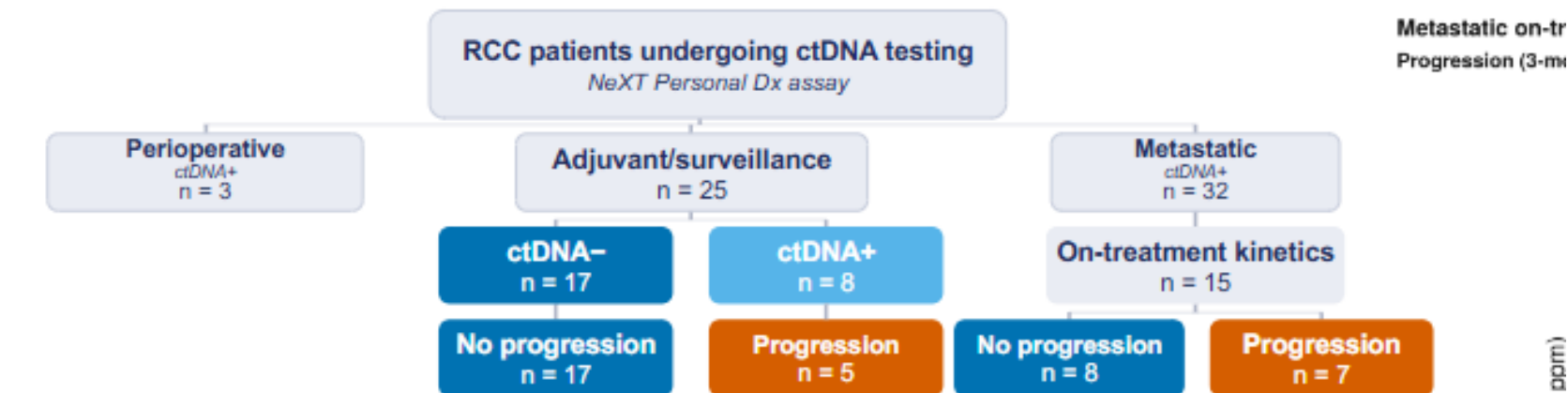
|                               | Number at risk<br>(events, censored) |               |                |               |             |
|-------------------------------|--------------------------------------|---------------|----------------|---------------|-------------|
| Molecular residual disease    | 47<br>(6, 0)                         | 41<br>(16, 9) | 22<br>(25, 13) | 9<br>(26, 17) | 4<br>(-, -) |
| No molecular residual disease | 31<br>(1, 1)                         | 29<br>(2, 6)  | 23<br>(7, 15)  | 9<br>(8, 19)  | 4<br>(-, -) |



|                               | Number at risk<br>(events, censored) |                |               |               |             |
|-------------------------------|--------------------------------------|----------------|---------------|---------------|-------------|
| Molecular residual disease    | 37<br>(10, 4)                        | 23<br>(18, 12) | 7<br>(21, 12) | 4<br>(21, 14) | 2<br>(-, -) |
| No molecular residual disease | 34<br>(0, 0)                         | 34<br>(3, 7)   | 24<br>(9, 17) | 8<br>(9, 22)  | 3<br>(-, -) |

## Ultrasensitive ctDNA prognostic pre and post XRT in RCC

Tang C, et al. *The Lancet Oncology*.  
2025



| Adjuvant/surveillance cohort |                              |                 |                 |                   |
|------------------------------|------------------------------|-----------------|-----------------|-------------------|
| Characteristic               | Adjuvant/surveillance (n=25) | ctDNA- (n=17)   | ctDNA+ (n=8)    | Progression (n=5) |
| n                            | 25                           | 17              | 8               | 5                 |
| Median age                   | 64 (44–80)                   | 64 (44–80)      | 67 (52–77)      | 64 (52–74)        |
| Male                         | 20/25 (80%)                  | 14/17 (82%)     | 6/8 (75%)       | 3/5 (60%)         |
| ccRCC                        | 21/25 (84%)                  | 14/17 (82%)     | 7/8 (88%)       | 4/5 (80%)         |
| pT3 stage                    | 24/25 (96%)                  | 16/17 (94%)     | 8/8 (100%)      | 5/5 (100%)        |
| Node positive                | 0/25 (0%)                    | 0/17 (0%)       | 0/8 (0%)        | 0/5 (0%)          |
| Median tumor size (cm)       | 6.5 (2.0–18.7)               | 5.7 (2.0–12.4)  | 8.2 (6.0–18.7)  | 9.2 (6.8–17.1)    |
| Rhabdoid/Sarcomatoid         | 9/25 (36%)                   | 3/17 (18%)      | 6/8 (75%)       | 4/5 (80%)         |
| Necrosis                     | 12/25 (48%)                  | 5/17 (29%)      | 7/8 (88%)       | 4/5 (80%)         |
| WHO/ISUP grade 4             | 9/25 (36%)                   | 3/17 (18%)      | 6/8 (75%)       | 4/5 (80%)         |
| Adjuvant therapy             | 12/25 (48%)                  | 7/17 (41%)      | 5/8 (62%)       | 3/5 (60%)         |
| Median f/u (mo)              | 12.2 (4.3–20.9)              | 11.7 (4.3–20.1) | 12.8 (4.7–20.9) | 17.2 (12.2–20.9)  |

Log2 (T3/T0 ctDNA, ppm)

ctDNA swimmer plot — Perioperative cohort & Adjuvant/surveillance cohort | n = 28 patients

◆ ctDNA+, active disease ◆ ctDNA+, stable/responsive disease ◆ ctDNA+, NED ◆ ctDNA-, NED/responsive disease ◆ ctDNA unknown ★ Progression ▲ Pembrolizumab start

Perioperative cohort (ctDNA+ pre-nephrectomy, T=0)

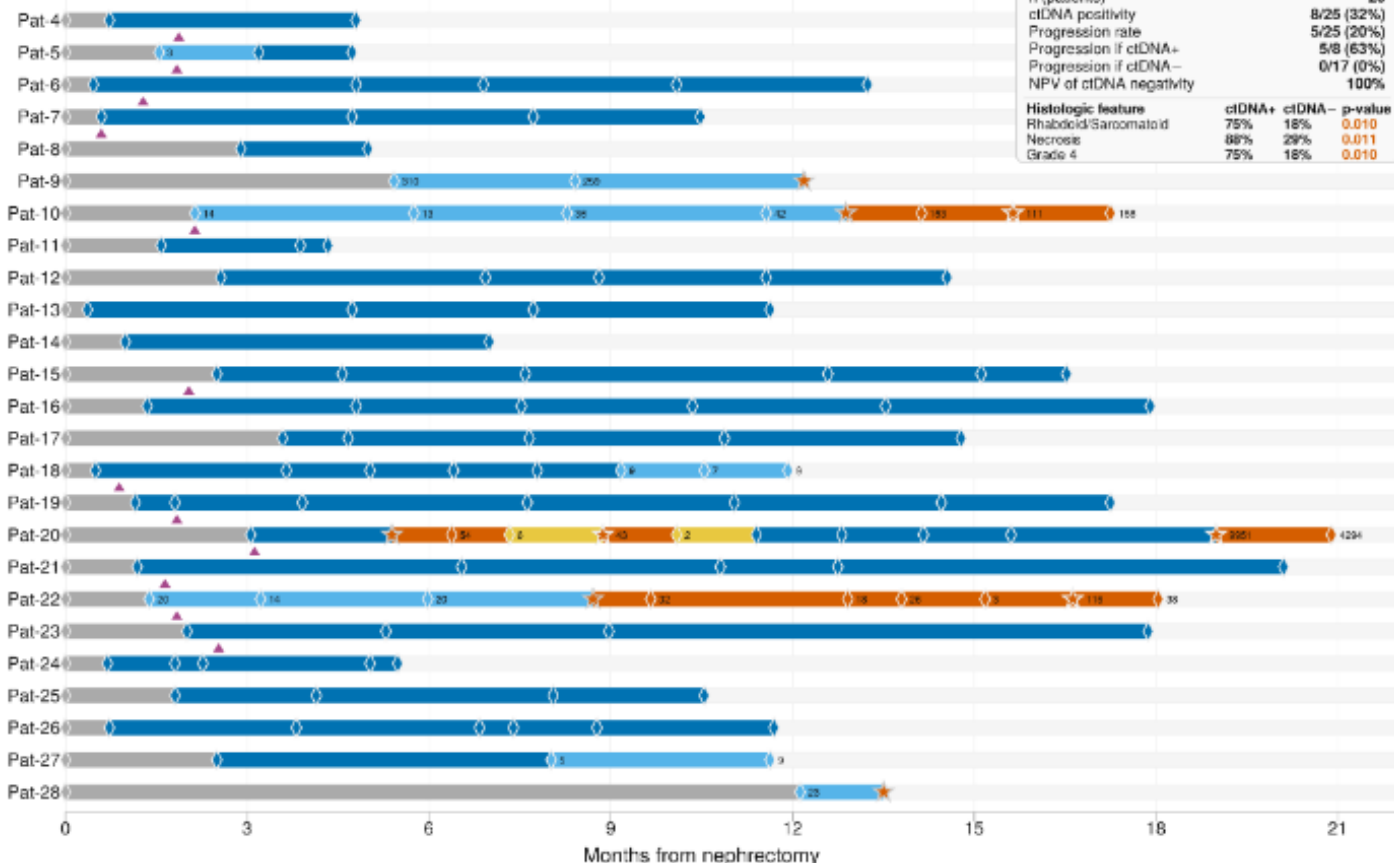
# ctDNA swimmer plot — Perioperative cohort & Adjuvant/surveillance cohort | n = 28 patients

◆ ctDNA+, active disease ◆ ctDNA+, stable/responsive disease ◆ ctDNA+, NED ◆ ctDNA-, NED/responsive disease ◆ ctDNA unknown ★ Progression ▲ Pembrolizumab start

## Perioperative cohort (ctDNA+ pre-nephrectomy, T=0)



## Adjuvant/surveillance cohort (ctDNA unknown pre-nephrectomy, T=0)



| Adjuvant/Surveillance Cohort Summary |        |        |         |
|--------------------------------------|--------|--------|---------|
| n (patients)                         | 25     |        |         |
| ctDNA positivity                     | 8/25   | 32%    |         |
| Progression rate                     | 5/25   | 20%    |         |
| Progression if ctDNA+                | 5/8    | 63%    |         |
| Progression if ctDNA-                | 0/17   | 0%     |         |
| NPV of ctDNA negativity              | 100%   |        |         |
| Histologic feature                   | ctDNA+ | ctDNA- | p-value |
| Rhabdoid/Sarcomatoid                 | 75%    | 18%    | 0.010   |
| Necrosis                             | 88%    | 29%    | 0.011   |
| Grade 4                              | 75%    | 18%    | 0.010   |

## ctDNA Findings by Disease Setting

Adjuvant/surveillance (n = 25; median follow-up 12.2 mo)

**100%**  
Sensitivity  
(progression)  
5/5 patients

**100%**  
NPV  
(no progression)  
17/17 patients

**5/8**  
MRD+ with  
progression to date  
(1 cleared on adjuvant therapy,  
2 recent MRD+ still on f/u)

**7/8**  
Ultrasensitive  
(patients with 1st draw ≤100  
ppm)  
median 17 ppm

Metastatic setting (n = 32; kinetics cohort n = 15)

**100%**  
Detection  
(all patients)  
32/32 patients

**23/32**  
Ultrasensitive  
(patients with ≥1 draw ≤100  
ppm)  
median 95 ppm

**7/7**  
ctDNA rise  
(in progressors, kinetics  
cohort)

**87%**  
Concordance  
(ctDNA vs. imaging, kinetics  
cohort)  
13/15 patients

# Next steps for hsctDNA in RCC

- Strategies needed to improve sensitivity ( 85%→ 95%)
  - Whole genome sequencing + high plex variants
  - High intensity serial testing (MRD GATE)
- Validation as prognostic factor in advanced disease
  - De-escalation studies for low ctDNA shedders

# ctDNA in Penile and Urethral Cancers

Utility of ctDNA for Monitoring Treatment Response in Penile and Primary Urethral Cancers

Sol Moon MD<sup>1</sup>, Kartik Patel BS<sup>2</sup>, Luke Shumaker MD<sup>1</sup>, James Ferguson III MD, PhD<sup>1,3</sup>, Arnab Basu MD<sup>3,4</sup>, Charles Peyton MD<sup>1,3</sup>

<sup>1</sup> Department of Urology, University of Alabama at Birmingham Heersink School of Medicine

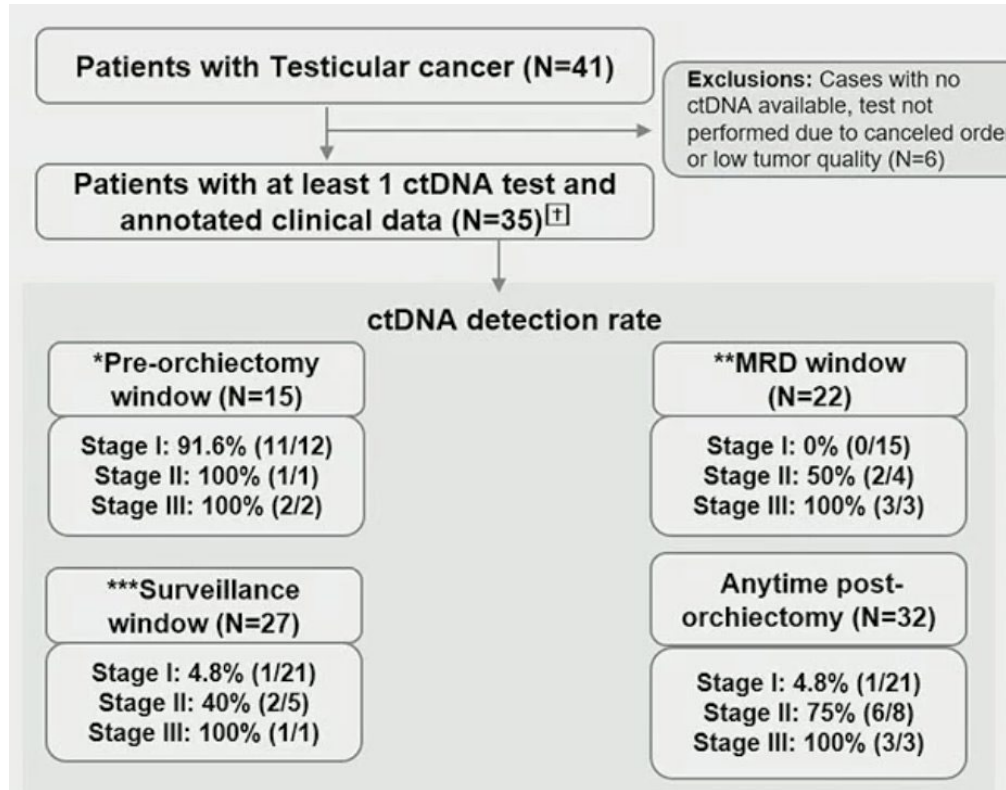
<sup>2</sup> University of Alabama at Birmingham Heersink School of Medicine

<sup>3</sup> O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham

<sup>4</sup> Department of Medicine, Division of Hematology-Oncology, University of Alabama at Birmingham Heersink School of Medicine

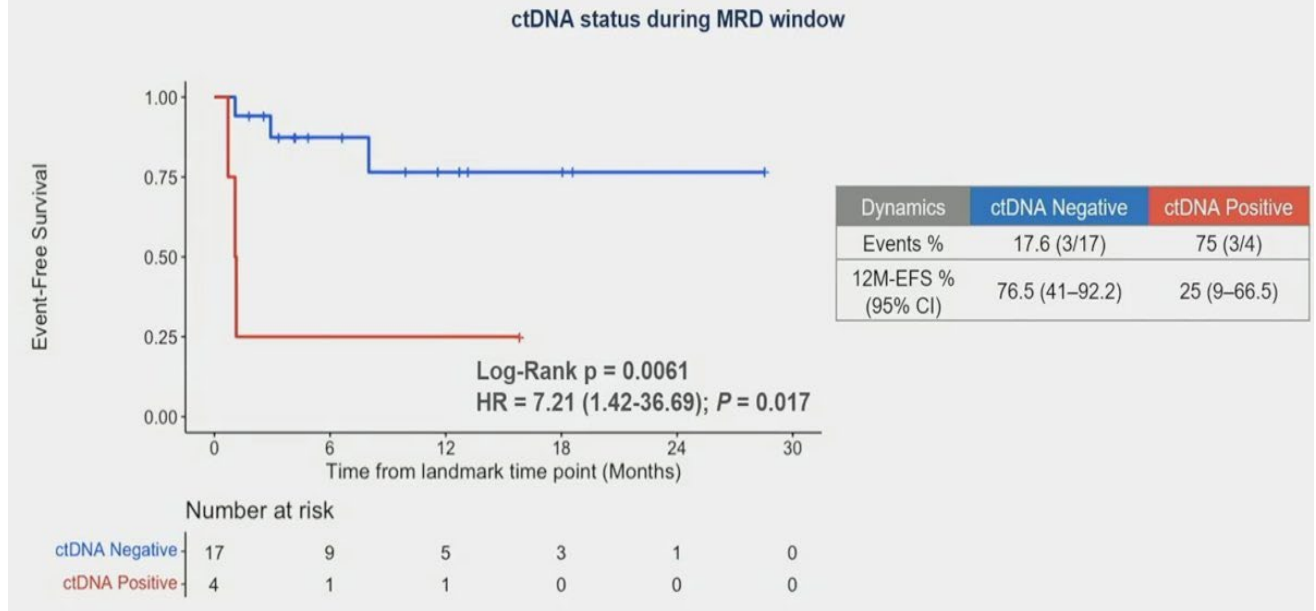
- 13 patients with penile and urethral cancers were included in a RWE retrospective cohort
- 58 serial samples were analyzed
- Median follow-up was 44 weeks
- Included patients treated with NAC, Upfront surgery and advanced settings
- **(96%) of results were concordant ( decrease / response)** , with radiographic response or pathologic downstaging depending on setting (  $p= 0.007$ ).
- Positive ctDNA tests had an odds ratio for progression of 1.92 (1.45-3.27,  $p=0.0015$ ).

# ctDNA in Testicular Cancer

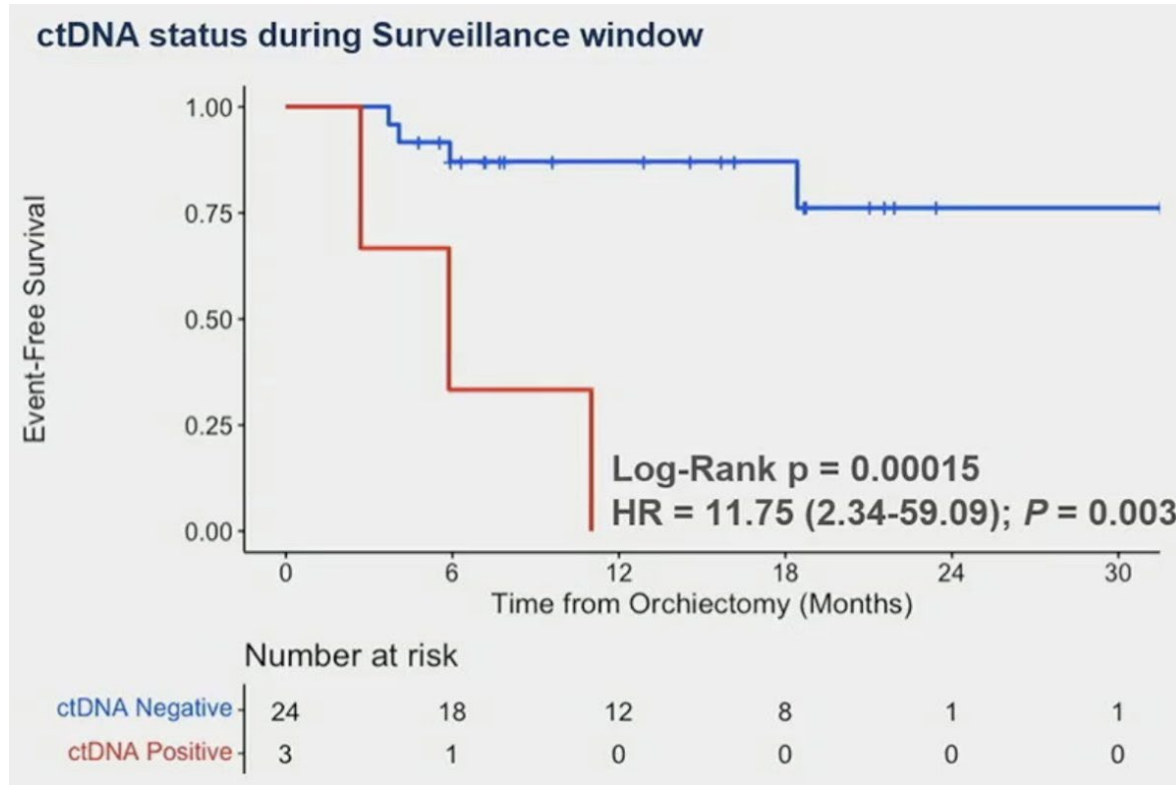


## Results: ctDNA-positivity at the MRD window is associated with inferior EFS

MRD window: 1-12 weeks post-orchietomy, prior to start of any first line therapy - Landmark 12 weeks



# Signatera in Testicular Cancer



| Association of ctDNA status with STM markers |                        |                      |
|----------------------------------------------|------------------------|----------------------|
| Status                                       | ctDNA Negative         | ctDNA Positive       |
| Normal Markers                               | 19 (3 relapsed, 15.8%) | 2 (2 relapsed, 100%) |
| Elevated Markers                             | 2 (0 relapsed, 0%)     | 1 (1 relapsed, 100%) |

# Summary

- Ultrasensitive ctDNA is a strong prognostic factor for recurrence in both neoadjuvant and adjuvant settings for MIBC
- Sustained ctDNA negative patients have excellent prognosis in MIBC after surgery irrespective of pathologic staging.
- ctDNA guided therapy can improve overall survival in adjuvant setting compared to observation.
- Newer techniques are improving detection rates and actionability of MRD in a low shedding disease like RCC but prospective studies are needed.

- Thank you , Questions?