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The Emerging role of ctDNAin the treatment of bladder cancer

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Terminology

- cfDNA : Circulating Free DNA
  - Free DNA circulating in the blood originates both from normal and tumor cells
- ctDNA : Circulating Free Tumor DNA
  - Free DNA circulating in the blood originated specifically from tumor cells
- CTC – Circulating Tumor Cells
  - Whole tumor cells shed from tumor to the blood

Precision Medicine – The Promise of ctDNA

- Selecting patients for different treatment modalities (i.e. adjuvant therapy)
- Detecting patients that are in high risk for recurrence at earlier stages
- Monitoring response to neoadjuvant treatment
- Monitoring response to systemic treatments (chemotherapy, immunotherapy)

What is ctDNA

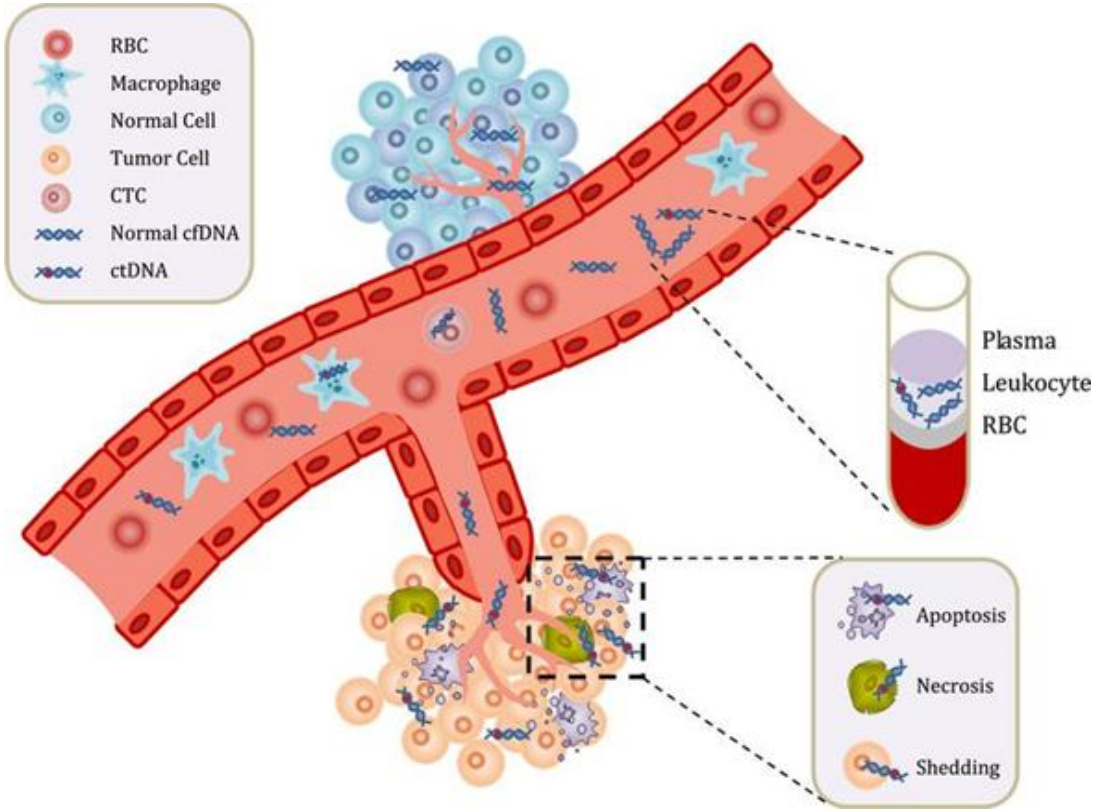
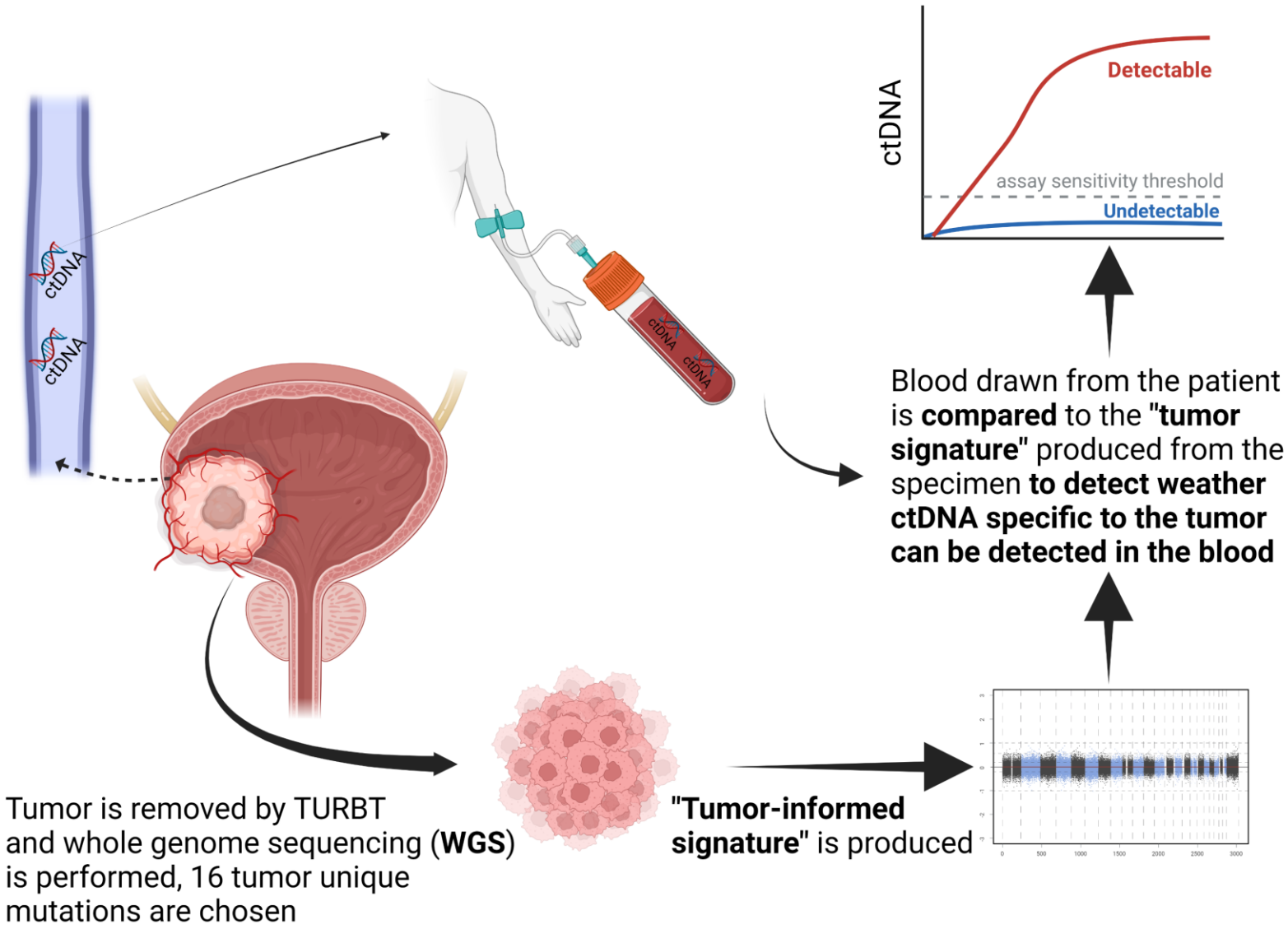


TABLE 1.
COMPARISON OF AVAILABLE CIRCULATING TUMOR DNA ASSAYS

COMPANY	ASSAY	TYPE OF ASSAY	TYPE OF SPECIMEN	CANCER TYPE	INSURANCE COVERAGE	FINANCIAL ASSISTANCE PROGRAMS
Guardant Complete	Guardant Reveal™	Tumor-agnostic	Plasma	Available for patients with colorectal, breast, and lung cancers (primarily stages II and III, although will accept patients with stages I and IV cancer [oligometastatic]); additional cancer types are undergoing research.	Insurance billed, accepts Medicare and Medicaid but does not guarantee payment; Medicare approval for patients with stages II and III colorectal cancer whose first test is ordered 3–13 weeks post–curative-intent treatment	Available; if insurance denies coverage, there is an appeals process. The patient may be billed for co-pay and deductible.
Haystack Oncology	Haystack MRD™ Test	Tumor-informed	Tumor, plasma, whole blood	Colorectal	Uncertain	Uncertain
Invitae	Personalized Cancer Monitoring™ Baseline Test and Personalized Cancer Monitoring™ Test	Tumor-informed	Tumor, plasma, whole blood	Colorectal, other	Insurance billed, accepts Medicare and Medicaid but does not guarantee payment	Available
Natera	Signatera™	Tumor-informed	Tumor, plasma, whole blood	Primarily colorectal; also indicated for bladder, breast, lung, skin, gynecologic, and immunotherapy treatment response monitoring for all cancer types	Insurance billed, accepts major carriers but does not guarantee payment; covered by Medicare and Medicare Advantage for colorectal, breast, and muscle-invasive bladder cancer, and immunotherapy treatment response monitoring; limited commercial and Medicaid insurance coverage	Available
NeoGenomics	RaDaR®	Tumor-informed	Tumor, plasma, whole blood	Colorectal, breast, head and neck, lung	Uncertain	Available; self-pay with financial assistance
Roche	Avenio	Tumor-informed and tumor-agnostic	Plasma	Colorectal, lung	Uncertain	Uncertain

Note. Based on information from Guardant Complete, 2023; Haystack Oncology, 2023; Invitae, 2023; Natera, 2023; NeoGenomics, 2023; Roche, 2023.

Figure. Bespoke Tumor-Informed ctDNA Analysis of Bladder Tumors



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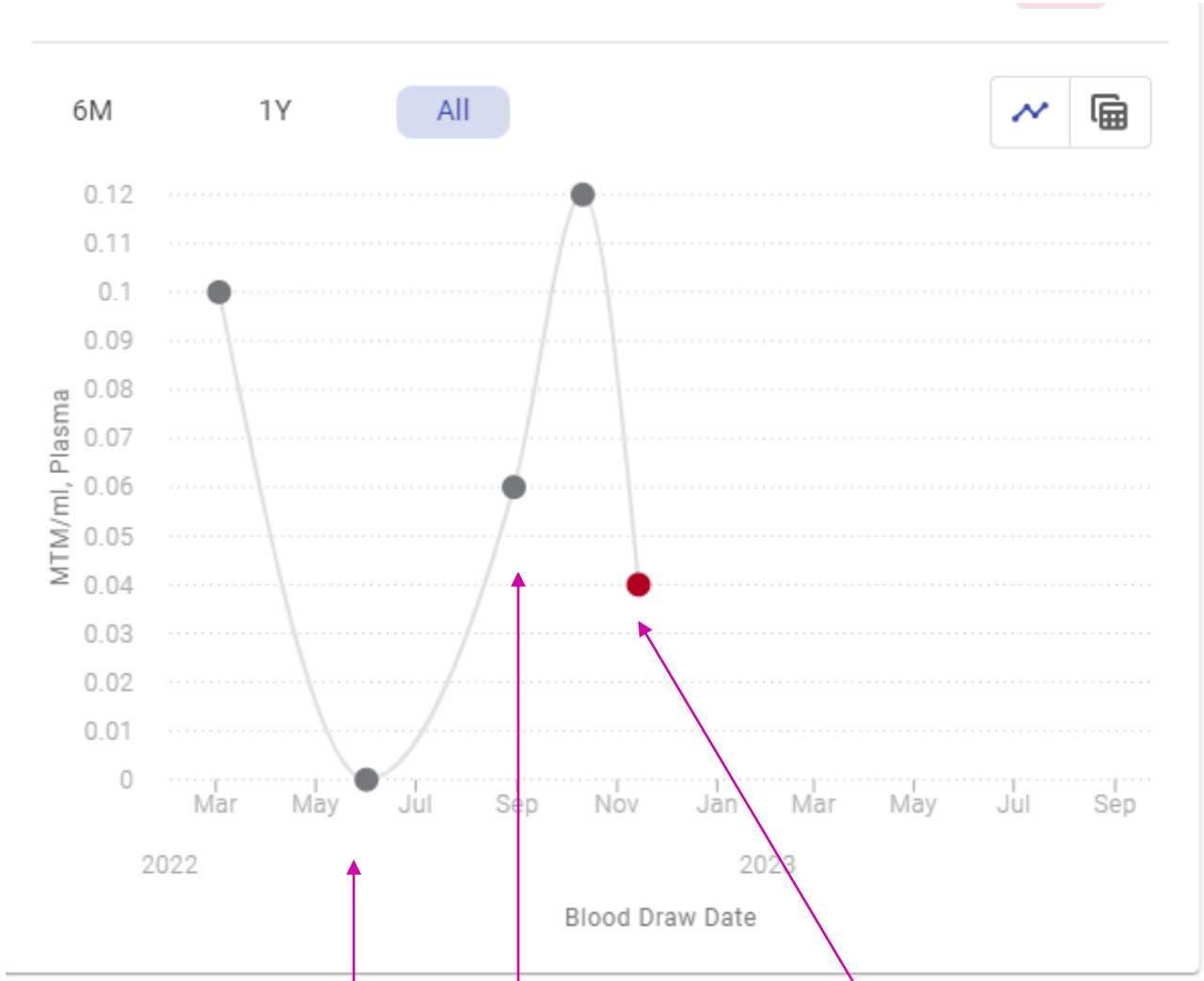


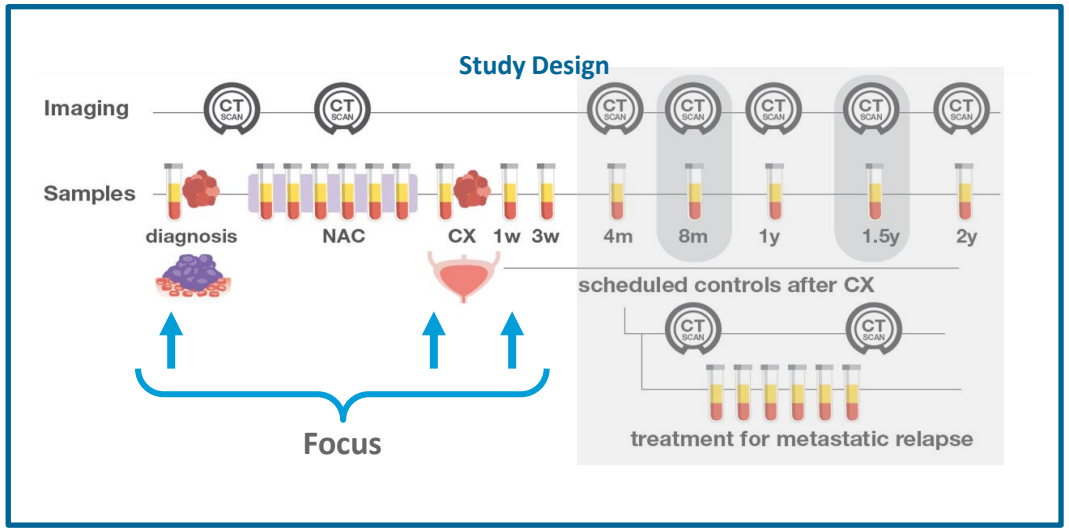
Surgery

Recurrence

Response to Systemic Therapy

ctDNA in NAC treated Patients

- **68 patients** with locally advanced muscle-invasive bladder cancer (MIBC)
- **656 plasma samples** longitudinally collected before, during, and after therapy and analyzed using Signatera™ assay



Key Findings

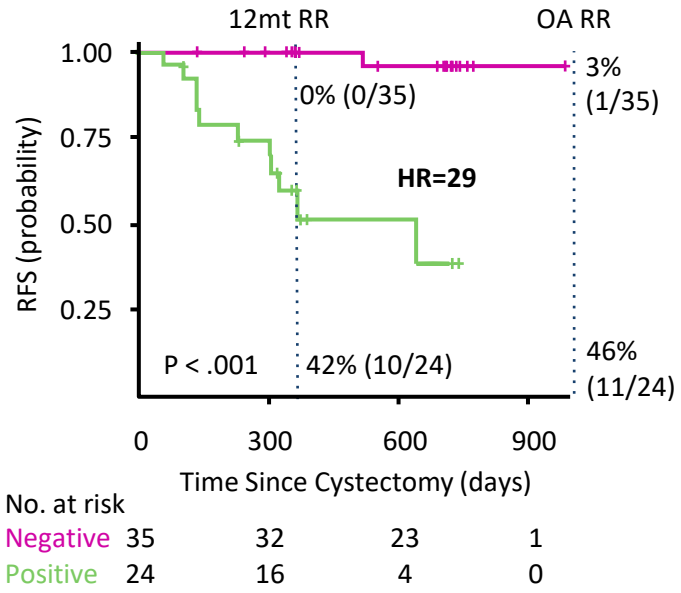
- ctDNA is a prognostic marker after TURBT and before neoadjuvant treatment
- Lack of ctDNA clearance during neoadjuvant treatment is a better predictor of recurrence than pathologic response
- Lead time to clinical recurrence of median 96 days
- Patients with serial negative ctDNA after cystectomy had 100% OS

Christensen E, Birkenkamp-Demtröder 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. doi:10.1200/JCO.18.02052

Lack of ctDNA clearance during neoadjuvant treatment is a better predictor of recurrence free survival

Single time point before neoadjuvant chemo

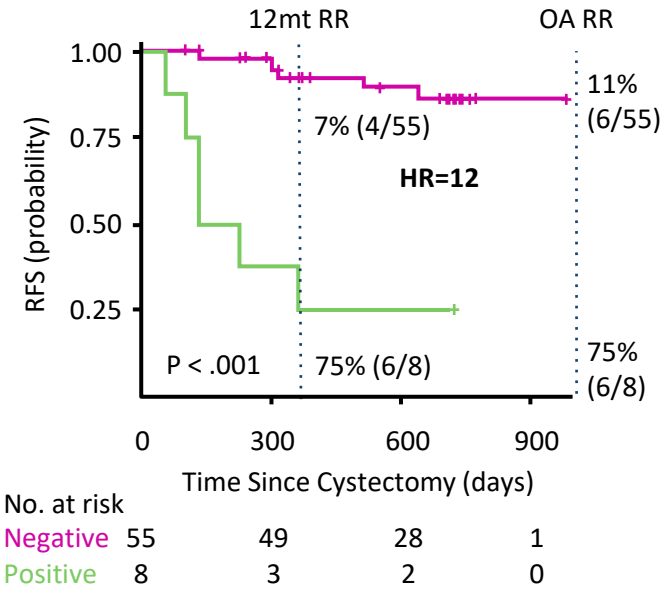
Recurrence Free Survival (RFS)



No. at risk				
Negative	35	32	23	1
Positive	24	16	4	0

Single time point after completion of neoadjuvant chemo

Recurrence Free Survival (RFS)



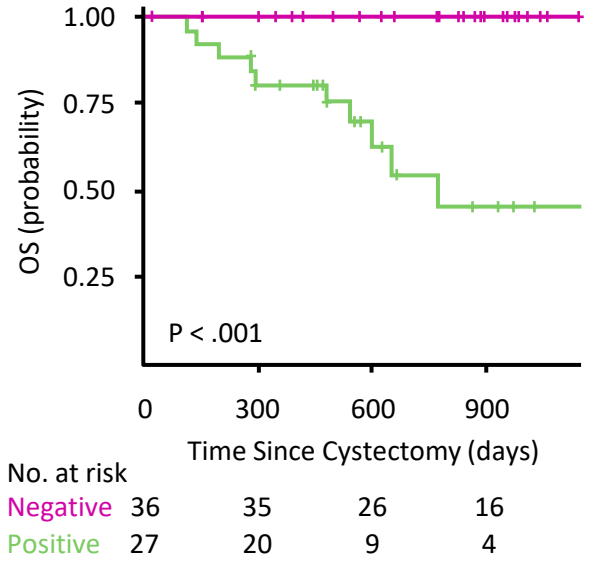
No. at risk				
Negative	55	49	28	1
Positive	8	3	2	0

Christensen et al. *JCO 2019*

Lack of ctDNA clearance during neoadjuvant treatment is a better predictor of Overall Survival

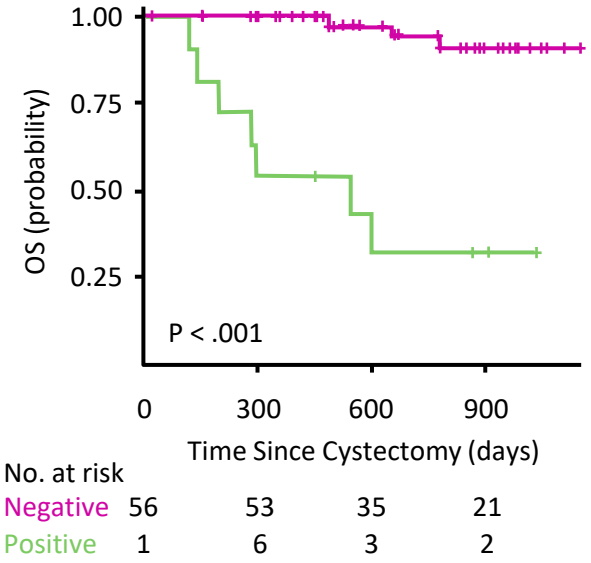
Single time point before neoadjuvant chemo

Overall Survival (OS)



Single time point after completion of neoadjuvant chemo

Overall Survival (OS)

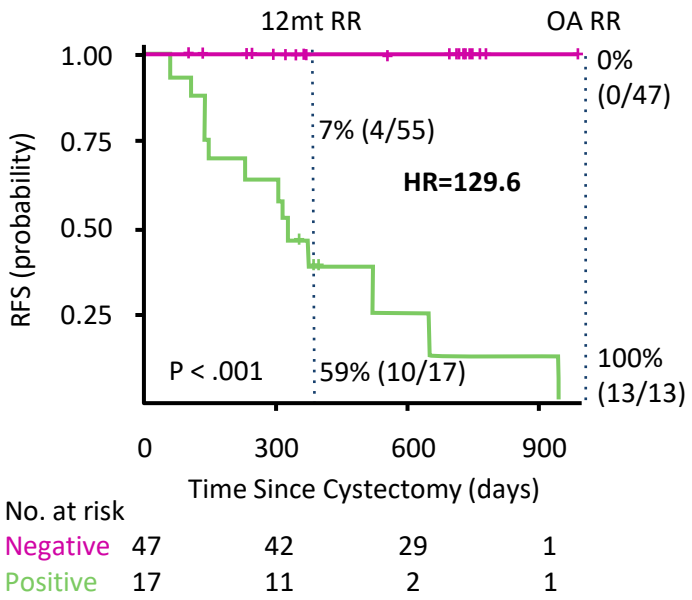


Christensen et al. *JCO 2019*

ctDNA-positive patients were likely to recur – serially ctDNA-negative patients did not

Longitudinal testing after cystectomy

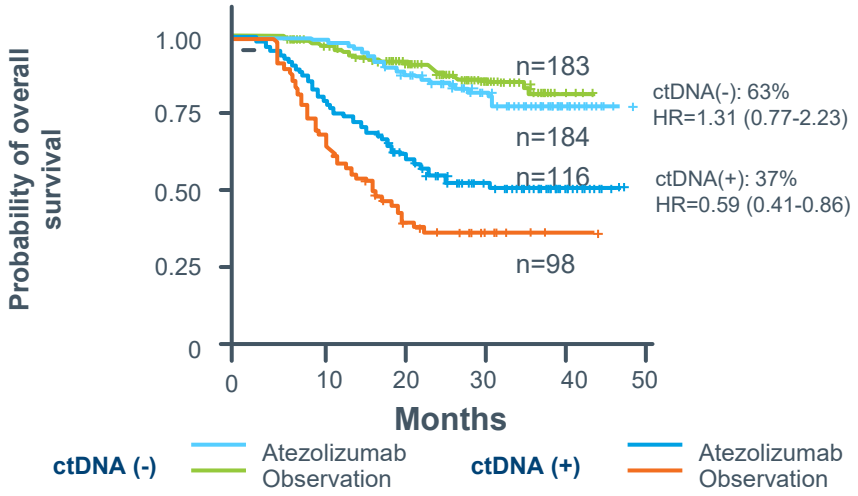
Recurrence free survival (RFS)



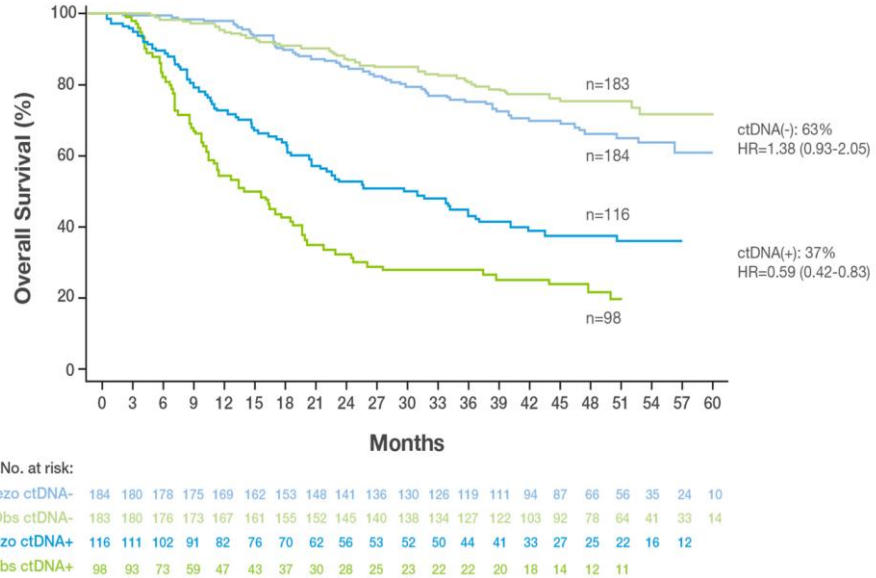
- Sensitivity = 100%; Specificity = 98%
- Highly prognostic with RFS HR=129.6
- Median lead time = 96 days
- Patients with serial negative ctDNA after cystectomy had 100% OS
- More reliable as a predictive factor than lymph node status or pathologic staging

Christensen et al. *JCO 2019*

Ph III IMvigor 010 showing predicative ability of ctDNA



Atezolizumab vs Observation: Extended Follow-up[2]



EUROPEAN UROLOGY

Powles et al. *Nature* 2021

Powles et al. *Eur Urol* 2023

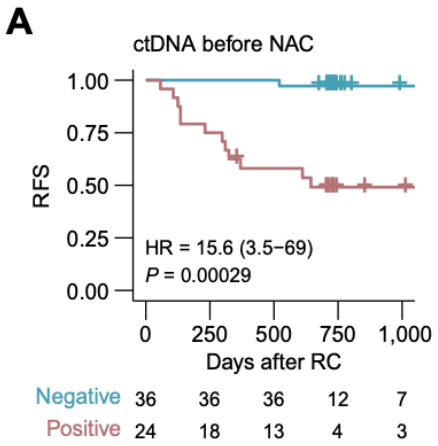
Circulating Tumor DNA Analysis in Advanced Urothelial Carcinoma: Insights from Biological Analysis and Extended Clinical Follow-up

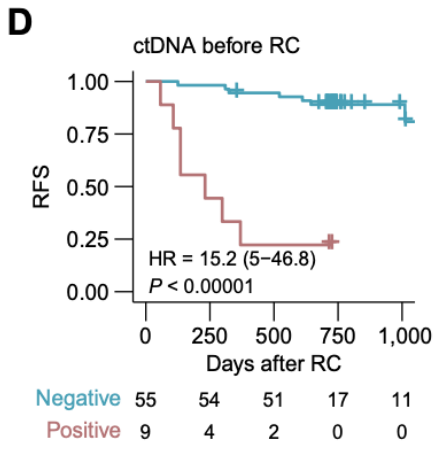
Sia V. Lindskrog[1,2], Karin Birkenkamp-Demtröder[1,2], Iver Nordentoft[1], George Laliotis[3], Philippe Lamy[1], Emil Christensen[1], Derrick Renner[3], Tine G. Andreasen[1,2], Naja Lange[1], Shruti Sharma[3], Adam C. ElNaggar[3], Minetta C. Liu[3], Himanshu Sethi[3], Alexey Aleshin[3], Mads Agerbæk[4], Jørgen B. Jensen[2,5], and Lars Dyrskjøt[1,2]

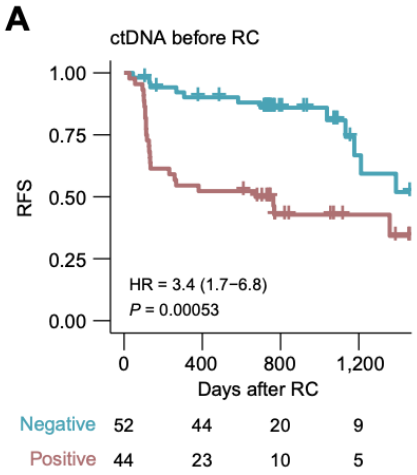
Table 1. Demographics and clinical characteristics of patient cohorts.

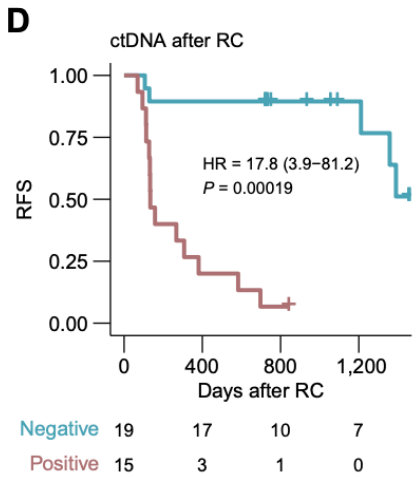
	NAC-treated cohort ($N = 68$)[a]	NAC-naïve cohort ($N = 102$)[a]	*P* value[b]
Age, years, median (IQR)	67 (59–71)	69 (62–75)	0.010
Sex			0.53
Female	12 (18%)	22 (22%)	
Male	56 (82%)	80 (78%)	
T-stage, TURBT			
Ta	0 (0%)	1 (1%)	<0.001
T1	2 (2.9%)	22 (22%)	
T2	59 (87%)	75 (74%)	
T3	1 (1.5%)	0 (0%)	
T4	6 (8.8%)	3 (3%)	
N-stage, TURBT			
N0	58 (85%)		
N1	7 (10%)		
N2	3 (4.4%)		
T-stage, RC			<0.001
T0/CIS/Ta	44 (67%)	27 (26%)	
T1	3 (4.5%)	8 (7.8%)	
T2	6 (9.1%)	23 (23%)	
T3	9 (14%)	35 (34%)	
T4	4 (6.1%)	9 (8.8%)	
N-stage, RC			0.059
N0	59 (88%)	79 (80%)	
N1	2 (3%)	13 (13%)	
N2	3 (4.5%)	6 (6.1%)	
N3	3 (4.5%)	1 (1%)	
Pathologic downstaging after NAC	44 (66%)		
Complete response after NAC	42 (63%)		
Recurrence	18 (28%)	44 (44%)	0.035
12-month recurrence	10 (15%)	29 (30%)	0.034
RFS, months, median (IQR)	24 (23–25)	24 (5–32)	0.10
2-year survival	57 (86%)	73 (72%)	0.037
5-year survival	48 (73%)	58 (57%)	0.049
OS, months, median (IQR)	68 (54–82)	72 (17–92)	0.042

Abbreviations: CIS, carcinoma in situ; IQR, interquartile range.
[a]Median (IQR); *n* (%).
[b]Wilcoxon rank sum test; Person's χ^2 test; Fisher exact test; Cox regression analysis.









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ctDNA Status in the Precystectomy Stage

- Precystectomy ctDNA status is informed from the TURBT specimen, it is predictive of both final pathological outcomes and long term prognosis irrespective of clinical staging
- Precystectomy ctDNA status can be used for treatment de-escalation or escalation already at the neoadjuvant setting.
- Patients with non-muscle invasive disease may harbor an aggressive disease and can be classified by ctDNA status to more aggressive treatments

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available at www.sciencedirect.com
journal homepage: euoncology.europeanurology.com

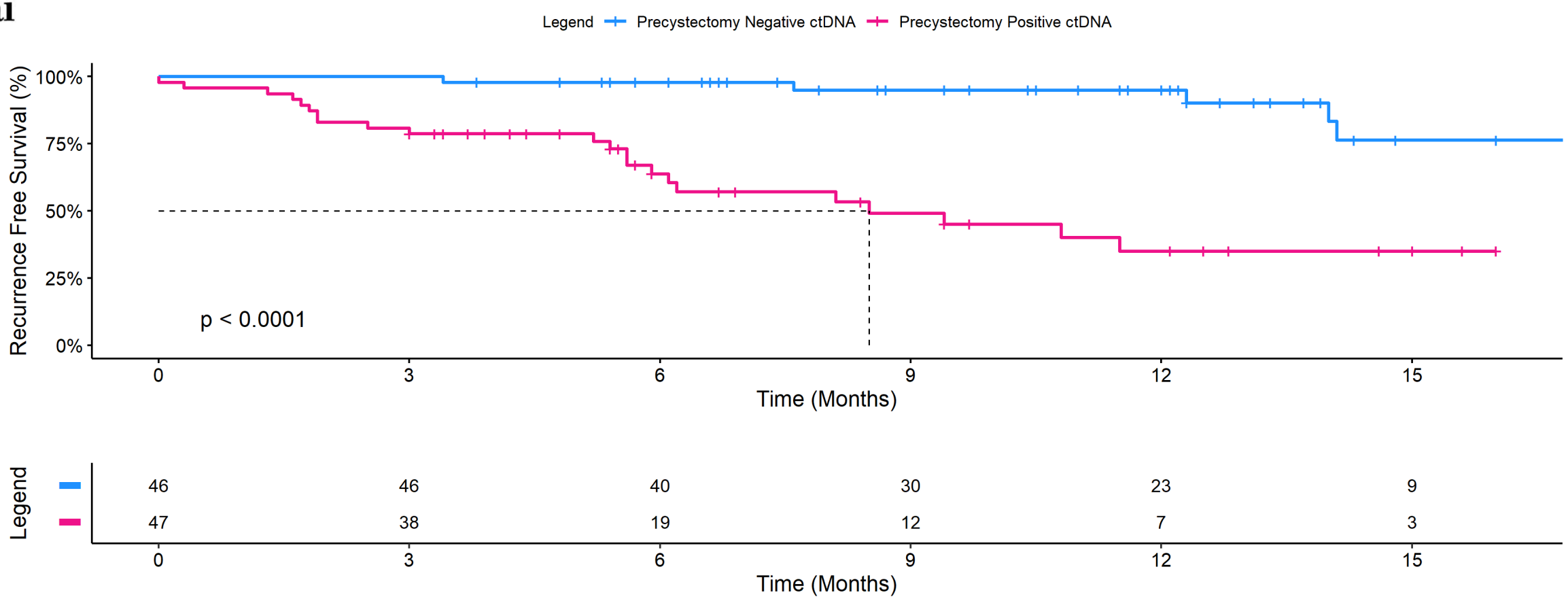
European Association of Urology

Longitudinal Tumor-informed Circulating Tumor DNA Status Predicts Disease Upstaging and Poor Prognosis for Patients Undergoing Radical Cystectomy

Reuben Ben-David [a,*], *Neeraja Tillu* [a], *Shivaram Cumarasamy* [a], *Parissa Alerasool* [b], *Jordan M. Rich* [a], *Basil Kaufmann* [a], *Yuval Elkun* [a], *Kyrollis Attalla* [a], *Reza Mehrazin* [a], *Peter Wiklund* [a], *John P. Sfakianos* [a]

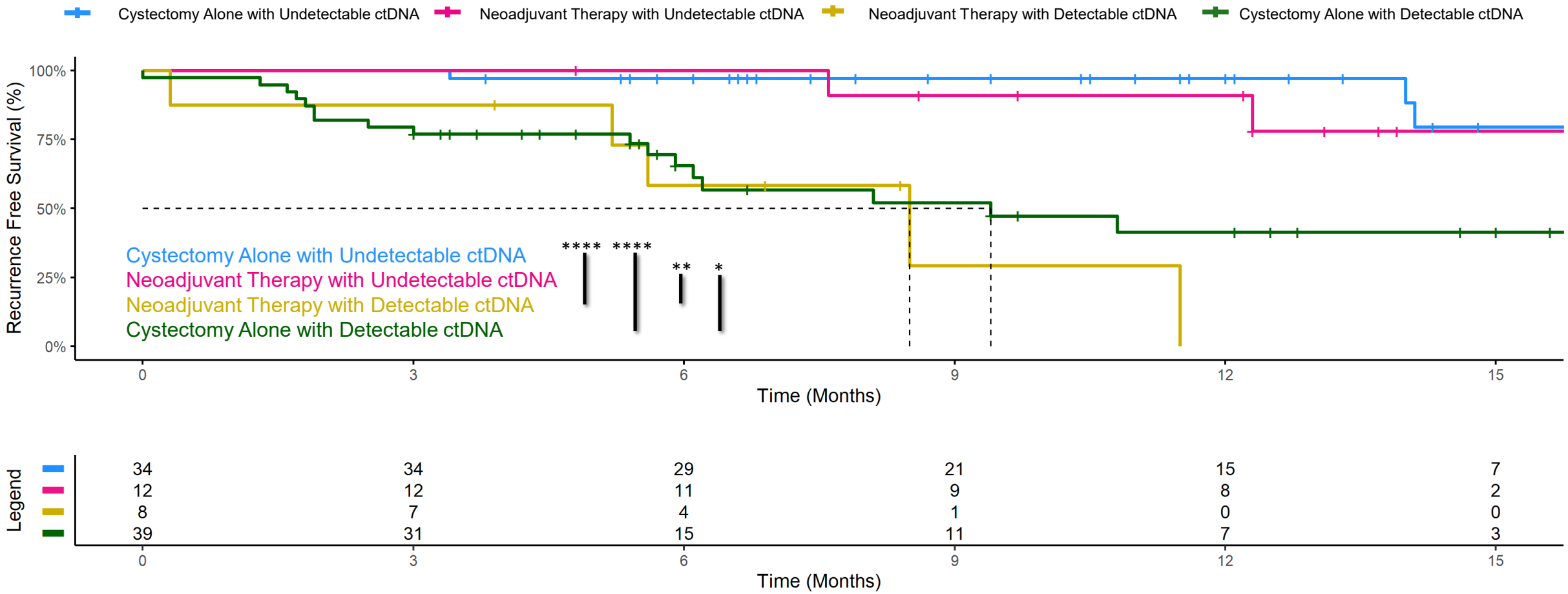
[a] *Urology Department, Icahn School of Medicine at Mount Sinai, New York, NY, USA;* [b] *The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Precystectomy ctDNA status and Prognosis

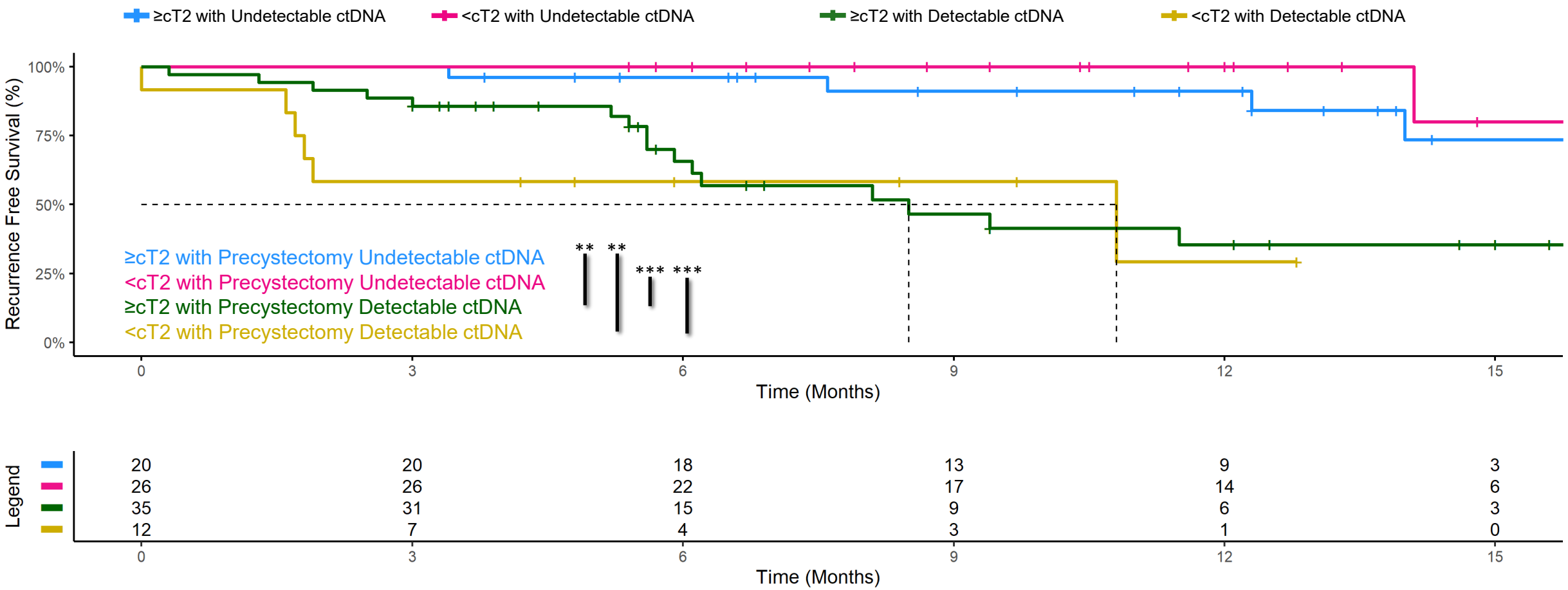


	Recurrence Free Survival at 6 Months	Recurrence Free Survival at 12 Months	Recurrence Free Survival at 15 Months
Precystectomy Undetectable ctDNA	98%	95%	76%
Precystectomy Detectable ctDNA	64%	35%	35%

RFS according to ctDNA status and neoadjuvant/cystectomy alone



RFS according to ctDNA status and clinical stage



ctDNA Precystectomy Status as Predictor of pN+ and ≥pT3 Disease

Positive Lymph Node Disease				Univariate Analysis			Multivariate Analysis	
Characteristic		N	Event N	OR [95% CI][1]	p-value	q-value	OR [95% CI][1]	p-value
Variant Histology on TURBT	No	108	29	Ref			Ref	
	Yes			3.3 [1.3, 8.5]	0.014	**0.014**	3.4 [1.2, 10]	**0.02**
clinical Stage	<T2	109	29	Ref			Ref	
	≥T2			3.6 [1.3,11.4]	0.011	**0.014**	2.7 [0.9, 9.4]	0.1
Precystectomy ctDNA	Undetectable	109	29	Ref			Ref	
	Detectable			6.8 [2.5, 22]	<0.001	**<0.001**	5.4 [1.9, 18.2]	**0.003**

[1] OR = Odds Ratio, CI = Confidence Interval

Locally Advanced Disease (≥pT3)				Univariate Analysis			Multivariate Analysis	
Characteristic		N	Event N	OR [95% CI][1]	p-value	q-value	OR [95% CI][1]	p-value
Age		112	44	1.06 [1.02, 1.12]	0.005	**0.008**	1.04 [0.99, 1.11]	0.1
Sex	Male	112	44	Ref			Ref	
	Female			1.5 [0.6, 4]	0.39	0.49	1.6 [0.5, 4.7]	0.4
Variant Histology on TURBT	No	111	44	Ref			Ref	
	Yes			1.3 [0.5, 3.1]	0.56	0.56	1.1 [0.4, 3.1]	0.8
clinical Stage	<T2	112	44	Ref			Ref	
	≥T2			5 [2,13.6]	<0.001	**<0.001**	3.6 [1.4, 10.2]	**0.013**
Precystectomy ctDNA	Undetectable	112	44	Ref			Ref	
	Detectable			4.9 [2.1, 11.6]	<0.001	**<0.001**	3.6 [1.5, 9]	**0.005**

[1] OR = Odds Ratio, CI = Confidence Interval

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ctDNA at the MRD Window

- ctDNA status at the MRD window can be used to refine our selection process to different adjuvant treatments vs observation-only
- ctDNA status can decrease overtreatment rates
- ctDNA status may stratify patients to treatment arm while not having “qualifying” stage to adjuvant treatment, yet are harboring a micrometastatic disease, potentially increasing the treatment benefit for these patients

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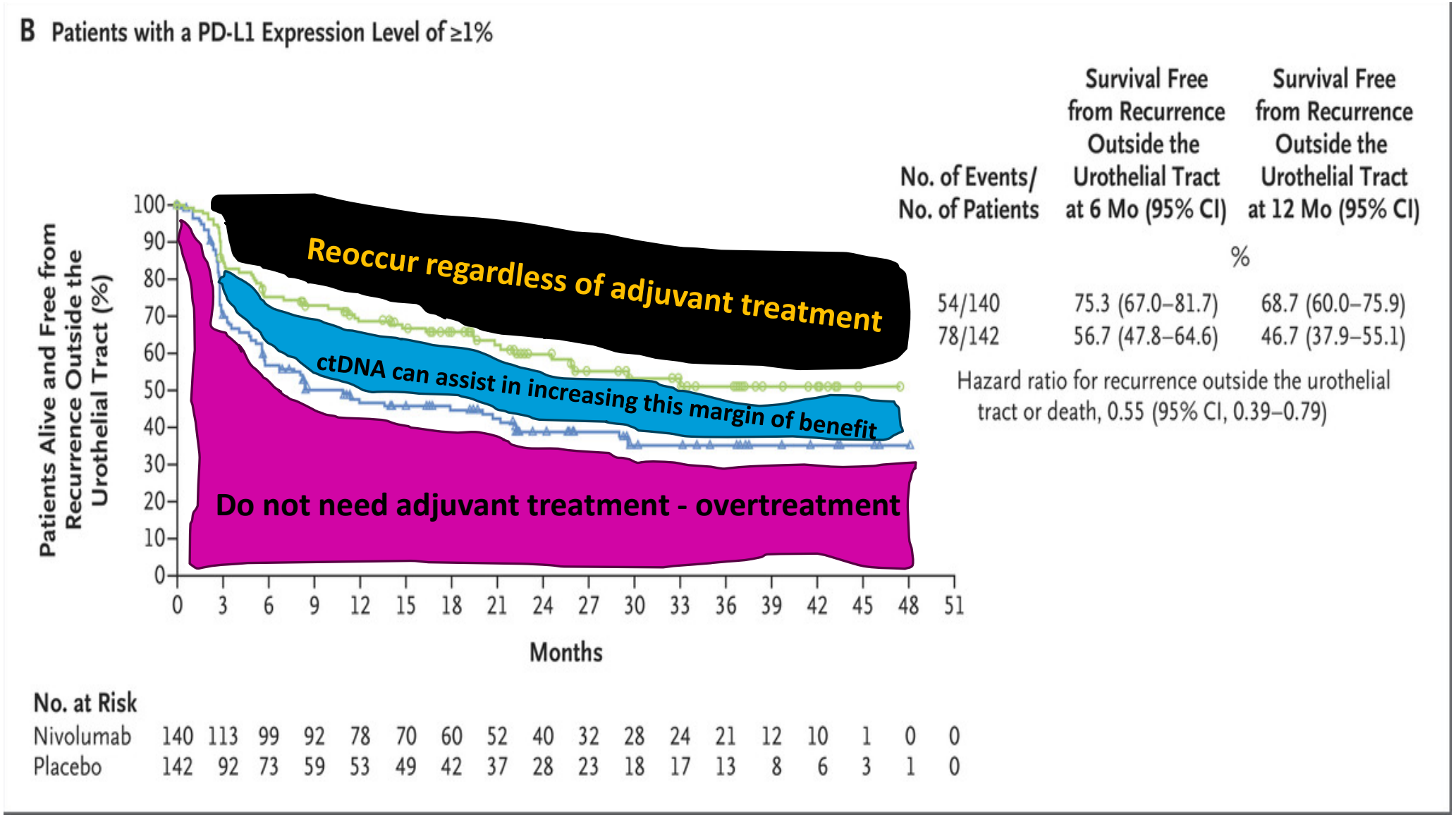
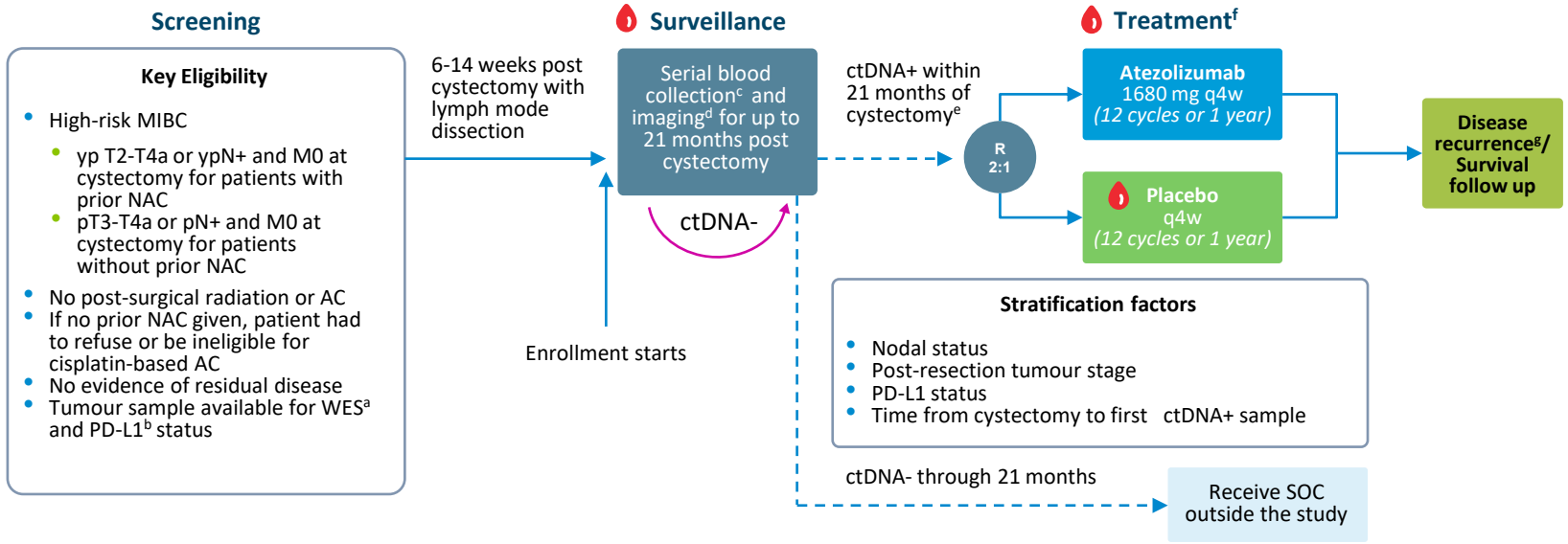


Bajorin et al., NEJM 2021, Adjuvant Nivolumab versus Placebo in Muscle Invasive Urothelial Carcinoma

IMvigor011

▸ctDNA-based rule-in trial criteria



ctDNA-, circulating tumor DNA negative; ctDNA+, circulating tumor DNA positive; q4w every 4 weeks; SOC, standard of care; WES, shole-exome sequencing.
[a]Evaluable WES data for development of personalized multiplex PCR (mPCR) ctDNA assay from post-surgical blood samples (Signatera assay) are required.
[b]Per the VENTANA SP 142 IHC assay.
[c]Every 6 weeks up to 36 weeks and q12 (every 12 weeks) up to 21 months.
[d]q12w up to Week 84or until 21 months from date of cystectomy, whichever occurs first.
[e]ctDNA positivity is defined as ≥2 mutations per ctDNA mPCR assay. Patients will be randomized to treatment at first ctDNA+ sample; full recovery from cystectomy and no evidence of disease recurrence within 28 days of treatment initiation is requires.
[f]Imaging and blood draws q9w (every 9 weeks) starting at Week 9 up to Week 54.
[g]Assessed q9w up to Year 3; less often up to Year 6.

European Urology
Volume 85, Issue 2, February 2024, Pages 114-122

Platinum Priority – Urothelial Cancer – Editor's Choice

Editorial by Natacha Naoun, Yohann Loriot on pp. 123–124 of this issue

Updated Overall Survival by Circulating Tumor DNA Status from the Phase 3 IMvigor010 Trial: Adjuvant Atezolizumab Versus Observation in Muscle-invasive Urothelial Carcinoma

Thomas Powles [a †], Zoe June Assaf [b †], Viraj Degaonkar [b], Petros Grivas [c], Maha Hussain [d], Stephane Oudard [e], Jürgen E. Gschwend [f], Peter Albers [g], Daniel Castellano [h], Hiroyuki Nishiyama [i], Siamak Daneshmand [j], Shruti Sharma [k], Himanshu Sethi [k], Alexey Aleshin [k], Yi Shi [b], Nicole Davarpanah [b], Corey Carter [b], Joaquim Bellmunt [l ‡], Sanjeev Mariathasan [b ‡]

IMvigor010 Trial

- Landmark trial - Phase 3 trial of adjuvant atezolizumab vs. observation for MIBC
- While atezolizumab failed in achieving overall survival benefits over observation-only, retrospective ctDNA analysis at the MRD window demonstrated important insights
- Atezolizumab demonstrated higher ctDNA clearance vs. observation-only (18% vs. 3.8%)
- ctDNA positive patients benefited from atezolizumab treatment vs observation (HR 0.59 [95% CI 0.42-0.83]), while patients with ctDNA negative have similar outcomes between the study arms
- Further investigation is made in the IMvigor011 trial

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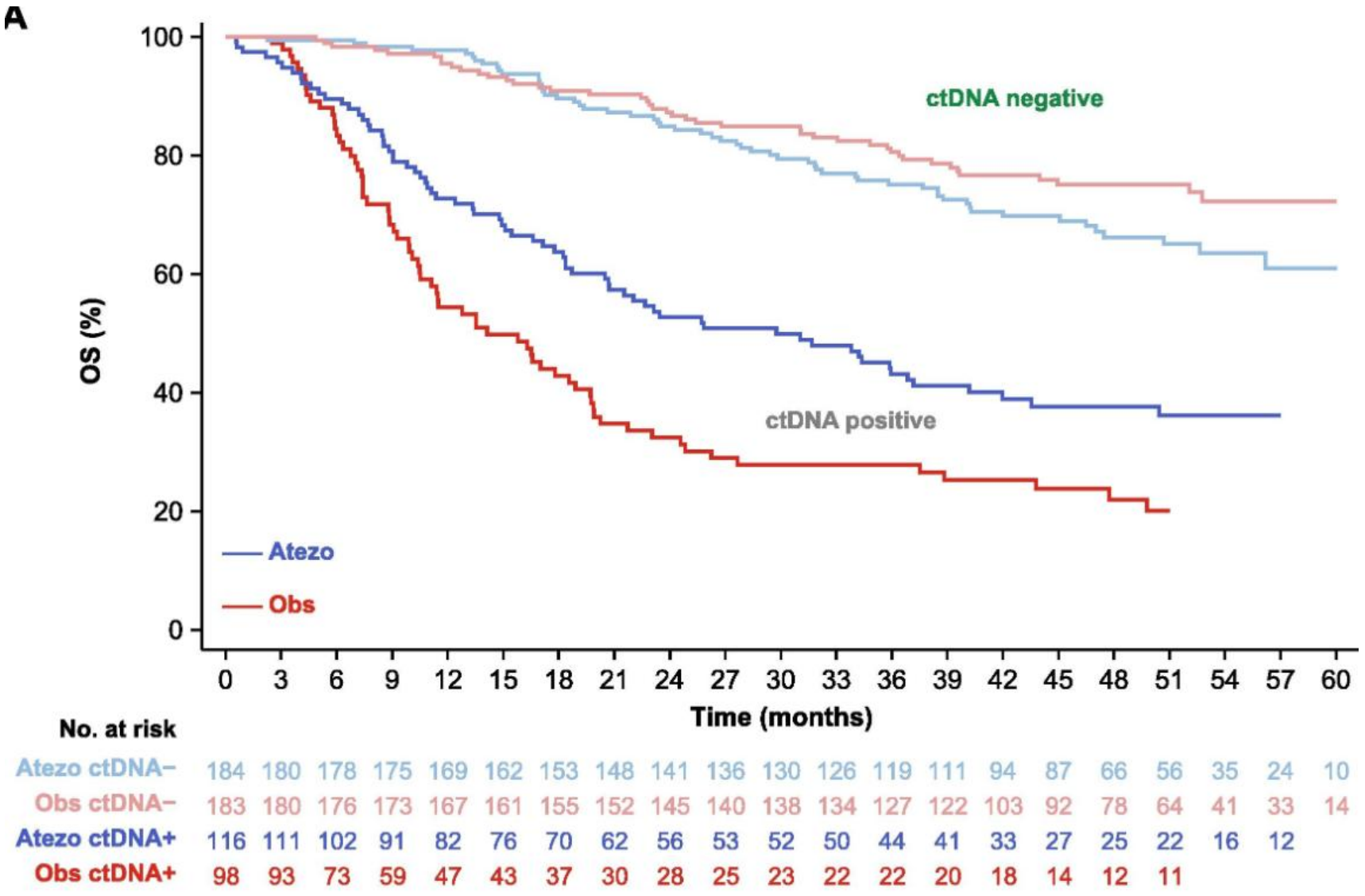
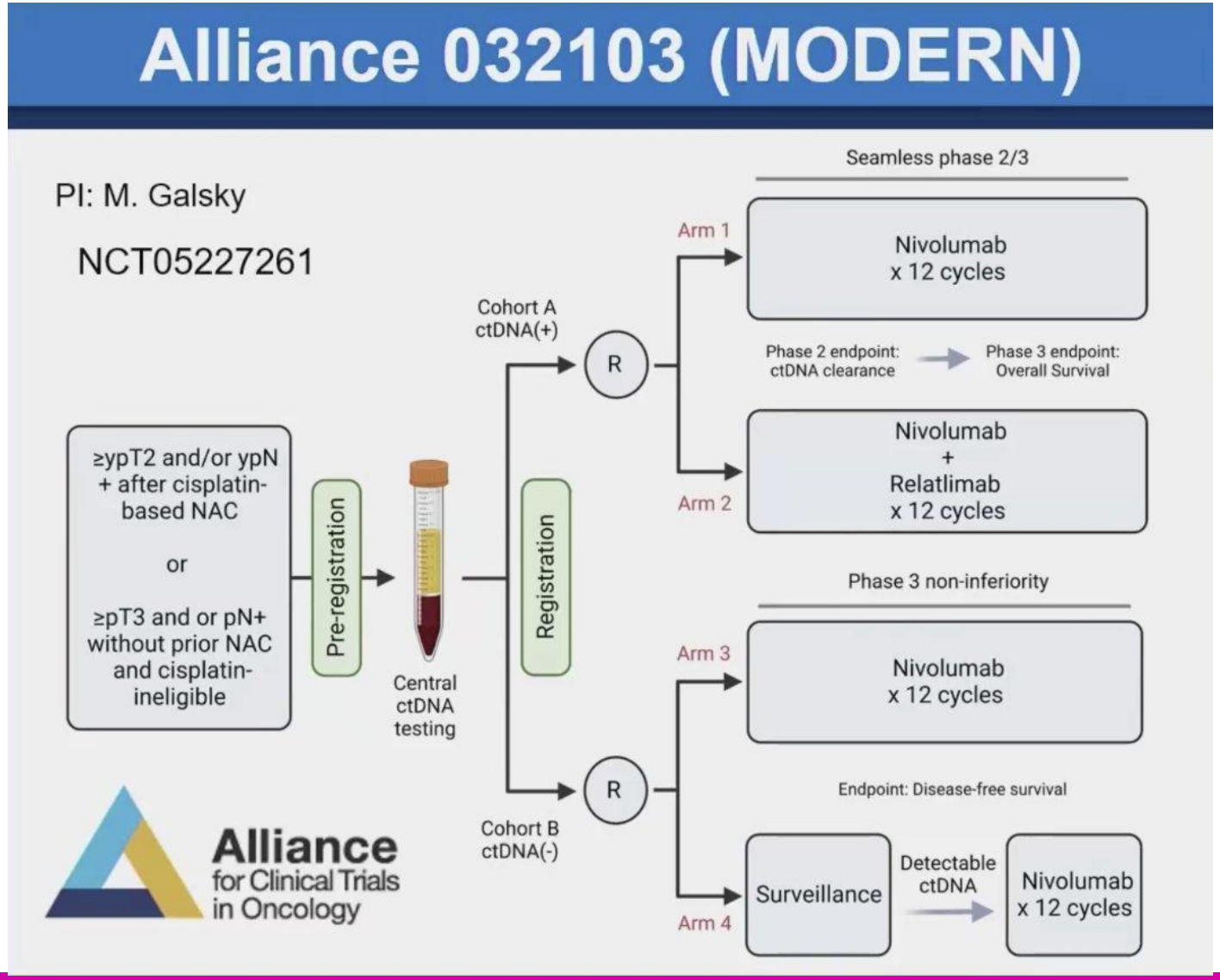

A
100
80
60
40
20
0
OS (%)
ctDNA negative
ctDNA positive
Atezo
Obs
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60
Time (months)
No. at risk
Atezo ctDNA− 184 180 178 175 169 162 153 148 141 136 130 126 119 111 94 87 66 56 35 24 10
Obs ctDNA− 183 180 176 173 167 161 155 152 145 140 138 134 127 122 103 92 78 64 41 33 14
Atezo ctDNA+ 116 111 102 91 82 76 70 62 56 53 52 50 44 41 33 27 25 22 16 12
Obs ctDNA+ 98 93 73 59 47 43 37 30 28 25 23 22 22 20 18 14 12 11

MODERN


Alliance 032103 (MODERN)
PI: M. Galsky
NCT05227261
≥ypT2 and/or ypN + after cisplatin-based NAC
or
≥pT3 and or pN+ without prior NAC and cisplatin-ineligible
Pre-registration
Central ctDNA testing
Registration
Cohort A ctDNA(+)
Cohort B ctDNA(-)
R
R
Seamless phase 2/3
Arm 1
Nivolumab x 12 cycles
Phase 2 endpoint: ctDNA clearance
Phase 3 endpoint: Overall Survival
Arm 2
Nivolumab + Relatlimab x 12 cycles
Phase 3 non-inferiority
Arm 3
Nivolumab x 12 cycles
Endpoint: Disease-free survival
Arm 4
Surveillance
Detectable ctDNA
Nivolumab x 12 cycles
Alliance for Clinical Trials in Oncology

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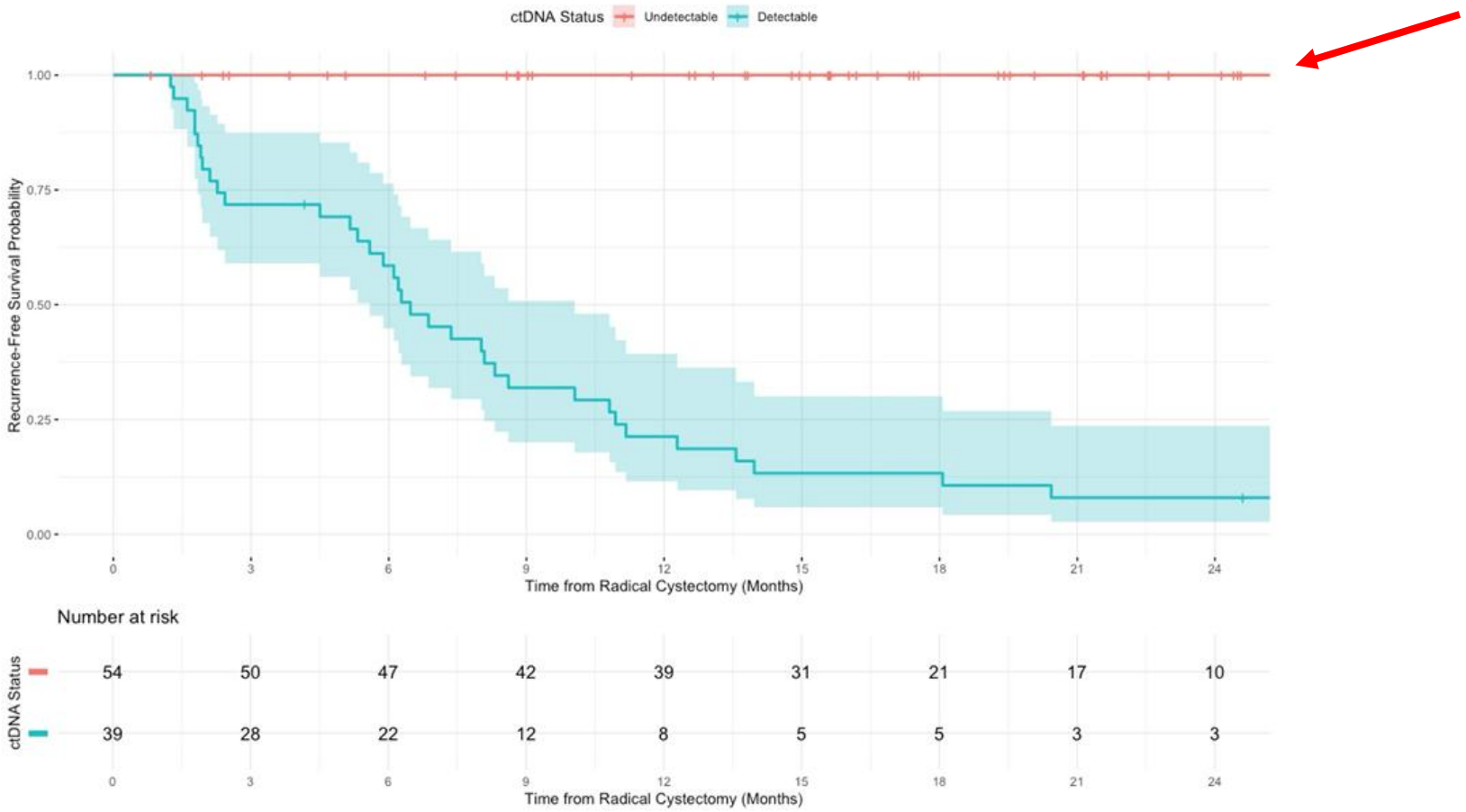
Future Perspectives of ctDNA Use

- ctDNA is a prognostic marker
- ctDNA has been incorporated in current clinical trials to assess if predictive (i.e. IMvigor11, AMBASSADOR and MODERN trial)
- It appears that treatment de-escalation can be aided by ctDNA status e.g., avoiding neoadjuvant treatment, avoiding adjuvant treatment
- ctDNA can be incorporated in novel bladder-sparing approaches, adding another layer of certainty to clinical complete response (cCR) stage

Future Perspectives of MRD ctDNA Status

Kaplan-Meier survival curves for recurrence-free survival (RFS) stratified by postoperative ctDNA status. Patients with undetectable ctDNA had significantly improved RFS compared to those with detectable ctDNA. The accompanying table summarizes survival probabilities at key time points (6, 12, 18, and 24 months) with 95% confidence intervals. Statistical significance was assessed using the log-rank test (**p < 0.0001**)



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Personalized cancer care has never been closer.

We are in the path of adding another layer of staging on-top of the current TNM staging for improved clarity on the patient's the actual stage