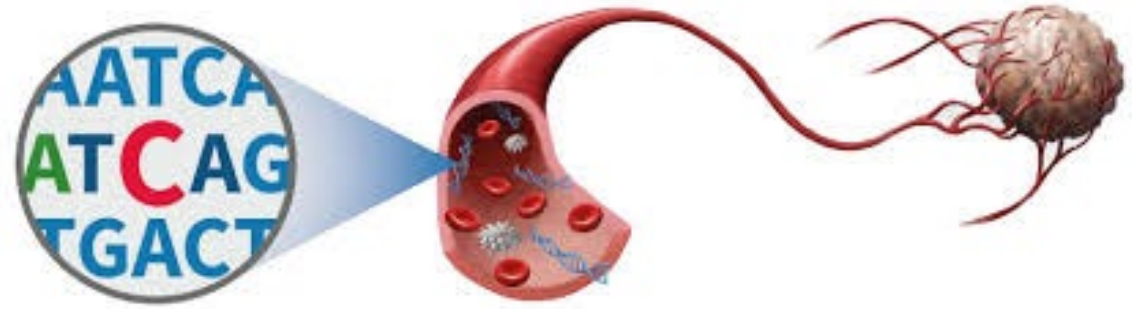


ctDNA, Ready for Clinical Practice

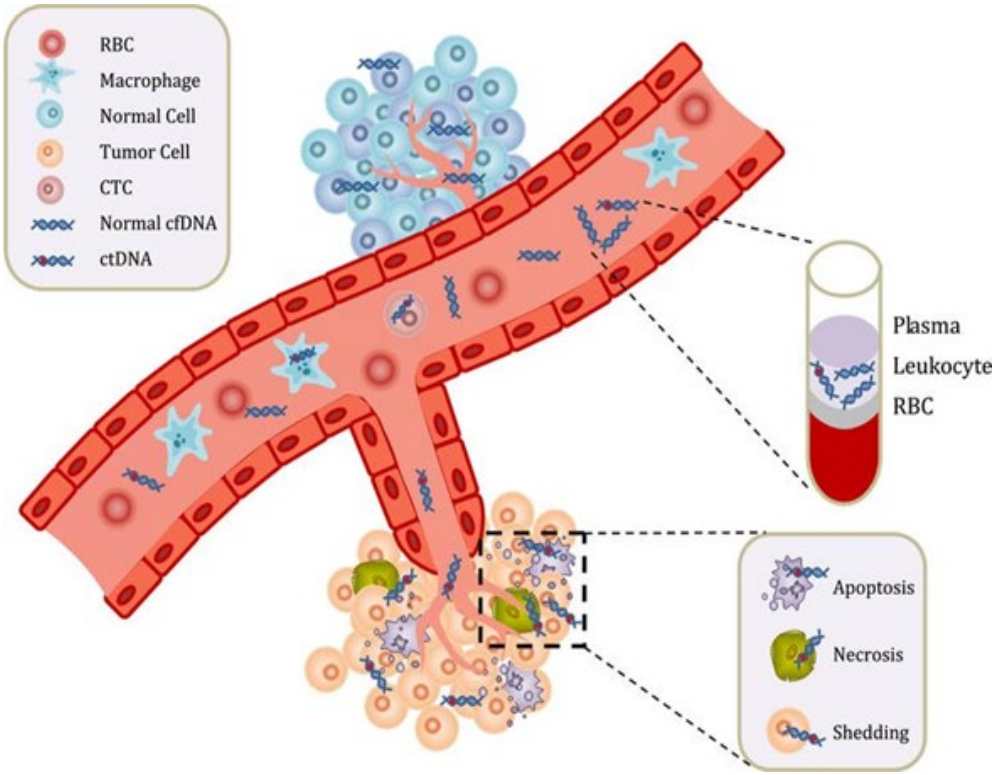
Adel Kardosh MD PhD

Knight Cancer Network Symposium

3/3/2023

Circulating Tumor DNA (ctDNA)

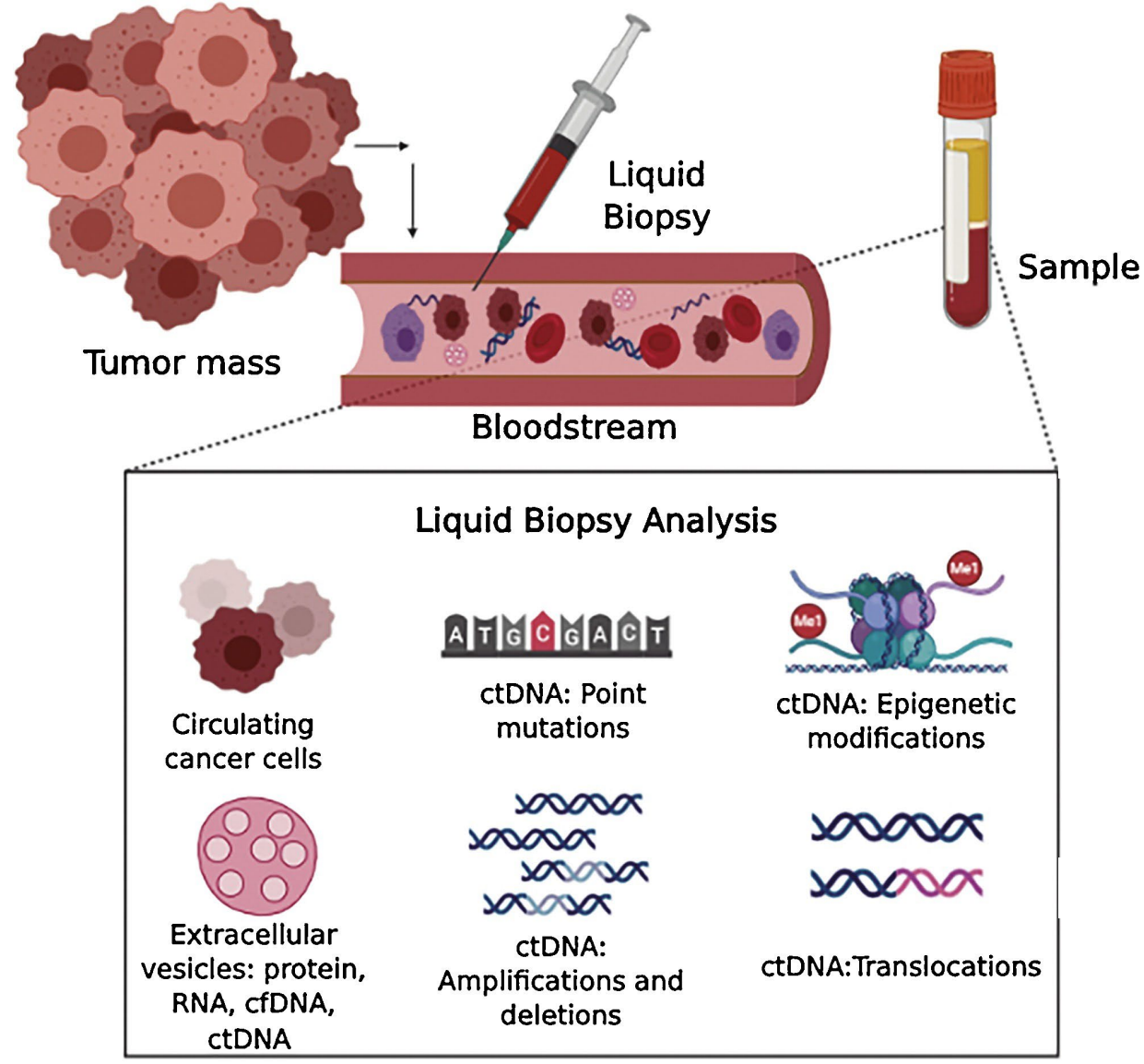
- **cfDNA**; Cell-free DNA → small DNA Fragment (160-200 bp) in circulation
- Released in Bloodstream due to cell death
  - Heathy persons – mainly form hematopoietic cells
- **ctDNA**; circulating tumor DNA → small DNA fragments (143-145 bp)in circulation released form cancer
- Half life is very short → approximate 2 hours



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

Consideration before testing

- Specimen type
- Sample volume
- Timing
- Storage/processing

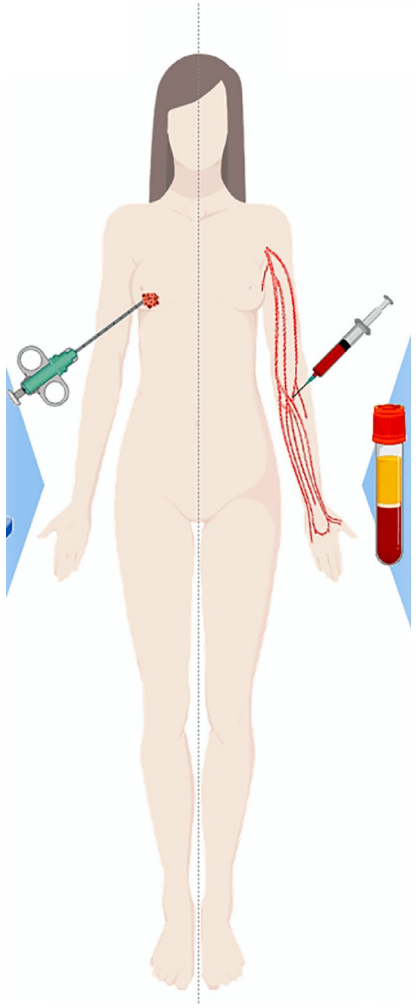


Pessoa et al. European school of Oncology. 2020

Blood Vs Tissue

Tumor Tissue

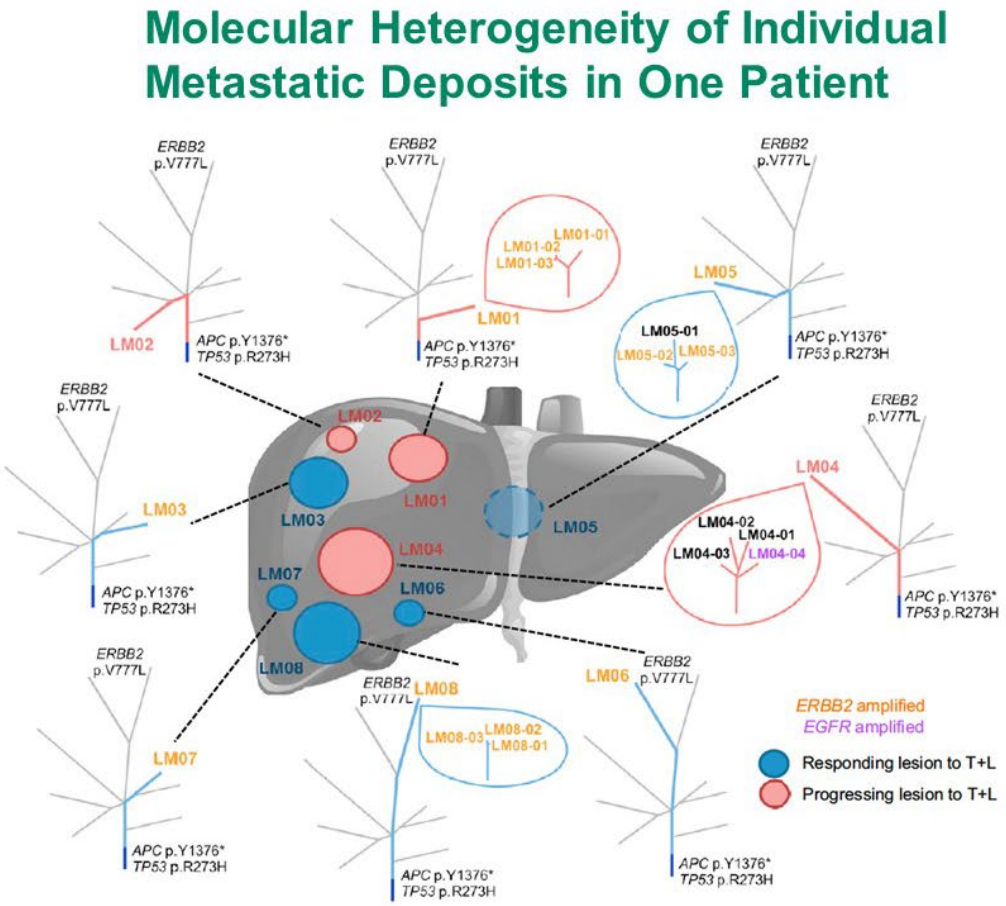
- Biopsy, invasive, side effects, multiple high risk
- Heterogeneity of tumor tissue
- No assessment of tumor load
- Utilizes existing tissue processing approaches

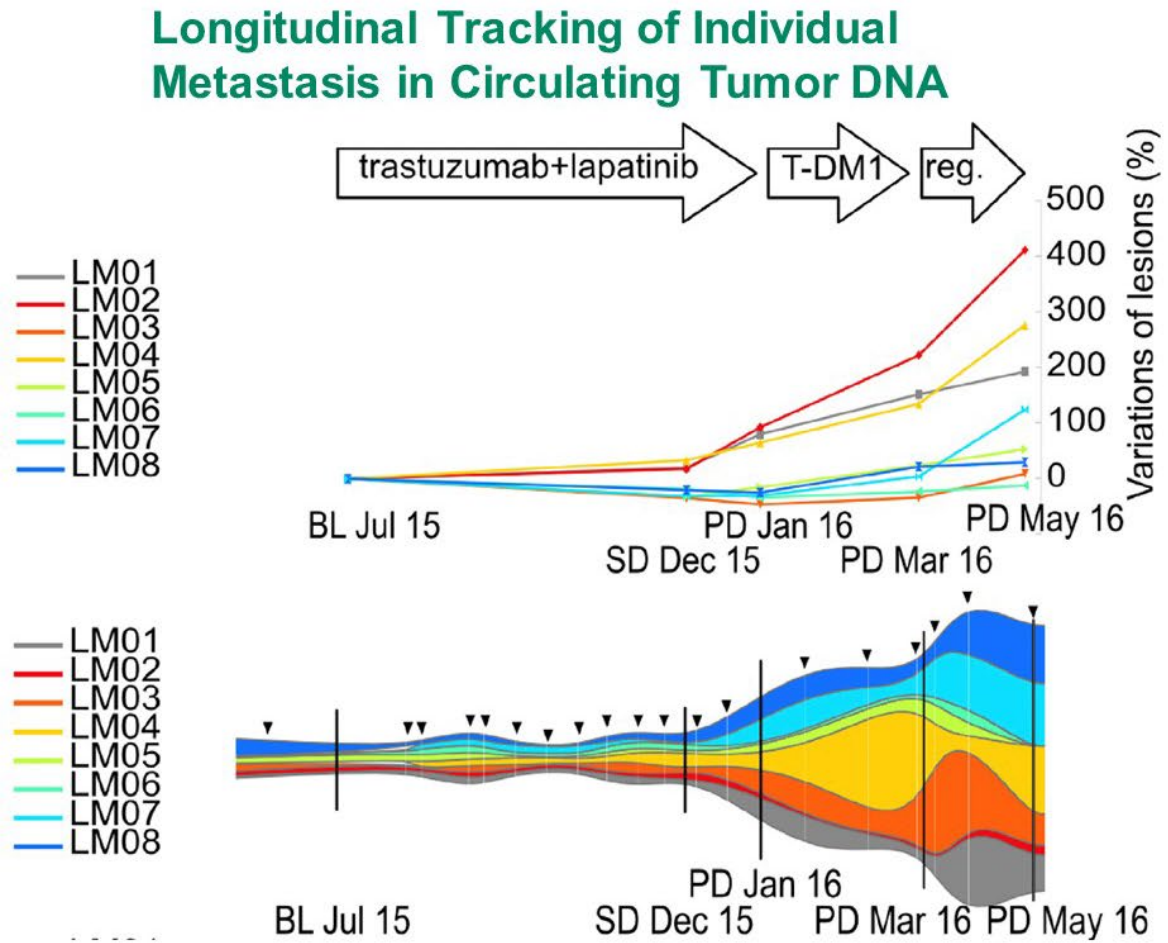


ctDNA

- Less invasive, serial testing (easy)
- Better representation of tumor and metastatic Heterogeneity
- Quantitative analysis of correlates with tumor load
- Requires special processing

Tumoral Heterogeneity





Siravegna G, et al. Cancer Cell 2018;34(1):148-162.

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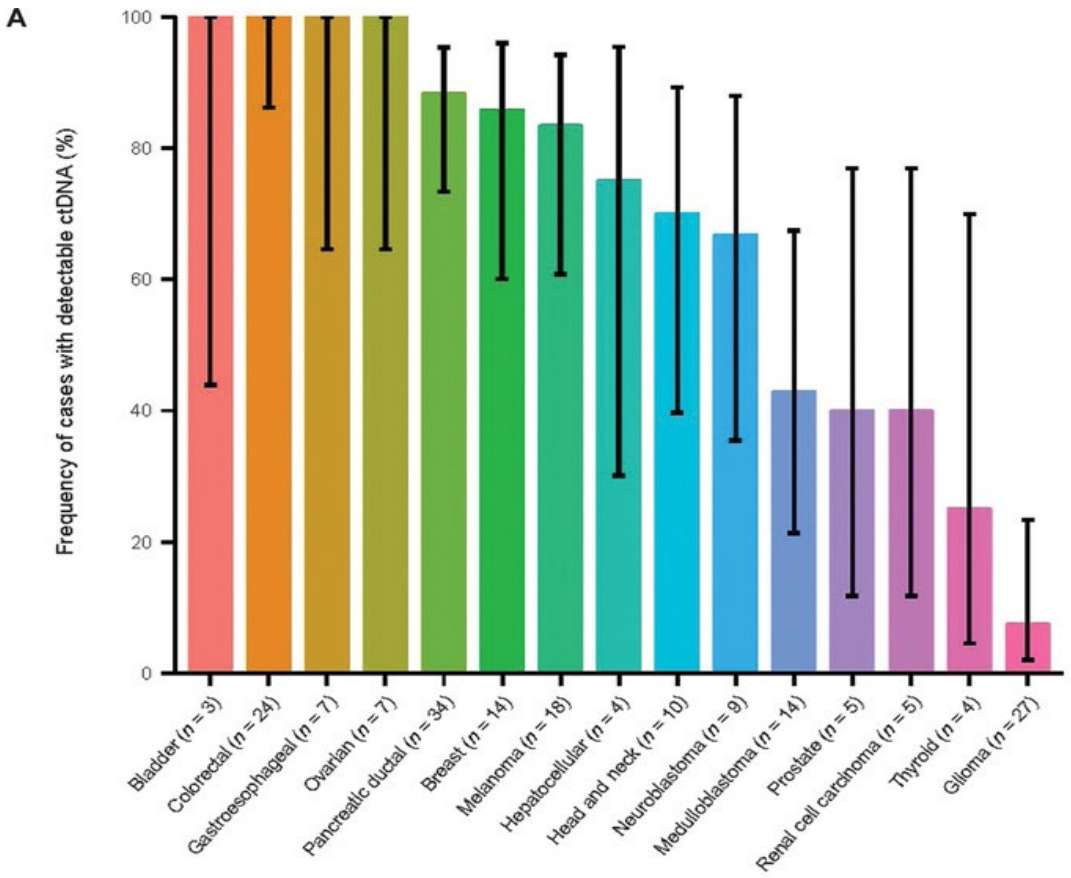
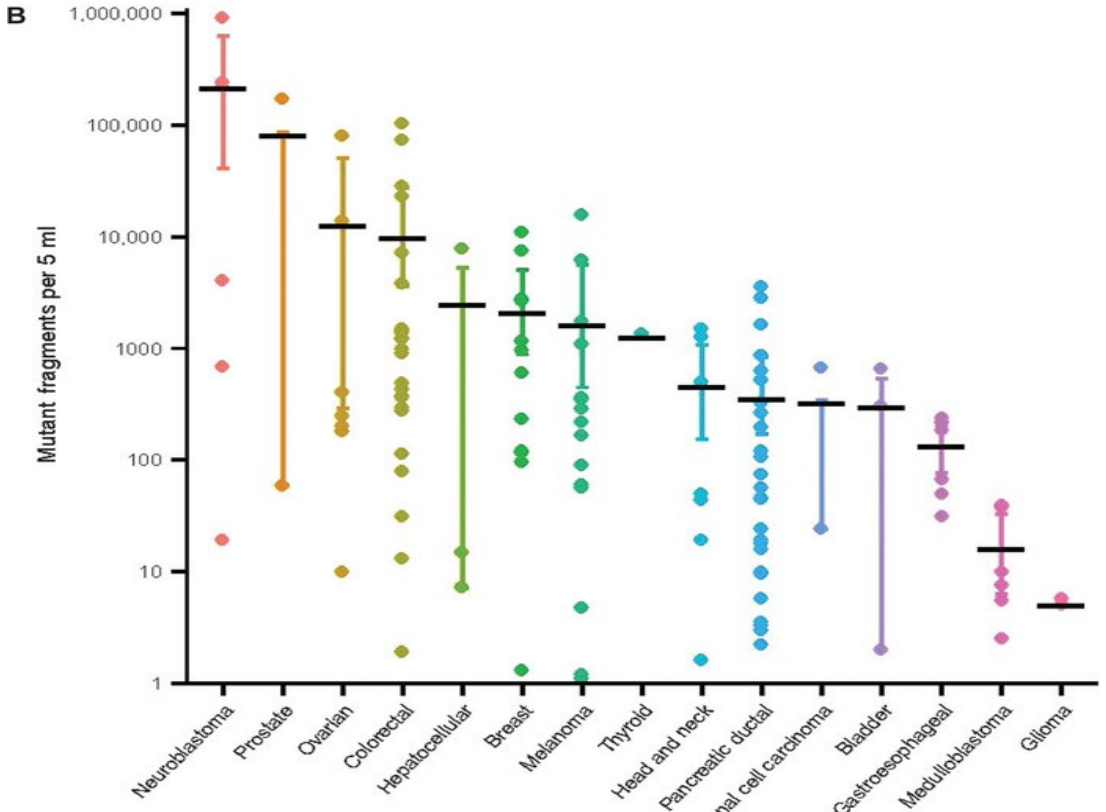
No All Are Created Equal

Detection rate are different across metastatic cancers



ctDNA levels are different across cancers and within the same cancer



Bettegowda et al. Science Medicine 2014

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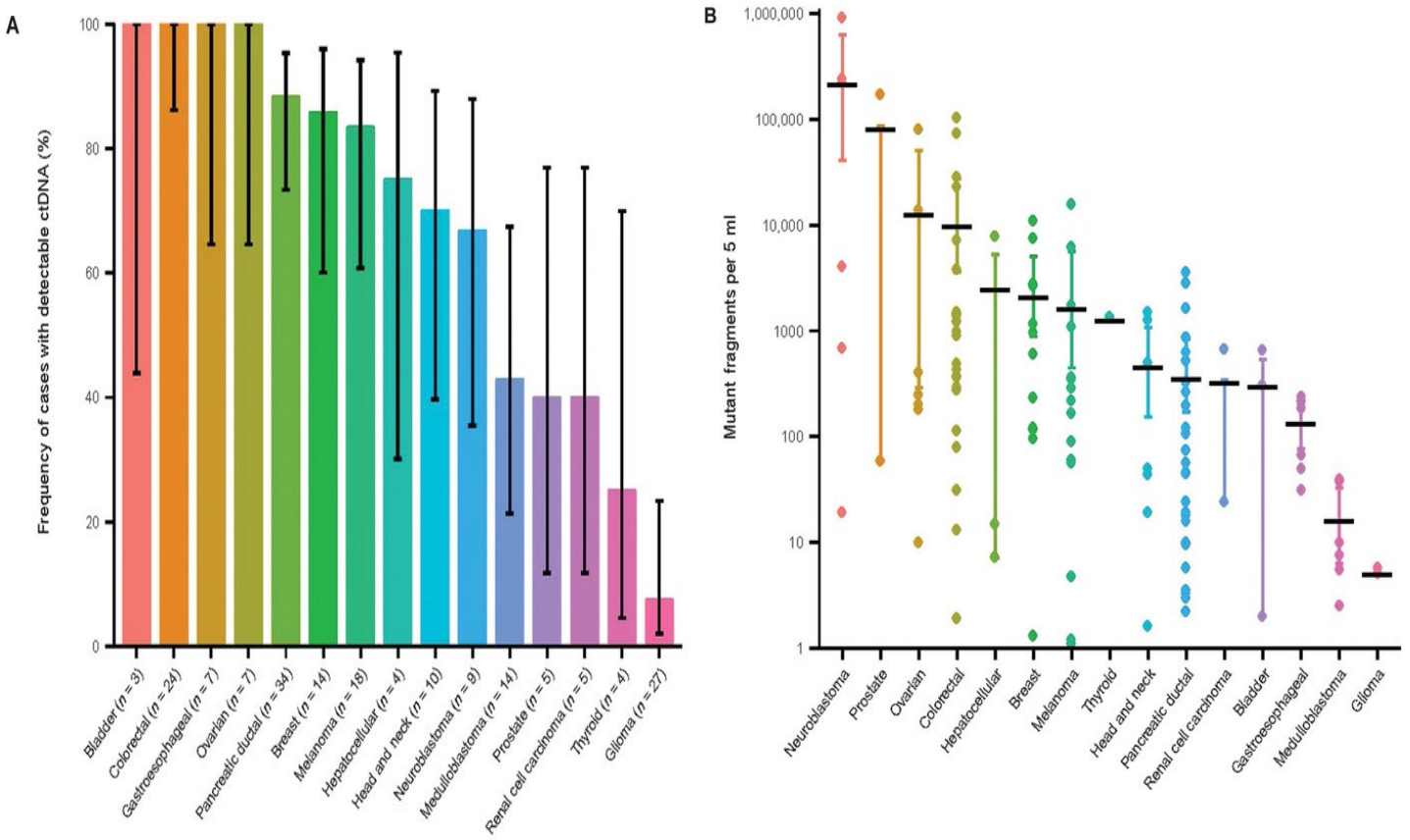
Factors Affecting ctDNA Levels and Detection



- **Timing to blood collection**
  - In association to treatment
- **Disease sites**
  - Liver > lung, peritoneal, bone, nodes
- **Tumor burden**
  - Volume vs MRD
- **Disease status**
  - Responding, stable, progressing
- **Cell type**
  - Squam > Adeno > Mucinous

Bettegowda et al. Science Medicine 2014

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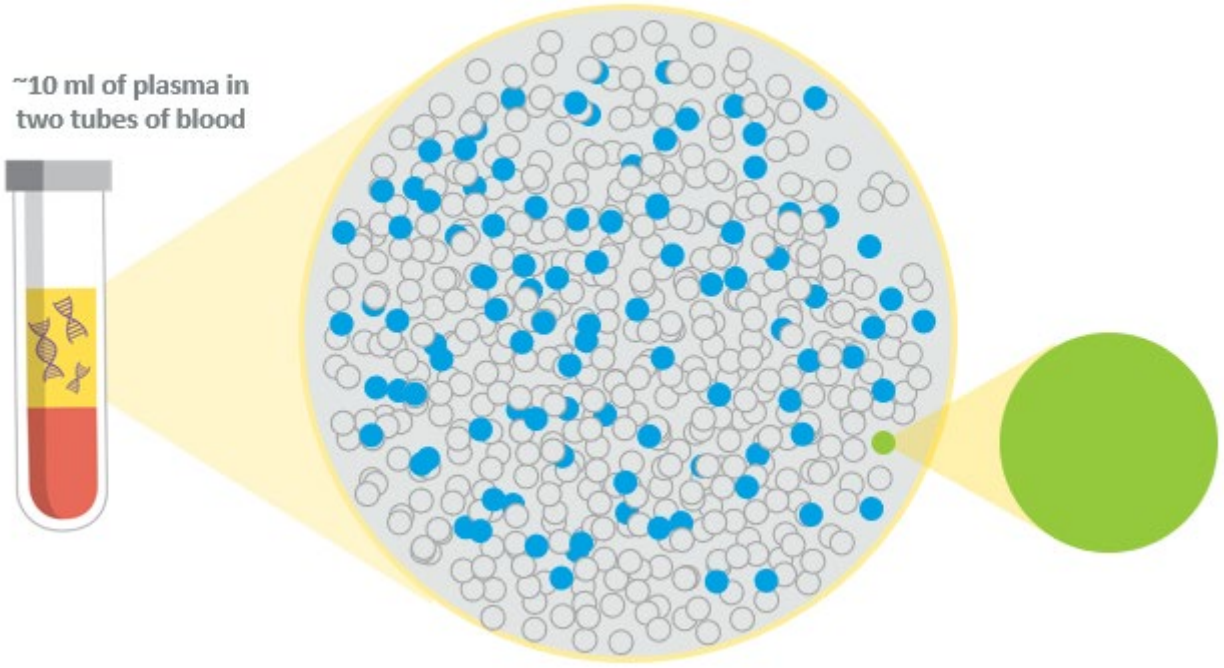
Needle in a Haystack

- 1-5 mutant tumor DNA fragments in 10,000 self DNA fragments



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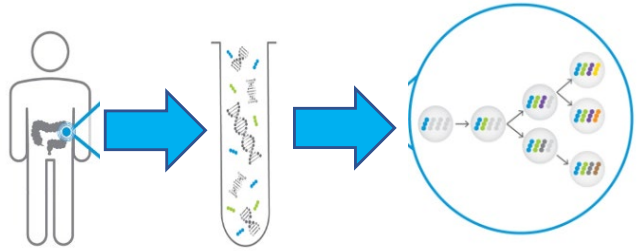
Methods

Target	Method	Advantage	Limitation
Point mutation /single locus	Digital PCR	High sensitivity Minimal bioinformatics Fast, inexpensive	Detect only known hotspots mutation (BRAF V600E)
Gene panel (NGS)	PCR amplification sequencing	High sensitivity Cost-effective (as compare with other NSG)	Less comprehensive than other NGS methods
	Hybrid capture sequencing	Covers large genomic regions Detects copy number variations/ rearrangements	Requires high DNA input more complex workflow
Comprehensive (NGS)	Whole exome or genome sequencing	Identifies novel mutations	Low sensitivity Expensive Longer turnaround

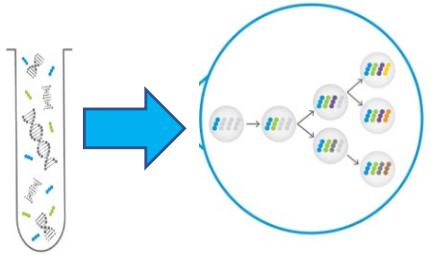
Adapted form Tie ASCO2022

ctDNA Approach



	Tumor naïve	Tumor informed
Methods	De novo from plasma (Same panel for all patients)	Identify mutations in tumor tissue Then track in plasma (Personalized)
Advantage	No tissue required Short turnaround time	Higher sensitivity
Disadvantage	Lower sensitivity (Multiple hypothesis testing)	Required tumor tissue Long turnaround time
Application	Noninvasive genotyping Detect emerging resistant mutation Cancer screening	Minimal residual disease (MRD) Response monitoring Surveillance

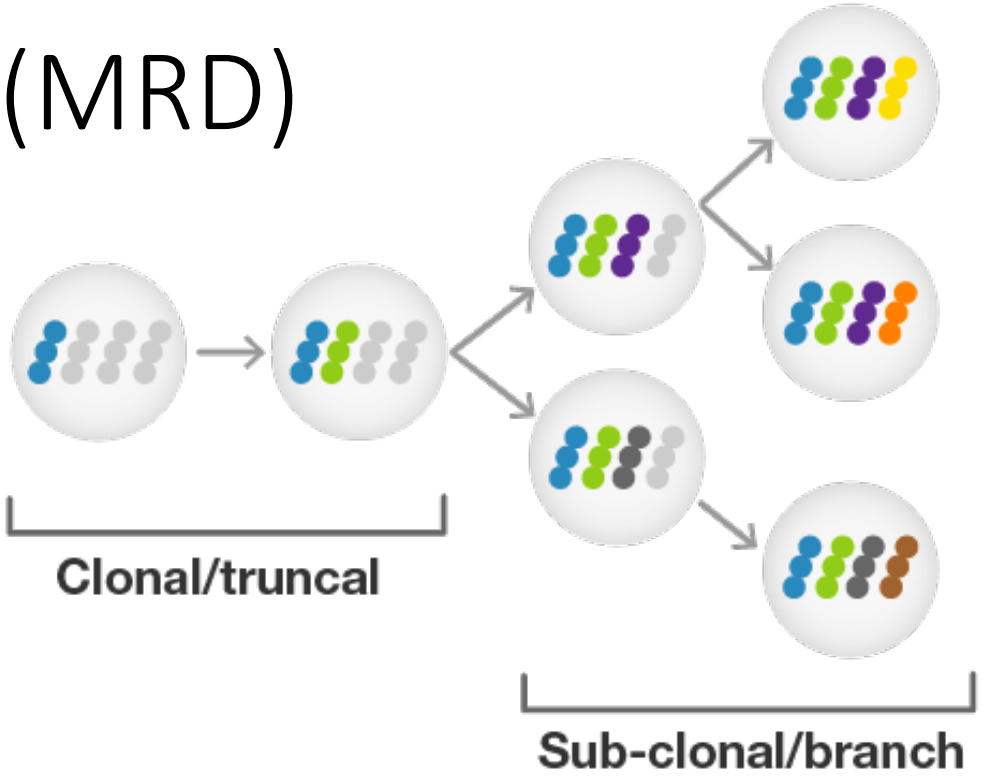
Tumor Naïve ctDNA Use in Current Guidelines

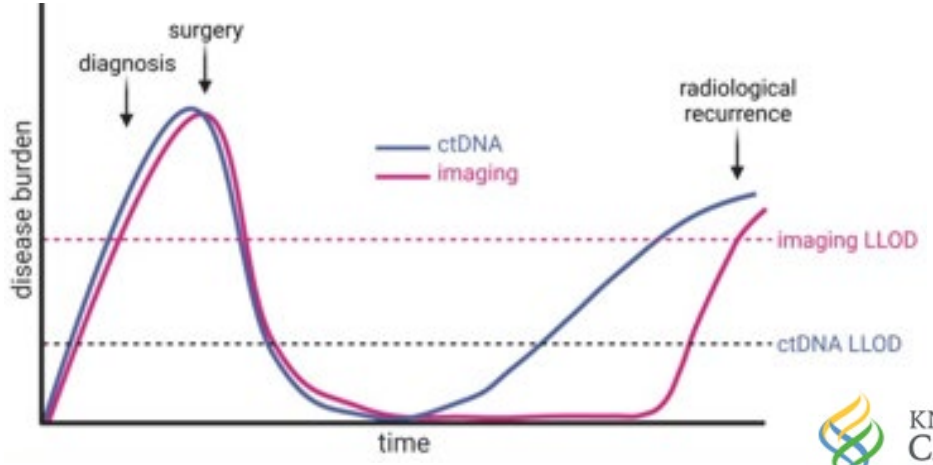


- Approved as standard of care and advanced NSCLC
  - EGFR mutations
  - PCR-based assays have high specificity but lower sensitivity, Therefore in some cases may still require tumor testing.
- NGS assays
  - Commercial platforms (e.g. FoundationOne Liquid CDX, Guardant360)
  - Academic platforms

Minimal Residual Disease (MRD)

- Selecting clonal mutation that persist through tumor evolution
- Designable mutation for and a multiplex PCR
- Detectable mutation with low background noise





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Commercial ctDNA **MRD** Assays

	Natera/FMI	ArcherDx	Inivata	Haystack	Guardant
MRD Assay	Signatera	Personalized cancer monitoring*	RaDaR	Haystack Duo	Guardant reveal
Approach	Tumor-informed	Tumor-informed	Tumor-informed	Tumor-informed	Tumor- agnostic
Biospecimen	Tumor, whole blood, plasma	Tumor, whole blood, plasma	Tumor, whole blood, plasma	Tumor, whole blood, plasma	Plasma only
Panel Size	Tissue: WES 324 genes ≤ 16 variants	Tissue: WES ≤ 50 variants	Tissue: WES ≤ 48 variants	Tissue: WES ≤ 50 variants	Fixed panel: 40genes
Turn-around time	Assay design: 3-4 weeks Plasma: 1-2weeks	N/A	Assay design: 4 weeks Plasma: 1 weeks	Assay design: 4 weeks Plasma: 1 weeks	1 week

*kit

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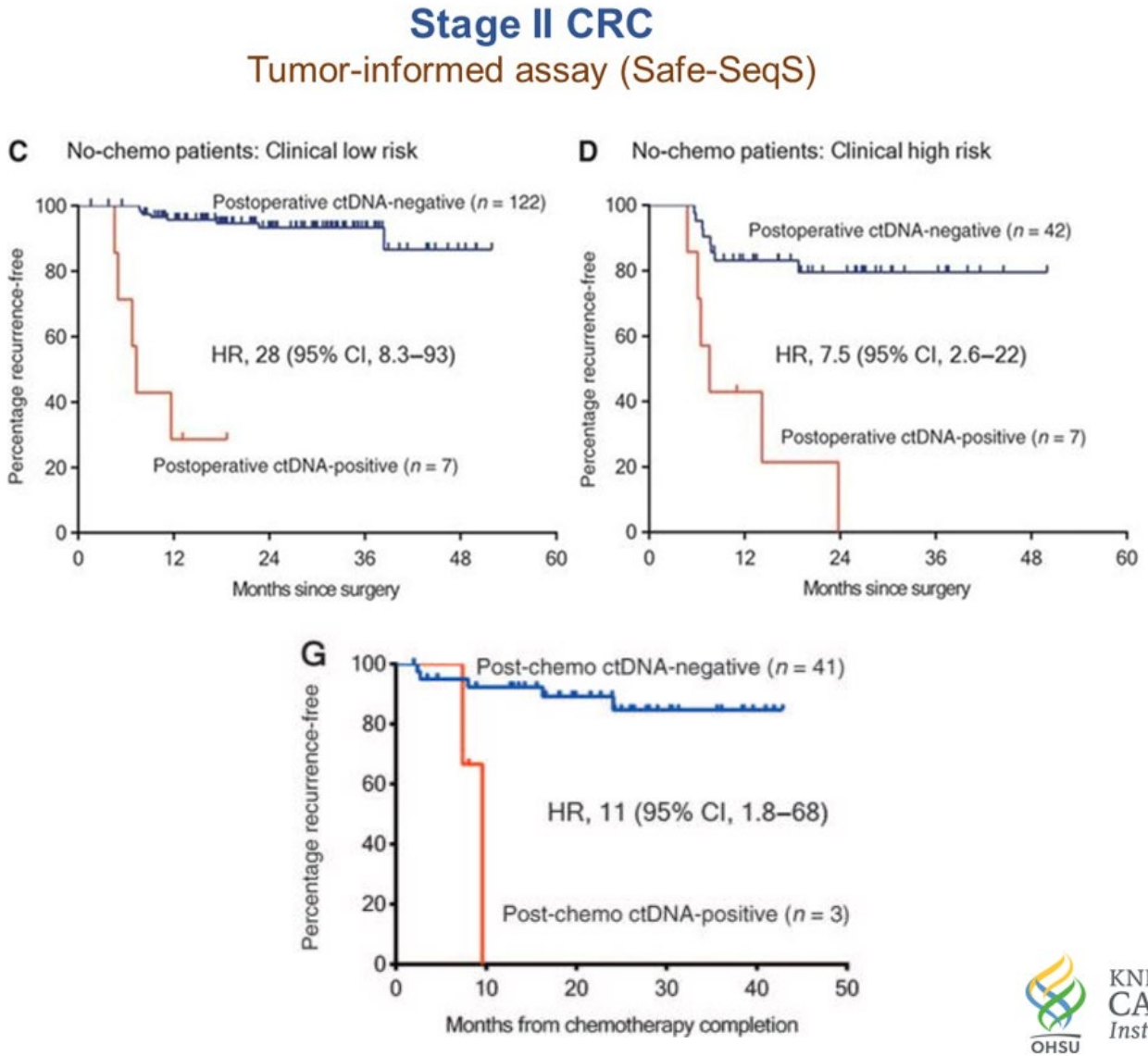
- **What is the data is CRC and MRD?**

MRD a Prognostic Biomarker in Stage II CRC

- ACT associated with poor RFS if ctDNA positive
- Median interval between ctDNA detection and radiological recurrence 5.5months



Tie et al. Sci Transl Med 2016

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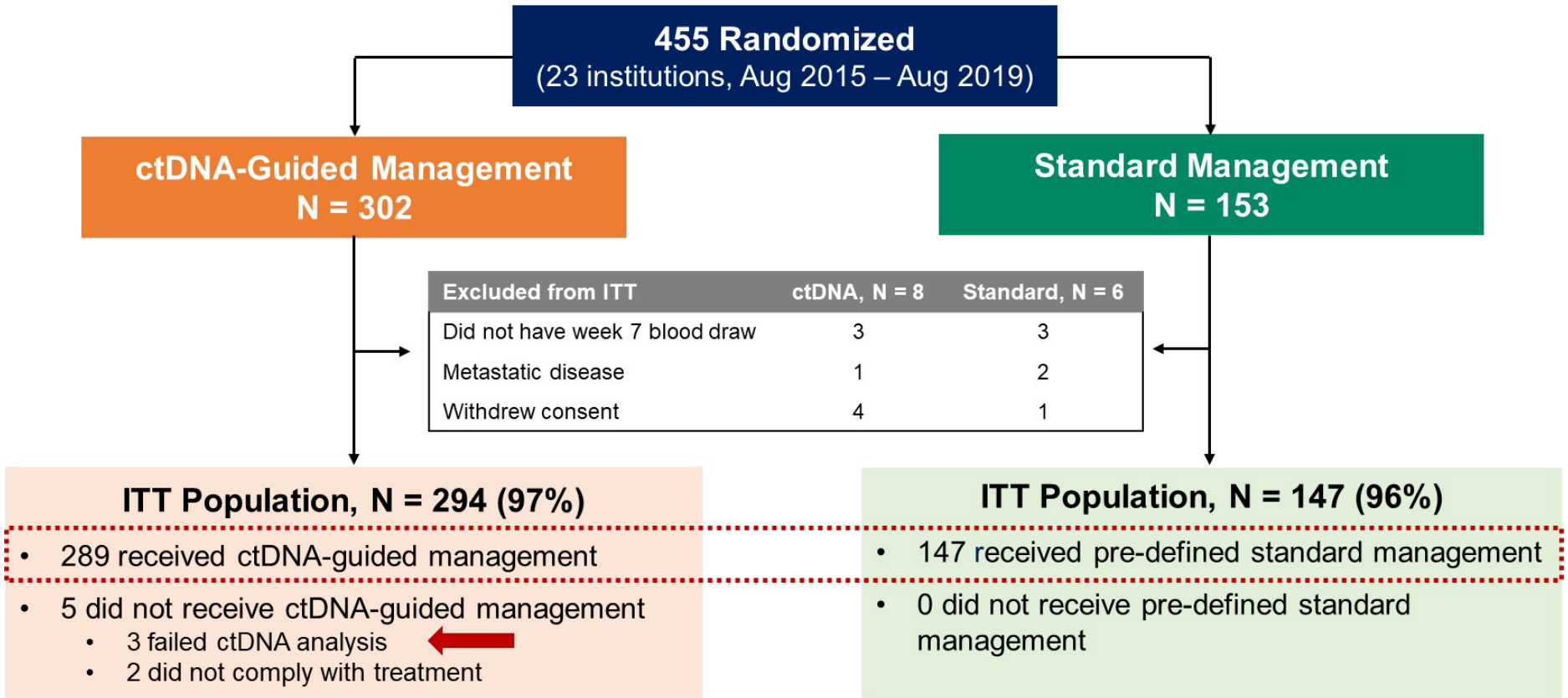
ORIGINAL ARTICLE

Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer



Tie ASCO 2022
Tie et al. NEJM 2022

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Baseline Characteristics

Characteristics	ctDNA-Guided Management N = 294, N (%)	Standard Management N = 147, N (%)
Age, median (range), years	65 (30 , 94)	62 (28 , 84)
Sex, Male	154 (52)	81 (55)
ECOG, 0	226 (77)	124 (84)
Center type, metropolitan	240 (82)	121 (82)
Primary tumor site, left-sided	126 (43)	78 (53)
Tumor stage, T3	250 (85)	127 (86)
Tumor differentiation, poor	43 (15)	17 (12)
Lymph node yield, < 12	13 (4)	7 (5)
Lymphovascular invasion, present	82 (28)	38 (26)
MMR, deficient	59 (20)	27 (18)
Clinical risk group, high*	116 (40)	60 (41)

*High clinical risk = proficient MMR + ≥1 high-risk feature (T4, poor tumor differentiation, <12 lymph node yield, LVI, tumor perforation and/or bowel obstruction)

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Adjuvant Treatment

Treatment Information	ctDNA-Guided N = 294	Standard Management N = 147	P-value
Adjuvant Chemotherapy received, n	45 **(15%)**	41 **(28%)**	0.0017
Chemotherapy regimen received, n			
Oxaliplatin-based doublet	28/45 **(62%)**	4/41 **(10%)**	
Single agent fluoropyrimidine	17/45 **(38%)**	37/41 **(90%)**	<.0001
Time from surgery to commencing chemotherapy, median (IQR), days	83 (76, 89)	53 (49, 61)	<.0001
Treatment duration, median (IQR), weeks	24 (19, 24)	24 (21, 24)	0.9318
Completed planned treatment, n	38 (85%)	32 (78%)	0.7036
Percentage of full dose delivered, median (IQR)	78 (56, 100)	84 (64, 100)	0.6194

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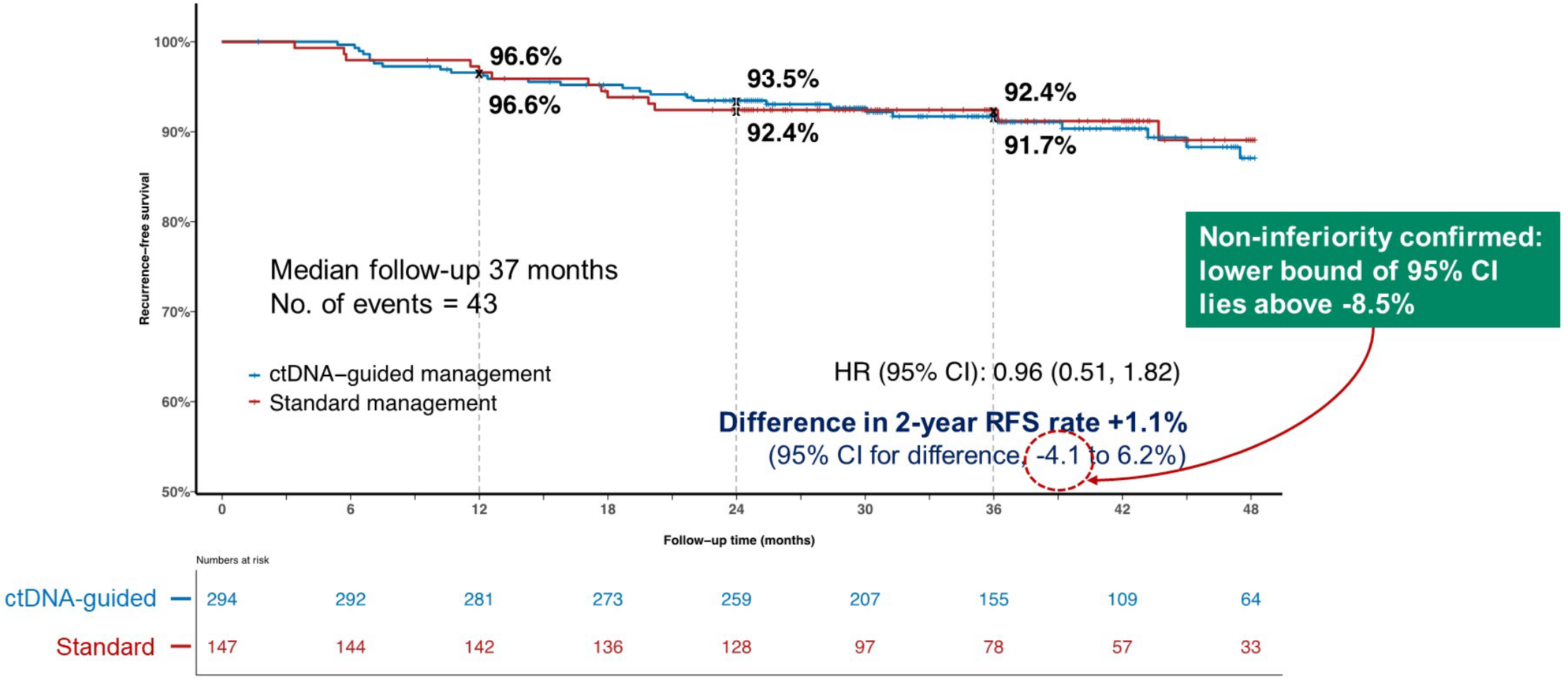
Recurrence Free Survival



Tie ASCO 2022
Tie et al. NEJM 2022

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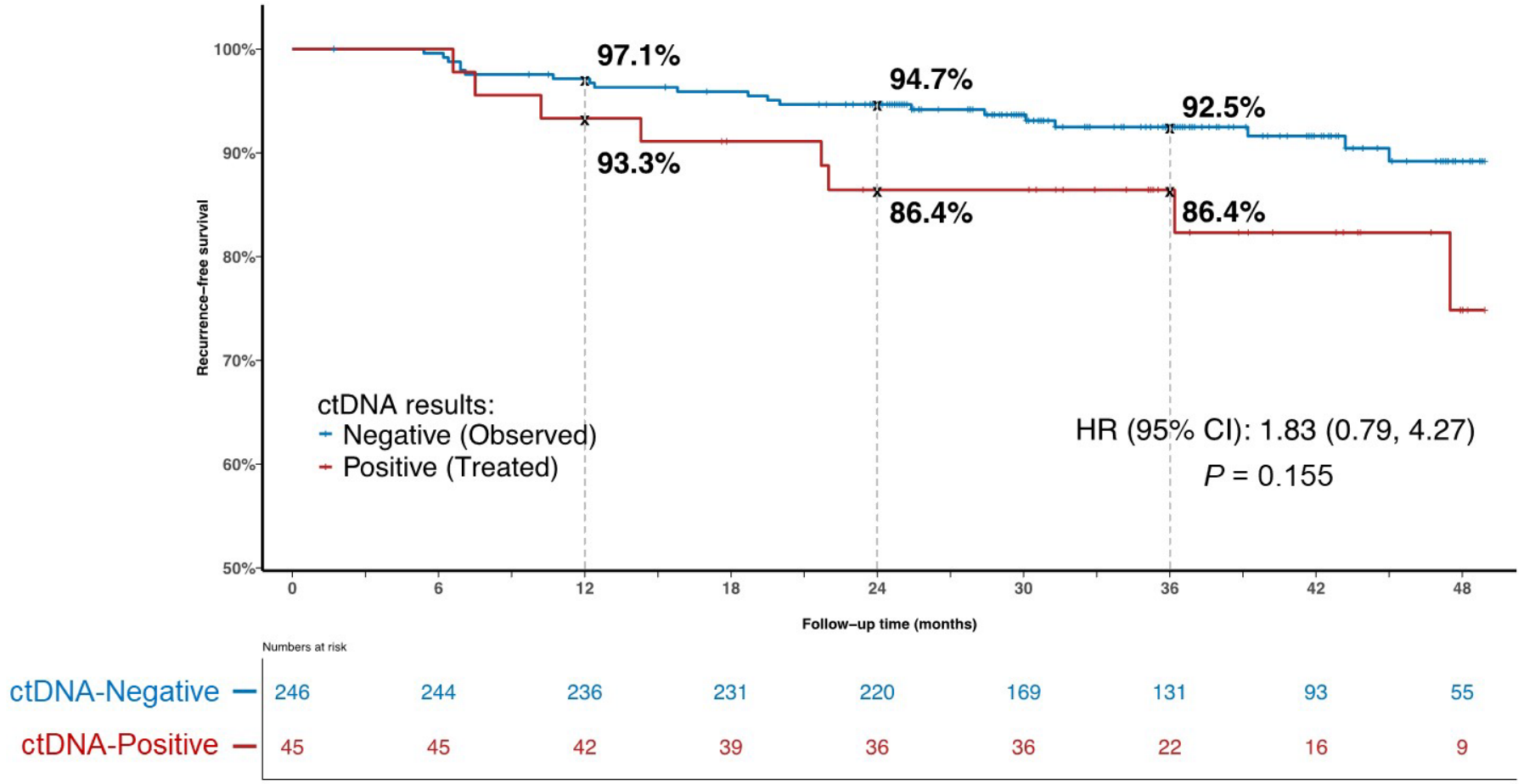
Recurrence Free Survival in ctDNA-Guided Management



Tie ASCO 2022
Tie et al. NEJM 2022

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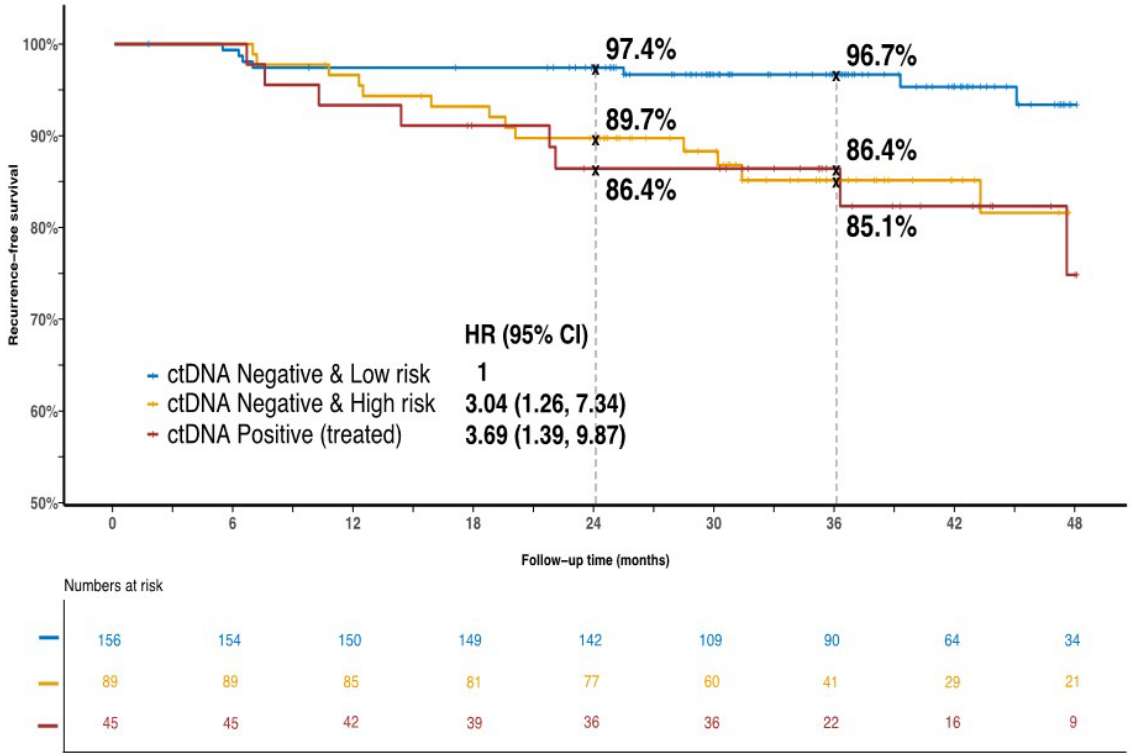
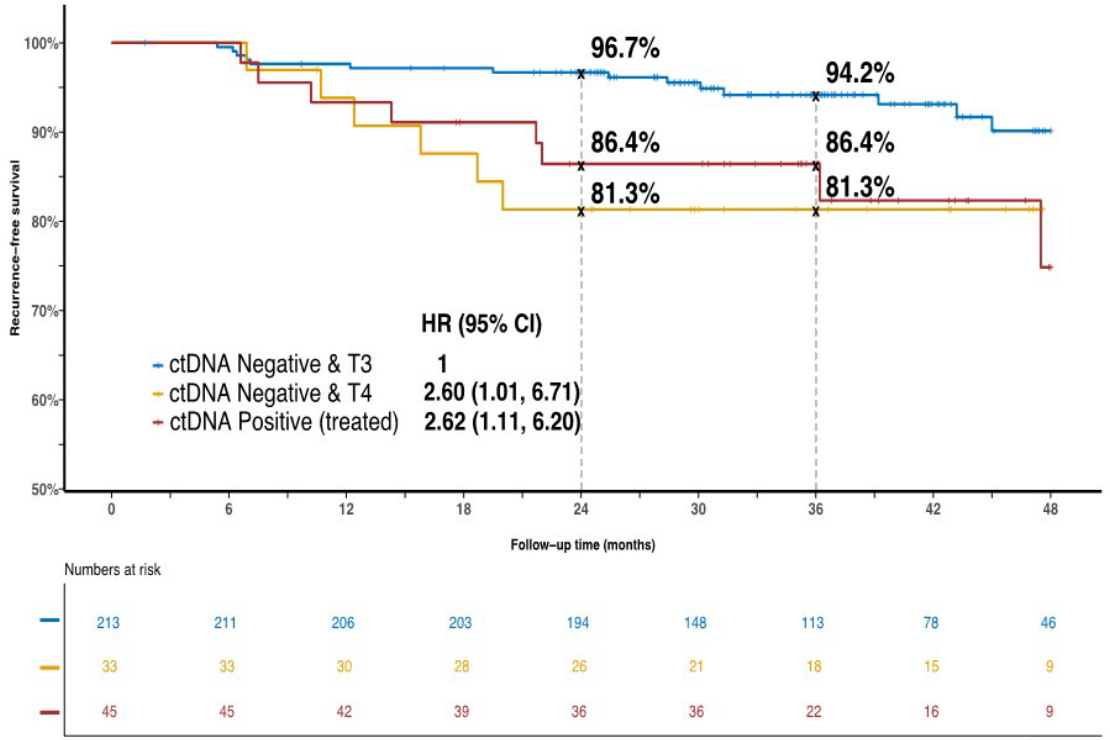
Recurrence Free Survival in ctDNA-Guided Management

ctDNA, Clinical Risk and T Stage

ctDNA and Clinical Risk



ctDNA and T Stage



Tie ASCO 2022
Tie et al. NEJM 2022

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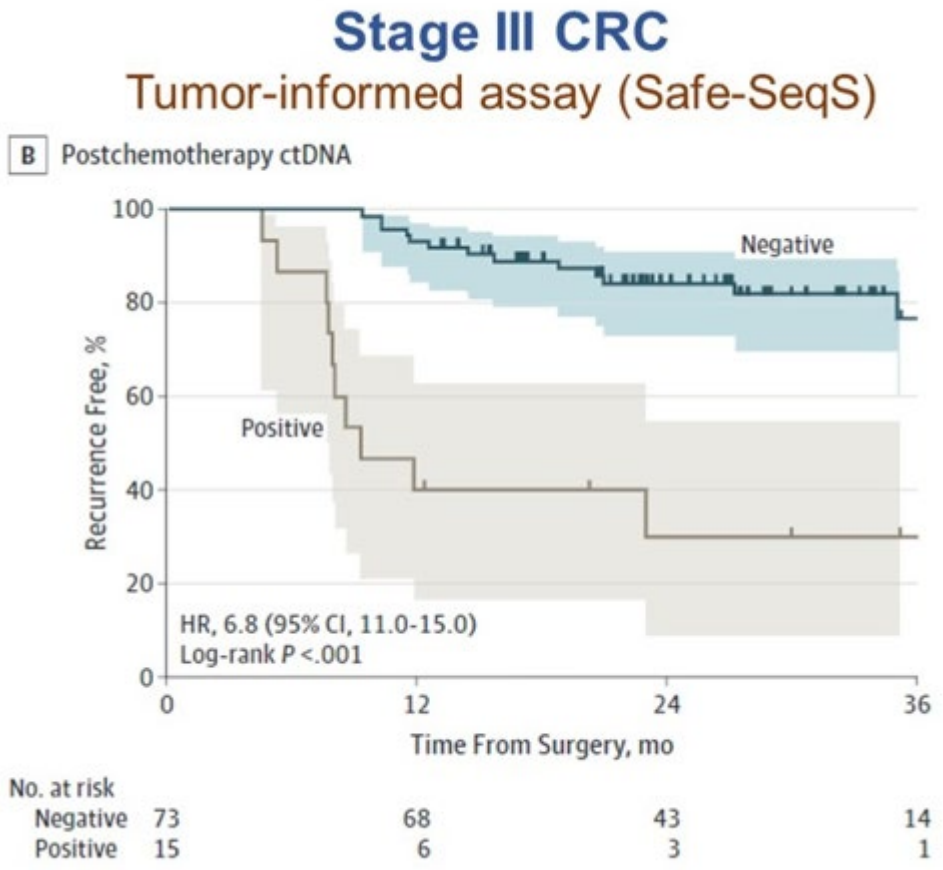
Summary

- ctDNA guided strategy in stage II colon cancer did not compromise RFS (2yesr RFS; 93.5% vs 92.4%)
- However, ctDNA negative high risk patients had similar outcome as ctDNA positive
- ctDNA Negative have low recurrence risk without adjuvant chemotherapy (3-year RFS 92.5%)
- However, low risk stage II typically don’t receive chemotherapy and 5FU alone is not a standard for unless…

MRD a Prognostic Biomarker in Stage III CRC

- Post-chemotherapy 3-year RFS
  - ctDNA positive 30%
  - ctDNA Negative 77%
- Post surgical ctDNA status independently associated with RFI



Tie et al. JAMA Onc 2019

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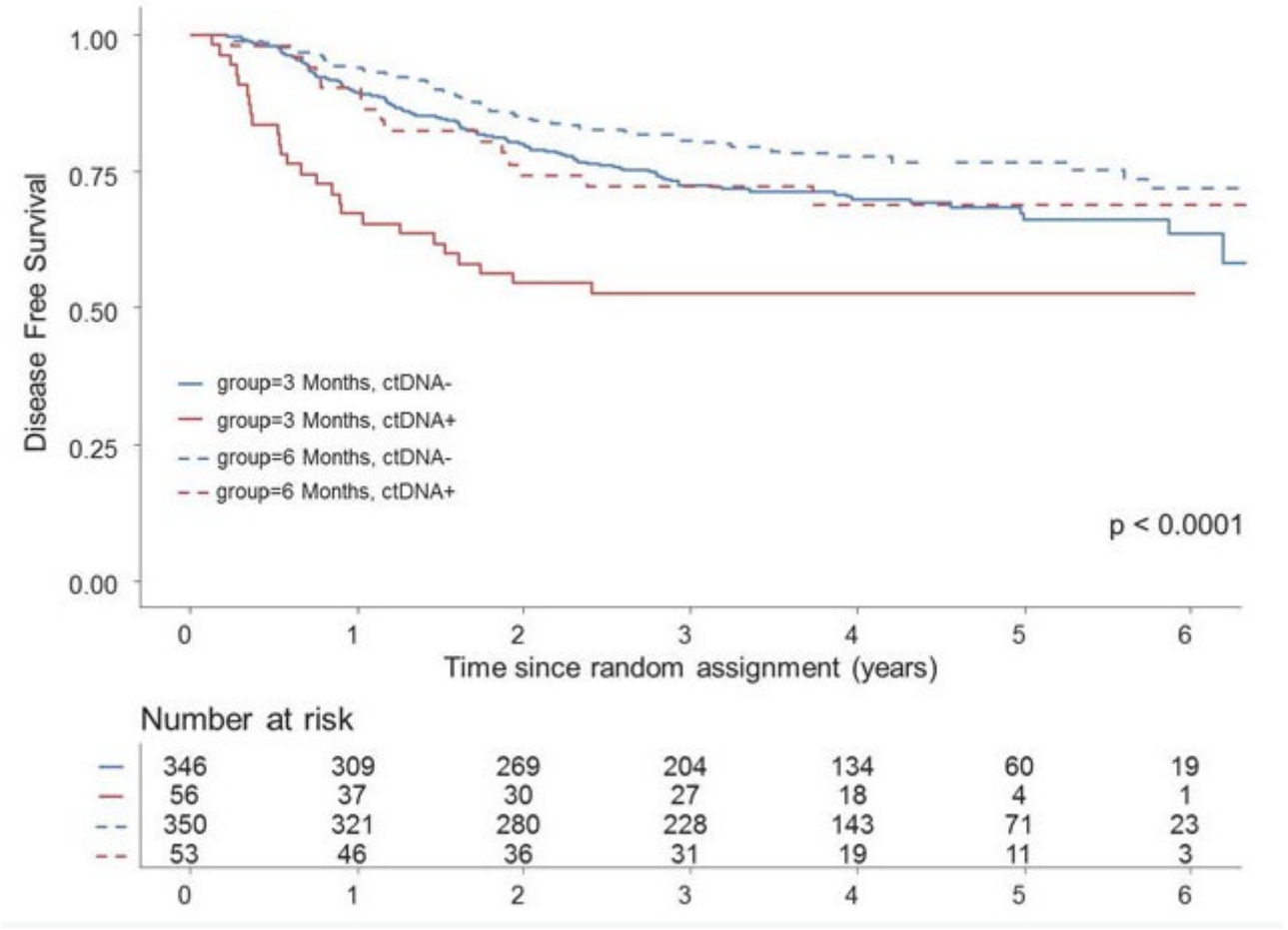
ctDNA a Marker for DFS in Stage III CRC

- IDEA-FRANCE 3 vs 6 months, N=805, 696 ctDNA negative and 109 (13.5%) ctDNA positive
- 2-year DFS rates in patients with ctDNA positive was 64% vs 82% and ctDNA negative
- 3-year DFS was 75.7% for patients receiving 6 months and 72.1% with the 3-month regimen



Taieb et al. ESMO2019

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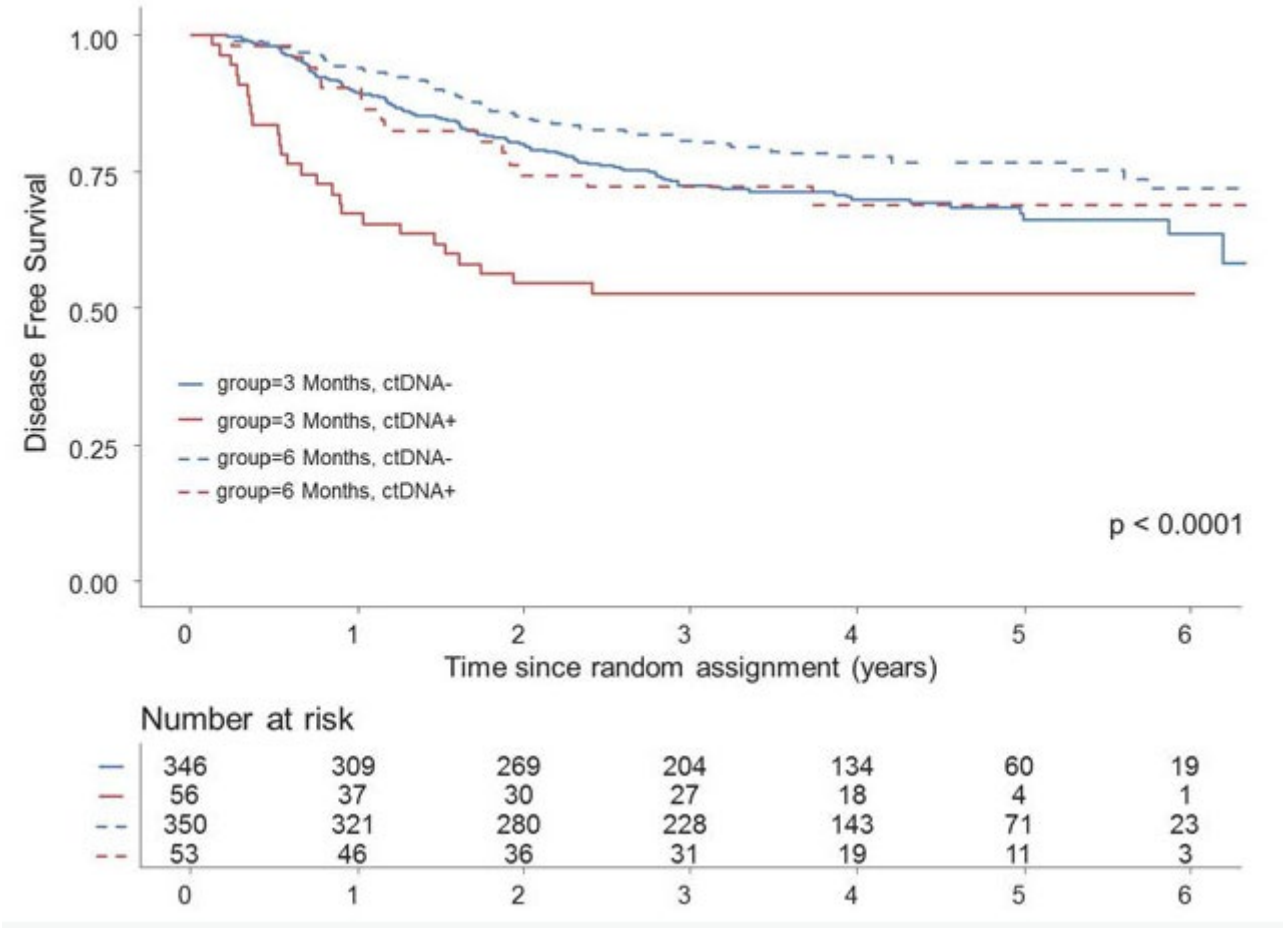
ctDNA a Marker for DFS in Stage III CRC

- ACT 6 months was superior to 3 months for both ctDNA negative and ctDNA positive
- ctDNA positive ACT x6 months had similar prognosis with ctDNA negative ACT x3 months



Taieb et al. ESMO2019

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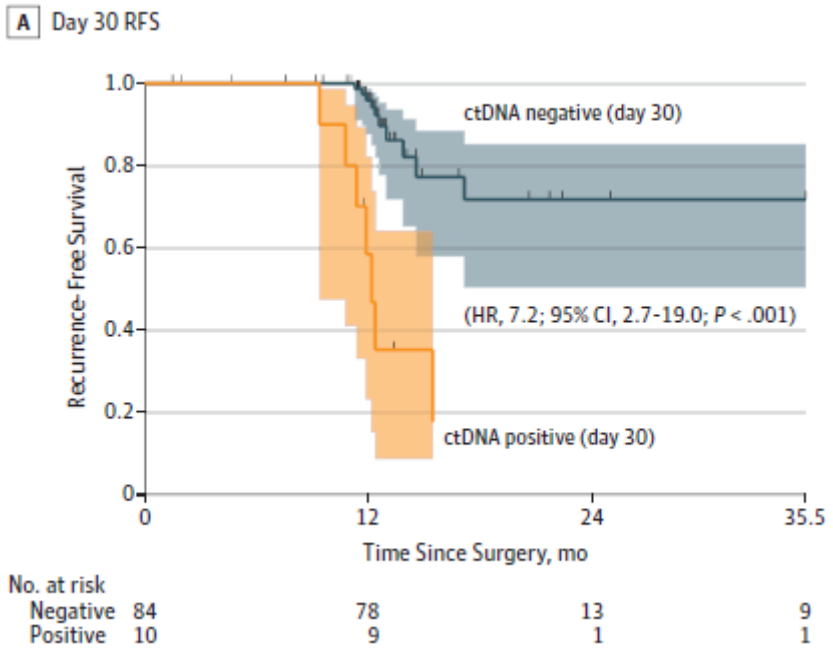
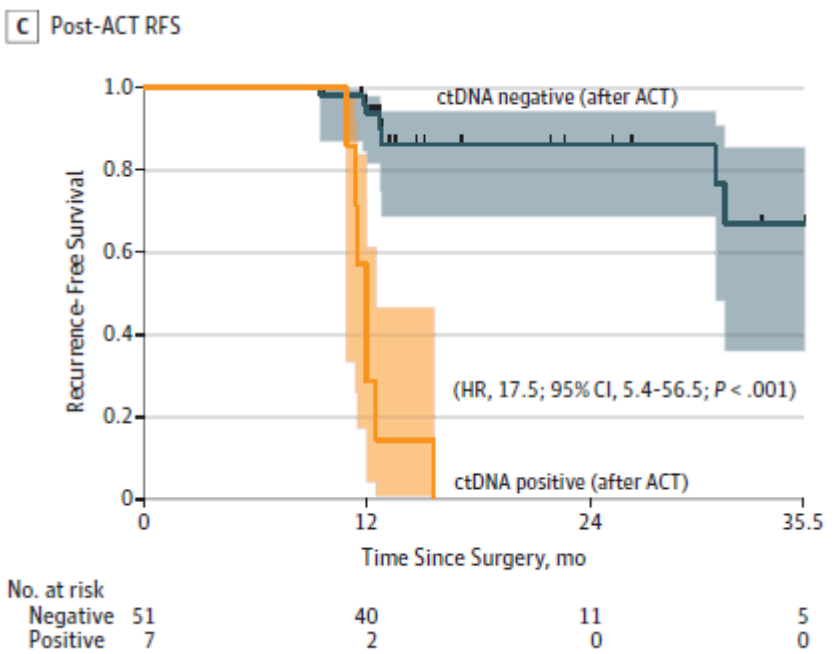
ctDNA in Stage I-III CRC

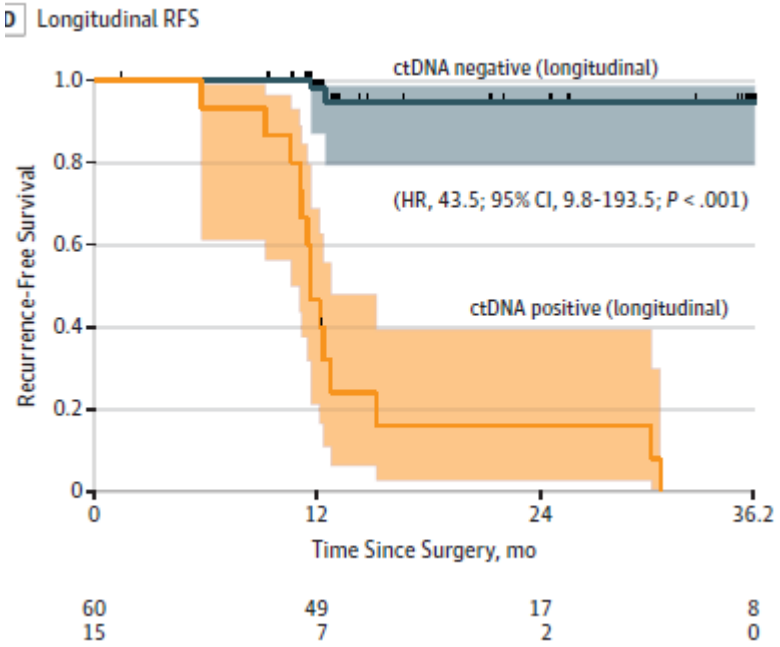
30 days postop ctDNA+ 7xmore likely to relapse

Immediate post ACT ctDNA+ 17xmore likely to relapse

Surveillance ctDNA+ 40xmore likely to relapse







Reinert et al. JAMA onc 2019

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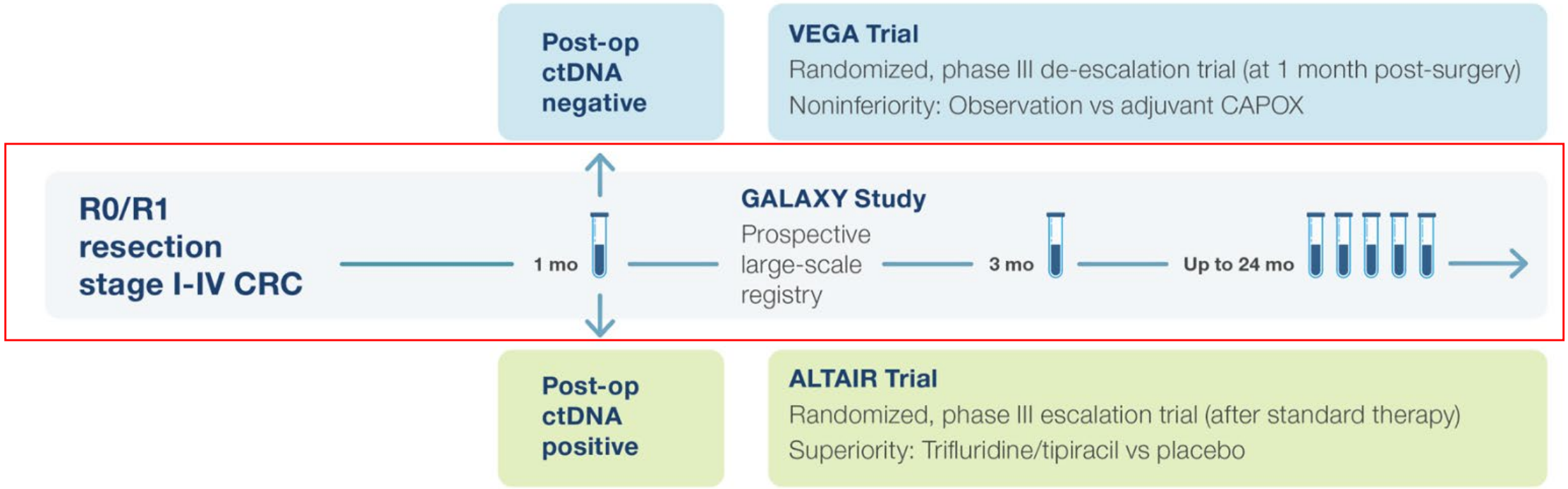
GALAXY arm



Kotaka et al.. ASCO GI 2022.

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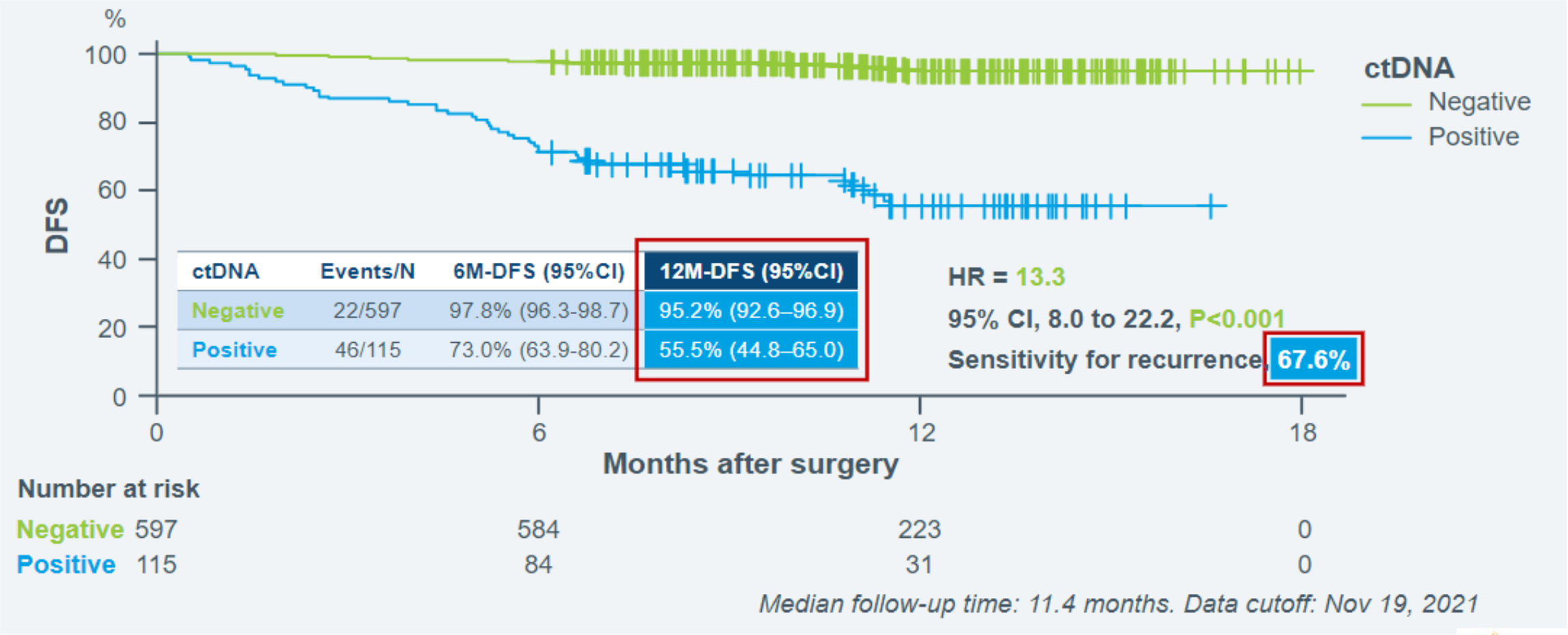
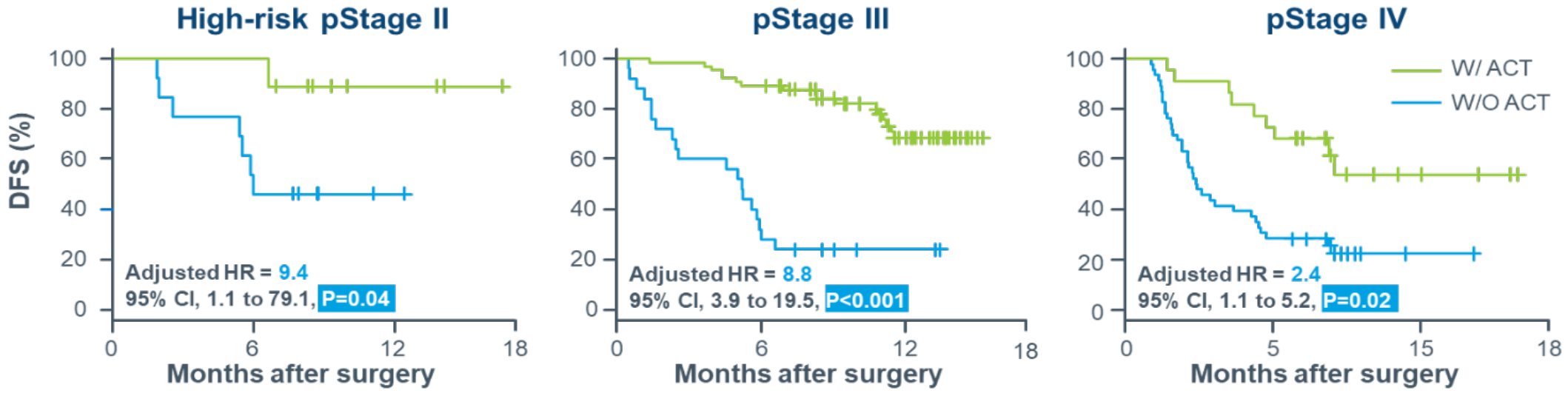
ctDNA Positivity is Associated with worse DFS



Kotaka et al.. ASCO GI 2022.

Adjuvant Chemotherapy in ctDNA Positive Patients



Number at risk

W/ ACT	9	9	3	0
W/O ACT	13	7	1	0

W/ ACT	65	58	24	0
W/O ACT	25	8	2	0

W/ ACT	22	16	4	0
W/O ACT	46	13	1	0

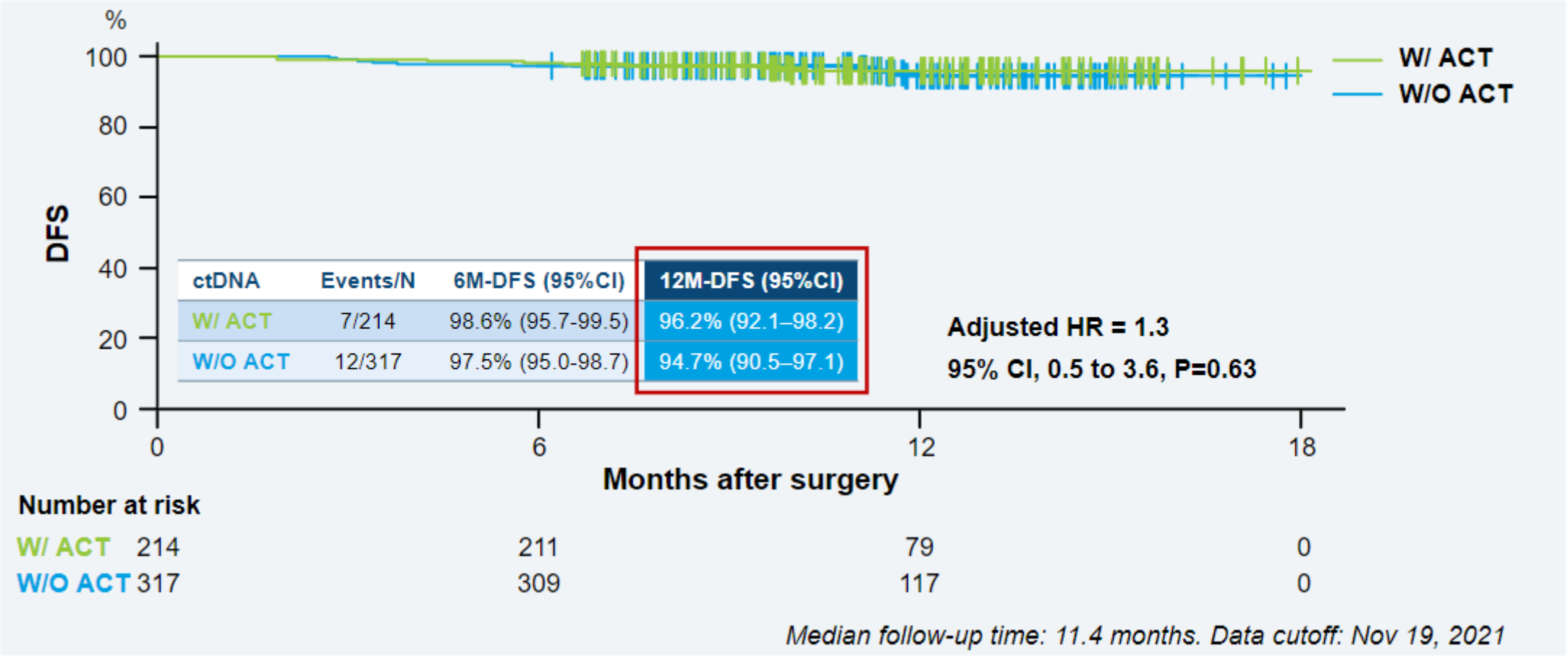
	Events/N	6M-DFS (95%CI)	12M-DFS (95%CI)
W/ ACT	1/9	100% (100–100)	88.9% (43.3–98.4)
W/O ACT	7/13	53.8% (24.8–76.0)	46.2% (19.2–69.6)

	Events/N	6M-DFS (95%CI)	12M-DFS (95%CI)
W/ ACT	17/65	89.2% (78.7–94.7)	68.3% (53.4–79.2)
W/O ACT	19/25	32.0% (15.2–50.2)	24.0% (9.8–41.7)

	Events/N	6M-DFS (95%CI)	12M-DFS (95%CI)
W/ ACT	9/22	72.7% (49.1–86.7)	53.7% (28.4–73.6)
W/O ACT	35/46	28.3% (16.2–41.6)	22.3% (11.2–35.7)

Kotaka et al.. ASCO GI 2022.

No significant benefit for MRN Negative Patients



Kotaka et al.. ASCO GI 2022.

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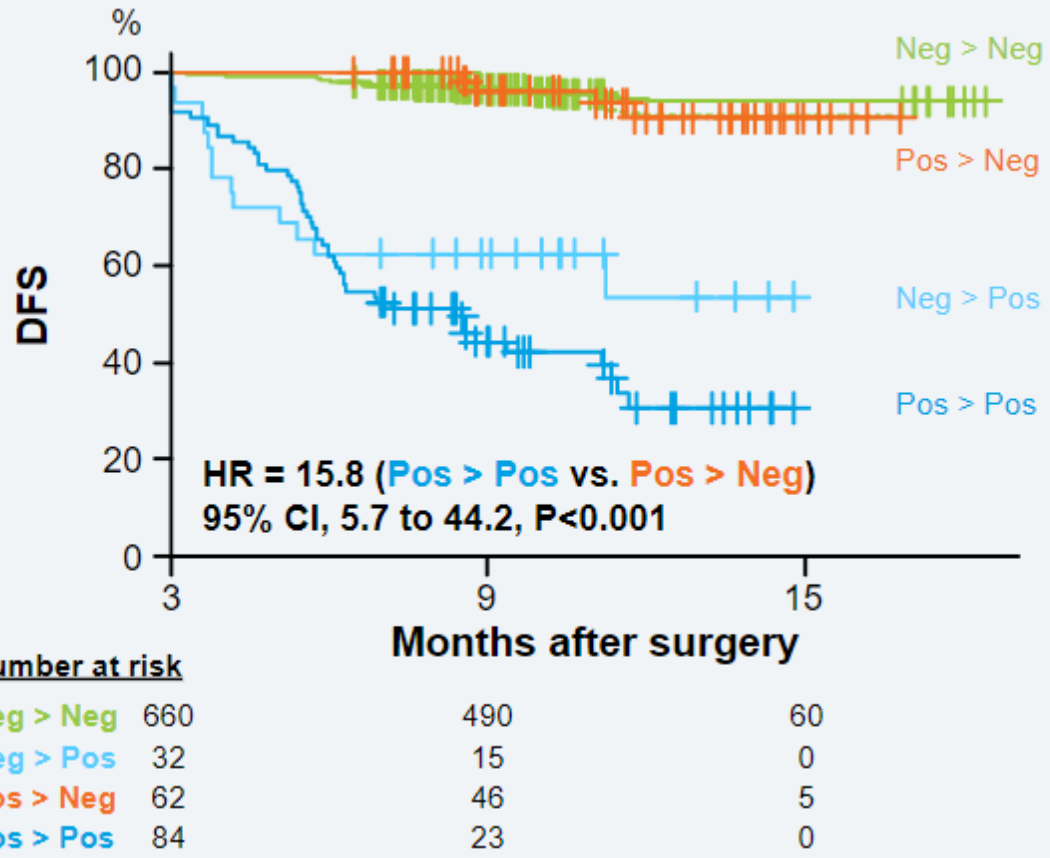
DFS by ctDNA Dynamics



Dynamics	Neg > Neg	Neg > Pos	Pos > Neg	Pos > Pos
Events/N	31/660	13/32	4/62	50/84
6M-DFS	98.0%	62.5%	100%	58.3%
HR	0.8	9.2	Reference	15.8
95%CI	0.27–2.15	3.0–28.4	-	5.7-44.2
P	0.60	<0.001	-	<0.001

Median follow-up time: 11.4 months. Data cutoff: Nov 19, 2021

Kotaka et al.. ASCO GI 2022.

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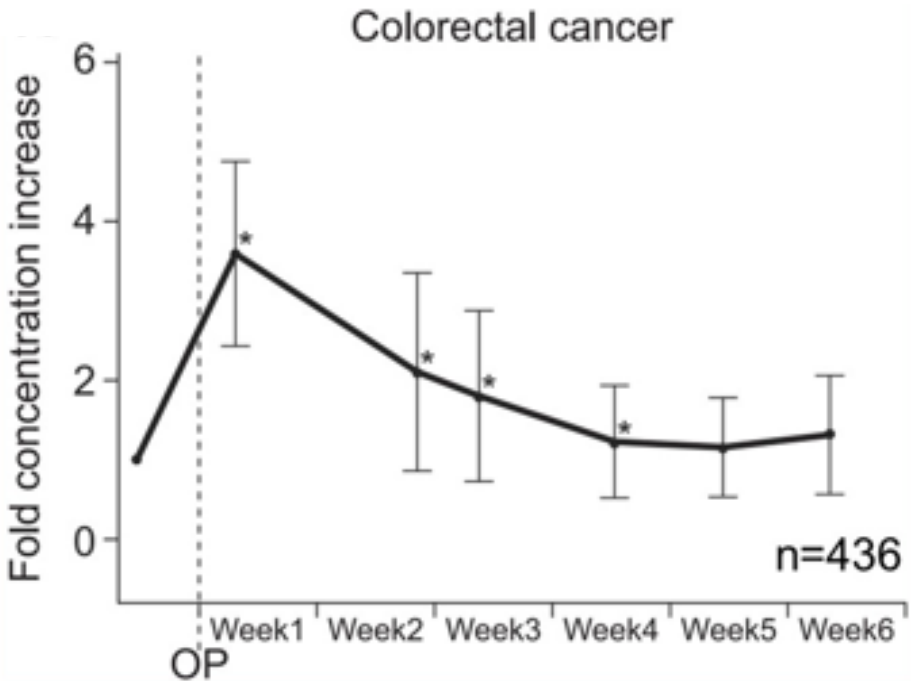
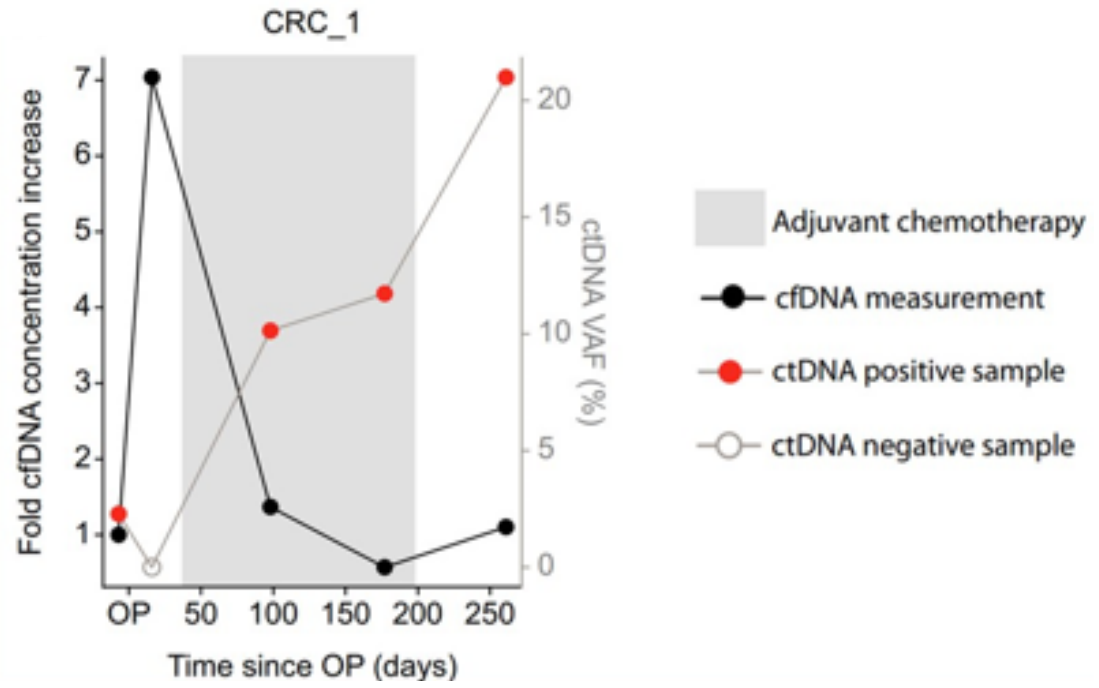
cfDNA Changes After Surgery and Chemotherapy





Henriksen et al. Mole Onco 2020

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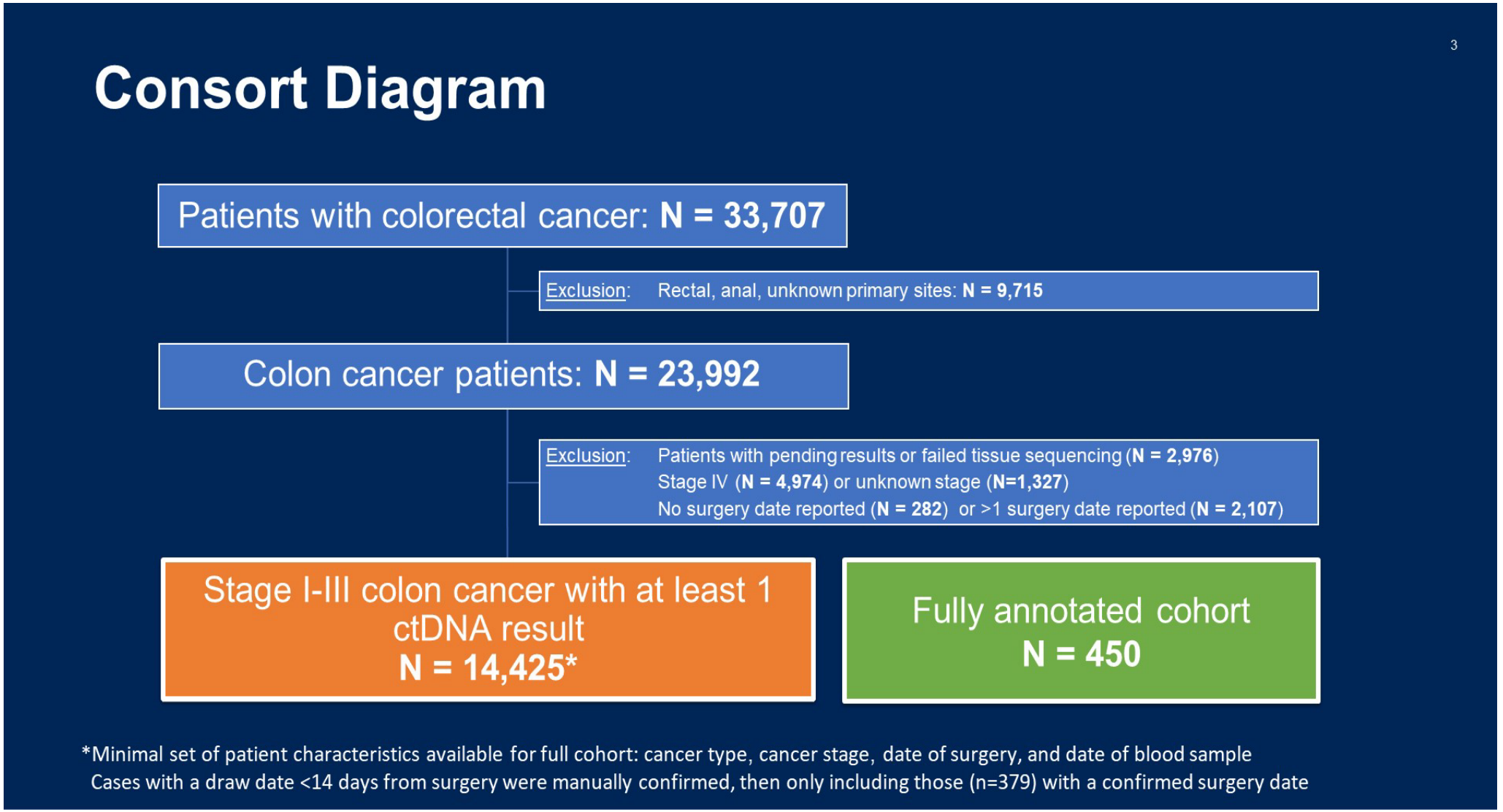
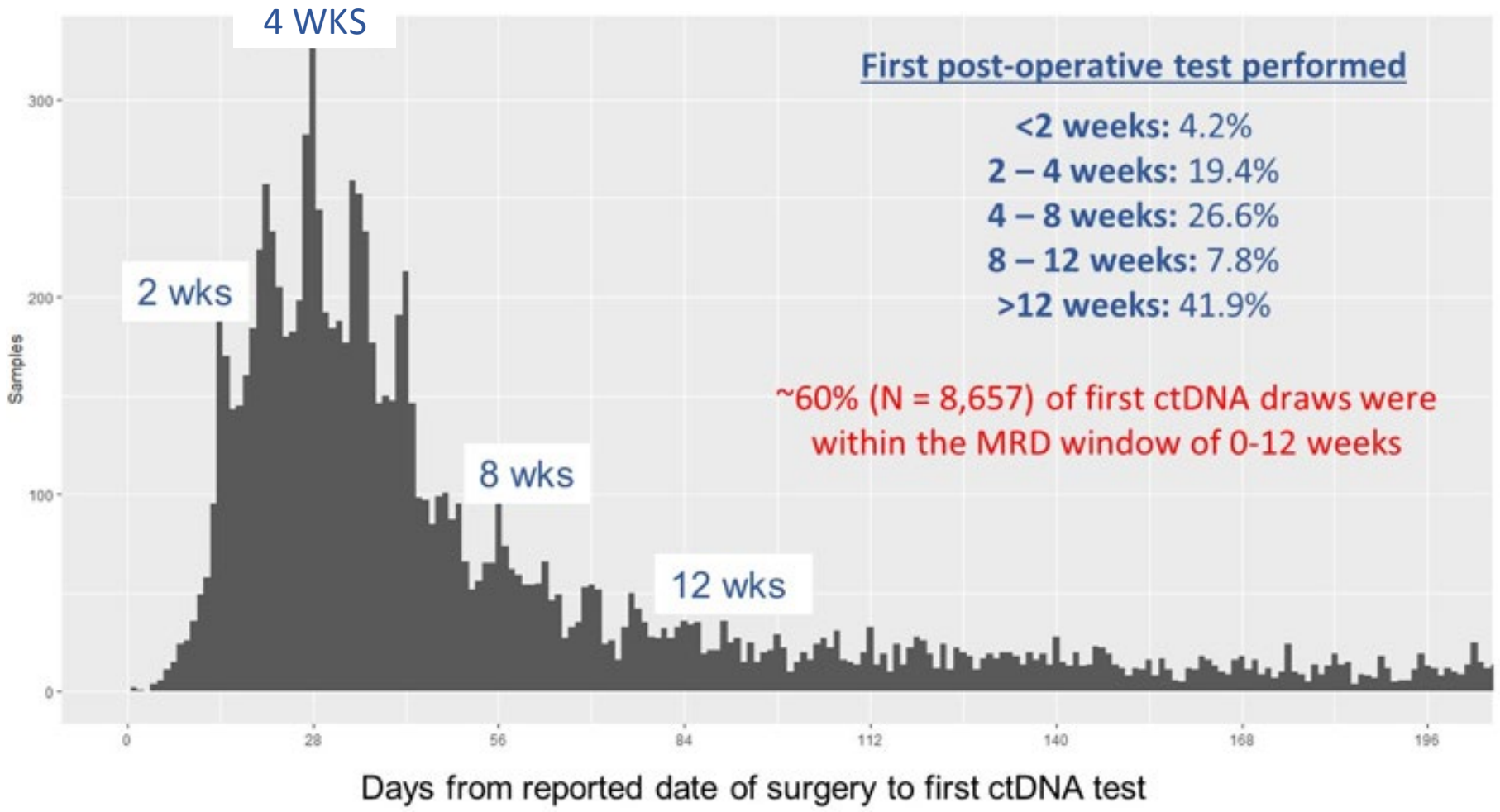
ct DNA Dynamics



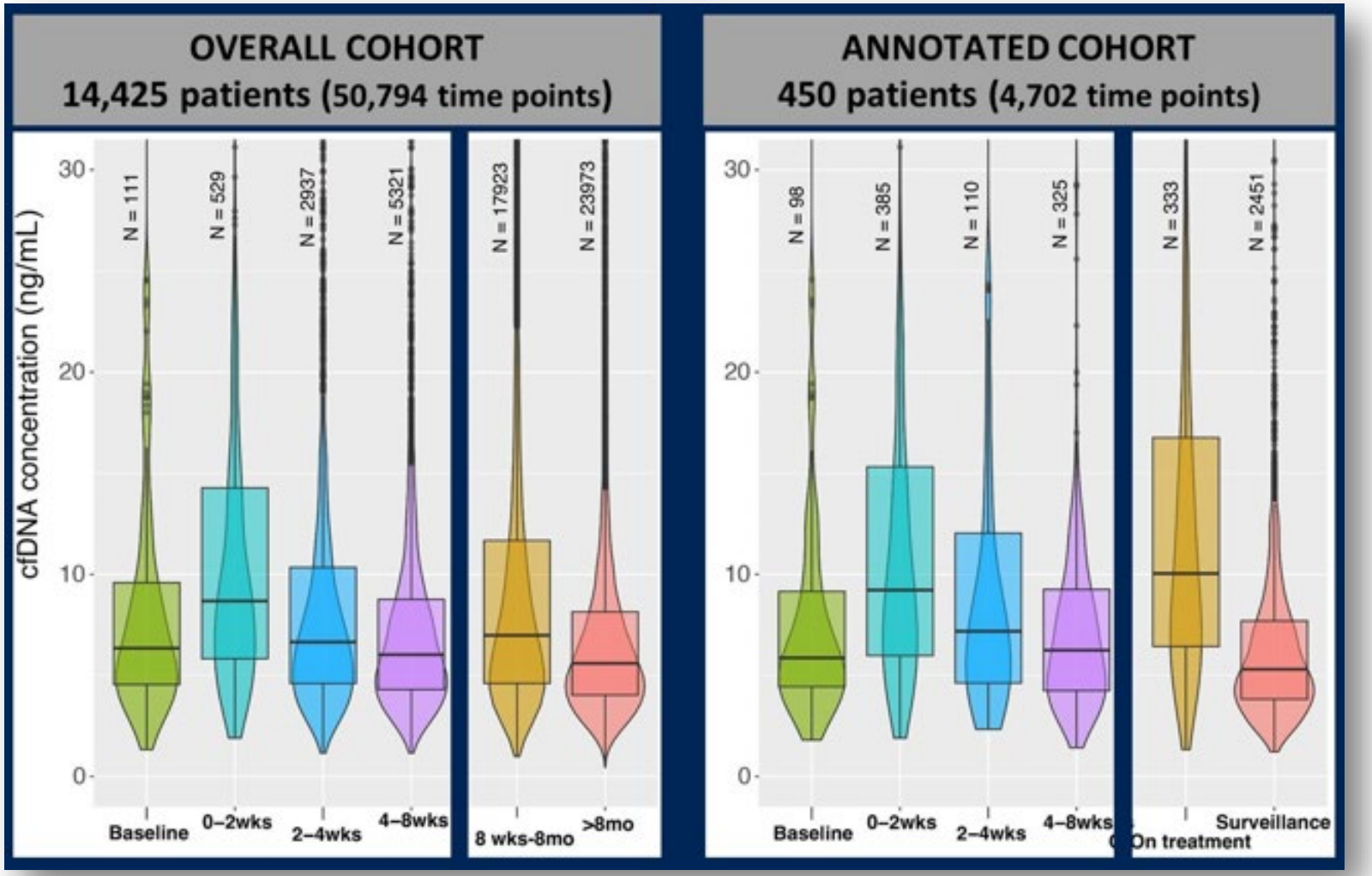
First time point for ctDNA testing



Cohen et al, GI ASCO 2023

Post-Op cfDNA dynamics

- Higher cfDNA at 0-2wks and while on ACT- possible shedding
- *Does high cfDNA affect detection of ctDNA?*



Cohen et al, GI ASCO 2023

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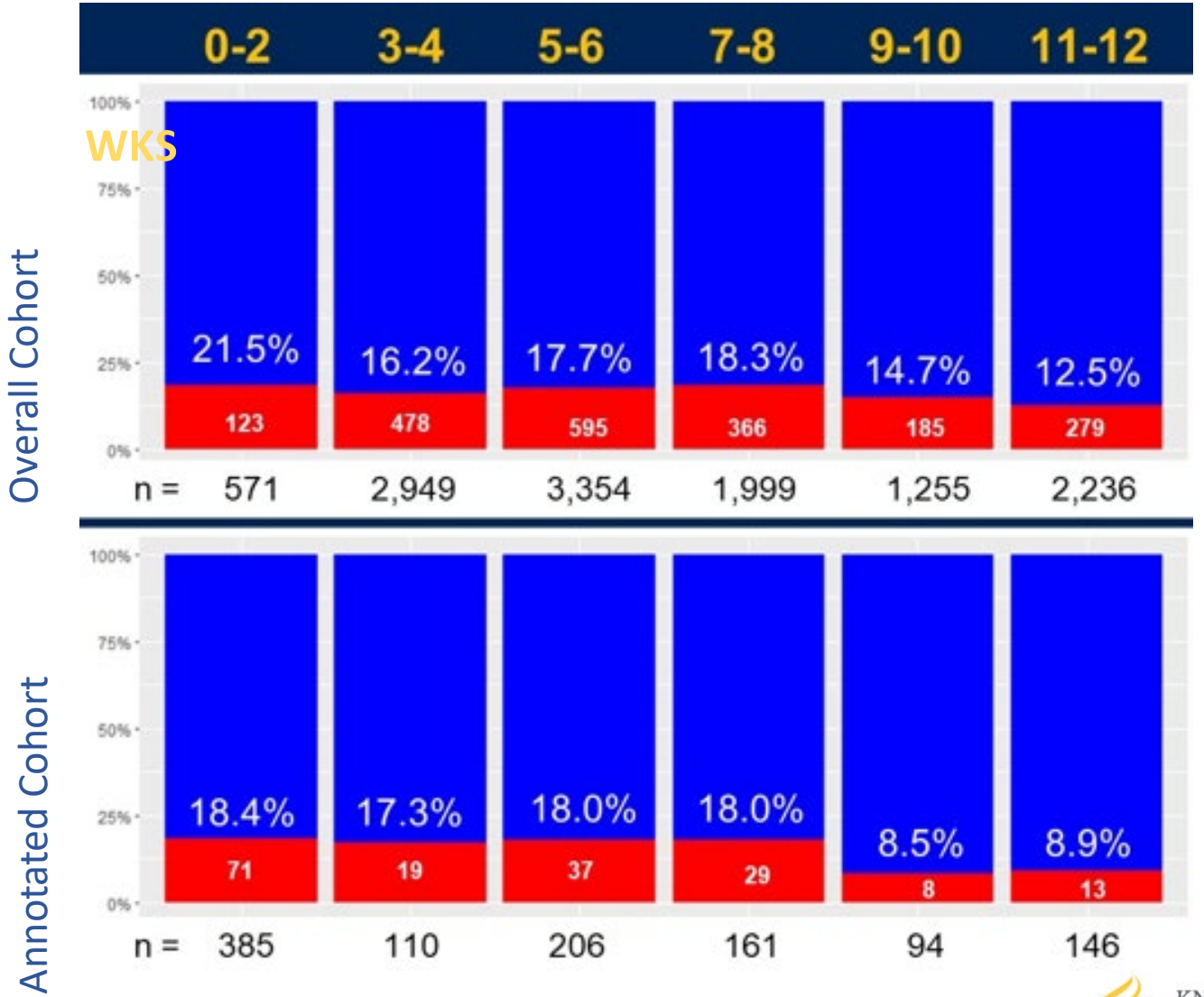
- Increase frequency of ctDNA positivity duing WKS 0-2
- Similar results for other weeks



Cohen et al, GI ASCO 2023

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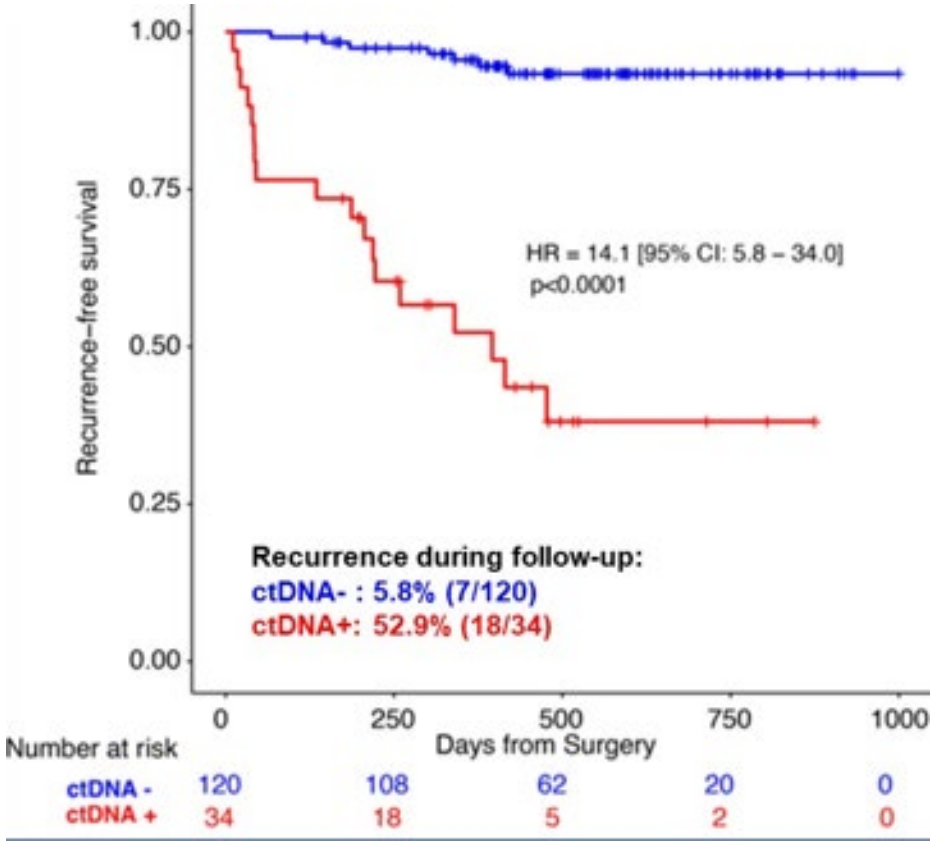
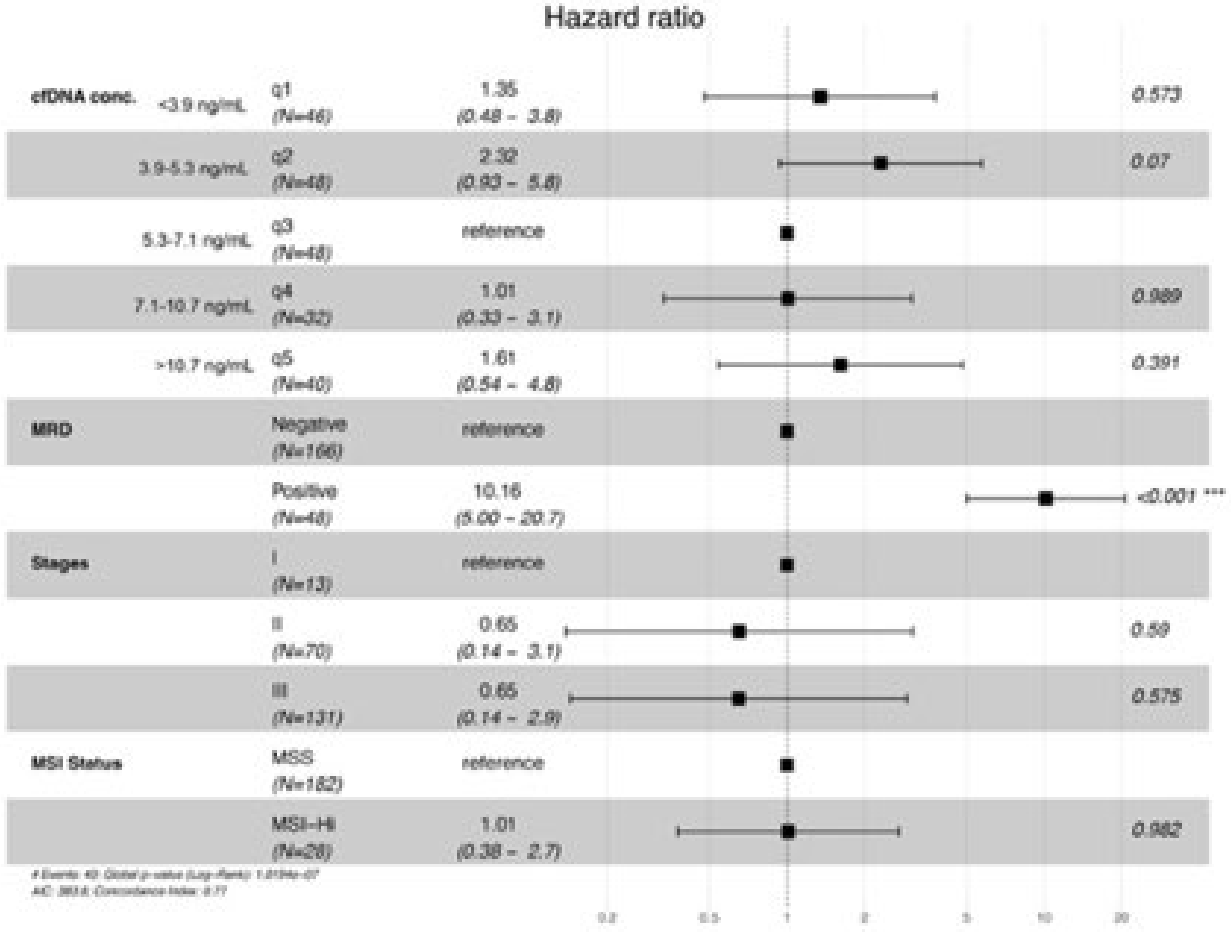
ctDNA Positivity is Associated with Shorter RFS





Cohen et al, GI ASCO 2023

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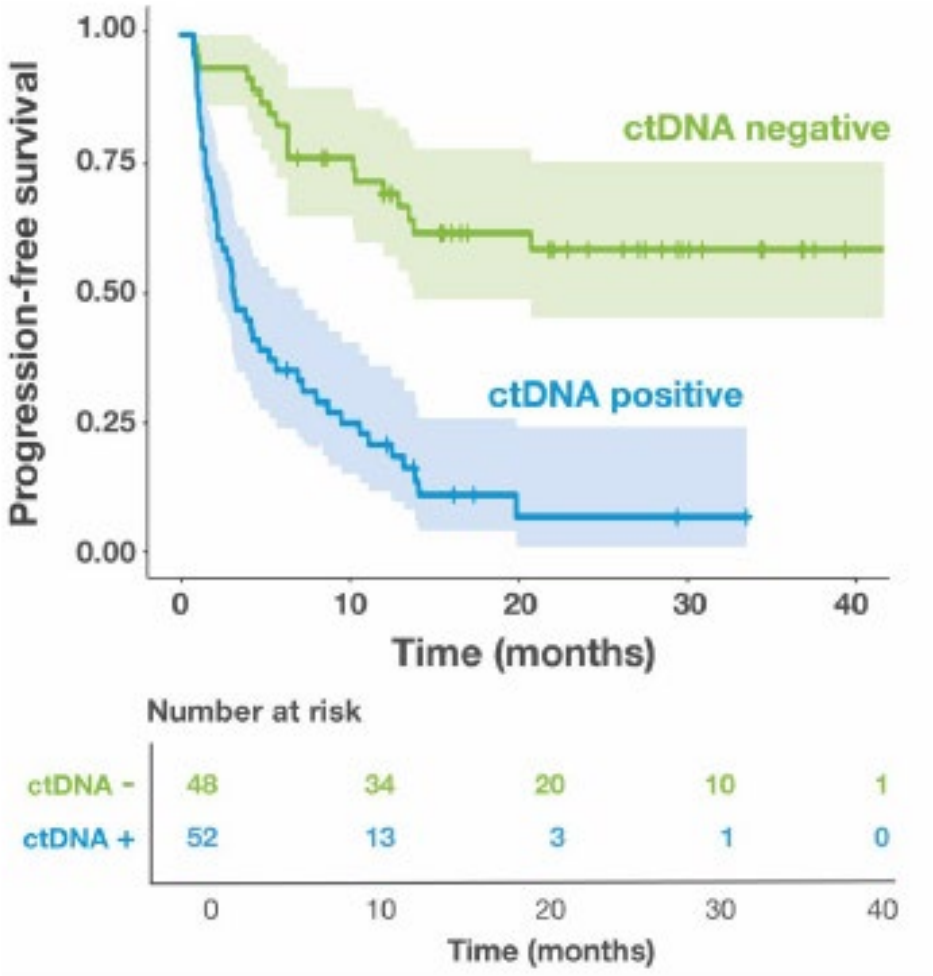
Can ctDNA detect patient that will benefit from treatment?

- Patient with resectable oligometastatic disease.
- Synchronous = 51%, (Liver= 58%, Lung= 21%, Peritoneum=14%, Others= 7%)
- HR:4.6; 95% CI: 2.6-8.1; P<0.001



Loupakis et al, ESMO 2020

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Single-center comparative surveillance strategies of ctDNA, imaging, and CEA

- Retrospectively evaluated, sensitivity (ss), specificity (sp), positive predictive value (ppv) and negative predictive value (npv) of **ctDNA, imaging (Im), and CEA** in curatively resected stage II, III, IV pts against **True Disease Recurrence (TDR)**
  - TDR= positive ctDNA that is confirmed by path or imaging
- 48 pts underwent curative resection (31 stage II-III, 17 stage IV). 15 patients recurred during surveillance (6 stage II-III, 9 stage IV)

Sandhu et al, ASCO2022

Single-center comparative surveillance strategies of ctDNA, imaging, and CEA

- ctDNA sensitivity was poor for lung and CNS only recurrences.
- 2 Pts with negative imaging at SR developed subsequent liver metastases
- 2 Pts, counted recurrent by ctDNA remain NED without any therapy, by CEA and Imaging > 1.5 years

Stage	S %			Im %			CEA %			Im or CEA %		
	II-III	IV	II-IV	II-III	IV	II-IV	II-III	IV	II-IV	II-III	IV	II-IV
ss	66.7	44.4	53.3	33.3	77.8%	60	50	11.1	26.7	83.3	77.8	80
sp	100	100	100	96	100	96.9	88	100	90.9	84	100	87.8
ppv	100	100	100	66.7	100	90	50	100	57.1	55.6	100	75
npv	92.6	61.5	82.5	85.7	80	84.2	88	50	73.2	95.5	80	90.6

Sandhu et al, ASCO2022

Summary

- ctDNA is a good prognostication marker for recurrence in stage III CRC
- However, the role of adjuvant chemotherapy and prognostication is unclear
- Conflicting results GALAXY vs IDEA-FRANCE
- ctDNA dynamics maybe predictive
- Higher concentration of cfDNA does not impact ctDNA detection
- Testing for MRN between weeks 2-8 showed similar sensitivity
- What is the role of ctDNA is stage IV CRC?

	Design	Population	N	Time of ctDNA analysis	Primary endpoint	Secondary/exploratory endpoints
CIRCULATE/PROGIGE70 NCT04120701	Phase III	Resectable stage II CRC	1980	≥ 2 week post op <8	3-year DFS and ctDNA positive patient	2-year DFS, OS, time to recurrent and toxicity
COBRA/NR-GI005 NCT0406810	Phase II/III	Stage IIa CRC after surgery	1408	Post op	Clearance of ctDNA RFS and CT and a positive patient	OS, time to recurrence, compliance Incidence of ctDNA positive post resection Cost effectiveness versus standard of care
TRACC NCT04050345	Phase II/III	High risk stage II, stage III CRC Subset of rectal cancer	1621	Preop, postop, 3 months afterACT, 3 months after maintenance	3-year DFS	OS, toxicity, quality of life, health economics
MEDOCC-CrEATE	Phase III	Stage II CRC	1320	Immediately after surgery interventional	Proportion of patient receiving chemotherapy when ctDNA is detectable after resection	2-year rate of recurrence, OS, DFS, cost effectiveness
CIRCULATE AIO-KRK-0217 NCT04089631	Phase II	Stage II CRC	4812	Within 5 weeks after resection	DFS and ctDNA positive patients	
VEGA	Phase III	ctDNA negative high risk stage II, low risk stage III CRC	1240	Postop week 4, end of chemotherapy (3-month)	RFS and ctDNA negative patients	ctDNA clearance, OS

MRD Challenges

- False negative or false positive results
  - Insufficient sample, low shedding tumor, low sensitivity, sequencing error

- No all studies have shown benefit
  - Sandhu et al[1] ;no benefit to ctDNA over standard imaging
  - Low sensitivity to low-volume disease and certain metastatic sites
  - True predictivity with adjuvant therapy
  - When to treat patients ?

Sandhu et al. JCO. 2022

Conclusion

- ctDNA has a significant potential in the treatment colorectal disease. (adjuvant and metastatic)
- ctDNA is useful for molecular/genomic analysis and difficult to biopsy lesions
- MRD post resection has the potential to improve risk stratification and guide systemic therapy
- ctDNA positive is associated with high risk for recurrence
  - Would patients benefit from adjuvant? (on going trials)
- ctDNA negative is unclear
  - Dynamics improve outcome

Future Questions

- Is there a benefit to changing treatment based on ctDNA?
- ctDNA vs CEA vs imaging
- Utilizing ctDNA to track tumor mutation (e.g. EGFR, HER2)
  - Example; conformation of EGFR resistance vs rechallenge
- Cost

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Thank you…