GI ASCO 2024
Highlights Webinar
with Dr. Van Morris and Research Advocate Lee Jones

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GI ASCO 2024 Highlights Webinar

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TODAY'S PRESENTERS

Van Morris, MD

Associate Professor, Department of Gastrointestinal (GI) Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston TX

Lee Jones

Fight CRC Research Advocacy Training and Support (RATS)

TODAY'S HIGHLIGHTS

NEST-1	Neoadjuvant botensilimab plus balstilimab in resectable mismatch repair proficient and deficient colorectal cancer: NEST-1 clinical trial.
CHECKMATE 8HW	Nivolumab (NIVO) plus ipilimumab (IPI) vs chemotherapy (chemo) as first-line (1L) treatment for microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC): First results of the CheckMate 8HW study.
DYNAMIC-RECTAL	Circulating tumor DNA analysis informing adjuvant chemotherapy in locally advanced rectal cancer: The randomized AGITG DYNAMIC-Rectal study.
BESPOKE	Circulating tumor DNA (ctDNA) for informing adjuvant chemotherapy (ACT) in stage II/III colorectal cancer (CRC): Interim analysis of BESPOKE CRC study.
GALAXY	Circulating tumor DNA (ctDNA) dynamics in patients with colorectal cancer (CRC) with molecular residual disease: Updated analysis from GALAXY study in the CIRCULATE-JAPAN.
COBRA	Phase II results of circulating tumor DNA as a predictive biomarker in adjuvant chemotherapy in patients with stage II colon cancer: NRG-GI005 (COBRA) phase II/III study.

NEST-1

ASCO Gastrointestinal Cancers Symposium

Neoadjuvant botensilimab plus balstilimab (BOT/BAL) in resectable mismatch repair proficient and deficient colorectal cancer: NEST-1 clinical trial

Pashtoon Murtaza Kasi, MD, MS

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Authors: *Pashtoon Murtaza Kasi, Mehraneh D. Jafari, Heather Yeo, Lea Lowenfeld, Uqba Khan, Alana Nguyen, Despina Siolas, Brandon Swed, Sahrish Khan, Madeleine Wood, Allyson J. Ocean, Elizabeta C. Popa, Kelly A. Garrett, Encouse Golden, Preethi Guniganti, Xi K. Zhou, Alessio Pigazzi, Manish A. Shah, Erika Hissong#, Manuel Hidalgo#

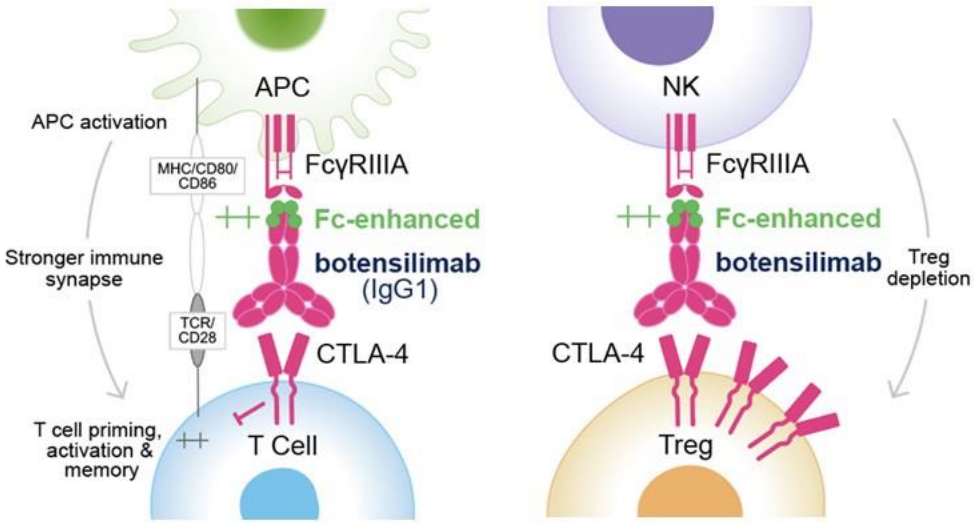
#authors share senior authorship

Background/Methods

- Effective therapies for colorectal cancer (CRC), particularly in those ~85-95% with **proficient mismatch repair/ microsatellite stable (pMMR/MSS)** cancer, are a critical unmet need.[1]
- **Botensilimab (BOT),** a multifunctional next-generation **anti-CTLA-4 antibody**, with balstilimab (BAL), an anti-PD-1 antibody, has a response rate of >20% in patients with heavily pretreated pMMR/MSS metastatic CRC.[2]
- NEST-1 (NCT05571293) is the first study to evaluate **neoadjuvant** BOT and BAL in CRC patients eligible for surgery.
- **Investigator-initiated trial supported by Agenus Inc.**

Botensilimab (BOT)[3]

Fc-enhanced CTLA-4 Inhibitor



- ↑ T cell priming, expansion, memory
- ↑ Frequency of activated APCs
- ↑ Treg depletion
- ↓ Complement mediated toxicity

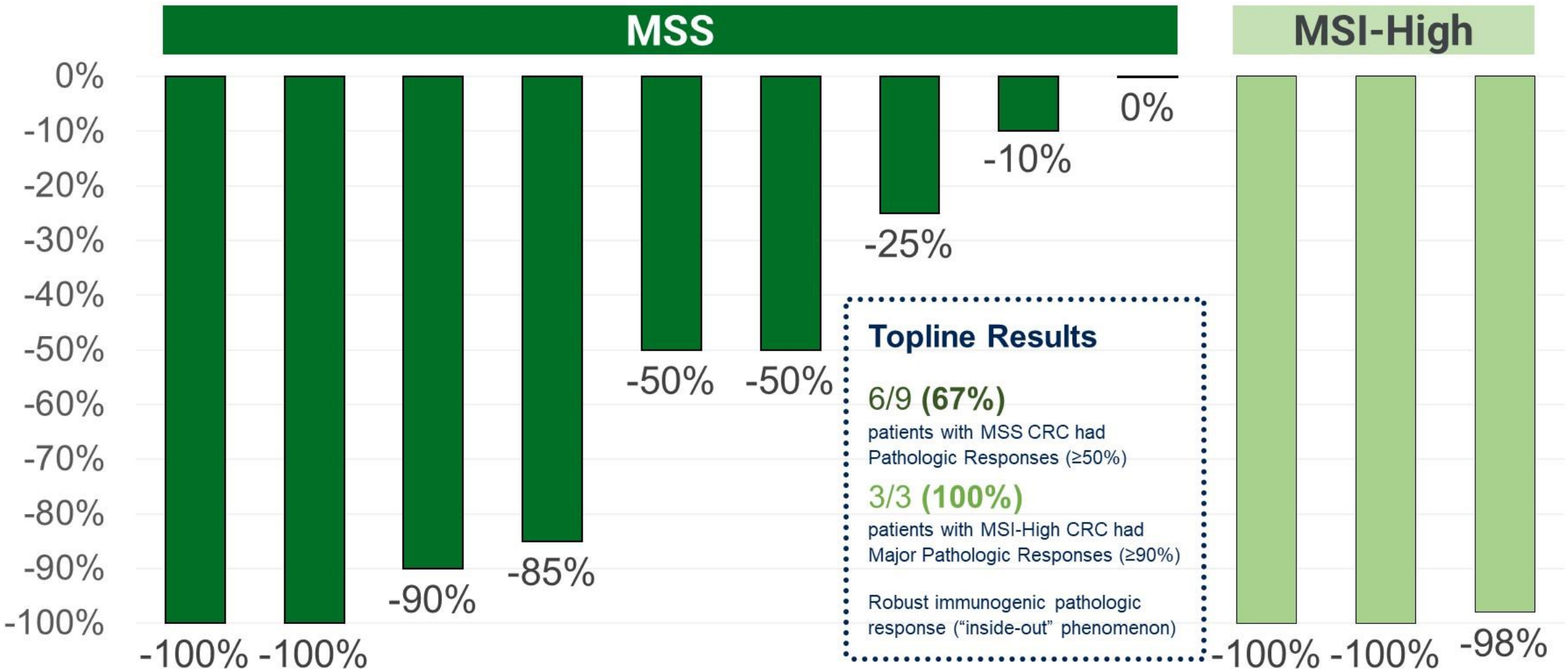
Study Schema[1]

@pashtoonkasi

1. Kasi PM et al. Oncogene. 2023 Oct; 42 (44): 3252-3259.
2. El-Khoueiry AB. Journal of Clinical Oncology 2023 41:4_suppl, LBA8
3. Adapted from Wilky B, et al. Oral Presentation at CTOS 2023. Dublin, Ireland. Paper 31

PRESENTED BY: Pashtoon Kasi, MD, MS

NEST-1 Clinical Trial: Pathologic Tumor Reductions (%) by Patient



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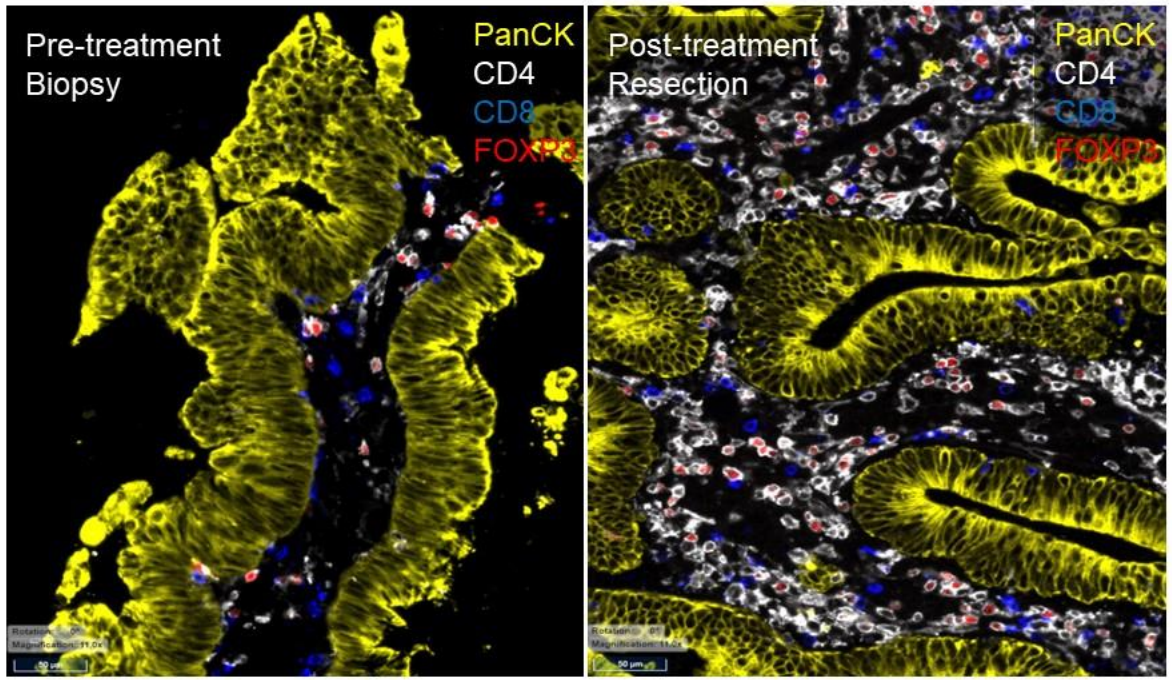
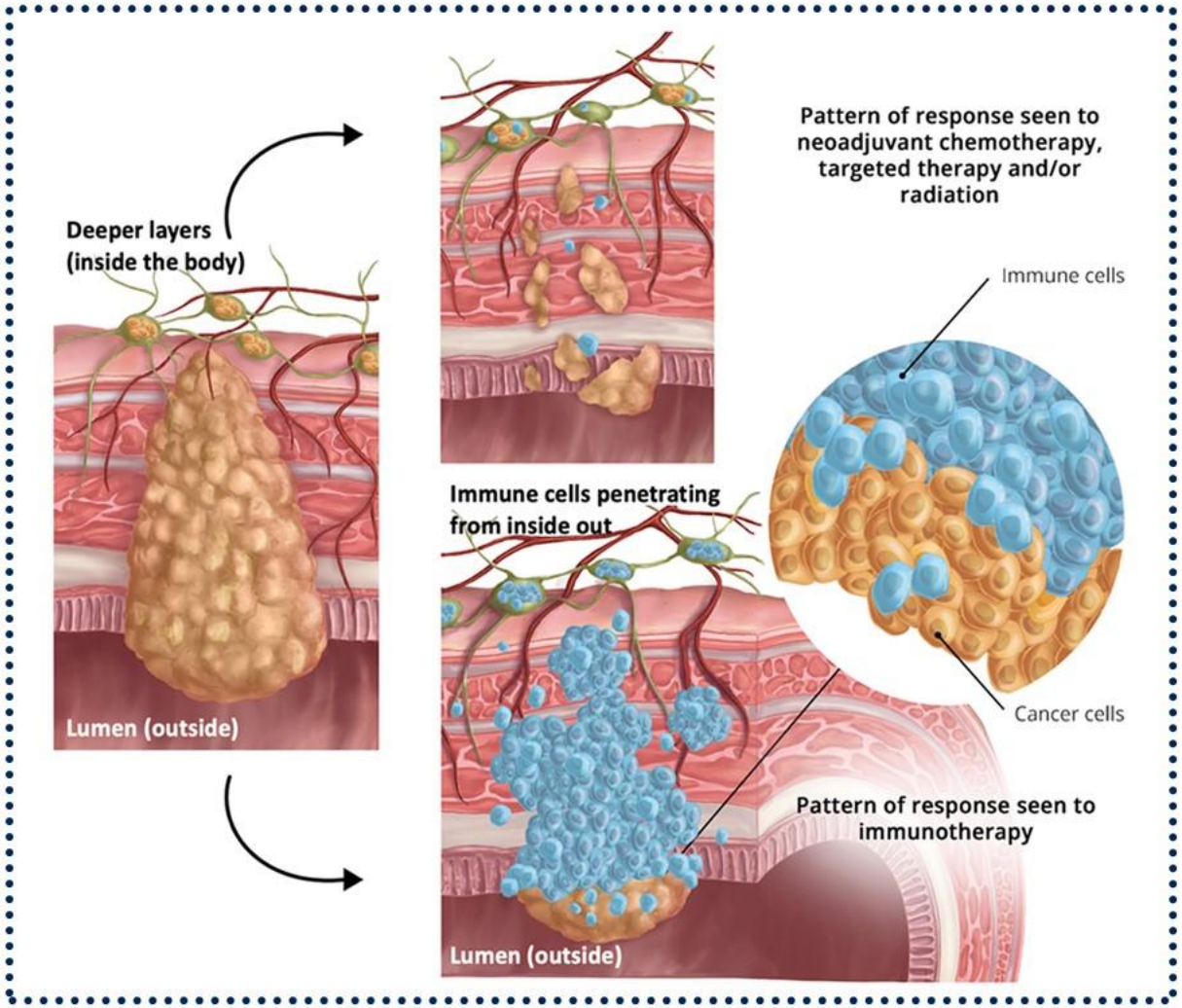
Results





Tissue immune-microenvironment correlates assessed pre- and post-treatment with immunotherapy by *RareCyte Inc.* (Seattle WA) using their 13-marker immune-oncology panel on colon and rectal cancer samples show a robust immunogenic pathologic response ("inside-out" phenomenon; serosa-to-mucosa pattern of response).

Acknowledgments: RareCyte Inc. for the pre-and post-images/analyses; DrawImpacts for the illustration.

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Conclusions/Take-Away

- **The study met its primary endpoints**.
  - Neoadjuvant BOT/BAL is a **safe** and **active** regimen in both pMMR/MSS and dMMR/MSI-H CRC.
  - 6/9 (67%) pMMR/MSS patients with ≥50% reduction, 2/9 with CR.
  - 3/3 (100%) dMMR/MSI-H with deep response (≥98% reduction), 2/3 with CR.
- **No surgery was delayed** due to any treatment-related adverse events (TRAEs).
- All patients positive for ctDNA at screening **cleared ctDNA** (7/7 – 100%). 11/11 (100%) tested post-operatively have remained **ctDNA/MRD negative** for more than 30 draws cumulatively.
- Post-treatment tumor IHC/IF demonstrates **robust T cell infiltration**, T reg depletion, and dendritic cells/myeloid repolarization.
- **Clinical downstaging** and deep pathological responses provide a framework for reduced reliance on surgery and/or adjuvant chemotherapy in future studies.
- **NEST-1 trial (NCT05571293) has expanded enrollment** to evaluate an 8-weeks course over the current minimum 3-week course for MSS, and the necessity for surgery for MSI-High.

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My take-aways

- Window of opportunity neoadjuvant trials are clinically feasible and scientifically important to understand clinically efficacy in previously untreated, curative-intent settings.

- Not all patients had detectable ctDNA at baseline – these analyses should continue in the exploratory setting, but clearance of ctDNA as a decision making tool in treatment of neoadjuvant colon cancer is VERY early for drawing definitive conclusions.

- The clinical signal with regards to degree of pathologic response is intriguing. Further data – including safety/toxicity profile, clinical/radiographic characteristics of the primary tumors, etc -- are needed before we make a more informed interpretation.

CHECKMATE 8HW

ASCO Gastrointestinal Cancers Symposium

Nivolumab plus ipilimumab vs chemotherapy as first-line treatment for microsatellite instability-high/ mismatch repair-deficient metastatic colorectal cancer: first results of the CheckMate 8HW study

Thierry Andre,[1] Elena Elez,[2] Eric Van Cutsem,[3] Lars Henrik Jensen,[4] Jaafar Bennouna,[5] Guillermo Ariel Mendez,[6] Michael Schenker,[7] Christelle de la Fouchardiere,[8] Maria Luisa Limon,[9] Takayuki Yoshino,[10] Jin Li,[11] Heinz-Josef Lenz,[12] Jose Manzano Mozo,[13] Giampaolo Tortora,[14] Rocio Garcia-Carbonero,[15] Elvis Cela,[16] Yingsi Yang,[16] Ming Lei,[16] Lixian Jin,[16] Sara Lonardi[17]

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Abstract number LBA768

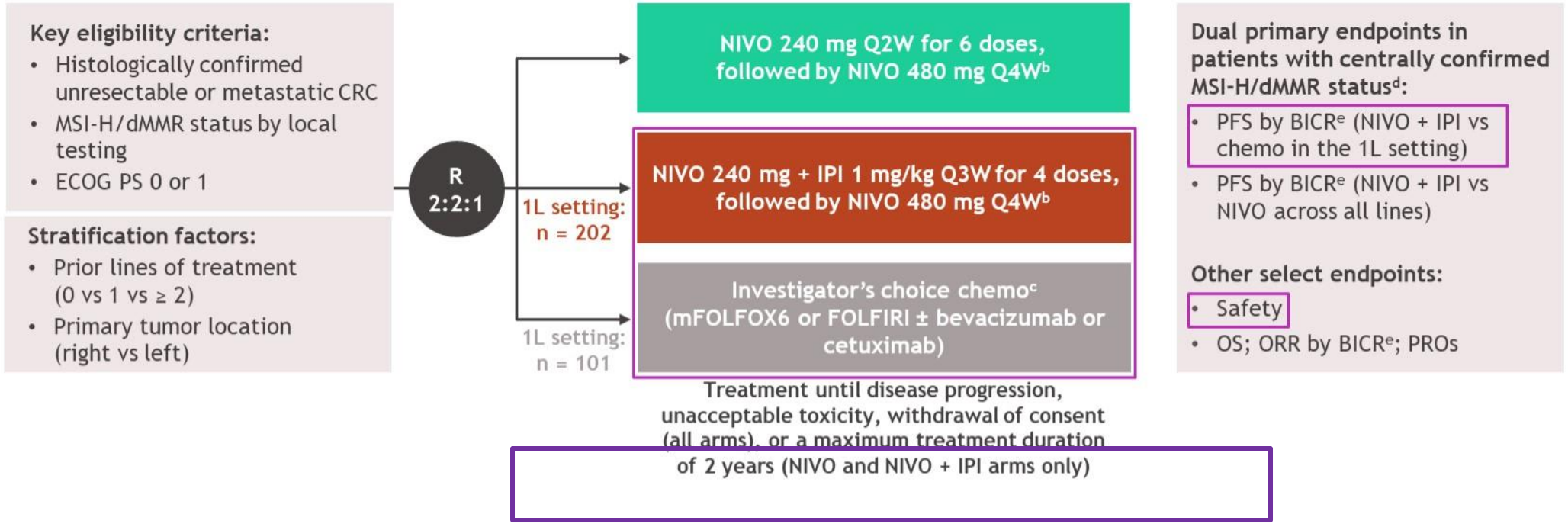
Introduction

- Patients with MSI-H/dMMR mCRC have poor outcomes with standard chemo with or without targeted therapies[1]
- Despite global approval of pembrolizumab monotherapy for MSI-H/dMMR mCRC in the 1L setting,[2,3] the 2-year and 5-year PFS rates were 48% and 34%, respectively[4,5]; an unmet need still exists for these patients
  - Less PFS benefit was seen with pembrolizumab monotherapy vs chemo in patients with *KRAS* or *NRAS* mutations compared with the overall study population[4]
- NIVO and IPI are immune checkpoint inhibitors with distinct but complementary mechanisms of action[6]
- NIVO alone or in combination with IPI are approved in previously treated patients with MSI-H/dMMR mCRC in many countries, based on the phase 2 CheckMate 142 study[7-9]
- CheckMate 8HW is a randomized phase 3 study comparing NIVO + IPI with NIVO or chemo in patients with MSI-H/dMMR mCRC
- We report first results from the prespecified interim analysis for NIVO + IPI vs chemo in the 1L setting

1. Venderbosch S, et al. *Clin Cancer Res* 2014;20:5322-5330. 2. KEYTRUDA® (pembrolizumab) [prescribing information]. Rahway, NJ: Merck & Co., Inc.; December 2023. 3. KEYTRUDA® (pembrolizumab) [summary of product characteristics]. Haarlem, The Netherlands: Merck Sharp & Dohme B.V.; October 2023. 4. Andre T, et al. *N Engl J Med* 2020;383:2207-2218. 5. Shiu K-K, et al. *Ann Oncol* 2023;34:S1271-S1272. 6. Das R, et al. *J Immunol* 2015;194:950-959. 7. OPDIVO® (nivolumab) [summary of product characteristics]. Dublin, Ireland: Bristol Myers Squibb Pharma EEIG; September 2023. 8. OPDIVO® (nivolumab) [prescribing information]. Princeton, NJ: Bristol Myers Squibb; October 2023. 9. OPDIVO® (nivolumab) [prescribing information]. Osaka, Japan: Ono Pharmaceutical Company, Ltd; December 2023.

CheckMate 8HW study design

- CheckMate 8HW is a randomized, multicenter, open-label phase 3 study[a]



- At data cutoff (October 12, 2023), the median follow-up[f] was 24.3 months

[a]ClinicalTrials.gov. NCT04008030. [b]Patients with ≥ 2 prior lines are randomized only to the NIVO or NIVO + IPI arms. [c]Patients receiving investigator's choice of chemotherapy are eligible to receive NIVO + IPI upon progression (crossover treatment). [d]Confirmed using either immunohistochemistry and/or polymerase chain reaction-based tests. [e]Evaluated using RECIST v1.1. [f]Time between randomization and last known date alive or death.

Baseline characteristics

Characteristic (1L all randomized patients)	Category	NIVO + IPI (n = 202)	Chemo (n = 101)
Age	Median (range), years	62 (21-86)	65 (26-87)
	< 65 years	117 (58)	46 (46)
Sex	Male	95 (47)	45 (45)
Region	US/Canada/Europe	133 (66)	71 (70)
	Asia	19 (9)	11 (11)
	Rest of world	50 (25)	19 (19)
ECOG PS	0	111 (55)	52 (51)
Disease stage at initial diagnosis[a]	Stage IV	85 (42)	49 (49)
Tumor sidedness	Right	138 (68)	68 (67)
Sites of metastases[a,b]	Liver	76 (38)	42 (42)
	Lung	44 (22)	25 (25)
	Peritoneum	84 (42)	43 (43)
Centrally confirmed MSI-H/dMMR status	Yes	171 (85)	84 (83)
	No	31 (15)	17 (17)
Tumor cell PD-L1[d,e]	< 1%	145 (72)	80 (79)
	≥ 1%	43 (21)	12 (12)
BRAF, *KRAS*, *NRAS* mutation status[e,f]	*BRAF*/*KRAS*/*NRAS* all wild-type	47 (23)	23 (23)
	BRAF mutant	52 (26)	24 (24)
	KRAS or *NRAS* mutant	43 (21)	21 (21)
	Unknown	55 (27)	31 (31)
Clinical history of Lynch syndrome[e,g]	Yes	22 (11)	17 (17)
	No	135 (67)	49 (49)
	Reported as unknown	44 (22)	30 (30)
Prior surgery related to current cancer	Yes	174 (86)	84 (83)
	No	28 (14)	17 (17)

Data are shown as n (%) unless otherwise noted. [a]All patients had stage IV disease at study entry. [b]Per BICR. [c]Metastases not reported in 3 patients in the NIVO + IPI arm. [d]Tumor cell PD-L1 expression indeterminate, not evaluable, or not available: NIVO + IPI, n = 14; chemo, n = 9. [e]Percentages may not add up to 100% due to rounding. [f]*BRAF* and *KRAS*/*NRAS* mutant: NIVO + IPI, n = 5; chemo, n = 2. [g]Patients with Lynch syndrome not reported: NIVO + IPI, n = 1; chemo, n = 5.

Exposure and disposition

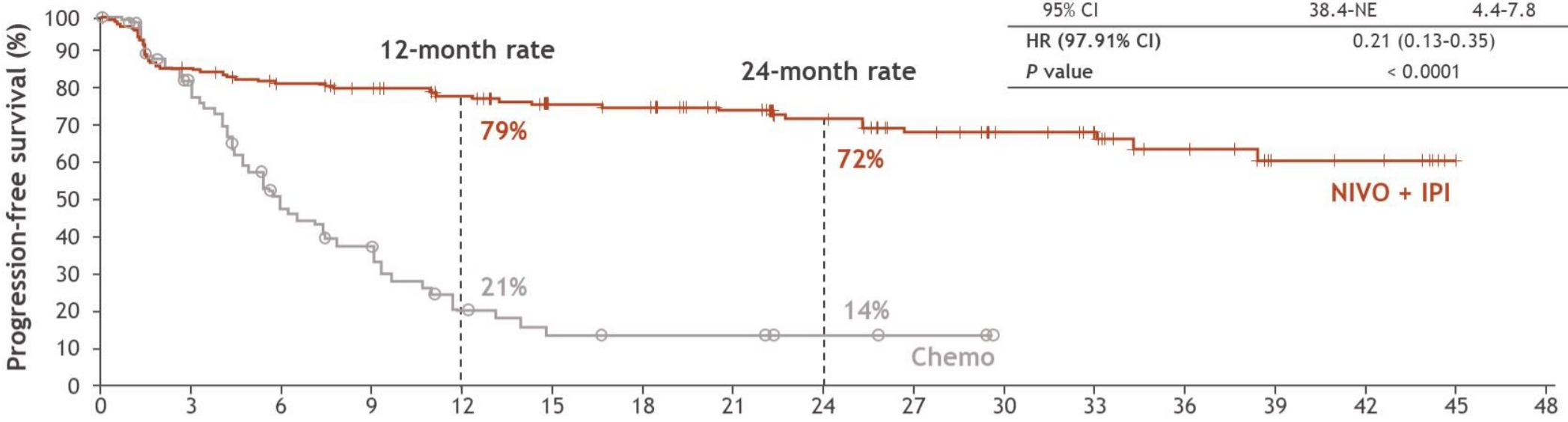
Disposition	NIVO + IPI	Chemo
All randomized patients, n	202	101
All treated patients, n	200	88
Ongoing treatment,[a] n (%)	42 (21)	6 (7)
Completed treatment,[a] n (%)	62 (31)	0
Discontinued treatment,[a] n (%)	96 (48)	82 (93)
Reasons for treatment discontinuation,[a] n (%)		
Disease progression	38 (19)	61 (69)
AE related to treatment	36 (18)	4 (5)
AE not related to treatment	12 (6)	5 (6)
Maximum clinical benefit	0	8 (9)
Other[b]	10 (5)	4 (5)

- Median duration of treatment was 13.5 months in the NIVO + IPI arm and 4.0 months in the chemo arm
  - In the NIVO + IPI arm, median duration of treatment for each component was 13.5 months for NIVO and 2.1 months for IPI
- Among patients treated with chemo, 66 patients (75%) received a biologic agent (bevacizumab, n = 56; cetuximab, n = 10)

In the chemo arm, 45 patients received crossover treatment upon disease progression by BICR. [a]Percentages shown are based on all treated patients. [b]Other reasons for discontinuation included death (n = 2), withdrawal of consent (n = 1), pregnancy (n = 1), patient no longer met study criteria (n = 1), and other reasons (n = 9).

Progression-free survival

1L centrally confirmed MSI-H/dMMR	NIVO + IPI (n = 171)	Chemo (n = 84)
Median PFS,[a,b] mo	NR	5.9
95% CI	38.4-NE	4.4-7.8
HR (97.91% CI)	0.21 (0.13-0.35)	
P value	< 0.0001	



No. at risk

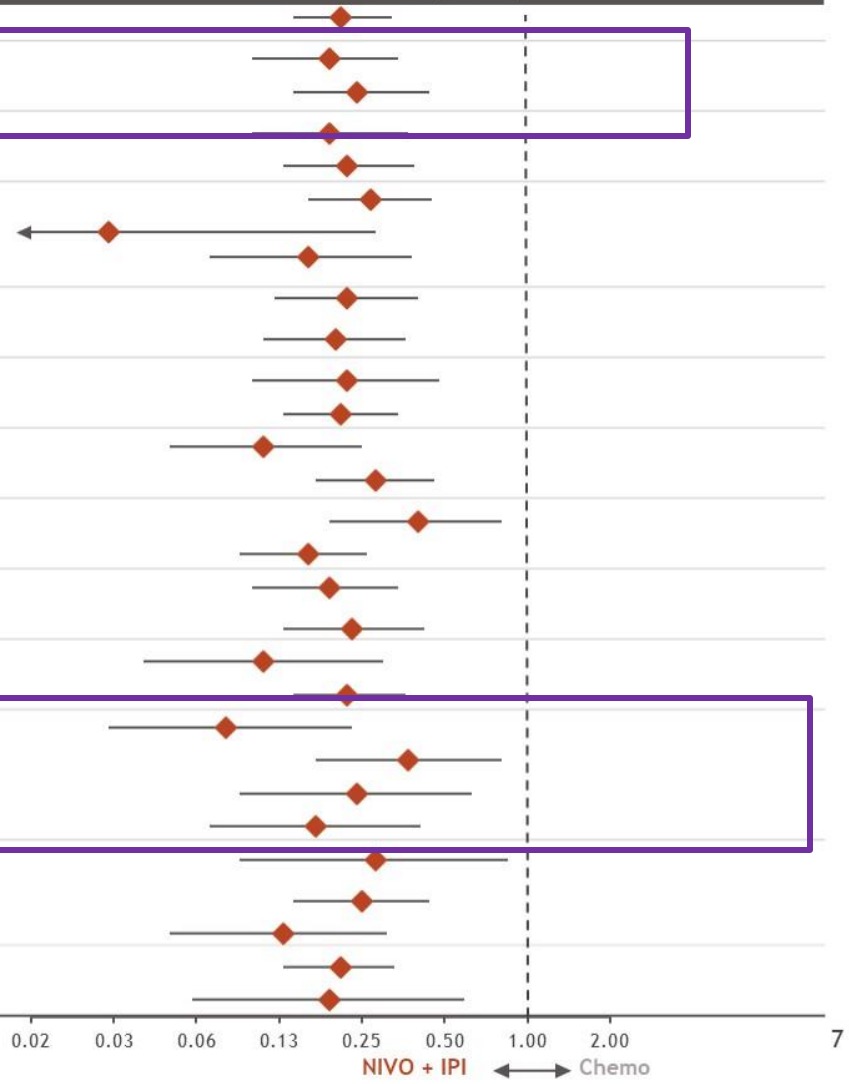
NIVO + IPI	171	144	132	122	108	95	92	77	64	53	42	37	22	10	9	1	0
Chemo	84	53	29	20	10	6	5	5	3	2	0	0	0	0	0	0	0

- PFS benefit with NIVO + IPI vs chemo was robust and consistent across the sensitivity analyses, including PFS by BICR in 1L all randomized patients (HR, 0.32; 95% CI, 0.23-0.46)

[a]Per BICR. [b]Median follow-up, 24.3 months.

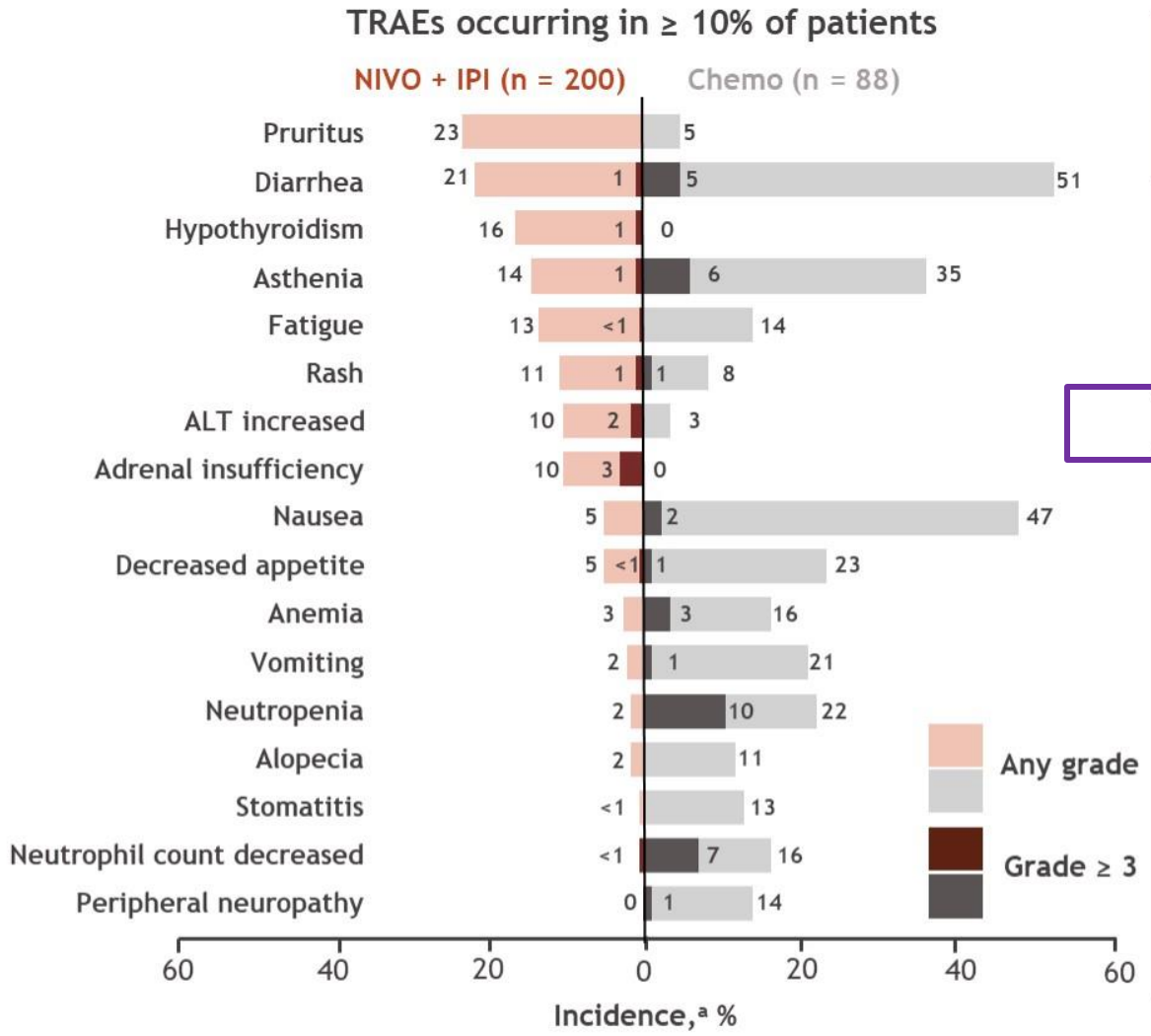
Progression-free survival subgroup analysis

Category (1L centrally confirmed MSI-H/dMMR)	Subgroup	Median PFS,[a] mo		Unstratified HR	Unstratified HR (95% CI)
		NIVO + IPI	Chemo		
Overall (N = 255)		NR	5.9	0.21	
Age, years	< 65 (n = 138)	NR	5.7	0.19	
	≥ 65 (n = 117)	NR	5.9	0.24	
Sex	Male (n = 117)	NR	5.9	0.19	
	Female (n = 138)	NR	6.2	0.22	
Region	US/Canada/Europe (n = 167)	NR	5.7	0.27	
	Asia (n = 28)	NR	7.4	0.03	
	Rest of world (n = 60)	NR	6.2	0.16	
ECOG PS	0 (n = 142)	NR	9.0	0.22	
	≥ 1 (n = 113)	NR	4.2	0.20	
Tumor sidedness	Left (n = 70)	NR	4.4	0.22	
	Right (n = 185)	NR	7.1	0.21	
Liver metastases[a]	Yes (n = 87)	NR	5.9	0.11	
	No (n = 166)	NR	5.4	0.28	
Lung metastases[a]	Yes (n = 53)	13.2	4.9	0.40	
	No (n = 200)	NR	6.2	0.16	
Peritoneal metastases[a]	Yes (n = 115)	NR	4.4	0.19	
	No (n = 138)	NR	7.4	0.23	
Tumor cell PD-L1 expression	≥ 1% (n = 55)	NR	3.4	0.11	
	< 1% (n = 191)	NR	6.5	0.22	
BRAF/KRAS/NRAS mutation status	*BRAF/KRAS/NRAS* all wild type (n = 58)	34.3	5.4	0.08	
	BRAF mutant (n = 72)	NR	9.2	0.37	
	KRAS or *NRAS* mutant (n = 45)	NR	5.7	0.24	
	Unknown (n = 74)	NR	4.9	0.17	
Lynch syndrome	Yes (n = 31)	NR	7.4	0.28	
	No (n = 152)	NR	6.2	0.25	
	Unknown (n = 66)	NR	5.5	0.13	
Prior surgery related to current cancer	Yes (n = 222)	NR	7.1	0.21	
	No (n = 33)	NR	3.0	0.19	



[a]Per BICR.

Treatment-related adverse events



1L all treated patients	NIVO + IPI (n = 200)		Chemo (n = 88)	
	Any grade	Grade 3/4	Any grade	Grade 3/4
TRAEs,[a] n (%)				
Any TRAEs	160 (80)	46 (23)	83 (94)	42 (48)
Serious TRAEs	38 (19)	32 (16)	17 (19)	14 (16)
TRAEs leading to discontinuation	33 (17)	23 (12)	28 (32)	9 (10)
Treatment-related deaths, n (%)	2 (1)[b]		0 (0)[c]	
IMAEs,[d] n (%)				
Non-endocrine events				
Diarrhea/colitis	13 (7)	9 (5)	1 (1)	0
Hepatitis	11 (6)	6 (3)	0	0
Rash	11 (6)	3 (2)	0	0
Pneumonitis	4 (2)	3 (2)	0	0
Endocrine events				
Hypothyroidism/thyroiditis	34 (17)	3 (2)	1 (1)	0
Adrenal insufficiency	21 (11)	7 (4)	0	0
Hyperthyroidism	18 (9)	0	1 (1)	0
Hypophysitis	10 (5)	5 (3)	0	0

[a]Includes events reported between first dose and 30 days after last dose of study therapy. [b]Includes 1 event each of myocarditis and pneumonitis. [c]One death (acute myocarditis) was related to crossover treatment. [d]Includes events reported within 100 days of last dose of study therapy reported in ≥ 2% of patients.

Summary

- NIVO + IPI demonstrated superior PFS vs chemo ± bevacizumab or cetuximab in previously untreated patients with centrally confirmed MSI-H/dMMR mCRC (HR, 0.21 [97.91% CI, 0.13–0.35]; *P* < 0.0001)
  - 79% reduction in the risk of disease progression or death
  - Early and sustained separation of PFS curves starting at approximately 3 months
  - 24-month PFS rates for NIVO + IPI vs chemo: 72% vs 14%
  - PFS benefit across all prespecified subgroups, including patients with *KRAS* or *NRAS* mutations and those with baseline liver, lung, or peritoneal metastases
- NIVO + IPI had a different safety profile compared with chemo, with fewer grade 3/4 TRAEs, and safety was consistent with the established profiles of each individual drug
  - No new safety signals were identified
- The study is ongoing to assess the other dual primary endpoint of PFS by BICR (NIVO + IPI vs NIVO across all lines), as well as secondary endpoints including OS
- **These results support NIVO + IPI as a standard-of-care 1L treatment option for patients with MSI-H/dMMR mCRC**

My take-aways

- Dual PD-1/CTLA-4 blockade is a proven, effective treatment for patients with dMMR/MSI-H metastatic CRC in front-line setting. Significant benefit was observed regardless of mutations status.

- The toxicity of PD1-/CTLA-4 combination therapy is significant (1% treatment-related death) and should be discussed when considering this option.

- It is too early to know if PD-1/CTLA-4 is superior to PD-1 blockade alone, and we will await further updates from the CHECKMATE 8HW study.

DYNAMIC-RECTAL

ASCO Gastrointestinal Cancers Symposium

AGITG
AUSTRALASIAN GASTRO-INTESTINAL TRIALS GROUP

Circulating Tumor DNA Analysis Informing Adjuvant Chemotherapy in Locally Advanced Rectal Cancer

The Randomized AGITG DYNAMIC-Rectal Study

Jeanne Tie

Peter MacCallum Cancer Centre and Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia

On behalf of the DYNAMIC-RECTAL Investigators

Joshua D Cohen, Yuxuan Wang, Chris Brown, Rachel Wong, Jeremy Shapiro, Rob Campbell, Fiona Day, Theresa Hayes, Morteza Aghmesheh, Christos Karapetis, Maria Popoli, Lisa Dobbyn, Janine Ptak, Natalie Silliman, Christopher Douville, Nickolas Papadopoulos, Kenneth Kinzler, Bert Vogelstein, Peter Gibbs

DYNAMIC-Rectal Study Design

ACTRN12617001560381

Locally Advanced Rectal Cancer

- cT3-4 and/or N+
- Pre-op LCCRT
- R0 TME surgery
- ECOG 0 – 2
- No metastasis on CT CAP at diagnosis
- Provision of adequate tumor tissue within 5 weeks post-op

ctDNA Analysis
Week 4 + 7 post-op

R
2:1

Stratification
ypN stage
Participating site

ctDNA-Informed Management

- **ctDNA-Pos → 4M Adjuvant Chemo** (oxaliplatin-based or single agent FP)
- **ctDNA-Neg + ypN0 → Observation**
- **ctDNA-Neg + ypN+ → Clinician's choice**

ctDNA-Positive = Positive result at week 4 and/or 7

Standard Management

Adjuvant treatment decisions at clinician's discretion

Endpoints

Primary
- Proportion receiving adjuvant chemo

Key Secondary
- 3-year RFS (non-inferiority)

Secondary
- OS
- ctDNA dynamics

- Target sample size 408: 80% power with 95% confidence to demonstrate non-inferiority margin of at most 10%
- Ceased recruitment early (due to COVID-19 and increasing adoption of TNT) → **230 eligible patients analyzed**

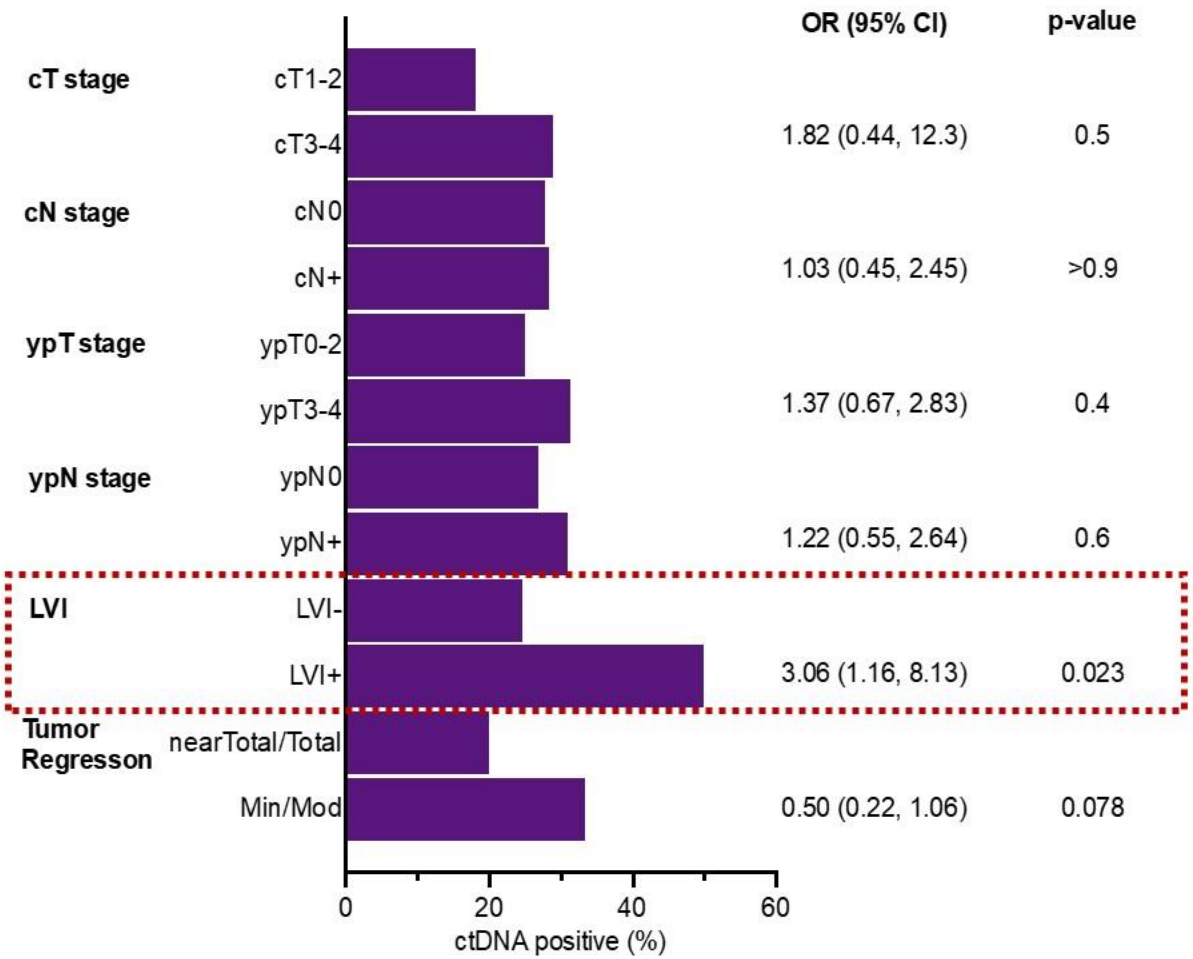
PRESENTED BY: Jeanne Tie, MBChB FRACP MD

Baseline Characteristics

Characteristics	ctDNA-Informed Management N = 155, N (%)	Standard Management N = 75, N (%)
Age, median (range), years	69 (29 - 85)	62 (34 - 84)
Sex, Male	109 (70)	57 (76)
ECOG, 0	96 (62)	50 (67)
Distance from anal verge, < 5 cm	39 (26)	15 (21)
Baseline T stage, cT4	17 (11)	6 (8)
Baseline N stage, cN2	39 (25)	15 (20)
Pathologic T stage, ypT4	2 (1.3)	2 (2.7)
Pathologic N stage, ypN+	45 (29)	23 (31)
Lymphovascular invasion, present	21 (14)	8 (11)
Pathologic complete response	26 (17)	9 (12)
Post-op CEA, > 5 ug/L	4 (2.6)	0 (0)
Surgery to randomization, median (range), days	38 (21 - 63)	39 (24 - 57)

ctDNA Detection in Key Subgroups

- ctDNA analysis completed in 150/155 **(97%)**
- ctDNA-positive in 42/150 **(28%)**

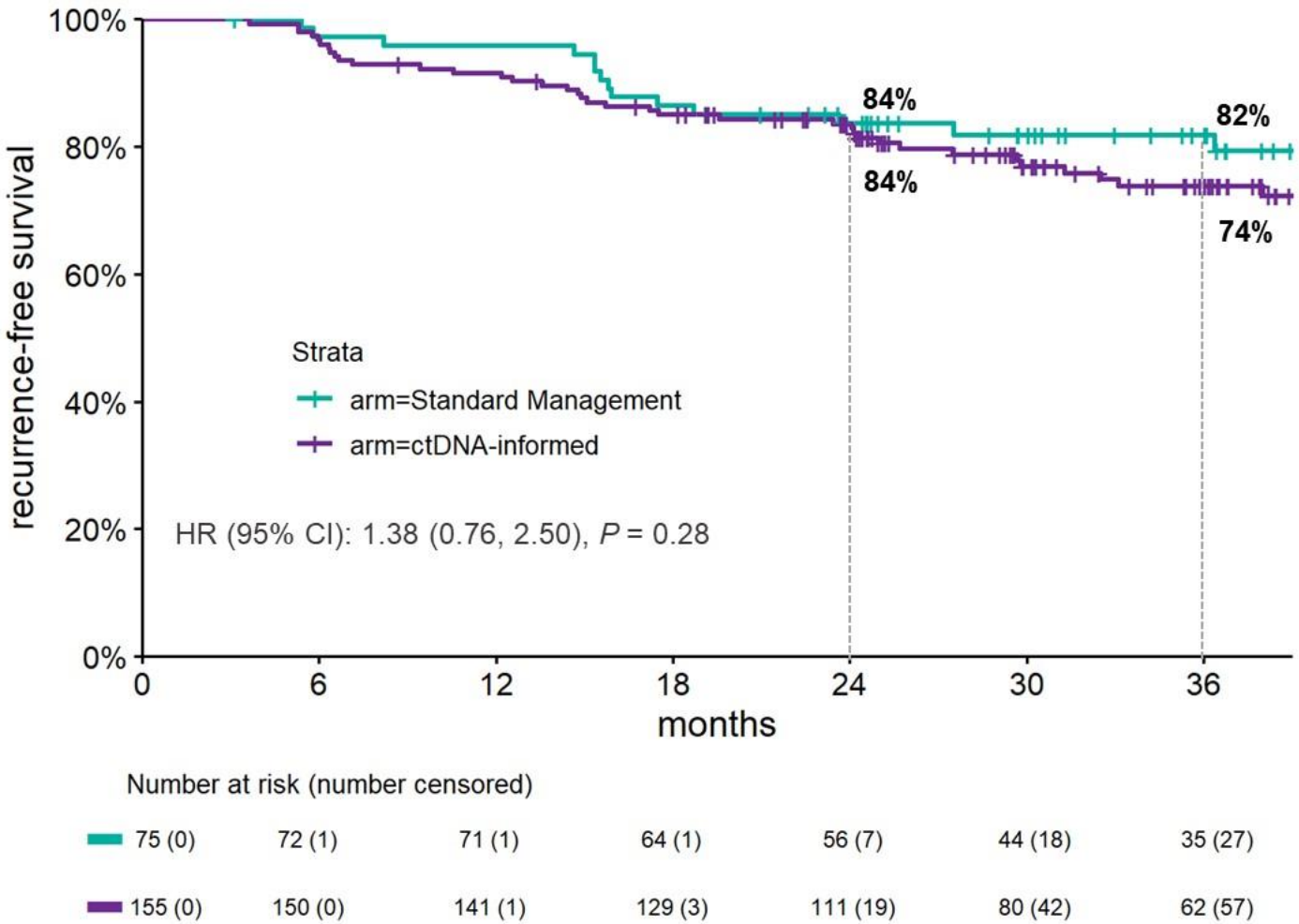


Adjuvant Treatment Delivery

Treatment Information	ctDNA-Informed N = 155	Standard N = 75	*P*
Adjuvant chemo commenced, n	71 **(46%)**	58 **(77%)**	<0.001
ctDNA +ve ctDNA –ve ctDNA unknown	42 (27%) 25 (16%) 4 (3%)		
Chemo regimen, n			
Oxaliplatin-based doublet	43/155 **(28%)**	19/75 **(25%)**	--
Single agent	28/155 **(18%)**	39/75 **(52%)**	
Time to commencing chemotherapy, median (IQR), days	69 (54, 80)	56 (49, 62)	--
Treatment duration, median (IQR), weeks	15 (11, 18)	14 (11, 17)	--
Completed planned treatment, n	57/71 (80%)	41/58 (71%)	--

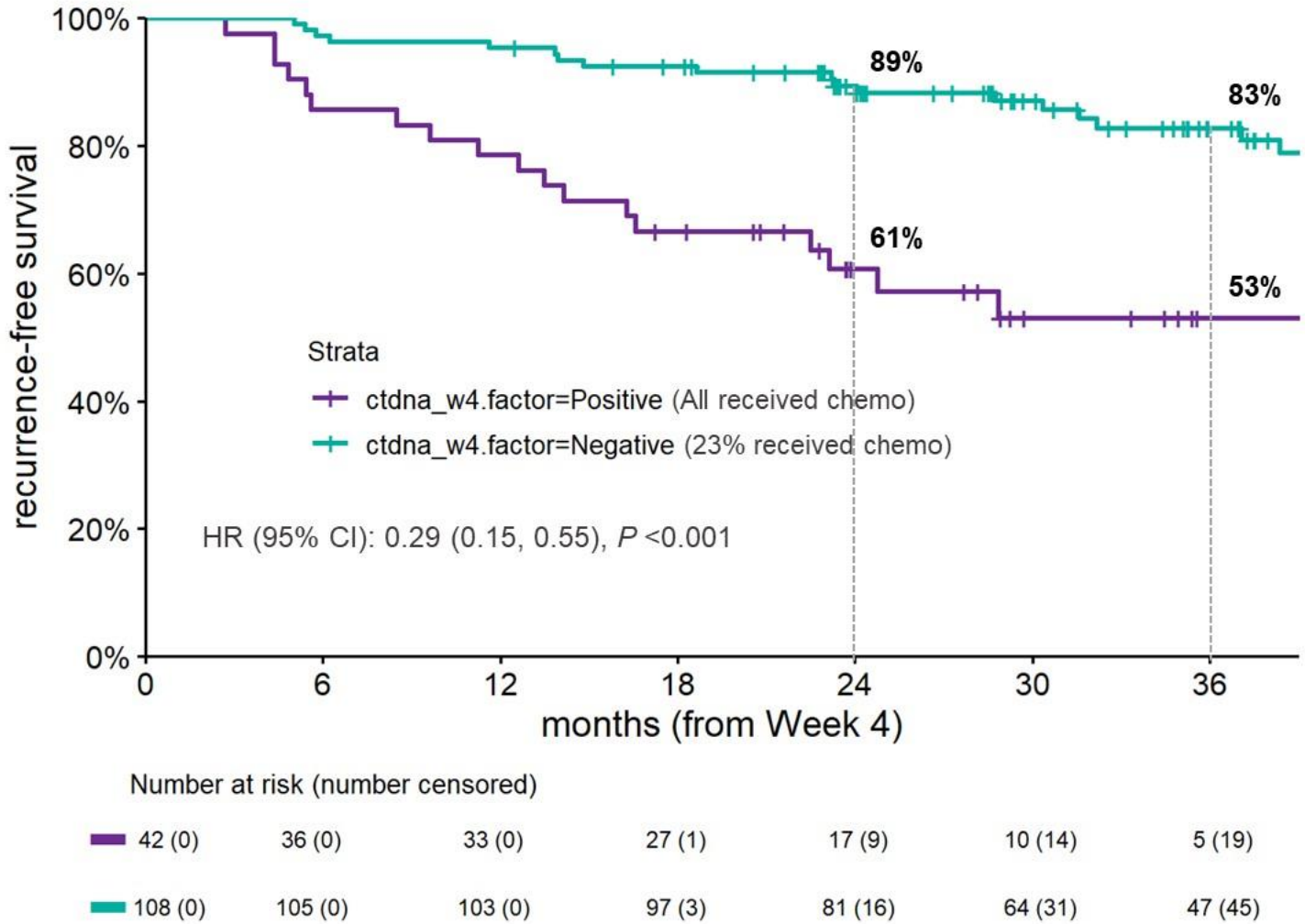
Recurrence-Free Survival

Median follow-up 36 months

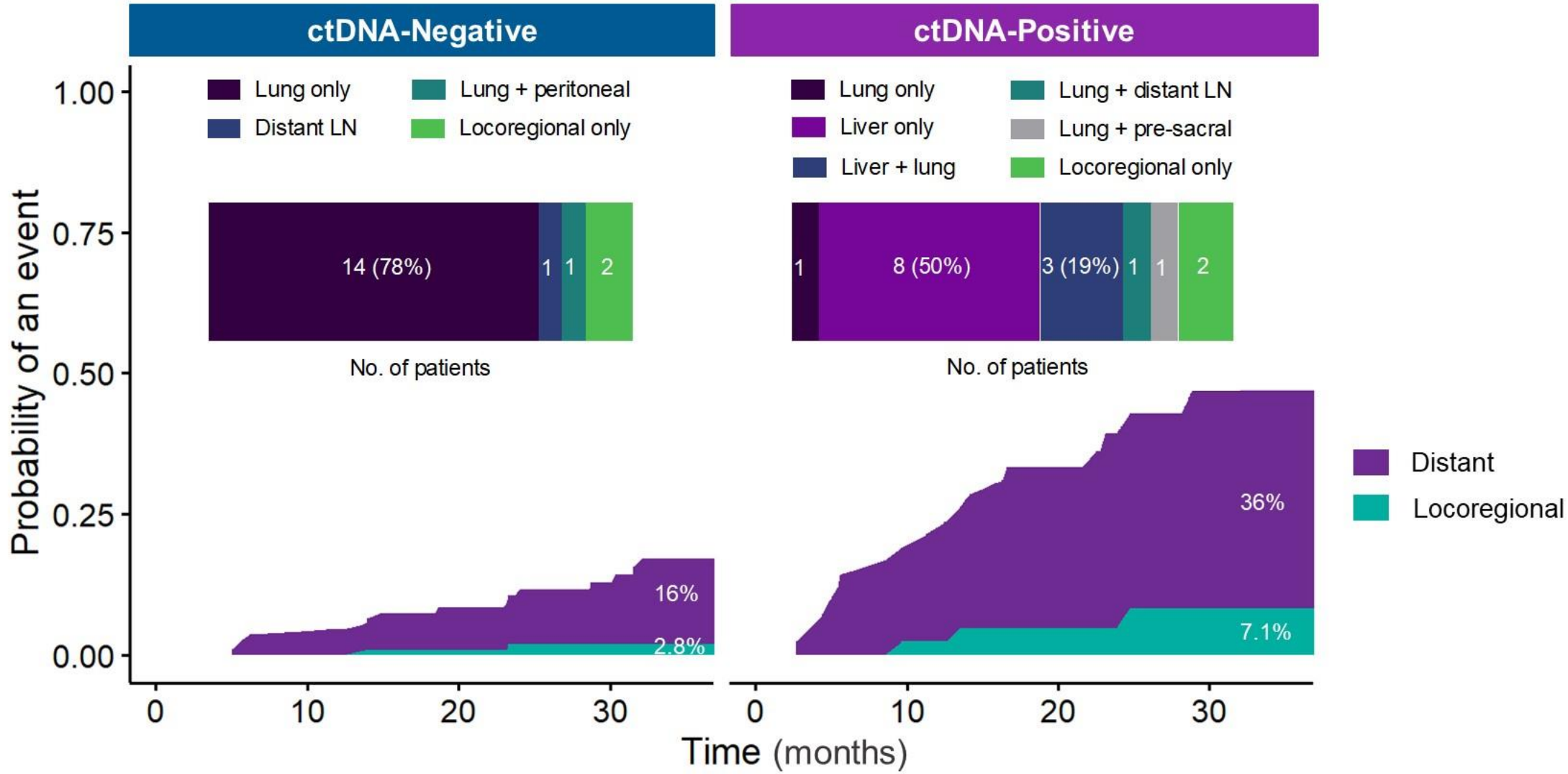


PRESENTED BY: Jeanne Tie, MBChB FRACP MD

Recurrence-Free Survival and ctDNA Status



Sites of Relapse by Post-Op ctDNA Status



DYNAMIC-Rectal Summary

- ctDNA-informed approach to adjuvant therapy for locally advanced rectal cancer following neoadjuvant chemoradiation and surgery was associated with a reduced rate of chemotherapy administration (46% vs 77%, p <0.001)
- A significant proportion of ctDNA-negative patients (23%) received adjuvant chemotherapy
- Small sample size precludes any conclusions to be drawn about the non-inferiority in recurrence-free survival of ctDNA-informed vs standard management
- Risk of recurrence was lower in post-op ctDNA-negative patients compared to ctDNA-positive patients (3-year RFS 83% vs 53%)
- The most common site of relapse in ctDNA-negative patients was the lung (83%) whilst liver (69%) was the dominant site of relapse in ctDNA-positive patients

PRESENTED BY: Jeanne Tie, MBChB FRACP MD

My take-aways

- Trial was done with a non-TNT treatment paradigm; despite "outdated" treatment approach for localized rectal cancer and early closure/low accrual, a "redo" trial evaluating ctDNA as a tool for localized rectal cancer is feasible and critically important.

- We should not assume that ctDNA-informed approach was "inferior" to standard treatment approach based on findings.

- What does a ctDNA(+) result after neoadjuvant therapy mean? We are getting more confident about early identification of liver mets (and less confident re lung mets?).

BESPOKE

ASCO Gastrointestinal Cancers Symposium

Circulating tumor DNA (ctDNA) for informing adjuvant chemotherapy (ACT) in stage II/III colorectal cancer (CRC): Interim analysis of BESPOKE CRC study

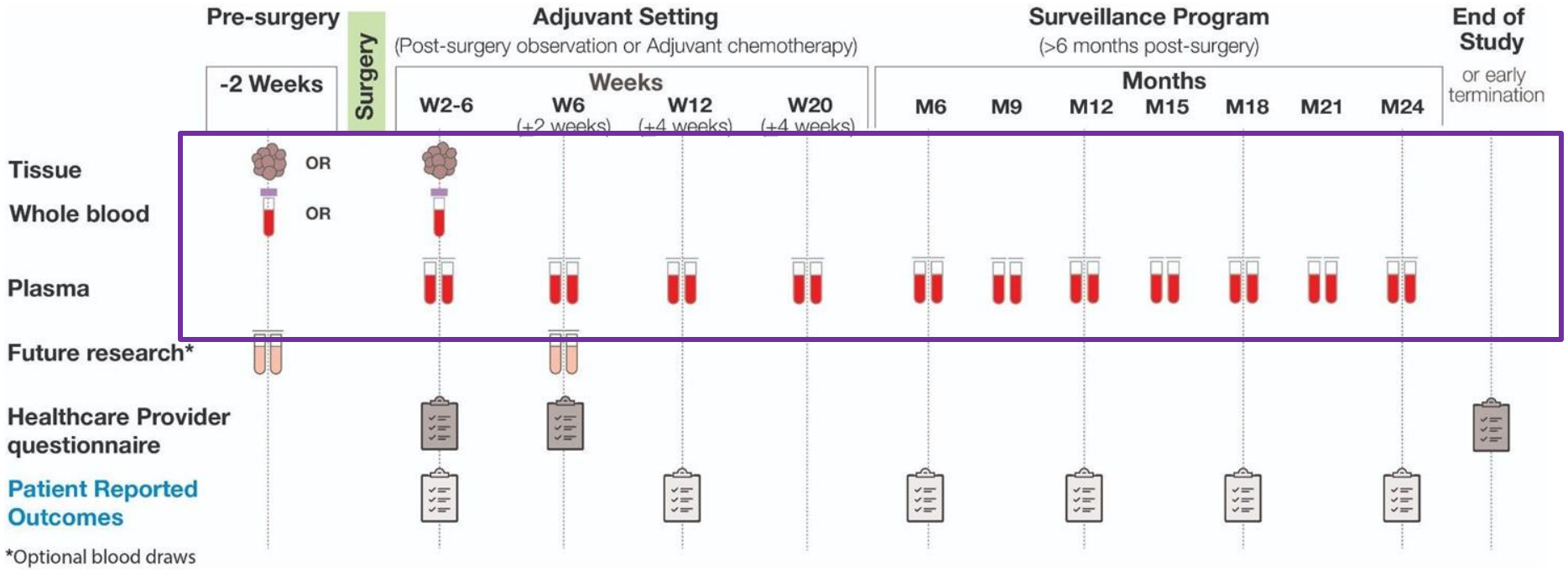
Presenting author: Pashtoon Kasi[1], MD, MS

Co-authors: Vasily N Aushev[2], Joe Ensor[2], Nathan Langer[3], Christopher Wang[4], Timothy Cannon[5], Lyudmyla Berim[6], Trevor Feinstein[7], Axel Grothey[8], Joseph McCollom[9], Sujith Kalmadi1[0], Ahmed Zakari[11], Farshid Dayyani[12], Don Gravenor[13], Janelle Meyer[14], Saima Sharif[15], Adham Jurdi[2], Minetta C Liu[2], Alexey Aleshin[2], Scott Kopetz[16]

[1]Department Weill Cornell Medicine, Englander Institute of Precision Medicine, New York Presbyterian Hospital, New York, NY; [2]Natera, Inc., Austin, TX; [3]Virginia Cancer Institute (QCCA), Richmond, VA; [4]Alabama Oncology, Birmingham, AL; [5]Inova Schar Cancer Institute, Fairfax, VA; [6]Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; [7]Piedmont Cancer Institue, Atlanta, GA; [8]West Cancer Center, Germantown, TN; [9]Parkview Cancer Institute, Fort Wayne, IN; [10]Ironwood Cancer & Research Centers, Chandler, AZ; [11]AdventHealth Cancer Institute, Montverde, FL; [12]Division of Haematology/Oncology, Department of Medicine, University of California Irvine, Orange, CA; [13]Baptist Cancer Center, Memphis, TN; [14]Hematology Oncology of Salem, LLP - Salem Office, Salem, OR; [15]University of Iowa, Iowa City, IA; [16]University of Texas MD Anderson Cancer Center, Houston, TX

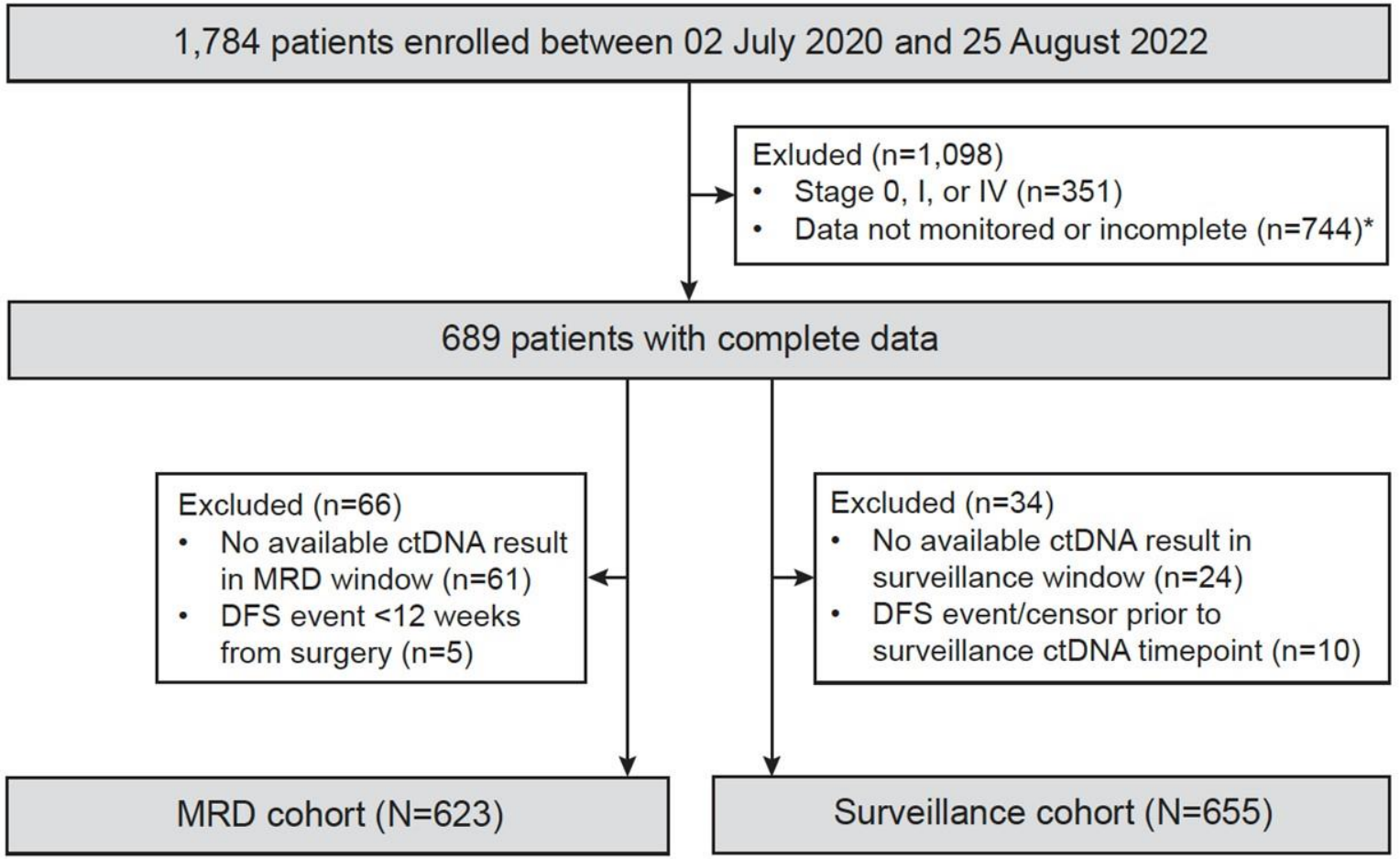
BESPOKE CRC study schema

BESPOKE CRC (NCT04264702) is a multicenter (133 US sites), prospective, observational study evaluating the ability of a tumor-informed, personalized ctDNA assay to inform ACT treatment decisions in patients with stage II/III CRC.[1]



[1]Kasi et al. *BMJ Open* 2021;11:e047831.

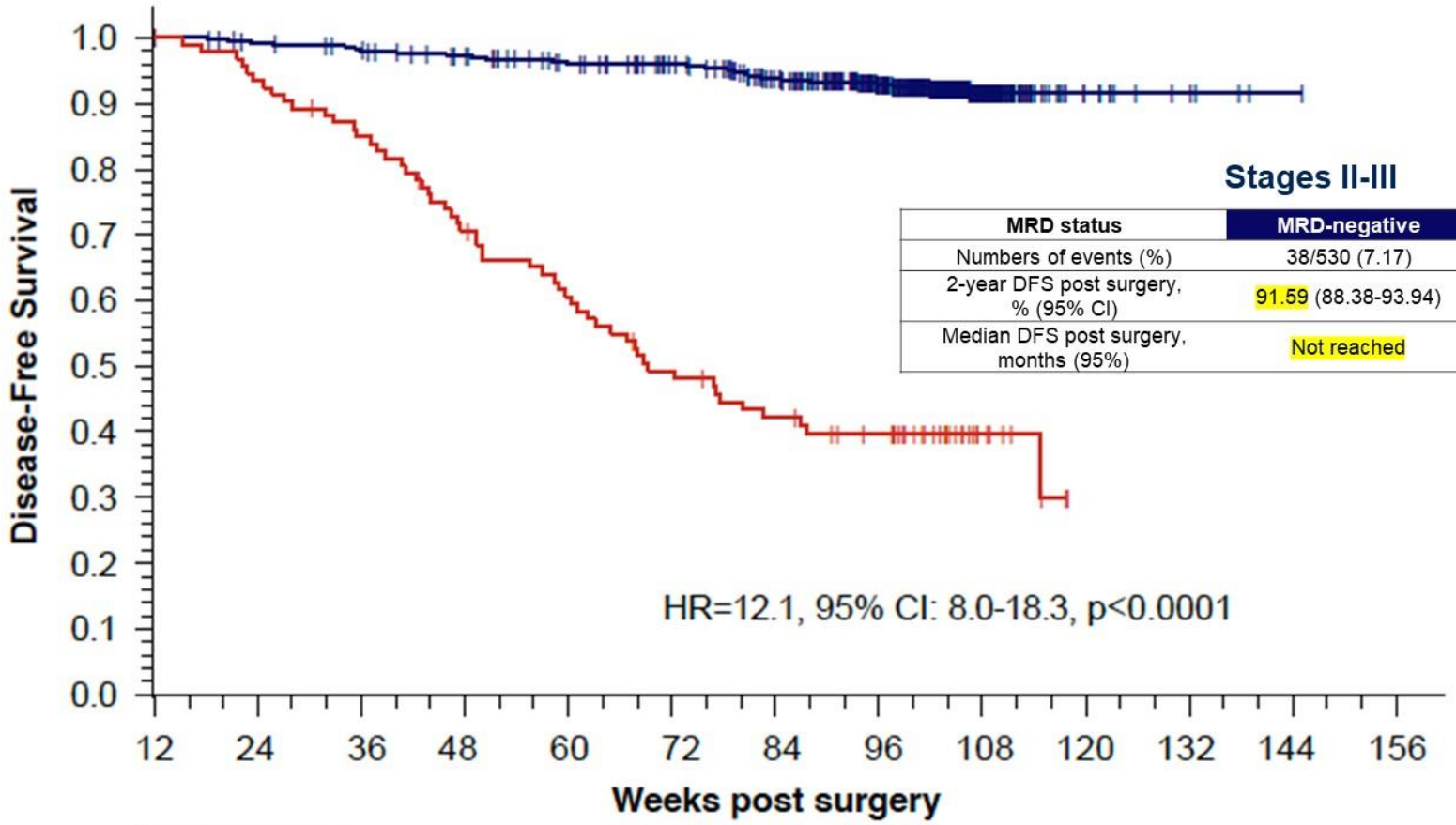
CONSORT diagram



MRD window:
2-12 weeks post-surgery, before the start of adjuvant chemotherapy (ACT)

Surveillance window: >2 weeks post-ACT or >12 weeks post-surgery if on observation

ctDNA-positivity at MRD time point is predictive of inferior DFS



Stages II-III

MRD status	MRD-negative	MRD-positive
Numbers of events (%)	38/530 (7.17)	55/93 (59.14)
2-year DFS post surgery, % (95% CI)	91.59 (88.38-93.94)	29.86 (13.26-48.54)
Median DFS post surgery, months (95%)	Not reached	15.98 (13.77-20.22)

Number at Risk

MRD-negative	530	522	513	499	482	464	429	353	101	12	5	1	0
MRD-positive	93	87	78	64	54	43	36	30	8	0			

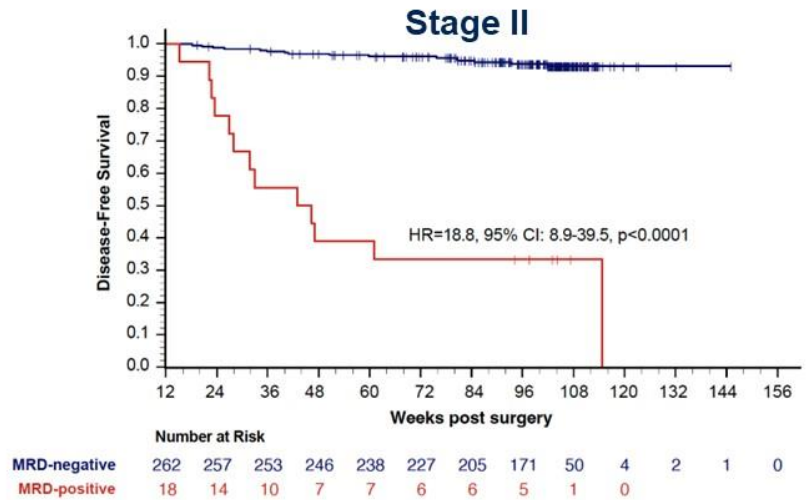
PRESENTED BY: Pashtoon Kasi, MD, MS

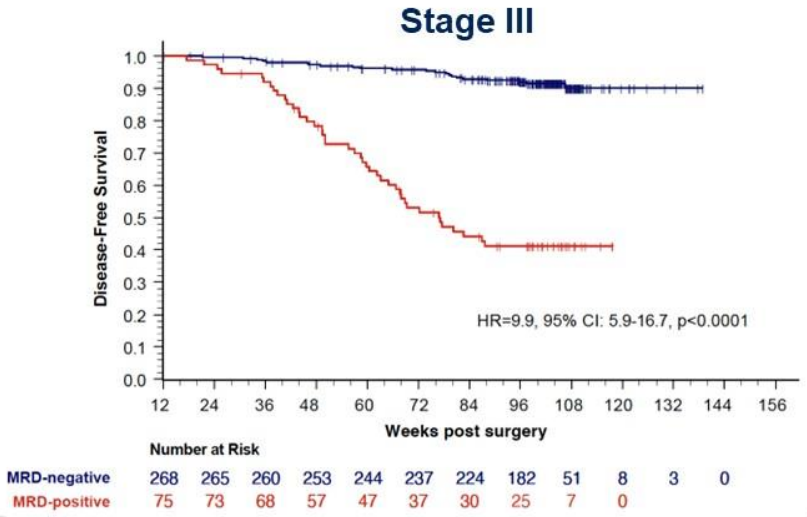
ctDNA-positivity at MRD time point is predictive of inferior DFS

MRD-positivity rate by stage II-III

Stage	Total, N	MRD-negative, n (%)	MRD-positive, n (%)	95% CI for positivity rate
II	280	262 (93.57)	**18 (6.43)**	4.10-9.93
III	343	268 (78.13)	**75 (21.87)**	17.82-26.54
Total	623	530	93	

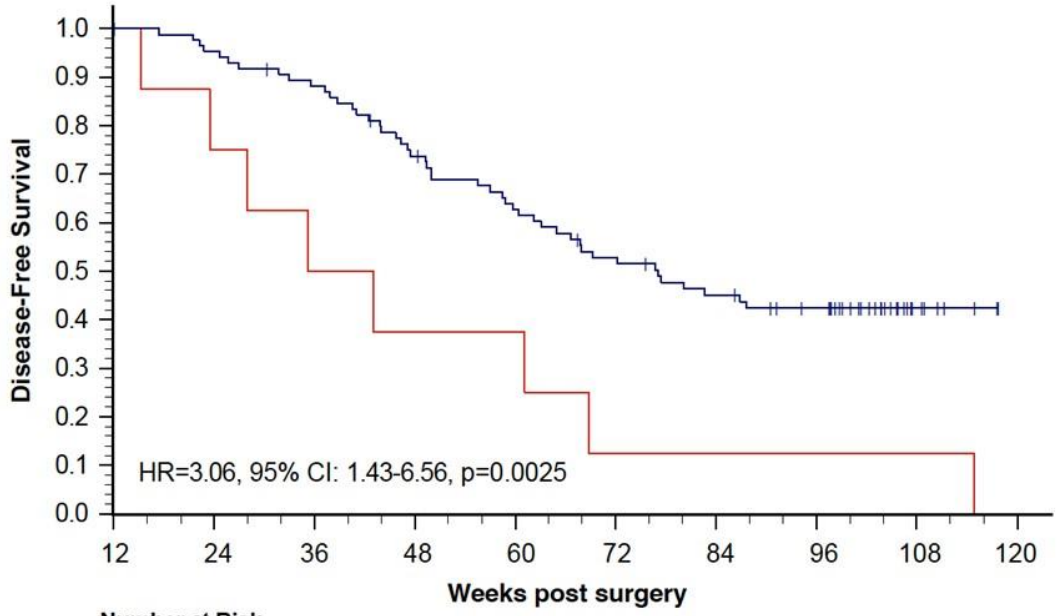
Benchmark for proportion (%) of patients who are MRD-positive with stage II and III colorectal cancer.





Benefit from ACT observed in MRD-positive but not MRD-negative patients

MRD-positive patients

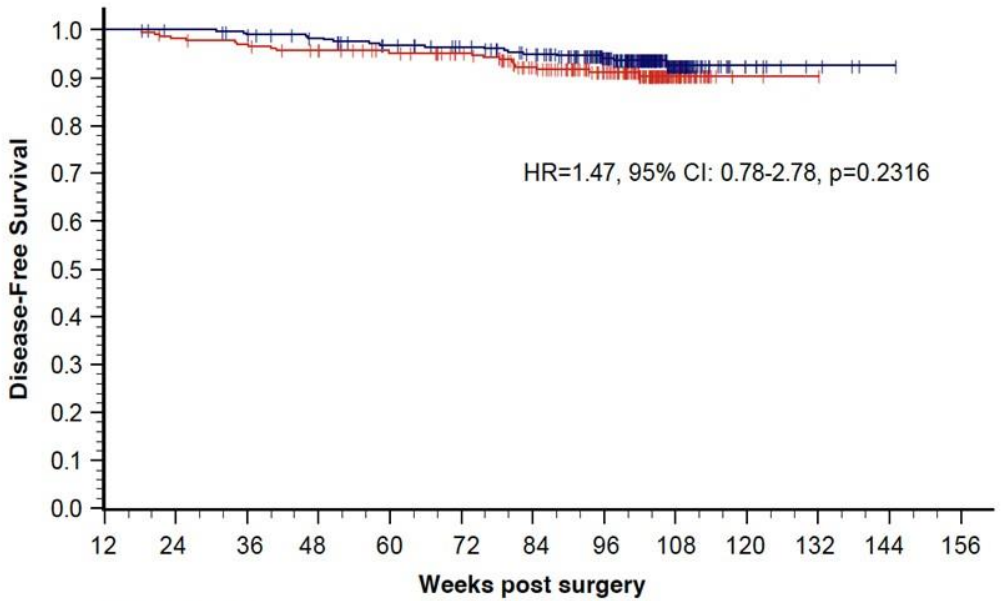


Number at Risk

ACT	85	80	72	56	48	39	32	18	2	0
Observation	8	6	4	3	2	1	1	1	0	

Adjuvant strategy	ACT	Observation
Numbers of events (%)	47/85 (55.29)	8/8 (100)
2-year DFS post surgery, % (95% CI)	42.44 (31.55-52.91)	12.50 (0.66-42.27)
Median DFS post surgery, months (95%)	17.78 (14.37-not reached)	7.52 (3.52-15.88)

MRD-negative patients



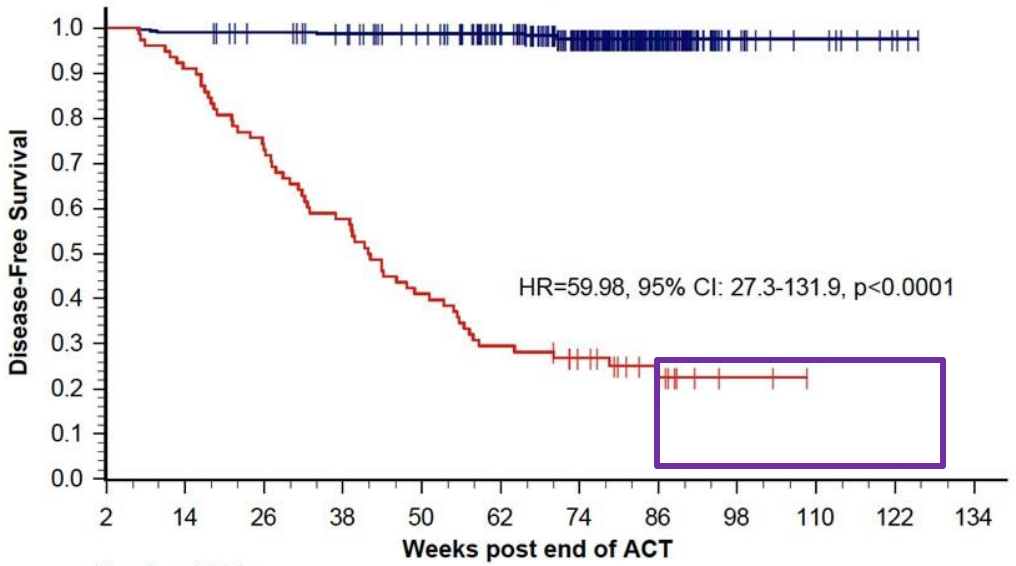
Number at Risk

ACT	296	293	288	281	271	263	251	207	61	10	4	1	0
Observation	234	229	225	218	211	201	178	146	40	2	1	0	

Adjuvant strategy	ACT	Observation
Numbers of events (%)	18/296 (6.08)	20/234 (8.55)
2-year DFS post surgery, % (95% CI)	93.70 (90.03-96.05)	90.39 (85.38-93.75)
Median DFS post surgery, months (95%)	Not reached	Not reached

ctDNA-positivity during surveillance is predictive of inferior DFS regardless of adjuvant therapy (ACT or observation)

Patients receiving ACT

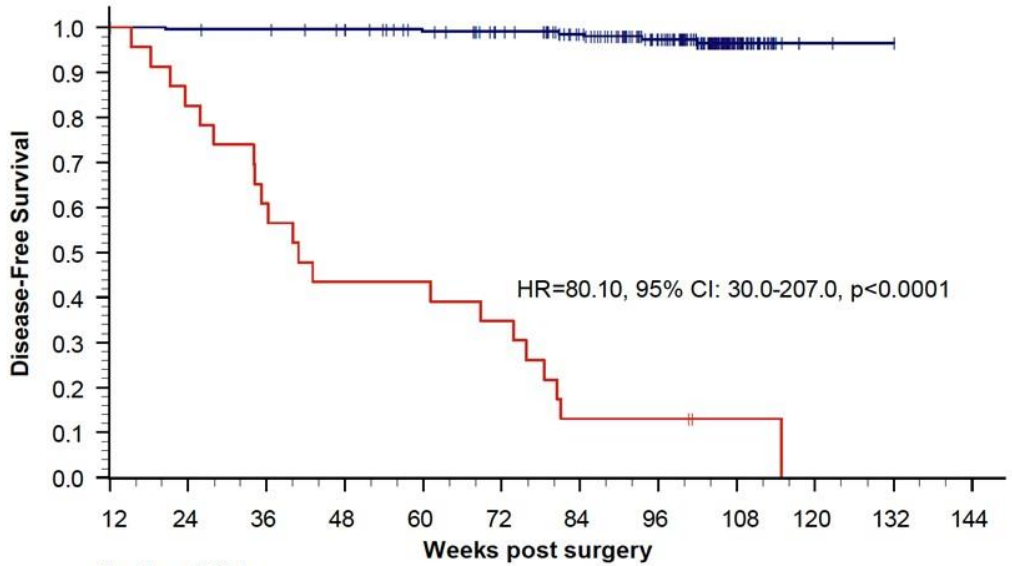


Number at Risk

ctDNA-negative	329	326	321	315	305	278	213	87	16	9	3	0
ctDNA-positive	78	71	57	45	32	23	17	8	2	0		

ctDNA status (surveillance)	ctDNA-negative	ctDNA-positive
Numbers of events (%)	7/329 (2.13)	59/78 (75.64)
2-year DFS post end of ACT, % (95% CI)	97.58 (94.96-98.84)	22.56 (13.49-33.08)
Median DFS post end of ACT, months (95%)	Not reached	9.70 (7.43-12.32)

Patients on observation



Number at Risk

ctDNA-negative	225	224	223	219	211	201	183	151	42	2	1	0
ctDNA-positive	23	19	14	10	10	8	3	3	1	0		

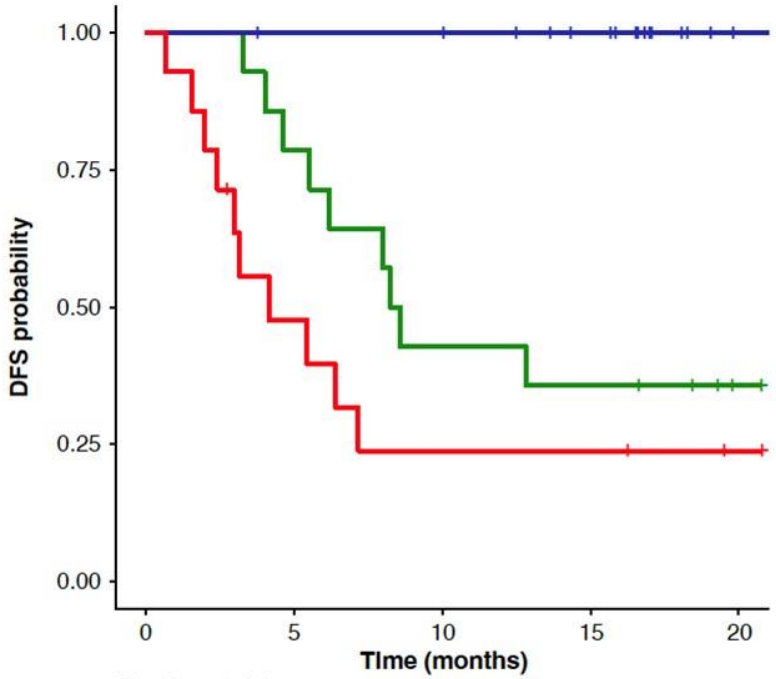
ctDNA status (surveillance)	ctDNA-negative	ctDNA-positive
Numbers of events (%)	6/225 (2.67)	21/23 (91.30)
2-year DFS post surgery, % (95% CI)	96.60 (92.44-98.49)	13.04* (3.27-29.72)
Median DFS post surgery, months (95%)	Not reached	9.44 (7.86-17.03)

*Most recurrences occurred within the1st year.

PRESENTED BY: Pashtoon Kasi, MD

Sustained ctDNA clearance is associated with superior DFS when compared to transient or no clearance

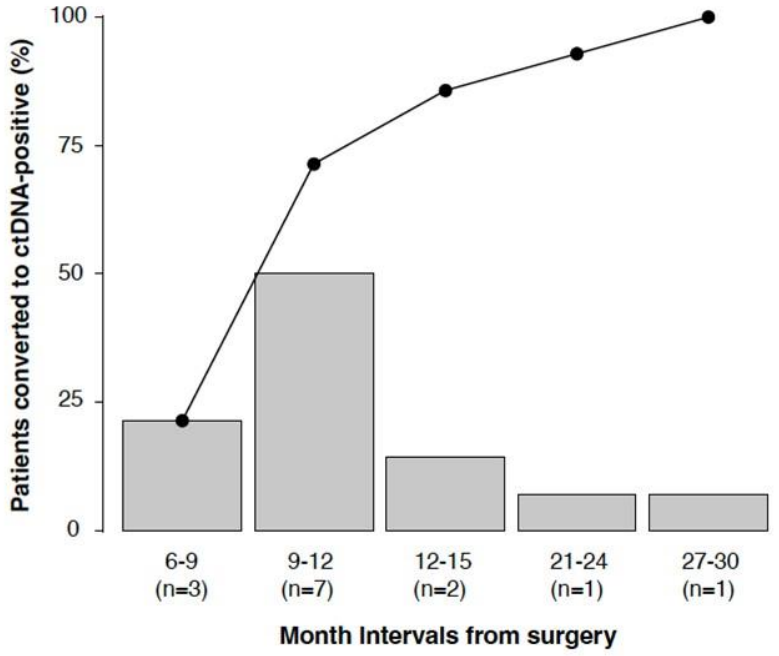
DFS by ctDNA clearance patterns



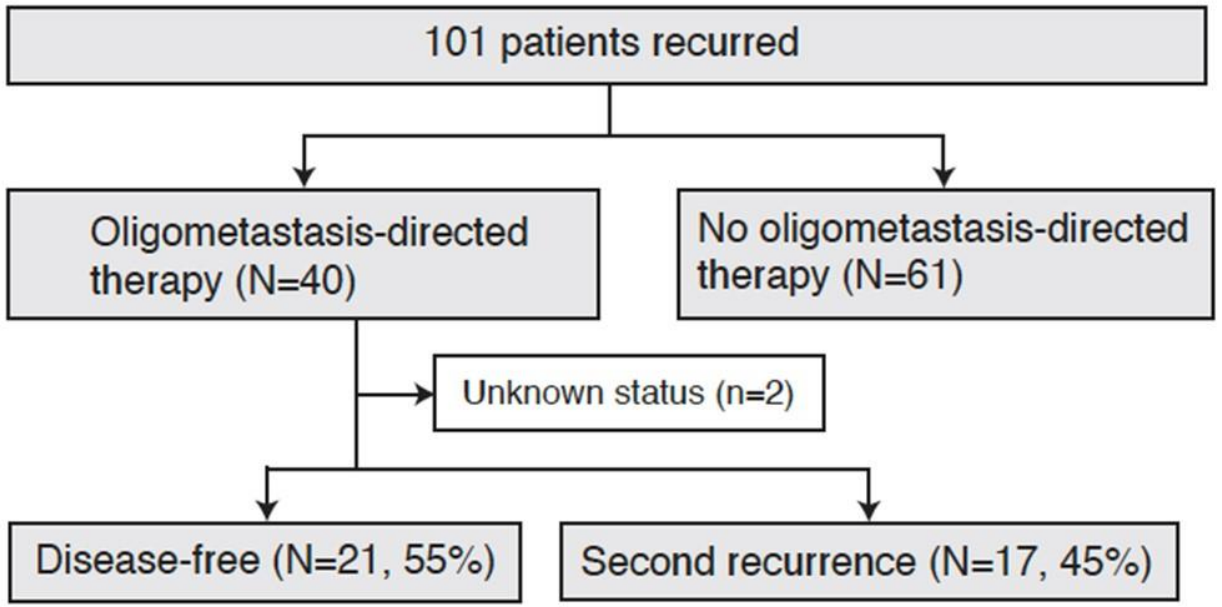
Number at risk

Sustained clearance	17	16	16	12	1
Transient clearance	14	11	6	5	1
No clearance	14	6	3	3	1

85% of patients with transient clearance develop molecular recurrence by the 15th month



ctDNA testing was associated with higher oligometastasis-directed therapy

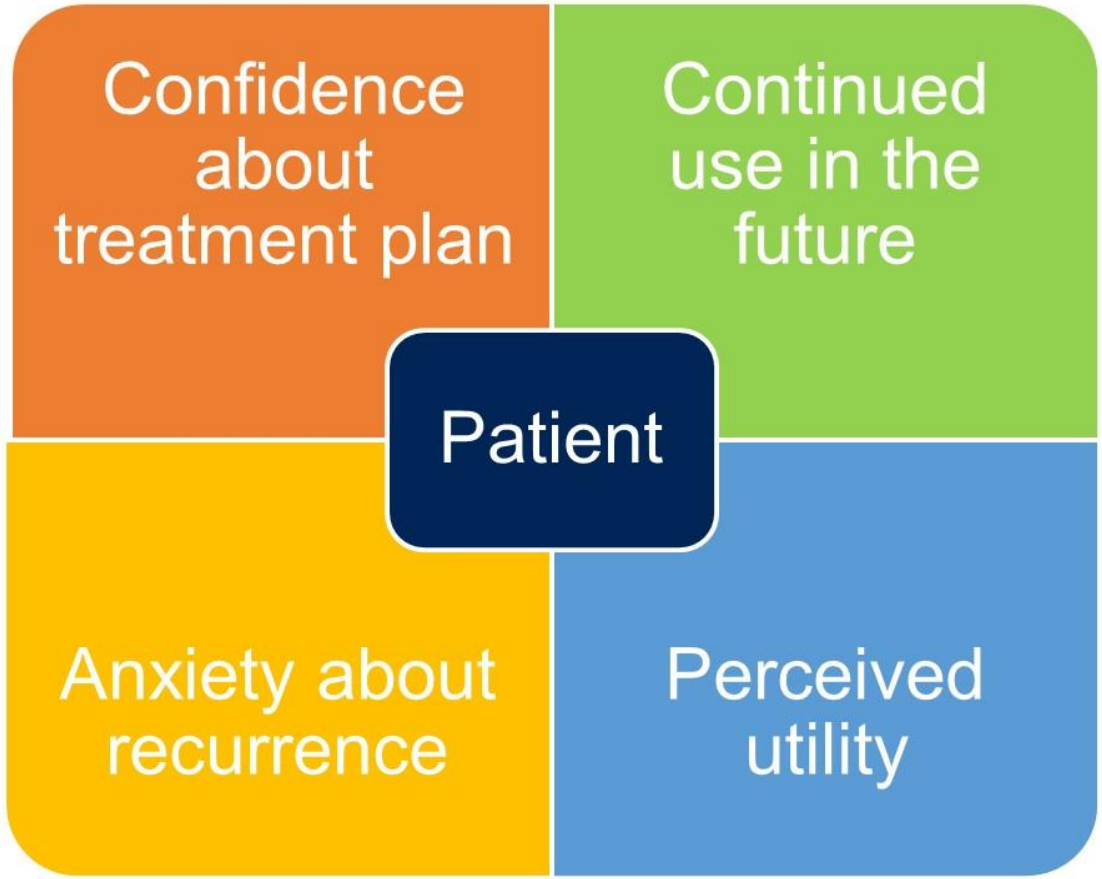


Oligometastasis-directed therapy type	N
Surgery	30
Radiofrequency Ablation (RFA)	3
Microwave Ablation (MWA)	2
Stereotactic Body Radiation Therapy (SBRT)	2
Y90 radiotherapy	2
Chemoradiation	1

Conclusions

- BESPOKE CRC is the first large, prospective, US-based trial (**133 sites – 1792 patients**) to report on the utility of tumor-informed ctDNA in patients with CRC after surgery.
- ctDNA detection of MRD is a powerful **prognostic** and **predictive** tool in patients with stage II and III CRC.
- ctDNA monitoring allowed for **oligometastasis-directed therapy** in 40% of patients who recurred.
- ctDNA status during **surveillance** was prognostic of outcomes regardless of whether the patients received ACT (DFS ~ 9 months).
- The patients' **perceived utility** of ctDNA testing and dimensions of **well-being** point towards general acceptance of ctDNA testing in patients with CRC.

ctDNA questionnaire: Perceived utility of ctDNA testing



73% reported ctDNA results reduced anxiety about cancer recurrence

87% felt they were receiving the right treatment after receiving their results

92% would continue using the ctDNA test to monitor cancer

96% valued the additional information received from ctDNA results

PRESENTED BY: Pashtoon Kasi, MD, MS

Conclusions

- BESPOKE CRC is the first large, prospective, US-based trial (**133 sites – 1792 patients**) to report on the patients' **perceived utility of ctDNA testing** and dimensions of **well-being**.
- Patients with positive ctDNA test results felt slightly more **anxious about CRC recurrence** (FCR4 and ctDNA questionnaire), had worse disease/treatment-related **symptoms** (FCSI-19), but had **similar anxiety and depression scores** (HADS) when compared with ctDNA-negative patients.
- Most responders valued the information they received through the results of their personalized, tumor-informed ctDNA tests and would **continue ctDNA testing** irrespective of positive or negative results.

My take-aways

- In a large, observational study of ~700 patients with stages II or III CC, ctDNA(+) status is prognostic for recurrence.
- A ctDNA(+) status may be able to help identify earlier patients with new, oligometastatic recurrence amenable to local therapies; no association with survival has been proven (yet).
- Among patients who participated in this study, testing for ctDNA was welcomed by the patient and associated with less anxiety .

GALAXY

ASCO Gastrointestinal Cancers Symposium

Circulating tumor DNA (ctDNA) dynamics in colorectal cancer (CRC) patients with molecular residual disease: Updated analysis from GALAXY study in the CIRCULATE-JAPAN

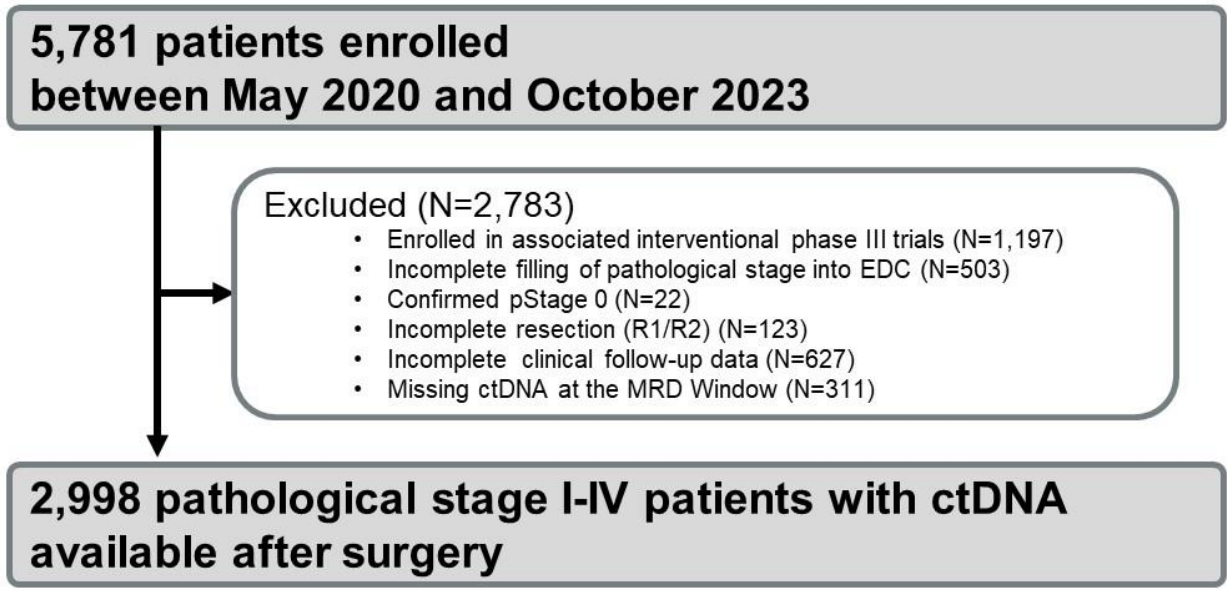
Presenting Author: Hiroki Yukami, MD, PhD

Co-authors: Yoshiaki Nakamura, Saori Mishima, Koji Ando, Hideaki Bando, Jun Watanabe, Keiji Hirata, Naoya Akazawa, Masataka Ikeda, Mitsuru Yokota, Kentaro Kato, George Laliotis, Vasily N. Aushev, Adham A. Jurdi, Minetta C. Liu, Daisuke Kotani, Eiji Oki, Ichiro Takemasa, Takeshi Kato, Takayuki Yoshino

Cancer Chemotherapy Center, Osaka Medical and Pharmaceutical University, Takatsuki, Japan; Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Japan; Department of Colorectal Surgery, National Cancer Center Hospital East, Chiba, Japan; Department of Surgery, Gastroenterological Center, Yokohama City University Medical Center, Kanagawa, Japan; The Committee of Hereditary Colorectal Cancer of the Japanese Society for Cancer of the Colon and Rectum, Tokyo, Japan; Department of Gastroenterological Surgery, Sendai City Medical Center Sendai Open Hospital, Sendai, Japan; Division of lower GI surgery, Department of Gastroenterological Surgery, Hyogo Medical University, Nishinomiya, Japan; Department of General Surgery, Kurashiki Central Hospital, Okayama, Japan; Department of Surgery, Teine-Keijinkai Hospital, Sapporo, Japan; Natera, Inc., Austin, TX; National Cancer Center Hospital East, Kashiwa, Japan; Kyushu University, Fukuoka, Japan; Department of Surgery, Surgical Oncology and Science, Sapporo Medical University, Sapporo, Japan; National Hospital Organization, Osaka National Hospital, Osaka, Japan; Division of Drug and Diagnostic Development Promotion, Department for the Promotion of Drug and Diagnostic Development, National Cancer Center Hospital East, Kashiwa, Japan

CONSORT diagram and patient characteristics

- Postoperative ctDNA-based molecular residual disease (MRD) is reported to be associated with a high risk of recurrence[1].
- Here, **we present an updated 24-month disease free survival (DFS)** analysis in stage I–IV patients with radically resected CRC participating in the prospective, observational GALAXY study (UMIN000039205).
- A personalized, tumor-informed assay (Signatera™, Natera, Inc.) was used for the detection and quantification of ctDNA in serial plasma samples collected at 1, 3, 6, 9, 12, 18, and 24 months after surgery until recurrence.
- We investigated the association between ctDNA status and recurrence.



Median Follow-up: 16.14 months (range: 0.23-42.14)

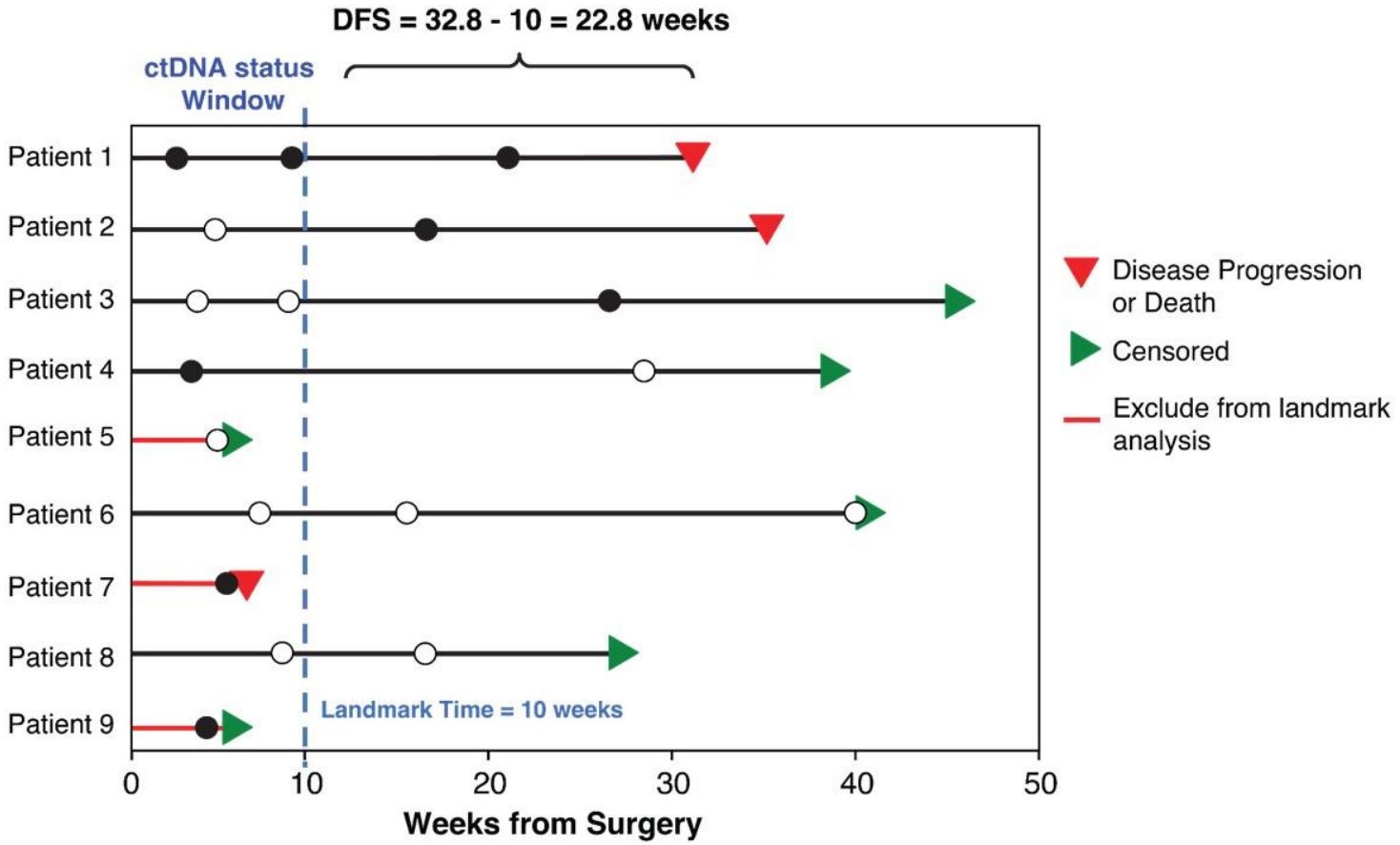
Characteristic	N = 2,998[1]	Characteristic	N = 2,998[1]
Age	69 (23 - 95)	**Neoadjuvant Treatment**	
Gender		Neoadjuvant Chemotherapy	315 (11%)
Male	1,622 (54%)	Upfront Surgery	2,683 (89%)
Female	1,376 (46%)	**Adjuvant Treatment**	
Performance Status		Adjuvant Chemotherapy	1,130 (38%)
0	2,700 (90%)	Observation	1,868 (62%)
1	298 (10%)	**Adjuvant Treatment Duration**	
Tumor Location		3 months	361 (32%)
Right-sided colon	938 (33%)	6 months	458 (40%)
Left-sided colon	1,376 (48%)	<3 or >6 months	311 (28%)
Rectum	553 (19%)	***BRAF* status**	
Unknown	131	*BRAF*^{wt}	2,638 (93%)
Pathological T Stage		*BRAF*^{V600E}	205 (7%)
T1-T2	592 (20%)	Unknown	155
T3-T4	2,351 (80%)	***RAS* status**	
Unknown	55	*RAS*^{wt}	1,622 (57%)
Pathological N Stage		*RAS*^{mut}	1,231 (43%)
N0	1,449 (49%)	Unknown	145
N1-N2	1,493 (51%)	**MSI status**	
Unknown	56	MSS or MSI-Low	2,686 (91%)
Pathological Stage		MSI-High	280 (9%)
I	415 (14%)	Unknown	32
II	901 (30%)	**Clinical or Radiological Recurrence**	
III	1,231 (41%)	Recurrence	530 (18%)
IV	451 (15%)	No Recurrence	2,468 (82%)
		Total Follow-up (months)	16.1 (0.2 - 42)

[1]Median (Range); n (%)

1)Daisuke K, et al. Nat Med 2023.

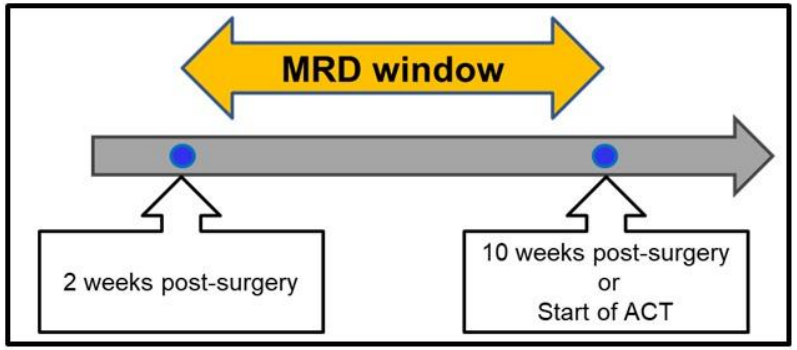
PRESENTED BY: Hiroki Yukami, MD, PhD

Landmark analysis for DFS



Through landmark analysis, we mitigate immortal time bias by partitioning follow-up time at a predefined point, enabling unbiased estimation of hazard ratios and hypothesis testing.

DFS according to status in the MRD window in all stage

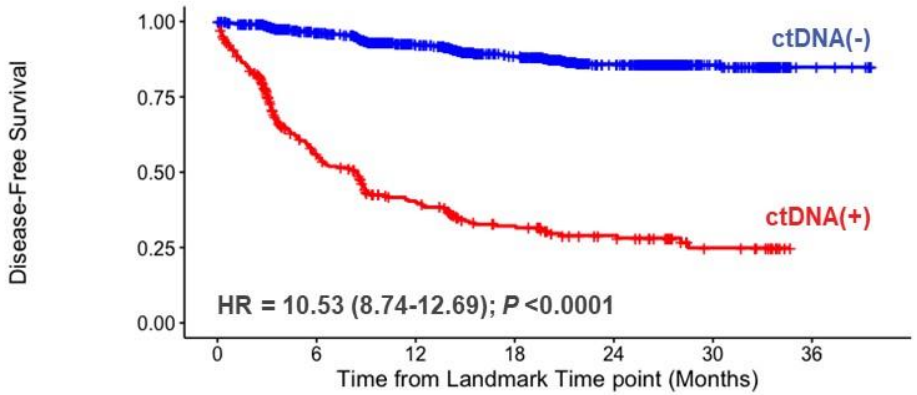


ACT: adjuvant chemotherapy

2,998 stage I-IV patients included in the outcome cohort

Excluded (N=138)
- DFS event prior to the 10 weeks landmark timepoint (n=138)

MRD Window analysis cohort (n=2,860)



Number at risk

ctDNA Negative	2491	2031	1441	1041	495	135	8
ctDNA Positive	369	165	98	59	35	13	0

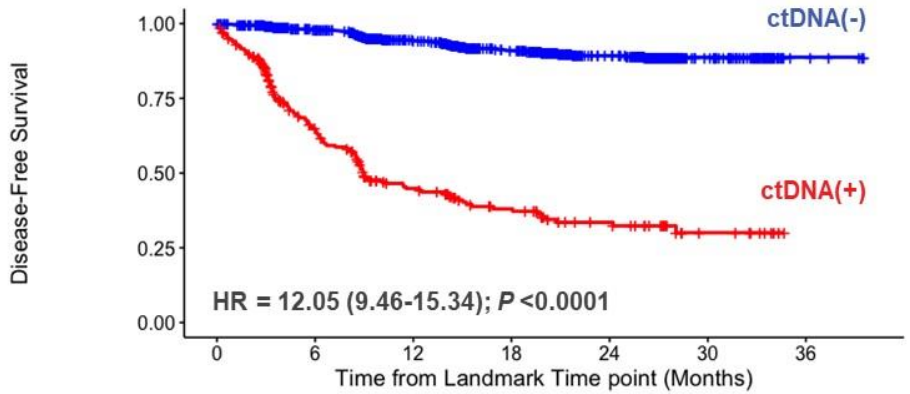
ctDNA status	Negative	Positive
Events %	9.4 (235/2491)	58.8 (217/369)
24M-DFS % (95% CI)*	85.9 (83.9–87.7)	28.9 (23.4–34.8)

**DFS % from landmark time point*

MRD window: 2-10 weeks post surgery, prior to start of any adjuvant therapy - Landmark 10 weeks post-surgery

ctDNA-positive in the MRD window is predictive inferior DFS

DFS according to status in the MRD window in pStage II/III

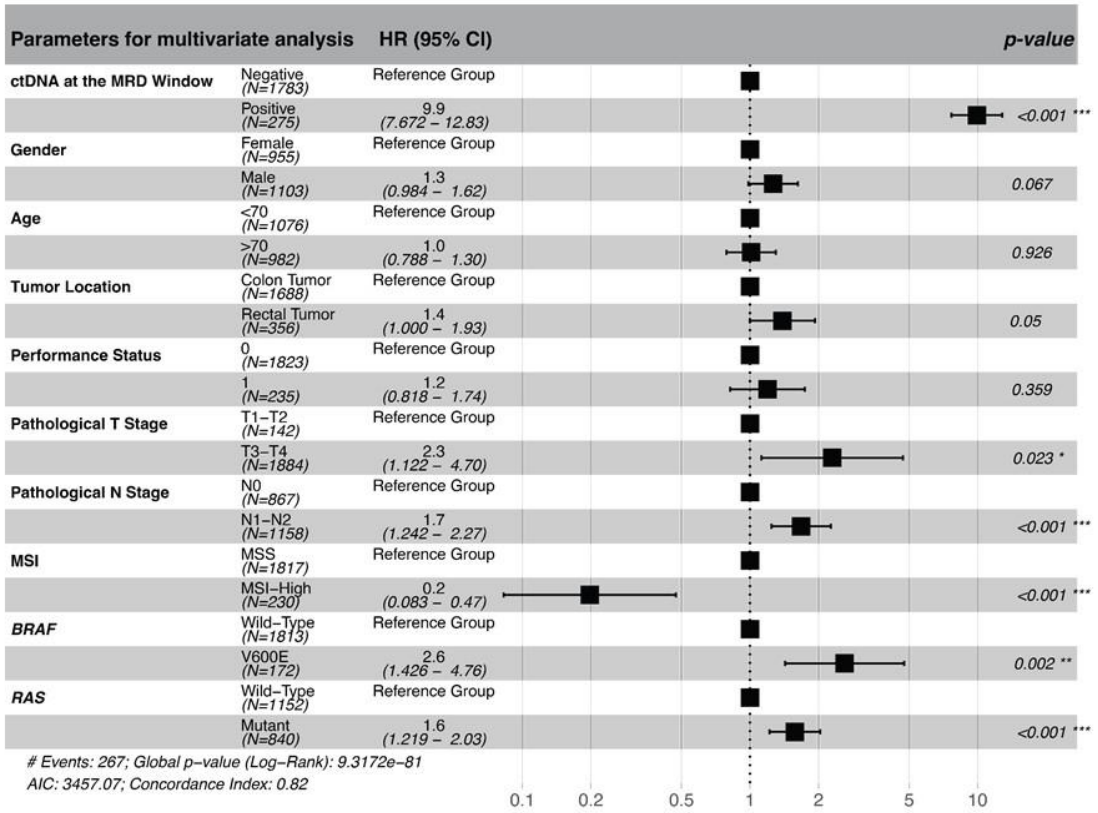


Dynamics	ctDNA Negative	ctDNA Positive
Events %	7.8 (126/1783)	56.5 (143/275)
24M-DFS % (95% CI)*	89.3 (87.2–91.1)	33.5 (26.5–40.7)

**DFS % from landmark time point*

MRD window: 2-10 weeks post surgery, prior to start of any adjuvant therapy - Landmark 10 weeks post-surgery

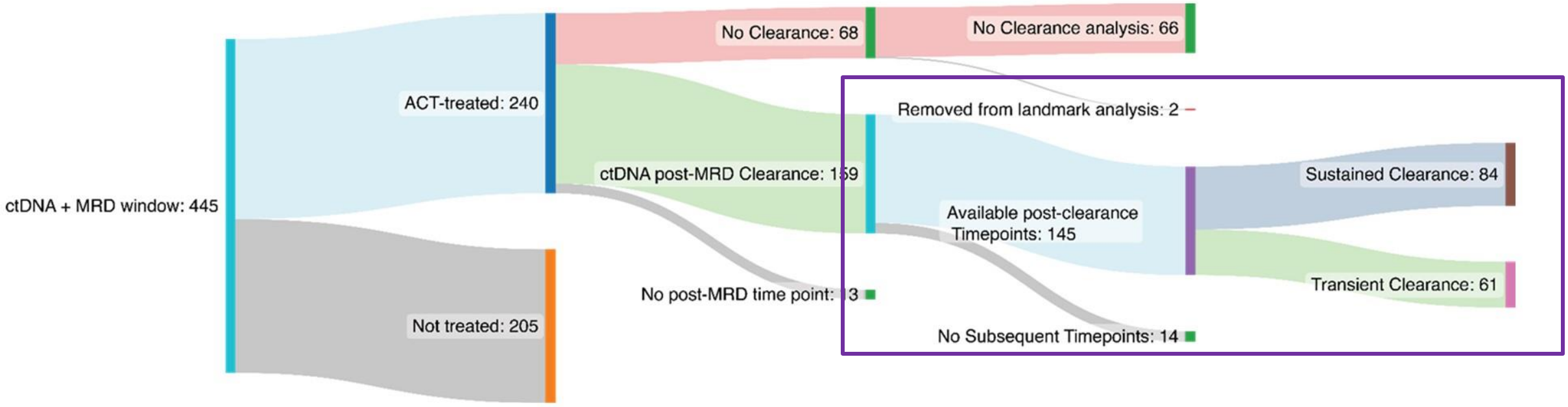
Multivariate Regression Model for DFS



ctDNA-positive in the MRD window is predictive of inferior DFS (pStage II/III)

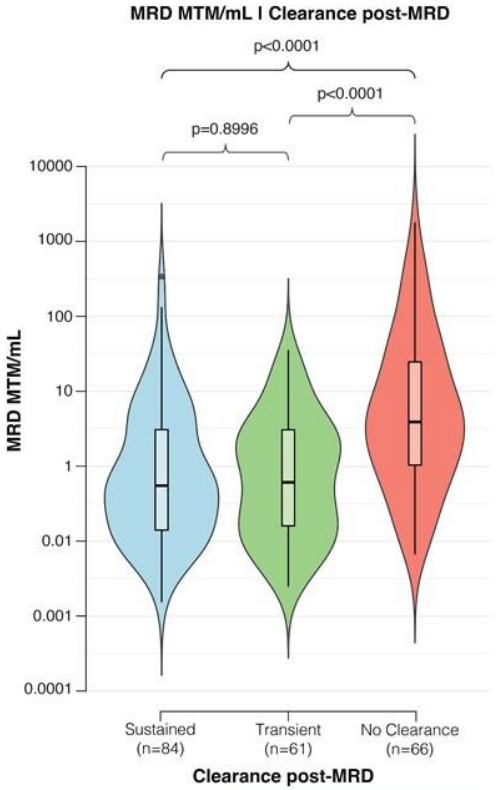
Sankey diagram of post MRD ctDNA clearance in the ACT treated cohort

Landmark 10 weeks post surgery



PRESENTED BY: Hiroki Yukami, MD, PhD

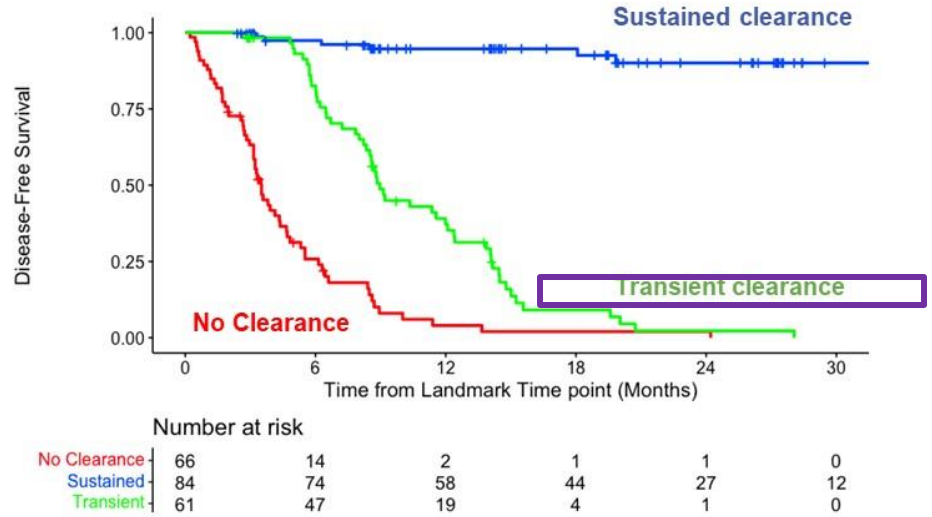
DFS according to ctDNA clearance in Patients with ctDNA positive in the MRD window



Group	Median MRD MTM/mL
Sustained	0.61
Transient	0.53
No Clearance	3.89

**P values from Wilcoxon rank-sum test*

Landmark 10 weeks post surgery



ctDNA Clearance	Sustained Clearance	Transient Clearance	No Clearance
Events %	7.1 (6/84)	85.2 (52/61)	89.4 (59/66)
Median DFS months (95% CI)	NR	9 (8.5–12.4)	3.5 (3.2–4.7)
24M-DFS % (95% CI)*	90.1 (78.6–95.6)	2.3 (0.02–10.3)	2 (0.02–9.2)
HR	**Reference**	**25.13**	**87.08**
95% CI	Not applicable	10.57–59.73	36.14–209.84
P	Not applicable	<0.0001	<0.0001

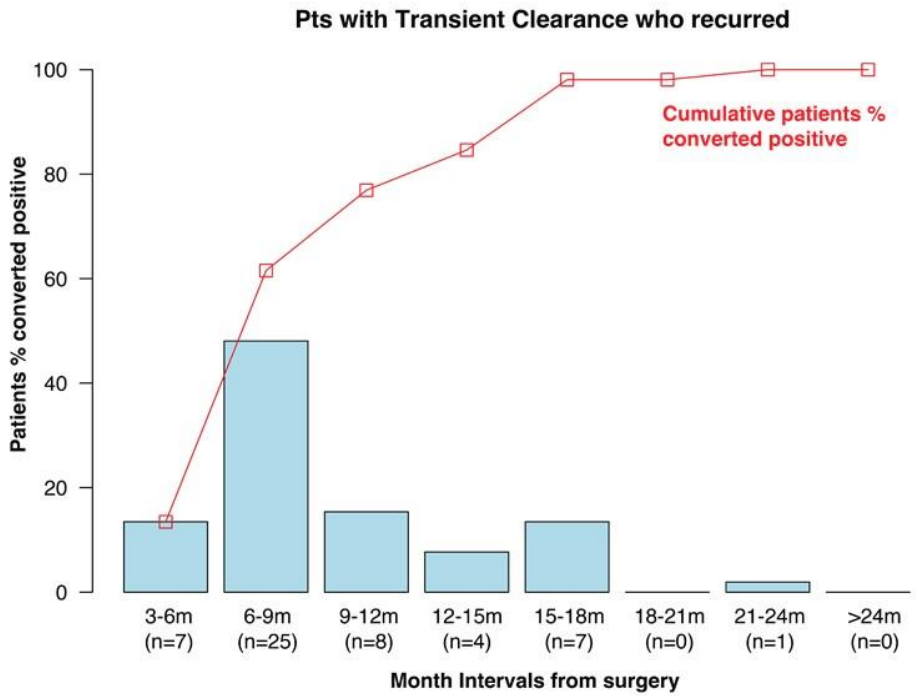
**DFS % from landmark time point*

Sustained clearance indicates superior DFS compared to Transient or No clearance

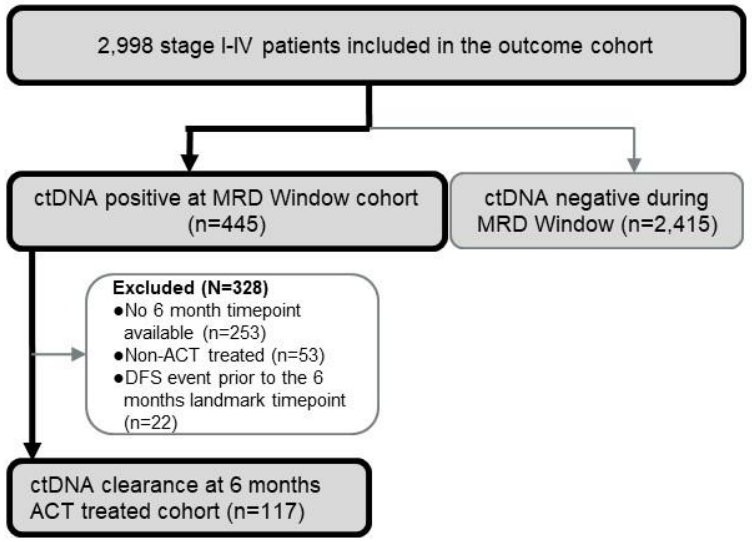
PRESENTED BY: Hiroki Yukami, MD, PhD

ctDNA dynamics of patients with transient clearance post-MRD with recurrence

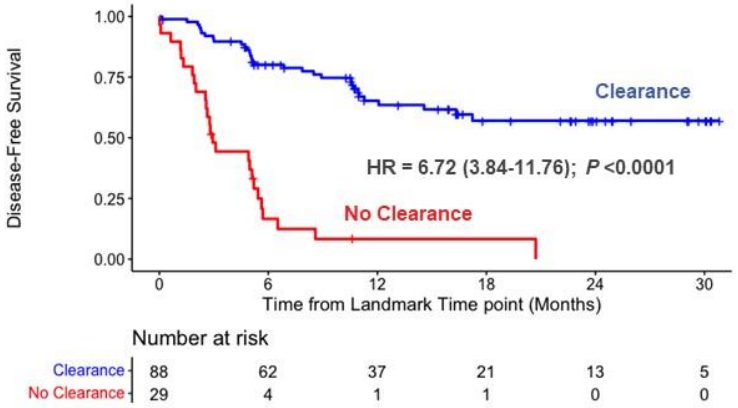
For recurrent pts with transient clearance, 98% of pts turned back positive by 18 months post-surgery.



Clearance and reduction in MTM/mL at 6 months in ACT treated patients

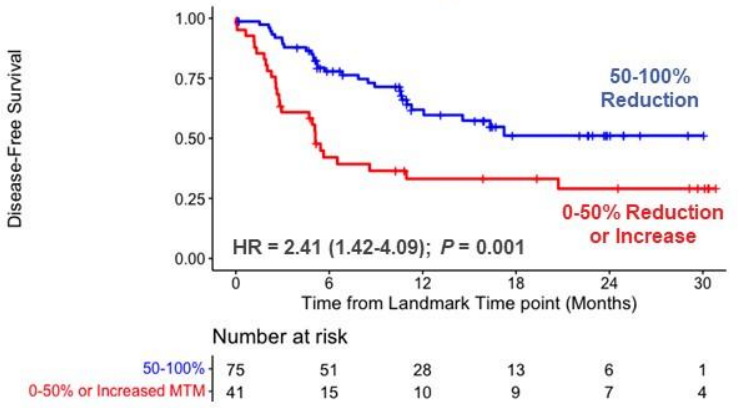


ctDNA clearance at 6 months



ctDNA Clearance	Clearance	No Clearance
Events %	35.2 (31/88)	89.7 (26/29)
24M-DFS % (95% CI)	57.1 (44–68.2)	NR

Positive at the MRD window to 6 months MTM/mL Reduction | ACT-treated



ctDNA Clearance	50-100% Reduction	0-50% Reduction or Increase
Events %	38.7 (29/75)	65.9 (27/41)
24M-DFS % (95% CI)	51.1 (36.4–64.1)	29 (15–44.6)

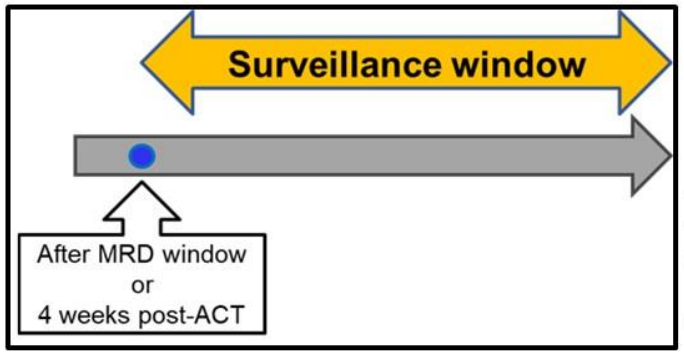
***DFS % from landmark time point**

Landmark 6 months post-surgery

ctDNA clearance and MTM/mL reduction on ACT is an indicator of treatment efficacy and results in better outcomes

PRESENTED BY: Hiroki Yukami, MD, PhD

DFS according to ctDNA status in the Surveillance window

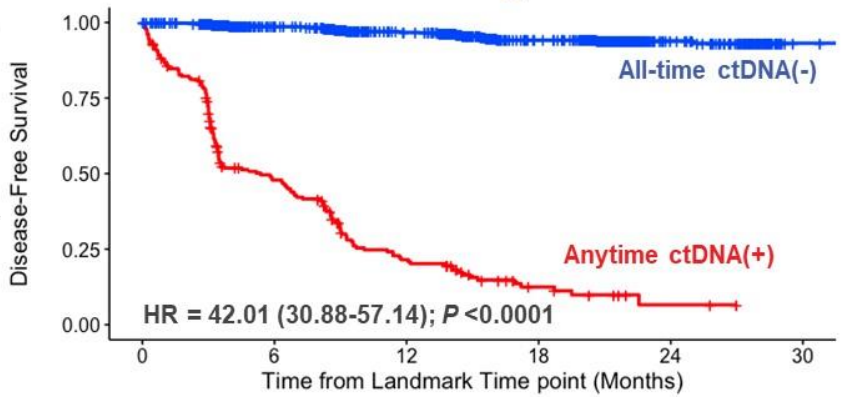


2,998 stage I-IV patients included in the outcome cohort

Excluded (N=1,212)
- No subsequent timepoints available (n=858)
- DFS event prior to the 8 months landmark timepoint (n=354)

Surveillance Window analysis cohort (n=1,786)

All stages

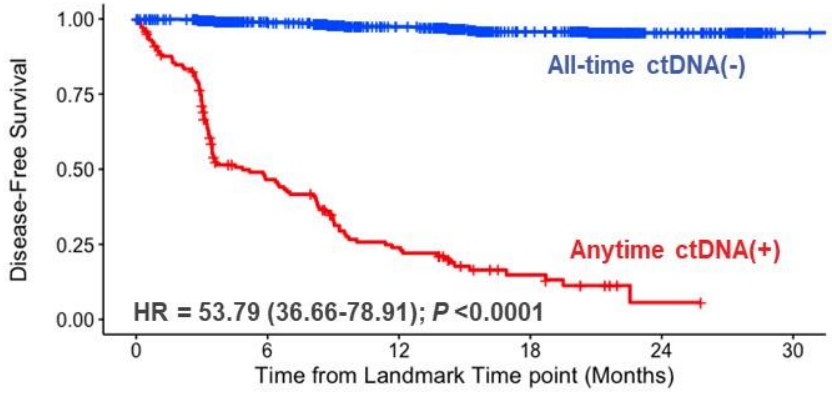


Number at risk

ctDNA Negative	1582	1211	885	432	125	8
ctDNA Positive	204	84	33	10	2	0

ctDNA status	All-time Negative	Anytime Positive
Events %	3.7 (58/1582)	77.5 (158/204)
24M-DFS % (95% CI)*	93.9 (92–95.4)	6.6 (2–14.9)

Stage II/III



Number at risk

ctDNA Negative	1326	1022	737	355	97	5
ctDNA Positive	146	57	26	9	1	0

ctDNA status	All-time Negative	Anytime Positive
Events %	2.7 (36/1326)	75.3 (110/146)
24M-DFS % (95% CI)*	95.4 (93.5–96.8)	5.6 (0.8–18.3)

**DFS % from landmark time point*

- *Surveillance window starts from 4 weeks post-ACT or at the end of MRD window if patient had no ACT, until the last follow up or relapse.*
- *Landmark 8 months post-surgery (2 months for ACT initiation + 6 months of ACT duration)*

ctDNA-positive in the surveillance window is predictive of inferior DFS

PRESENTED BY: Hiroki Yukami, MD, PhD

Conclusions

- Our data demonstrate the **prognostic value and predictive capabilities of ctDNA detection analyzed in ~3,000 patients at 24 months**. The importance of **ctDNA quantification by MTM/mL** is also shown.
- **Sustained ctDNA clearance** post-adjuvant chemotherapy is significantly **associated with >90% DFS**.
- **Transient ctDNA clearance** on ACT correlates with improved median DFS compared to non-cleared patients, but **prognosis remains very poor.**
  - Of the patients with transient ctDNA clearance who ultimately recurred clinically or radiographically, **98% experienced molecular recurrence by 18 months.**
- Stage I–IV patients with **ctDNA detected post surgery have significantly lower DFS** at 24 months than ctDNA negative patients (29% vs. 86%).
- **ctDNA status during surveillance** is significantly associated with DFS. **Reduction in ctDNA concentration (MTM/mL) at 6 months** can also be used to **predict clinical outcomes**.
- ctDNA-guided adjuvant strategies will further be established by **ongoing randomized interventional VEGA and ALTAIR** clinical trials that are part of CIRCULATE-Japan.

My take-aways

- In a large, observational study of ~3000 patients with stages I-IV CC, sustained clearance of ctDNA after adjuvant chemotherapy prognosticates a VERY favorable long-term outcome (most likely curative).

- Among patients with baseline ctDNA(+) status, "transient clearance" and "no clearance" outcomes by 24 months both lead to unfavorable outcomes. Further refinement of a definition of "transient clearance" is essential before incirporating this term in the clinical lexicon.

COBRA

NRG ONCOLOGY

Advancing Research. Improving Lives.™

Phase II results of circulating tumor DNA as a predictive biomarker in adjuvant chemotherapy in patients with stage II colon cancer: NRG-GI005 (COBRA) phase II/III study

Van K. Morris[1], Greg Yothers[2], Scott Kopetz[1], Shannon L. Puhalla[3], Peter C. Lucas[2], Atif Iqbal[4], Patrick M Boland[5], Dustin A. Deming[6], Aaron J. Scott[7], Howard J Lim[8], Theodore S. Hong[9], Norman Wolmark[2], Thomas J. George[10]

[1]The University of Texas -- MD Anderson Cancer Center; [2]NSABP Foundation, Inc.; [3]UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine; [4]Baylor College of Medicine; [5]Rutgers Cancer Institute of New Jersey; [6]University of Wisconsin; [7]University of Arizona Cancer Center; [8]BC Cancer - Vancouver, University of British Columbia; [9]Massachusetts General Hospital Cancer Center, Harvard Medical School; [10]UF Health Cancer Center, Gainesville, FL

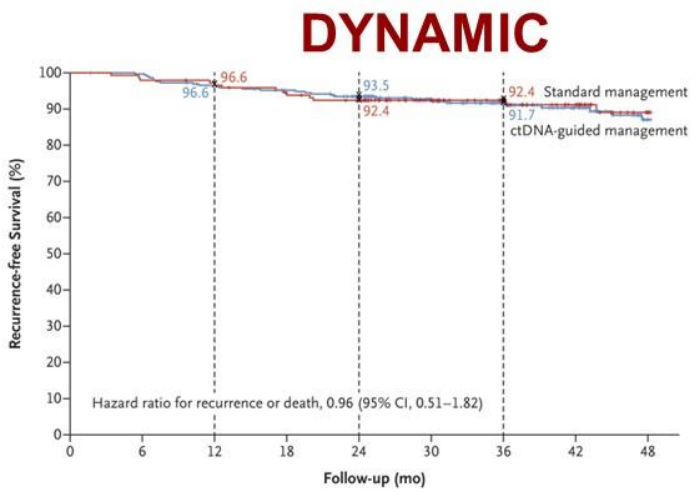
January 20, 2024

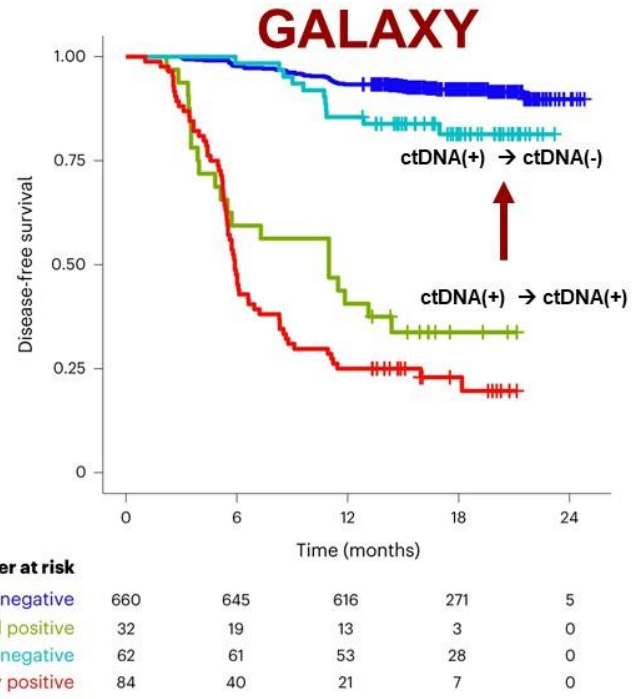
NCI National Clinical Trials Network — a National Cancer Institute program

NCI Community Oncology Research Program — A program of the National Cancer Institute of the National Institutes of Health

Background

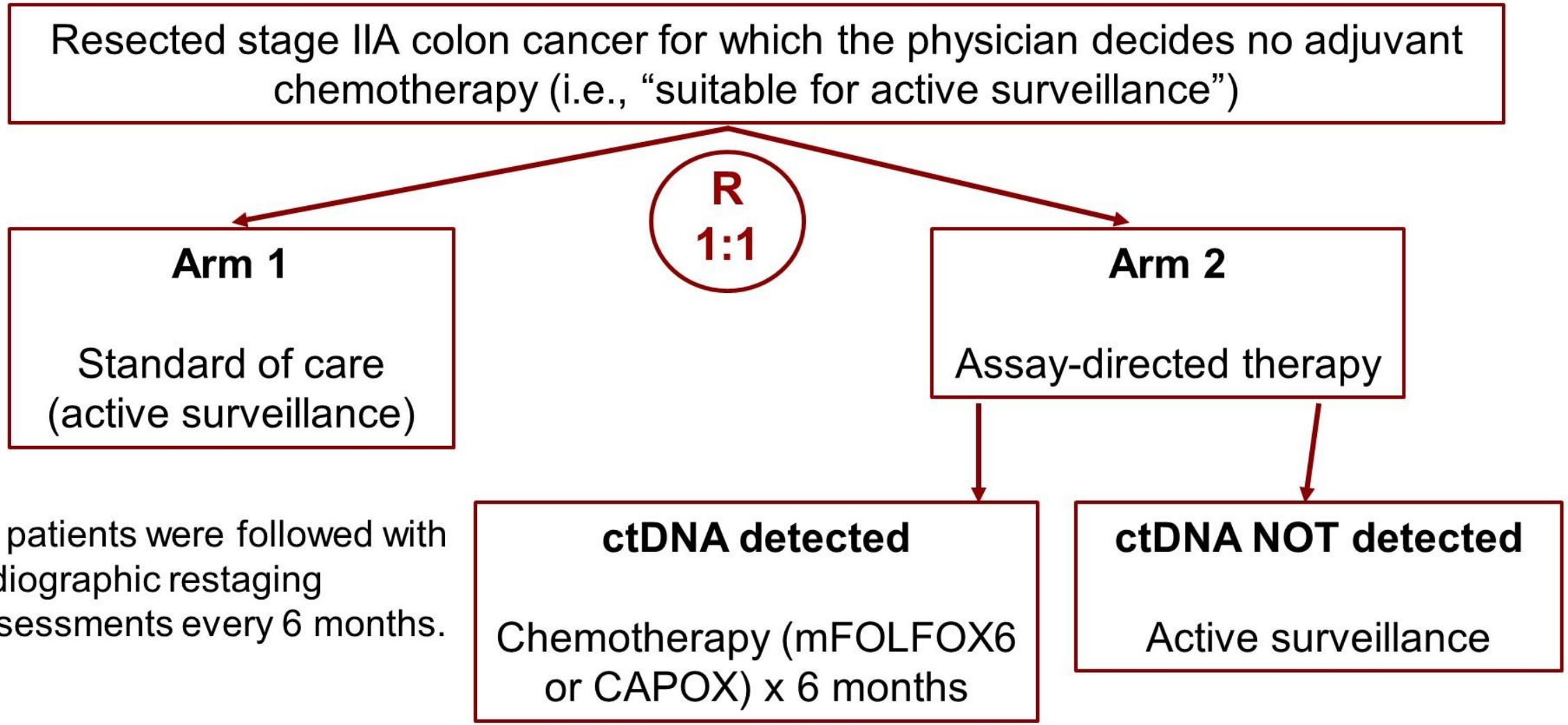
- Detection of ctDNA after curative-intent therapies is prognostic for recurrence for patients with all stages of colon cancer[1-3].
- Prospective RCT (DYNAMIC) has supported de-escalation therapies for patients with stage II colon cancer with no detectable ctDNA after surgical resection[4].
- Observational studies (GALAXY) using a tumor-informed ctDNA assay have suggested that cytotoxic chemotherapy may clear ctDNA and improve survival outcomes for patients with localizec colon cancer[5].
- Use of ctDNA as a predictive biomarker has not been demonstrated, due to the lack of validating prospective data.



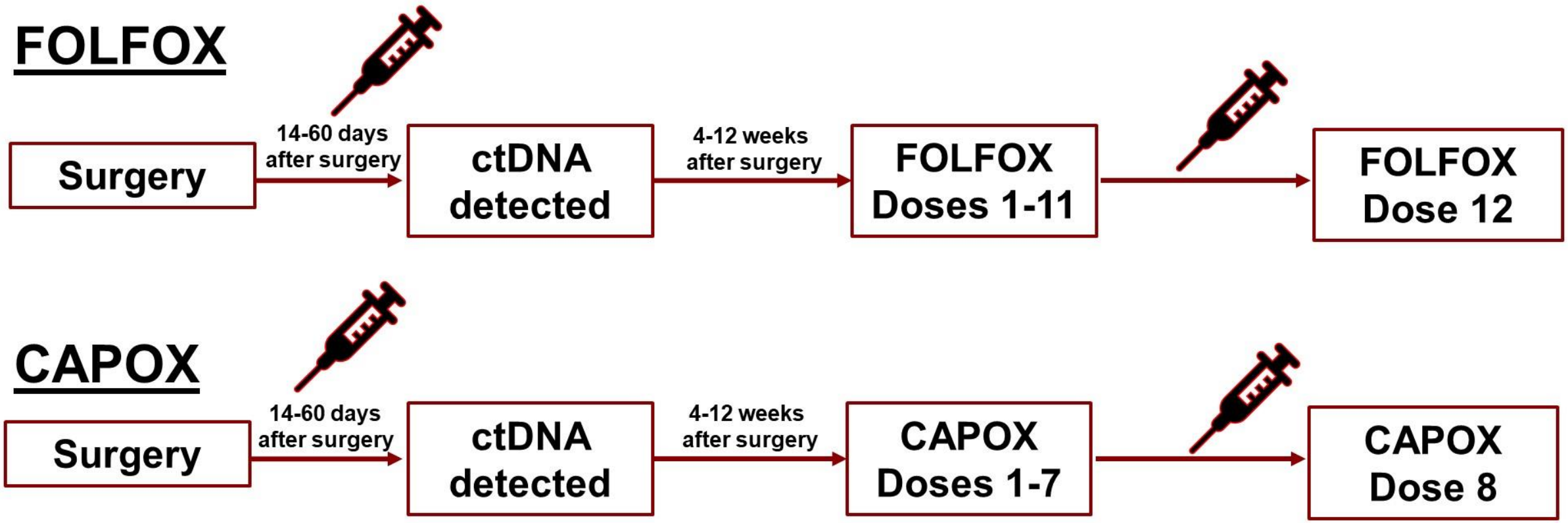


[1]Tie J et al. Sci Transl Med 2015; [2]Tie J et al JAMA Oncol 2019; [3]Newhook T et al Ann Surg 2022; [4]Tie J et al NEJM 2021; [5]Kotanti D et al Nature Medicine 2023

NRG-GI005 (COBRA) Study Schema

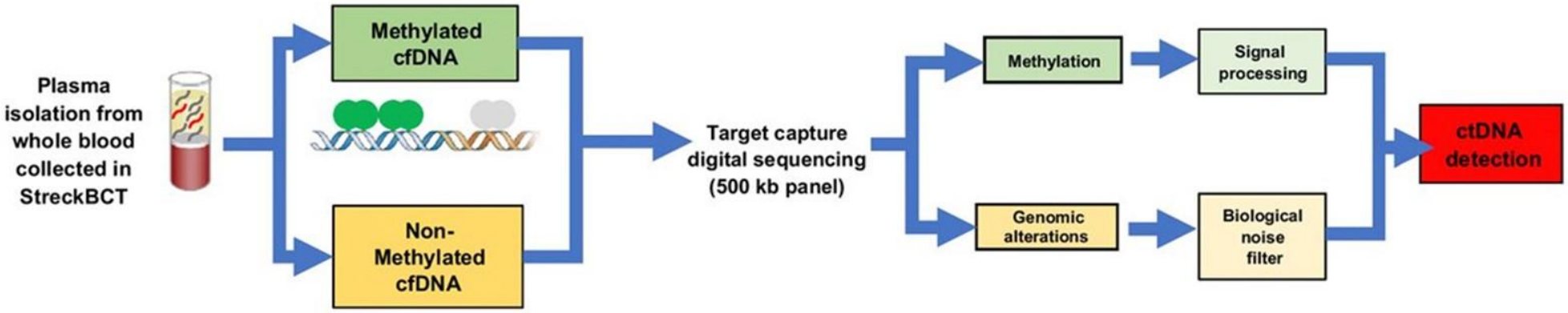


Treatment schema: Arm 2 “ctDNA detected”



The 6-month timepoint was collected two weeks after prior dose of chemotherapy/ immediately prior to the administration of the last dose of chemotherapy.

ctDNA assay



- Guardant LUNAR assay was selected for NRG GI005 through an open RFA and peer-reviewed process as a tissue-agnostic assay that incorporates mutation/genomic and methylation/epigenomic markers alike for detection of ctDNA.
- Guardant LUNAR had undergone previous clinical and analytic validation:
  - In a previously reported cohort of 70 patients with stage I-IV colorectal cancer, sensitivity and specificity for were 56% and 95% (100% for those with one year of follow-up), respectively, when drawn one month after completion of definitive therapy.
  - Adding epigenomic profiling improved sensitivity relative to mutation calling alone by 25%.

Parikh A et al, Clin Cancer Res 2021

Selected Eligibility Criteria

- pT3N0 stage IIA adenocarcinoma of the colon with ≥12 lymph nodes examined at surgical resection.
- Appropriate for active surveillance (i.e., no adjuvant chemotherapy) at the discretion of the evaluating oncologist based on current practice patterns.
- Complete resection of tumor within 14 to 60 days of study randomization.
- No clinical or radiographic evidence of metastatic disease.
- ECOG PS 0 or 1.
- Adequate hematologic function, hepatic function, renal function.
- No prior testing of ctDNA for colorectal cancer outside of study conduct prior to registration.
- No history of prior invasive colon/rectal malignancy, regardless of disease-free interval, AND no other invasive non-colorectal malignancy within 5 years before randomization.
- No prior (neoadjuvant) therapy administered as treatment for colon cancer.

Statistical Plan

Primary objective (phase II):

- Compare rates of ctDNA clearance between ctDNA (+) cohorts at 6 months after randomization.

Secondary objectives:

- Describe median OS, RFS, and TTR according to ctDNA status and treatment arm.
- Assess feasibility of trial design (compliance with adjuvant chemotherapy).
- Correlate ctDNA clearance/persistence with survival outcomes.

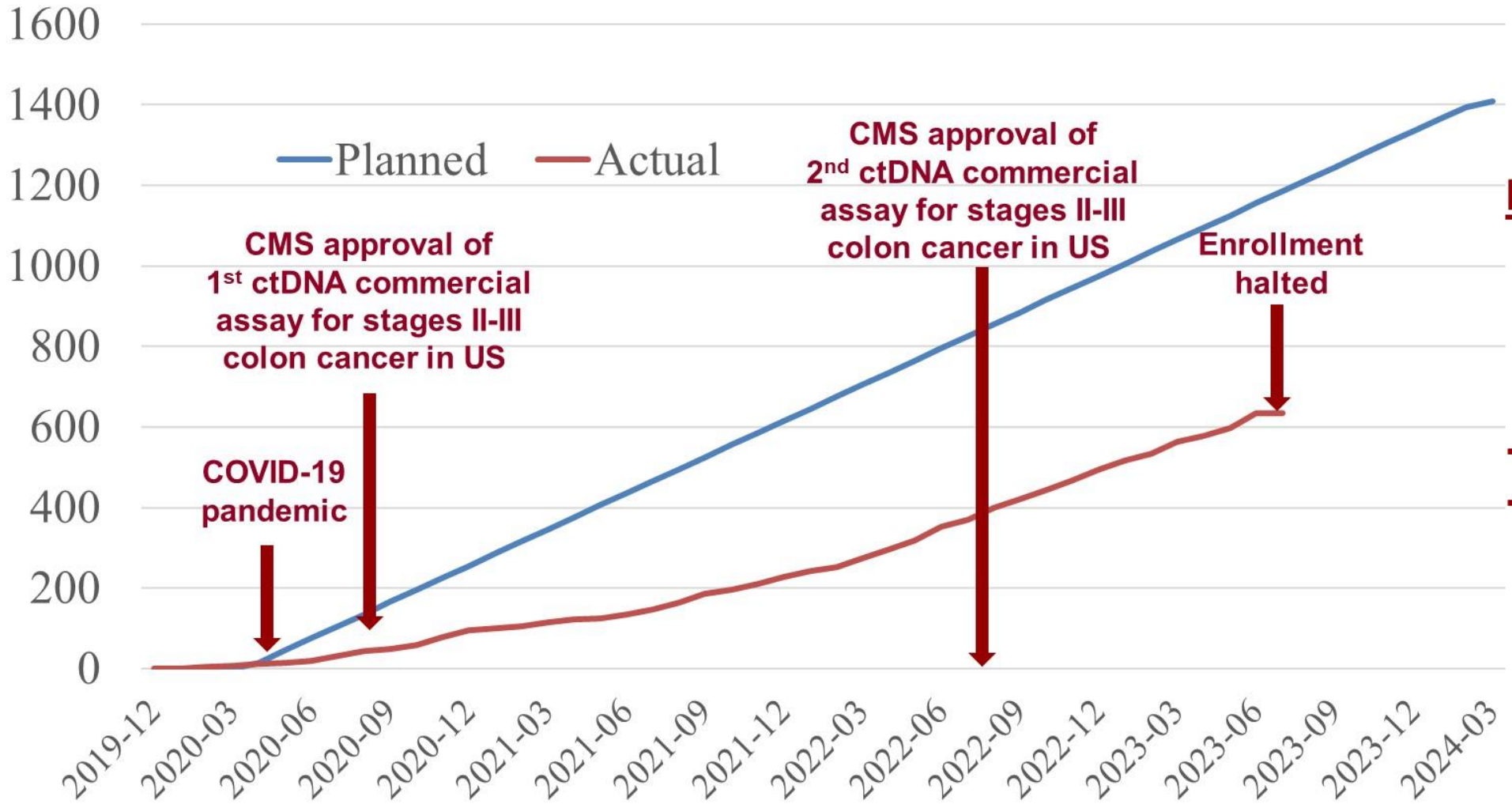
Statistical Design:

- For an assumed baseline ctDNA+ rate of 5.45%, the phase II/III decision rule (using a 1-sided p-value ≤ 0.35 from a Fisher exact test with 1-sided $\alpha = .072$ and power .909) based on intention to treat,
  H_0: clearance 10% in each arm
  H_a: clearance 60% vs 10% (Arm 2 versus Arm 1)

- 1408 patients were anticipated to provide 55 RFS events in the subset of patients ctDNA+ at baseline in order to provide 92% power at one-sided $\alpha = .025$ for the primary phase III endpoint.

- First 16 ctDNA(+) patients were evaluated after 6 months from randomization for phase II futility analysis.

Patient Accrual



Final accrual:

635/1408 participants

- **US: 458**
- **Canada: 177**

Demographics

	Standard of Care		Assay Directed		Total*	
	N=318	%	N=317	%	N=635	%
Gender						
Female	142	44.7	153	48.3	295	46.5
Male	176	55.3	164	51.7	340	53.5
Age (years)						
<50	57	17.9	68	21.5	125	19.7
50-59	86	27.0	73	23.0	159	25.0
60-69	105	33.0	97	30.6	202	31.8
>70	70	22.0	79	24.9	149	23.5
Race						
Asian	17	5.3	16	5.0	33	5.2
Black or African American	25	7.9	18	5.7	43	6.8
White	257	80.8	262	82.6	519	81.7
Other	1	0.3	3	0.9	4	0.6
Not reported / Unknown	18	5.7	18	5.7	36	5.7
Ethnicity						
Hispanic or Latino	26	8.2	34	10.7	60	9.4
Not Hispanic or Latino	283	89.0	270	85.2	553	87.1
Not reported / Unknown	9	2.8	13	4.1	22	3.5
ECOG Performance Status						
0	236	74.4	246	77.6	482	76.0
1	81	25.6	71	22.4	152	24.0

*No statistically significant differences in demographics between arms.

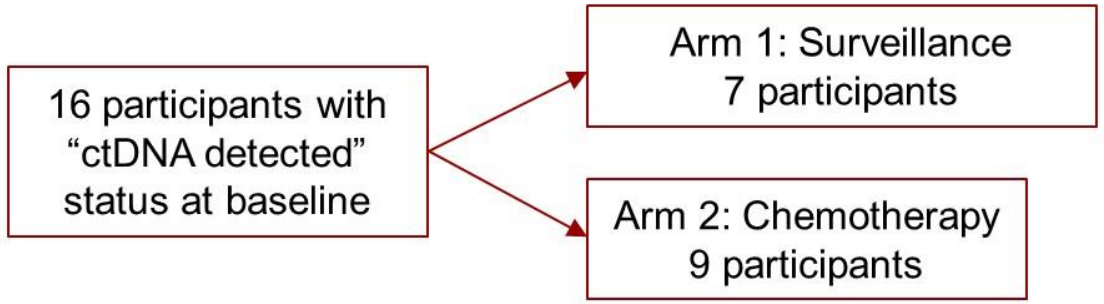
Toxicity (Arm 2)*

	CAPOX (N=4)				mFOLFOX6 (N=12)			
	N (%) of Patients by Grade				N (%) of Patients by Grade			
Grade	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
Overall Highest Grade	**2 (50)**	**2 (50)**	**0**	**0**	**2 (17)**	**5 (41)**	**1(8)**	**0**
Neutropenia	0	0	0	0	2 (17)	1 (8)	1 (8)	0
Neuropathy	0	0	0	0	4 (33)	2 (17)	0	0
Increased LFTs	0	0	0	0	1 (8)	1 (8)	0	0
Diarrhea	1 (25)	2 (50)	0	0	1 (8)	1 (8)	0	0
Hyperkalemia	0	0	0	0	0	1 (8)	0	0

***at time of study closure**

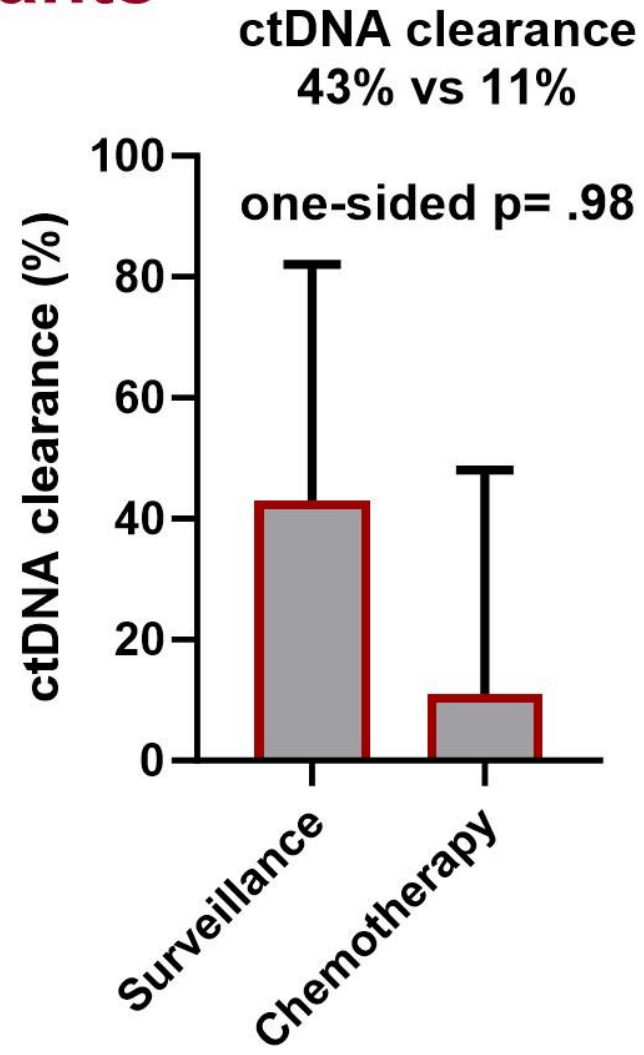
Phase II Endpoint Analysis: ctDNA(+) baseline participants

- Among 596 participants with baseline ctDNA status available, ctDNA(+) detection was observed in 33 (5.54%).



- Clearance of ctDNA at 6 months among ctDNA(+) participants at baseline was observed in:
  - **Arm 1 (surveillance):** 3 of 7 (43%, 95% CI 10 - 82%) participants
  - **Arm 2 (chemotherapy):** 1 of 9 patients (11%, 95% CI 0.3 - 48%) participants

- **Because the 1-sided Fisher's Exact Test yields p = 0.98 exceeded 0.35, H_o was not rejected, and the decision rule calls for early stopping due to futility.**



Conclusions

- Using the selected ctDNA assay in this clinically low-risk population, we did not observe an improvement in ctDNA clearance with 6 months of adjuvant chemotherapy relative to surveillance for patients with “low-risk” stage IIA colon cancer in a subset of patients with detected ctDNA at baseline.

- Prospective RCTs assessing ctDNA as a surrogate for minimal residual disease are feasible and necessary in order to confirm clinically relevant hypotheses in oncology.

- Future clinical trial design should account for the continuous evolution and refinement of ctDNA methodologies and assay performance in order to provide answers to specified clinically relevant questions.