Novel Frontiers in the Use of ctDNA in Oncology

Updates in Cancer Therapies: An ASCO/ESMO Review

October 2022

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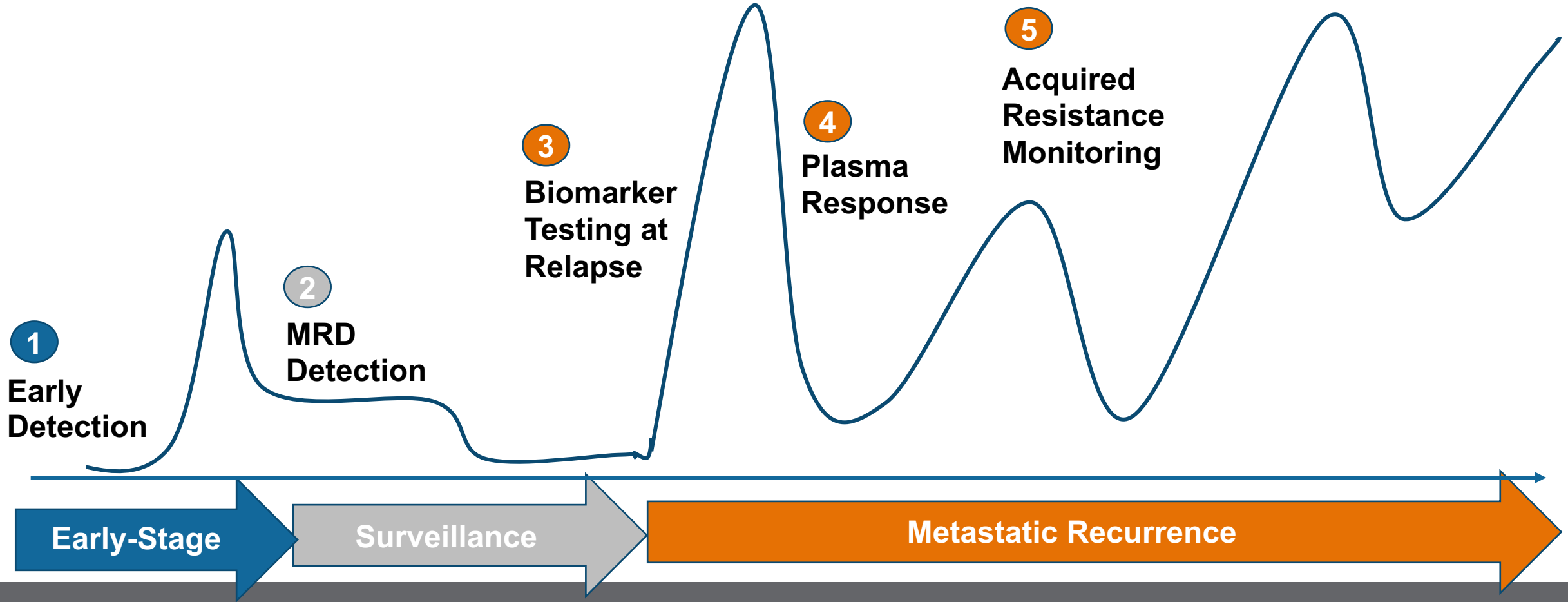
Dana-Farber Cancer Institute

Cell-free DNA Across Many Phases of Disease



Tumor-informed vs. tumor-naïve assays

Tumor-Informed	Tumor-naïve
Requires tissue biopsy	No need for biopsy
Personalized assay	Off the shelf assay
Longer turnaround time	Shorter turnaround time
Does not account for tumor heterogeneity	Can detect clonal variants that emerge during follow-up
Potential for better sensitivity and specificity	Variable sensitivity and specificity

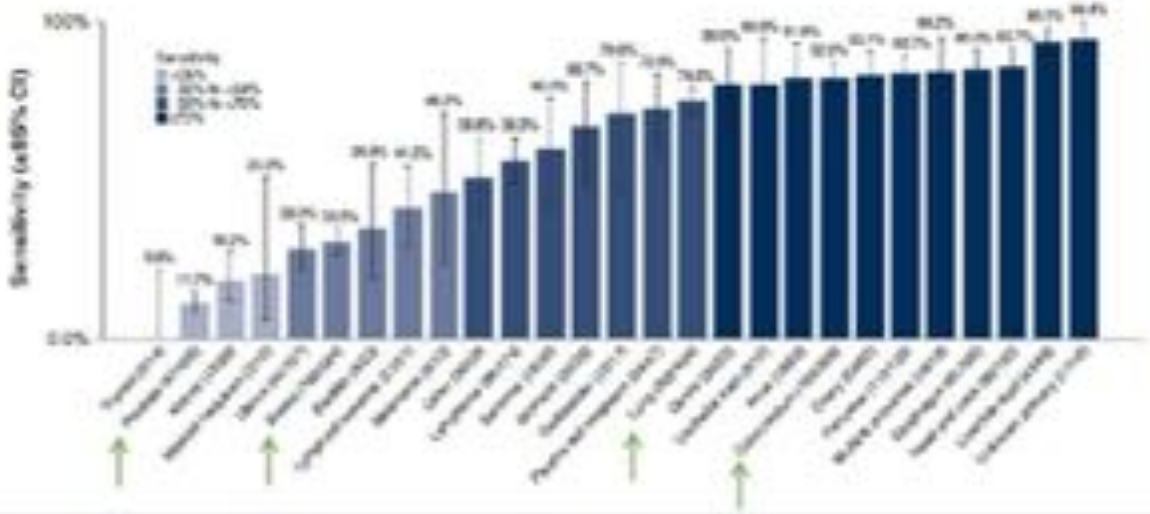
Pellini B and Chaudhuri A. *J Clin Oncol*. 2022

ctDNA methylation for early cancer detection

	Cancer	Non-cancer	Total
	2823	1254	4077
Test positive	1453	6	1459
Test negative	1370	1248	2618
	Sensitivity = 1453/2823 51.5% (49.6%-53.3%)	Specificity = 1248/1254 99.5% (99.0%-99.8%)	

Two-sided 95% Wilson confidence intervals were calculated

Targeted methylation assay
Tumor-naïve



Sensitivity varies with **cancer** type, **histology**, and **stage**

Klein EA et al. *Ann Oncol*. 2021

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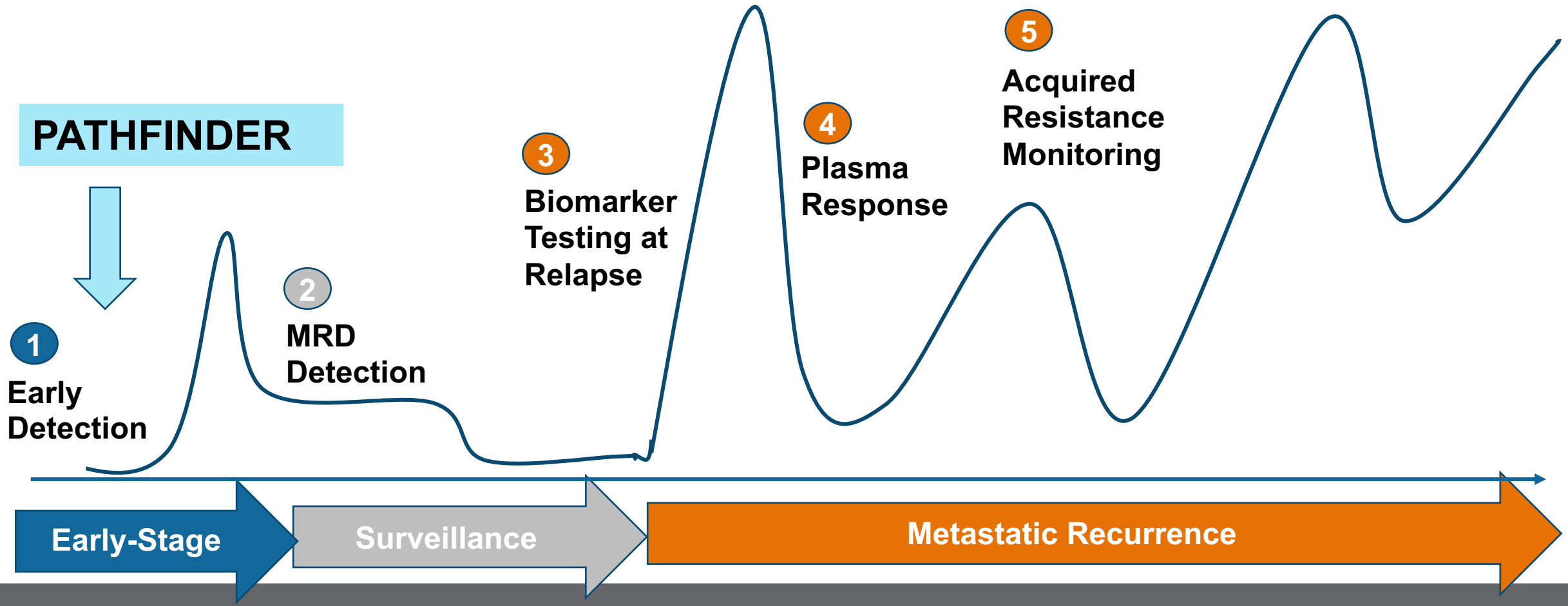
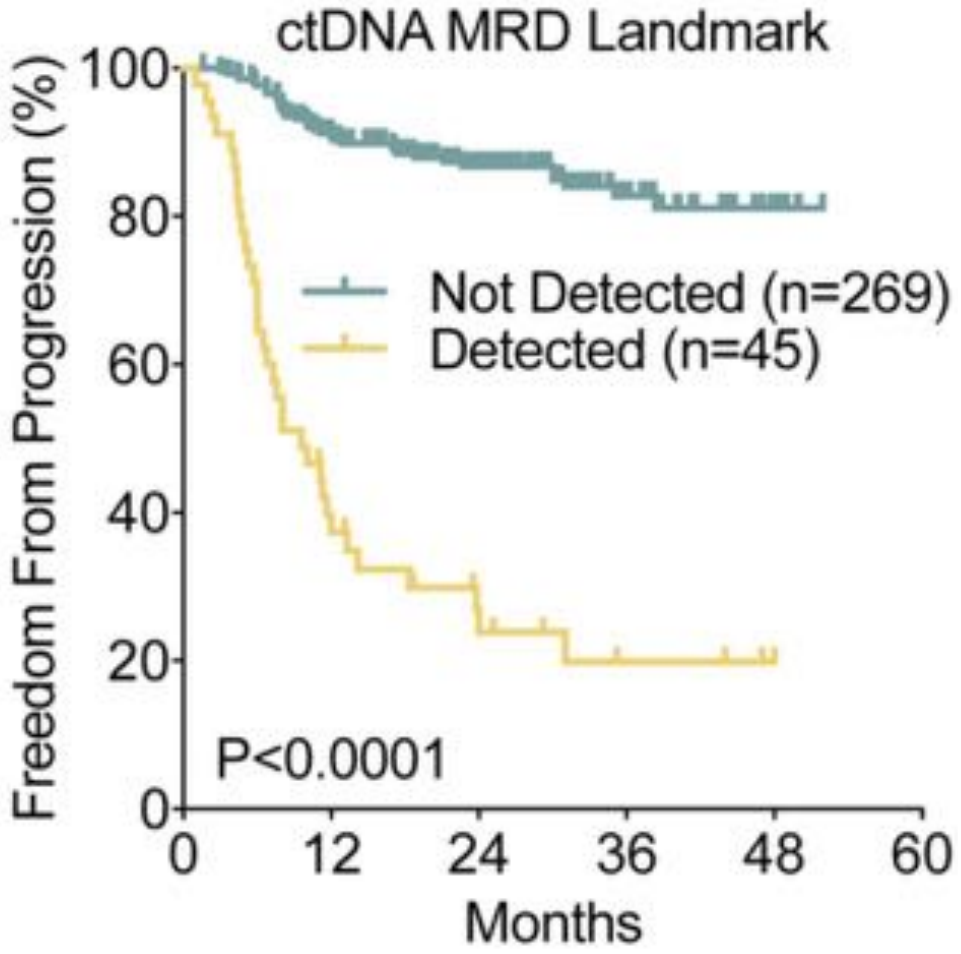
Bruna Pellini, MD
Assistant Member, Department of Thoracic Oncology, Moffitt Cancer Center

ASCO

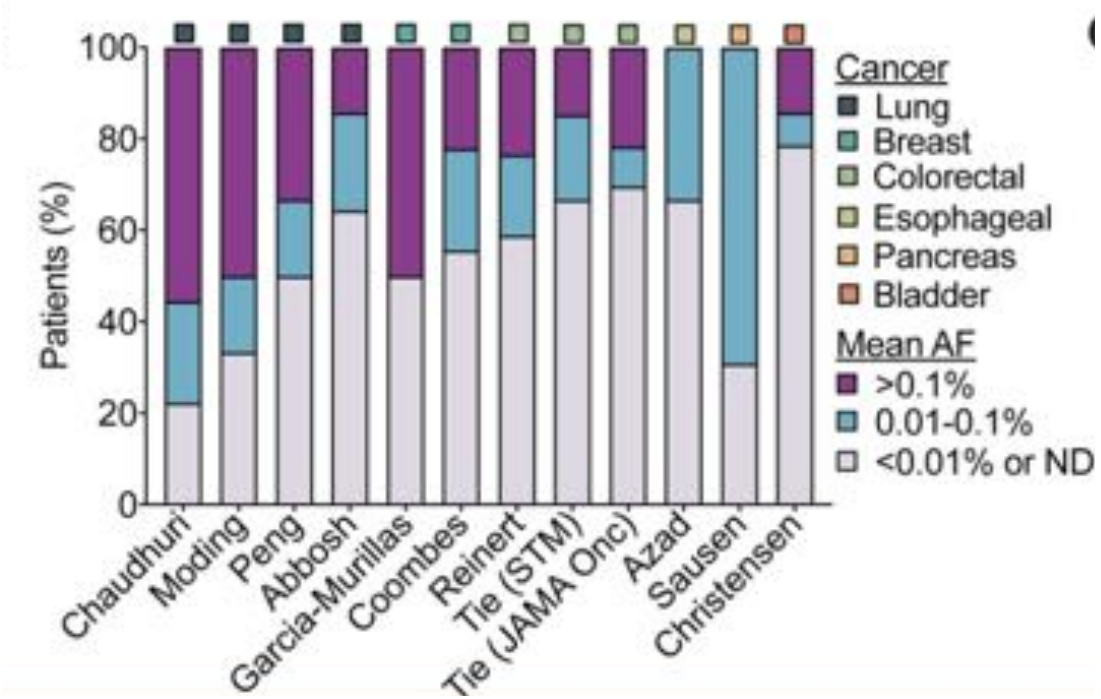
Cell-free DNA Across Many Phases of Disease



Pooled MRD Analysis – Solid Tumors



What degree of sensitivity is needed to declare a patient MRD negative? To modify therapy based on a MRD negative status?

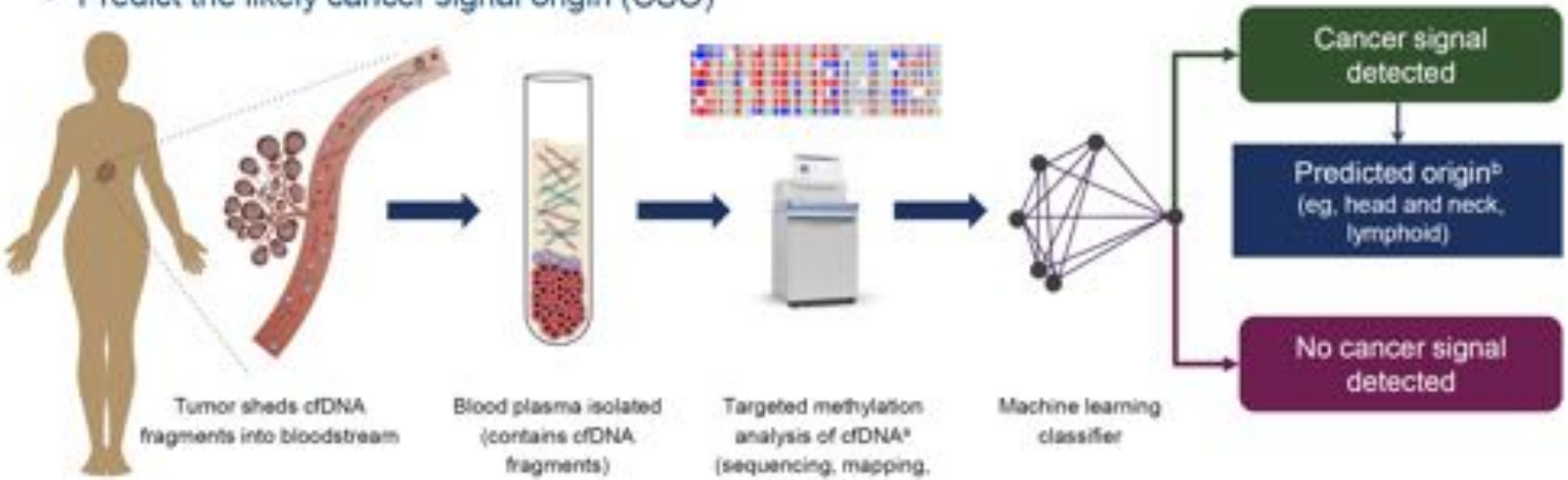


Modig et al. Cancer Discovery. 2021;11(12):2968-86.

Background: Multi-Cancer Early Detection (MCED) Blood Assays

MCED testing uses a targeted methylation, next-generation sequencing (NGS)-based assay to:

- Detect and analyze cfDNA in the bloodstream
- Deploy machine learning to detect a cancer signal
- Predict the likely cancer signal origin (CSO)



cfDNA, cell-free DNA. [a]Bisulfite treatment; targeted probes pull out fragments matching regions of interest. [b]For a detected signal, the MCED test predicts 1-2 cancer signal origins (CSO) that can be either an anatomic site (eg, colorectal) or a cellular lineage (eg, lymphoid). Adapted from Liu MC, et al. *Ann Oncol*. 2020;31(6):745-759. PMID: 33506768

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PATHFINDER Eligibility Criteria

Inclusion:

- **Adults ≥50 years** who were eligible for either:
  - With Additional Risk Cohort
  - Without Additional Risk Cohort
- Eligibility for With Additional Risk Cohort:
  - Lifetime history of smoking at least 100 cigarettes
  - Hereditary cancer predisposition[a]
  - A history of cancer with no treatment for >3 years[b]
- Eligibility for Without Additional Risk Cohort:
  - None of the above risk factors

Exclusion:

- Clinical suspicion of malignancy
- Undergoing diagnostic evaluation for malignancy
- History of invasive or hematologic malignancy diagnosed <3 years before enrollment
- Definitive treatment for invasive or hematologic malignancy <3 years before enrollment[b]

[a]Genetic cancer predisposition, hereditary cancer syndrome, or meeting criteria for germline testing based on NCCN guidelines.
[b]Personal history of invasive or hematologic malignancy, with definitive treatment completed >3 years prior to enrollment. Adjuvant hor

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Deb Schrag, MD, MPH

Content of this presentation

Primary Objective: Understand extent of diagnostic testing to achieve diagnostic resolution
-Time to resolution
-Number and type of tests

Participant Characteristics

	With Additional Risk[a] n = 3,681	Without Additional Risk n = 2,940	Total N = 6,621
Age[b], in years, mean (SD)	64.7 (8.7)	61.6 (8.1)	63.4 (8.6)
Female	65%	62%	63%
White, Non-Hispanic	93%	89%	92%
College Degree or Higher	59%	71%	65%
Up to Date With Standard Cancer Screening Prior to MCED Testing			
Colorectal Cancer[c]	91%	92%	92%
Breast Cancer[d]	78%	83%	80%

[a]Previous history of cancer, smoking, and hereditary risk.
[b]Participants >85 were eligible to participate, but to protect confidentiality, 85 years was the maximum age recorded and used in calculations for participants ≥85 years of age.
[c]Participants ≤75 years old, up to date with USPSTF colorectal cancer screening recommendations (n=4888 total eligible with complete information).
[d]Women 50-74 years old up to date with breast cancer screening recommendations (USPSTF, MRI, or ultrasound; n=3547 total eligible with complete information).

Fraction of Patients with Positive Signal

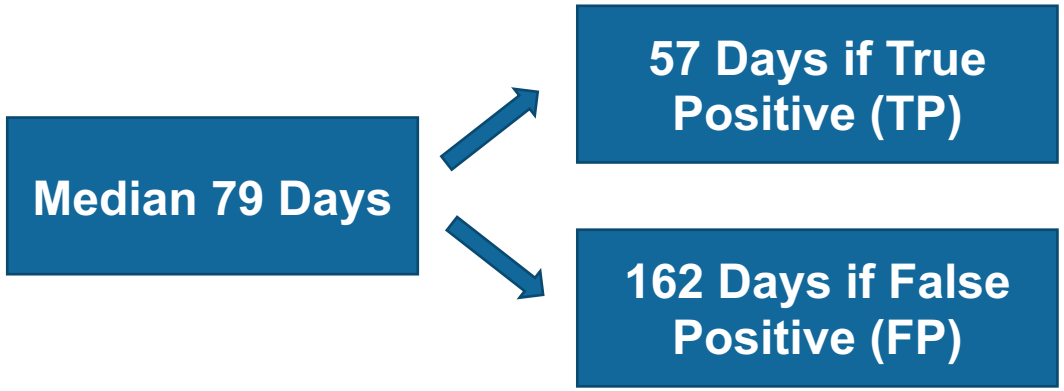
	With Additional Risk[a] n = 3,681	Without Additional Risk n = 2,940	Total N = 6,621
Signal Detected	1.5%	1.2%	**1.4%**
No Signal Detected	98.5%	98.8%	98.6%

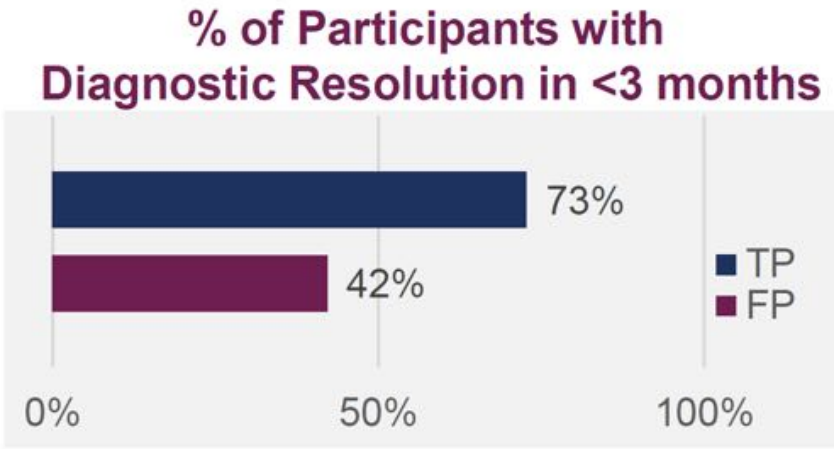
N=6621 analyzed

Schrag et al. ESMO 2022. #9030

Primary Objective: Achieving Diagnostic Resolution

Time Required to Achieve a Diagnostic Resolution





Extent of Testing to Achieve a Diagnostic Resolution

Imaging Procedure 92% (similar TP and FP)

Any Invasive Procedure: 82% TP 30% FP

Schrag et al. ESMO 2022. #9030

Secondary Objective: Accuracy of Predicted Cancer Origin

Test Performance: Ability to Predict Origin of Malignancy

	TP	FP	Total
Participants, n	35	57	92
Determinate predicted origin	**34**	53	87
Indeterminate predicted origin	1	4	5

Predicted Origin Accuracy	
First Predicted Origin,[a] n	29/34[b]
% (95% CI)	**85.3** (69.9-93.6)
First or Second Predicted Origin,[a,c] n	33/34[b]
% (95% CI)	**97.1** (85.1-99.8)

- **The predicted origin helped to direct diagnostic workups**

CI, confidence interval.
[a]For a detected signal, the MCED test predicts cancer signal origins (CSO) that can be either an anatomic site (eg, colorectal) or a cellular lineage (eg, lymphoid).
[b]Excludes 1 participant with indeterminate origin prediction from the true positive per study protocol.
[c]Proportion of first or second origin correctly predicted among true positive participants.

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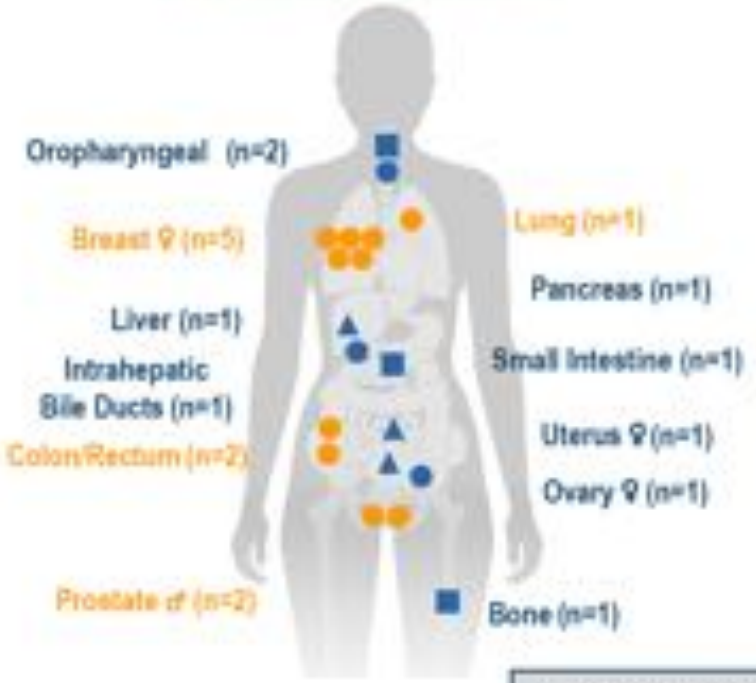
Cancers Diagnosed After a True Positive MCED Signal

18 people diagnosed with Solid Tumors

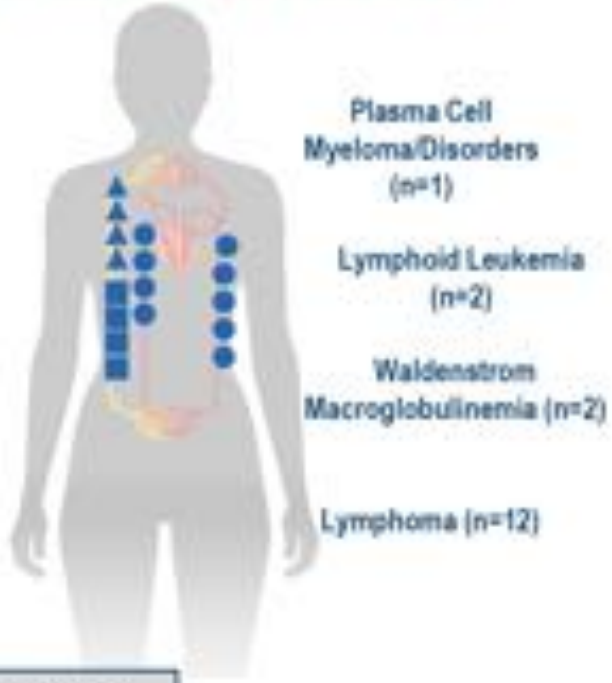


35 people were diagnosed with 36 cancers

- 24 in high-risk cohort
- 11 in not-high-risk cohort

- 7 recurrent cancers
- 14 early-stage cancers
- 26 cancers lacking standard screening

17 People diagnosed with Hematologic Cancers



AJCC Staging: ▲Stage I ■Stage II ●Stage III/IV/No Stage/Recurrent

Available Screening: USPSTF cancer screening or No standard screening

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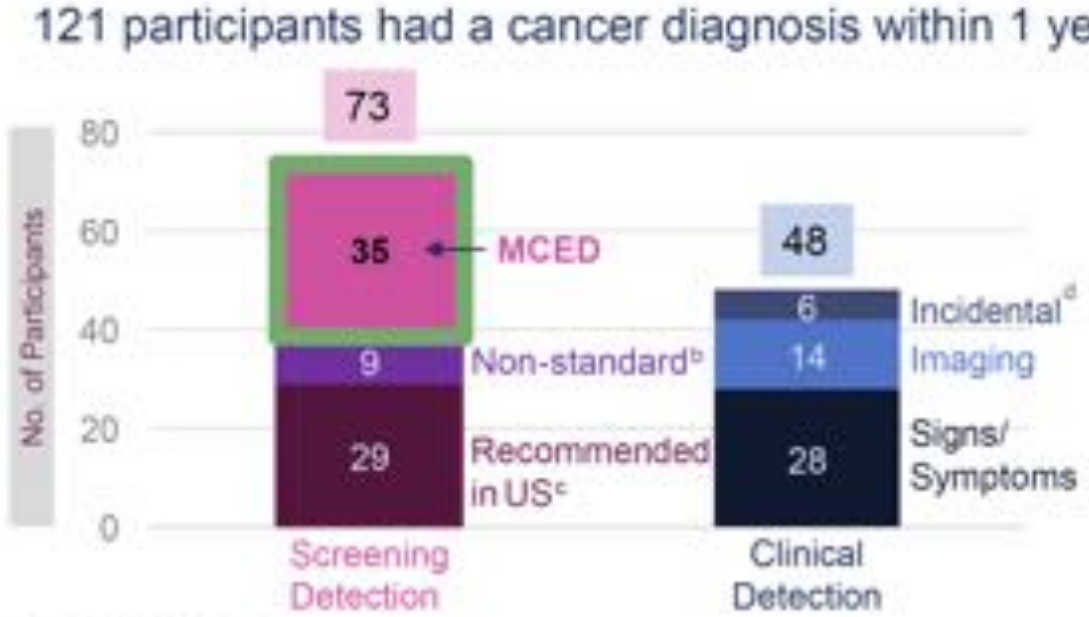
Cancers Identified Within One Year of MCED Testing

Participants with Cancers Detected by Either Screening or Clinical Findings



- 35/121 (29%) had cancer diagnosed and positive MCED

Number needed to screen to detect one cancer: 189

MCED, multi-cancer early detection.
[a]Based on participants with cancer status assessment at the end of the study.
[b]3 thyroid and 6 melanoma.
[c]Breast, cervical, colorectal, lung, and prostate cancer.
[d]1 incidental radiology finding, 1 incidental finding on routine physical exam, 2 changed lab values, 1 surveillance of prior cancer, 1 follow-up after MGUS diagnosis.

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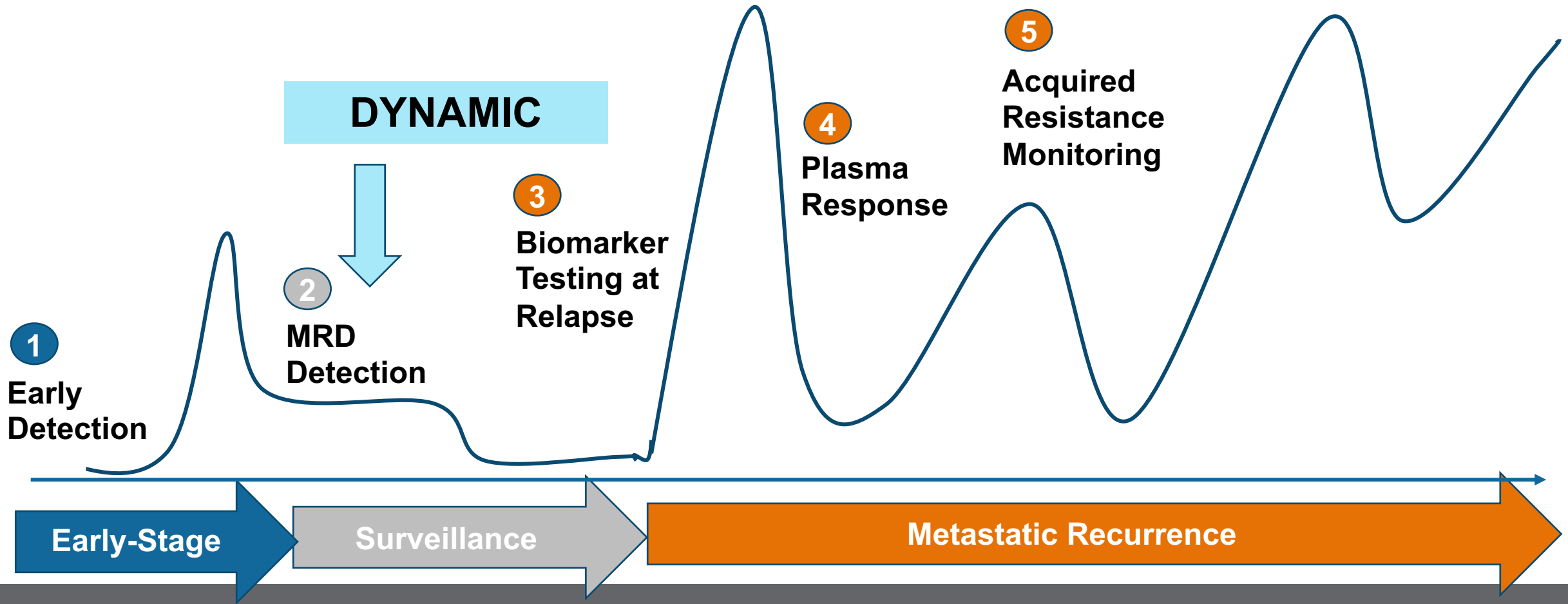
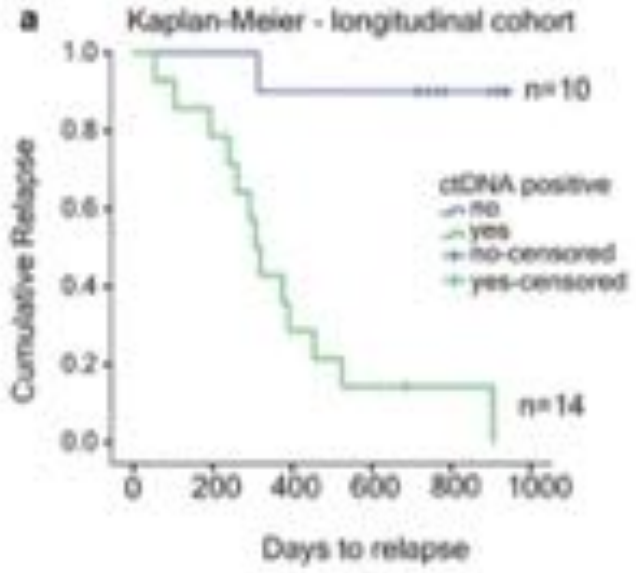
Cell-free DNA Across Many Phases of Disease

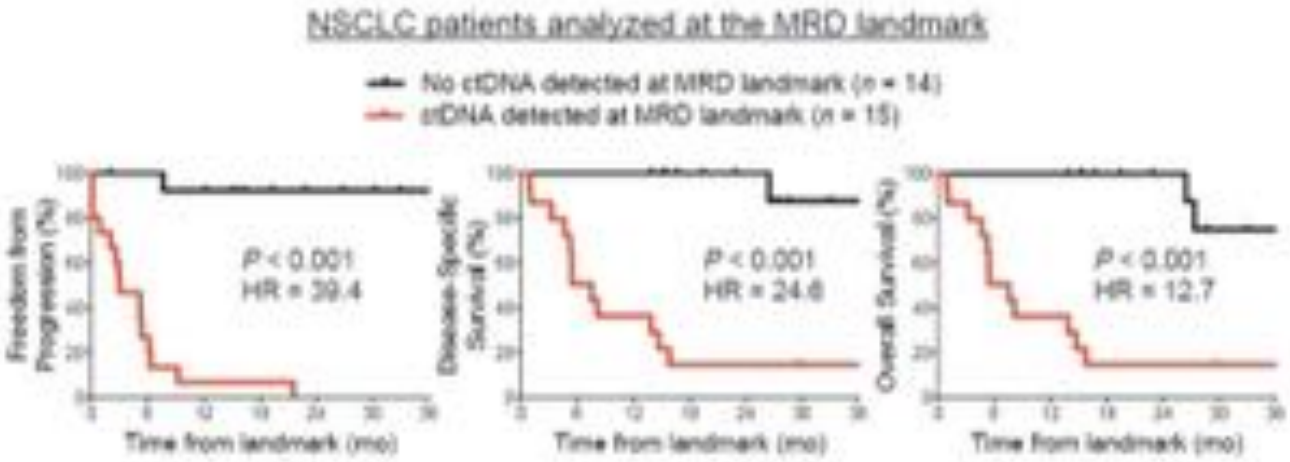


ctDNA can detect minimal residual disease (MRD) and it is a prognostic biomarker

Stages I-III NSCLC
Tumor-informed assay



Stages I-III NSCLC
Tumor-naive assay (CAPP-Seq)

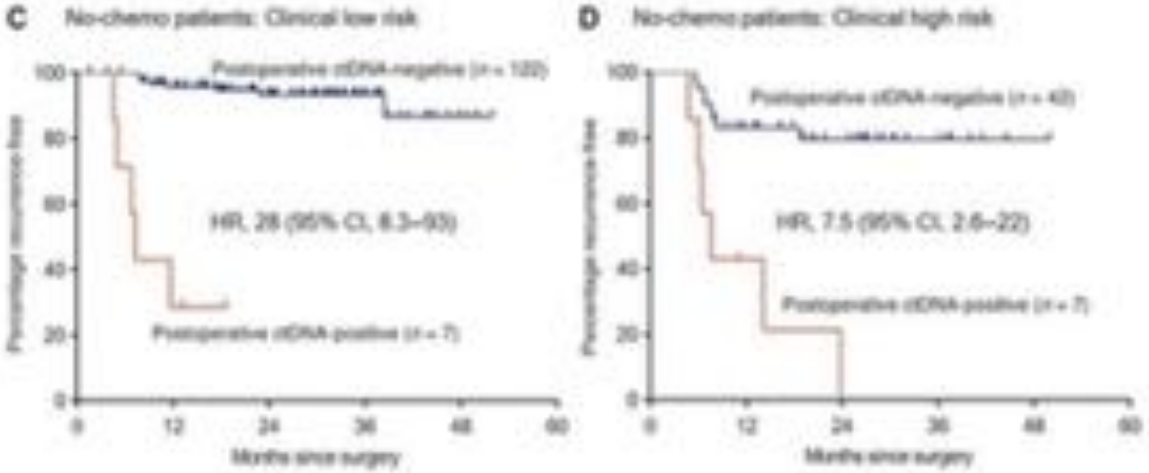


Abbosh C et al. *Nature*. 2017
Chaudhuri A et al. *Cancer Discov*. 2017

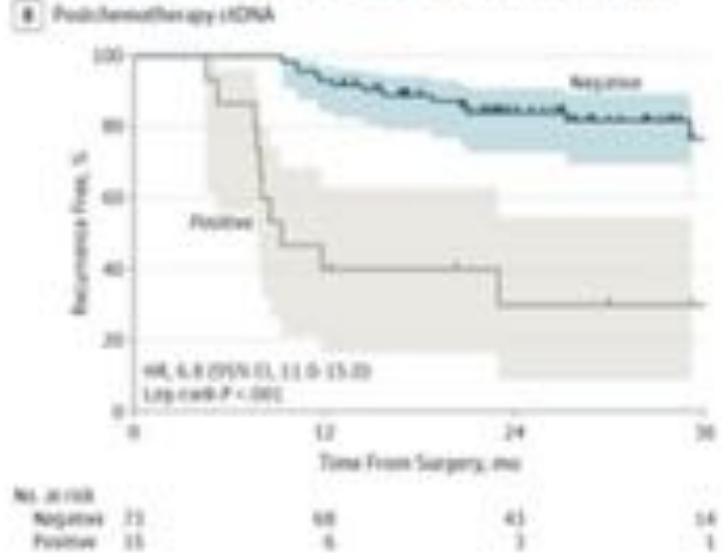
Bruna Pellini, MD
Assistant Member, Department of Thoracic Oncology, Moffitt Cancer Center

ctDNA can detect minimal residual disease (MRD) and it is a prognostic biomarker

Stage II CRC
Tumor-informed assay (Safe-SeqS)



Stage III CRC
Tumor-informed assay (Safe-SeqS)



Tie J et al. *Sci Transl Med*. 2016
Tie J et al . *JAMA Oncol*. 2019

Bruna Pellini, MD
Assistant Member, Department of Thoracic Oncology, Moffitt Cancer Center

DYNAMIC Study: Using ctDNA to Guide Adjuvant Chemotherapy In Stage II Colon Cancer

Can adjuvant chemotherapy be optimized for stage II disease?

- ❑ Many will be cured by surgery alone (<5% survival benefit)
- ❑ Variability in use of adjuvant chemotherapy for stage II colon cancer
- ❑ Adjuvant chemotherapy to be considered if with high-risk features

DYNAMIC: Can a tumor-informed ctDNA-guided approach safely reduce use of adjuvant chemotherapy?

DYNAMIC Study Design

ACTRN12615000381583

Stage II Colon Cancer

- R0 resection
- ECOG 0 – 2
- Staging CT within 8 weeks
- Provision of adequate tumor tissue within 4 weeks post-op
- No synchronous colorectal cancer

Plasma Collections
Week 4 + 7 post-op

R
2:1

ctDNA-Guided Management

- ctDNA-Positive → Adjuvant Chemo (oxaliplatin-based or single agent FP)
- ctDNA-Negative → Observation

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

Standard Management

Adjuvant treatment decisions based on conventional clinico-pathologic criteria

Endpoints

Primary
- RFS rate at 2 years

Key Secondary
- Proportion receiving adjuvant chemo

Secondary
- RFS by ctDNA status for ctDNA-guided arm
- TTR
- OS

Stratification Factors
- T stage (T3 vs T4)
- Type of participating center (metropolitan vs regional)

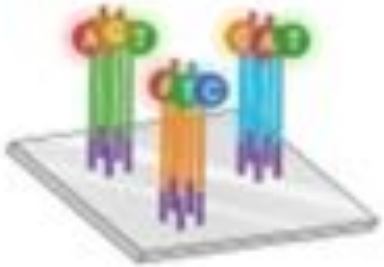
Surveillance:
- CEA → 3-monthly for 24M, then 6-monthly for 36M
- CT C/A/P → 6-monthly for 24M, then at 36M

ctDNA Analysis: Tumor-Informed Personalized Approach

Resected tumor tissue

FFPE tissue from primary tumor

Targeted sequencing identifies mutation(s) unique to that cancer

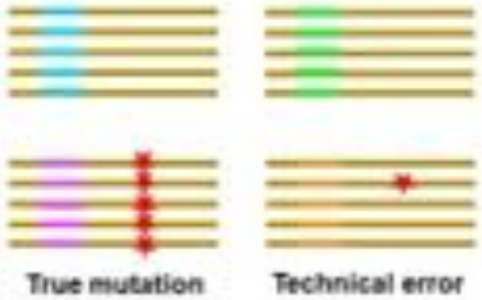


15 recurrently mutated genes in colorectal cancer

(APC, TP53, KRAS, PIK3CA, FBXW7, BRAF, SMAD4, RNF43, POLE, CTNNB1, ERBB3, NRAS, PPP2R1A, AKT1, HRAS)

Week 4 + 7 plasma

At least one patient-specific mutation assessed in plasma



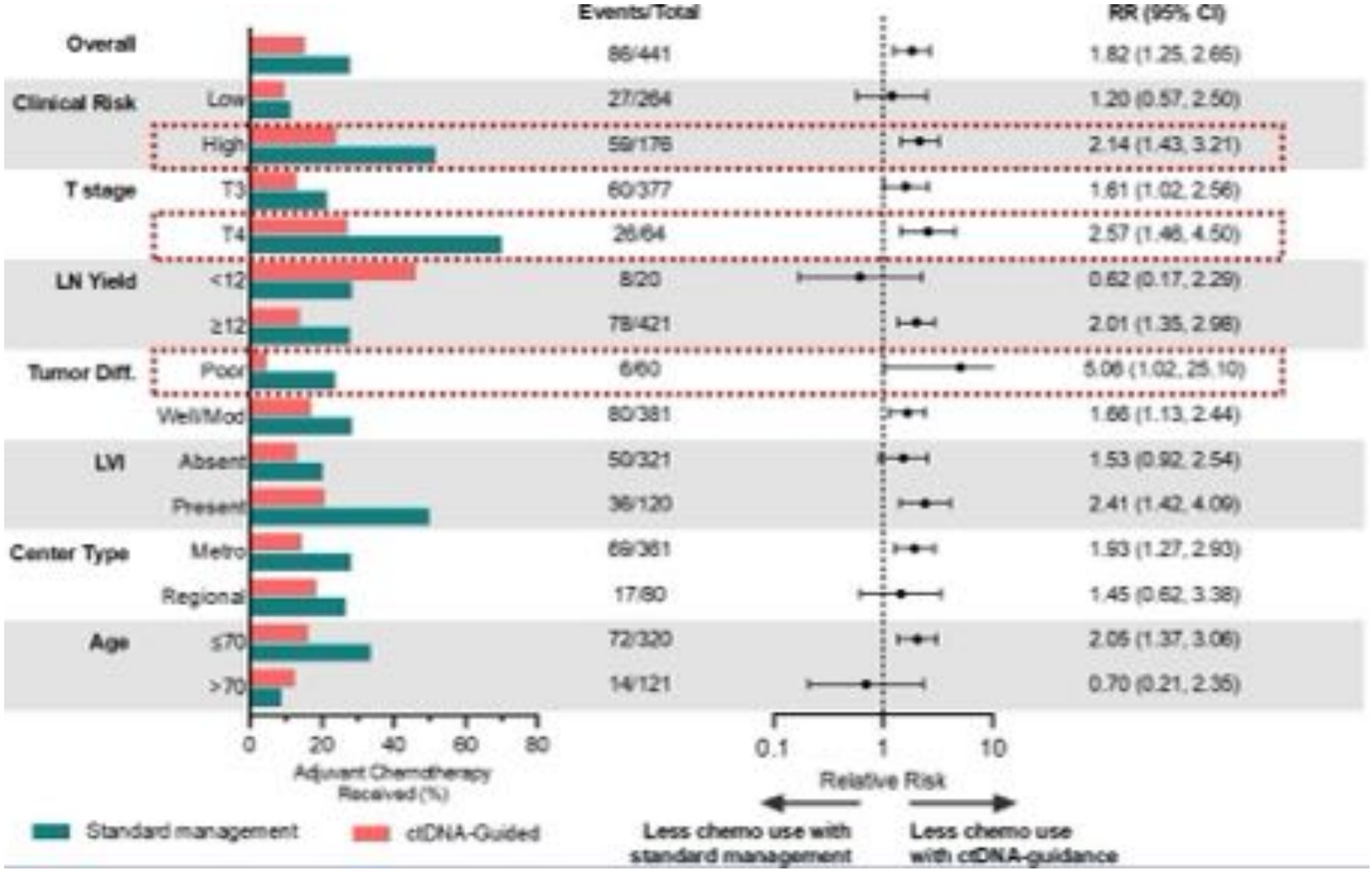
ctDNA detection by Safe-Sequencing System*

(error reduction technology designed to detect low frequency mutations using unique molecular identifier)

*Kinde et al. Proc Natl Acad Sci U S A. 2011;108(23):9530-5

Adjuvant Chemotherapy Delivery

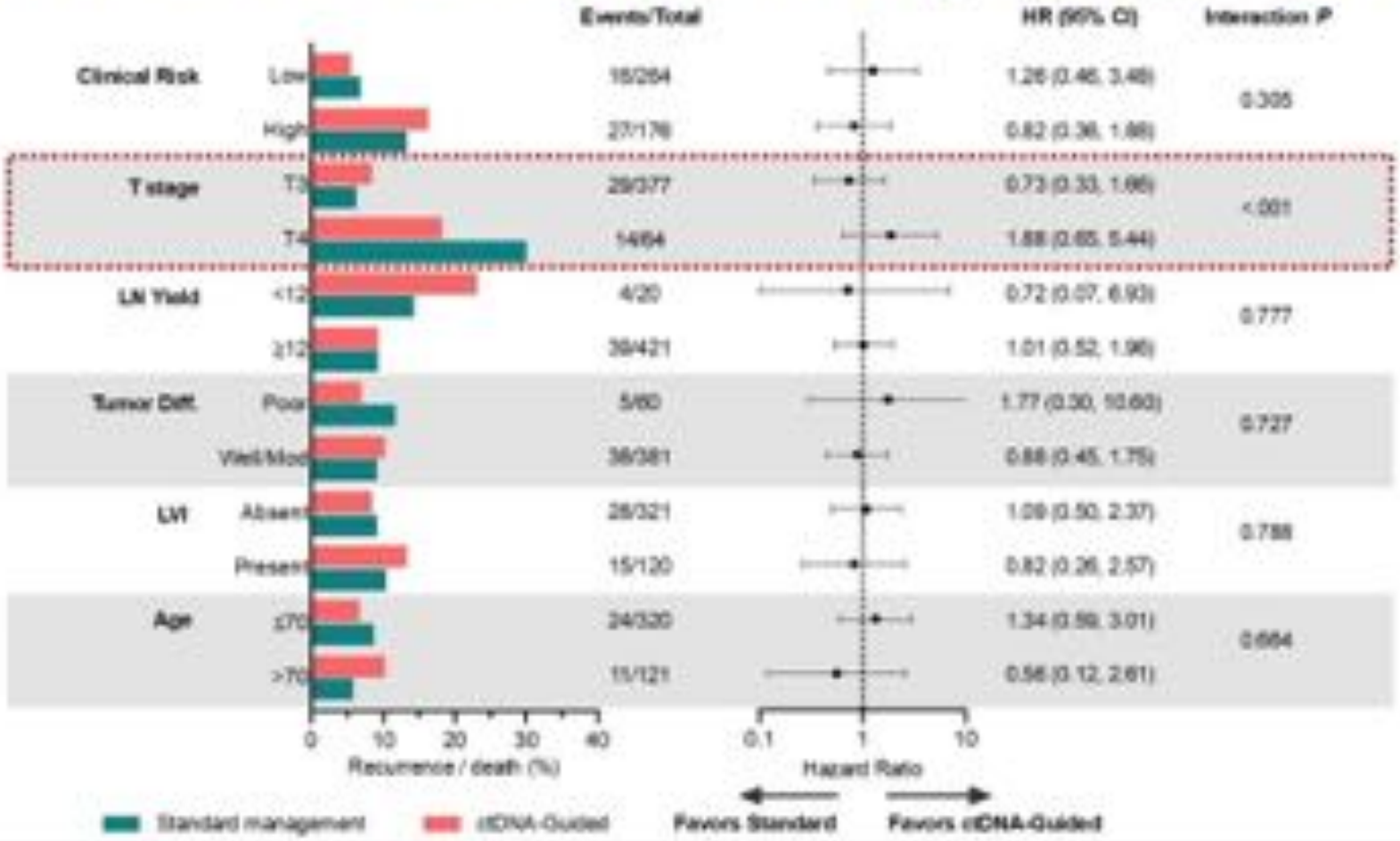
	ctDNA N = 294	Standard N = 147	P-value
Adjuvant Chemo Received n (%)	45 **(15%)**	41 **(28%)**	0.0017
Chemo Regimen			
Oxaliplatin-Based	62%	10%	<0.0001
Single Agent Fluoropyrimidine	38%	90%	



Tie et al. ASCO 2022. #LBA100

Recurrence-Free Survival

Recurrence-Free Survival in Key Subgroups



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Presented by: Jeanna Tie

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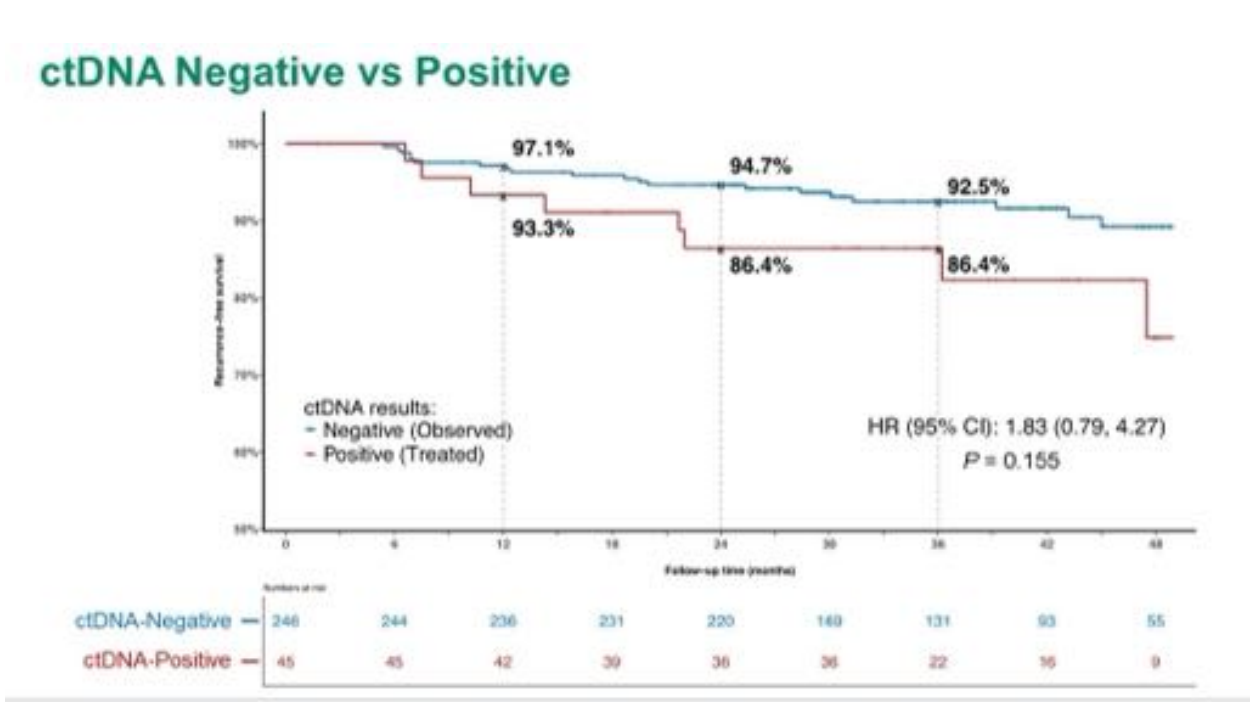
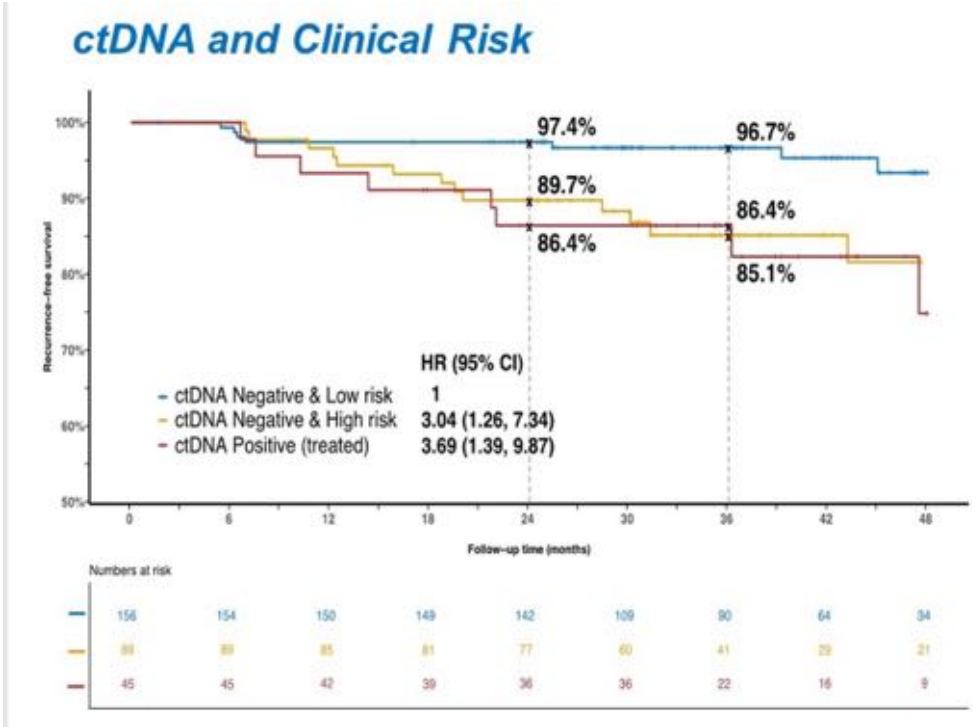
ctDNA Status and Recurrence-Free Survival



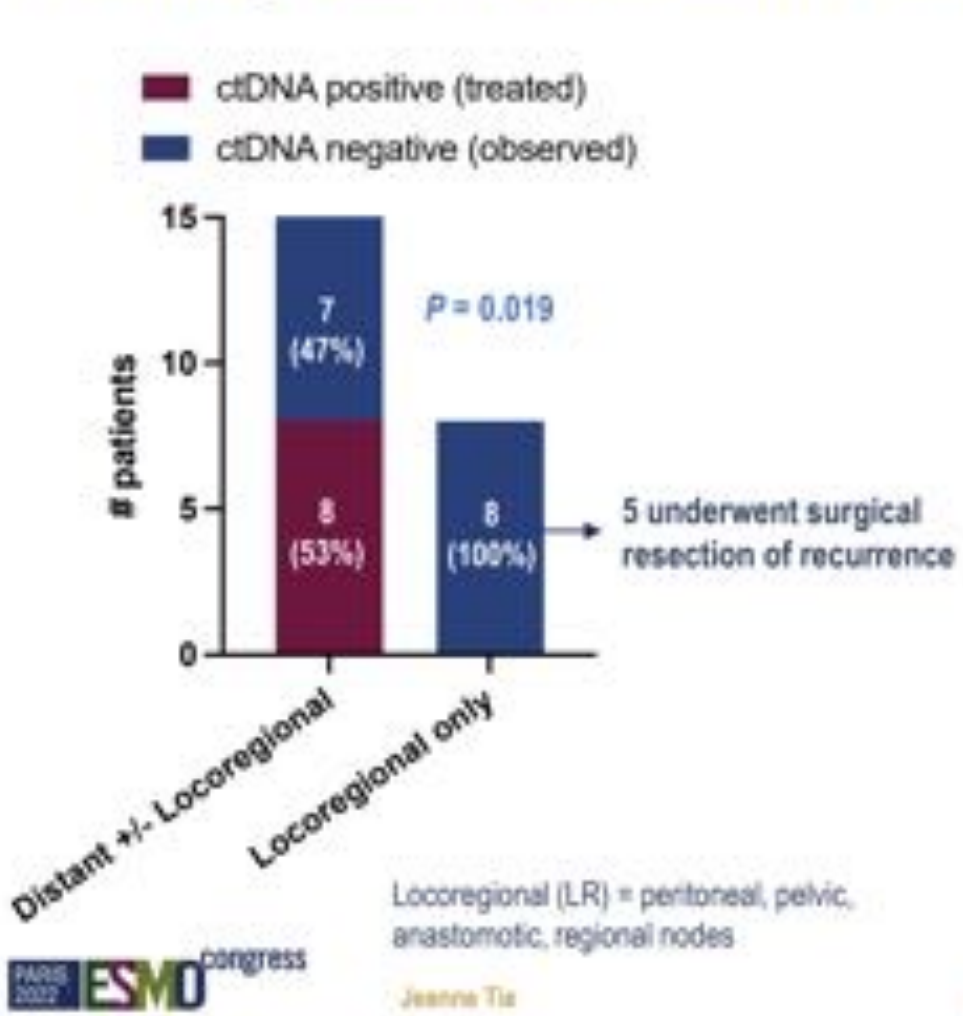


Tie et al. ASCO 2022. #LBA100

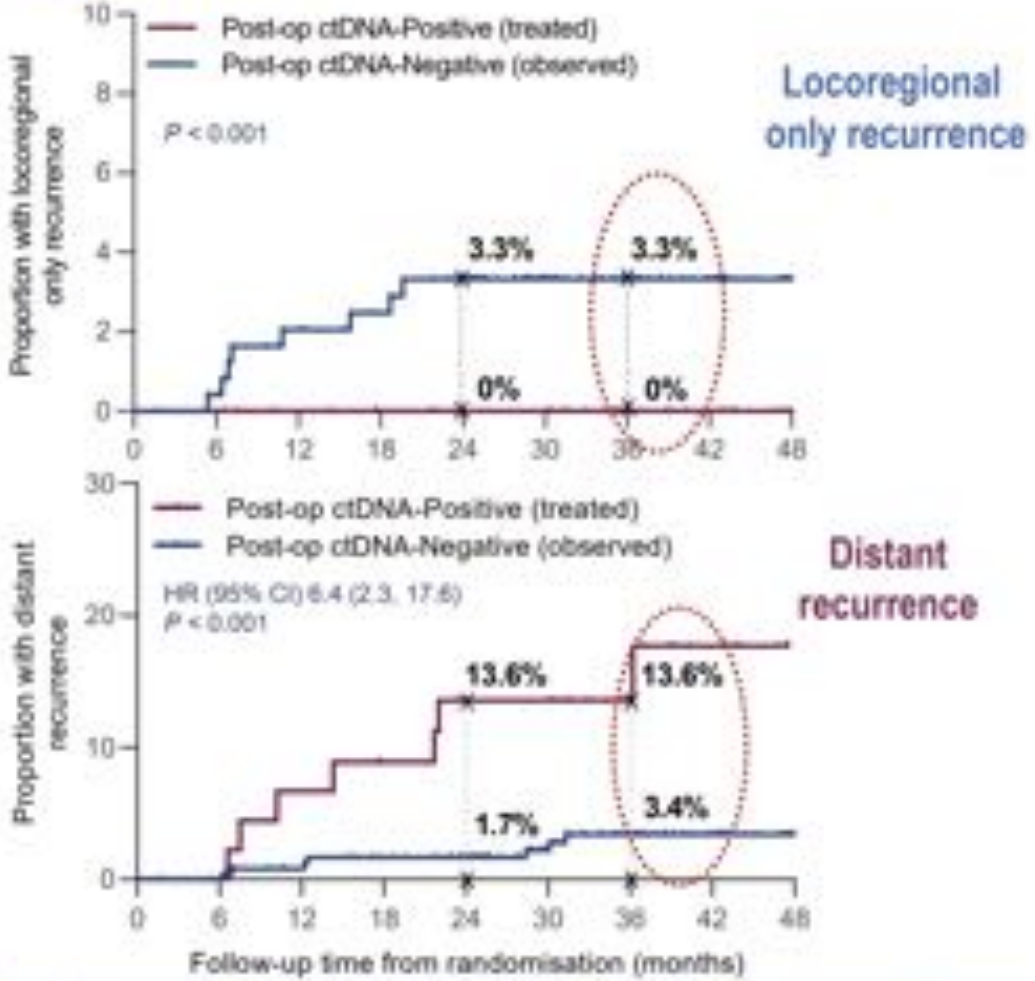
Summary

- **For patients with stage II colon cancer, a ctDNA-guided approach (treating only patients with a positive ctDNA after surgery) compared to standard-of-care**
  - Substantially reduced the proportion receiving adjuvant chemotherapy (28% → 15%)
  - Did not compromise recurrence-free survival (2-year RFS: 93.5% vs 92.4%)
- **Patients with a positive ctDNA after surgery may derive RFS benefit from adjuvant chemotherapy**
  - Favorable 3-year RFS in patients treated with adjuvant chemotherapy (86.4%) versus low RFS in historical series (< 20%) if untreated
  - Ongoing trials (e.g., COBRA, CIRCULATE, CIRCULATE-PRODIGE) will provide further guidance regarding the optimal use of ctDNA-informed management
- **ctDNA-negative patients have a low recurrence risk without adjuvant chemotherapy**
  - 3-year RFS 92.5% (clinical low risk: 96.7%; T3: 94.2%)

Post-op ctDNA and Sites of Recurrence

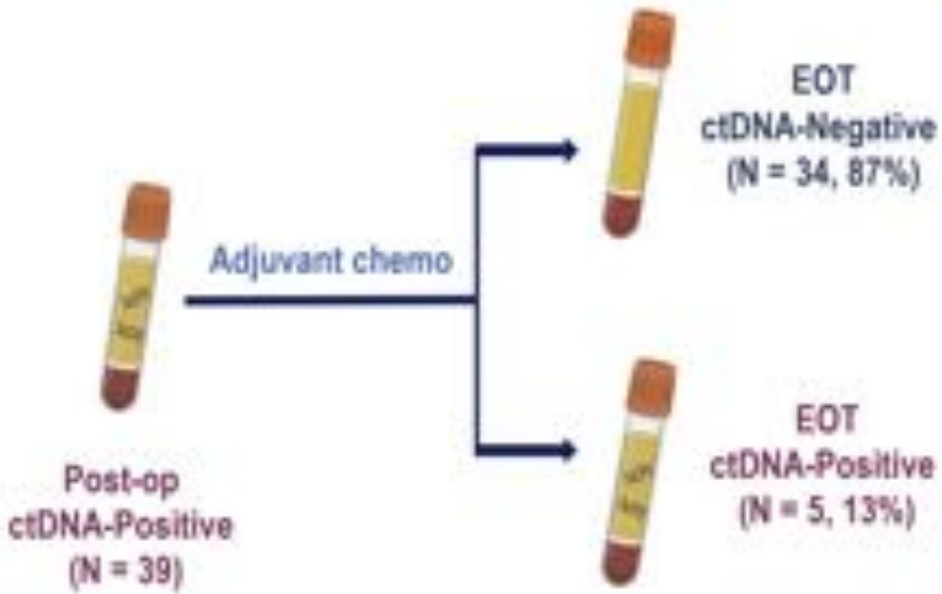


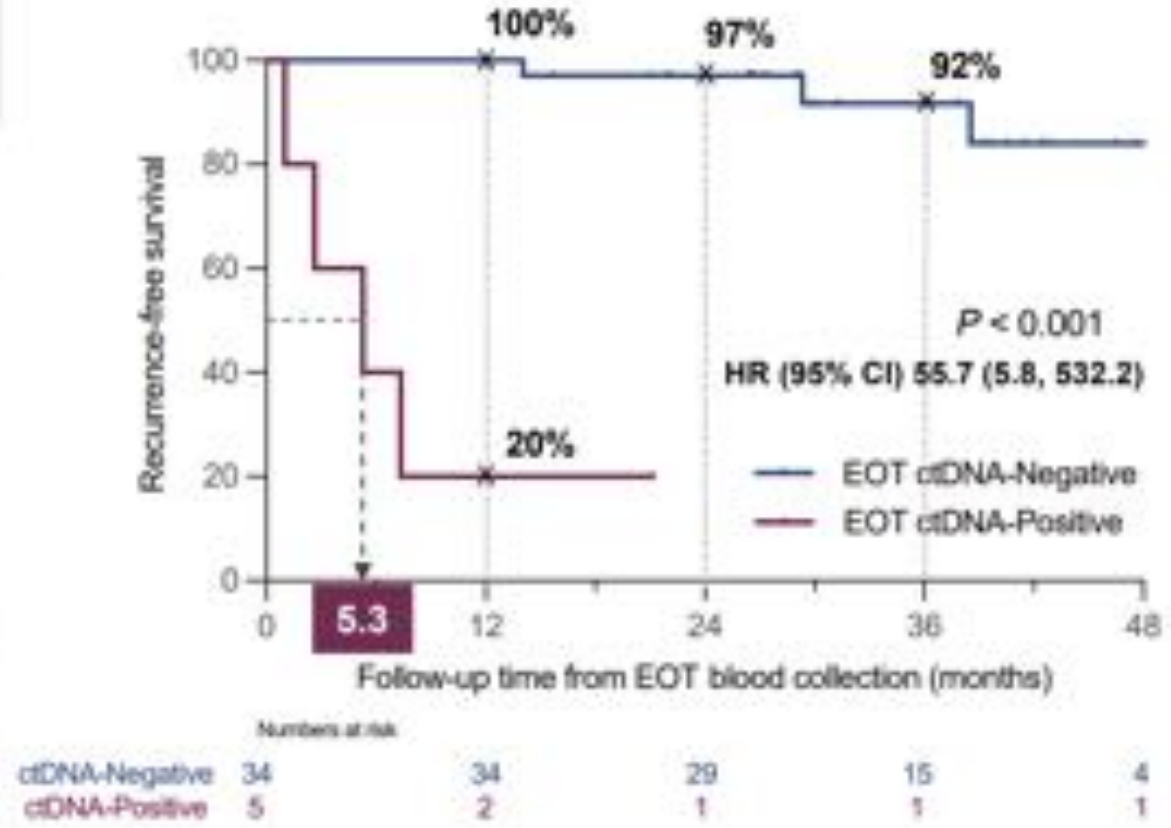
Loccoregional (LR) = peritoneal, pelvic, anastomotic, regional nodes



Post-op ctDNA-Positive: End-of-Treatment ctDNA and RFS

End-of-treatment (EOT) ctDNA collected in 39 of 44 post-op ctDNA-positive patients treated with adjuvant chemotherapy

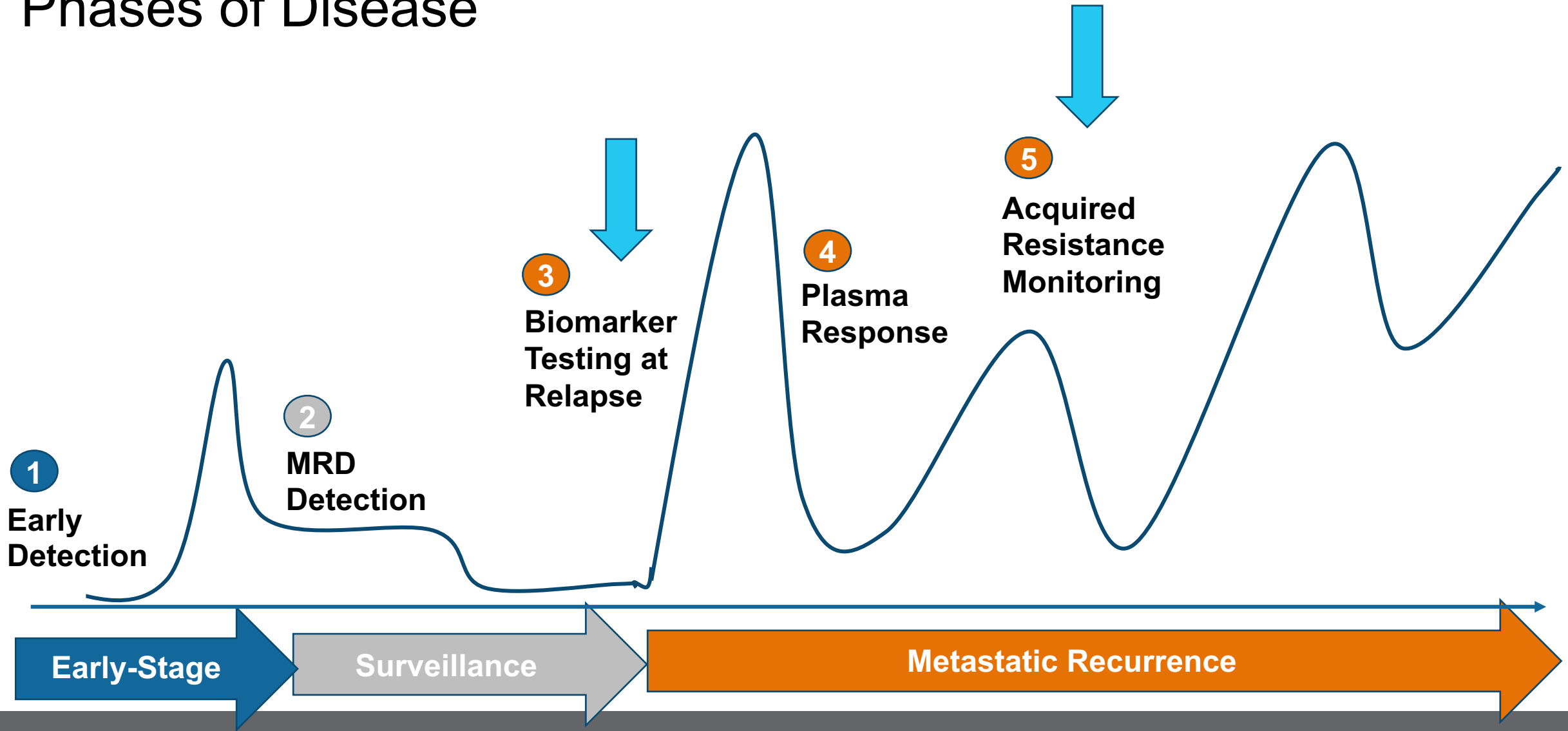




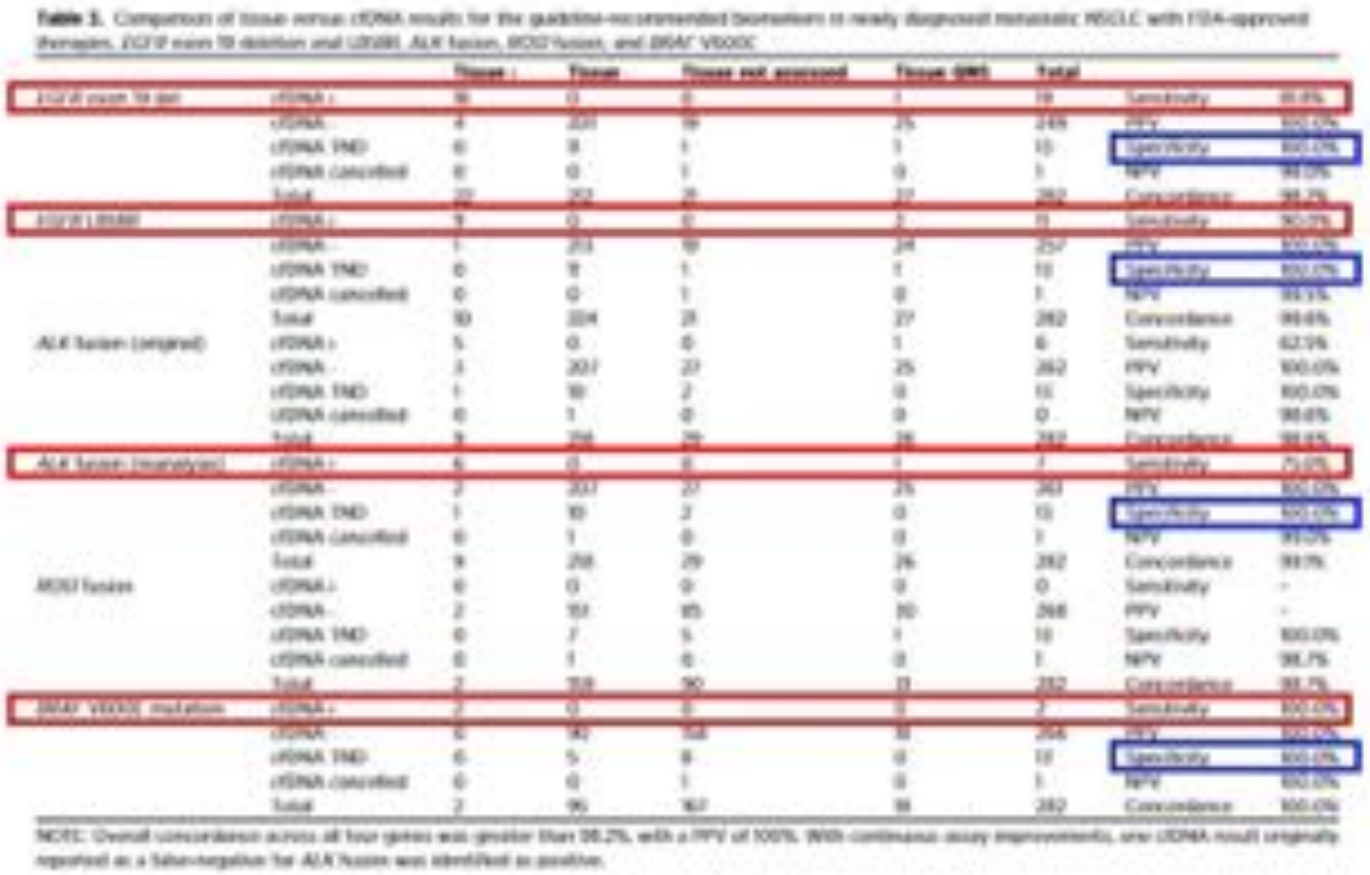
ESMO congress

Jeanne Tie

Cell-free DNA Across Many Phases of Disease



ctDNA sequencing has high sensitivity and specificity to identify actionable genomic alterations



Stage IV NSCLC
Tumor-naïve assay
(Guardant 360)

Leighl N et al. *Clin Cancer Res.* 2019

Bruna Pellini, MD
Assistant Member, Department of Thoracic Oncology, Moffitt Cancer Center

cfDNA for symptomatic patients hospitalized with a new diagnosis of lung cancer

METHODS

PATIENT ENROLLMENT
30 patients were enrolled from December 2021 to August 2022. Overall population received liquid biopsy, only 20 patients performed also conventional biopsy

PLASMA COLLECTION

For each patient plasma sample was collected at time of diagnosis, for patients with any molecular alterations, plasma sample was collected also at time of first revaluation after starting treatment and at time of disease progression

DEMOGRAPHIC AND CLINICAL PATIENT'S CHARACTERISTICS AT DIAGNOSIS	
Median age – yrs	73
Sex- n	14 M 16 F
Smoking status - n	8 Current smoker 11 Former smoker 11 Never smoker
Performance status (ECOG)	12 PS ECOG 1 6 PS ECOG 2 12 PS ECOG 3
Disease stage	28 stage IV 2 stage III
First Symptoms	11 Dyspnoea 8 Pain 4 Cough/Haemoptysis 7 Other

1/30 ALK+

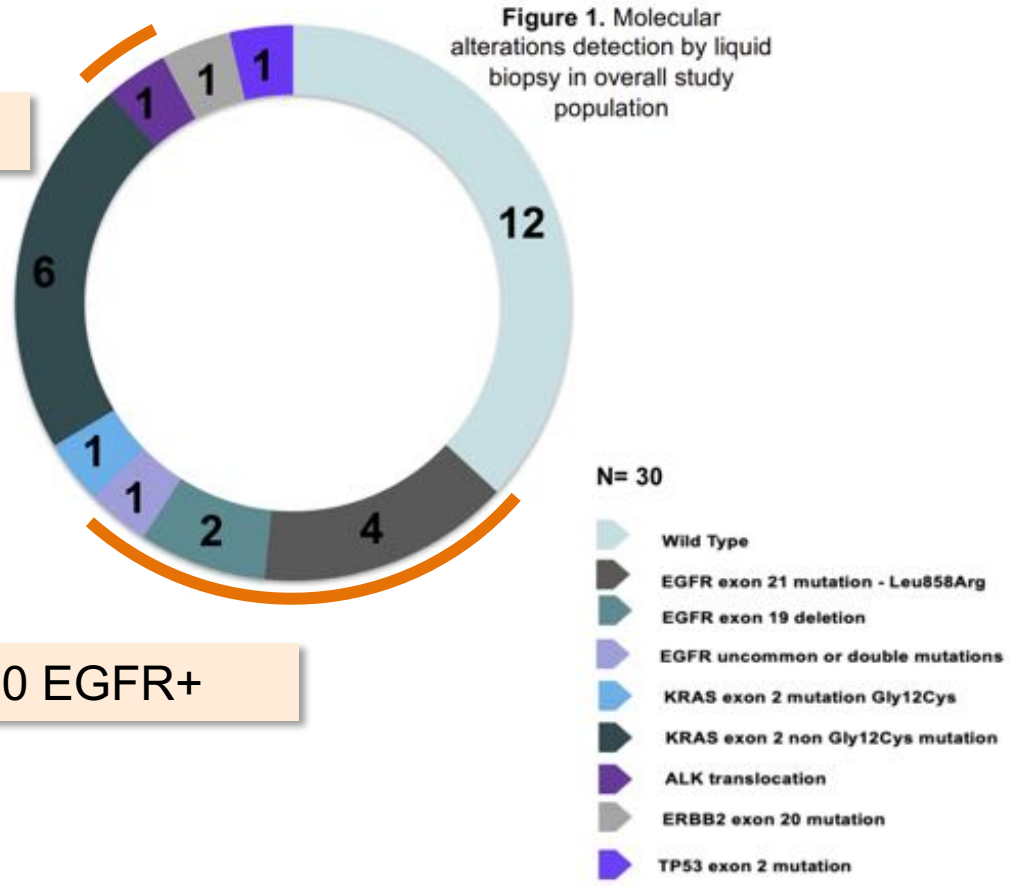
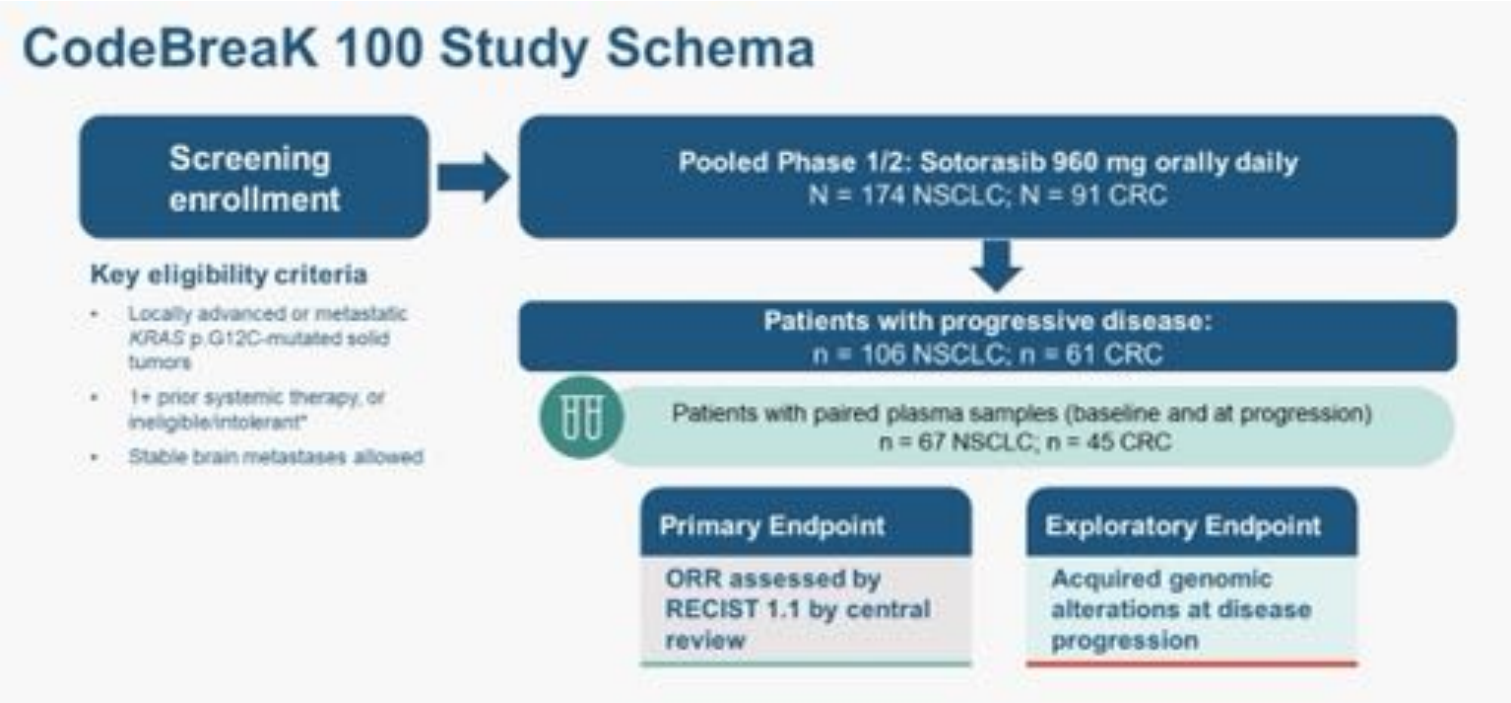


Figure 1. Molecular alterations detection by liquid biopsy in overall study population

7/30 EGFR+

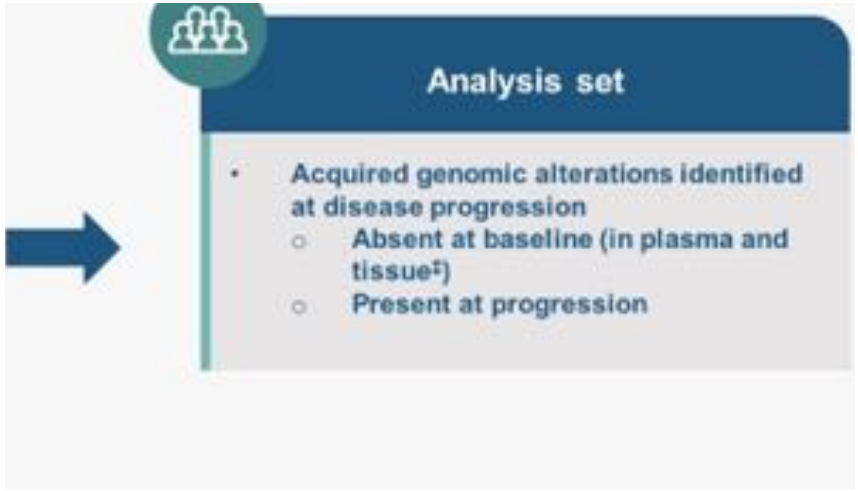
Median time (days) from assay to result
Liquid Biopsy 11 days
Conventional Biopsy 20 days

Parisi et al. ESMO 2022. #1099P



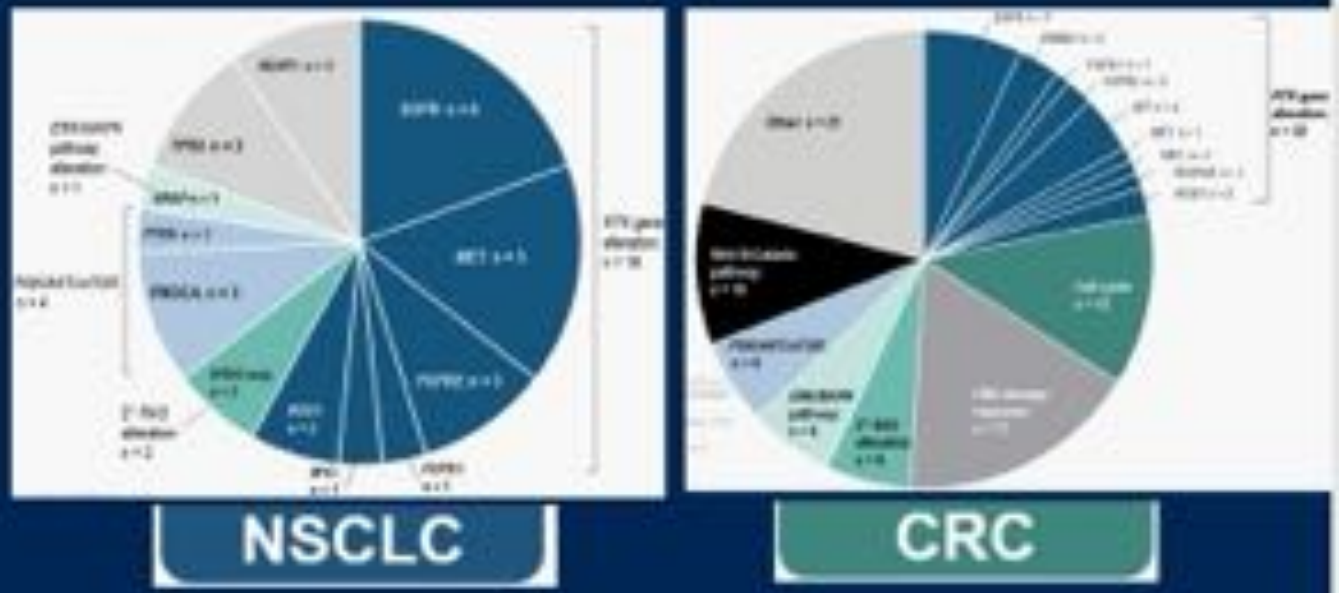
Presented by Bob Li. ASCO 2022.

cfDNA Assays



Largest evaluation of acquired resistance to sotorasib in *KRAS* p.G12C-mutated NSCLC and CRC: plasma biomarker analysis of CodeBreaK 100 Li et al.

- In both NSCLC and CRC patients, acquired resistance as detected by ctDNA was heterogenous
- Despite this, many mutations were in genes that have targeted therapies, particularly in RTKs
- This could lead to clinical utility studies combining sotorasib with other inhibitors.



PRESENTED BY:
Ben Ho Park MD, PhD
Professor of Medicine, VICC, VUMC

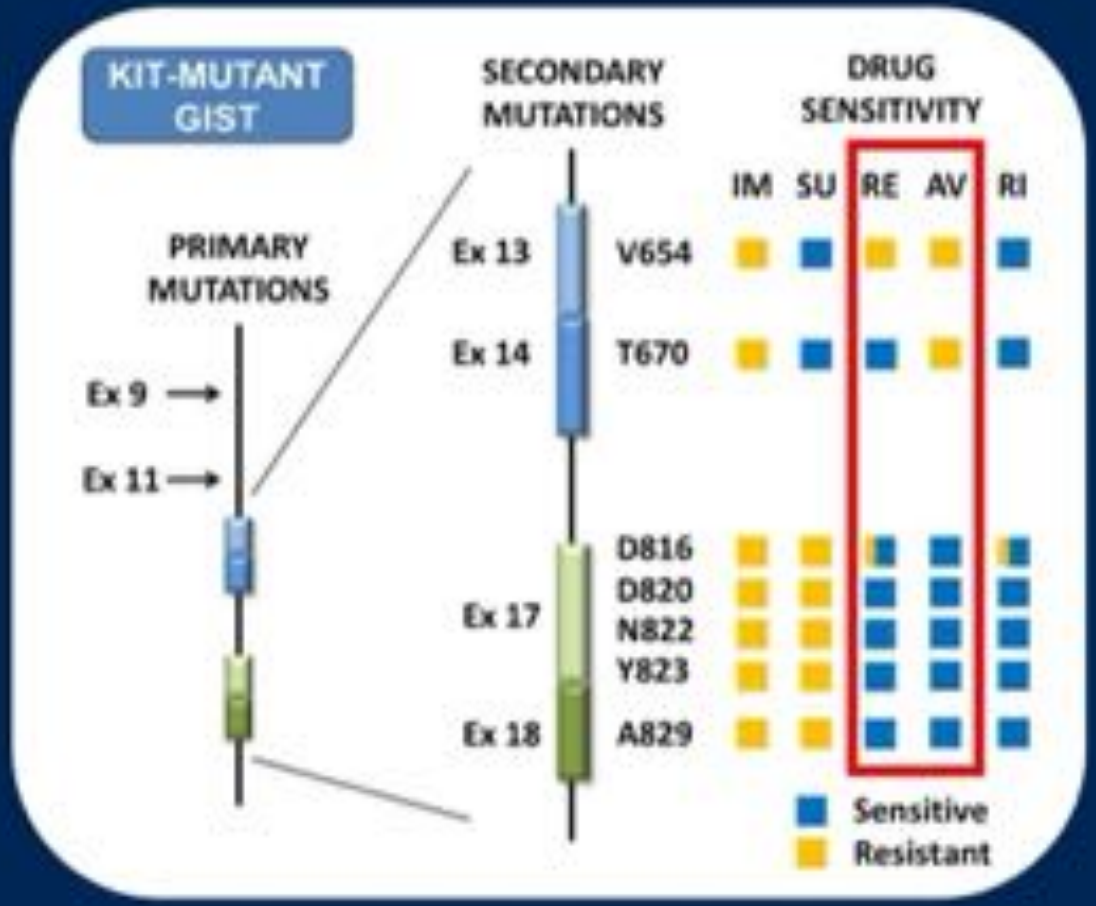
Background VOYAGER phase III clinical trial

VOYAGER

3L/4L GIST R, 1:1

- Avapritinib N=240
- Regorafenib N=236

Primary endpoint: PFS



2022 ASCO ANNUAL MEETING #ASCO22

César Serrano, MD PhD
Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

ASCO

Circulating tumor DNA (ctDNA) analyses of the phase III VOYAGER trial: KIT mutational landscape and outcomes in patients with advanced gastrointestinal stromal tumor (GIST)

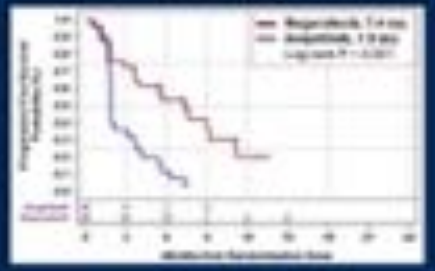
César Serrano et al.

- **ctDNA sequencing correlates with outcomes in pretreated GIST.** Identification of ATP binding pocket mutations in KIT negatively correlates with avapritinib activity.
- The **multikinase inhibitory nature of regorafenib** may be relevant for its clinical activity regardless the type of KIT secondary mutation by plasma.
- Potential clinical utility of selecting more targeted therapy in the absence of mutation

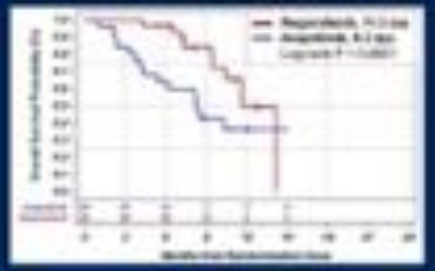
ctDNA mutations & outcomes: **ATP-binding pocket**

Shorter mPFS and mOS in patients with ctDNA+ ATP binding pocket mutations treated with AVAPRITINIB v. REGORAFENIB

Median PFS



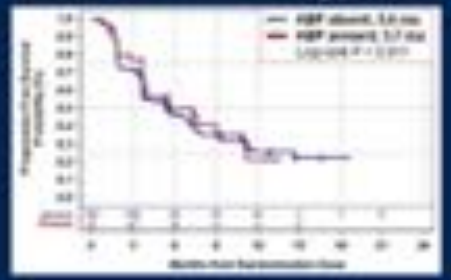
Median OS



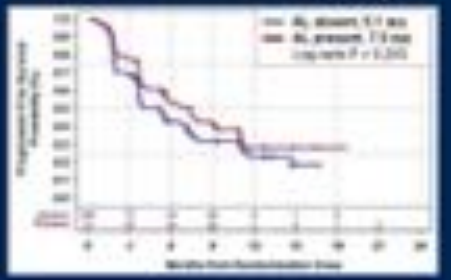
ctDNA mutations & outcomes: **Regorafenib**

REGORAFENIB showed similar activity regardless KIT mutational status and the location of KIT mutation

Median PFS – ATP binding pocket



Median PFS – Activation loop



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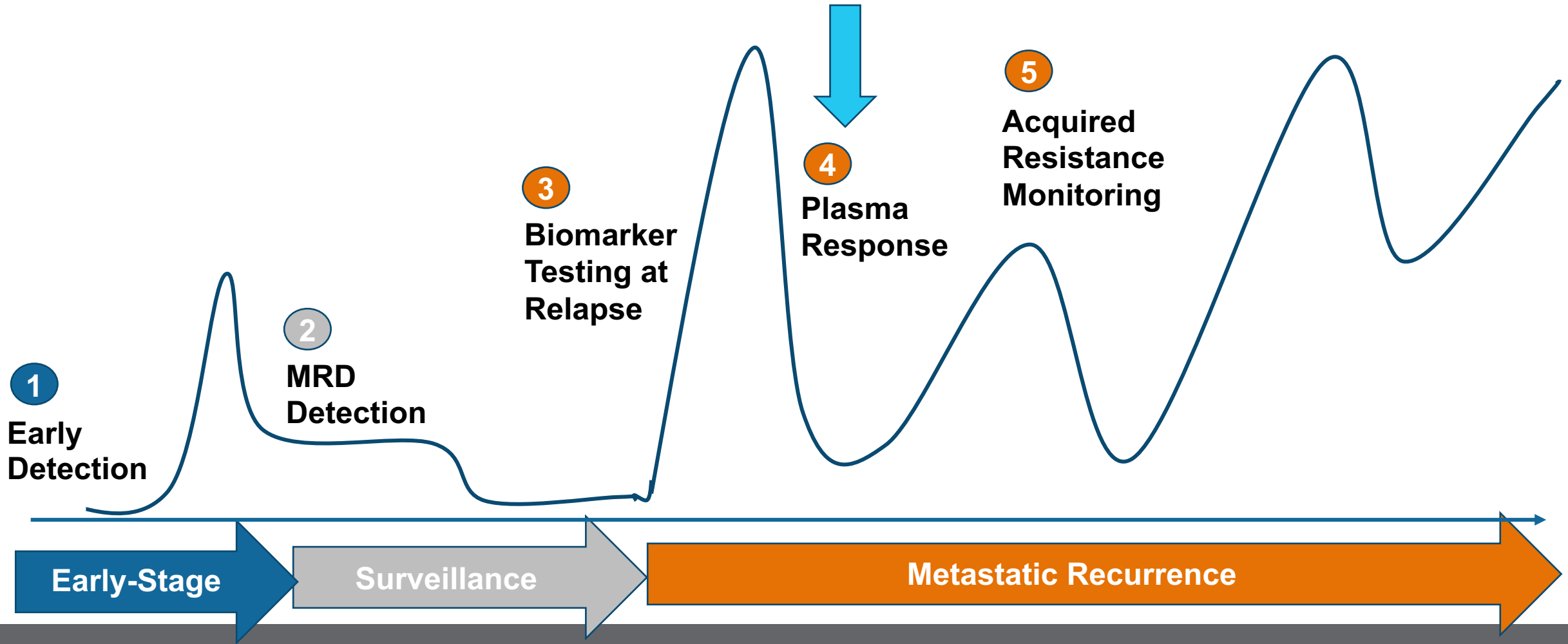
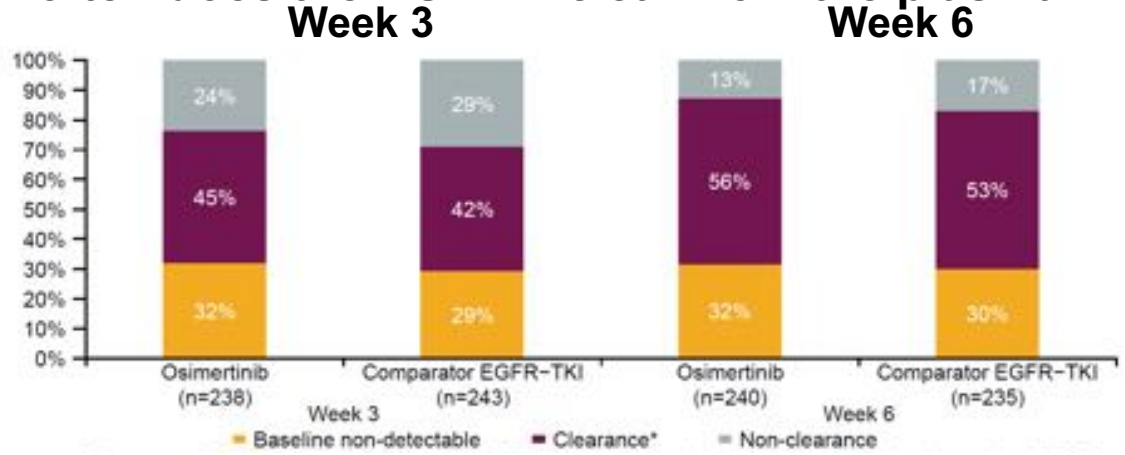
Ben Ho Park MD, PhD
Professor of Medicine, VICC, VUMC

Cell-free DNA Across Many Phases of Disease



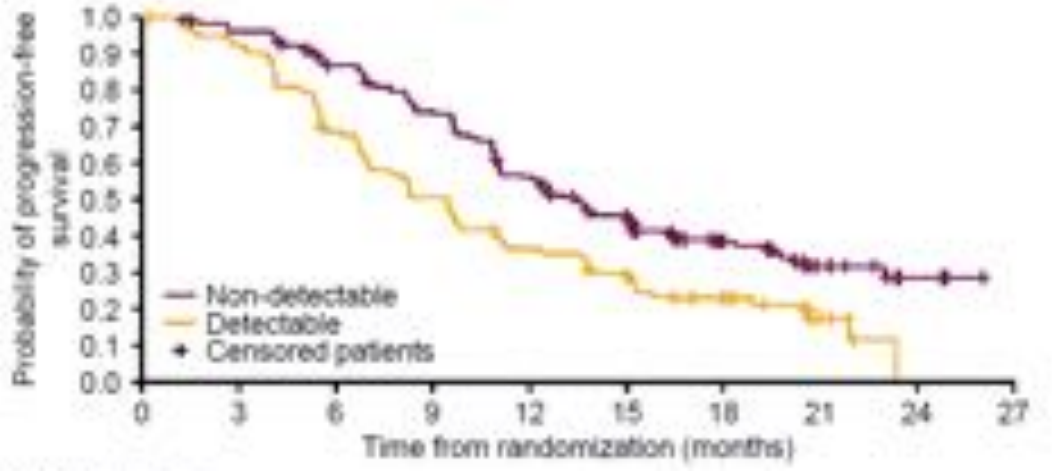
How often does the EGFRm clear from the plasma?



At 6 weeks osimertinib treatment

- 13% undetectable at baseline
- 56% convert to negative
- 32% remain detectable

Impact of positive week 3 plasma EGFR on PFS?

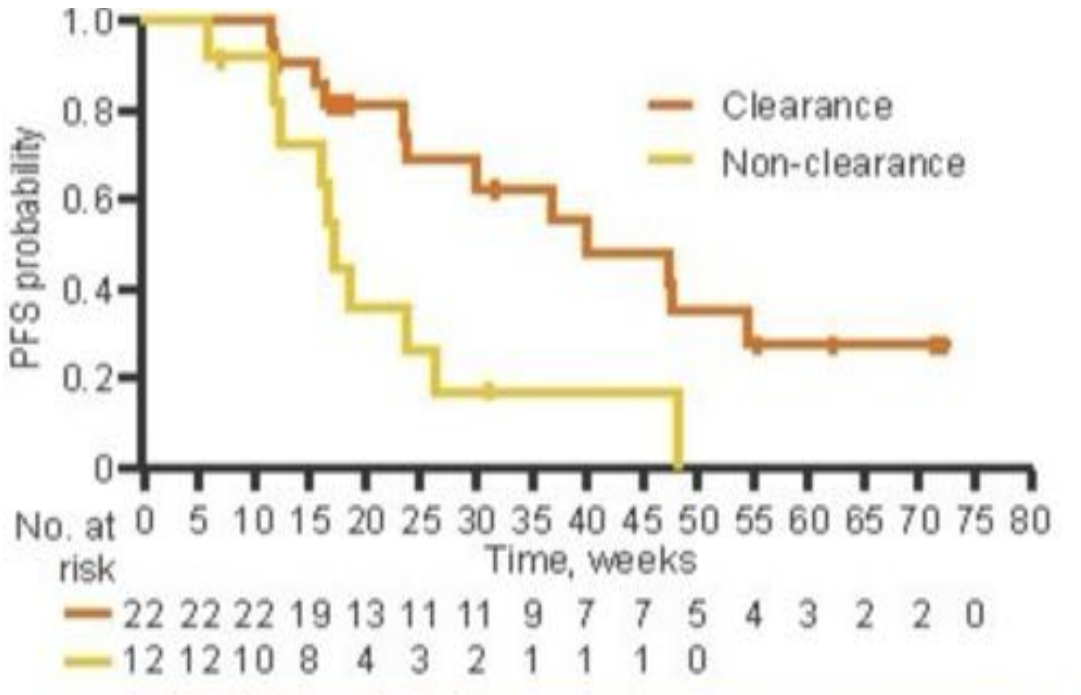


Plasma EGFR positive at 3 weeks
PFS 9.5 vs 13.5 months (HR 0.57, 0.4-0.7)

Plasma EGFR positive at 6 weeks
PFS 8.2 vs 13.5 months (HR 0.51, 0.4-0.7)

Zhou et al. ASCO 2019. Abstract #9020

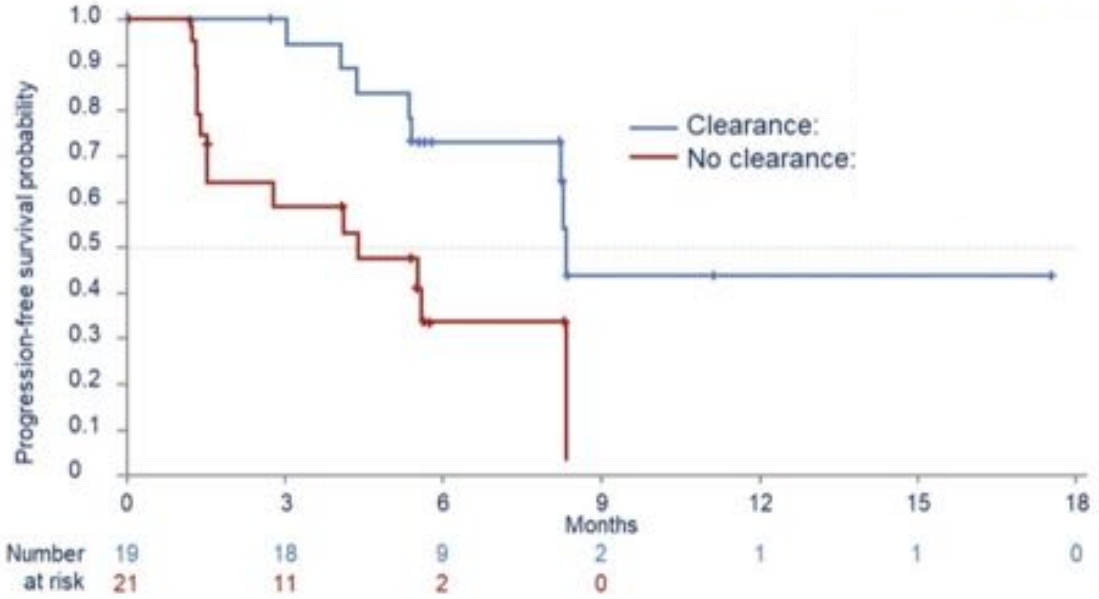
TATTON Savolitinib + Osimertinib for MET+ EGFR TKI Resistance



CfDNA status at cycle 3 or 4

PFS 3.9 vs 9.1 months
(HR 0.34, 0.14-0.81)

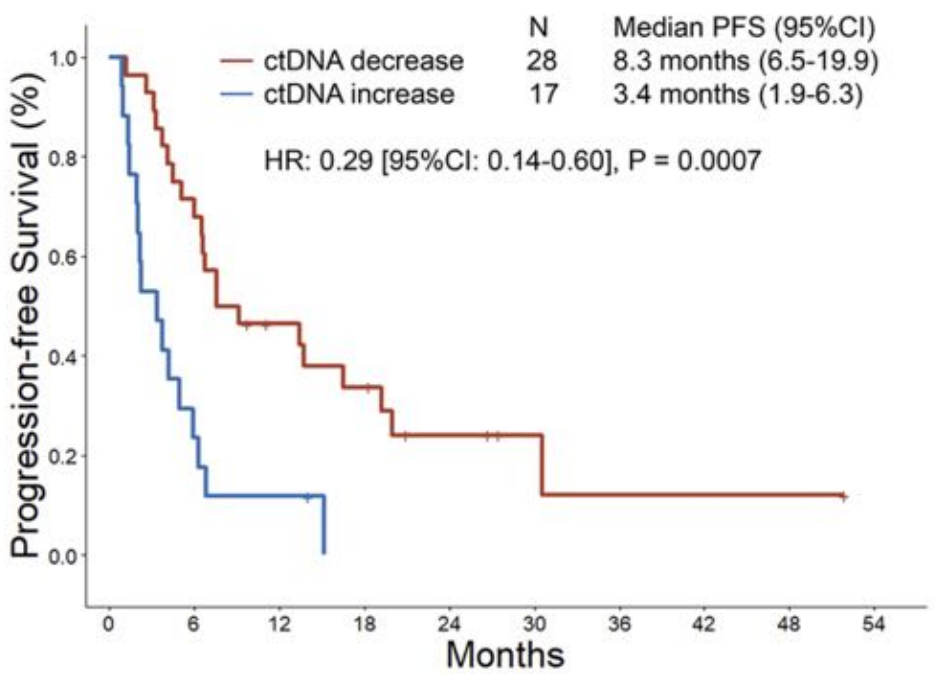
U3 1402-A-U102: HER3-ADC for EGFR TKI resistance



CfDNA status at week 3/6

PFS 4.4 vs 8.3 months
(HR 0.33, 0.13-0.81)

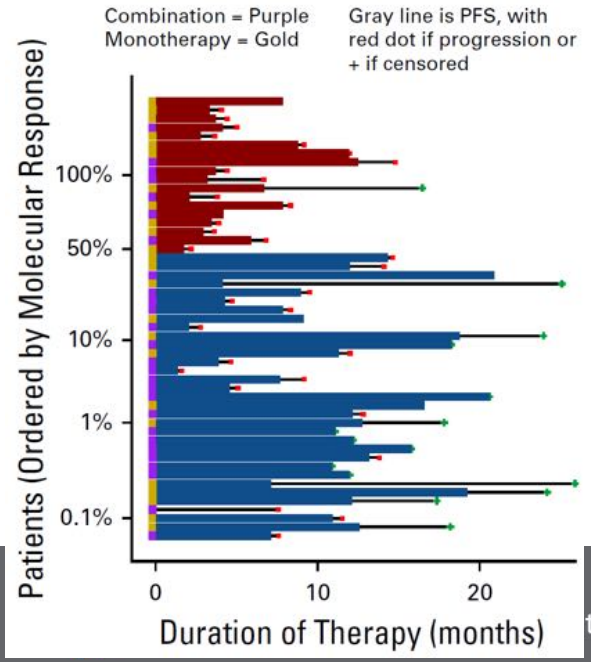
Hartmaier et al. AACR 2020. Abstract CT303; Jänne et al. ASCO 2021. Abstract #9007.

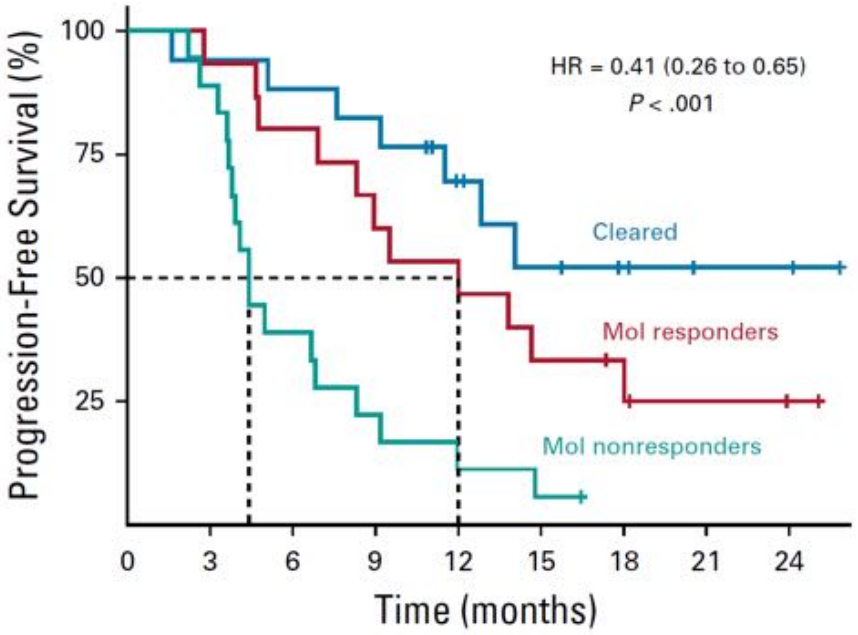


Early cfDNA Trends Predict Survival Outcomes on ICI Therapy

cfDNA status 1st follow up (mean 21 days):
8.3 vs 3.4 months (HR 0.29, 0.14-0.60)

Ricciuti et al. BMJ. 2021;9:3001504





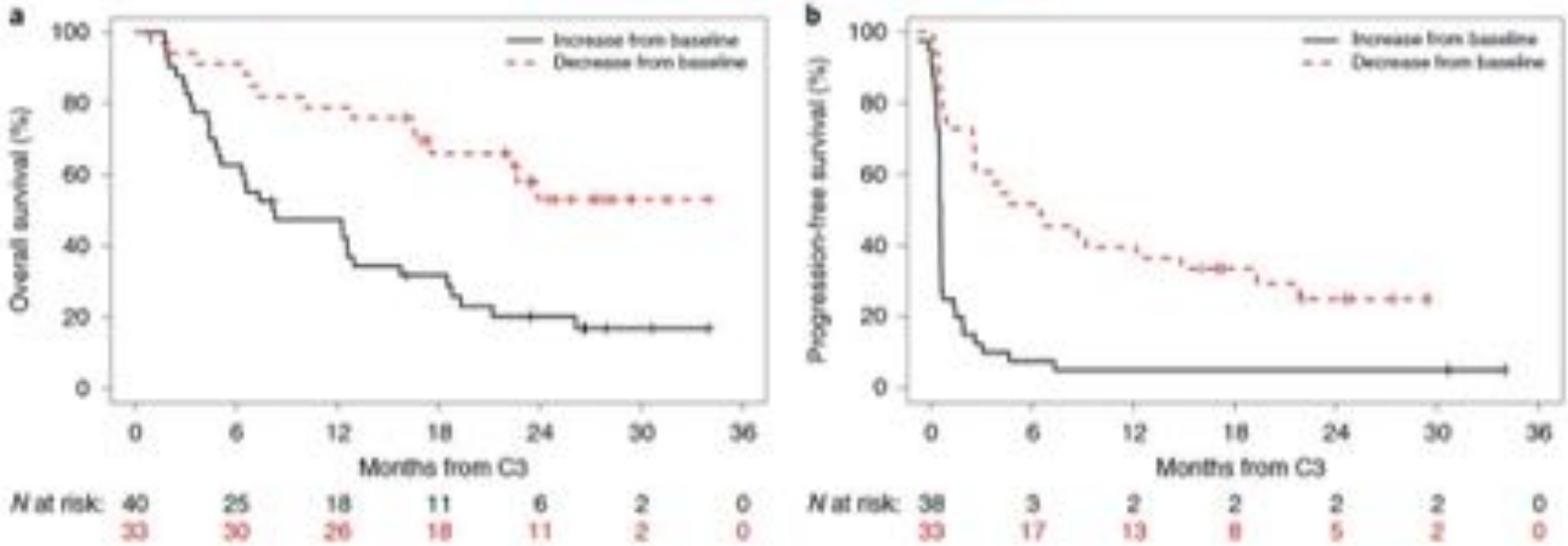
cfDNA status at 9 weeks:

12.8 vs 4.4 months
HR 0.20 (0.08-0.50)

Thompson et al. JCO Precis Oncol. 2021;5.

ctDNA decrease during pembrolizumab treatment is associated with favorable response to therapy and with better outcomes

Advanced HNSCC, TNBC, HGSOC, Melanoma, MST
Tumor-informed assay (Signatera™)



HNSCC, head and neck squamous cell carcinoma; TNBC, triple negative breast cancer; HGSOC, high-grade serous ovarian cancer; MST, mixed solid tumors

Bratman SV et al. *Nat Cancer*. 2020

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PRESENTED BY:
Bruna Pellini, MD
Assistant Member, Department of Thoracic Oncology, Moffitt Cancer Center

ASCO AMERICAN SOCIETY OF CLINICAL ONCOLOGY KNOWLEDGE CONQUERS CANCER

Summary

- cfDNA offers a tool to improve cancer therapy across disease stages, from early detection to management of acquired resistance in the metastatic setting
- Plasma MRD status may inform selection of high-risk patients for adjuvant systemic therapy in stage II colon cancer
- Plasma clearance can predict for treatment benefit in the early and advanced stage setting
- Rapid identification of actionable biomarkers via ctDNA may allow for more effective personalized treatment strategies.