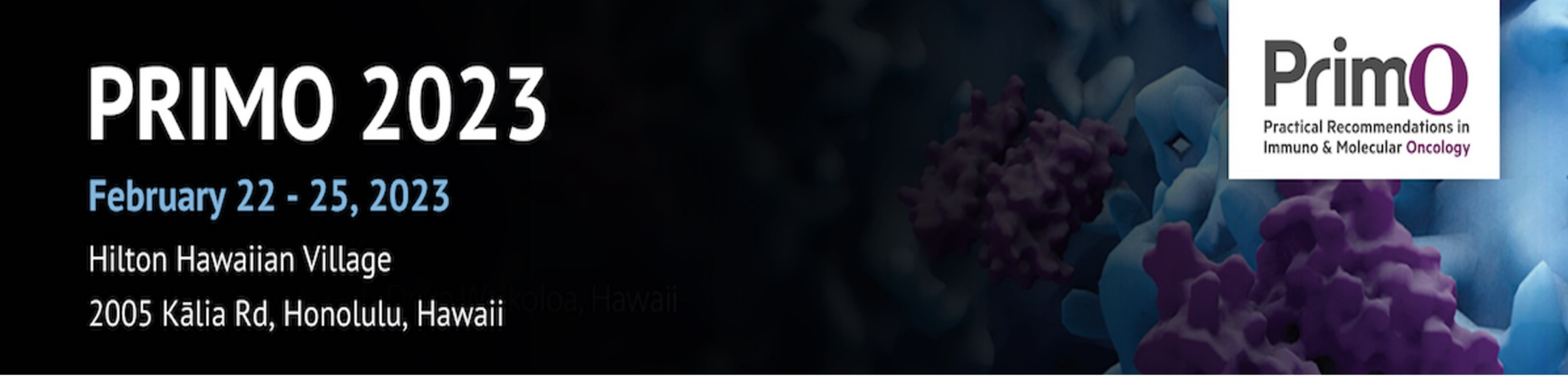


Liquid Biopsy: Advances in the Last Decade and Future Directions

Edgardo S. Santos Castillero, M.D., FACP
Genesis Care US
Medical Director of Research Services/Thoracic Oncology
Clinical Associate Professor
Charles E. Schmidt College of Medicine/Florida Atlantic University
FLASCO Treasurer & FLASCO Foundation President

February 24, 2023

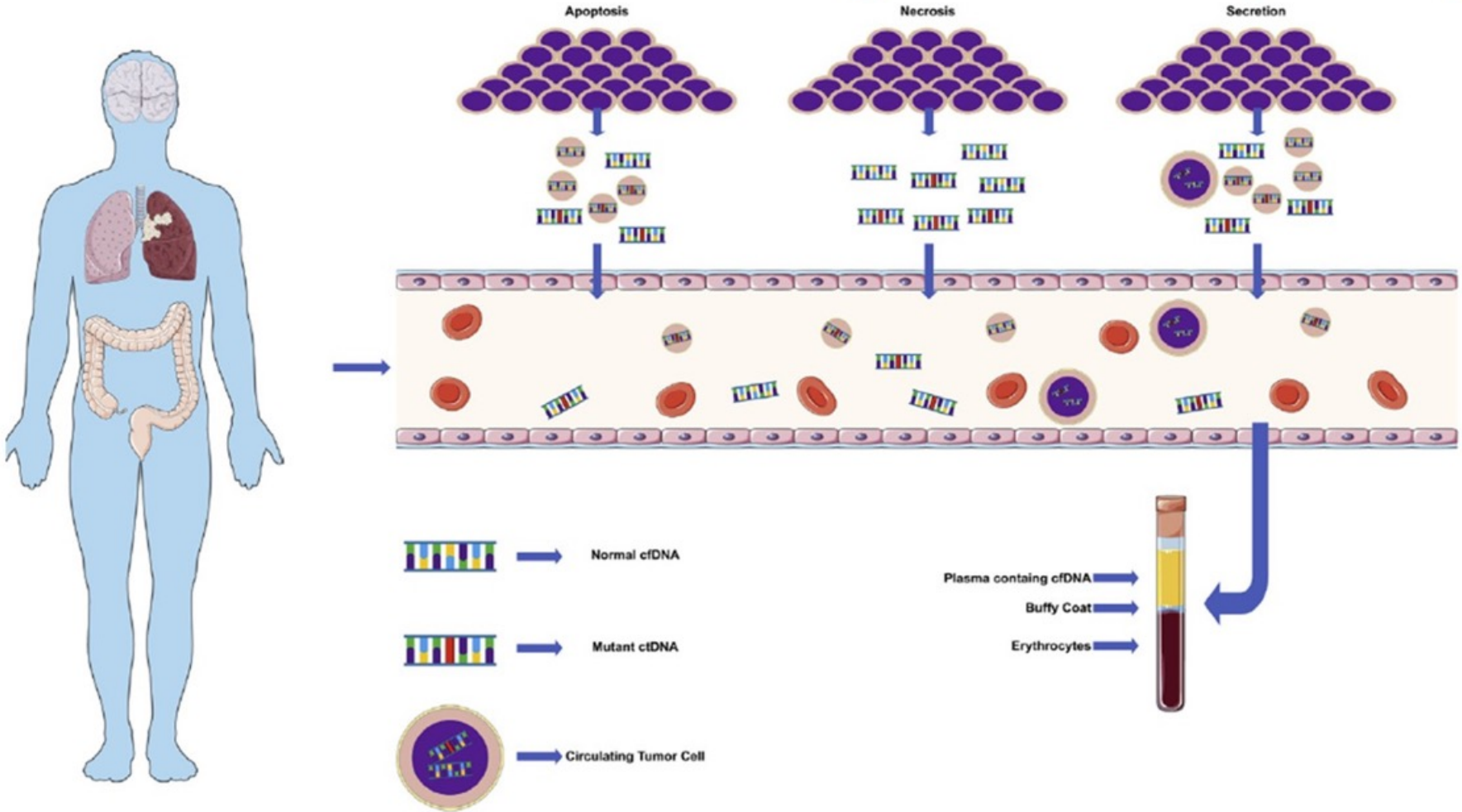
GenesisCare OLA Oncology Latin American Association FLASCO FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY

PrimO
Practical Recommendations in
Immuno & Molecular Oncology

Outline

- Cell-free DNA (cfDNA) and circulation tumor DNA (ctDNA) [liquid specimen]
- Background of cfDNA and ctDNA
- Tumor-informed vs tumor-naïve assays
- ctDNA applications in oncology
  - Current
  - Future Directions

Tumor-derived fragments of nucleic acids identified in the blood are called circulating tumor DNA (ctDNA)



Pellini B et al. Thorac Surg Clin. 2020

The history of cell-free DNA (cfDNA) & circulating tumor DNA (ctDNA)

1985: Kary B. Mullis invents PCR

↓

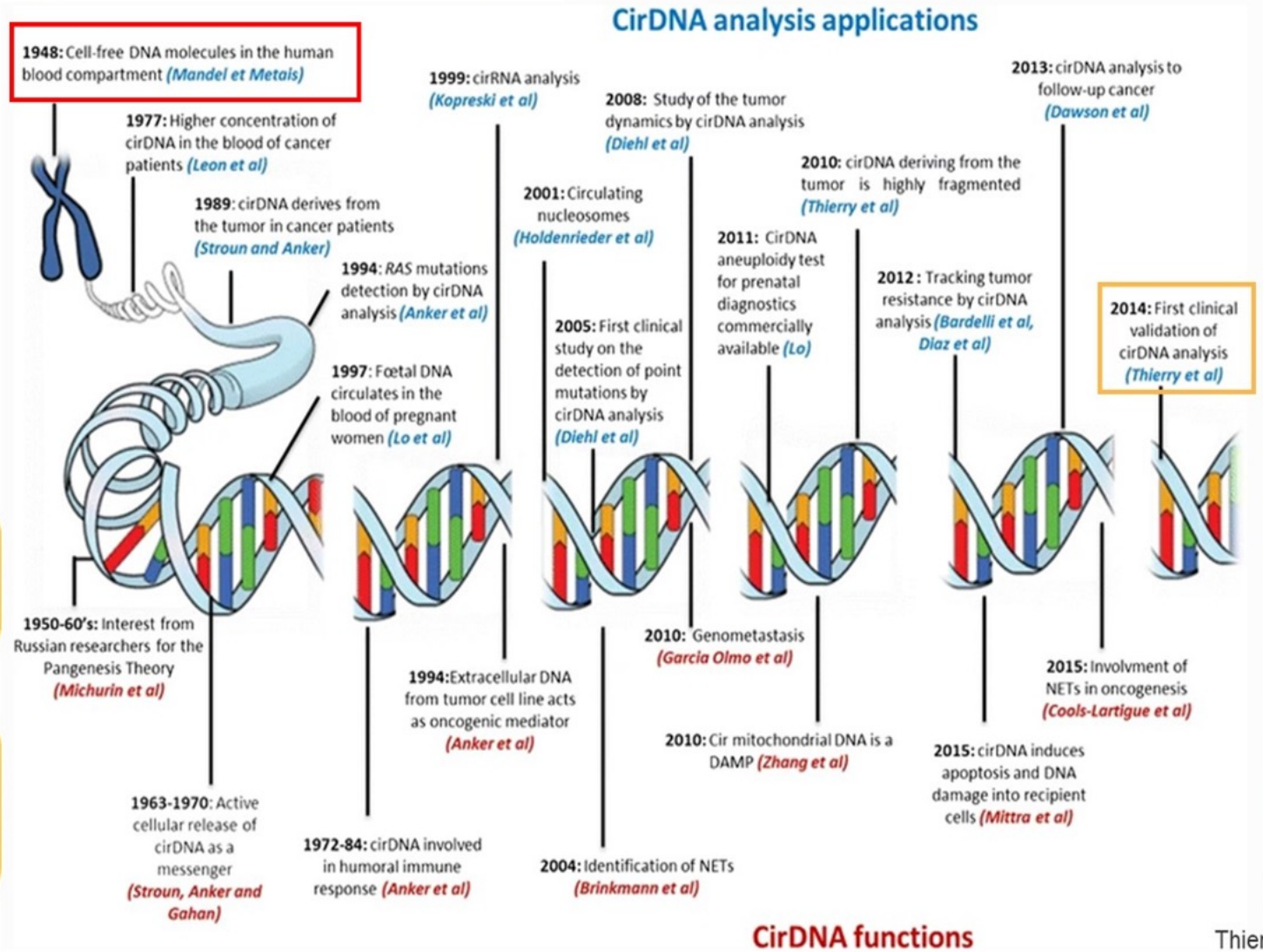
1988: PCR becomes widely available

↓

1992: dPCR concept is described by Sykes et al.

↓

1999: dPCR is developed by Vogelstein and Kinzler



2000: the first NGS technology is launched

↓

2008: 1st paper on human genome sequencing using NGS

↓

2016: first liquid biopsy test approval (cobas® EGFR)

↓

2020: approval of two targeted-NGS liquid biopsy platforms

Thierry AR et al. *Cancer Metastasis*. 2016

Mobley I. A brief history of Next Generation Sequencing. 2021

GenesisCare OLA FLASCO FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY

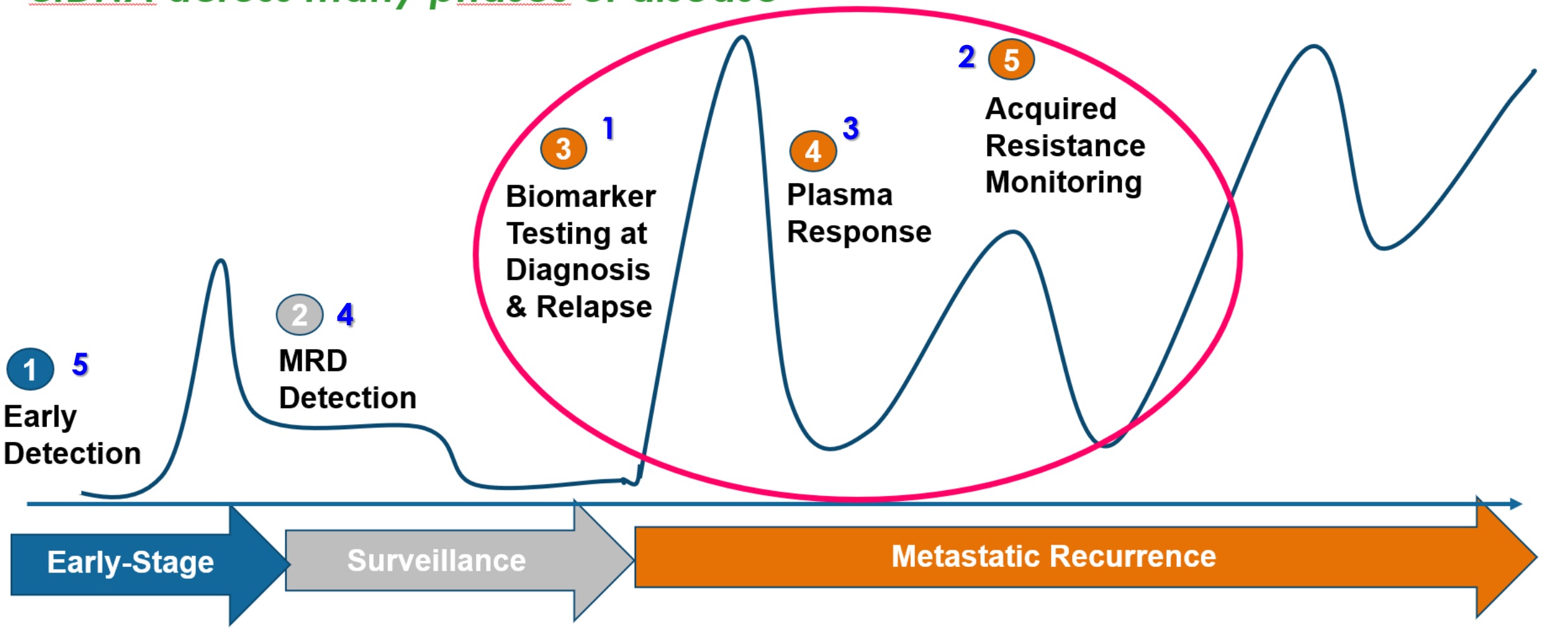
Tumor-informed vs. tumor-naïve assays

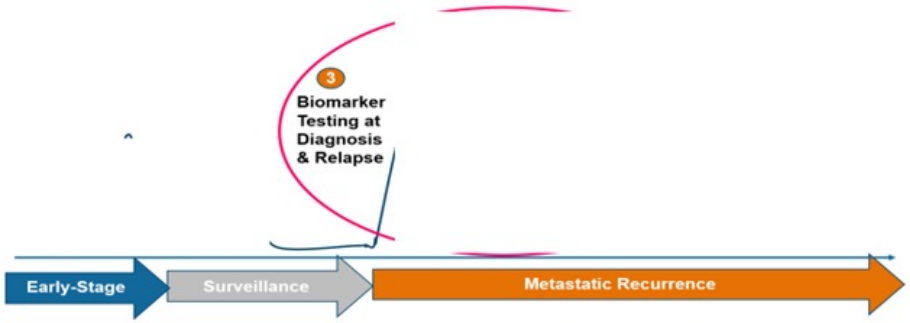
Tumor-Informed	Tumor-naïve
Requires tissue biopsy	No need for biopsy
Personalized assay	Off the shelf assay
Longer turnaround time	Shorter turnaround time
Does not account for tumor heterogeneity	Can detect clonal variants that emerge during follow-up
Potential for better sensitivity and specificity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

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

ctDNA Applications in Oncology

cfDNA across many phases of disease





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

Table 3. Comparison of tissue versus cfDNA results for the guideline-recommended biomarkers in newly diagnosed metastatic NSCLC with FDA-approved therapies, *EGFR* exon 19 deletion and L858R, *ALK* fusion, *ROS1* fusion, and *BRAF* V600E

		Tissue+	Tissue−	Tissue not assessed	Tissue QNS	Total		
EGFR exon 19 del	cfDNA+	18	0	0	1	19	Sensitivity	81.8%
	cfDNA−	4	201	19	25	249	PPV	100.0%
	cfDNA TND	0	11	1	1	13	Specificity	100.0%
	cfDNA cancelled	0	0	1	0	1	NPV	98.0%
	Total	22	212	21	27	282	Concordance	98.2%
EGFR L858R	cfDNA+	9	0	0	2	11	Sensitivity	90.0%
	cfDNA−	1	213	19	24	257	PPV	100.0%
	cfDNA TND	0	11	1	1	13	Specificity	100.0%
	cfDNA cancelled	0	0	1	0	1	NPV	99.5%
	Total	10	224	21	27	282	Concordance	99.6%
ALK fusion (original)	cfDNA+	5	0	0	1	6	Sensitivity	62.5%
	cfDNA−	3	207	27	25	262	PPV	100.0%
	cfDNA TND	1	10	2	0	13	Specificity	100.0%
	cfDNA cancelled	0	1	0	0	0	NPV	98.6%
	Total	9	218	29	26	282	Concordance	98.6%
ALK fusion (reanalysis)	cfDNA+	6	0	0	1	7	Sensitivity	75.0%
	cfDNA−	2	207	27	25	261	PPV	100.0%
	cfDNA TND	1	10	2	0	13	Specificity	100.0%
	cfDNA cancelled	0	1	0	0	1	NPV	99.0%
	Total	9	218	29	26	282	Concordance	99.1%
ROS1 fusion	cfDNA+	0	0	0	0	0	Sensitivity	-
	cfDNA−	2	151	85	30	268	PPV	-
	cfDNA TND	0	7	5	1	13	Specificity	100.0%
	cfDNA cancelled	0	1	0	0	1	NPV	98.7%
	Total	2	159	90	31	282	Concordance	98.7%
BRAF V600E mutation	cfDNA+	2	0	0	0	2	Sensitivity	100.0%
	cfDNA−	0	90	158	18	266	PPV	100.0%
	cfDNA TND	0	5	8	0	13	Specificity	100.0%
	cfDNA cancelled	0	0	1	0	1	NPV	100.0%
	Total	2	95	167	18	282	Concordance	100.0%

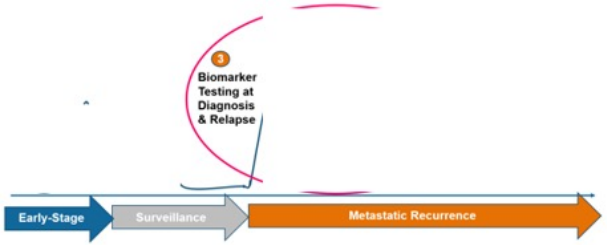
NOTE: Overall concordance across all four genes was greater than 98.2%, with a PPV of 100%. With continuous assay improvements, one cfDNA result originally reported as a false-negative for *ALK* fusion was identified as positive.

1L @ diagnosis

Stage IV NSCLC
Tumor-naïve assay

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

GenesisCare OLA FLASCO FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY



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

Parisi et al. ESMO 2022. #1099P

1/30 ALK+

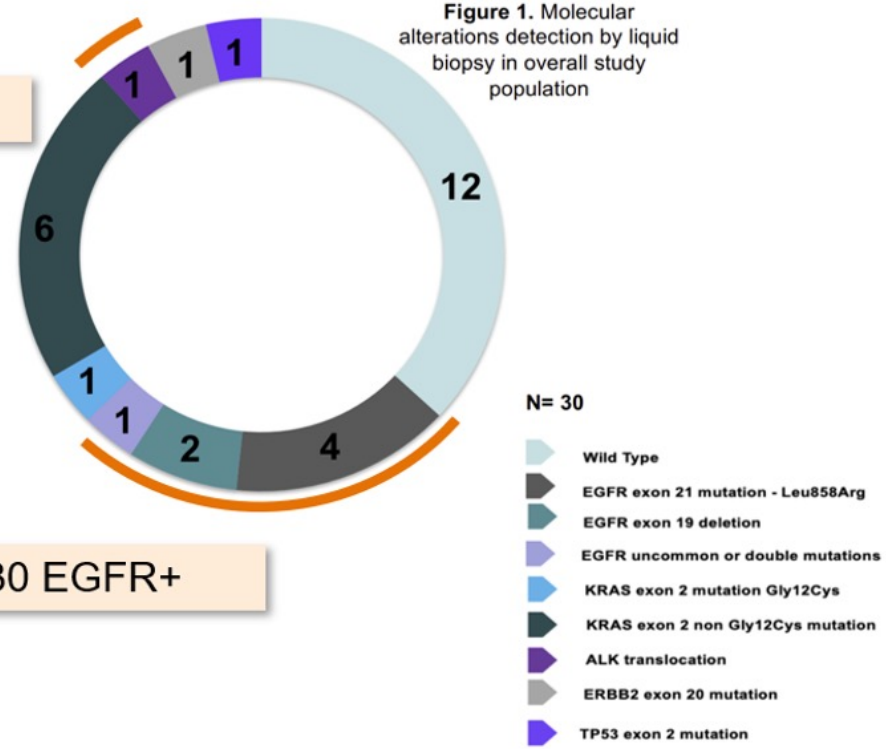
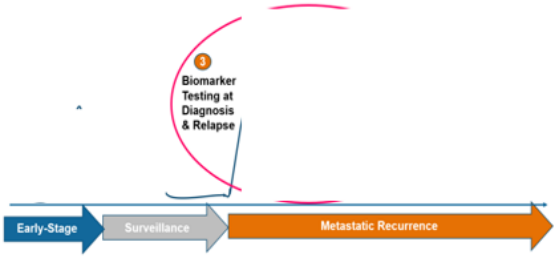


Figure 1. Molecular alterations detection by liquid biopsy in overall study population

7/30 EGFR+

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



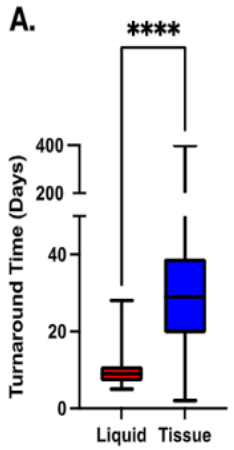
ORIGINAL STUDY | ARTICLES IN PRESS

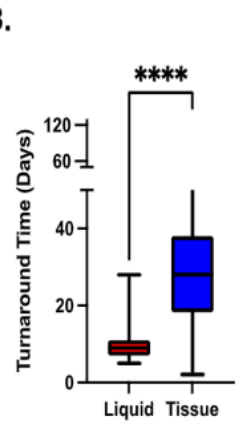
Liquid Biopsy Versus Tissue Biopsy to Determine Front Line Therapy in Metastatic Non-Small Cell Lung Cancer (NSCLC)

Luis E. Raez # • Kayla Brice # • Katerine Dumais • ... Paola A. Izquierdo • Edgardo S. Santos • Hermán W. Powery • Show all authors • Show footnotes

Published: November 25, 2022 • DOI: https://doi.org/10.1016/j.cllc.2022.11.007

Figure 2 Turnaround time (TAT) of liquid versus tissue biopsy NGS. (A) TAT of all samples (N = 170). Liquid biopsy NGS had a significantly faster TAT than tissue biopsy ($P < .0001$, 2-tailed unpaired student *t*-test). (B) Adjusted TAT for samples excluding patients with order dates >6 m between liquid and tissue (N = 165). Liquid biopsy NGS had a significantly faster TAT than tissue biopsy ($P < .0001$, 2-tailed unpaired student *t*-test)

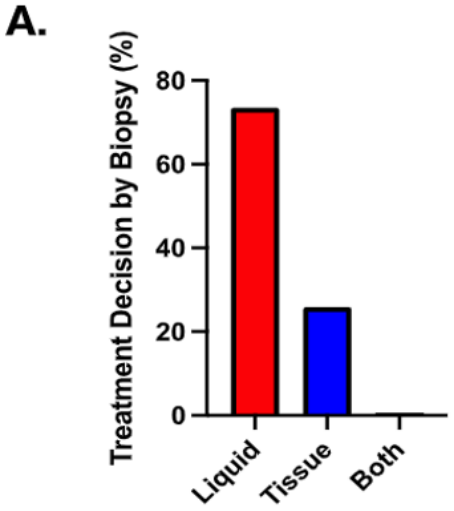




N=170	Liquid	Tissue
TAT Mean	9.6	36.4
TAT Median	9	29
Samples QNS	0	18 (10.6%)

N=165	Liquid	Tissue
TAT Mean	9.6	30.5
TAT Median	9	28
Samples QNS	0	18 (10.6%)

Frequency of treatment decision based on liquid biopsy versus tissue biopsy NGS.



	Liquid Guided	Tissue Guided	Both
Patients	119	42	1

GenesisCare

OLA Oncology Latin American Association

FLASCO

FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY

Table 2 Comparison of Liquid Versus Tissue Biopsy NGS Results for Guideline-Recommended Biomarkers in mNSCLC With FDA-Approved Therapies That Were Identified in Patients in This Study

EGFR		Tissue+	Tissue-	Sensitivity	66.7%
	Liquid +	14	18	Specificity	86.4%
	Liquid -	7	114	PPV	43.8%
	Total	21	132	NPV	94.2%
				Concordance	94.8%
BRAF		Tissue+	Tissue-	Sensitivity	0.0%
	Liquid +	0	2	Specificity	98.7%
	Liquid -	2	149	PPV	0.0%
	Total	2	151	NPV	98.7%
				Concordance	98.7%
ALK		Tissue+	Tissue-	Sensitivity	NA
	Liquid +	0	2	Specificity	98.7%
	Liquid -	1	150	PPV	0.0%
	Total	1	152	NPV	99.3%
				Concordance	99.3%
MET		Tissue+	Tissue-	Sensitivity	50.0%
	Liquid +	1	1	Specificity	99.3%
	Liquid -	1	150	PPV	50.0%
	Total	2	151	NPV	99.3%
				Concordance	99.3%
NTRK		Tissue+	Tissue-	Sensitivity	0.0%
	Liquid +	0	0	Specificity	100.0%
	Liquid -	1	152	PPV	NA
	Total	1	152	NPV	99.3%
				Concordance	99.3%
ROS1		Tissue+	Tissue-	Sensitivity	100.0%
	Liquid +	1	0	Specificity	100.0%
	Liquid -	0	152	PPV	100.0%
	Total	1	152	NPV	100.0%
				Concordance	100.0%

Raez LE et al. Clin Lung Cancer 2022

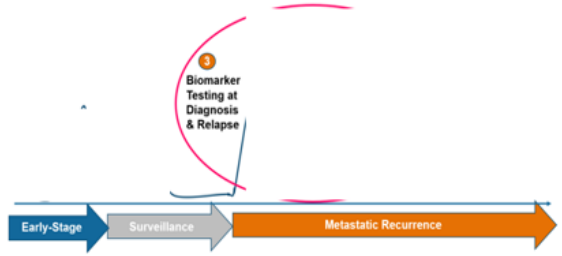
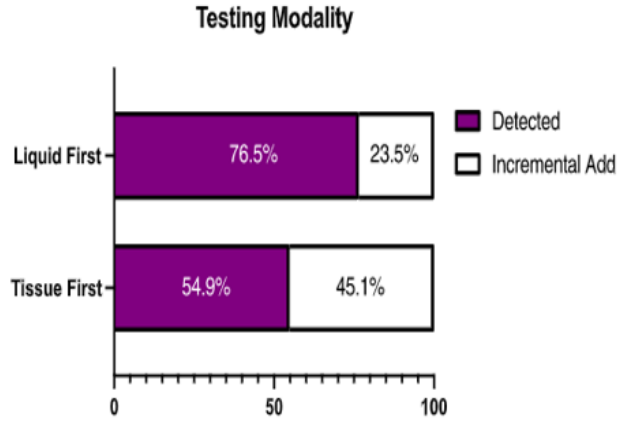
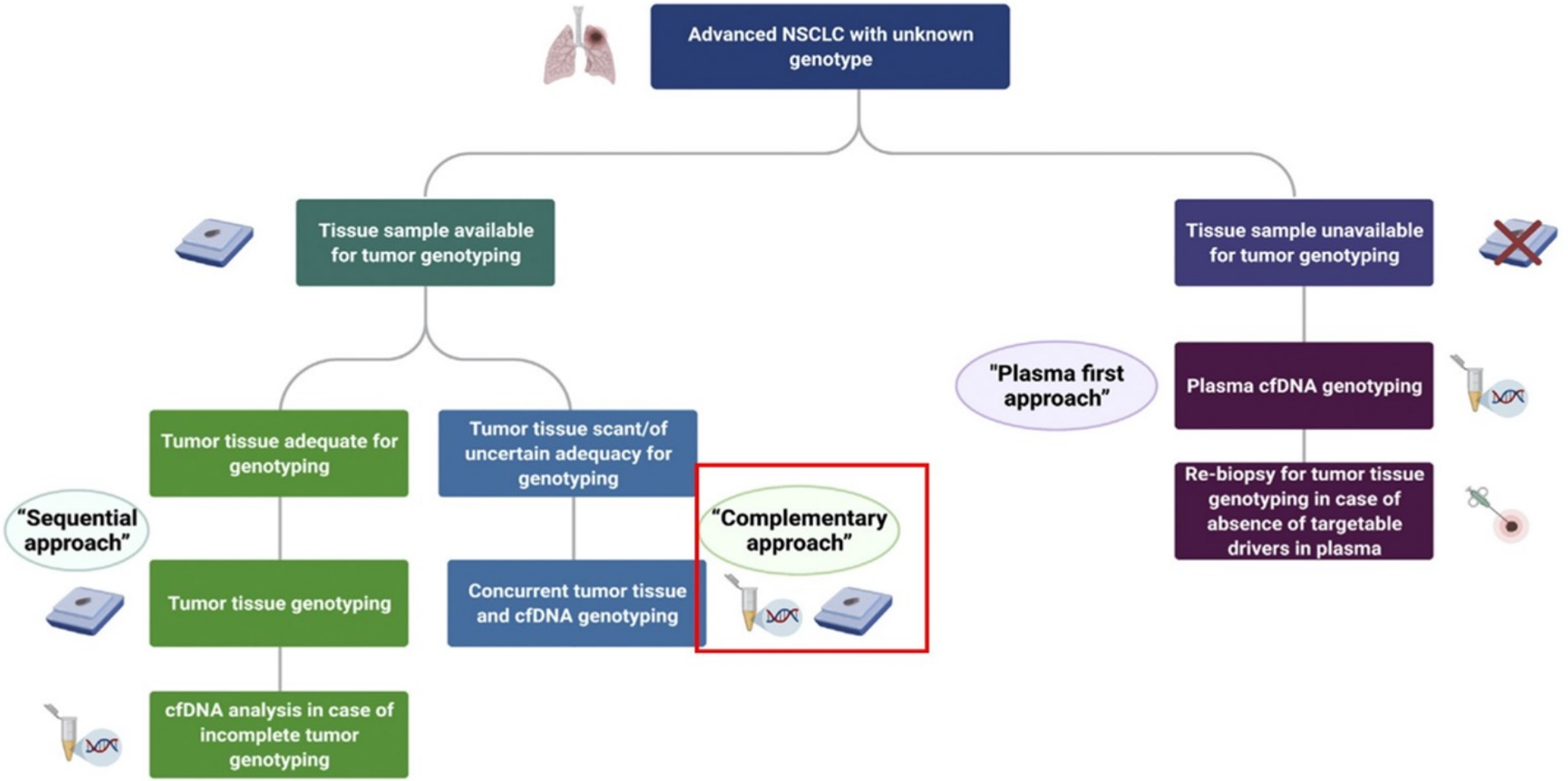


Figure 6 Frequency of guideline-recommended biomarkers detected by testing modality. In this cohort, leading with liquid testing, 76.5% of patients with a guideline-recommended biomarker would have been detected with 23.5% of patients identified on reflex tissue testing. If tissue biopsy was the first genomic testing modality, substantially less patients would have been identified

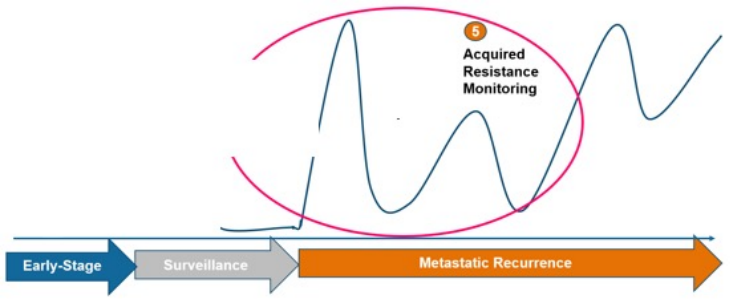


	Detected	Incremental Add	Total
Liquid First	39	12	51
Tissue First	28	23	51

Diagnostic algorithm for liquid biopsy use in treatment-naive advanced/metastatic NSCLC



Rolfo C et al. J Thorac Oncol. 2021



CodeBreaK 100 Study Schema

Screening enrollment

Pooled Phase 1/2: Sotorasib 960 mg orally daily
N = 174 NSCLC; N = 91 CRC

Key eligibility criteria

- Locally advanced or metastatic *KRAS* p.G12C-mutated solid tumors
- 1+ prior systemic therapy, or ineligible/intolerant*
- Stable brain metastases allowed

Patients with progressive disease:
n = 106 NSCLC; n = 61 CRC

Patients with paired plasma samples (baseline and at progression)
n = 67 NSCLC; n = 45 CRC

Primary Endpoint

ORR assessed by RECIST 1.1 by central review

Exploratory Endpoint

Acquired genomic alterations at disease progression

cfDNA Assays

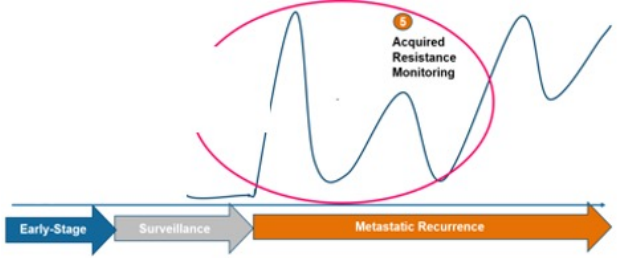
NSCLC (n = 67)*
23-gene Resolution Bioscience ctDx Lung test†
- With baseline tissue sample (n = 44; 66%)

CRC (n = 45)*
74-gene Guardant 360 ctDNA test†
- With baseline tissue sample (n = 32; 71%)

Analysis set

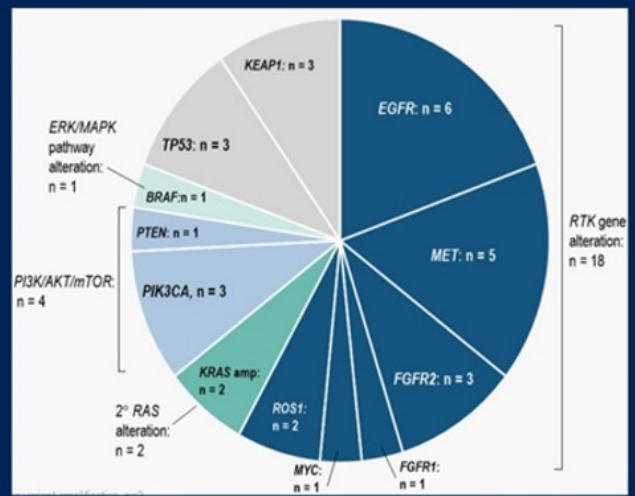
- Acquired genomic alterations identified at disease progression
  - Absent at baseline (in plasma and tissue‡)
  - Present at progression

Bob Li. ASCO 2022.

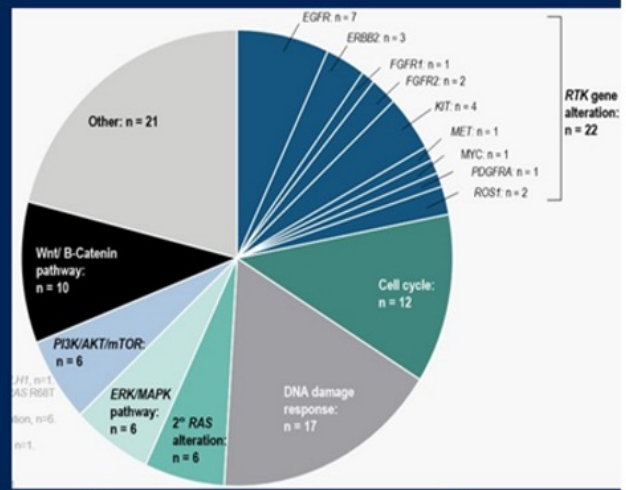


Largest evaluation of acquired resistance to sotorasib in *KRAS* p.G12C-mutated NSCLC and CRC: plasma biomarker analysis of CodeBreaK 100 Li et al.

- 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.

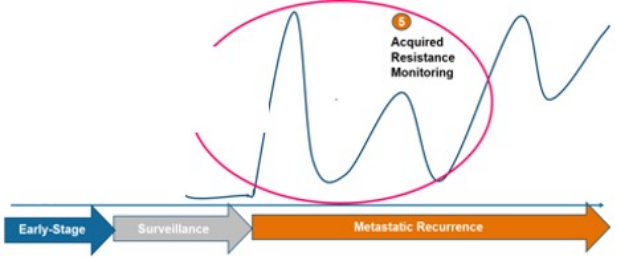


NSCLC



CRC

Ben Ho Park. 2022 ASCO.



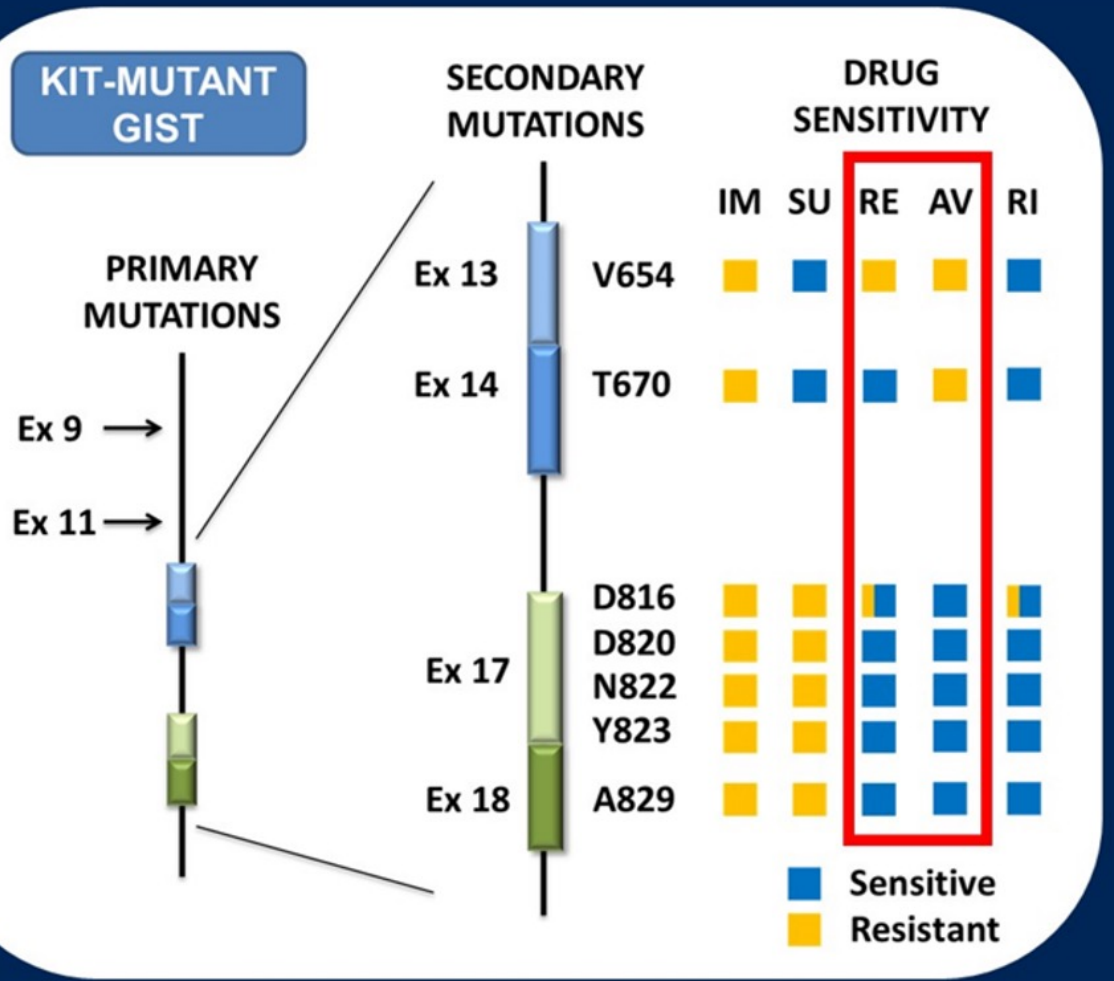
VOYAGER Clinical Trial

VOYAGER

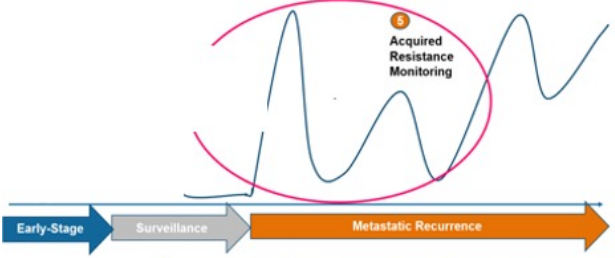
3L/4L GIST R, 1:1

- Avapritinib N=240
- Regorafenib N=236

Primary endpoint: PFS



Cesar Serrano. 2022 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)

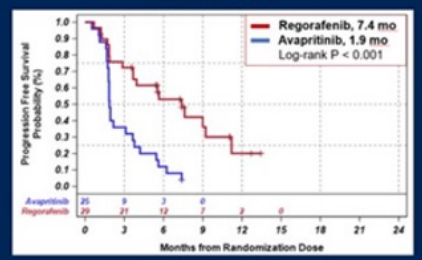
César Serrano et al.

- **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

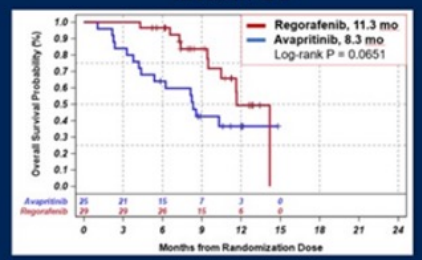
ctDNA mutations & outcomes: **ATP-binding pocket**

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

Median PFS



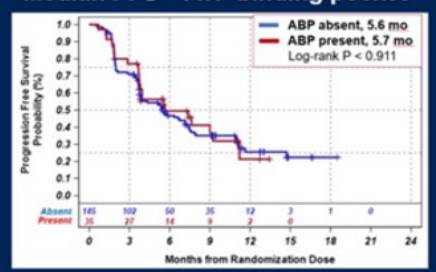
Median OS



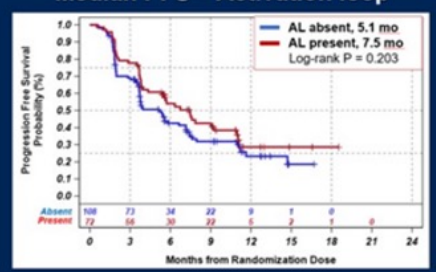
ctDNA mutations & outcomes: **Regorafenib**

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

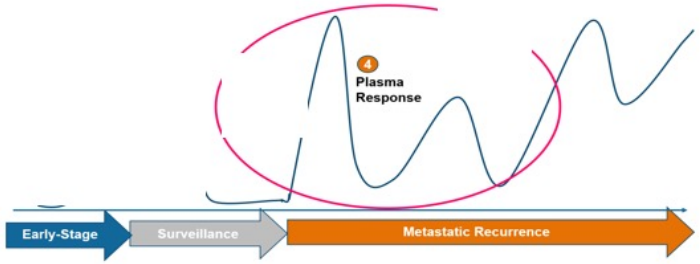
Median PFS – ATP binding pocket



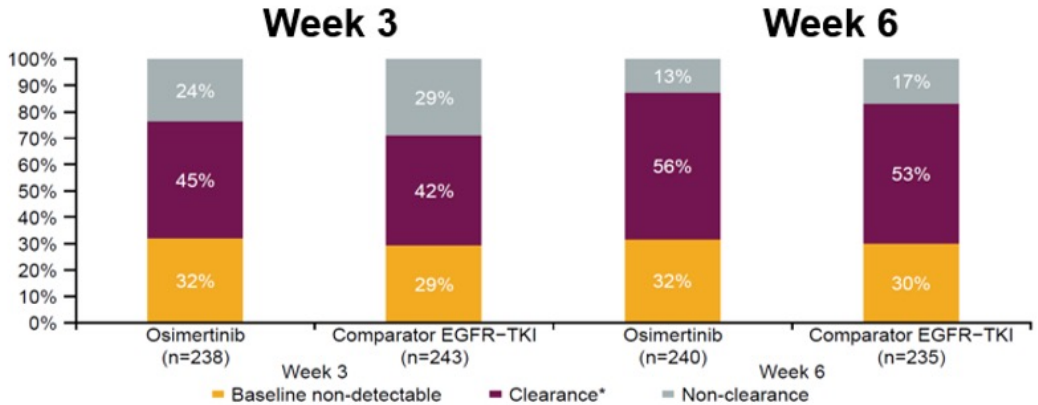
Median PFS – Activation loop



Ben Ho Park. 2022 ASCO.



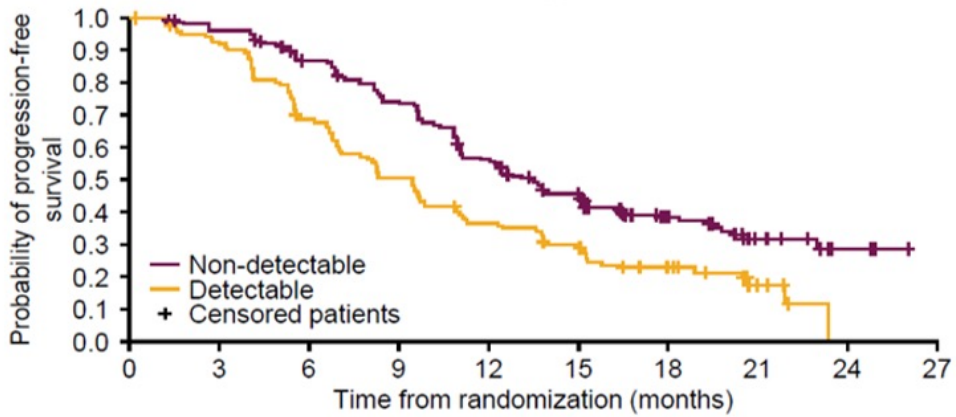
How often does the EGFRm clear from the plasma?



At 6 weeks osimertinib treatment:

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

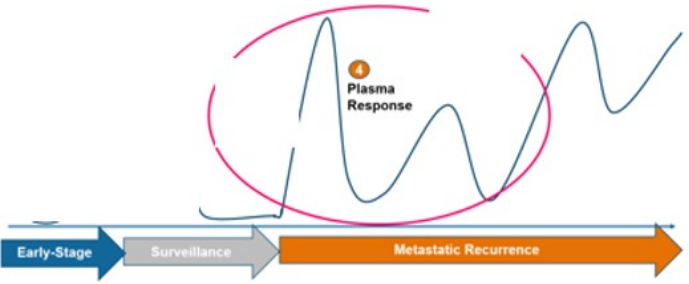
Impact of positive week 3 plasma EGFR on PFS?



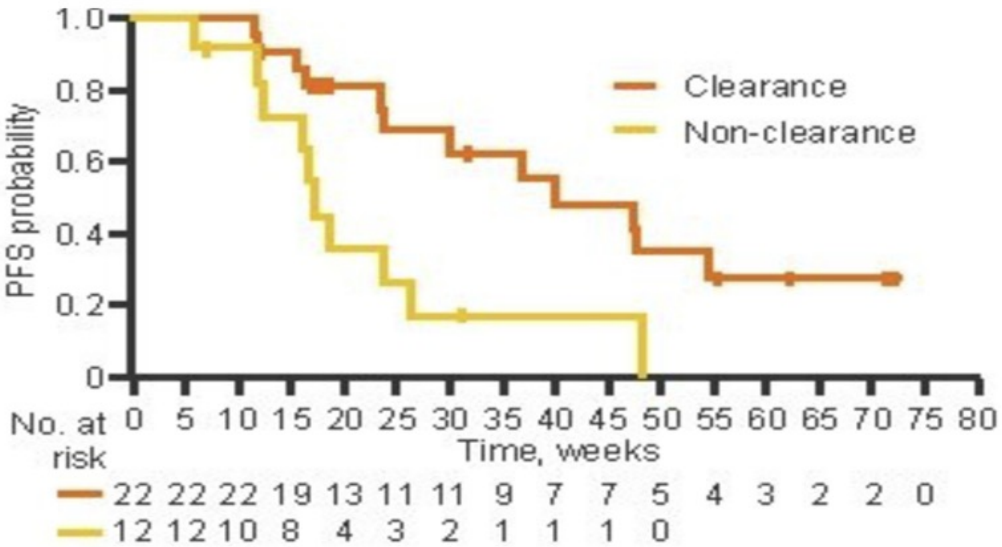
Plasma EGFR positive at 3 weeks
PFS 9.5 vs 13.5 months (HR 0.57, 0.4-0.7)

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



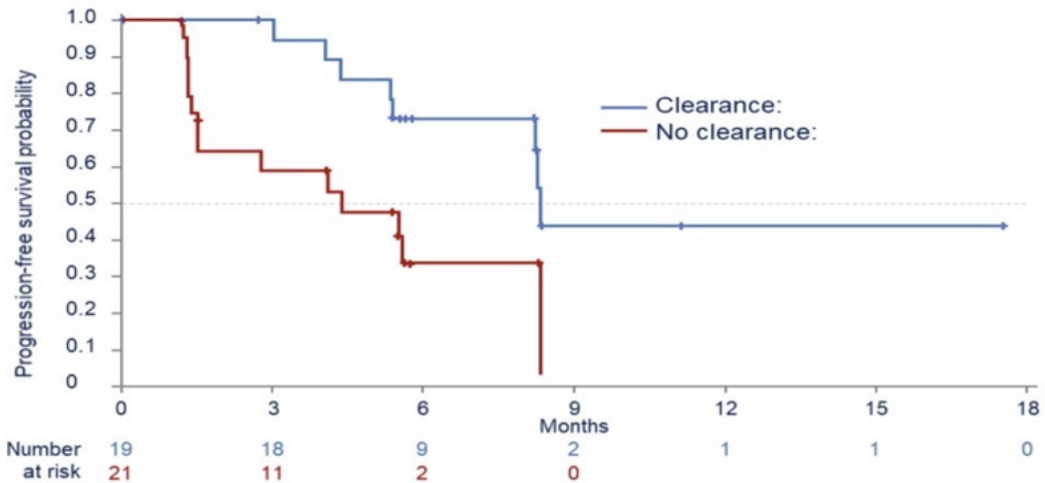
TATTON Savolitinib + Osimertinib for MET+ EGFR TKI Resistance



cfDNA status at cycle 3 or 4

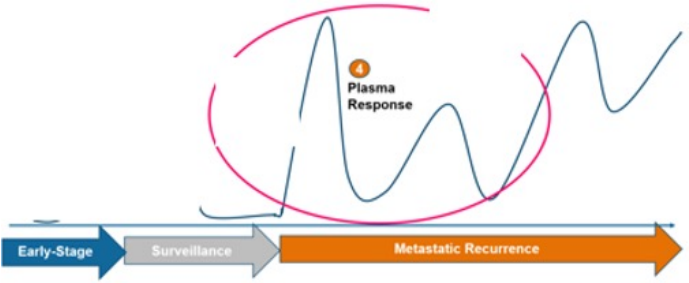
PFS 3.9 vs 9.1 months
(HR 0.34, 0.14-0.81)

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)

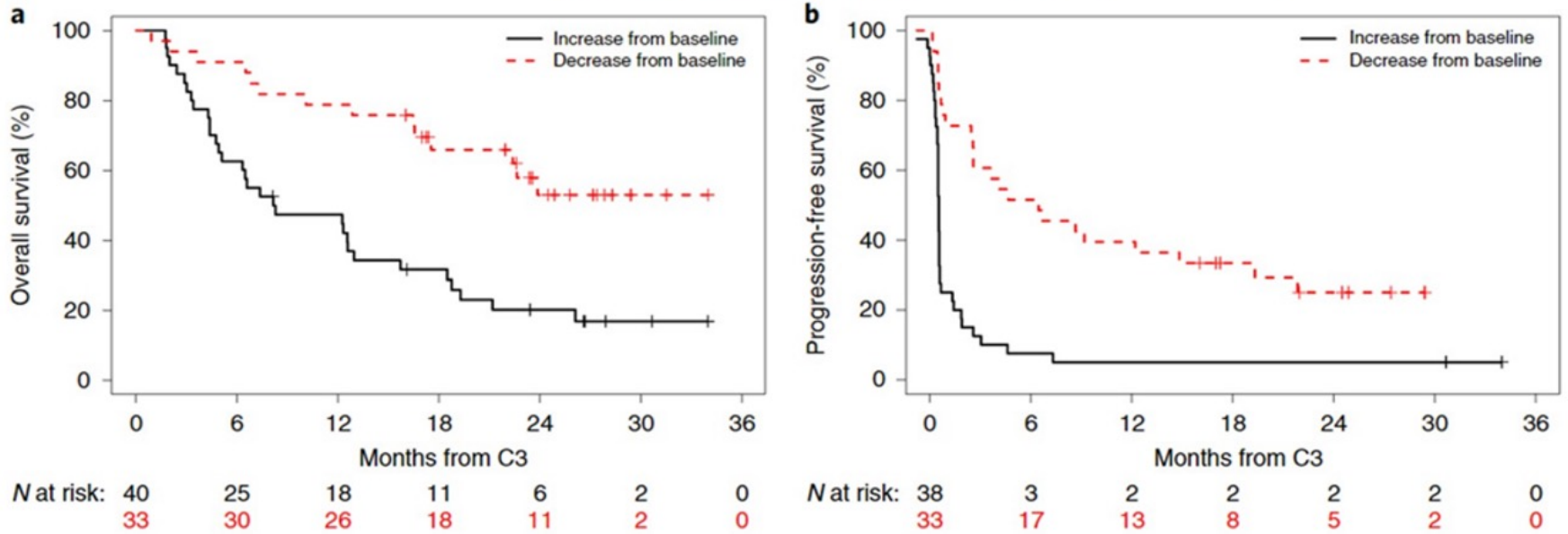


In the Era of Immunotherapy

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 **(Signature/Fingerprint in Blood)**

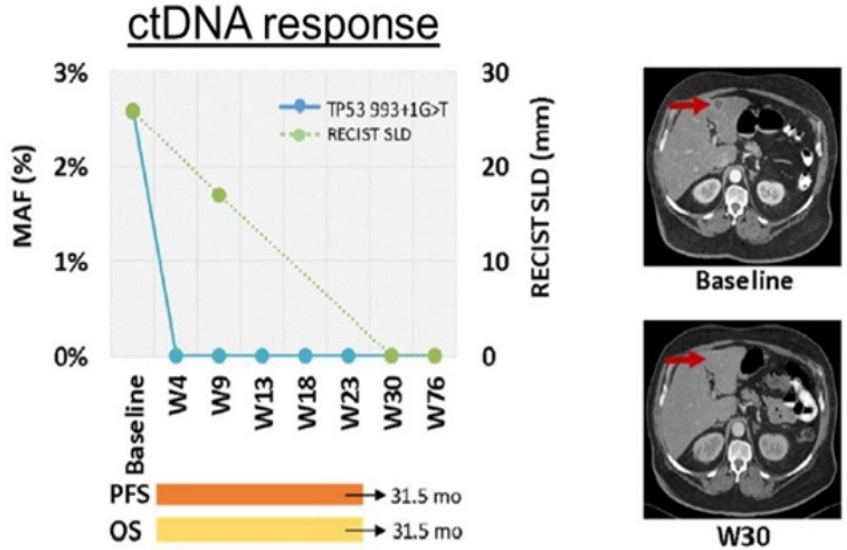


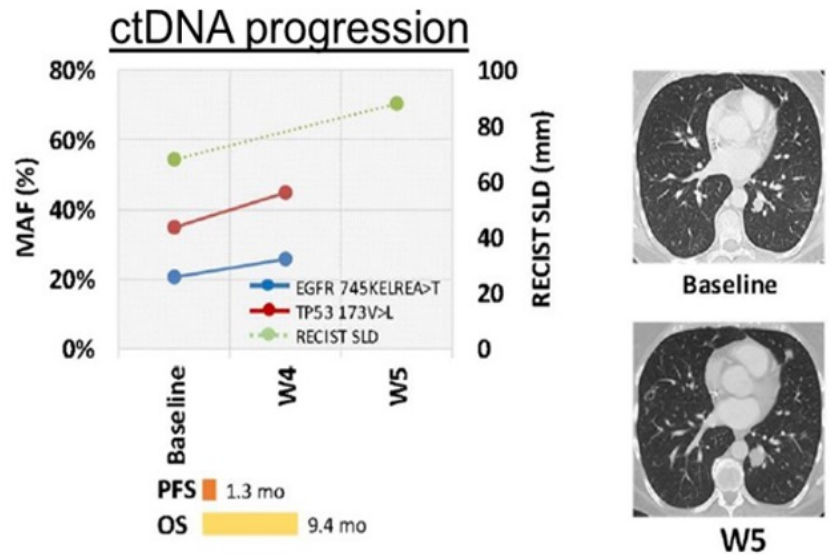
HNSCC, head and neck squamous cell carcinoma; TNBC, triple negative breast cancer; HGSOC, high-grade serous ovarian cancer, MST, mixed solid tumors

Bratman SV et al. *Nat Cancer*. 2020

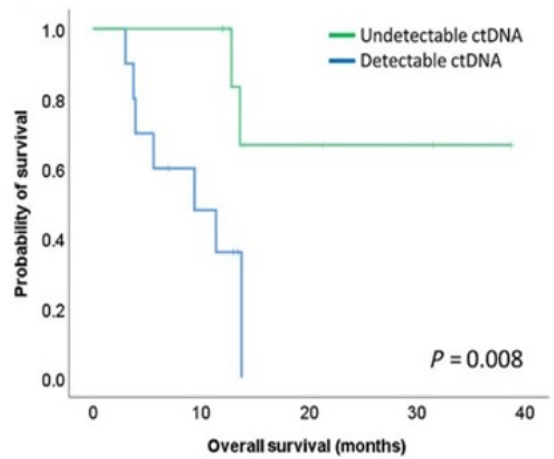
GenesisCare OLA FLASCO FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY

Patients with response to ICB had undetectable ctDNA and superior OS and PFS





Stage IV NSCLC
Tumor-naïve assay
(TEC-Seq)



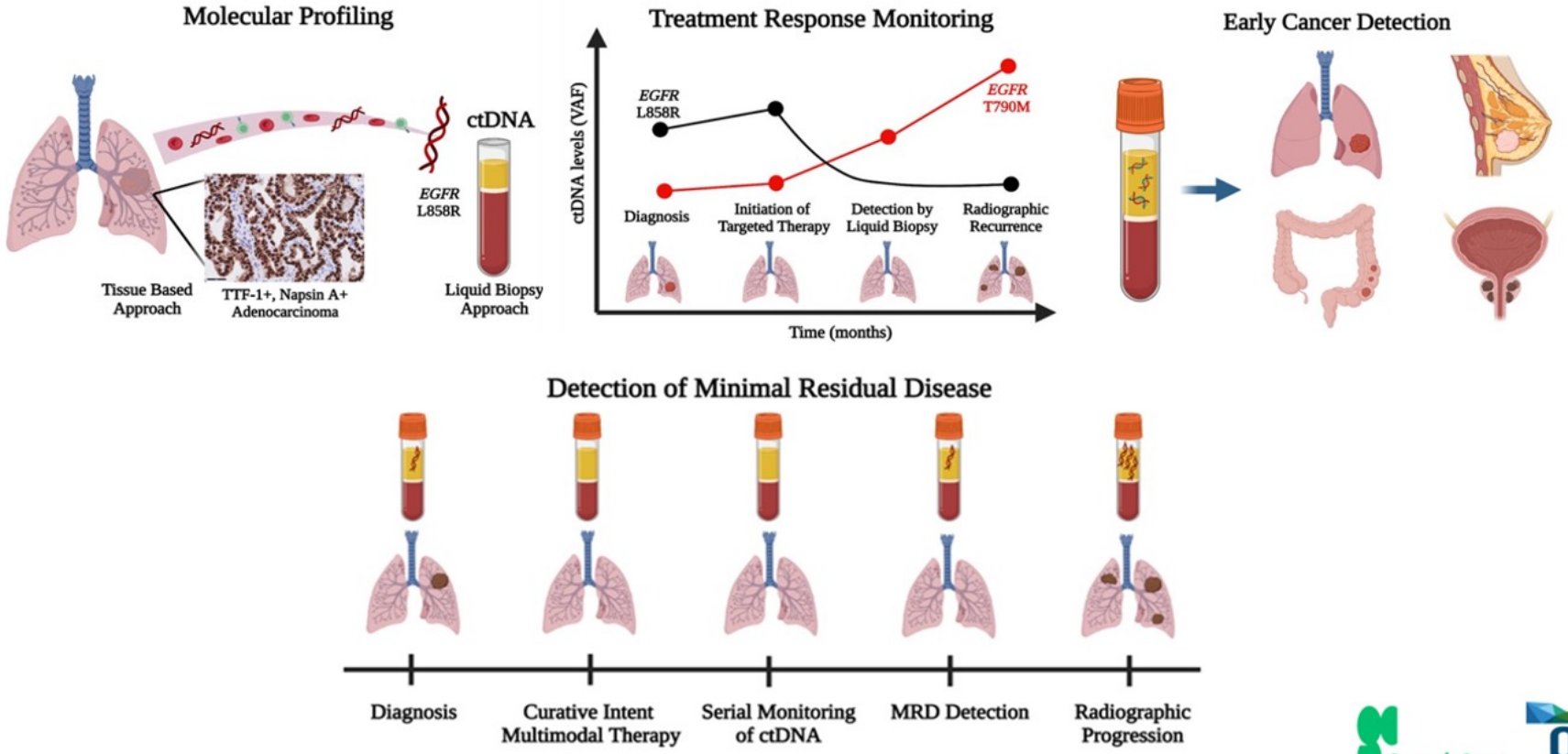
Molecular response is associated with improved survival

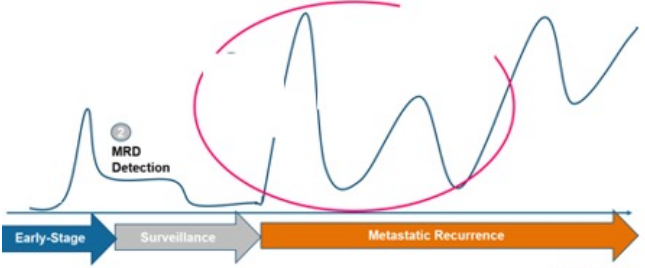
ICB, immune checkpoint blockade

Anagnostou V et al. *Cancer Res*. 2019

Pellini B. 2022 ASCO

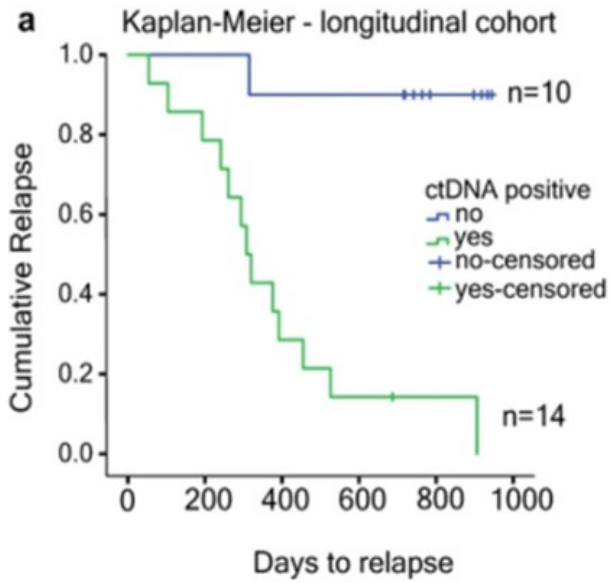
Future Directions of ctDNA



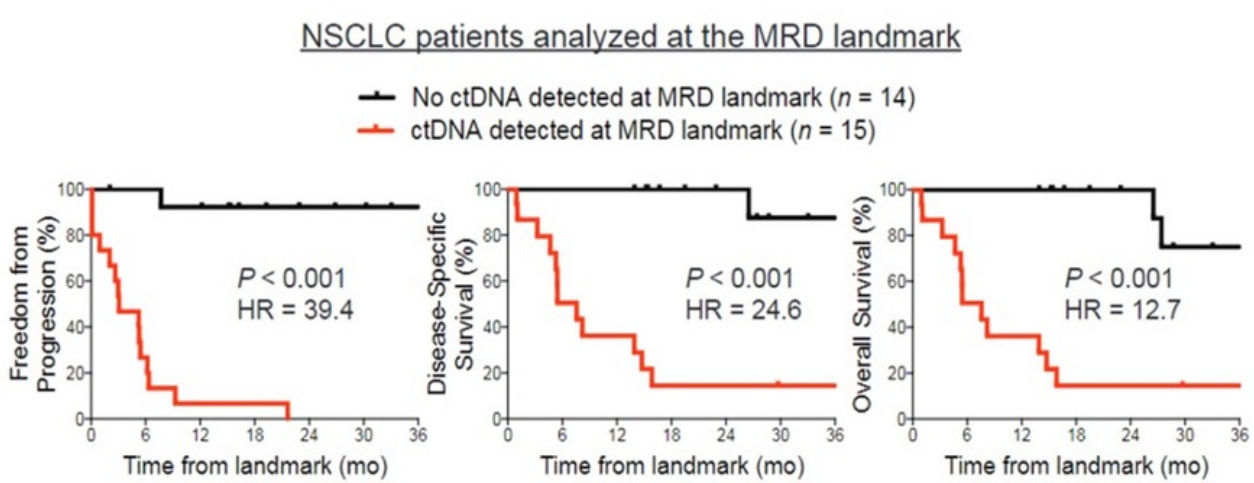


ctDNA can detect minimal residual disease (MRD) and it is a prognostic biomarker

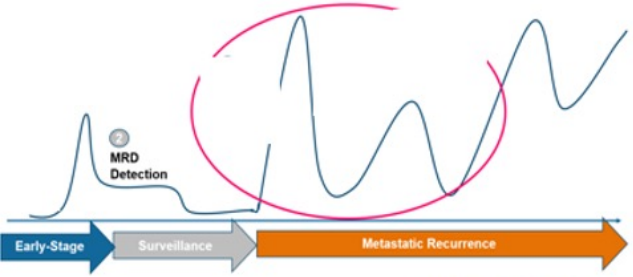
Stages I-III NSCLC
Tumor-informed assay



Stages I-III NSCLC
Tumor-naïve assay

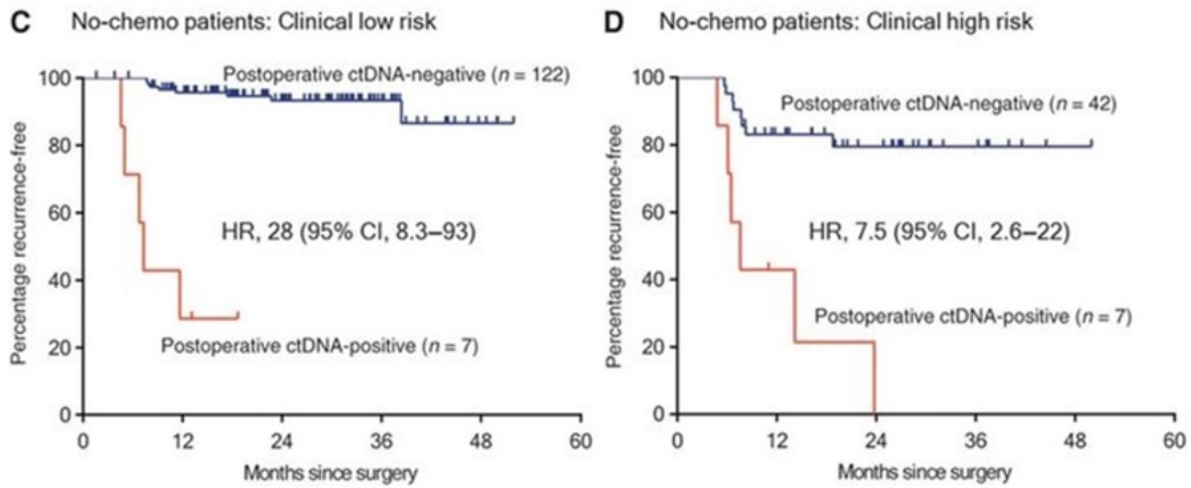


Abbosh C et al. *Nature*. 2017
Chaudhuri A et al. *Cancer Discov*. 2017

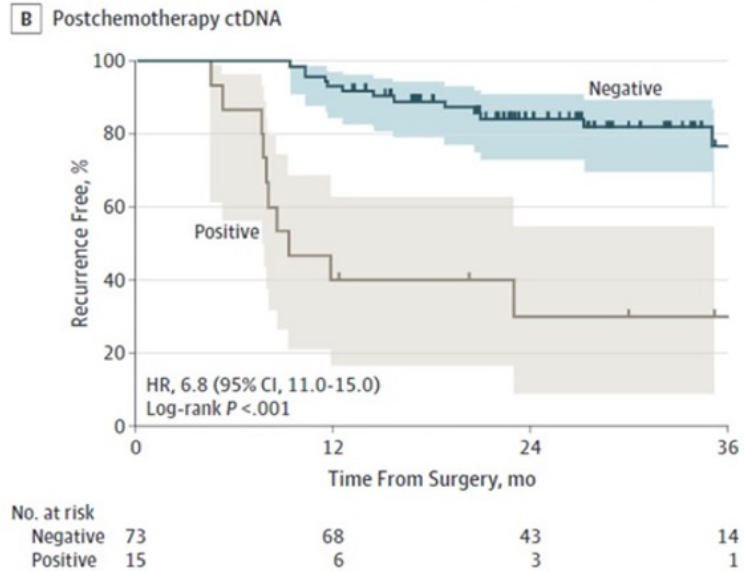


ctDNA can detect minimal residual disease (MRD) and it is a prognostic biomarker

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

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)

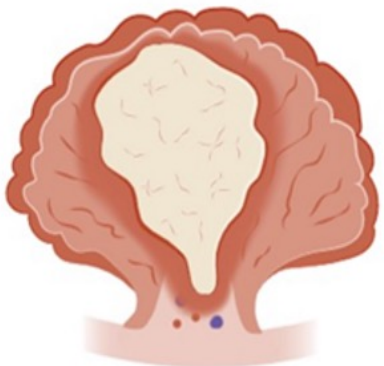
Surveillance:
- CEA → 3-monthly for 24M, then 6-monthly for 36M
- CT C/A/P → 6-monthly for 24M, then at 36M

Jeanne Tie. 2022 ASCO

GenesisCare OLA FLASCO FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY

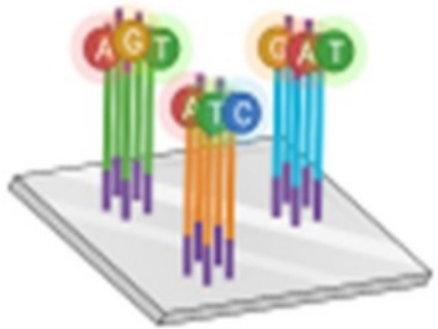
ctDNA Analysis: Tumor-Informed Personalized Approach

Resected tumor tissue



FFPE tissue from primary tumor

Targeted sequencing identifies mutation(s) unique to that cancer

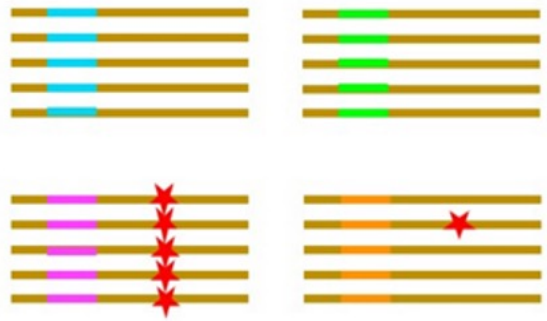


15 recurrently mutated genes in colorectal cancer

(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

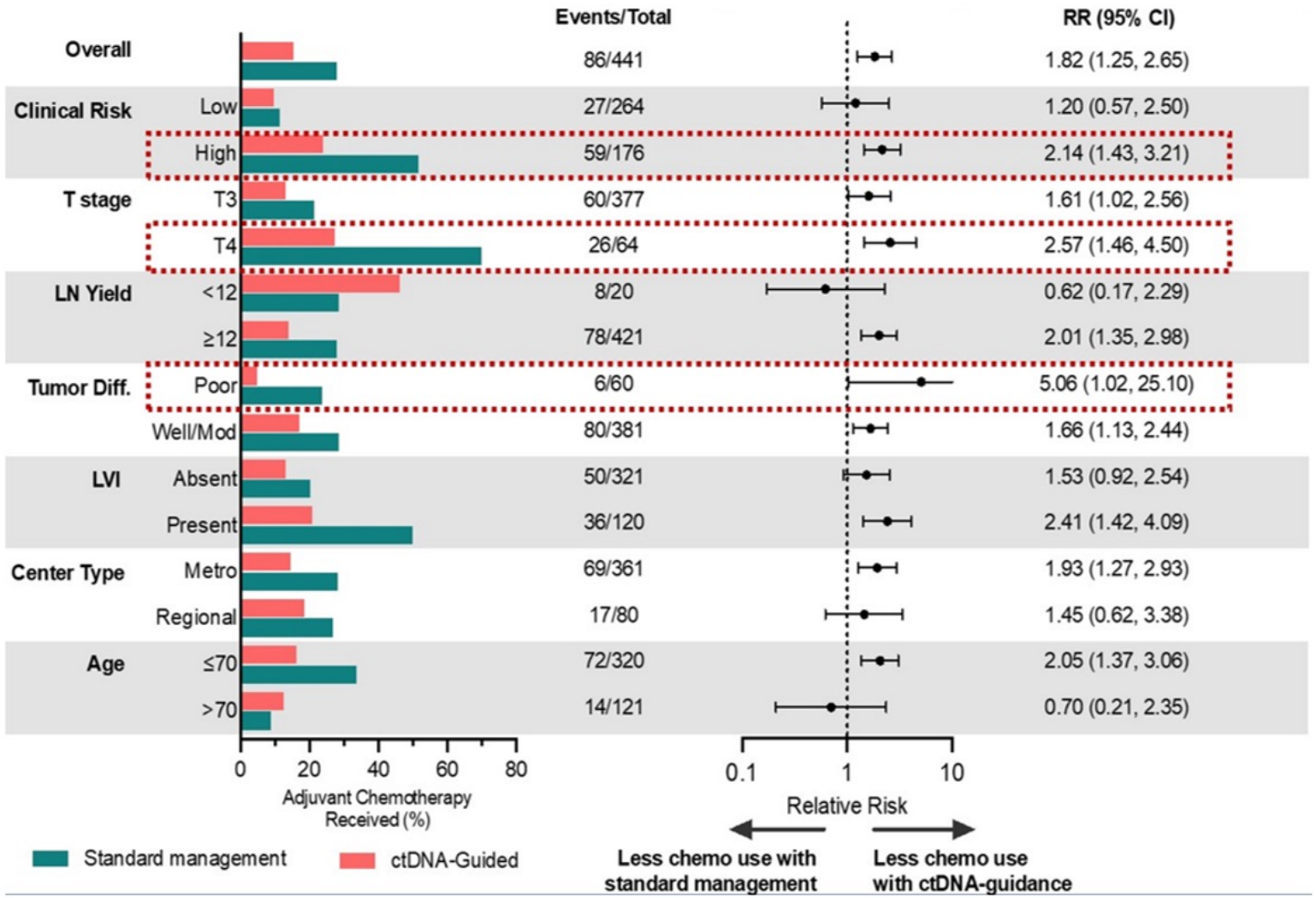


ctDNA detection by Safe-Sequencing System*

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

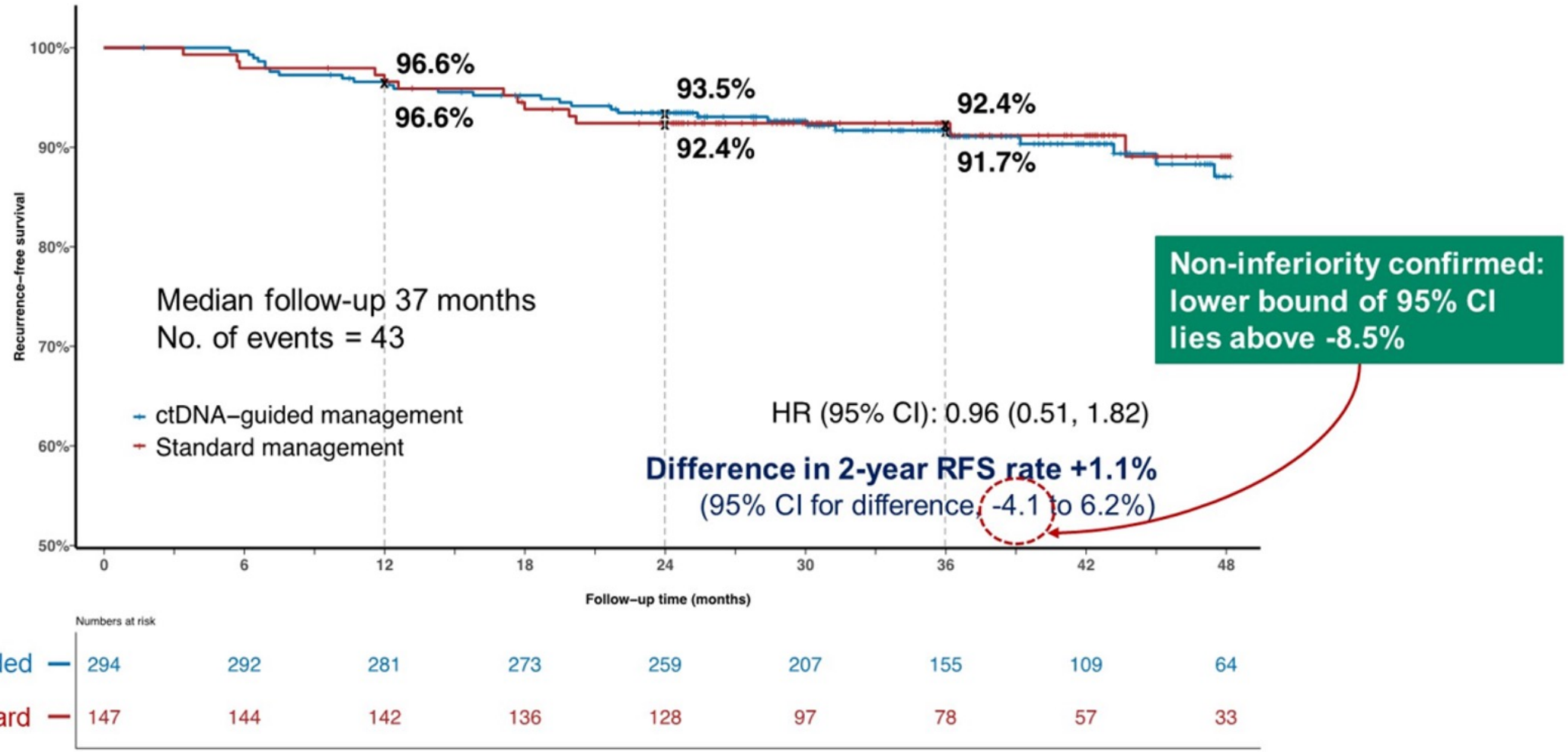
Adjuvant Chemotherapy Delivery

	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%	



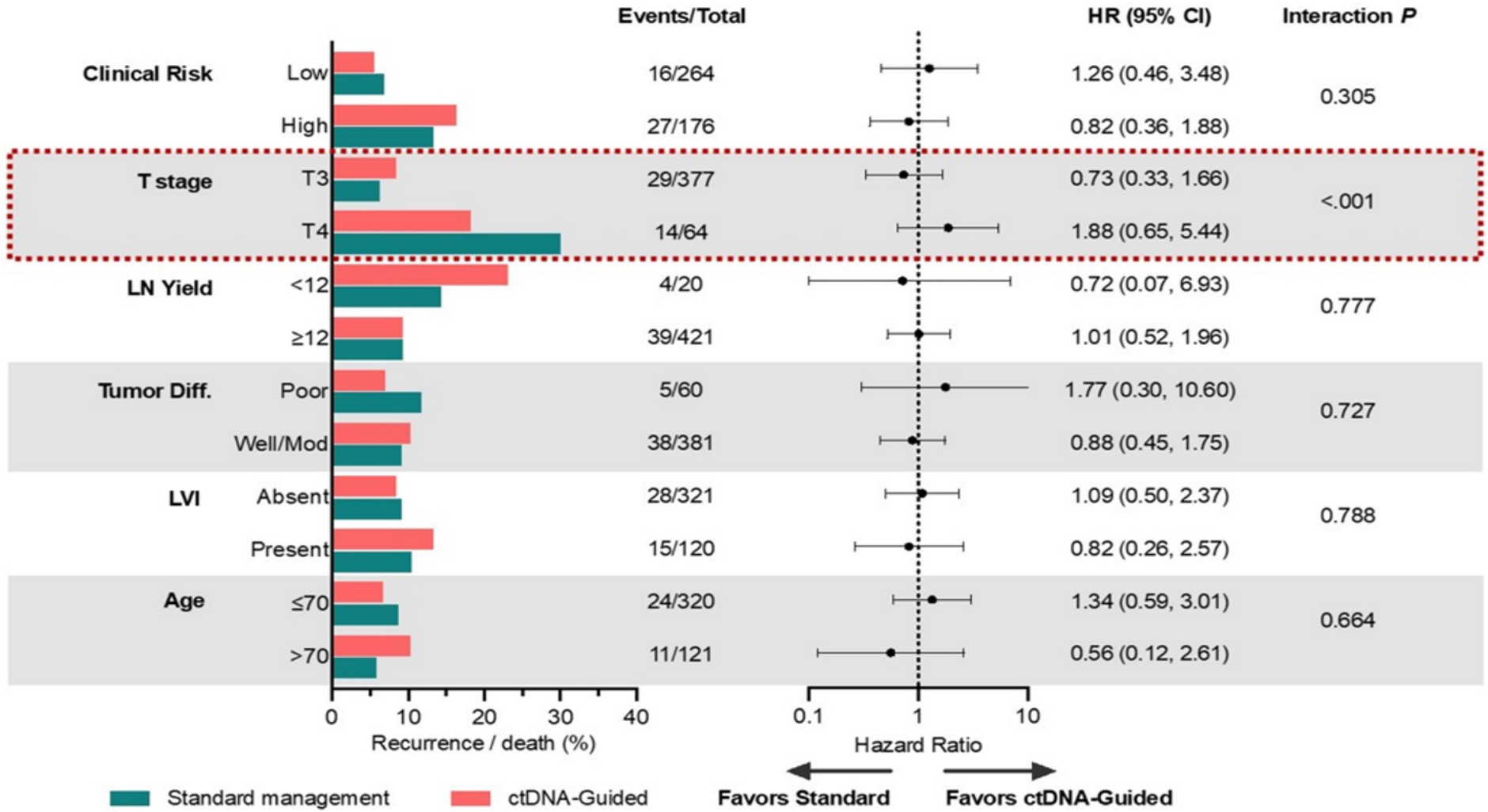
Tie et al. ASCO 2022. #LBA100

Recurrence-Free Survival



Tie et al. ASCO 2022. #LBA100

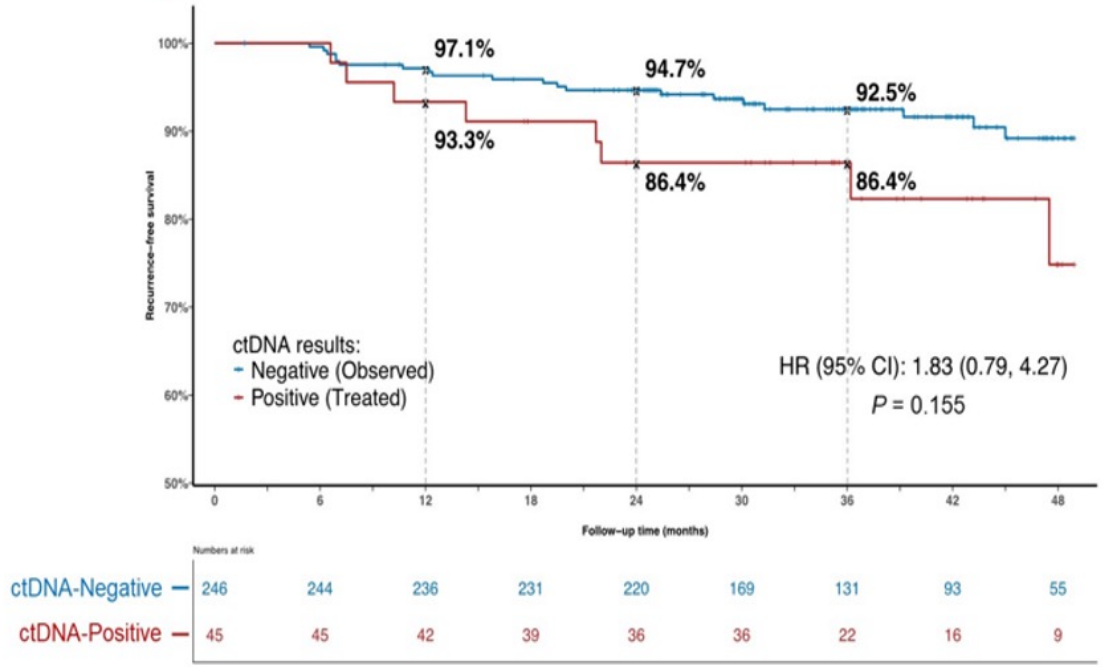
Recurrence-Free Survival in Key Subgroups



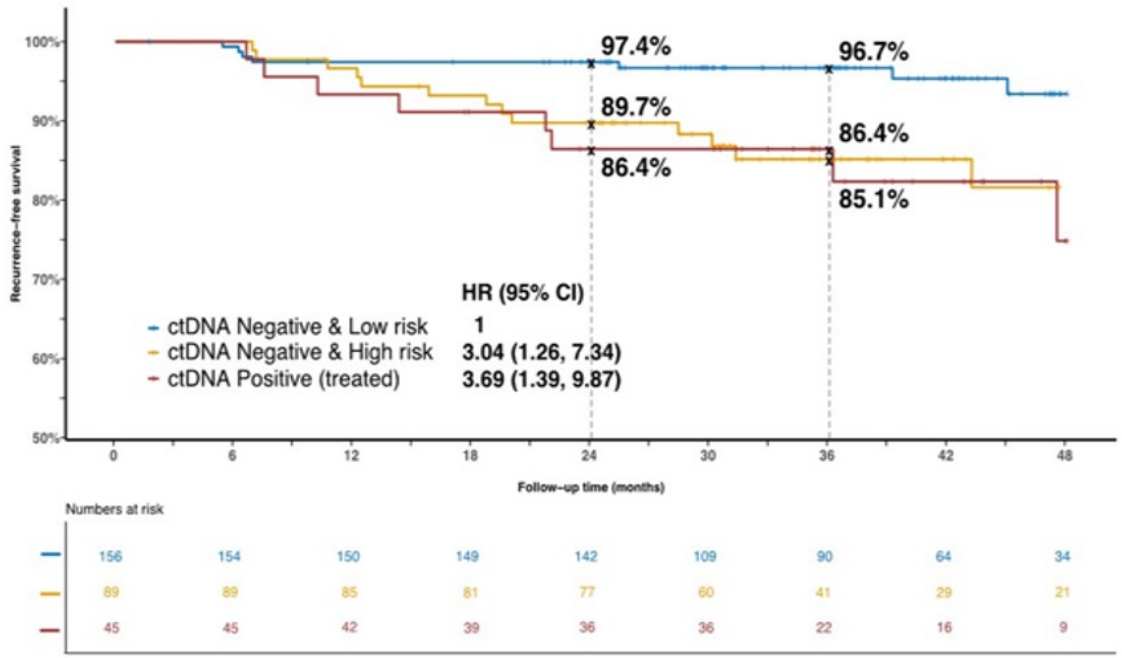
Tie et al. ASCO 2022. #LBA100

ctDNA Status and Recurrence-Free Survival

ctDNA Negative vs Positive



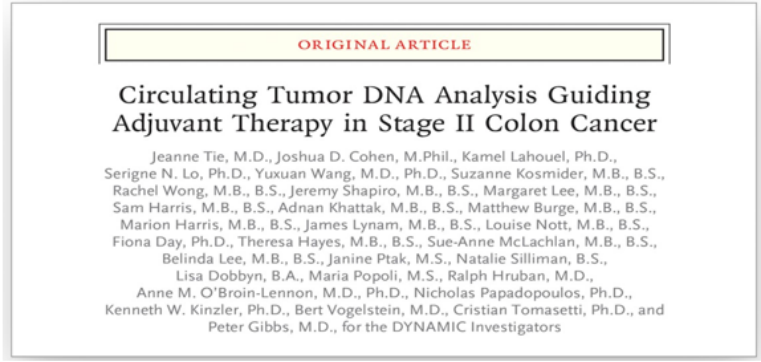
ctDNA and Clinical Risk



Tie et al. ASCO 2022. #LBA100

The NEW ENGLAND JOURNAL of MEDICINE

DYNAMIC study concluded:



ORIGINAL ARTICLE

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

Jeanne Tie, M.D., Joshua D. Cohen, M.Phil., Kamel Lahouel, Ph.D., Serigne N. Lo, Ph.D., Yuxuan Wang, M.D., Ph.D., Suzanne Kosmider, M.B., B.S., Rachel Wong, M.B., B.S., Jeremy Shapiro, M.B., B.S., Margaret Lee, M.B., B.S., Sam Harris, M.B., B.S., Adnan Khattak, M.B., B.S., Matthew Burge, M.B., B.S., Marion Harris, M.B., B.S., James Lynam, M.B., B.S., Louise Nott, M.B., B.S., Fiona Day, Ph.D., Theresa Hayes, M.B., B.S., Sue-Anne McLachlan, M.B., B.S., Belinda Lee, M.B., B.S., Janine Ptak, M.S., Natalie Silliman, B.S., Lisa Dobbyn, B.A., Maria Popoli, M.S., Ralph Hruban, M.D., Anne M. O'Broin-Lennon, M.D., Ph.D., Nicholas Papadopoulos, Ph.D., Kenneth W. Kinzler, Ph.D., Bert Vogelstein, M.D., Cristian Tomasetti, Ph.D., and Peter Gibbs, M.D., for the DYNAMIC Investigators

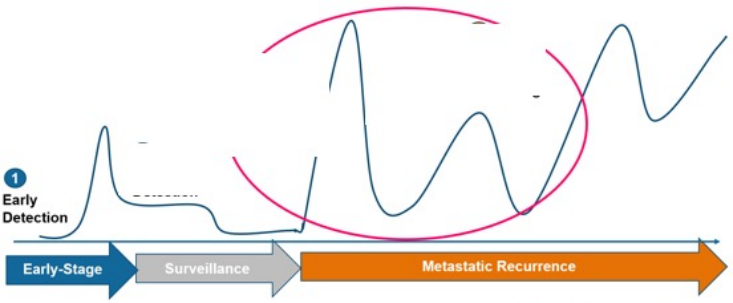
- **For patients with stage II colon cancer, a ctDNA-guided approach (treating only patients with a positive ctDNA after surgery) compared with standard-of-care**
  - Substantially reduced the proportion receiving adjuvant chemotherapy (28%→15%)
  - Did not compromise recurrence-free survival (2-year RFS: 93.5% vs 92.4%)

- **Patients with a + 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

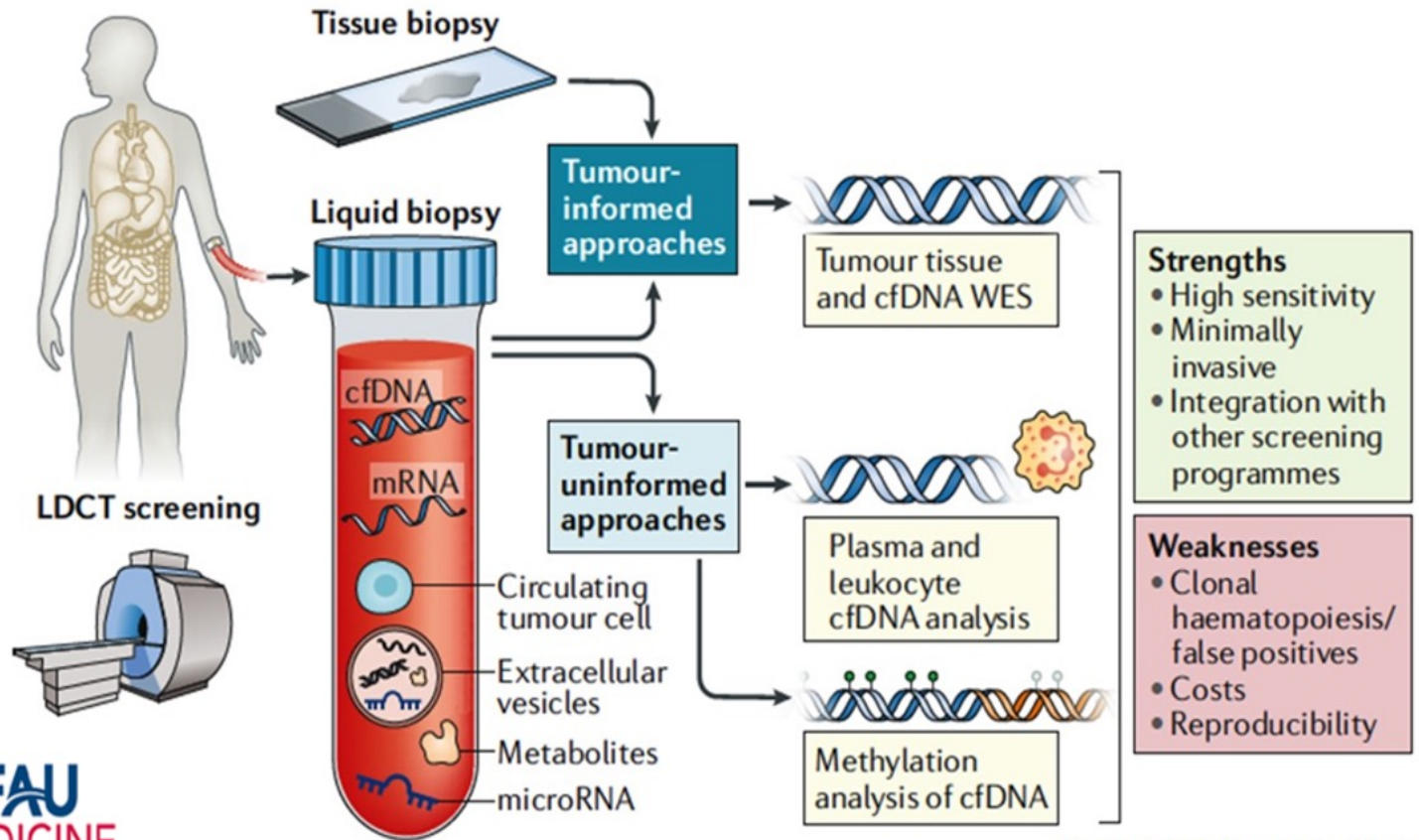
- **ctDNA-negative patients have a low recurrence risk without adjuvant chemotherapy**
  - 3-year RFS 92.5% (clinical low risk: 96.7%; T3: 94.2%)

Tie et al. ASCO 2022. #LBA100

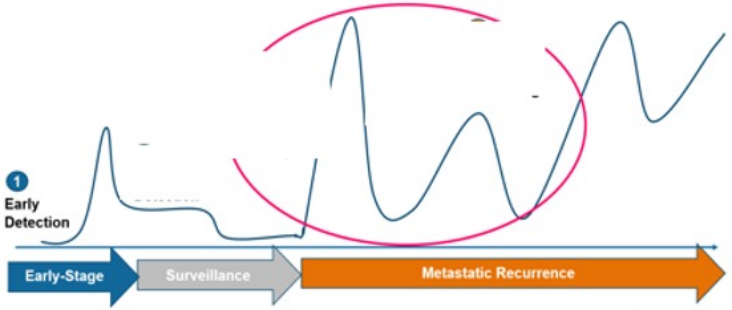
GenesisCare OLA FLASCO FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY



Challenges for ctDNA use for solid tumors early detection



Rolfo C & Russo A. Nat Rev Clin Oncol. 2020

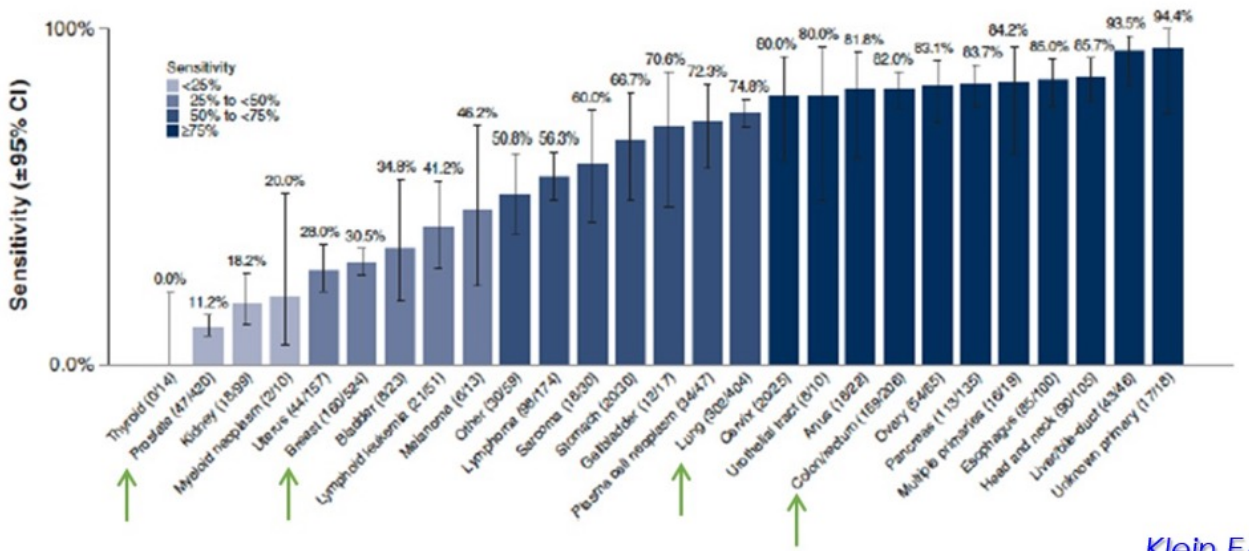


ctDNA methylation for early cancer detection

	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%)	

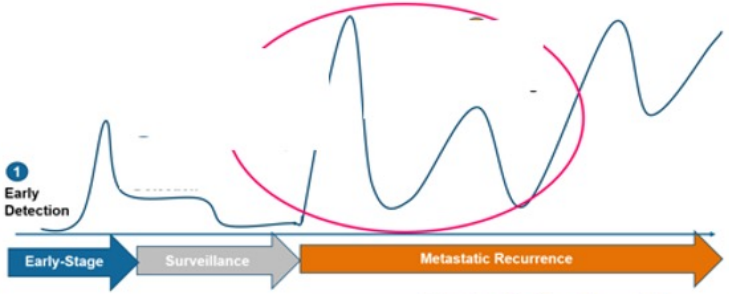
Two-sided 95% Wilson confidence intervals were calculated.

Targeted methylation assay
Tumor-naïve

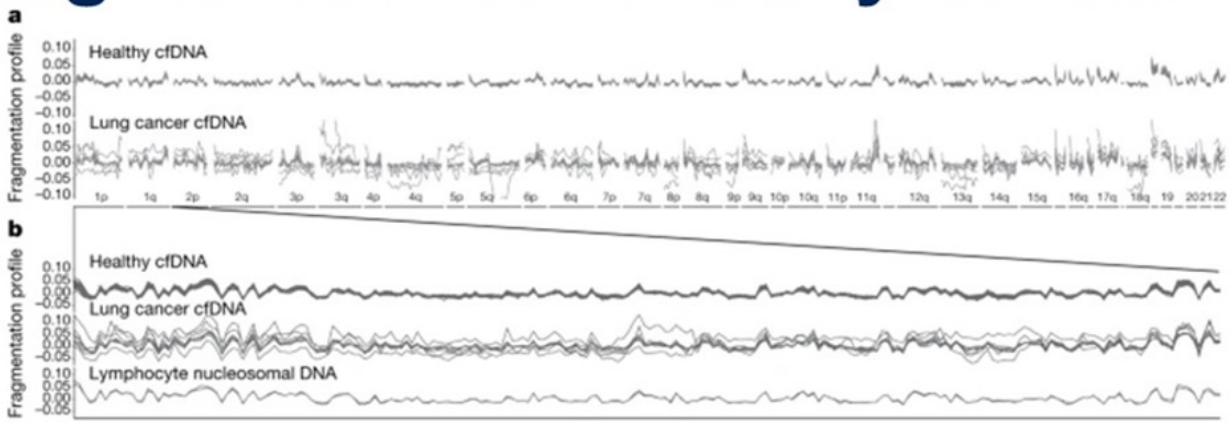


Sensitivity varies with **cancer** type, **histology**, and **stage**

Klein EA et al. Ann Oncol. 2021



ctDNA fragmentomics for early cancer detection



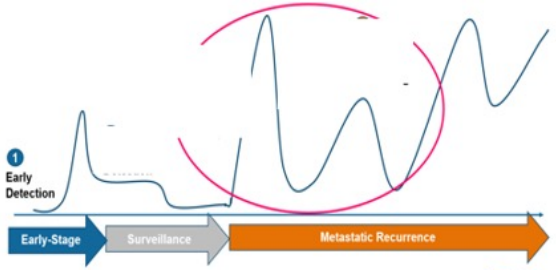
DNA evaluation of fragments for early interception

Tumor-naïve

Cancer Type	Patients Detected*	Top Prediction		Top Two Predictions		Random Assignment	
		Patients	Accuracy (95% CI)	Patients	Accuracy (95% CI)	Patients	Accuracy
Breast	42	32	76% (61%-88%)	38	91% (77%-97%)	9	22%
Bile Duct	23	10	44% (23%-66%)	15	65% (43%-84%)	3	12%
Colorectal	24	17	71% (49%-87%)	19	79% (58%-93%)	3	12%
Gastric	24	16	67% (45%-84%)	19	79% (58%-93%)	3	12%
Lung	30	16	53% (34%-72%)	23	77% (58%-90%)	2	6%
Ovarian	27	13	48% (29%-68%)	16	59% (38%-78%)	4	14%
Pancreatic	24	12	50% (29%-71%)	16	67% (45%-84%)	3	12%
Total	194	116	61% (53%-67%)	146	75% (69%-81%)	26	13%

*Patients detected are based on DELFI detection at 90% specificity. Lung cohort includes additional lung cancer patients with prior therapy.

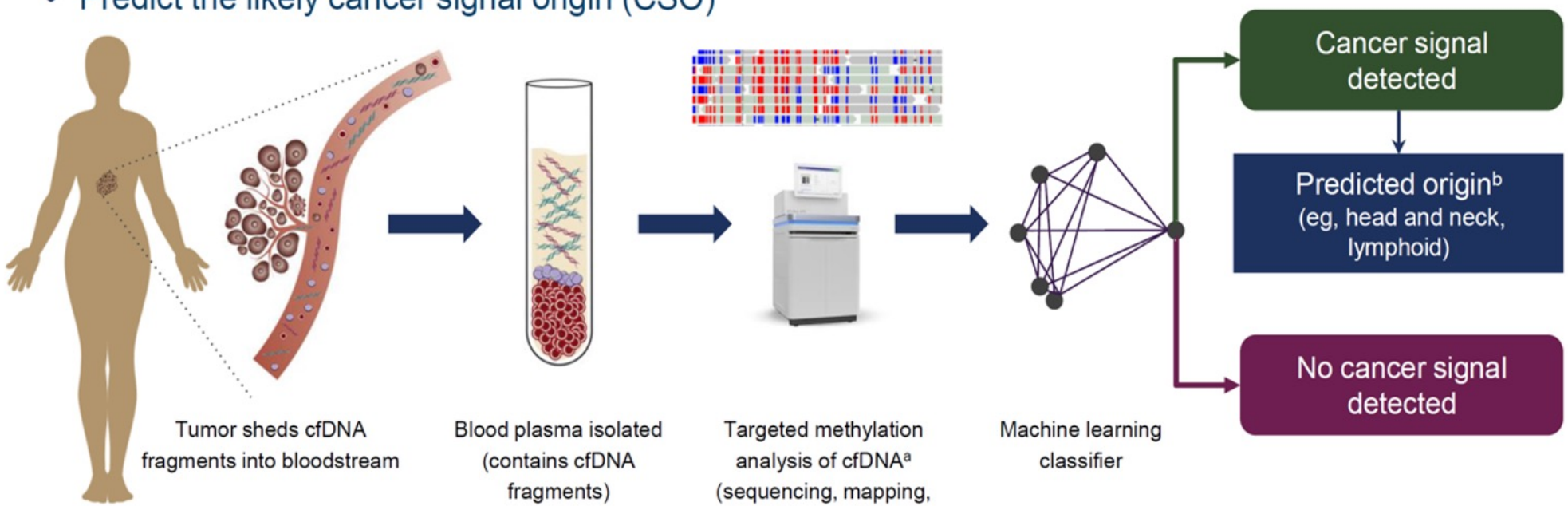
Cristiano S et al. Nature. 2019



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

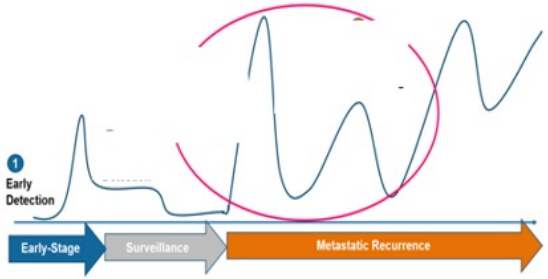
MCED testing uses a targeted methylation, next-generation sequencing (NGS)-based assay to:

- 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: 33506766

Deb Schrag. 2022 ESMO



PATHFINDER Eligibility Criteria

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

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 hormone therapy for breast cancer was permissible.

Deb Schrag. 2022 ESMO

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).

Deb Schrag. 2022 ESMO

GenesisCare OLA FLASCO FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY

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

Deb Schrag et al. ESMO 2022. #9030

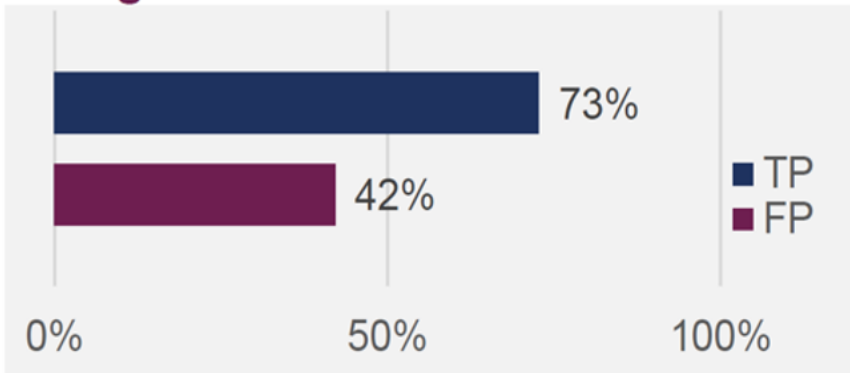
Primary Objective: Achieving Diagnostic Resolution

Time Required to Achieve a Diagnostic Resolution

Median 79 Days

- **57 Days if True Positive (TP)**
- **162 Days if False Positive (FP)**

% of Participants with Diagnostic Resolution in <3 months



Extent of Testing to Achieve a Diagnostic Resolution

Imaging Procedure 92% (similar TP and FP)

Any Invasive Procedure: 82% TP 30% FP

Deb 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).

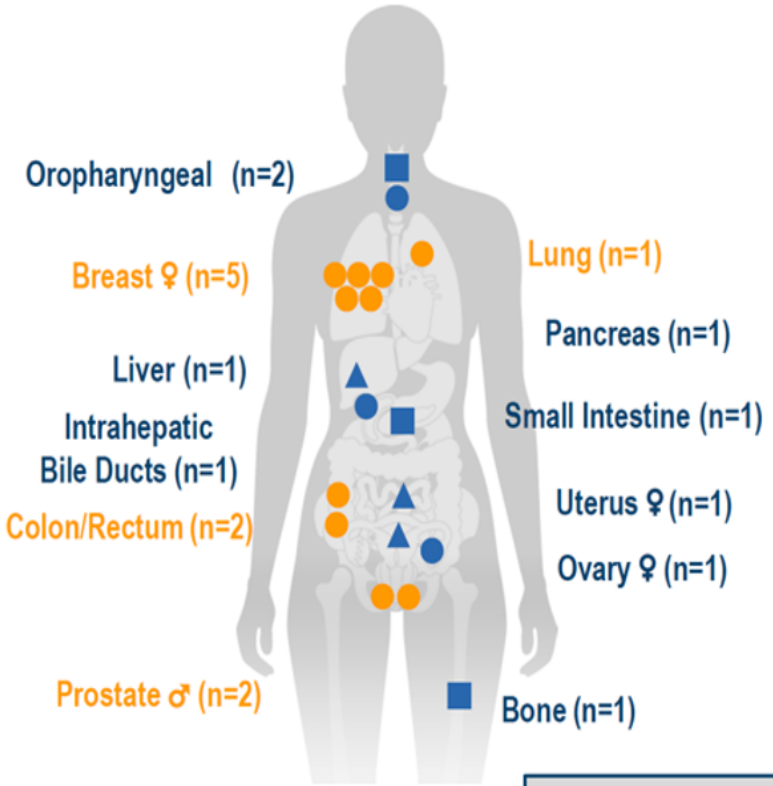
[b]Excludes 1 participant with indeterminate origin prediction from the true positive per study protocol.

[c]Proportion of first or second origin correctly predicted among true positive participants.

Deb Schrag. ESMO 2022.

Cancers Diagnosed After a True Positive MCED Signal

18 people diagnosed with Solid Tumors

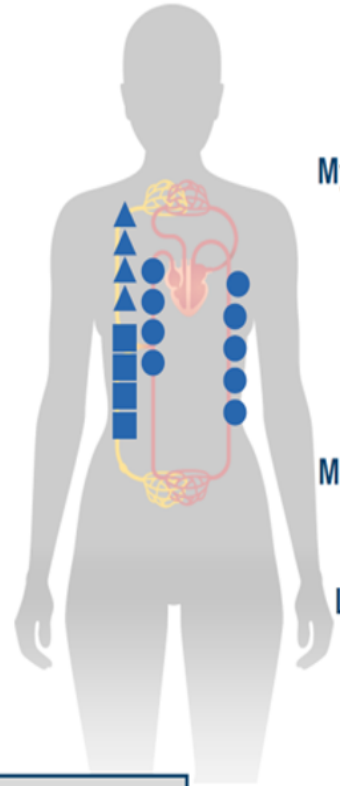


35 people were diagnosed with 36 cancers

- 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



Plasma Cell Myeloma/Disorders (n=1)

Lymphoid Leukemia (n=2)

Waldenstrom Macroglobulinemia (n=2)

Lymphoma (n=12)

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

Available Screening: USPSTF cancer screening or **No standard screening**

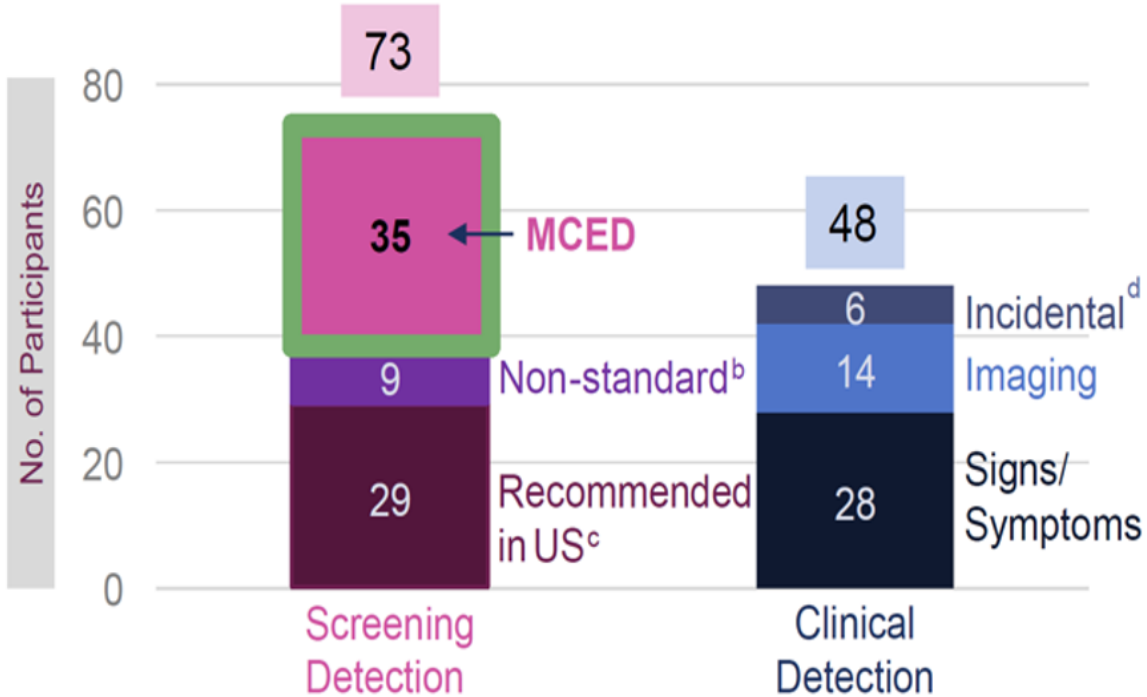
Deb Schrag. ESMO 2022.

GenesisCare OLA FLASCO FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY

Cancers Identified Within One Year of MCED Testing

Participants with Cancers Detected by Either Screening or Clinical Findings

121 participants had a cancer diagnosis within 1 year



- 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.

Deb Schrag. ESMO 2022.

GenesisCare OLA FLASCO FAU MEDICINE FLORIDA ATLANTIC UNIVERSITY

PATHFINDER Investigators concluded:

- MCED screening was safely implemented for adults with and without additional cancer risk.
- 1.4% of participants had a cancer signal detected.
- 0.5% of participants were diagnosed with cancer due to MCED signal detection.
- Median time to diagnostic resolution was 79 days.
- High accuracy of predicted origin enabled targeted diagnostic evaluations.
- Most diagnostic evaluations involved imaging, few required invasive procedures.
- *This study shows that it is **feasible to detect cancers early** using blood tests*

Deb Schrag. ESMO 2022

Future/Ongoing Work

- Optimization of MCED test performance characteristics.
- PATHFINDER2 is screening 20,000 individuals using the refined MCED test.
- NHS-Galleri (ISRCTN 91431511) is a randomized trial of 140,000 adults 50-77 years old in the UK's NHS. It will compare the incidence of advanced cancer diagnoses among participants assigned to undergo annual MCED screening for 3 years or alternatively, to usual care.

Take Home Message

- cfDNA offers a tool to improve cancer therapy and management across disease stages, from early detection to acquired mechanism of resistance in the metastatic setting.
- ctDNA can be used for molecular profiling in patients with advanced solid tumors to guide therapeutic decisions.
- ctDNA has the potential to monitor response to therapy (molecular response) at an early timepoint.
- Plasma clearance can predict for treatment benefit in the early & advanced stage setting.
- ctDNA can detect MRD; MRD has shown to be a prognostic biomarker.
- ctDNA methylation & fragmentomics are under investigation for early cancer detection; sensitivity rate may be a limiting factor.