Novel Frontiers in the Use of ctDNA (and other analytes and fluids) in Oncology

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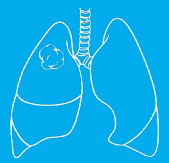


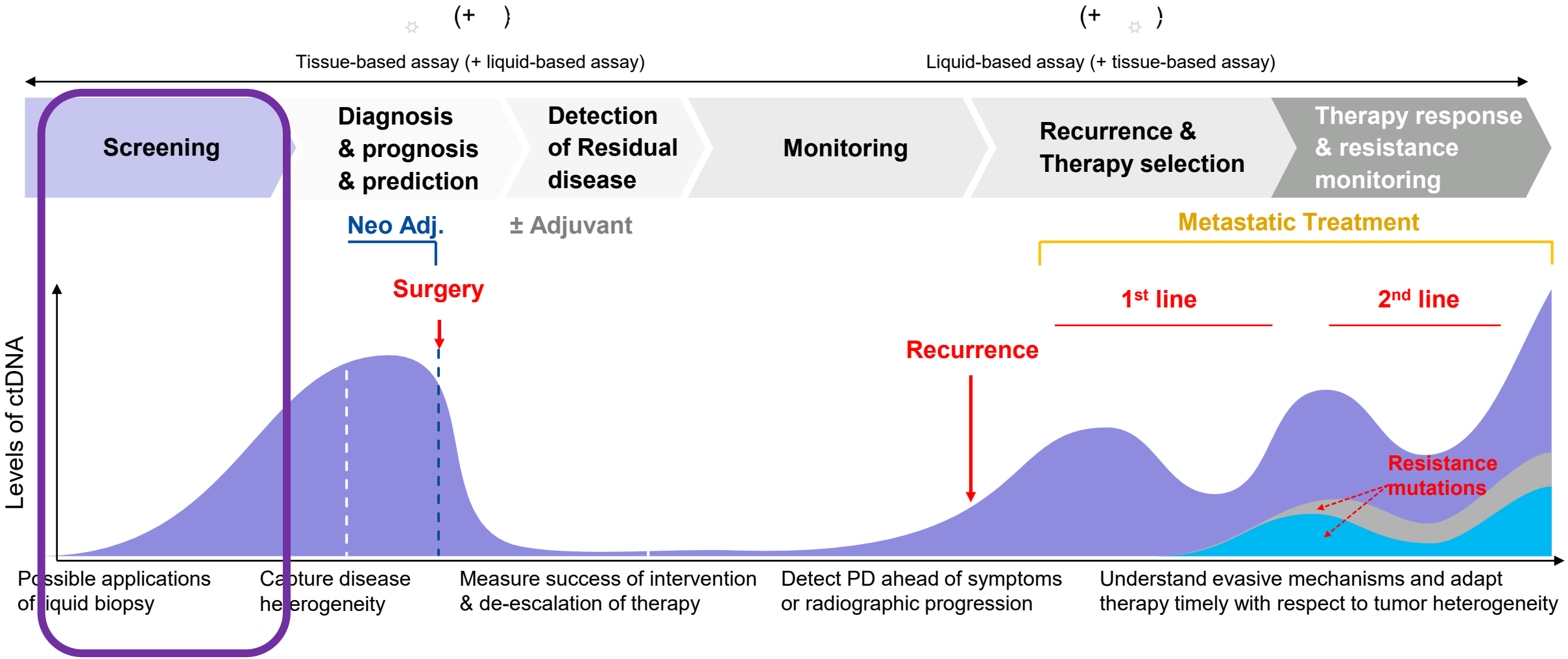
Center for Thoracic Oncology

Mount Sinai
The Tisch Cancer Institute

Declaration of Interests

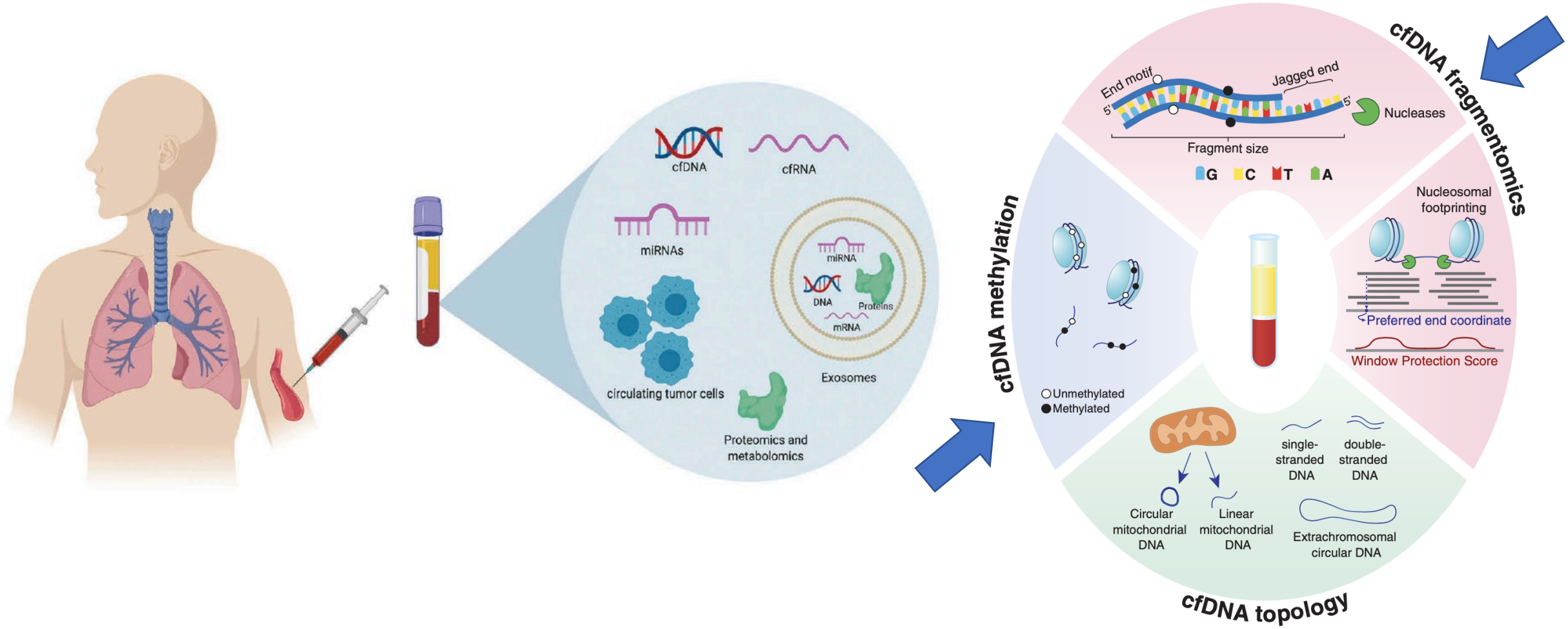
Research grants	**Lung Cancer Research Foundation-Pfizer Grant 2019** **NIH U54 grant**
Personal financial interests	**Speaker: MSD, Roche, Astra Zeneca**
	Advisory board: Inivata, ArcherDx, MD Serono, Novartis, Boston Pharmaceuticals, Pfizer, Eisai, Blueprint, Mirati, COR2ED, Astra Zeneca, Daiichi Sankyo.
Non-financial interests	**Research Collaboration: GuardantHealth**
Leadership roles	**Chair Educational Committee IALSC - President ISLB (International Society of Liquid Biopsy) - Educational Chair: OLA Oncology Latin American Association** **Scientific Committee Member at ESO (European School of Oncology).**

Liquid biopsy can provide clinically-valuable information along the whole patient journey



Adapted from Wan, J.C.M., et al., (2017) Nat Rev Cancer 17:223-38.

The liquid biopsy family in Early Detection



Russo A (Rolfo C), et al. Expert Rev Mol Diagn 2019 , Lo et al., Science 372, eaaw3616 (2021)

Evaluation of Cell-Free DNA Approaches for Multi-Cancer Early Detection

CCGA1 → **CCGA2** → **CCGA3**

Discovery

- Methylation Patterns
- Mutations
- Chromosome Alterations

Training / Validation

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

ORIGINAL ARTICLE

Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA

M. C. Liu[1†], G. R. Oxnard[2†], E. A. Klein[3], C. Swanton[4,5], M. V. Seiden[6*] & on behalf of the CCGA Consortium[†]

Validation

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

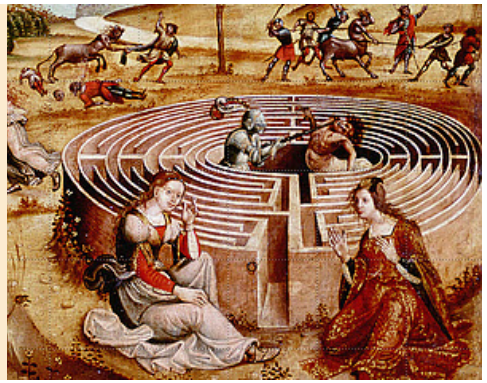
ANNALS OF ONCOLOGY driving innovation in oncology

ORIGINAL ARTICLE

Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set

E. A. Klein[1*], D. Richards[2], A. Cohn[3], M. Tummala[4], R. Lapham[5], D. Cosgrove[6], G. Chung[7], J. Clement[8], J. Gao[9], N. Hunkapiller[9], A. Jamshidi[9], K. N. Kurtzman[9], M. V. Seiden[10], C. Swanton[11,12] & M. C. Liu[13]

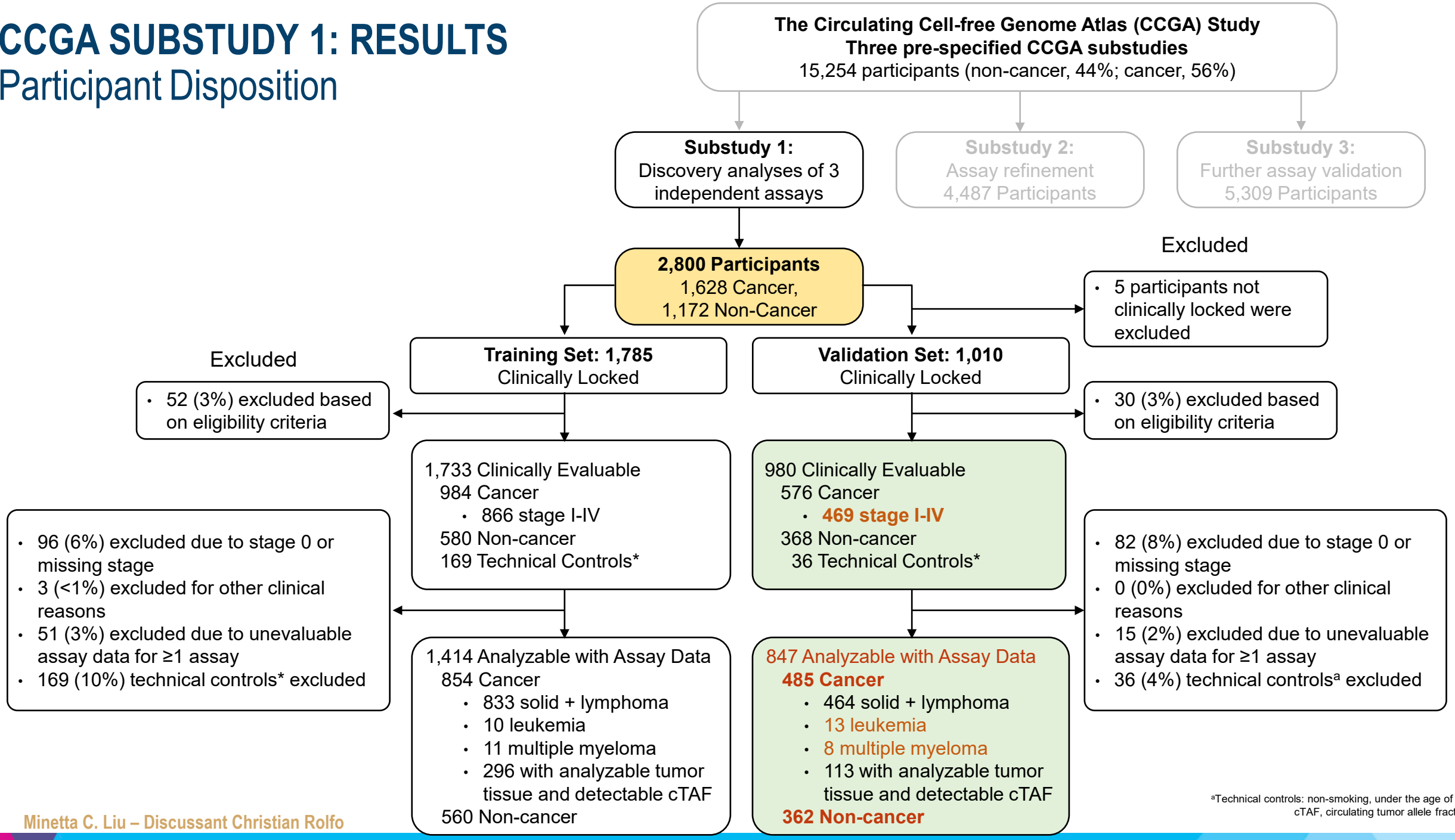
Ariadne's Thread



Liu et al, ESMO 2021

Liu et al Annals of Onc 2020, Klein EA, et al. Ann Oncol 2021

CCGA SUBSTUDY 1: RESULTS
Participant Disposition

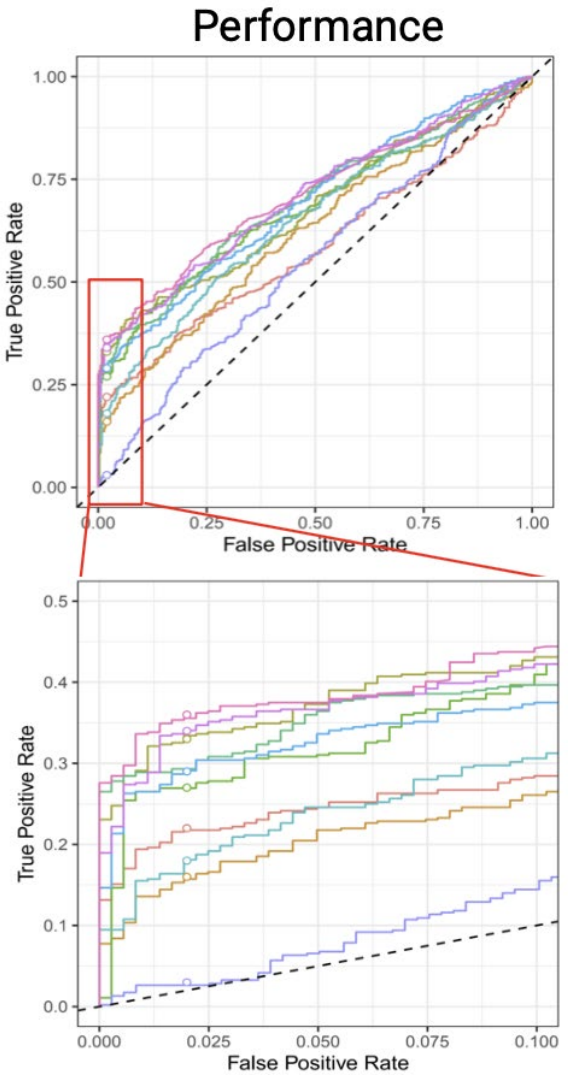


[a]Technical controls: non-smoking, under the age of 35.
cTAF, circulating tumor allele fraction

CCGA SUBSTUDY 1: RESULTS
Cancer Signal Detection

Assay	Classifier	Sensitivity at 98% specificity	
		% (95% CIs)	TP/Total samples, n
WGBS	**WG methylation**	**34% (30%-39%)**	**158/464**
TS	SNV	16% *** (13%-20%)	73/464
	SNV-WBC	**33% (29%-38%)**	**155/464**
WGS	SCNA	27% *** (23%-31%)	125/464
	SCNA-WBC	30% * (26%-34%)	139/464
	Fragment endpoints	18% *** (15%-22%)	84/464
	Fragment lengths	29% * (25%-34%)	135/464
	Allelic Imbalance	22% *** (18%-26%)	101/464
All three	**Pan-feature**	**36% (31%-40%)**	**165/464**
None	Clinical Data	2.6% ***(1.4%-4.5%)	12/457

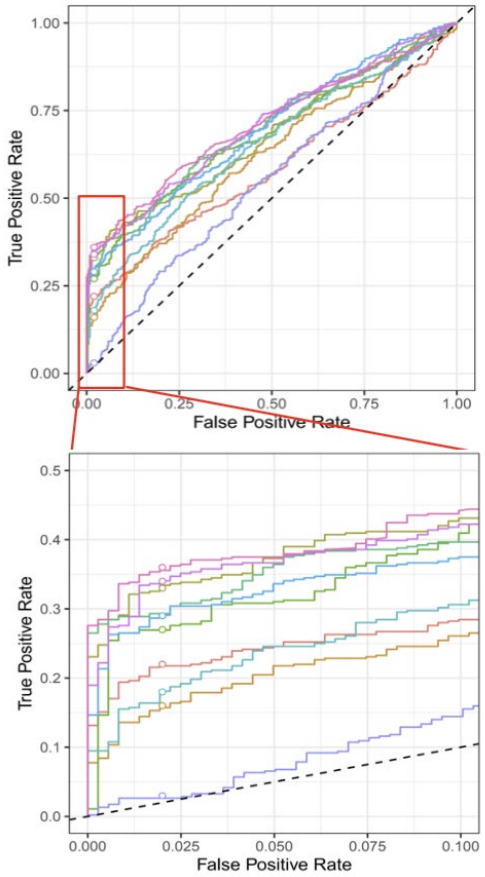
* $p<0.01$; ** $p<0.001$; *** $p<0.0001$. *p*-values were only computed for the validation set and represent paired McNemar analysis versus WG methylation.



CI, confidence interval; SCNA, somatic copy number alteration; SCNA-WBC, somatic copy number alterations with correction for clonal hematopoiesis noise; SNV, single nucleotide variant; SNV-WBC, single nucleotide variants with correction for clonal hematopoiesis noise; TP, true-positive; TS, targeted sequencing; WG, whole-genome; WGS, whole-genome sequencing

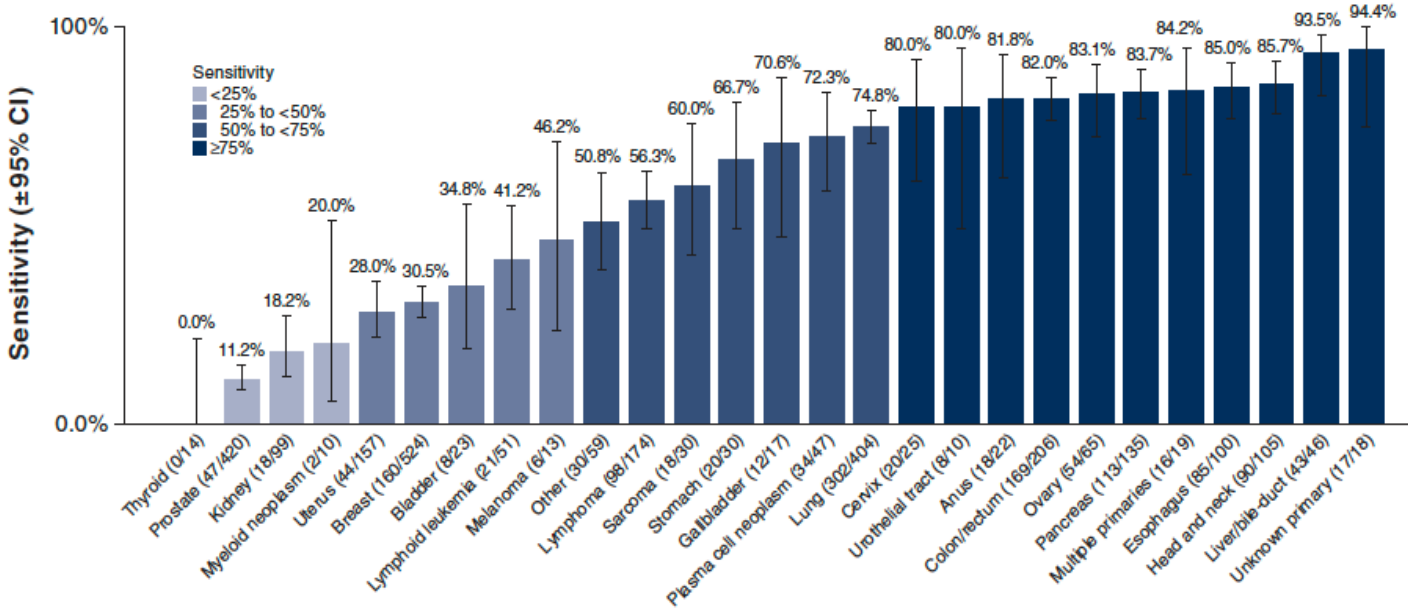
Sensitivity at 98% specificity	WGBS	WG methylation	34% (30%-39%)	158/464

Performance



	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%)	

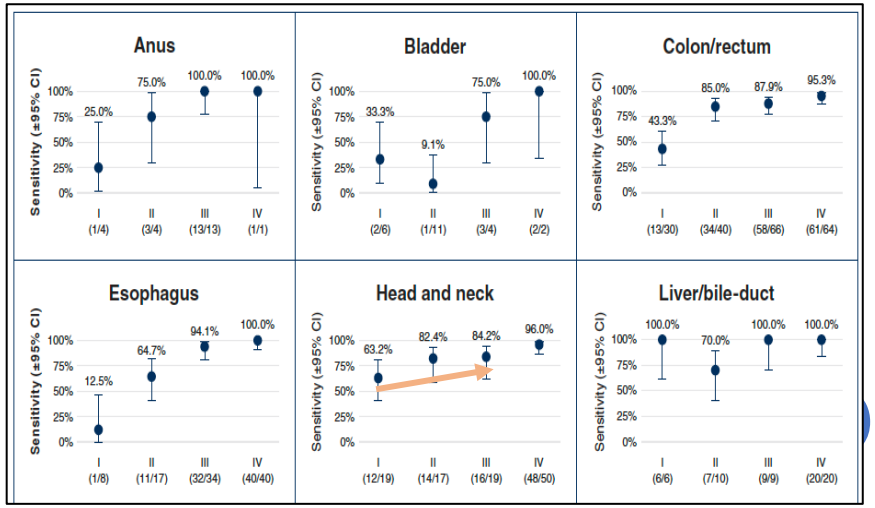
Two-sided 95% Wilson confidence intervals were calculated.

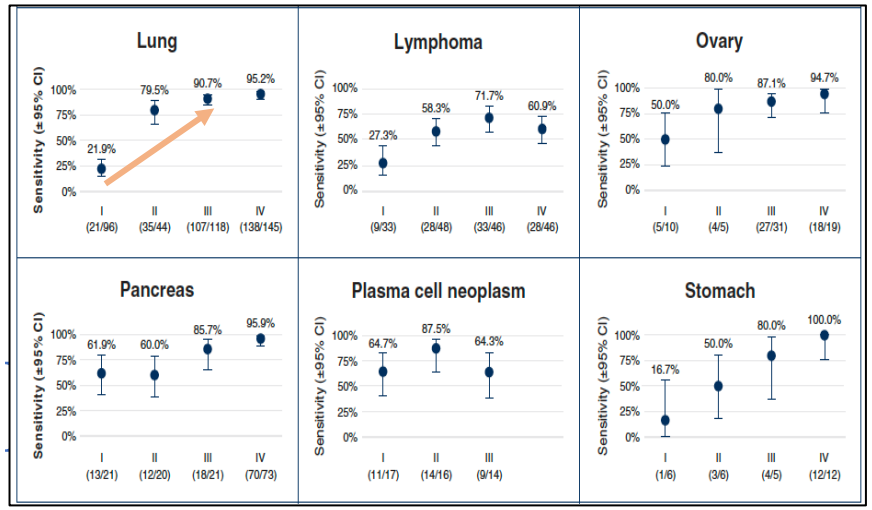


Confirmed status analysis set, *n* = 4077
cancer, *n* = 2823; non-cancer, *n* = 1254

Clinical cancer stage, *n* (%)	
I	849 (30.1)
II	703 (24.9)
III	566 (20.0)
IV	618 (21.9)

Klein EA, et al. Ann Oncol 2021

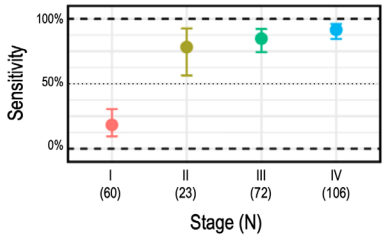




Clinical stage	Total *N*	Test positive	Sensitivity % (95% CI)[a]
All	2823	1453	51.5 (49.6% to 53.3%)
I	849	143	16.8 (14.5% to 19.5%)
II	703	284	40.4 (36.8% to 44.1%)
III	566	436	77.0 (73.4% to 80.3%)
IV	618	557	90.1 (87.5% to 92.2%)
I-II	1552	427	27.5 (25.3% to 29.8%)
I-III	2118	863	40.7 (38.7% to 42.9%)
I-IV	2736	1420	51.9 (50.0% to 53.8%)
III-IV	1184	993	83.9 (81.7% to 85.9%)
Not expected to be staged	67	23	34.3 (24.1% to 46.3%)
Missing	20	10	50.0 (29.9% to 70.1%)

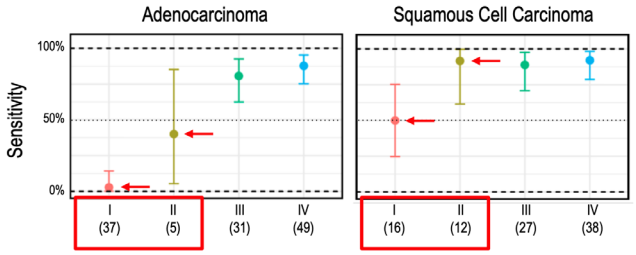
All subtypes have the same sensitivity?

Lung Cancer Detection Varies by Subtype at 99.4% Specificity

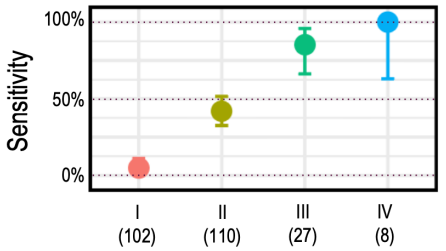


- Overall lung cancer sensitivity: 71.6% (95% CI: 65.8-77.0%)

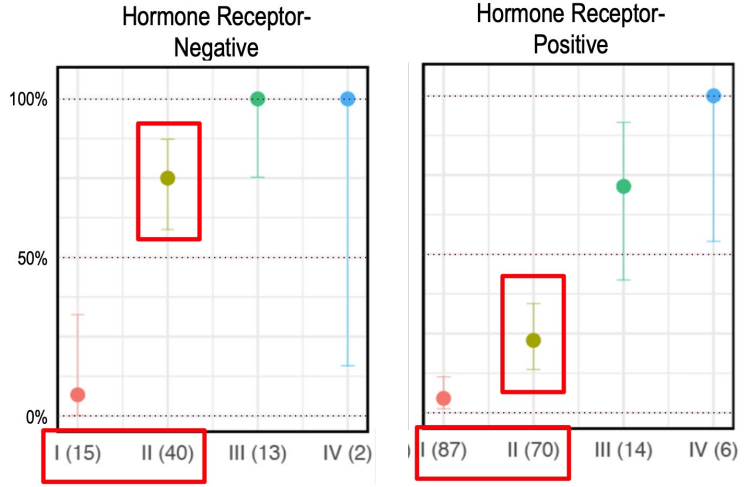
- Detection rate affected by early-stage adenocarcinomas
  - Detection higher in squamous cell carcinoma
- Consistent with prior report showing ctDNA detection was higher in squamous cell carcinoma than adenocarcinoma[1]



Breast Cancer Detection Varies by Subtype at 99.4% Specificity

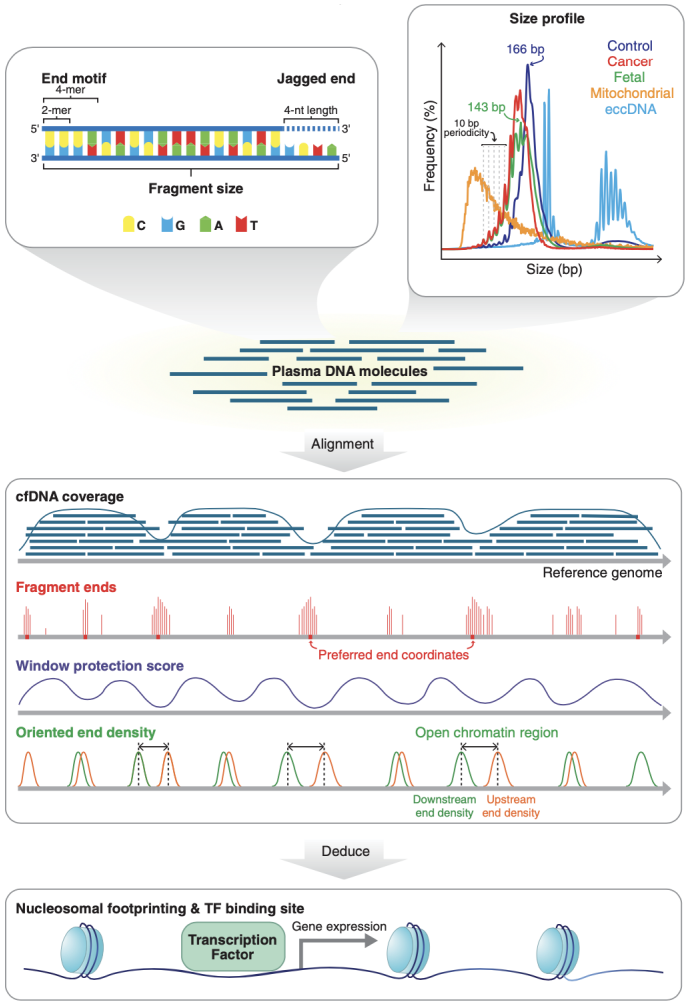


- Overall breast cancer sensitivity: 33.2% (95% CI: 27.4-39.4%)

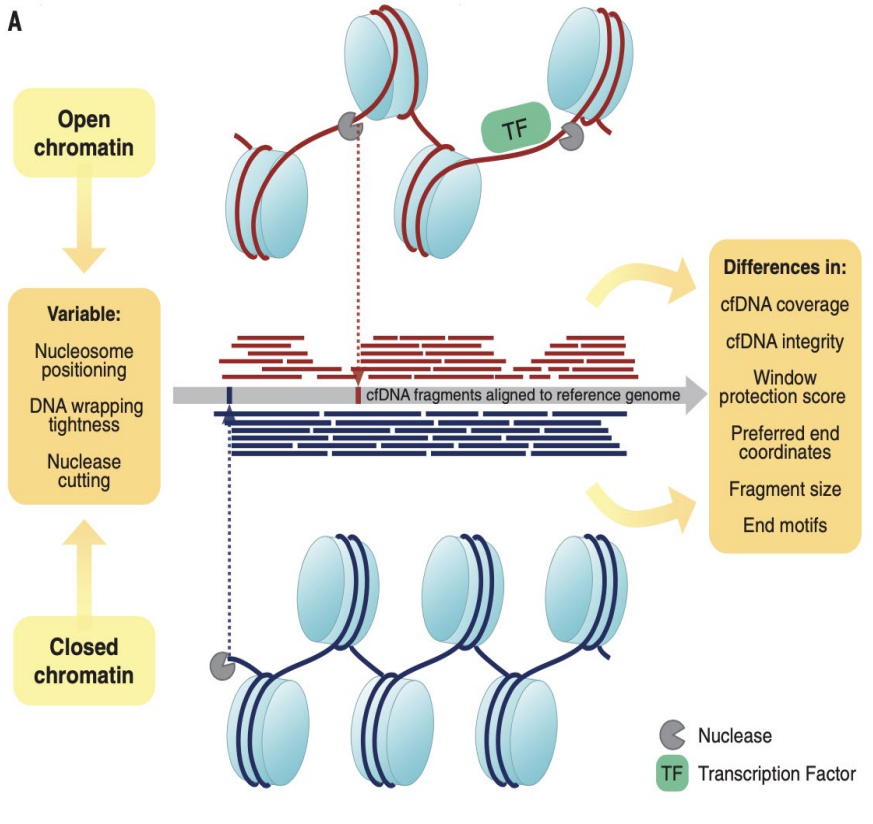


Oxnard et al, ESMO 2019, Klein EA, et al. Ann Oncol 2021

ctDNA Fragmentomics: Recognizing the complexity



In patients with cancer, fragmentation patterns in cfDNA appear to result from mixtures of nucleosomal DNA from both blood and neoplastic cells.



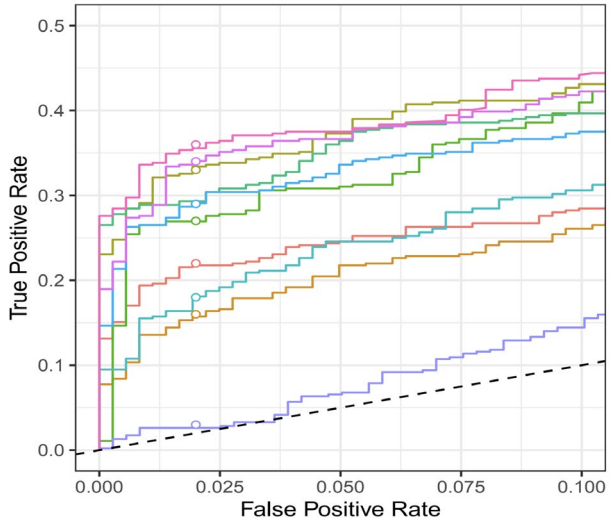
- Open or closed chromatin regions differ in **their nucleosome repeat lengths and gene expression levels** which in turn may increase or decrease nuclease accessibility.
- The majority of circulating DNA fragments are **mononucleosomal in length, and nucleosomal packaging** affects the cell-free DNA size.

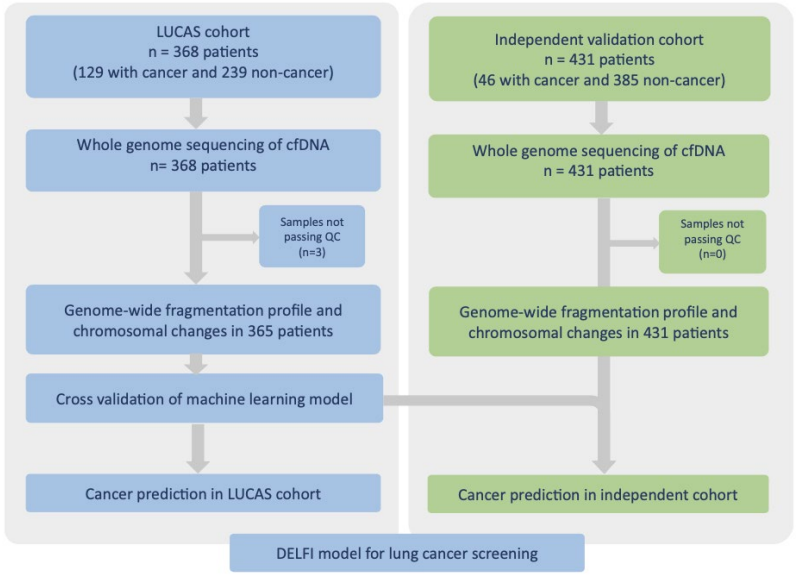
Lo et al., Science 372, eaaw3616 (2021)

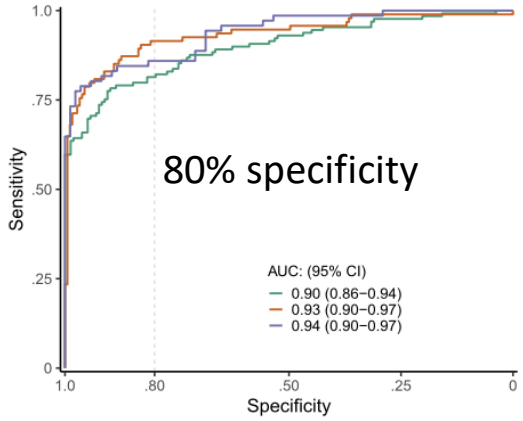
Fragmentomics in a Single-tumor test

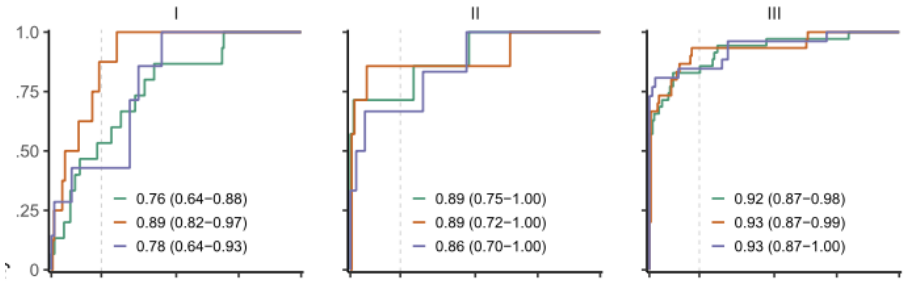
Sensitivity at 98% specificity			
	Fragment endpoints	18% *** (15%-22%)	84/464
	Fragment lengths	29% * (25%-34%)	135/464

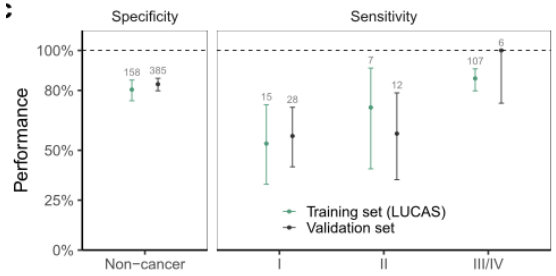
Non-cancer individuals: 236
Patients with Lung cancer 129



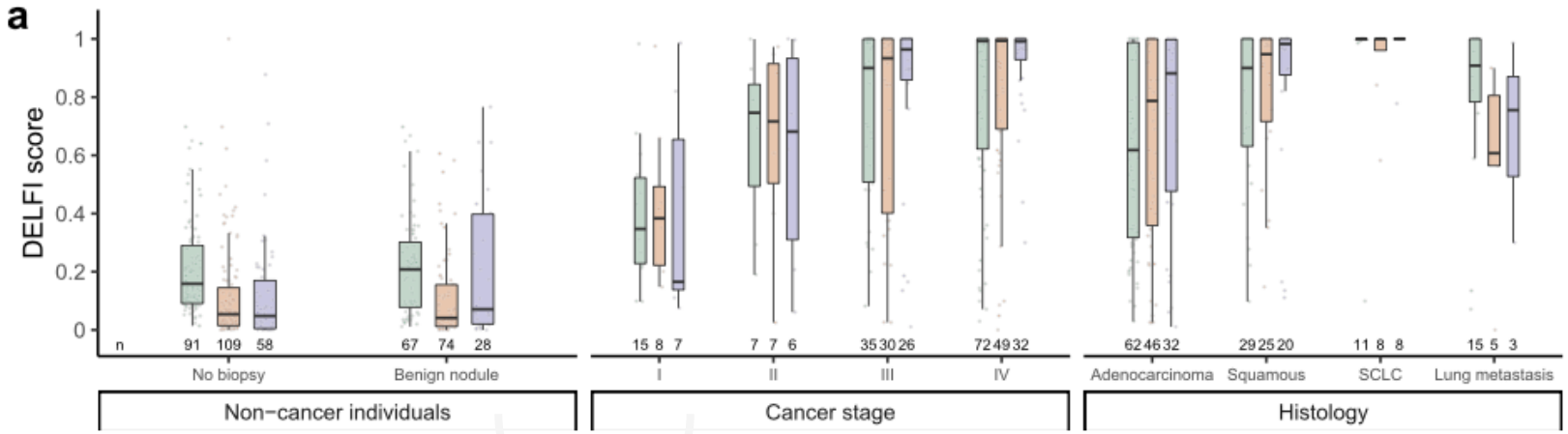








Cristiano S, et al. Nature 2019

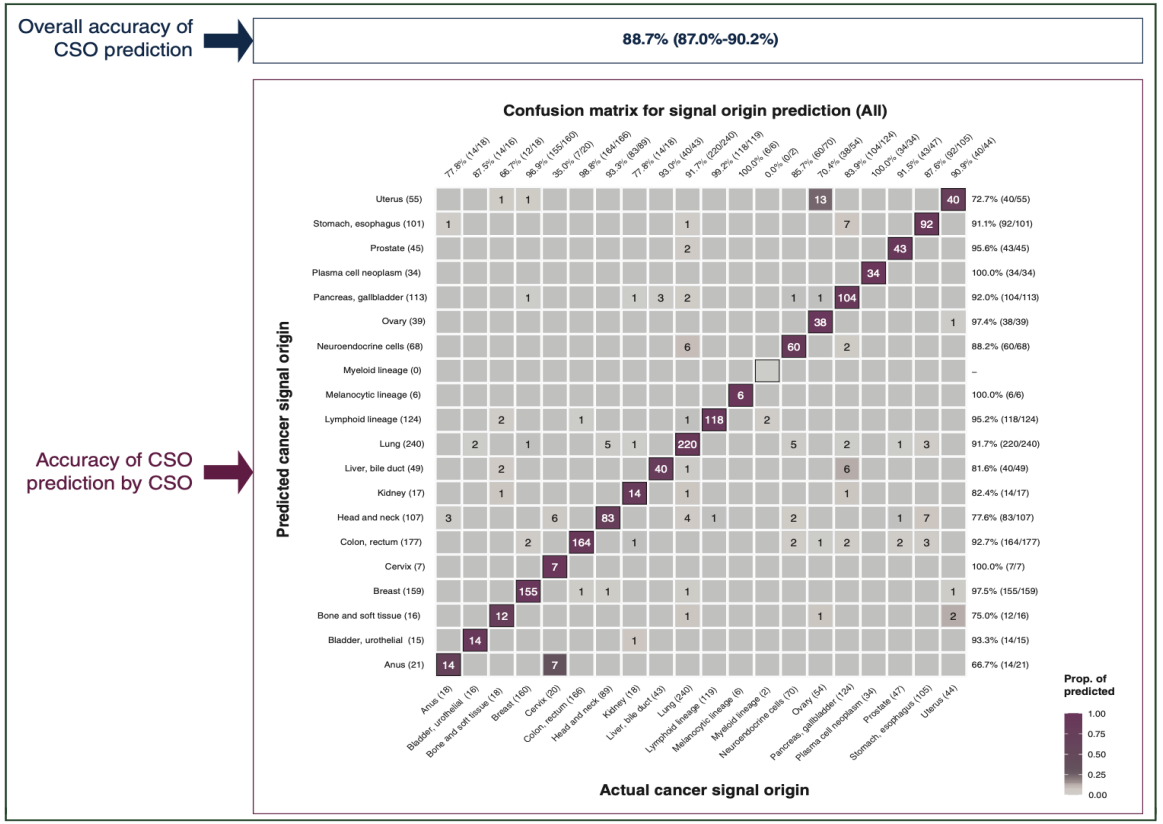


High risk population Missing in the current analysis and validation

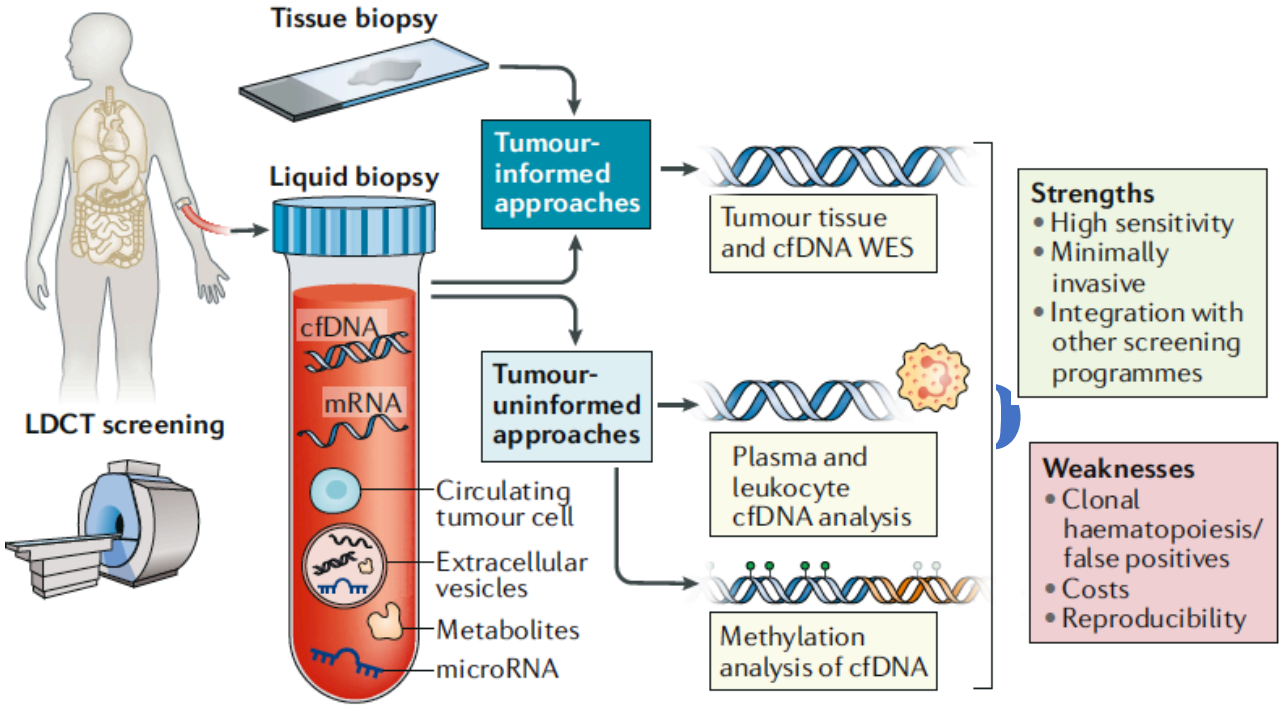
SURVIVAL IMPACT

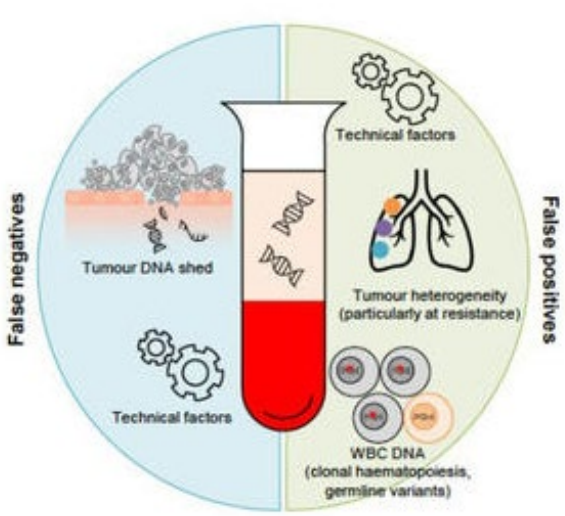
Cancer Signal Origin Prediction Accuracy

Assay	Classifier	Accuracy
WGBS	WG methylation	75% (95/127)



Cristiano S, et al. Nature 2019, Klein EA, et al. Ann Oncol 2021



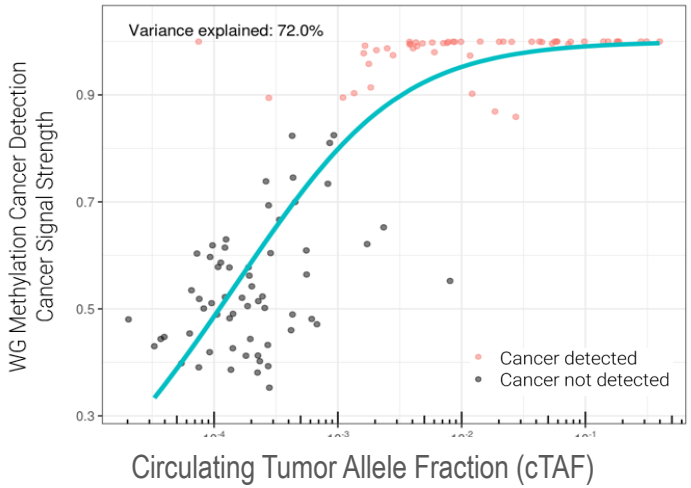


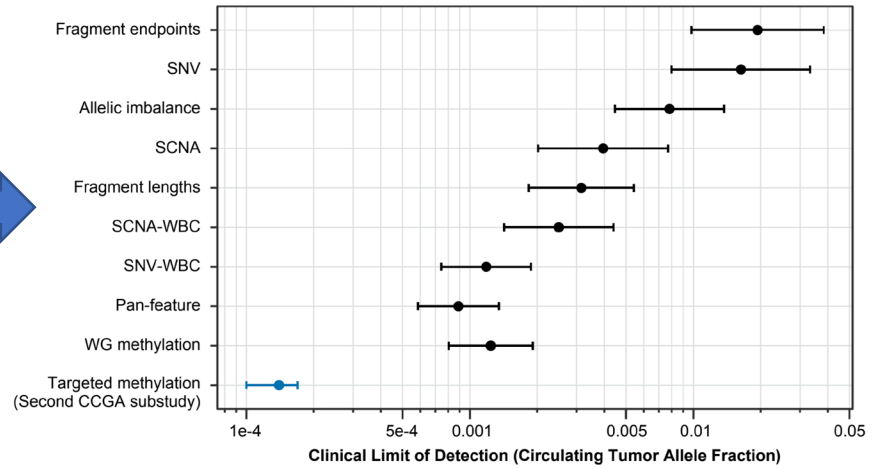
"False positives" in liquid biopsy

Technical factors (sample differences e.g. > 6 months from tissue to plasma sampling)

WBC contamination (germline variants, clonal haematopoiesis)

Tumour heterogeneity (positive plasma and negative tissue [assumes tissue is the "gold standard"])



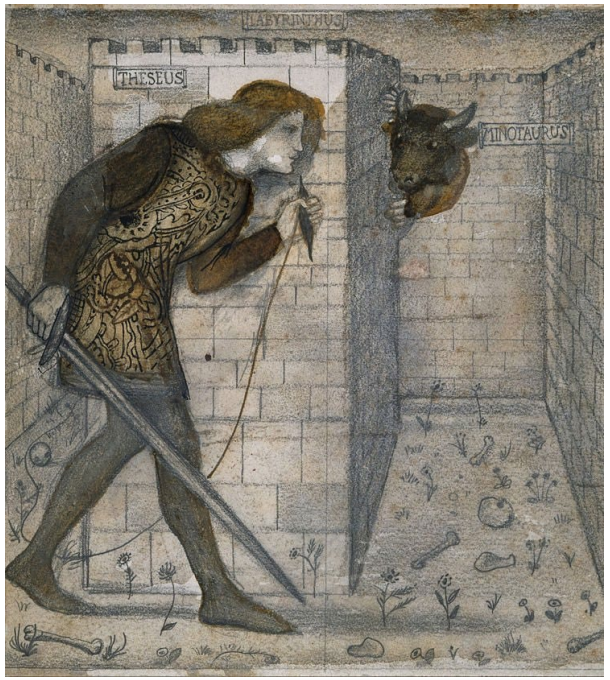


Clinical Limit of Detection

- Among the lowest clinical LOD measured
- Unlike SNV-WBC and pan feature, **does not require removal** of biological background from paired WBCs

Rolfo C & Russo A. Nat Rev Clin Oncol 2020

Take home message from Liu et al abstract:



Strengths:

- Impressive research in multi-omics to confirm WG methylation approach as the most promising one for MCED
- cfDNA WG Methylation is less complex and avoid confounding factors
- cfDNA WG Methylation has Validation at high specificity >>> FDA Approval

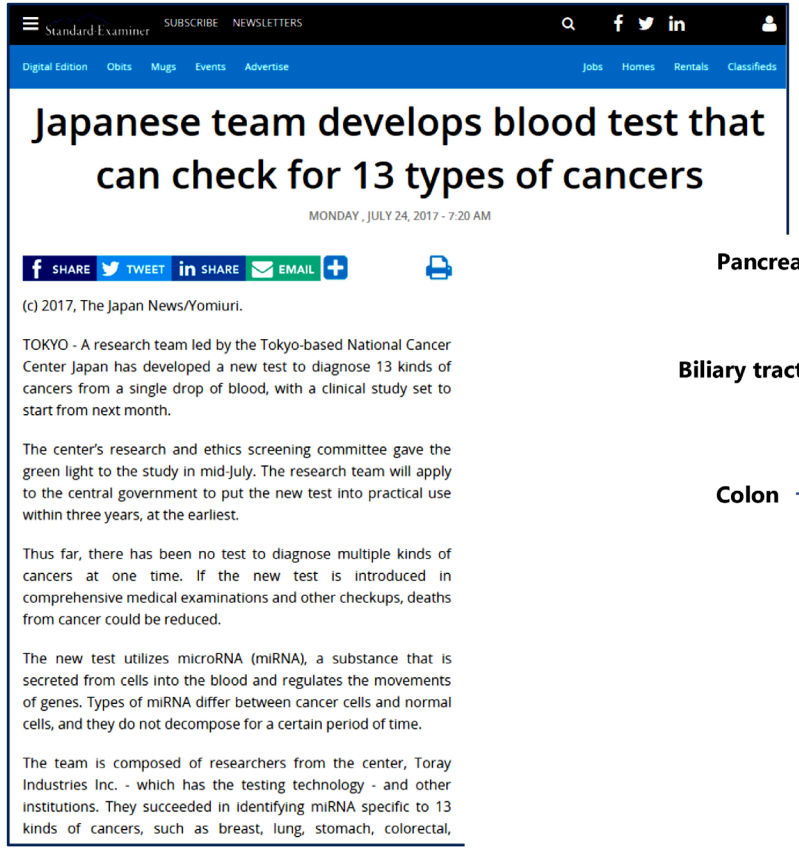
Questions still open:

- Heterogeneous population, including solid tumors and hematologic malignancies
- It's necessary to include Stage IV?
- Missing data of high risk population –nodules discrimination: Useful in this scenario?
- Differences in Histology and other variants for each tumor type will affect the performance?

Multi-Omics → **WG Methlyation** → **Multi-Omics Beyond cfDNA**

Machine Learning-Based Multiple Cancer Detections with circulating MiRNA Profiles in Blood

National Project to develop a circulating miRNA database in Japan (2014-2019)



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Japanese team develops blood test that can check for 13 types of cancers

MONDAY , JULY 24, 2017 - 7:20 AM

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(c) 2017, The Japan News/Yomiuri.

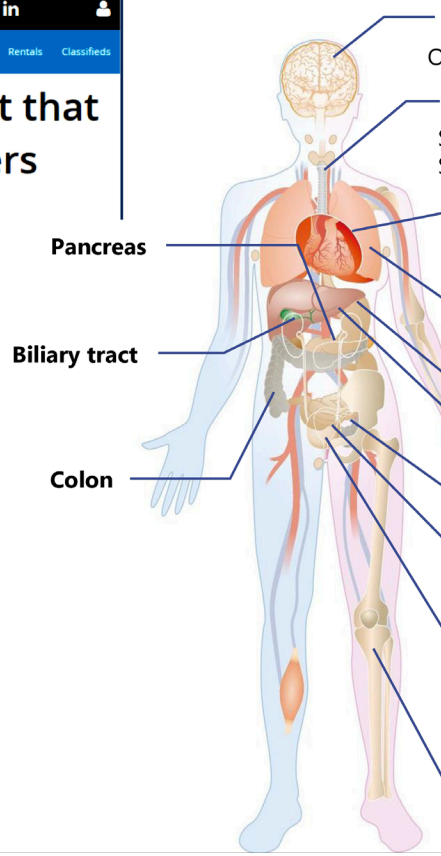
TOKYO - A research team led by the Tokyo-based National Cancer Center Japan has developed a new test to diagnose 13 kinds of cancers from a single drop of blood, with a clinical study set to start from next month.

The center's research and ethics screening committee gave the green light to the study in mid-July. The research team will apply to the central government to put the new test into practical use within three years, at the earliest.

Thus far, there has been no test to diagnose multiple kinds of cancers at one time. If the new test is introduced in comprehensive medical examinations and other checkups, deaths from cancer could be reduced.

The new test utilizes microRNA (miRNA), a substance that is secreted from cells into the blood and regulates the movements of genes. Types of miRNA differ between cancer cells and normal cells, and they do not decompose for a certain period of time.

The team is composed of researchers from the center, Toray Industries Inc. - which has the testing technology - and other institutions. They succeeded in identifying miRNA specific to 13 kinds of cancers, such as breast, lung, stomach, colorectal,



Brain
Ohno, Matsuzaki et al. ***JAMA Netw Open*** 2:e1916953, 2019

Esophagus
Sudo et al. ***JAMA Netw Open*** 2:e194573, 2019
Sudo, Matsuzaki et al. ***Jpn J Clin Oncol*** 50:114, 2020

Breast
Shimomura et al. ***Cancer Sci*** 107:326, 2016
Shiino, Matsuzaki et al. ***Clin Cancer Res*** 15:1817, 2019

Pancreas

Lung
Asakura, Kadota et al. ***Commun Biol*** 3:134, 2020

Biliary tract

Stomach
Abe, Matsuzaki et al. ***Gastric Cancer*** (online)

Liver
Yamamoto, Kondo, Matsuzaki et al. ***Hepatol Commun*** 4:284, 2020

Colon

Ovary
Yokoi, Matsuzaki et al. ***Nat Commun*** 9:4319, 2018

Bladder
Usuba et al. ***Cancer Sci*** 110:408, 2019

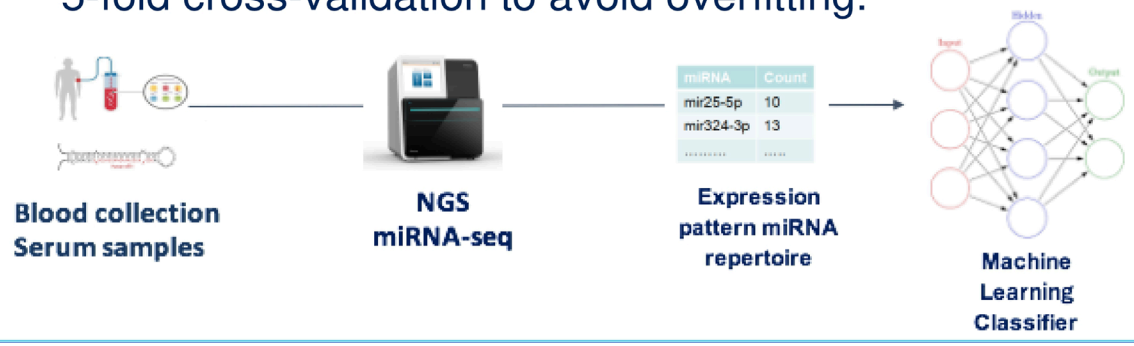
Prostate
Urabe, Matsuzaki et al. ***Clin Cancer Res*** 25:3016, 2019

Sarcoma
Asano, Matsuzaki et al. ***Nat Commun*** 10:1299, 2019

Matzuzaki et al, ASCO 2021

Prospective Validation with New Technologies

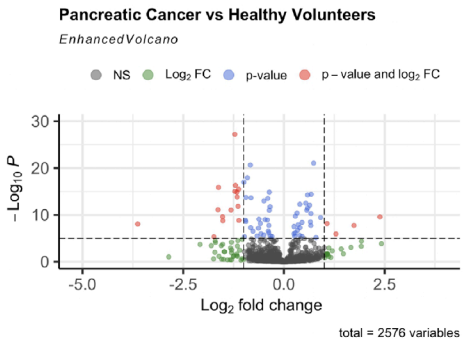
- Serum samples were prospectively collected with standard operating procedures.
- The entire miRNA expression profile was analyzed via NGS (Illumina NovaSeq 6000)
- The resulting total miRNA expression profile was used to train machine learning models.
- The machine learning model was trained with a training set to test set ratio of 4:1 and was carefully monitored by 5-fold cross-validation to avoid overfitting.

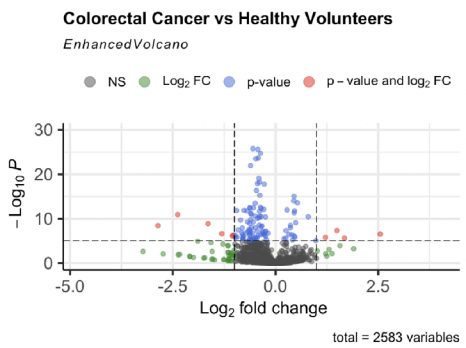


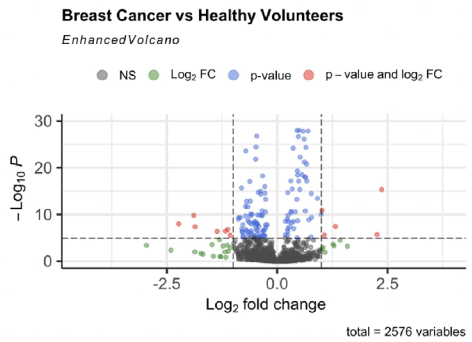
Cancer Type	Male	Female	Age [Mean (SD)]
Breast: 272	-	272	54.0 (11.8)
Lung: 223	133	90	68.3 (9.8)
Colorectal: 237	144	93	64.8 (11.9)
Stomach: 221	152	69	68.2 (10.6)
Pancreas: 99	60	39	64.7 (11.3)
Volunteers: 289	142	147	60.7 (12.0)

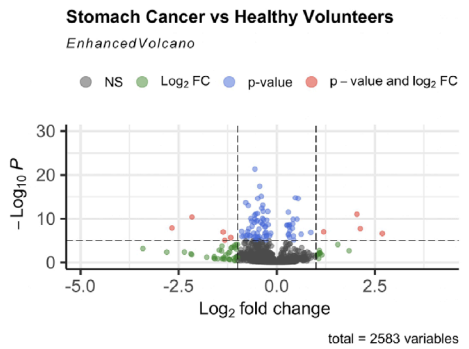
Matzuzaki et al, ASCO 2021

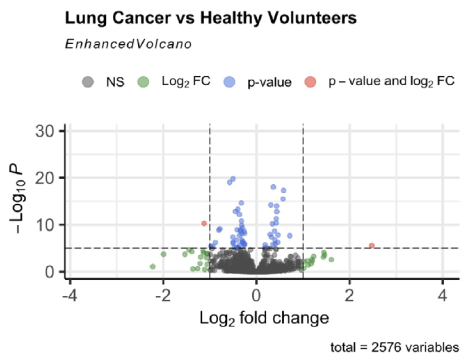
The diagnostic model provided 88% accuracy for all five cancer types

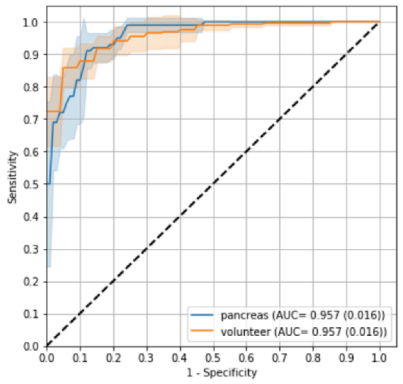


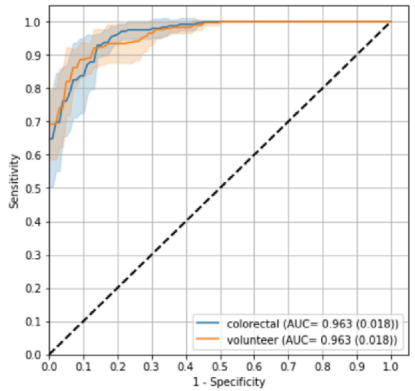


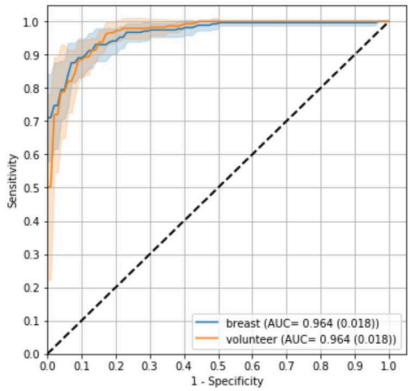


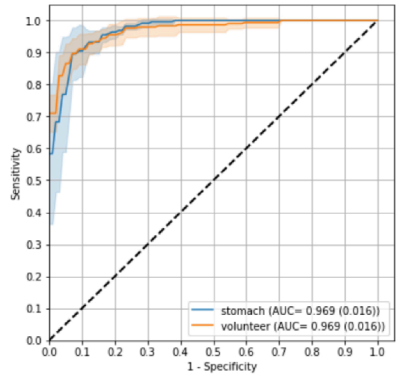


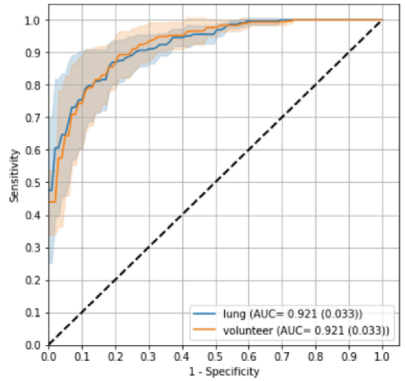






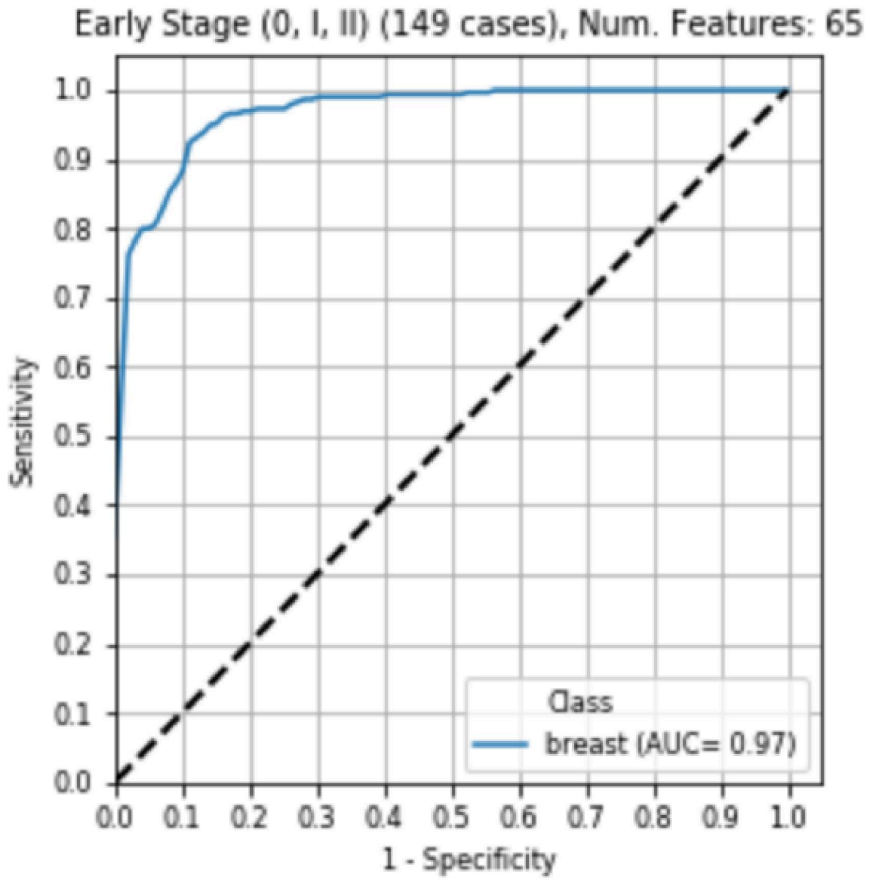


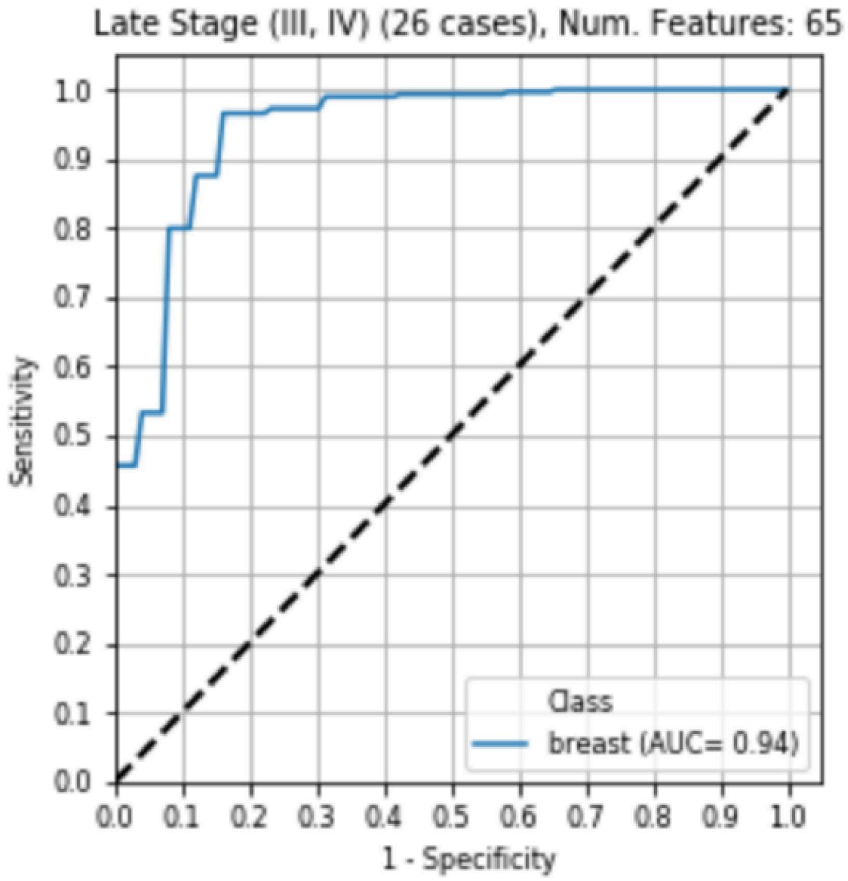




Matzuzaki et al, ASCO 2021

Early vs Late Stage Breast Cancer Detection





Matzuzaki et al, ASCO 2021

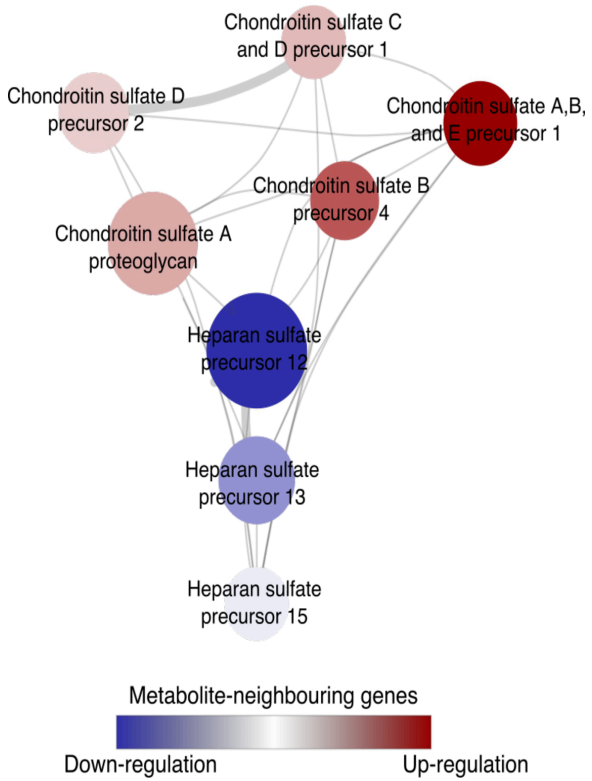
Conclusions

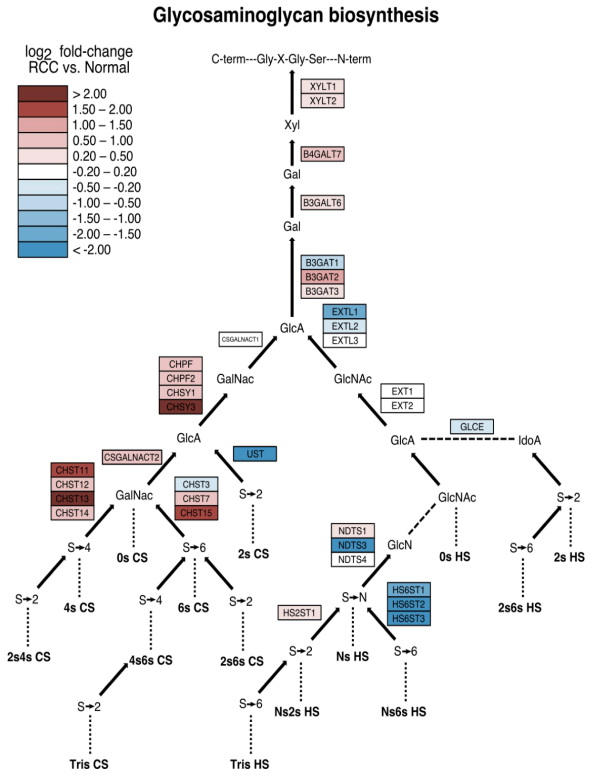
- The main advantage of miRNA-based cancer diagnosis is that they are more sensitive even in the early stages of cancer, compared to other diagnostic methods, such as cell-free DNA diagnostics, where the sensitivity of many types of cancer in the early stages still remains low.
- This approach is easily expandable to other cancer types.
- Given the potential value of early detection in fatal malignancies, further validation studies are justified in future population-based studies. Many cancer research institutes are currently conducting further clinical trials to validate this early cancer diagnosis based on miRNA expression profiles.
- Further basic research to elucidate the functionality of extracellular miRNAs is also required.

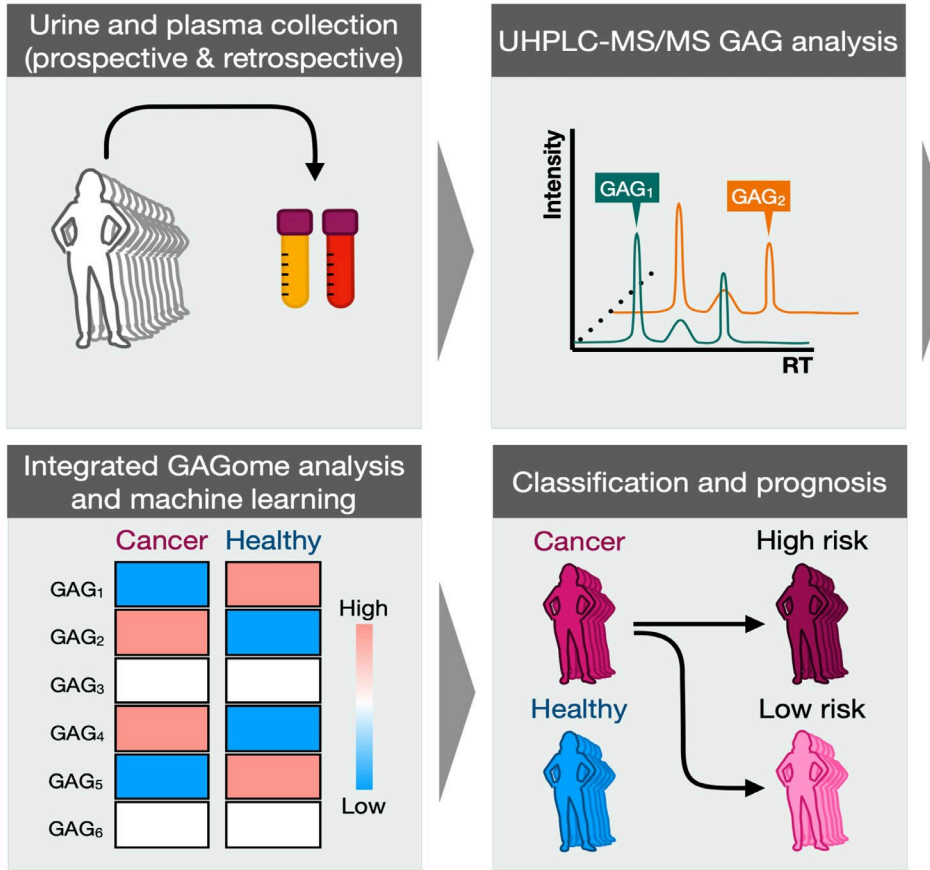
Matzuzaki et al, ASCO 2021

Detection of any-stage cancer using plasma and urine glycosminglycans

Do GAGomes change from Normal levels in any cancer type? At which stage?

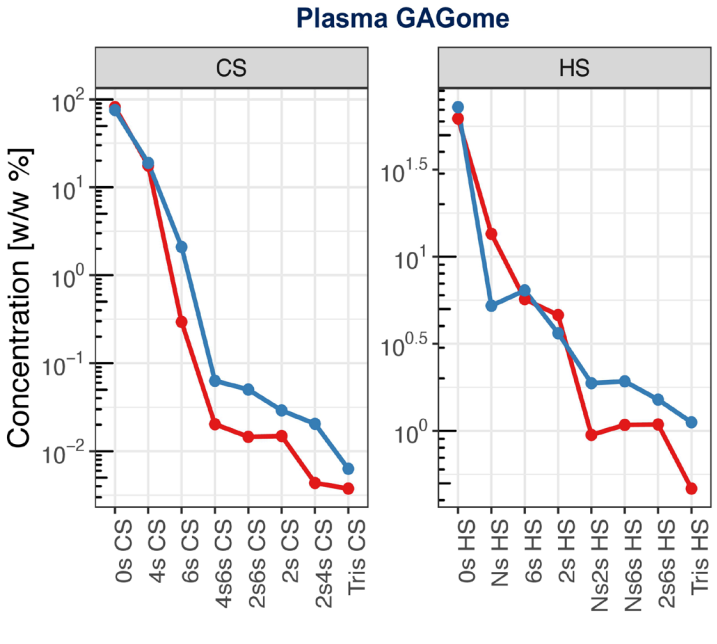


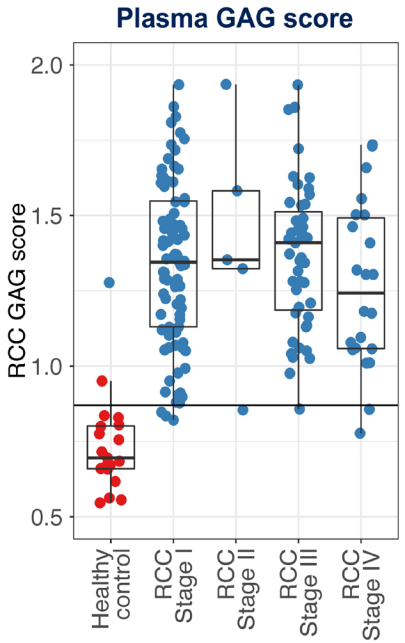




Harnessing Deregulated GAG metabolism led to the development of GAG scores

Plasma & Urine GAGome in 1500+ samples from 553 Cancer Patients vs 426 helathy





	C (N=553)	H (N=426)
Age		
Mean (SD)	64.3 (12.6)	57.4 (13.8)
Median [Min, Max]	67.0 [21.0, 91.0]	59.0 [22.0, 78.0]
Gender		
Female	253 (45.8%)	246 (57.7%)
Male	300 (54.2%)	180 (42.3%)
Group		
H	0 (0%)	426 (100%)
BC	28 (5.1%)	0 (0%)
BCa	47 (8.5%)	0 (0%)
CRC	27 (4.9%)	0 (0%)
CST	28 (5.1%)	0 (0%)
DG	40 (7.2%)	0 (0%)
EC	30 (5.4%)	0 (0%)
GNET	14 (2.5%)	0 (0%)
HN	17 (3.1%)	0 (0%)
LL	18 (3.3%)	0 (0%)
NHL	30 (5.4%)	0 (0%)
NSCLC	83 (15.0%)	0 (0%)
OV	30 (5.4%)	0 (0%)
PCa	104 (18.8%)	0 (0%)
RCC	57 (10.3%)	0 (0%)
Stage		
Stage I/Low-grade	187 (33.8%)	0 (0%)
Stage II	56 (10.1%)	0 (0%)
Stage III	59 (10.7%)	0 (0%)
Stage IV/High-grade	238 (43.0%)	0 (0%)
Unspecified stage/grade	13 (2.4%)	0 (0%)
Healthy controls	0 (0%)	426 (100%)

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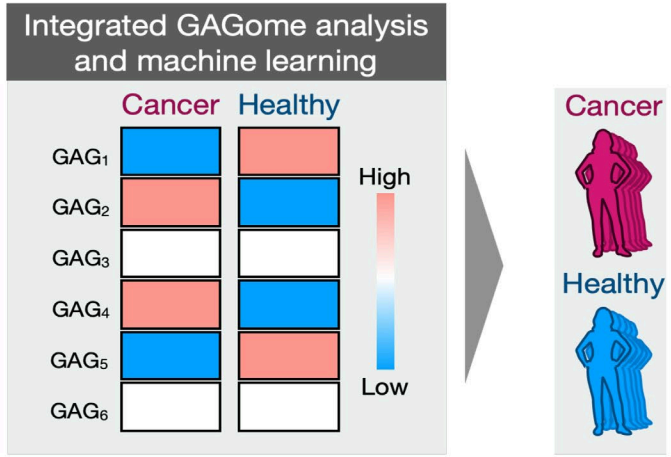
Alterations of Plasma & Urine GAGome in Cancer Used to develop GAG Scores



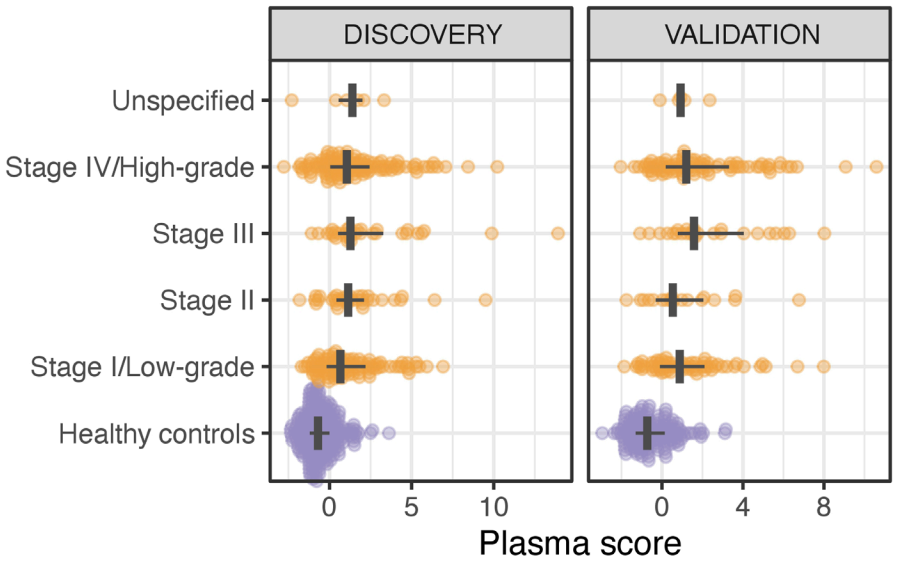
	Combined (N=523)		Plasma (N=942)		Urine (N=560)	
	DISCOVERY (N=314)	VALIDATION (N=209)	DISCOVERY (N=567)	VALIDATION (N=375)	DISCOVERY (N=337)	VALIDATION (N=223)
Stage						
Healthy controls	204 (65.0%)	135 (64.6%)	255 (45.0%)	170 (45.3%)	205 (60.8%)	135 (60.5%)
Stage I/Low-grade	23 (7.3%)	21 (10.0%)	110 (19.4%)	68 (18.1%)	29 (8.6%)	24 (10.8%)
Stage II	10 (3.2%)	6 (2.9%)	36 (6.3%)	18 (4.8%)	11 (3.3%)	7 (3.1%)
Stage III	11 (3.5%)	8 (3.8%)	32 (5.6%)	25 (6.7%)	12 (3.6%)	9 (4.0%)
Stage IV/High-grade	66 (21.0%)	39 (18.7%)	128 (22.6%)	89 (23.7%)	79 (23.4%)	47 (21.1%)
Unspecified stage/grade	0 (0%)	0 (0%)	6 (1.1%)	5 (1.3%)	1 (0.3%)	1 (0.4%)

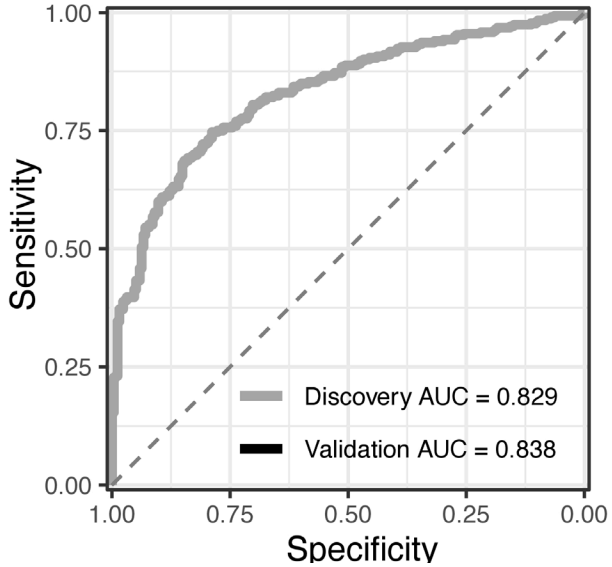
Francesco Gatto, ASCO 2021

Plasma, Urine and combined Pan-Cancer GAG Scores Achived AUC=0.82-0.86

Plasma GAG score

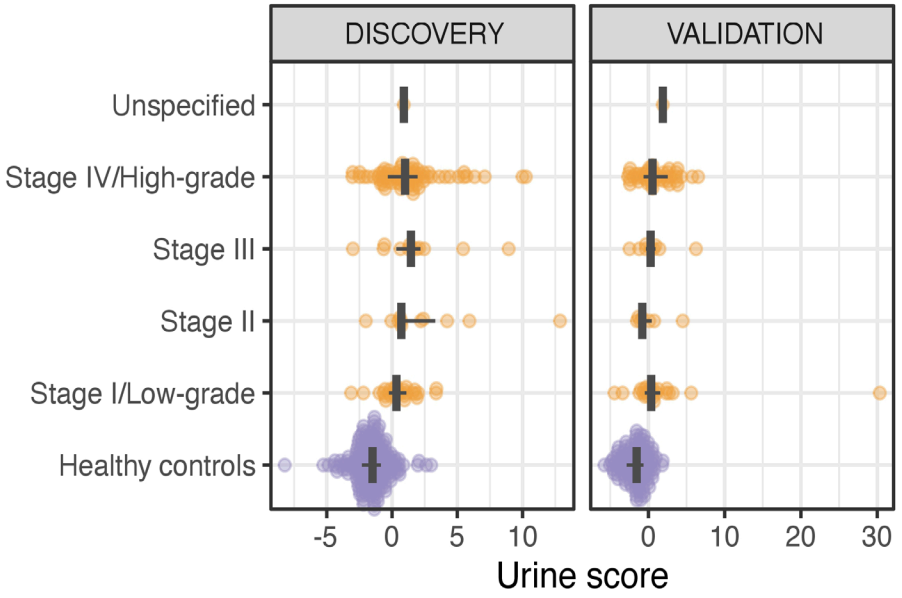
- Validated AUC = 0.84
- 35% sensitivity at 98% specificity
- 31% sensitivity to stage I/low-grade* at 98% specificity

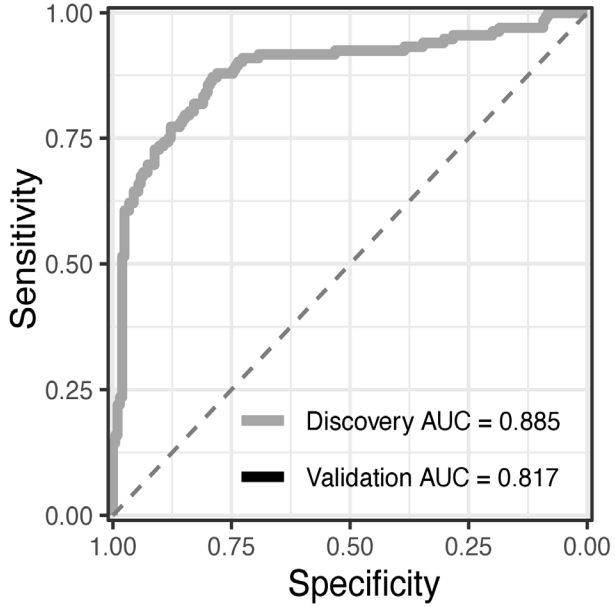




Urine GAG score

- Validated AUC = 0.82
- 39% sensitivity at 98% specificity
- 33% sensitivity to stage I/low-grade* at 98% specificity





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Combined GAGomes Could predict tissue-of-origin with 74.3% Balanced accuracy

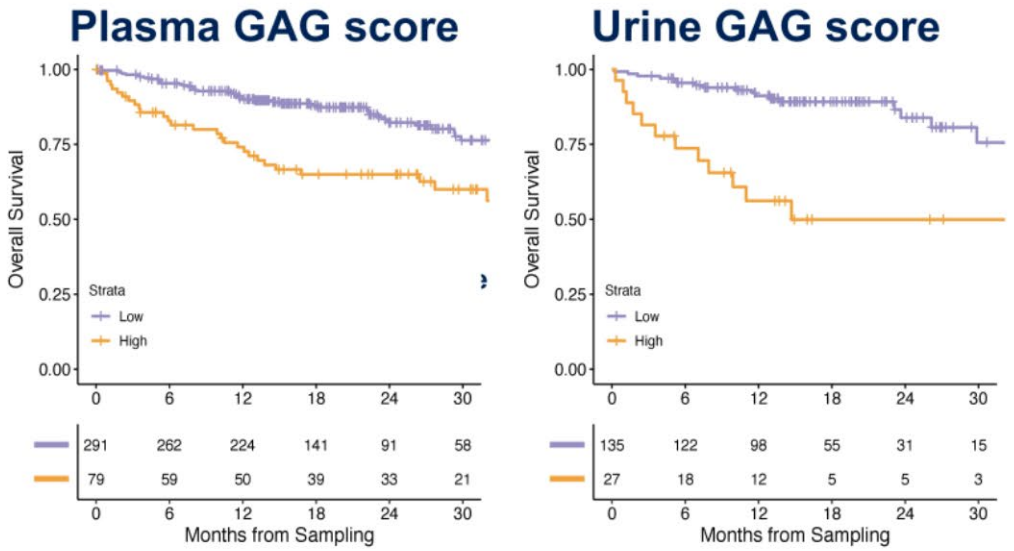
Bayesian Additive Regression Tree model

- Trained in N = 110 cancer combined GAGomes
- Validated in N = 74 (5 types)
- Balanced accuracy 74.3%
- 89.2% accuracy for genitourinary vs. respiratory tract tumors

Plasma & Urine GAG Scores independently correlate with OS

Kaplan-Meier survival analysis

- Adjusted for age, gender, cancer type and stage IV/high-grade
- Plasma: HR = 1.87 [95% CI = 1.36-2.57], p < 0.001, N = 370, 13 types
- Urine: HR = 2.50 [95% CI = 1.50-4.16], p < 0.001, N = 162, 4 types)

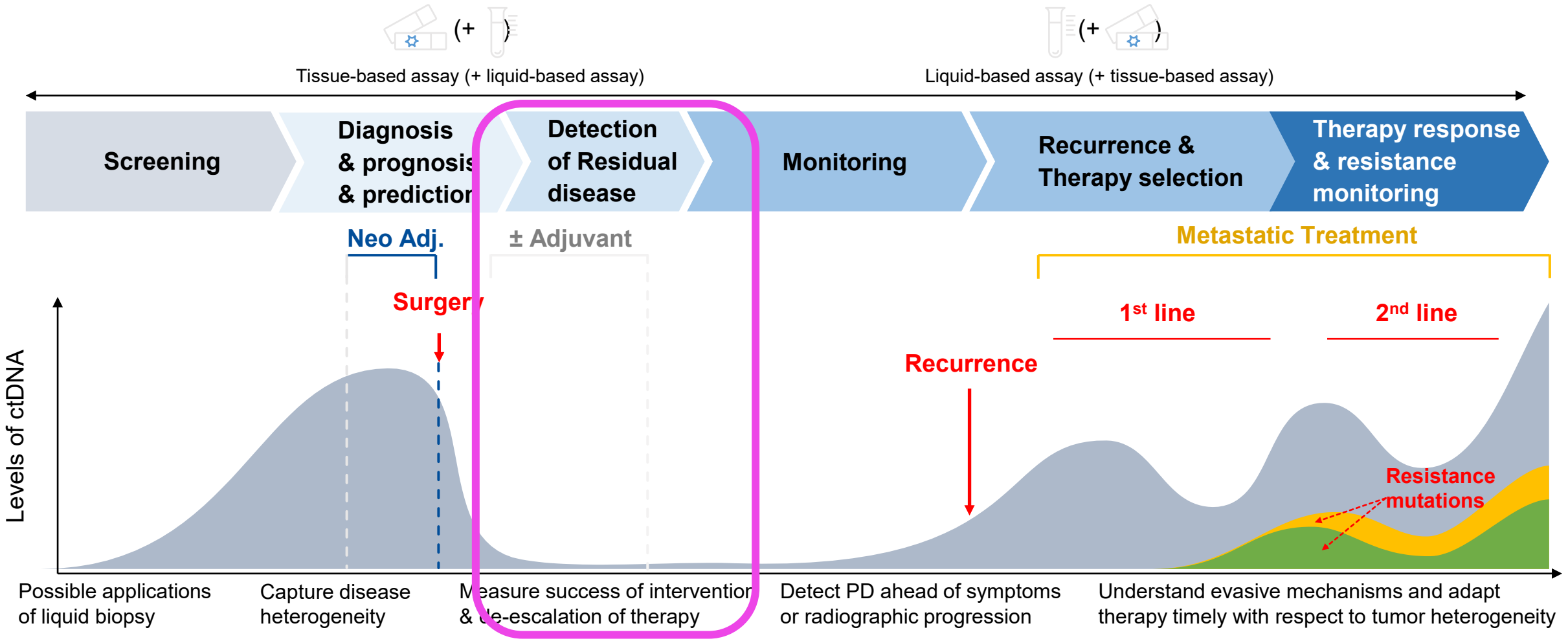


Francesco Gatto, ASCO 2021

Conclusions and Future Directions

- Urine and plasma GAG scores were robust and versatile liquid biomarkers for early multicancer detection based on tumor metabolism
- GAG scores detected up to 33% stage I/low grade cancers as well as brain and genitourinary tumors - historically missed by genomics-based liquid biopsies.
- Required external validation in studies with patient population representative for early multicancer detection

Liquid biopsy can provide clinically-valuable information along the whole patient journey



Adapted from Wan, J.C.M., et al., (2017) Nat Rev Cancer 17:223-38.

Clinical Performance of Methylation-Based Liquid Biopsy test

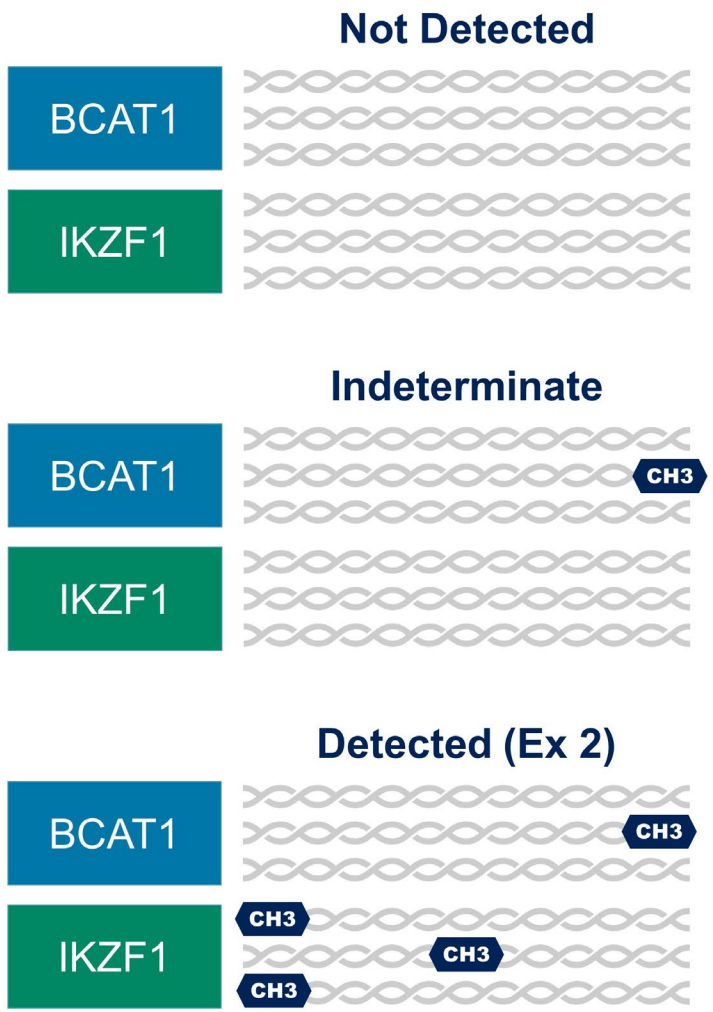
Background

Clinical guidelines recommend surveillance for patients who complete primary treatment for colorectal cancer (CRC) with the aim of detecting recurrence when amenable to curative intent treatment.

- Current surveillance methods (imaging and CEA) have limitations both in sensitivity and specificity.
- Liquid biopsy tests that detect circulating tumor DNA (ctDNA) have improved performance over CEA.

This is a laboratory-developed, real-time PCR test that detects DNA methylation of BCAT1 and IKZF1 genes in blood (two genes are hypermethylated in 95% of CRC tissue).

- Previously, presence of any replicate of either target gene was reported as "detected". In the current study, test reports "detected" result when at least one replicate of IKZF1 or multiple replicates of either IKZF1 and/or BCAT1 are present.



Study Design

- The study evaluated the impact of optimizing the assay's qualitative reporting method on actionability and clinical performance for recurrence detection in CRC surveillance setting.
- Two previously described cohorts of CRC patients (N=322[1] and N=144[2]) who completed primary treatment and were undergoing surveillance were evaluated.
- Imaging and blood collections were performed at, or adjacent to, a standard of care visit.
- Sensitivity, specificity, and diagnostic odds ratio (DOR) for CRC recurrence detection from a single time-point blood sample was determined using radiological imaging as clinical reference standard.
- Performance was compared for 2 category result reporting (detected/not detected) vs. 3 category result reporting (detected/not detected/indeterminate).

1. Musher, BL et al. CEBP. 2020; 29(12): 2702-2709
2. Symonds, EL et al. Cancer. 2020; 126: 1460-1469

Results

	Cohort 1 N=322		Cohort 2 N=144	
	Detected/Not Detected[1]	Detected/Not Detected/Indeterminate	Detected/Not Detected[2]	Detected/Not Detected/Indeterminate
Recurrence (N/%)	27/322 (8.4%)		50/144 (34.7%)	
Test Positivity Rate	13.0% 42/322	6.5% 21/322	29.1% 42/144	26.4% 38/144
Sensitivity (95% CI)	63% (42.4-80.6%)	59.3% (38.8-77.6%)	66% (51.2-78.8%)	62% (47.2-75.4%)
Specificity (95% CI)	91.5% (87.7-94.4%)	98.3% (96.1-99.5%)	90.4% (82.6-95.5%)	92.6% (85.3-97%)
Negative Predictive Value (NPV)	96.4% 270/280	96.4% 290/301	83.3% 85/102	82.08% 87/106
Positive Predictive Value (PPV)	44.7% 17/38	76.2% 16/21	78.6% 33/42	81.6% 31/38

1. Musher, BL et al. CEBP. 2020; 29(12): 2702-2709
2. Symonds, EL et al. Cancer. 2020; 126: 1460-1469

Conclusions

Increased confidence in positive test results

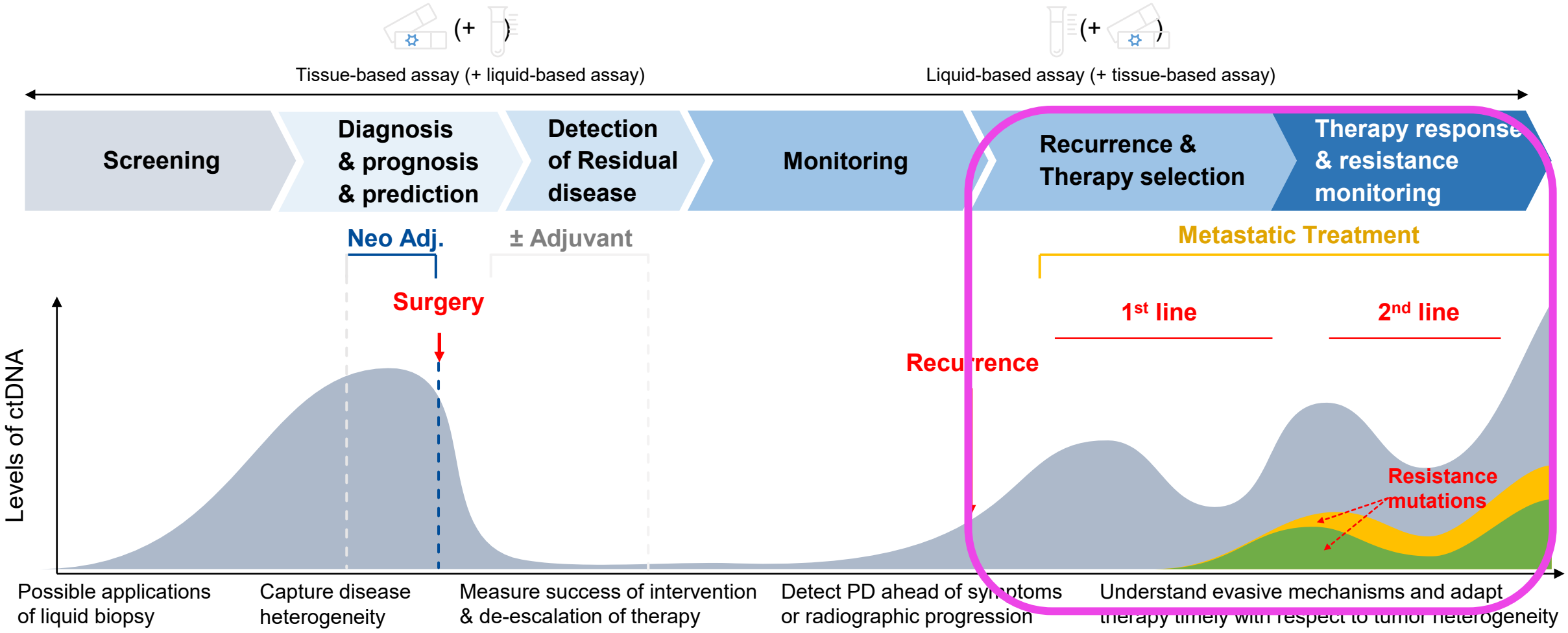
- Overall, it can be expected to see lower number of patients with reported positive test results.
- Improved positive predictive value (PPV) to >76% (from 44.7%) means that over ¾ of patients with detectable [redacted] levels will have confirmed recurrence on imaging at timepoint closest to the blood draw.
- Sensitivity (true positive results) is not significantly changed.

Maintaining confidence in negative test results

- Improved specificity - 98% of patients without imaging detected recurrence were correctly identified as not having detectable [redacted] results.
- High NPV - only 4% of patients with not detectable ctDNA levels will have recurrence.

Optimization of [redacted] test results improved clinical utility and actionability when used in surveillance for detection of recurrent CRC.

Liquid biopsy can provide clinically-valuable information along the whole patient journey



Adapted from Wan, J.C.M., et al., (2017) Nat Rev Cancer 17:223-38.

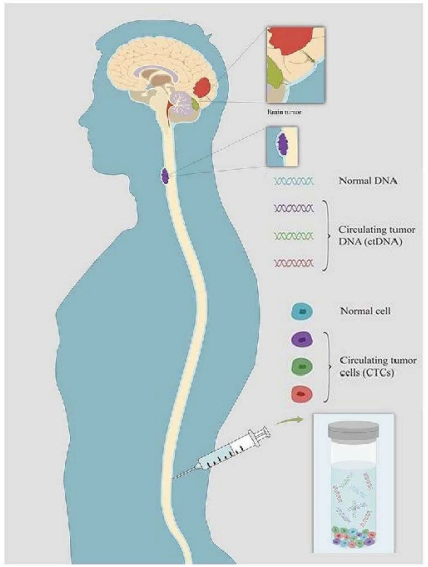
Current Status of CSF Liquid Biopsies

- ✓ Presence in CSF of tumor-derived DNA/tumor cells confirmed in many patients with primary or secondary CSF cancers (Adult Diffuse Glioma, DMG/DIPG, CNS Lymphoma, Medulloblastoma, CNS Metastases/Leptomeningeal Disease)
- ✓ Technical feasibility of many approaches (CTCs, ddPCR, targeted exome, sWGA) documented in retrospective series
- ➢ **Unknown feasibility and utility of CSF liquid biopsies for evaluation of treatment response in prospectively collected CSF samples**

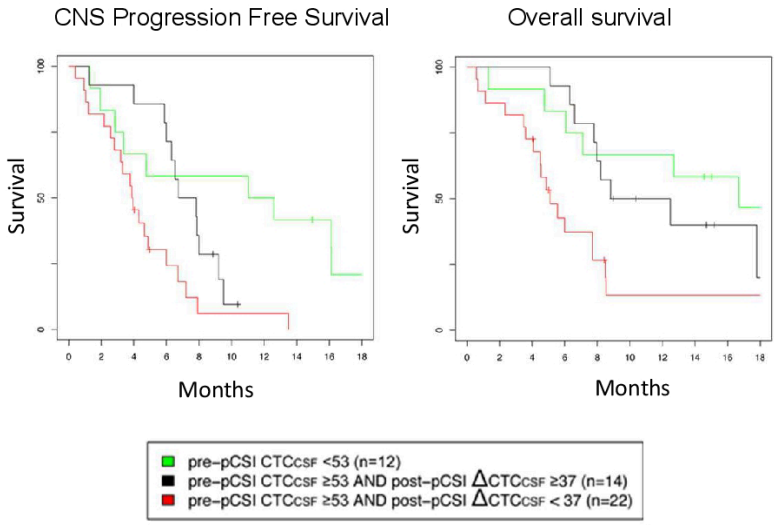
Cantor et al., Abstract ID:2012 (343245)

- **Clinical Trials.gov Identifier:** NCT03416530; multicenter, open-label, seven arm, dose escalation, phase I study of oral ONC201 in pediatric patients with newly diagnosed Diffuse Intrinsic Pontine Glioma (DIPG) and recurrent/refractory H3 K27M gliomas
- **Therapeutic Intervention:** ONC201
- **Measurement:** cell-free tumor DNA (CSF and Plasma); 17 patients with >2 sample time points; 62 plasma samples/186 replicates; 29 CSF samples/87 replicates
- **Result:** No correlation between change in tumor area and VAF, but decrease/increase in ct-DNA associated with response/resistance

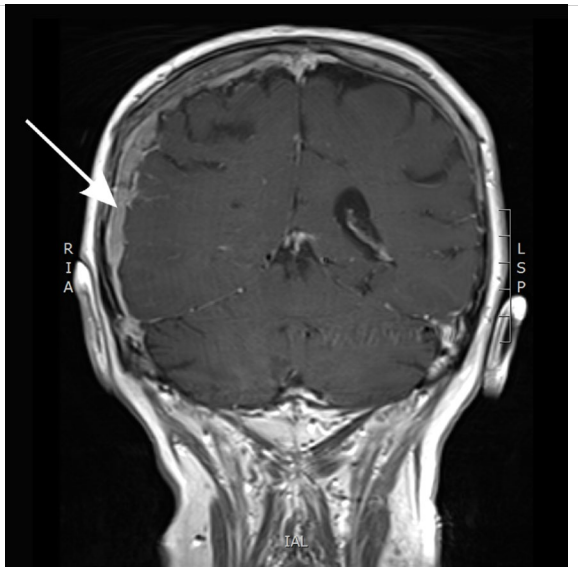
Cerebrospinal Fluid CTCs for proton carnial irradiation for solid tumor leptomeningeal mets



- **Patients/Clinical Trials.gov:** builds on Phase Ib Study With Dose Expansion Cohort of Proton Craniospinal Irradiation (pCSI) for Leptomeningeal Metastases From Solid Tumors (24 participants; NCT03520504) --- overall 58 LM patients (lung 27, Breast 22, other 9) who received pCSI
- **Therapeutic Intervention:** Proton Craniospinal Irradiation
- **Measurement:** CSF circulating tumor cells (CellSearch®) prior to and after pCSI
- **Result:** Pre-pCSI CTC CSF <53 cells/mL associated with improved CNS PFS and a trend toward improved OS; ΔCTC-CSF ≥37 cells/3mL associated with improved CNS PFS



1. **Most favorable group:** pre-pCSI CTC_{CSF} <53 cells/3mL (median CNS PFS=12 months, OS= 17 months)
2. **Favorable group:** pre-pCSI CTC_{CSF} ≥53 cells/3mL and $\Delta_{CTC\text{-}CSF}$ ≥37 cells/3mL post-pCSI (median CNS PFS=7 months, OS=11 months)
3. **Unfavorable group:** pre-pCSI CTC_{CSF} ≥53 cells/3mL and $\Delta_{CTC\text{-}CSF}$ <37 cells/3mL post-pCSI (median CNS PFS=4 months, OS=5 months)



Wijetunga et al, Discussant I. Mellinghoff

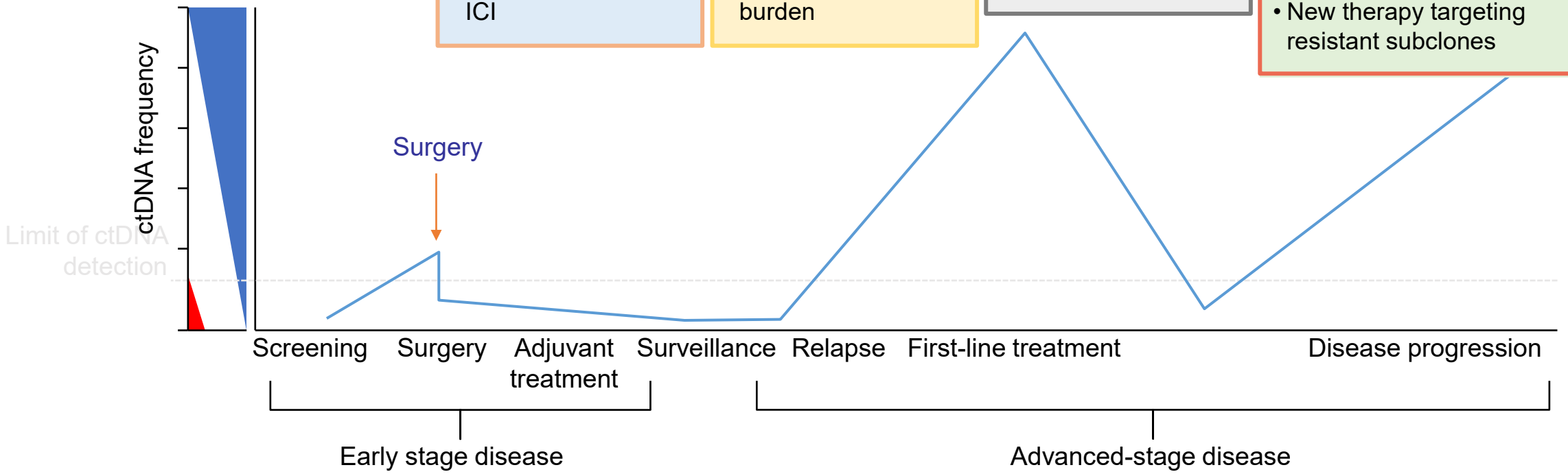
Clinical application of liquid biopsy in immunotherapy

Detection of minimal residual disease
- Tailoring adjuvant ICI

Early detection of relapse
- Initiating first line ICI at a low tumour burden

Early monitoring of response
- ICI stop/switch in resistant cancers

Detection of secondary resistance
- Characterisation of resistance mechanisms
- New therapy targeting resistant subclones



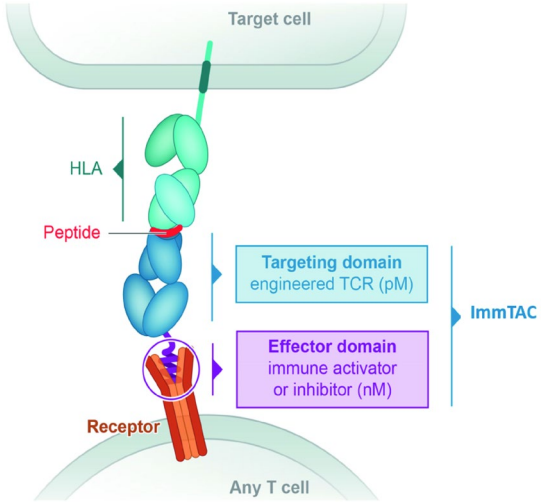
Not so easy!!

ctDNA: circulating tumour DNA; ICI: immune checkpoint inhibitor.

Cabel, L., et al. (2018) *Nat Rev Clin Oncol* 15:639-50.

Tebentafusp versus investigator choice in 1L mUM

While ORR was only 5%, OS was promising relative to historical published data

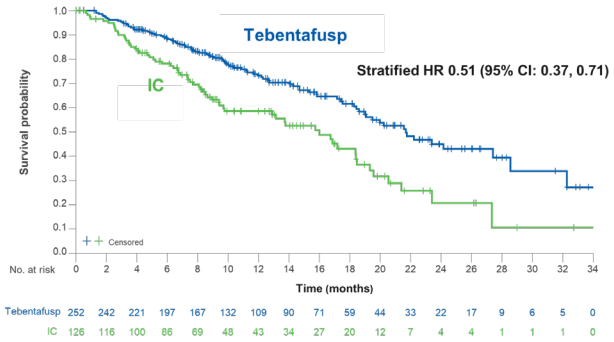


Tebentafusp

- Bispecific soluble TCR therapeutic
- Affinity-enhanced TCR fused to anti-CD3
- Designed to redirect T cells to gp100+ melanocytic cells

The authors claim that the **radiographic assessment** of tumors **may under-estimate**

	N=127	Historical 2L+[1]
Response rate	5%	-
Duration of response	8.7 month	-
OS, median months	16.8 month	7.8 month
1-yr OS rate	62%	37%
2-yr OS rate	37%	15%



- **RECIST response rate: 9.1%**
- **Progression free survival: HR 0.73 (95% CI: 0.58, 0.94)**

Shoushtari et al, ESMO 2021, Discussant C. Rolfo

Plasma total cfDNA is a surrogate biomarker for tumor burden in melanoma patients

N=127

↓

ctDNA, any time point

N=118

↓

ctDNA, at baseline

N=116

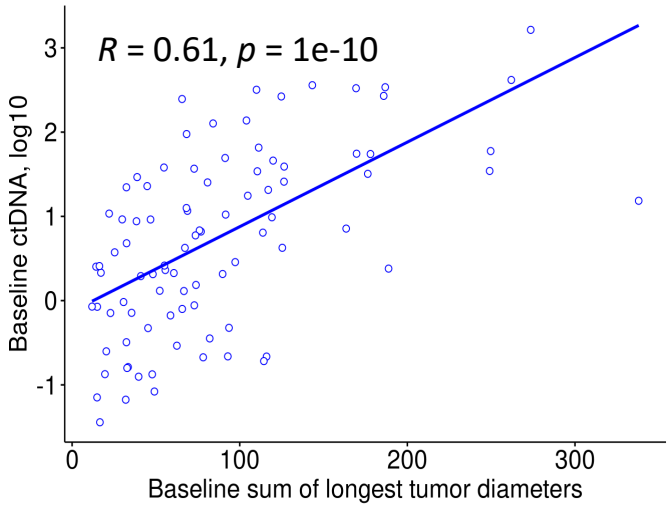
↓

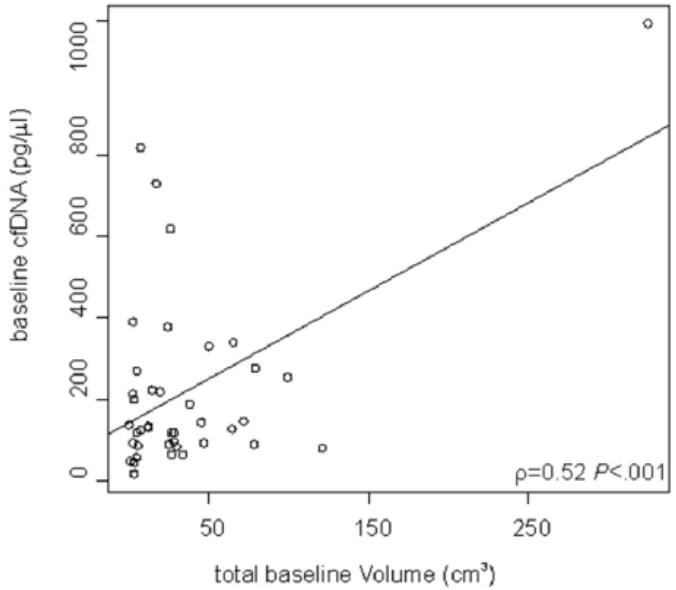
ctDNA, at baseline & by Week 9

N=99

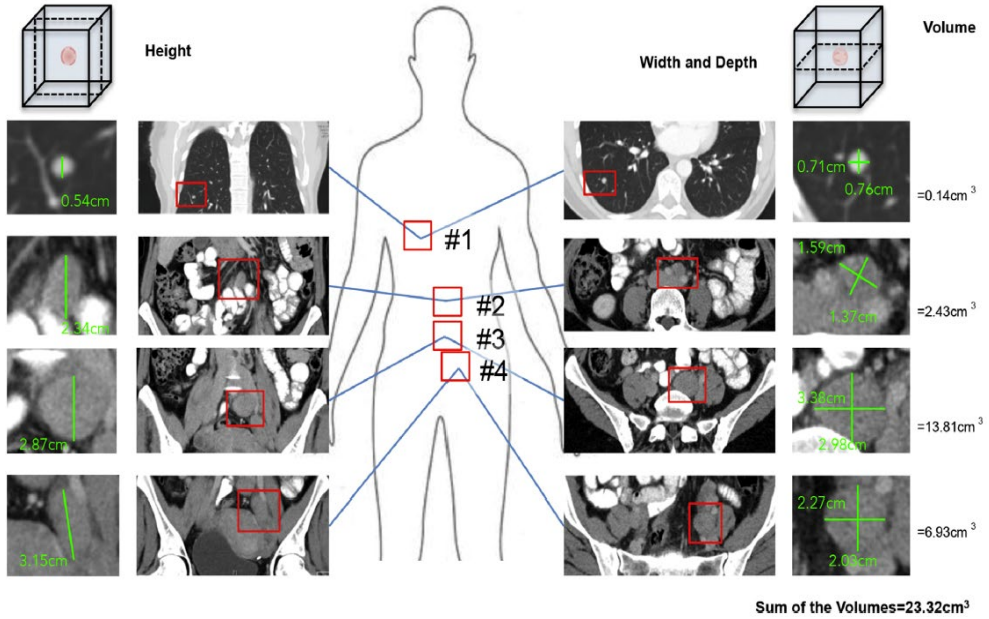
- New custom panel to detect melanoma ctDNA using **multiplex PCR followed by NGS**
- Including **UM specific genes**: GNAQ, GNA11, SF3B1, PLCB4, CYSLTR2, EIF1AX

Baseline ctDNA vs. tumor size





Using dDPCR and NGS



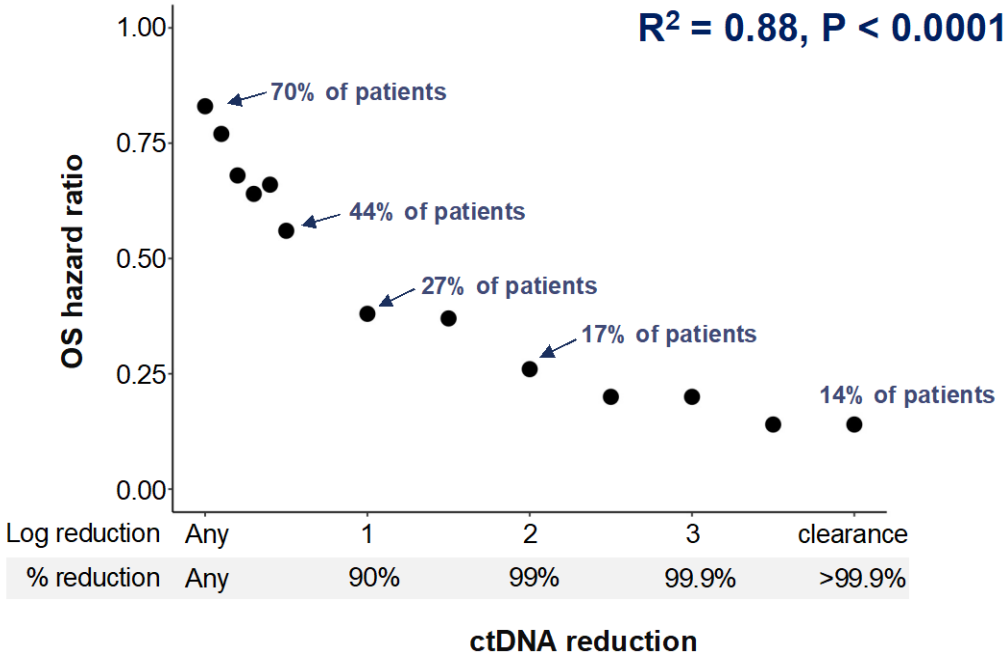
Correlation between baseline cfDNA concentration and pre-treatment tumor burden in pts with melanoma

Shoushtari et al, ESMO 2021, Discussant C. Rolfo

Valpione S, et al. Eur J Cancer 2018

ctDNA changes and outcome with Tebentafusp

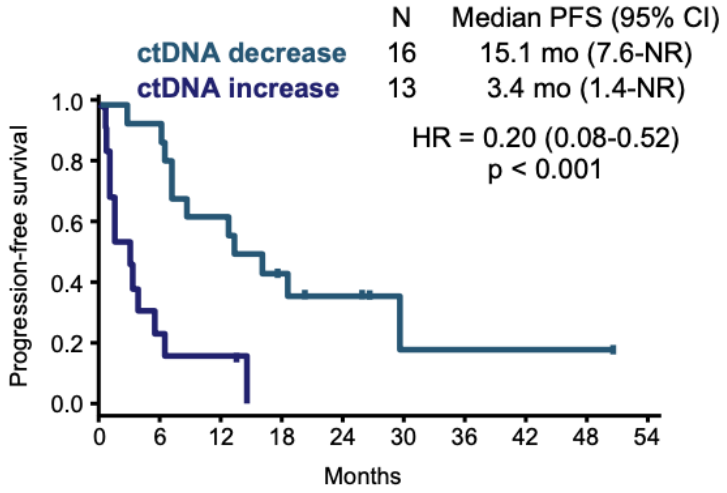
Linear correlation between ctDNA reduction and better OS



Identification of early plasma ctDNA changes to predict response to first-line pembrolizumab +/- chemotherapy in aNSCLC patients[1]

Blood samples were collected on 1st day of treatment and at each subsequent cycle

A 36-gene panel NGS* **detected early quantitative changes** across a wide range of variants



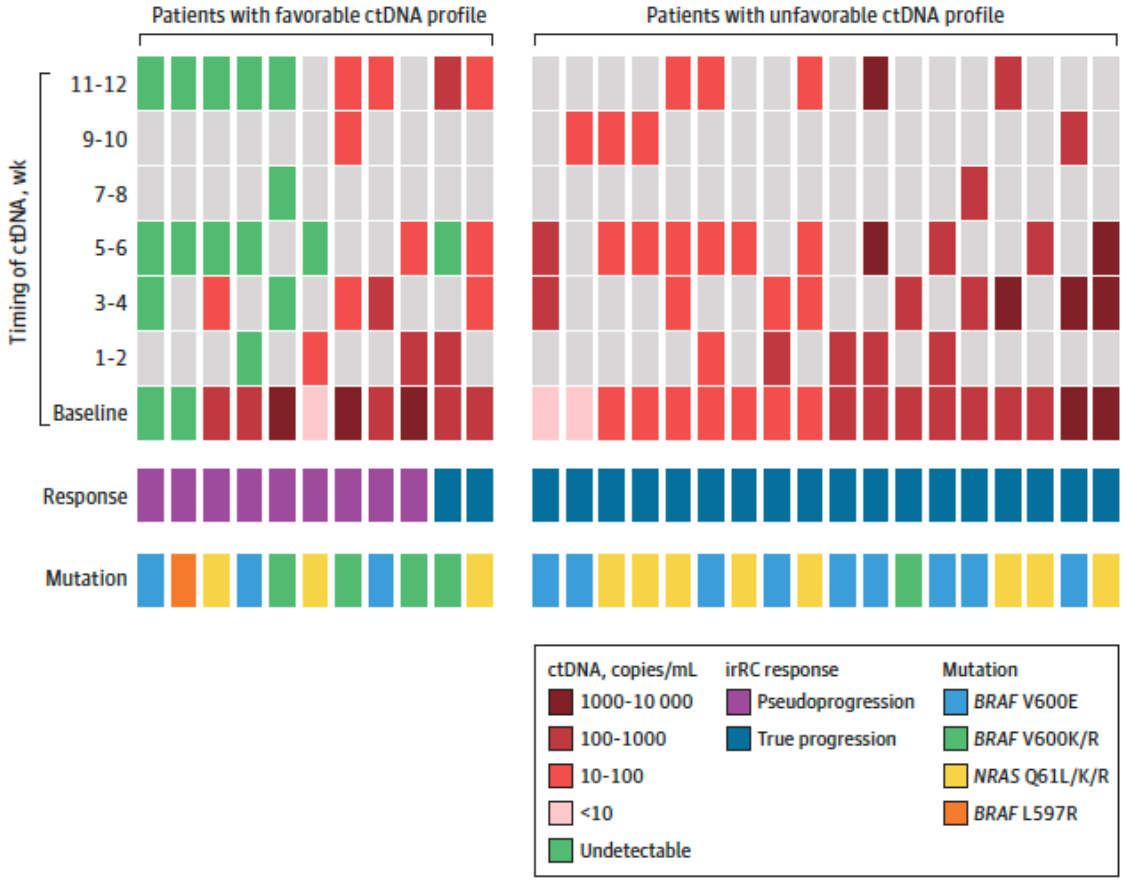
Rapid **decrease of ctDNA** correlated with **clinical benefit**, while **increase** correlated with **PD**

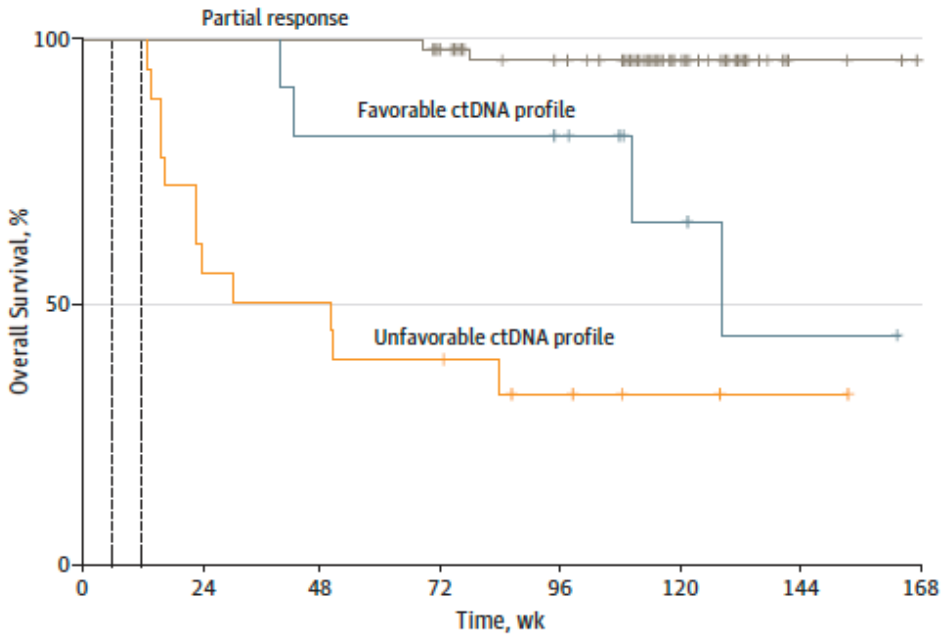
Shoushtari et al, ESMO 2021, Discussant C. Rolfo

Ricciuti, P.C., et al. (2020) ASCO poster 3518

Utility of ctDNA for unclear radiographic scenarios: Differentiating Pseudoprogression from PD in Patients With Metastatic Melanoma Treated With PD-1 inhibitors

Using dDPCR





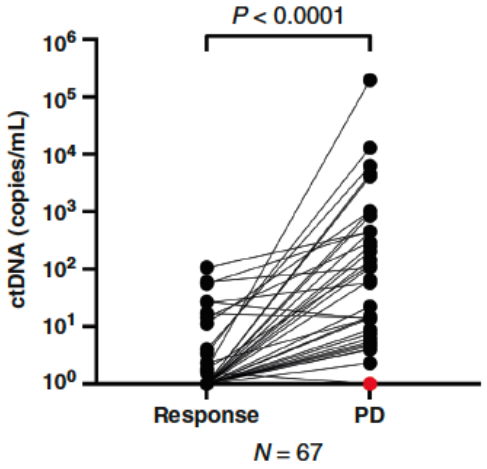
No. at risk

Partial response	54	54	54	52	44	24	4	1
Unfavorable ctDNA profile	18	11	10	8	5	3	2	1
Favorable ctDNA profile	11	11	10	10	9	5	3	1

Shoushtari et al, ESMO 2021, Discussant C. Rolfo

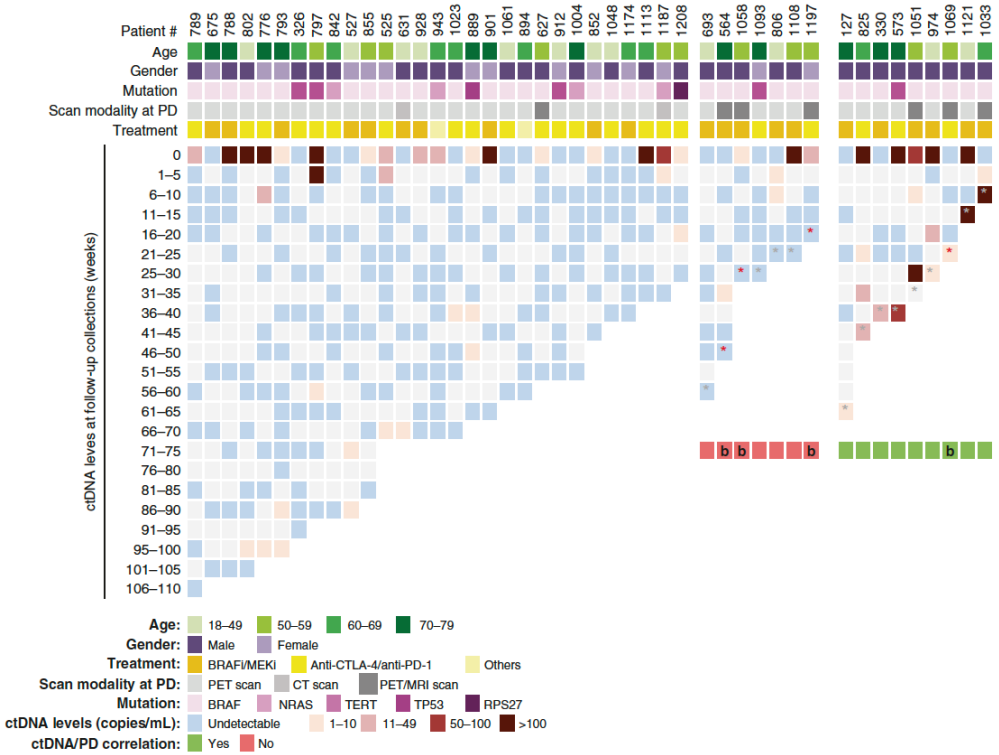
Lee JH, et al. JAMA Oncol 2018

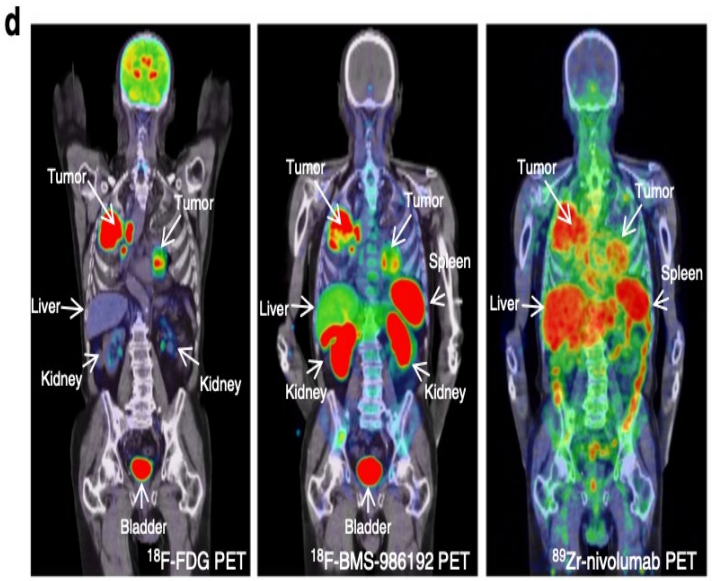
ctDNA vs. radiographic response in melanoma patients treated with ICIs and BRAFi + MEKi



Changes in ctDNA levels between the time of response and the time of progression.

Using dDPCR and NGS





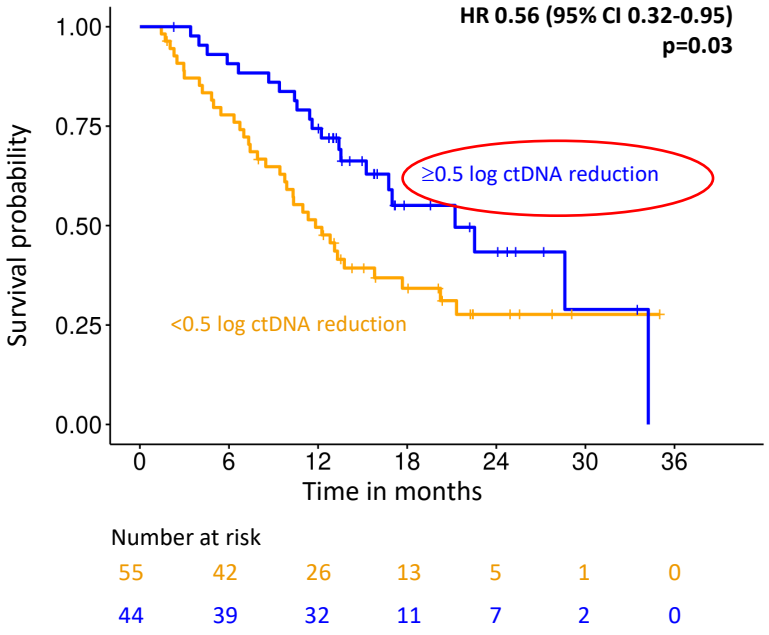
These results highlight the low efficacy of ctDNA to detect disease progression in melanoma when compared mainly to **standard PET imaging**

Shoushtari et al, ESMO 2021, Discussant C. Rolfo

Marsavela G, et al. Br J Cancer 2021, Niemeijer ey al, Nature Comm 2018

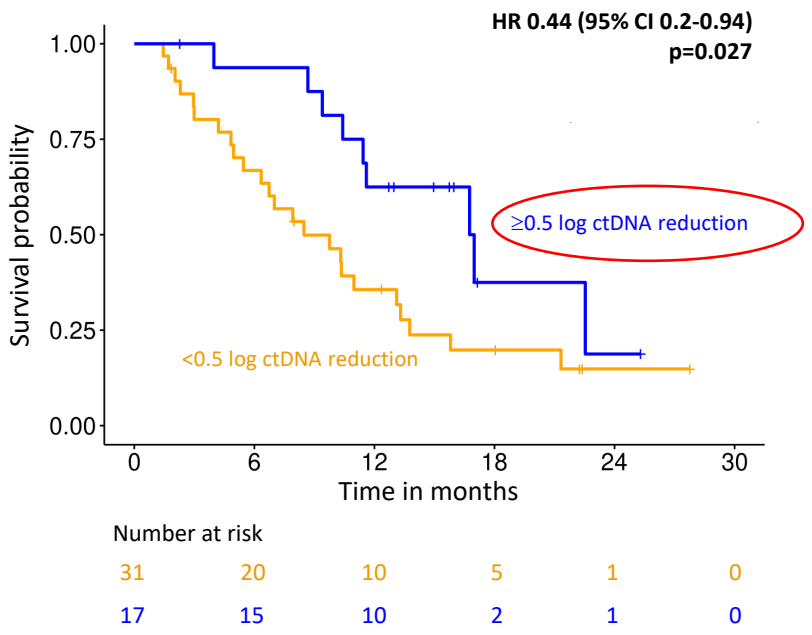
ctDNA reduction identifies patients with OS benefit, regardless best RECIST response

All evaluable patients



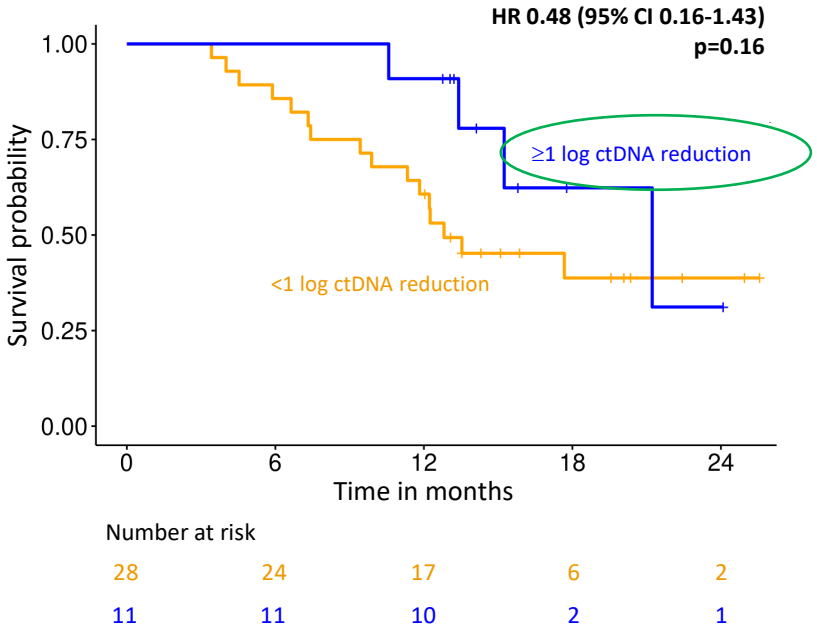
44% of these patients had ≥ 0.5 log reduction ctDNA

Patients with best response progressive disease



35% of these patients had ≥ 0.5 log reduction ctDNA

Patients with best response stable disease



28% of these patients had ≥ 1 log reduction ctDNA

Shoushtari et al, ESMO 2021, Discussant C. Rolfo

Take home message from Shoushtari et al abstract:

Strengths:

- Drug and tumor specific study evaluating the role of ctDNA as surrogate of response
- Important correlation of ctDNA levels and OS.
- RR better evaluable with ctDNA

Questions still open:

- It's RECIST Criteria a good comparator for biological response?
- New approach as Immuno-PET to be correlated with ctDNA
- This benefit could be also observed beyond second line?
- Blood first approach a new assessment tool delaying CT scan in IO?

Shoushtari et al, ESMO 2021, Discussant C. Rolfo

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Thanks