Liquid biopsy for treatment monitoring and minimal residual disease detection in breast cancer

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Disclosures

AJW is an inventor on a pending patent application (WO2023018791A1), filed by Cornell University, that is directed to methods of detecting SNVs for the purposes of MRD detection and other plasma-based cancer monitoring.

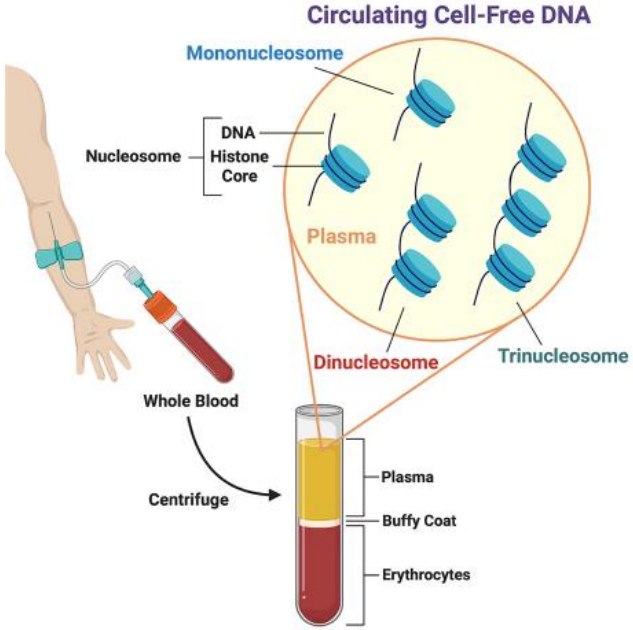
AJW is an inventor on a pending patent application (WO2023133093A1), filed by Cornell University, that is directed to methods of detecting CNVs for the purposes of MRD detection and other plasma-based cancer monitoring

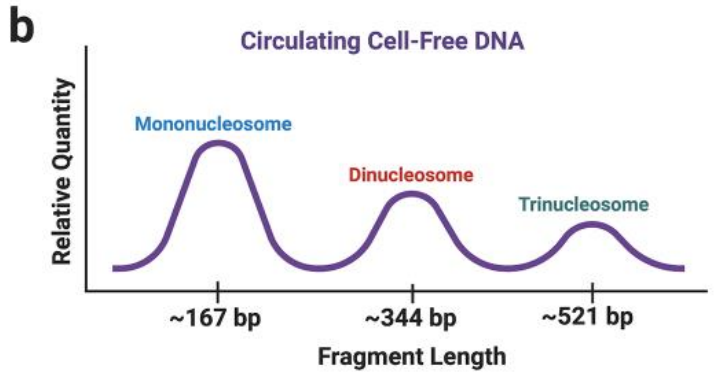
Outline

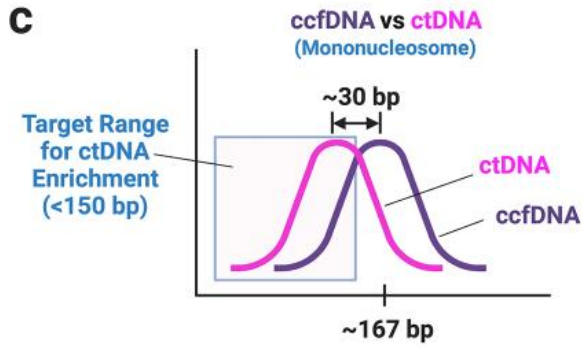
- State of the art in liquid biopsy for circulating tumor DNA in breast cancer
- Plasma whole genome sequencing for ultrasensitive ctDNA detection

Cell-free DNA (cfDNA)
Circulating tumor DNA (ctDNA)
biology

- Constant release mainly through apoptosis and clearance by liver >> kidney > circulating nucleases
- Very short half-life 30min –2h
- Short DNA fragment (attached to nucleosomes), size of the fragment associated with gene expression, etc
- Among cell-free DNA: frequency extremely variable <0.01% - >50%
- ctDNA fraction correlated with disease burden, cell turnover (high proliferation rate and apoptosis), site of disease



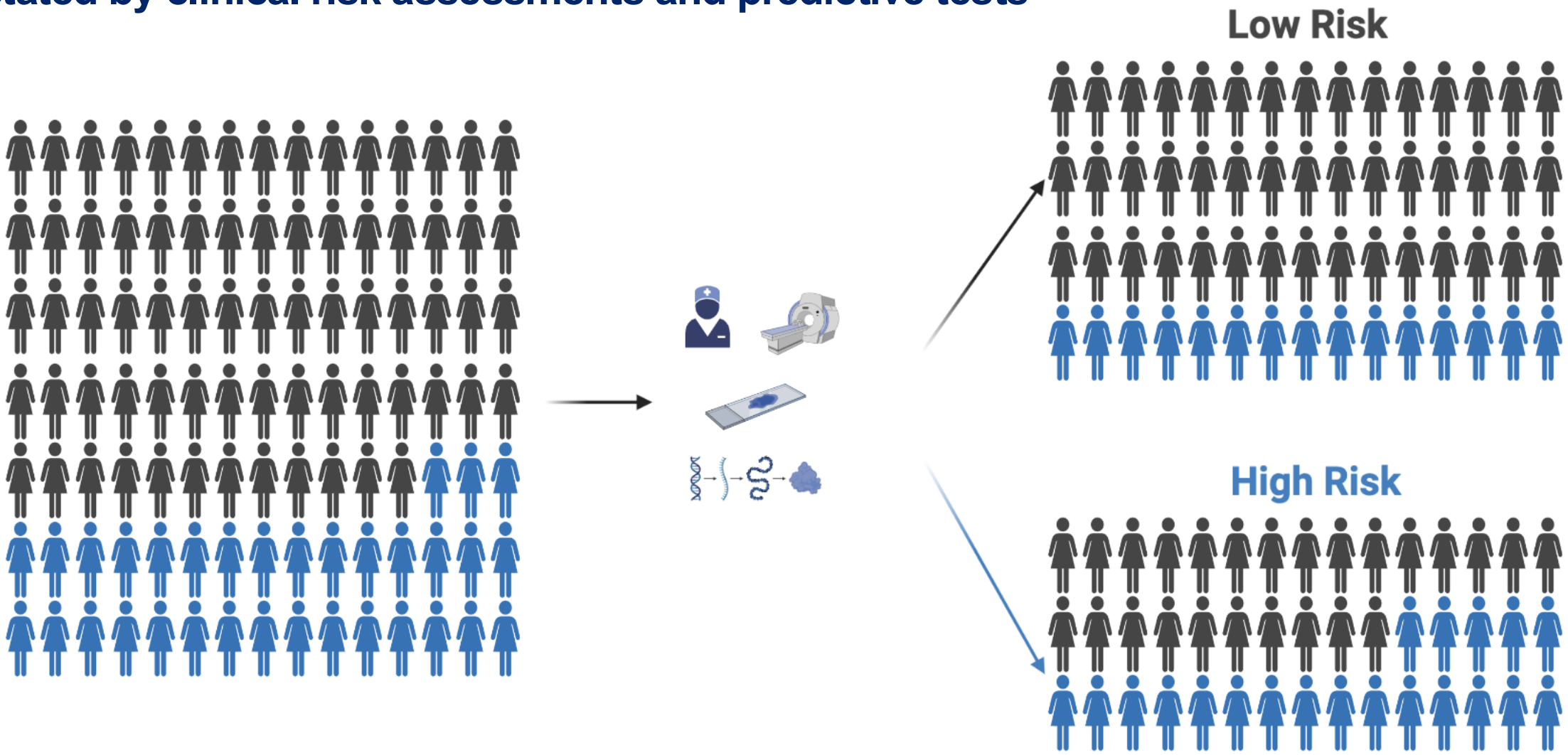




Leveraging the Fragment Length of Circulating Tumour DNA to Improve Molecular Profiling of Solid Tumour Malignancies with Next-Generation Sequencing: A Pathway to Advanced Non-invasive Diagnostics in Precision Oncology?

Current paradigm

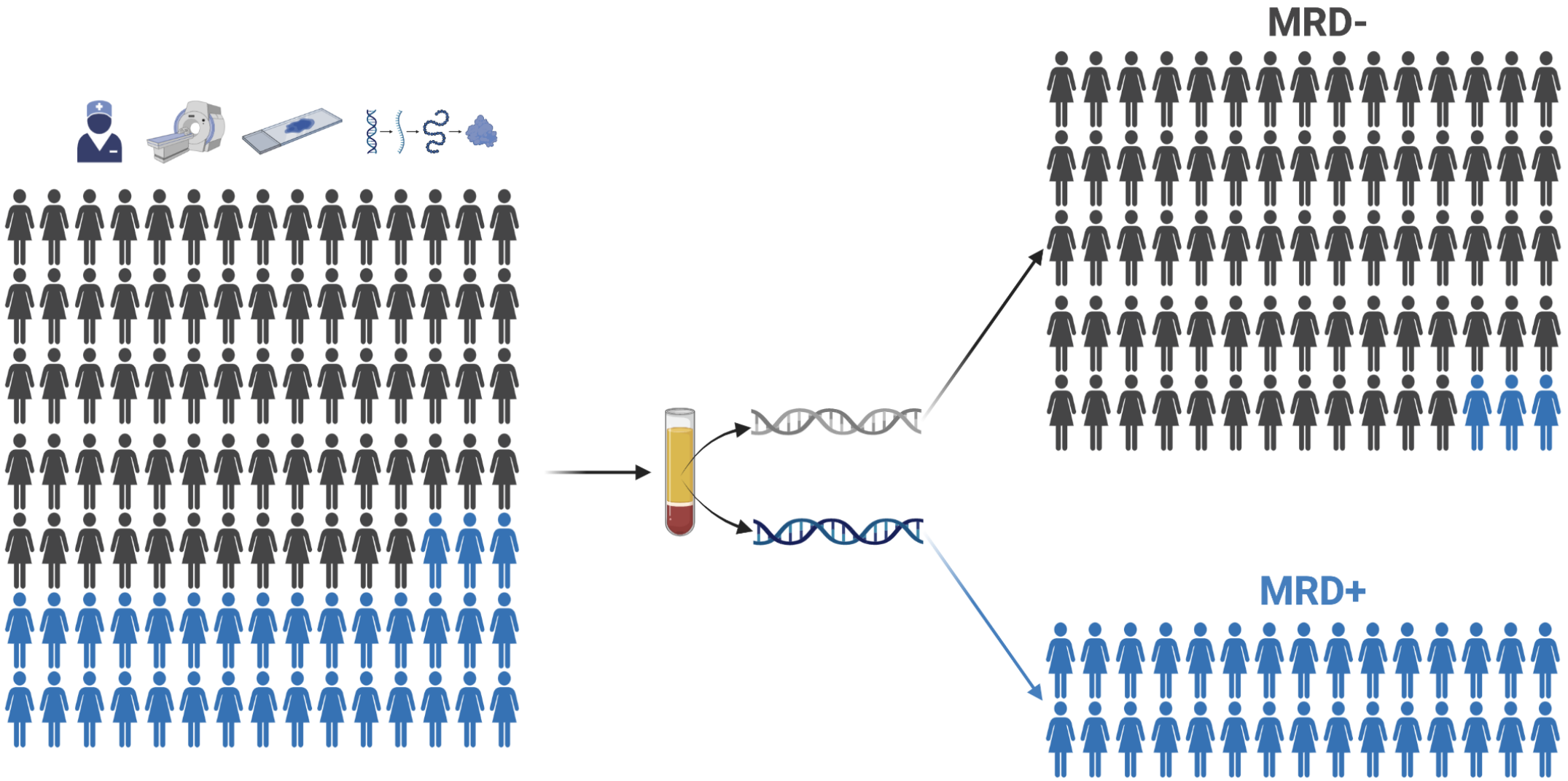
Treatment of early-stage patients with choice of therapy dictated by clinical risk assessments and predictive tests



Adapted from Pedram Rezavi, MD, PhD

Adaptive paradigm

ctDNA MRD assessment in the context of baseline risk stratification



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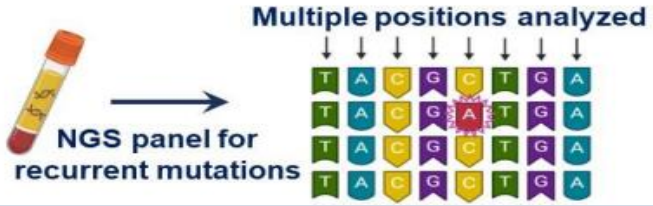
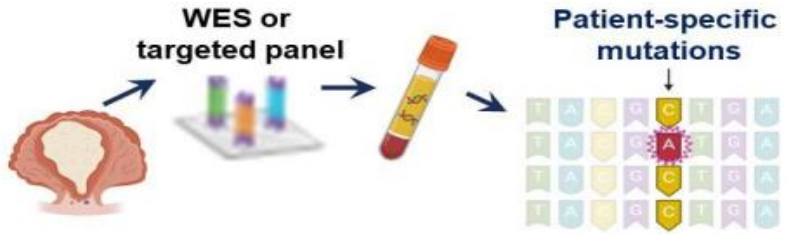
ctDNA detection

Tumor Naïve versus Tumor-informed approach

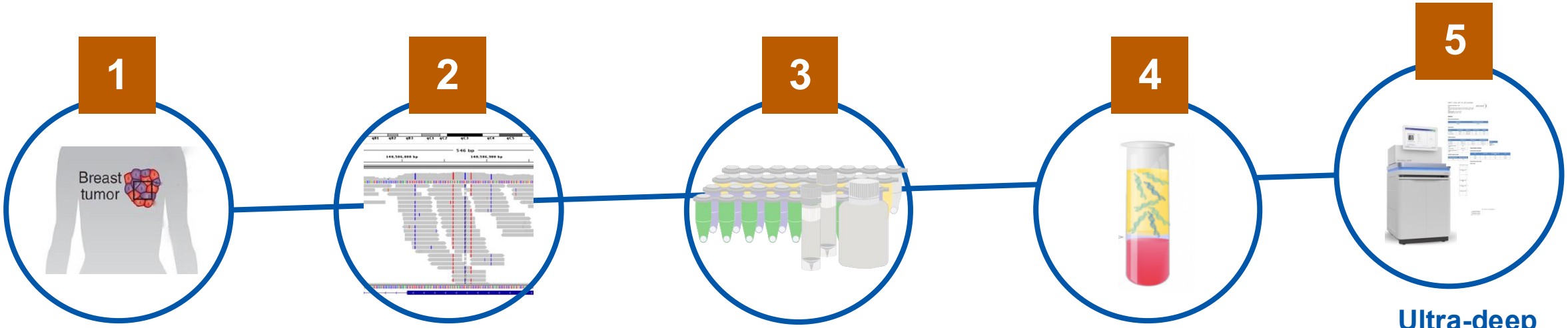




	Tumor Naive	Tumor Informed
Method	Detect de novo mutations or epigenomic alterations in plasma	Identify mutations in tumor tissue => track in plasma (personalized panel)
Key Advantage	**Fast / No Tumor Tissue**	**Higher Sensitivity/Specificity**
Key Disadvantage	Lower sensitivity	Tumor Tissue
Applications	• Non-invasive genotyping • Detection of new genomic alterations • Screening	• **Minimal residual disease detection** • Surveillance • Response monitoring

Adapted from Halpern N 2022 ASCO

Personalized tumor-informed ctDNA assays



1. Tumor tissue sampling
2. WES or WGS of tumor and matched normal
   Select tumor-derived variants
3. Patient-specific ctDNA Panel
4. cfDNA extraction
5. Ultra-deep sequencing
   error correction for mutation calling and MRD assessment

↑ Threshold of detection <0.01%

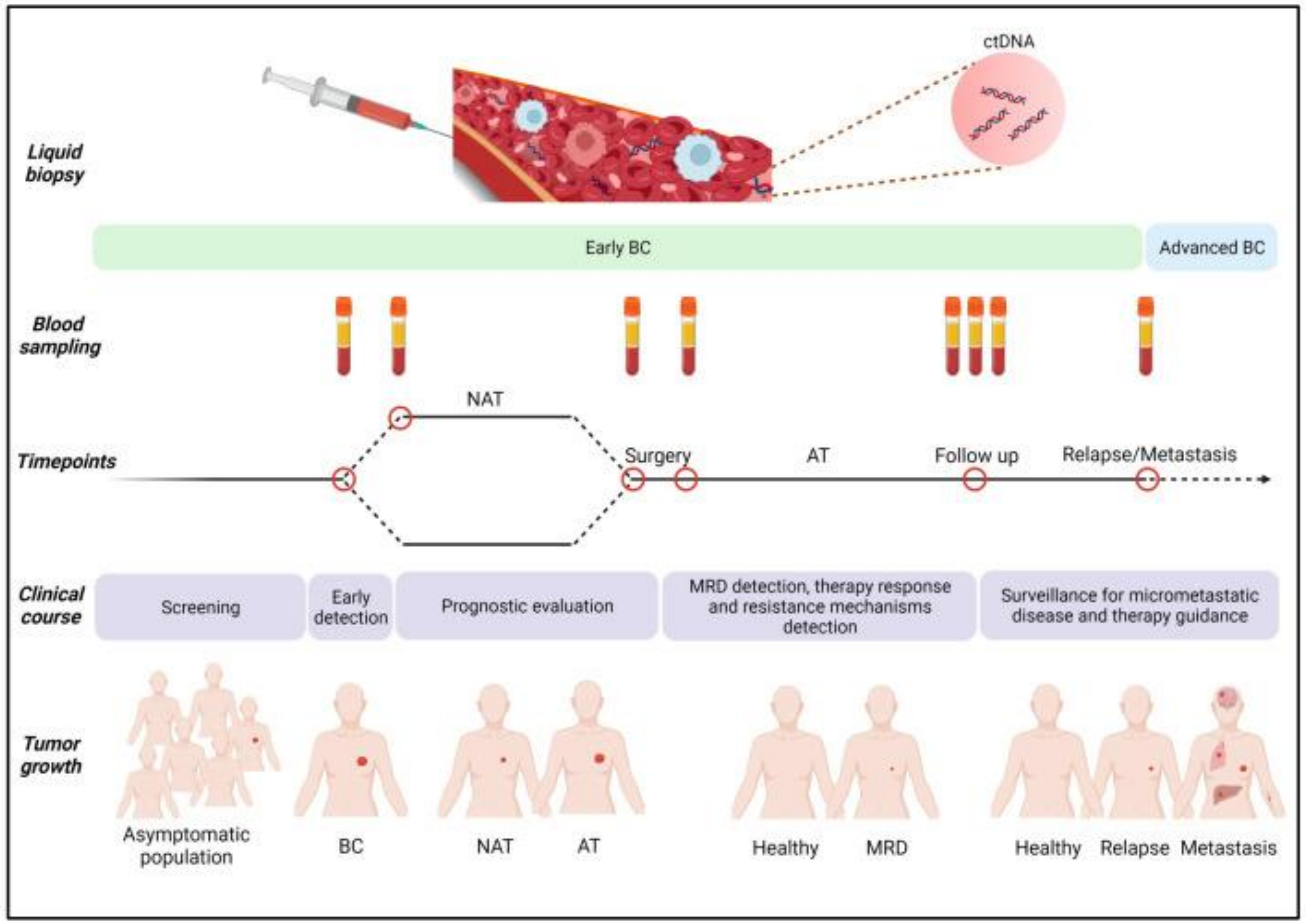
↑ Number of mutations tracked
1-2 mutations to 100s

↓ Sequencing noise

↓ CH-related false positives

ctDNA detection

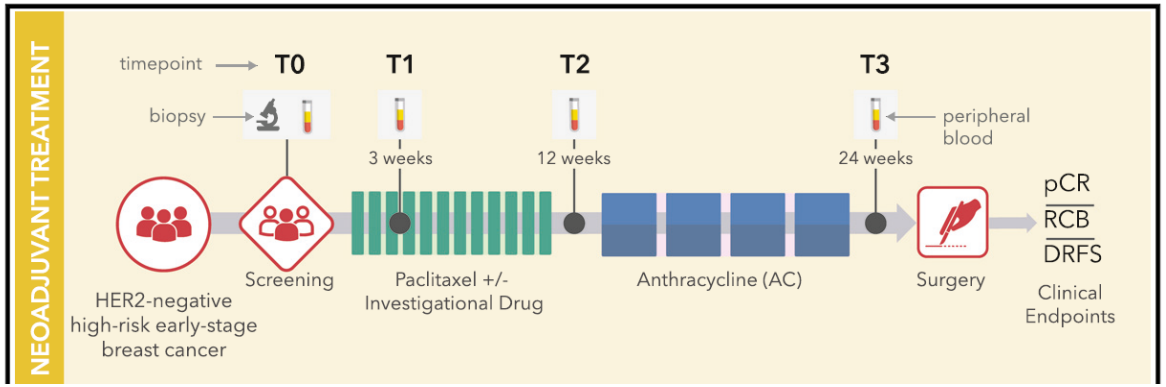
Ideal paradigm



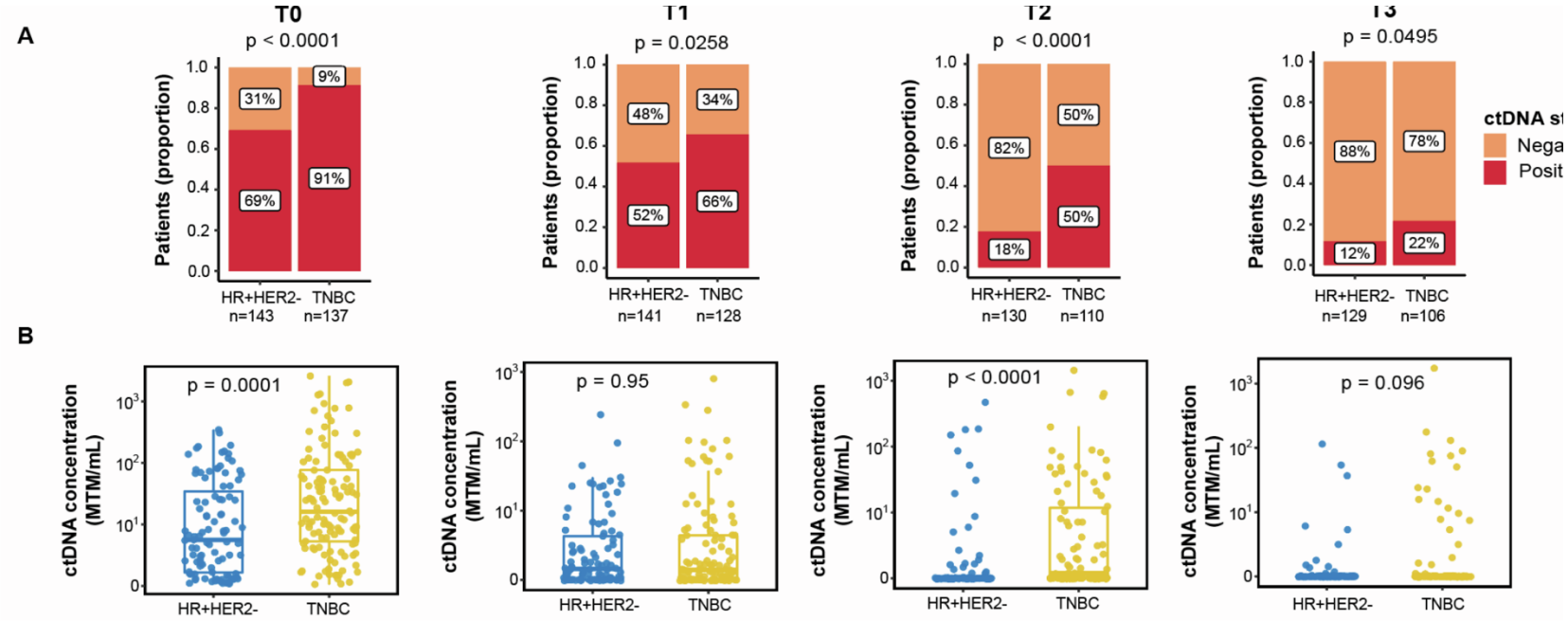
Vlataki cells 2023

Neoadjuvant

I SPY2 Signatera test



- More ctDNA+ (detection rate/concentration) in TNBC at baseline vs HR+/HER2- (more proliferative)

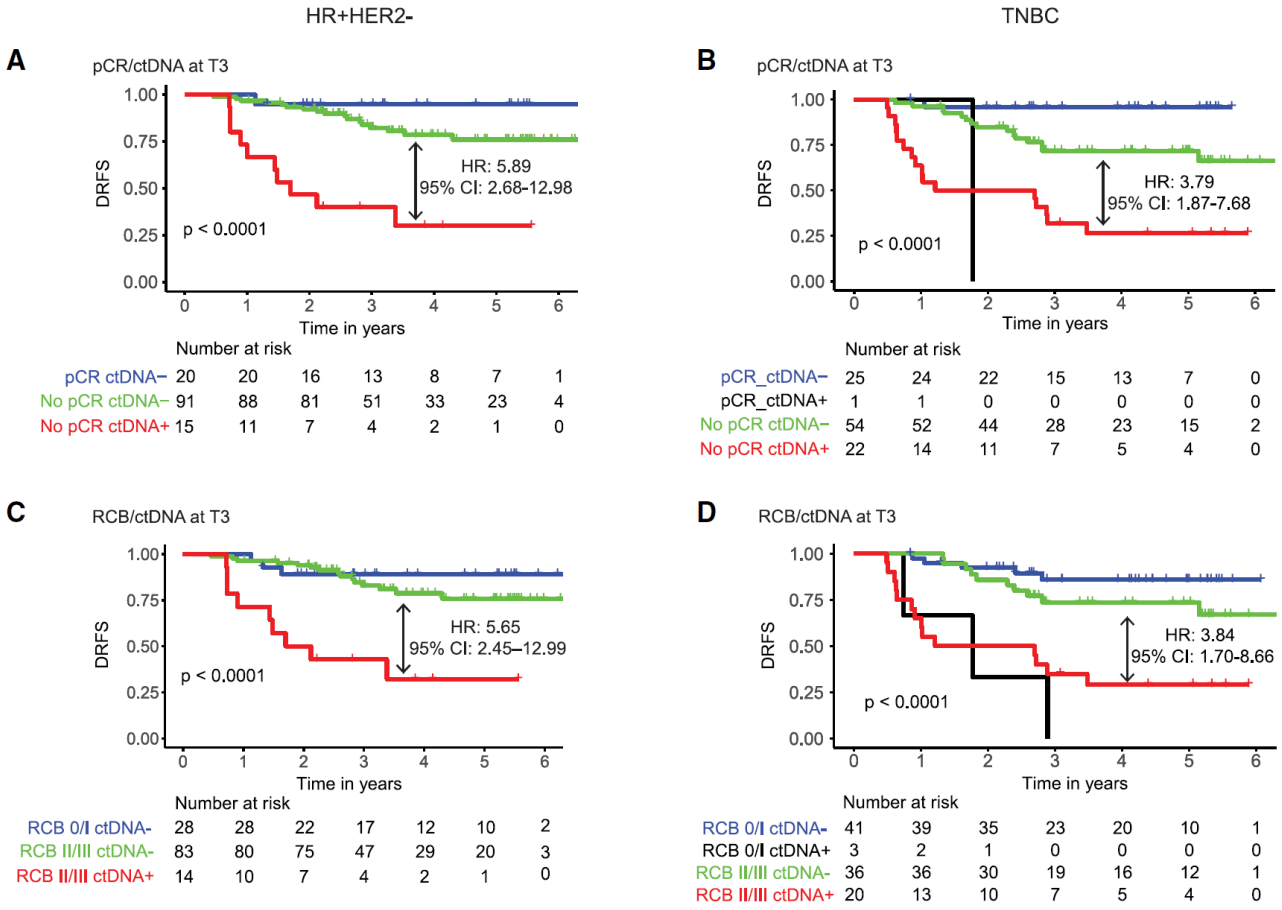


- Higher ctDNA detection in higher T/N/Grade

Clinical significance and biology of circulating tumor DNA in high-risk early-stage HER2-negative breast cancer receiving neoadjuvant chemotherapy
Mark Jesus M. Magbanua Cancer cell. 2024

ctDNA before surgery

- **ctDNA + before surgery was a major prognostic factor in both HR+ / TNBC in patients without pCR**
- ctDNA+ in patients with pCR is very rare (but they relapsed)
- Validated in others studies
- Not shown here but Early clearance was associated with pCR in TNBC



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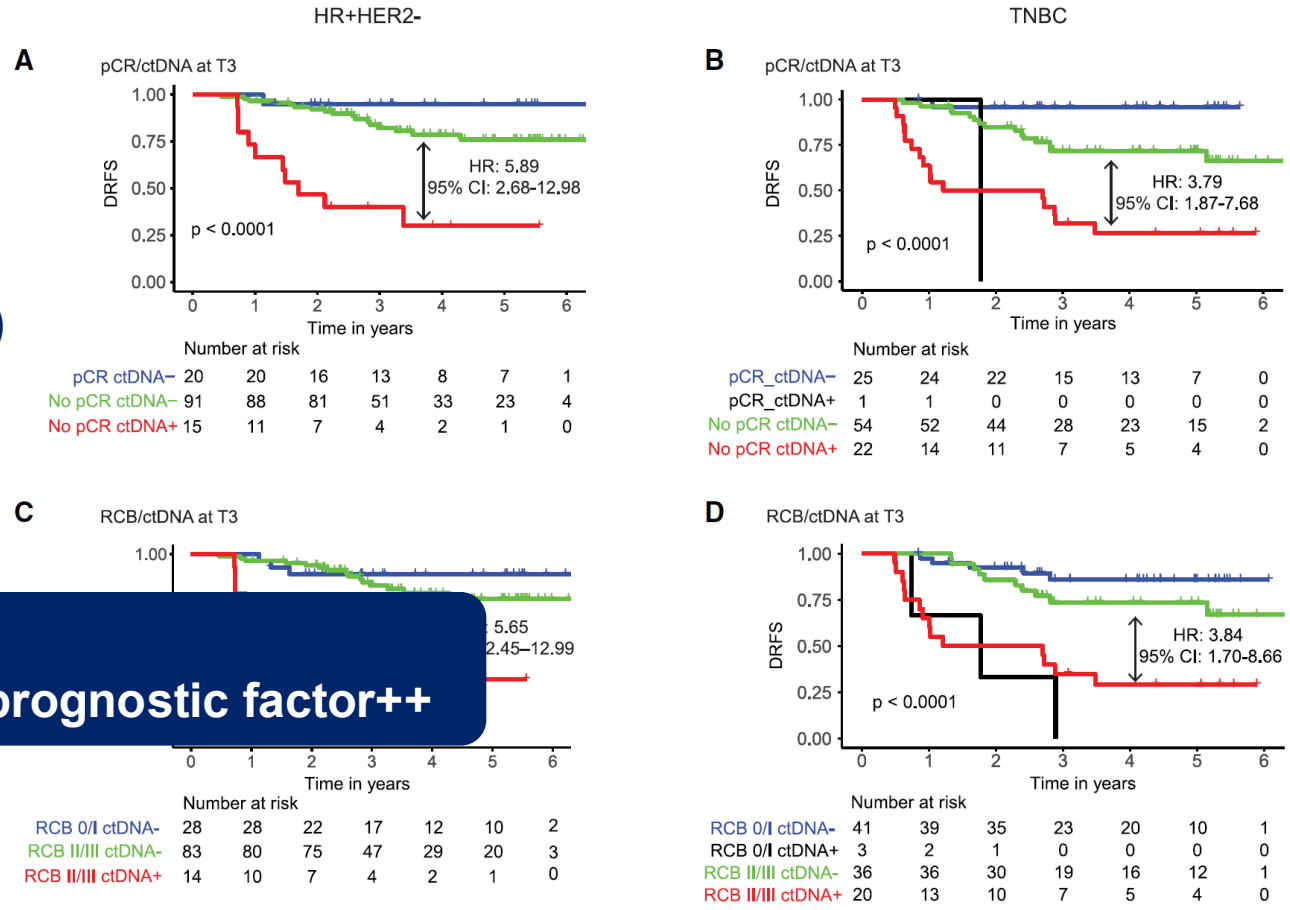
Key concept
In case of non-pCR, ctDNA+ presurgery is a major prognostic factor++



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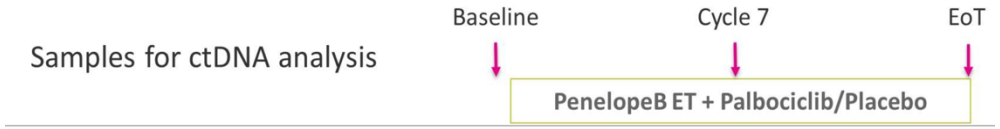
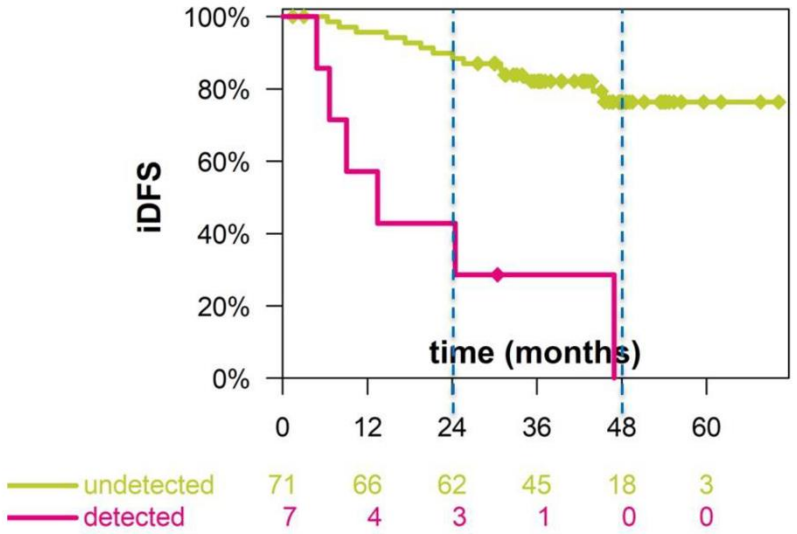
MRD Post Surgery PENELOPE B



RaDaR assay: Palbo vs Placebo post neoadjuvant CT in high-risk HR+/HER2-

- 129/1250 endocrine naïve, 78 patients with ctDNA

- **Baseline ctDNA detection 9% (7/78)**, 5% were ctDNA+ later
- **Higher sensitivity (49%) for relapses <12 mo**
- **Low sensitivity (36%) for early relapses** < 24 mo
- **Very Low sensitivity** for relapses > 48 months
- **Becoming ctDNA detected** (N=3) => relapse, becoming undetected (N=2) no relapse



monarchE trial

Signatera / high-risk BC HR+/HER2-

- CtDNA before ET +/- abema and at 24 months
- 178 pts, **10 (5.6%) baseline ctDNA+** before first initiating Tx
- 7/10 remained ctDNA+ : all relapses
- 3/10 cleared: no relapse yet
- **Becoming ctDNA+ 35 pts (20%) at 24 mo : all relapses**

Key concept in HR+/HER2- with first generation of personalized assay

- **ctDNA detection is rare post surgery/adjuvant CT in luminal BC**
- **ctDNA detection is a strong prognostic factor in high risk luminal**
- **ctDNA detection mostly predicts early relapse**
- **If ctDNA- :No Tx de-escalation in this setting due to this low sensitivity ++**

SABCS 2023 PS06-01 Results from a pilot study exploring ctDNA detection using a tumor-informed assay in the monarchE trial of adjuvant abemaciclib with endocrine therapy in HR+, HER2-, nodepositive, high-risk early breast cancer S. Graff

Early Breast Cancer

Adjuvant ex OXEL study in TNBC with residual cancer

- 45 patients treated with cape / nivo
- ctDNA+ was highly associated with relapse
- 13 pts ctDNA+ (30%) , 4 cleared ctDNA at 6 weeks
- **3 /4 patients who became MRD-** negative at 6 weeks have **not experienced a recurrence to date.**
- **9 patients ctDNA+ without clearance 6weeks : all relapses**

Key concept

-ctDNA kinetics add information that is orthogonal and complementary to radiographic imaging

-Can demonstrate adjuvant therapy efficacy

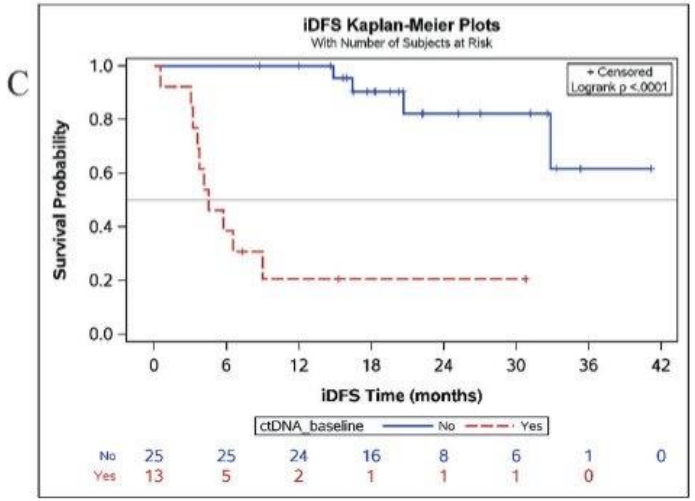
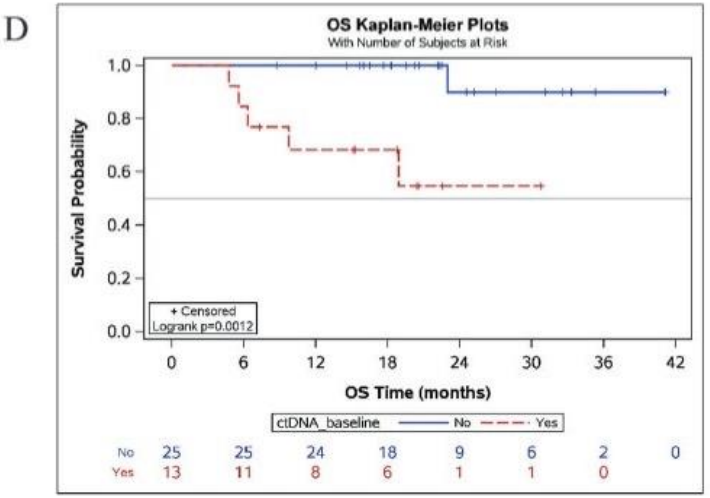


Fig. 2 | Median invasive disease-free survival (iDFS) and overall survival (OS). iDFS (**A**) and OS (**B**) stratified by treatment arm. iDFS (**C**) and OS (**D**) stratified by the presence or absence of circulating tumor DNA (ctDNA) at landmark in patients



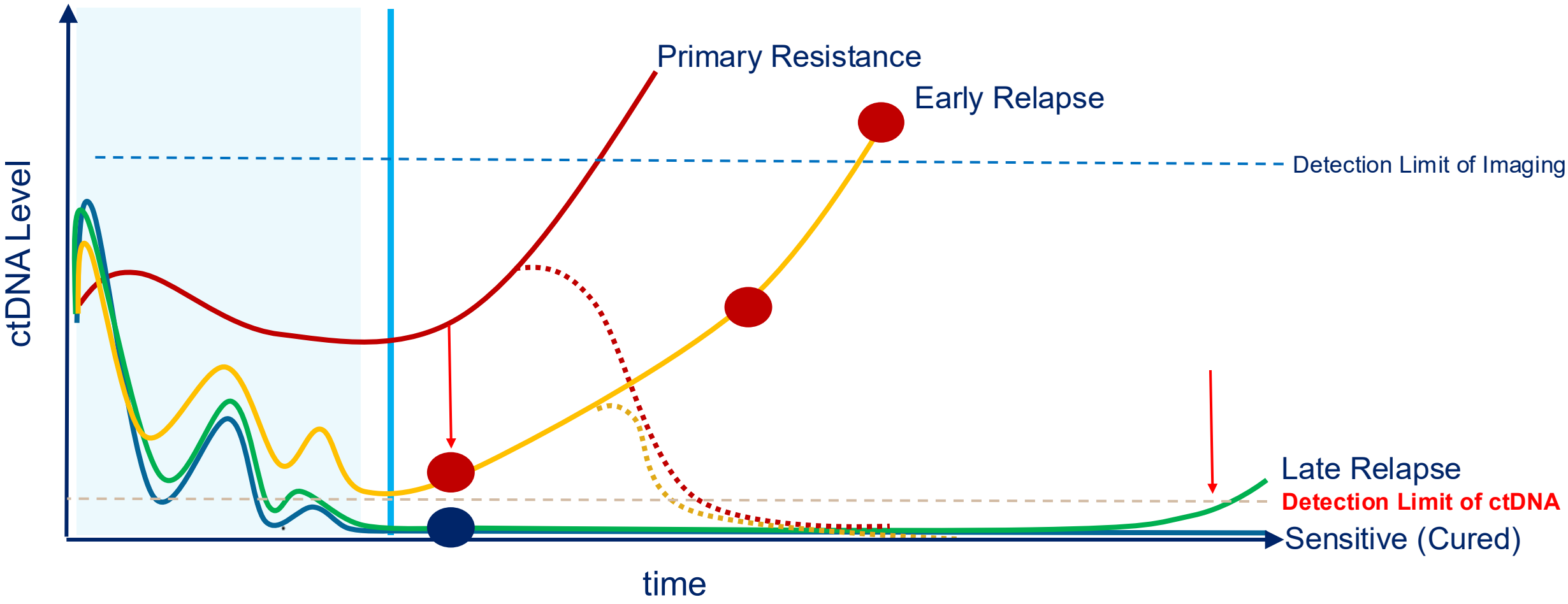
enrolled in Arms A, B, and C combined. Blue line: Arm A (nivolumab); Red line: arm B (capecitabine); Green line: Arm C (combination of nivolumab and capecitabine). Source data are provided as a source data file.

Adjuvant nivolumab, capecitabine or the combination in patients with residual triple-negative breast cancer: the OXEL randomized phase II study

Filipa Lynce Nat Comm 2024

With a better assay we hope to detect more patients

Decrease of the detection limit of ctDNA



ctDNA early breast clinical utility

ctDNA is a strong prognostic factor, should we use it ?

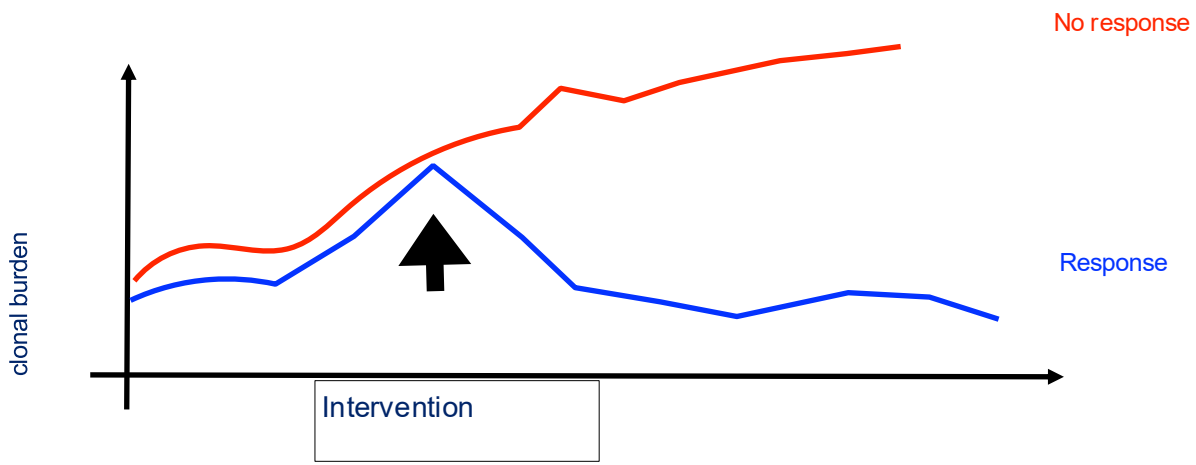
Key Message

There is no prospective data demonstrating that :

- **ctDNA-guided therapy improves outcome**
- **Earlier detection of metastatic relapse with ctDNA versus symptoms improves outcome**

Plasma whole genome sequencing for ultrasensitive ctDNA detection

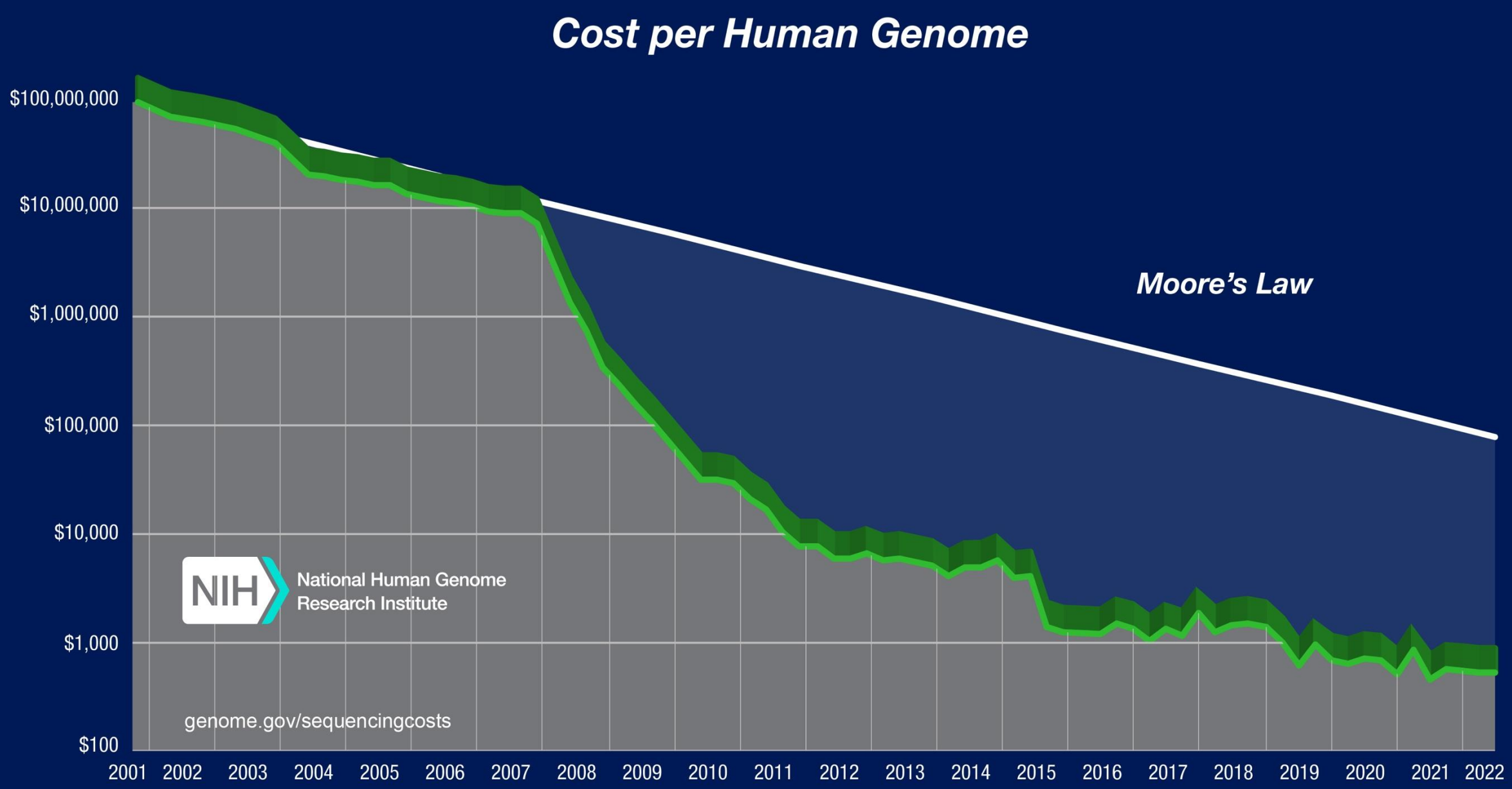
Urgent unmet need: Ultra-sensitive liquid biopsy for solid tumor management



Implementation barriers

1. Inadequate sensitivity
2. Key workflow barriers- bespoke targeted panels and matched tumor tissue dependency

The road less traveled: plasma WGS


Cost per Human Genome
$100,000,000
$10,000,000
$1,000,000
$100,000
$10,000
$1,000
$100
Moore's Law
NIH
National Human Genome
Research Institute
genome.gov/sequencingcosts
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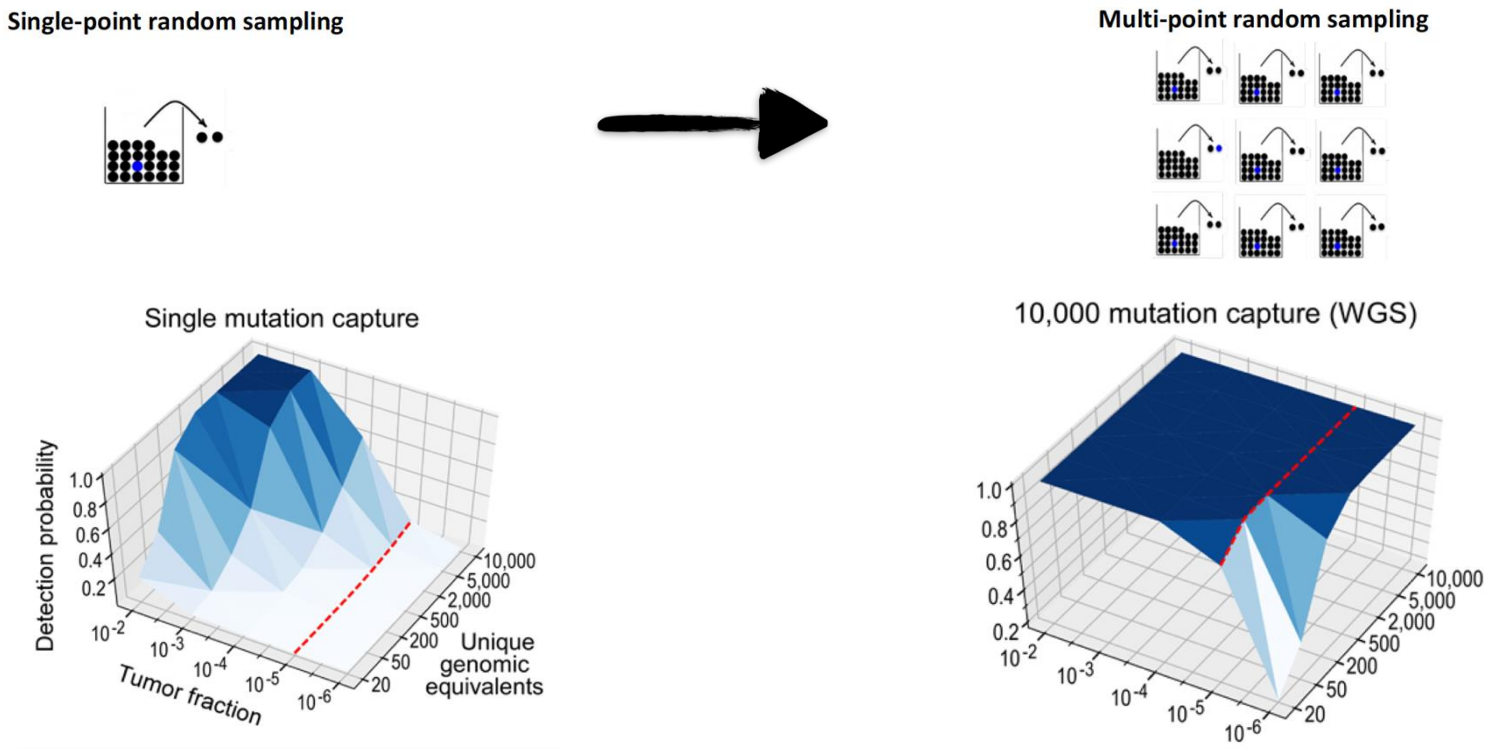
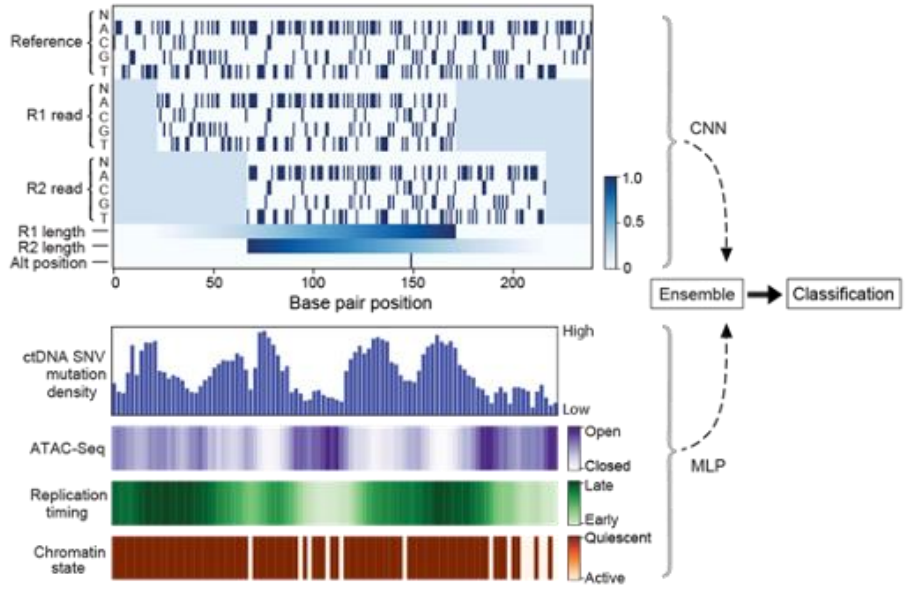
Whole genome and the input problem: breadth vs. depth



1. Overcomes input material ceiling on targeted seq
2. Major operational advantages for broad application

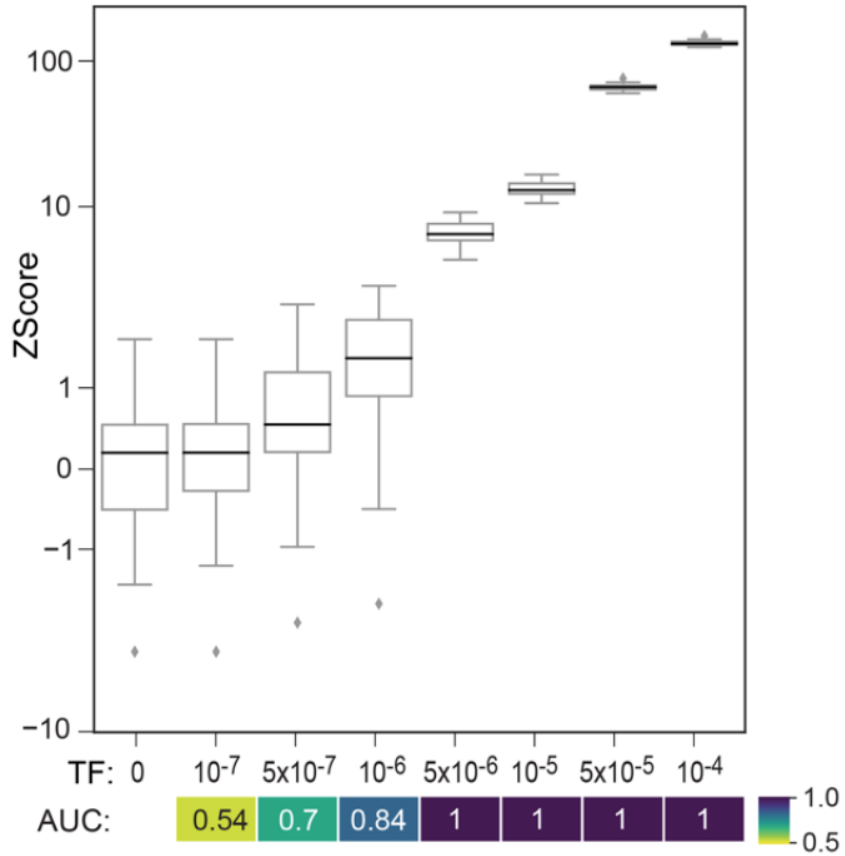
Zviran et al, Nature Medicine, 2020

ctDNA training sets & advanced ML architecture for complex feature space enables individual fragment classification



Widman et al., Nature Medicine, in press

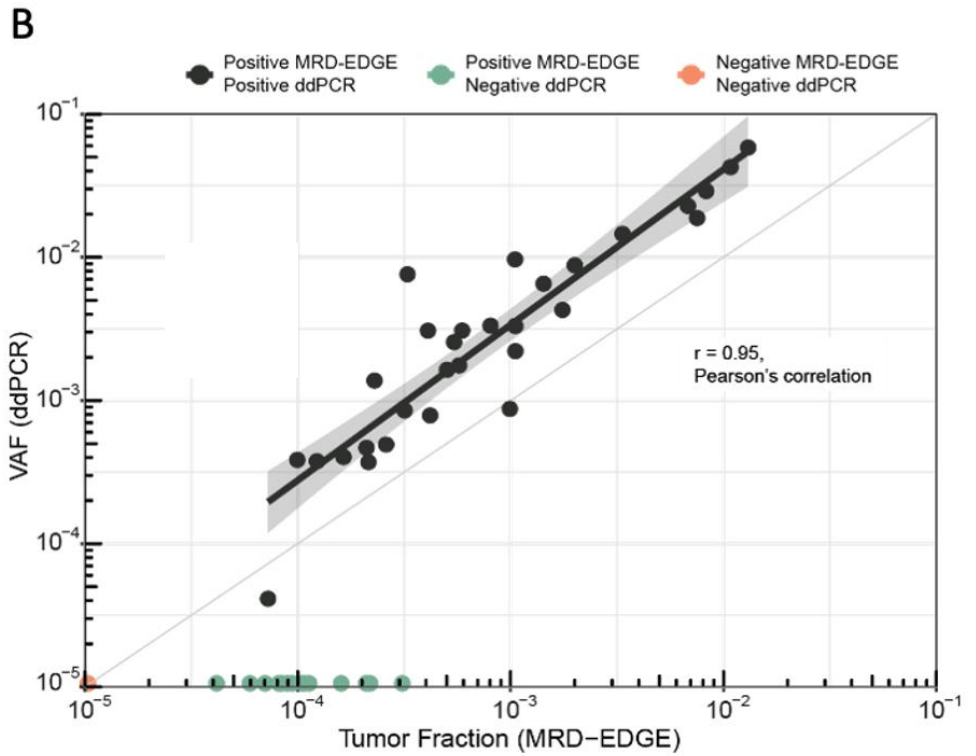
in silico admixtures shows parts per million detection in tumor informed setting



Widman et al Nature Medicine, in press

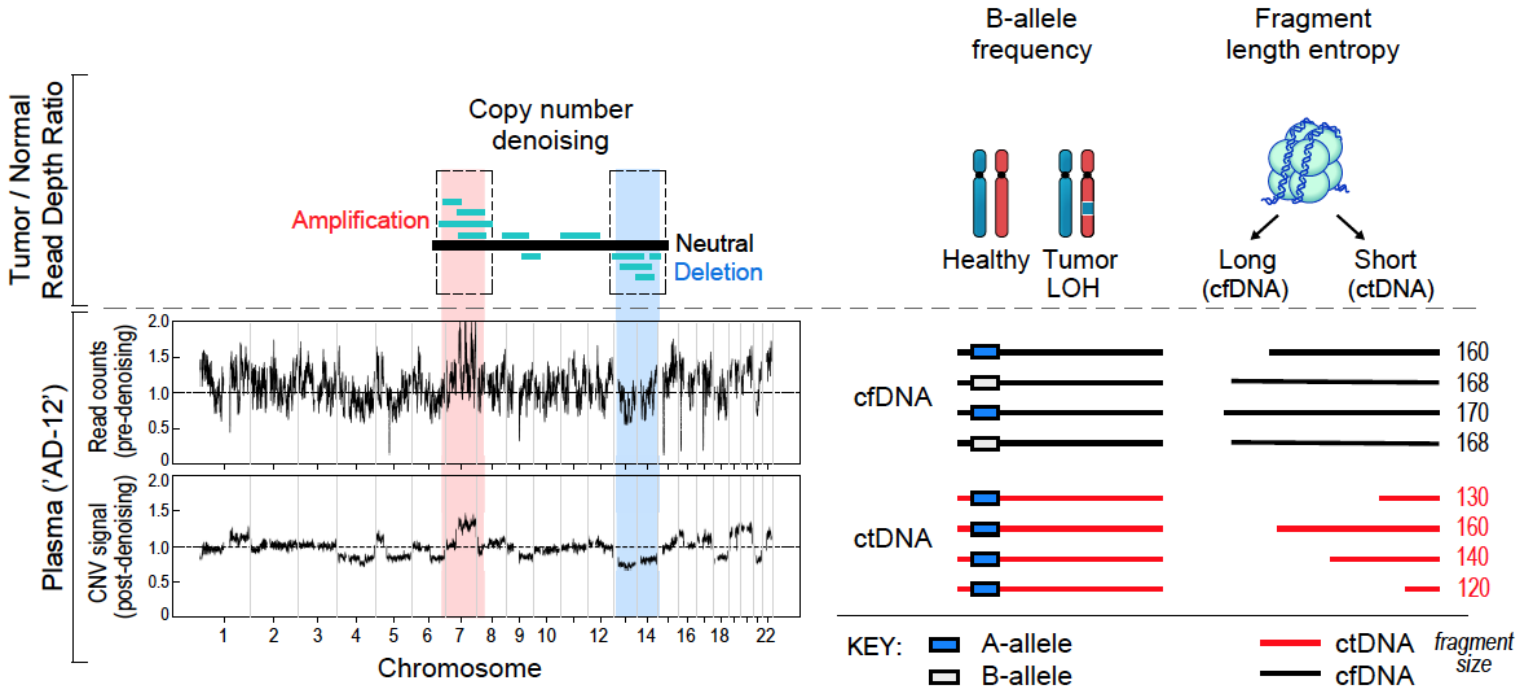
Benchmarking to ddPCR and Signatera

NO panels….

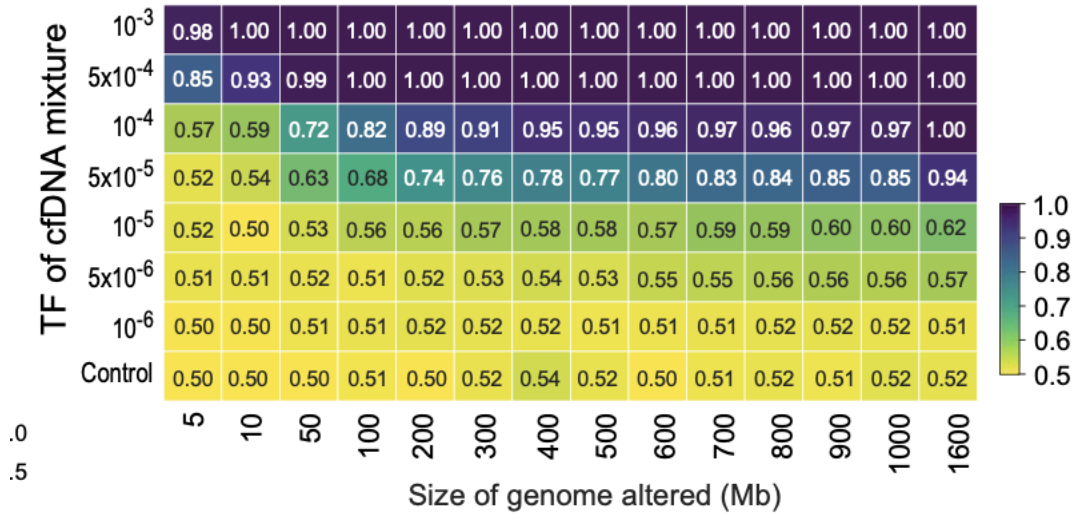


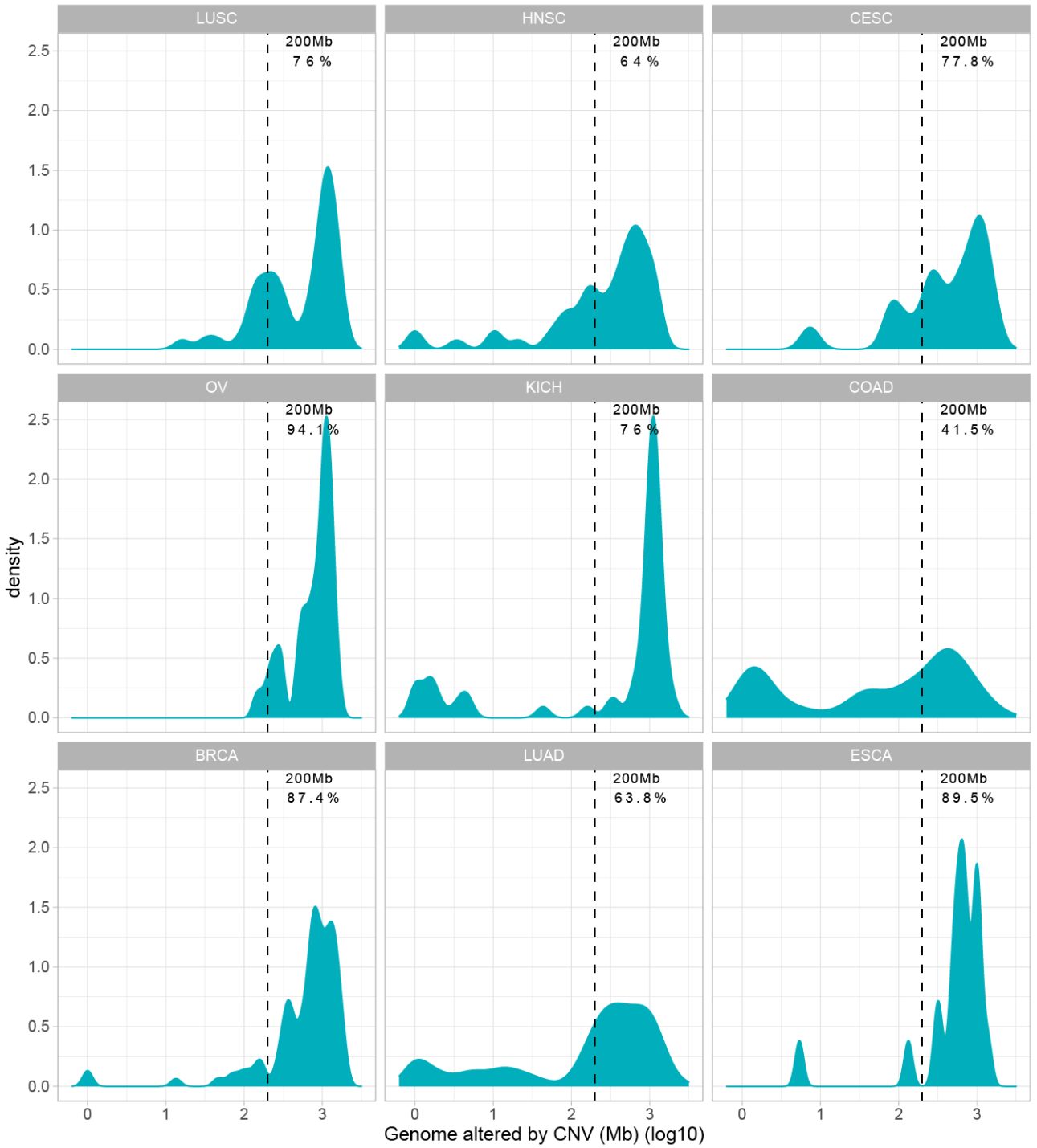
Widman et al Nature Medicine, in press

Expanding feature space and ML integration enhances CNV detection



Widman et. al, Nature Medicine, in press

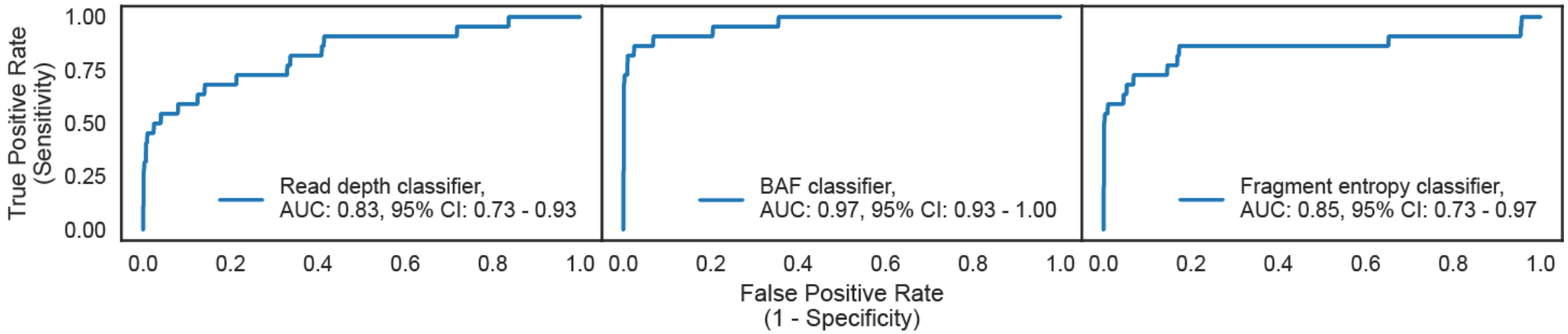




Widman et al., Nature Medicine in press

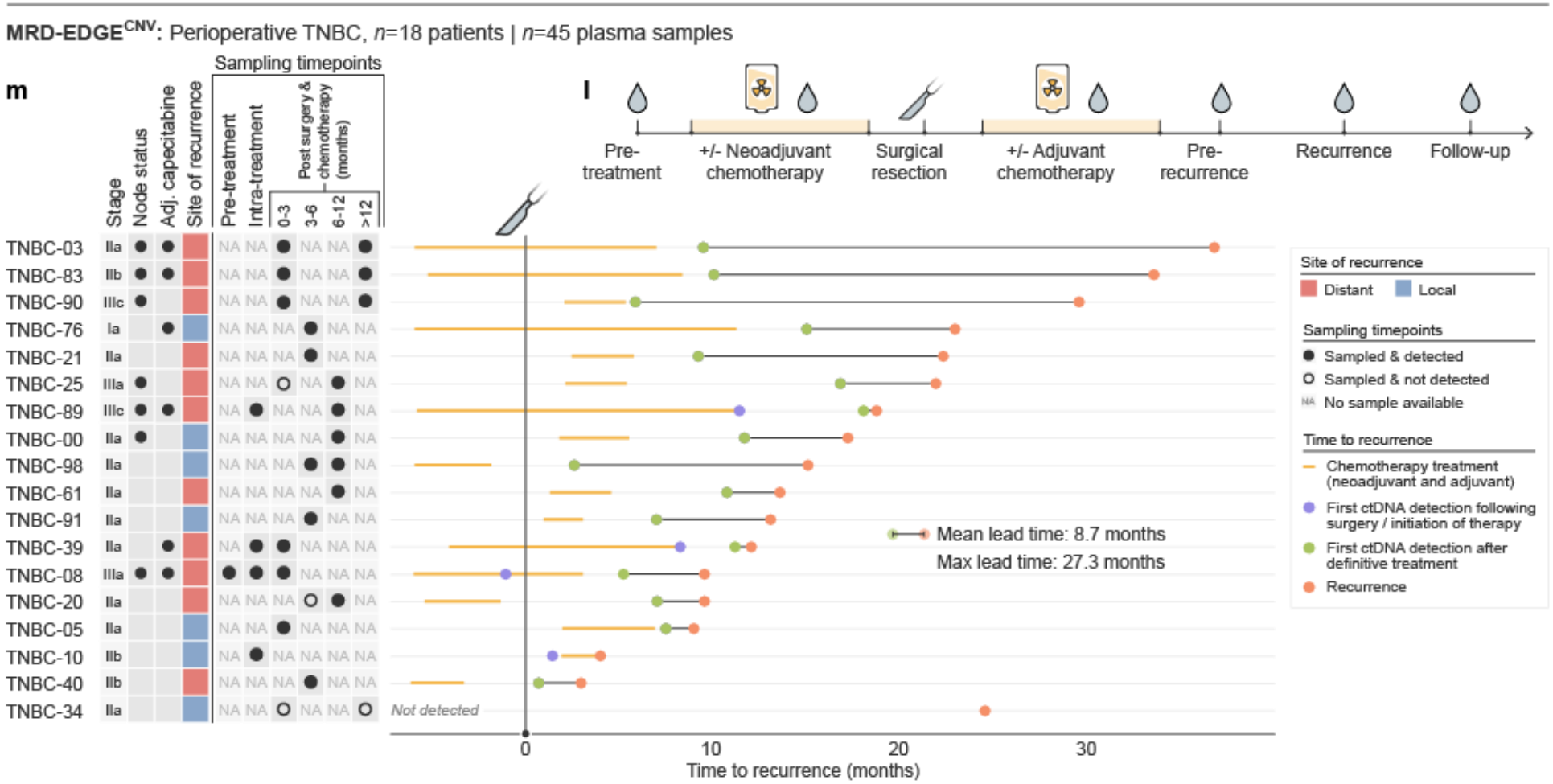
Tumor-informed detection of preoperative disease through aneuploidy

22 early-stage NSCLC



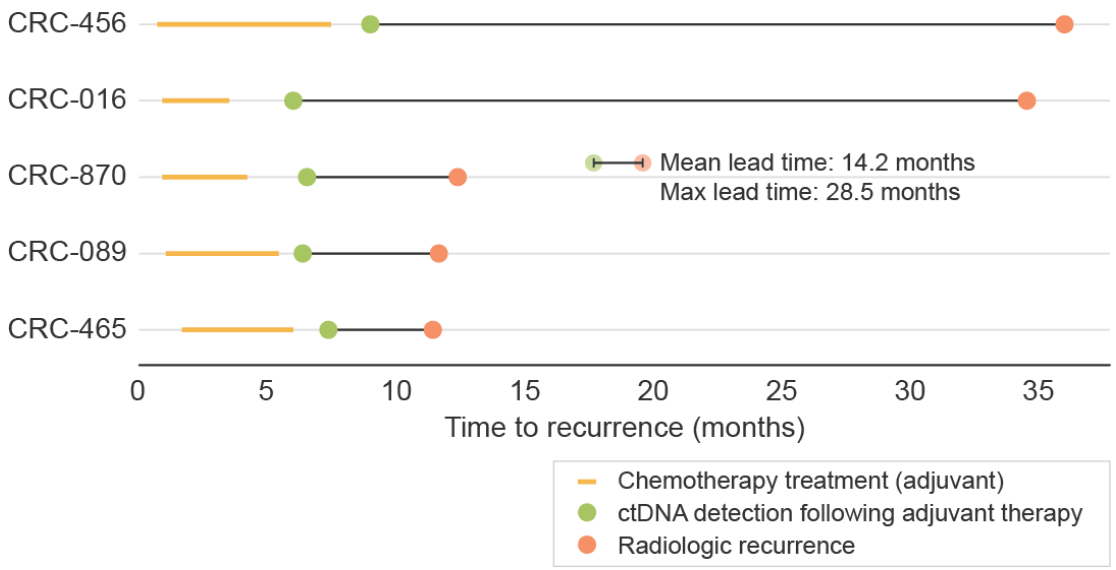
Widman et al., Nature Medicine in press

Strong sensitivity and lead times in TNBC patients with disease recurrence



Widman et al Nature Medicine in press

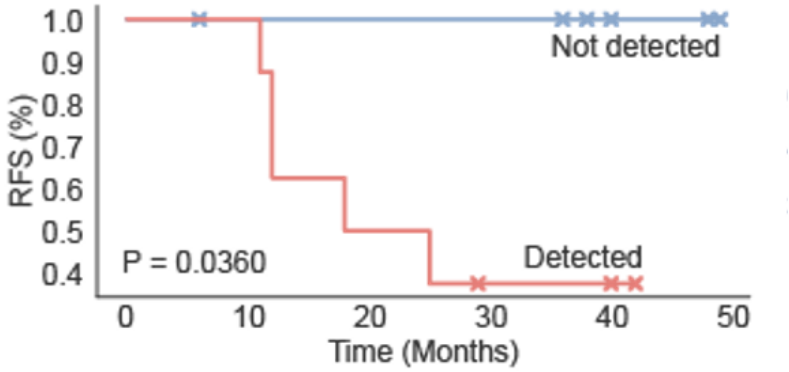
Powerful MRD ready for clinical application



CRC residual dz post adjuvant (mean lead time 14 months, max 28 months)

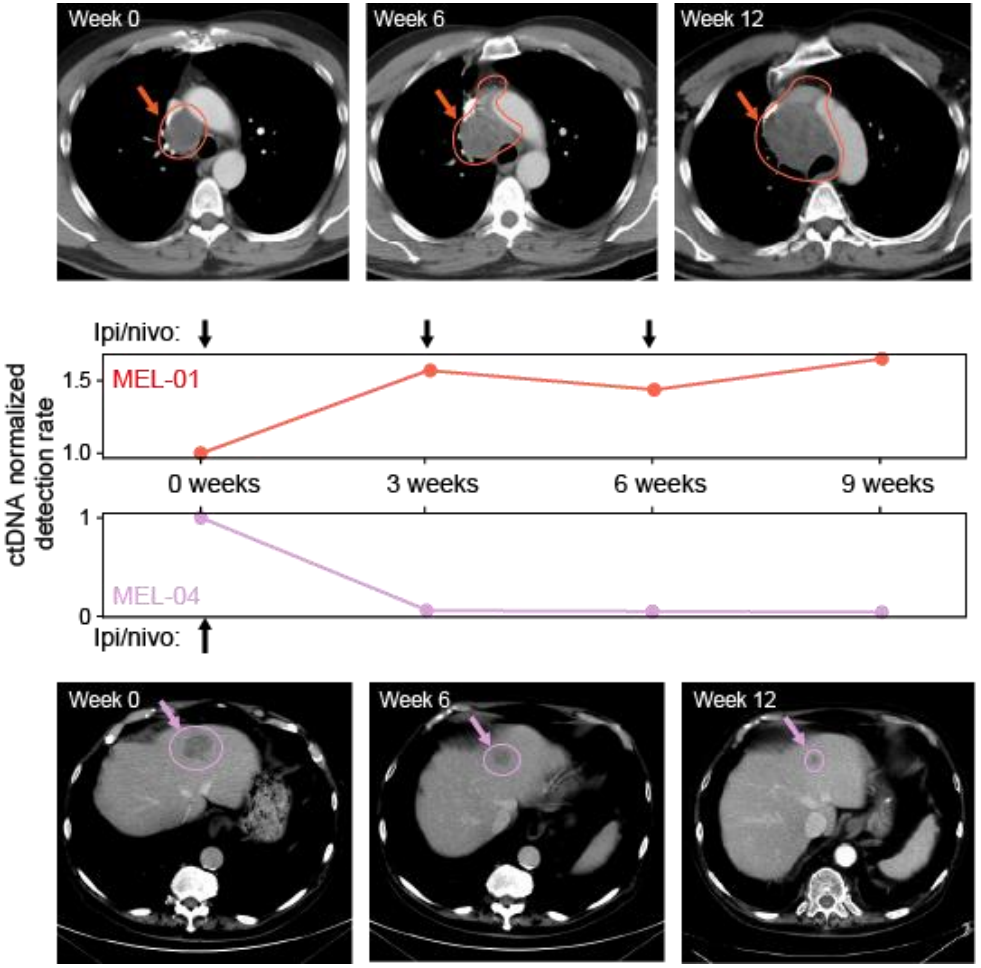
MRD in early-stage resected NSCLC

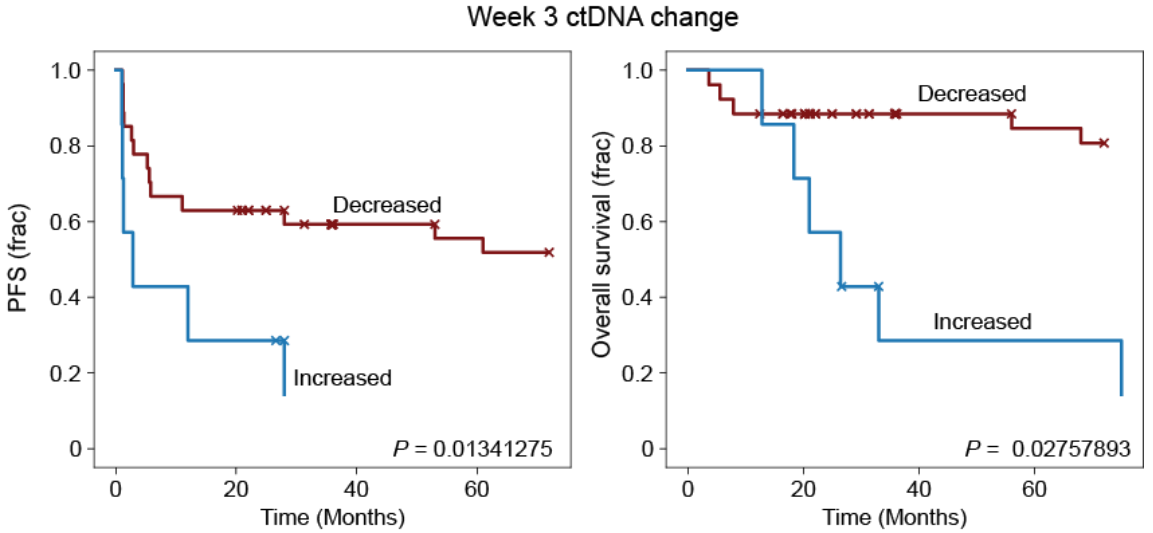
Neoadjuvant treatment monitoring in NSCLC



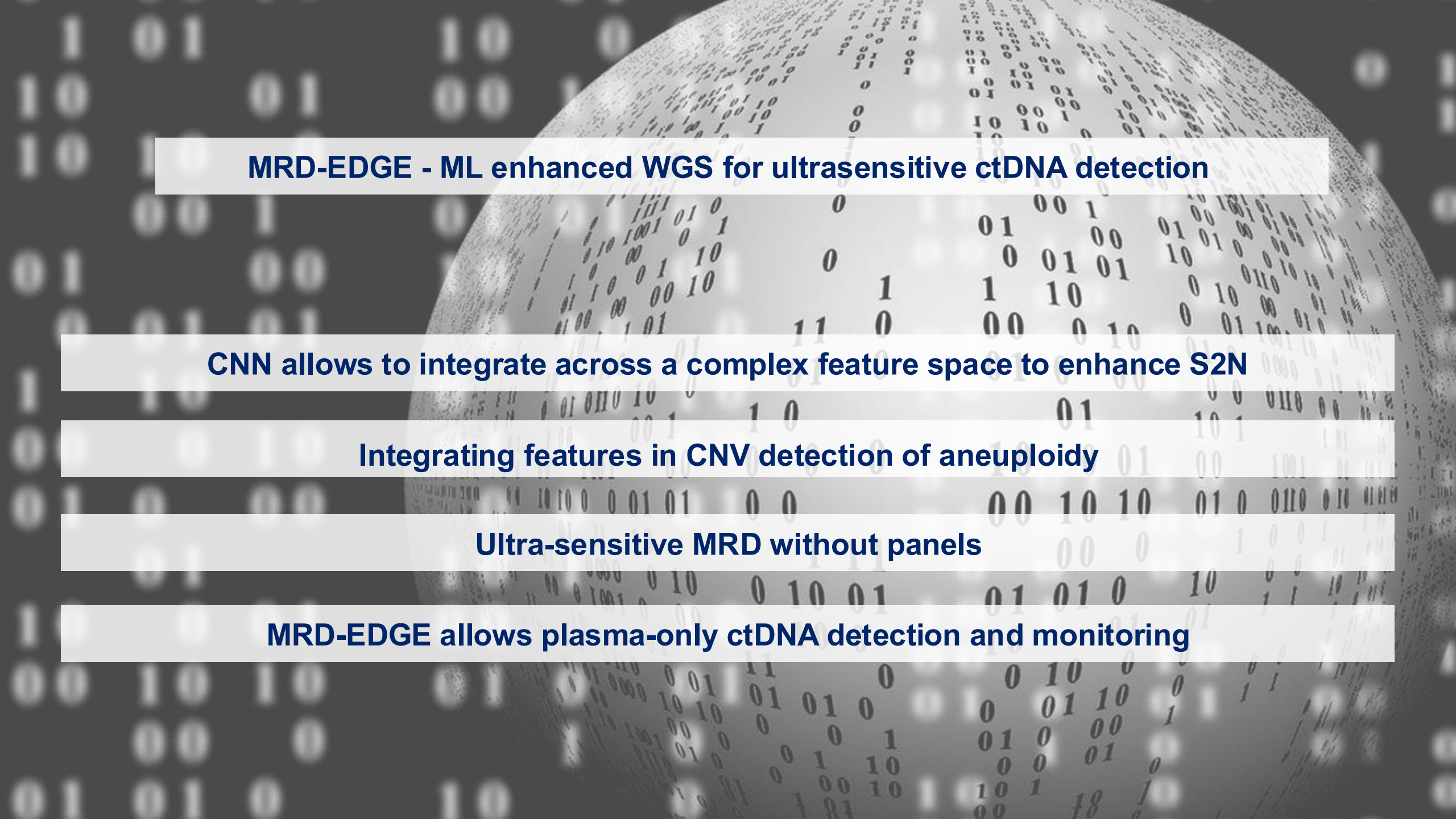
Widman et. al, Nature Medicine, in press

Plasma-only immunotherapy response prediction within 3 weeks





Widman et. al, Nature Medicine, in press



MRD-EDGE - ML enhanced WGS for ultrasensitive ctDNA detection

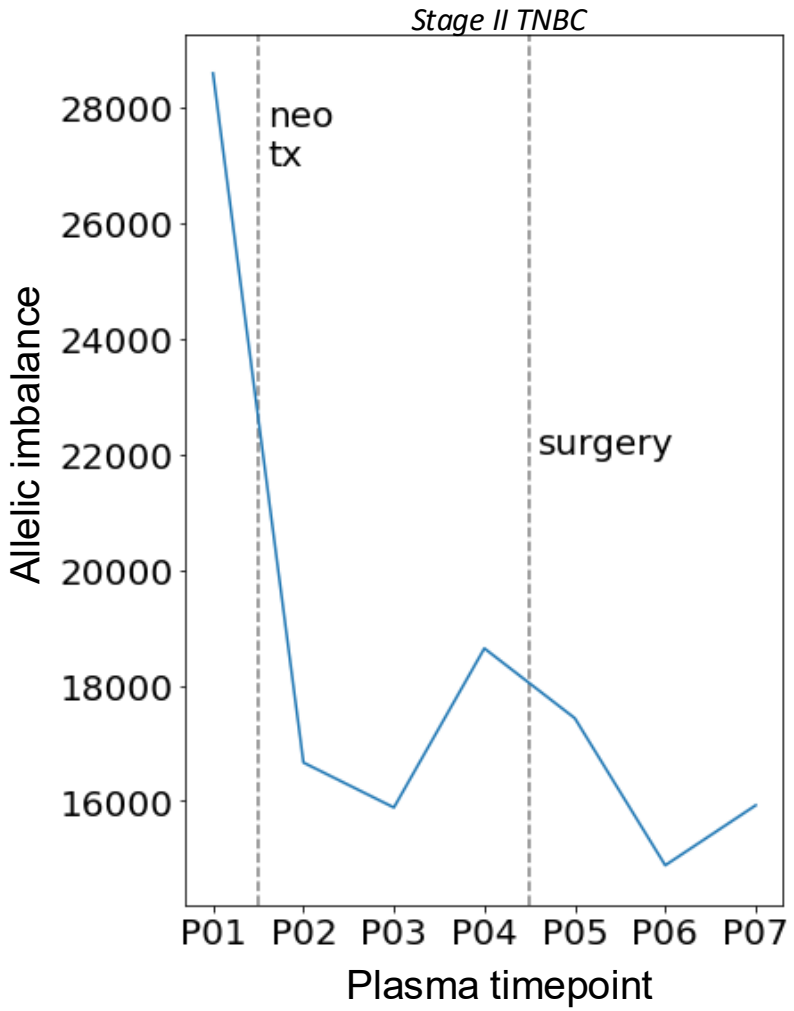
CNN allows to integrate across a complex feature space to enhance S2N

Integrating features in CNV detection of aneuploidy

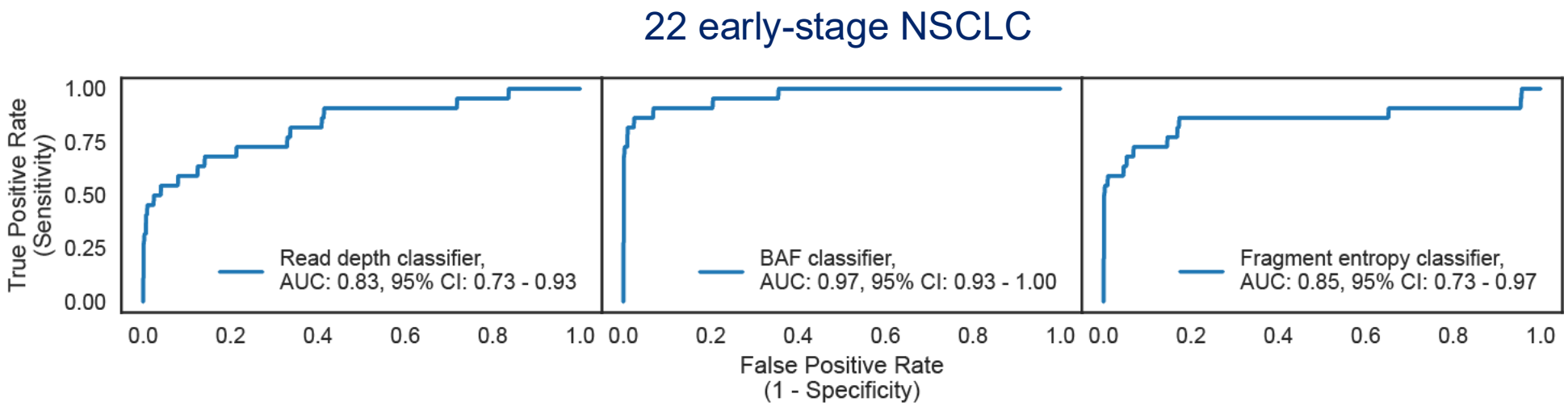
Ultra-sensitive MRD without panels

MRD-EDGE allows plasma-only ctDNA detection and monitoring

“High depth WGS for plasma-only early detection of breast and ovarian cancer”



Tumor-informed detection of preoperative disease



Widman et al., Nature Medicine in press


pilot-1
Allelic imbalance
80000
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Plasma timepoint
pilot-2
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Plasma timepoint
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Allelic imbalance
13200
13100
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12900
12800
12700
surgery
P01
P02
Plasma timepoint

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Julia An

MSKCC Pediatrics
Neerav Shukla
Paola Angelini

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Mike Postow

MSKCC Colorectal Surgery
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Tommy Chan

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Bishoy Faltas

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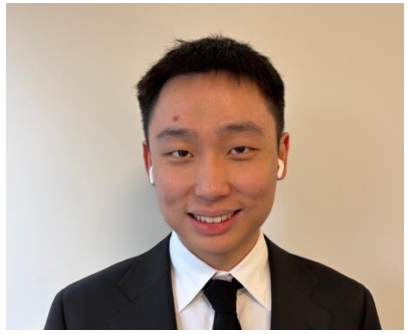
Laura Andersen
Malene Ostergaard



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LUSTGARTEN Pancreatic Cancer Research

Tina's Wish

Our patients and their families

Melanoma Research Alliance

CONQUER CANCER THE ASCO FOUNDATION

AACR American Association for Cancer Research

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