MRD: Tissue-agnostic vs. Tumor-informed

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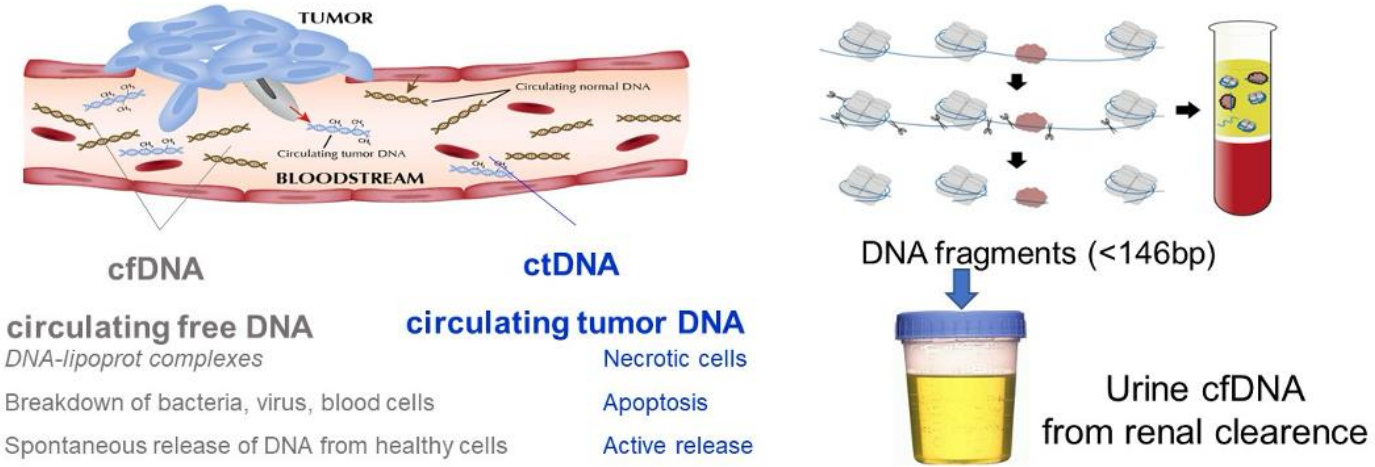
Circulating tumor DNA (ctDNA)



- Highly tumor specific
- Early detection/screening
- Actionable genomic alterations
- Disease monitoring (MRD, prognosis)

cfDNA half life: <2 hours → real time monitoring of tumor burden

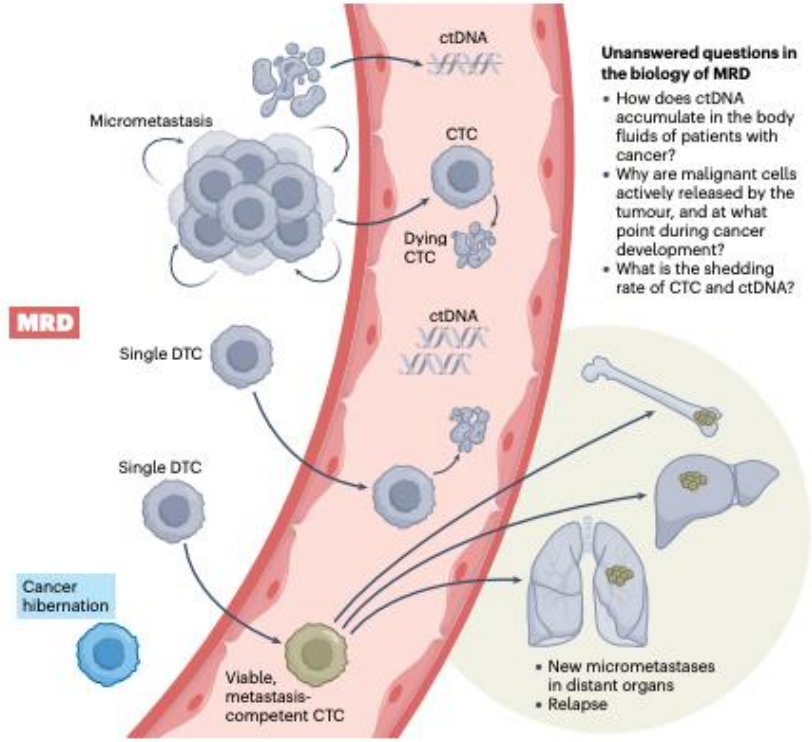
Leary et al, Sci Transl med, 2012

Minimal Residual Disease (MRD)

Microscopic tumor burden remaining in the body after treatment in patients who have no clinical evidence of disease

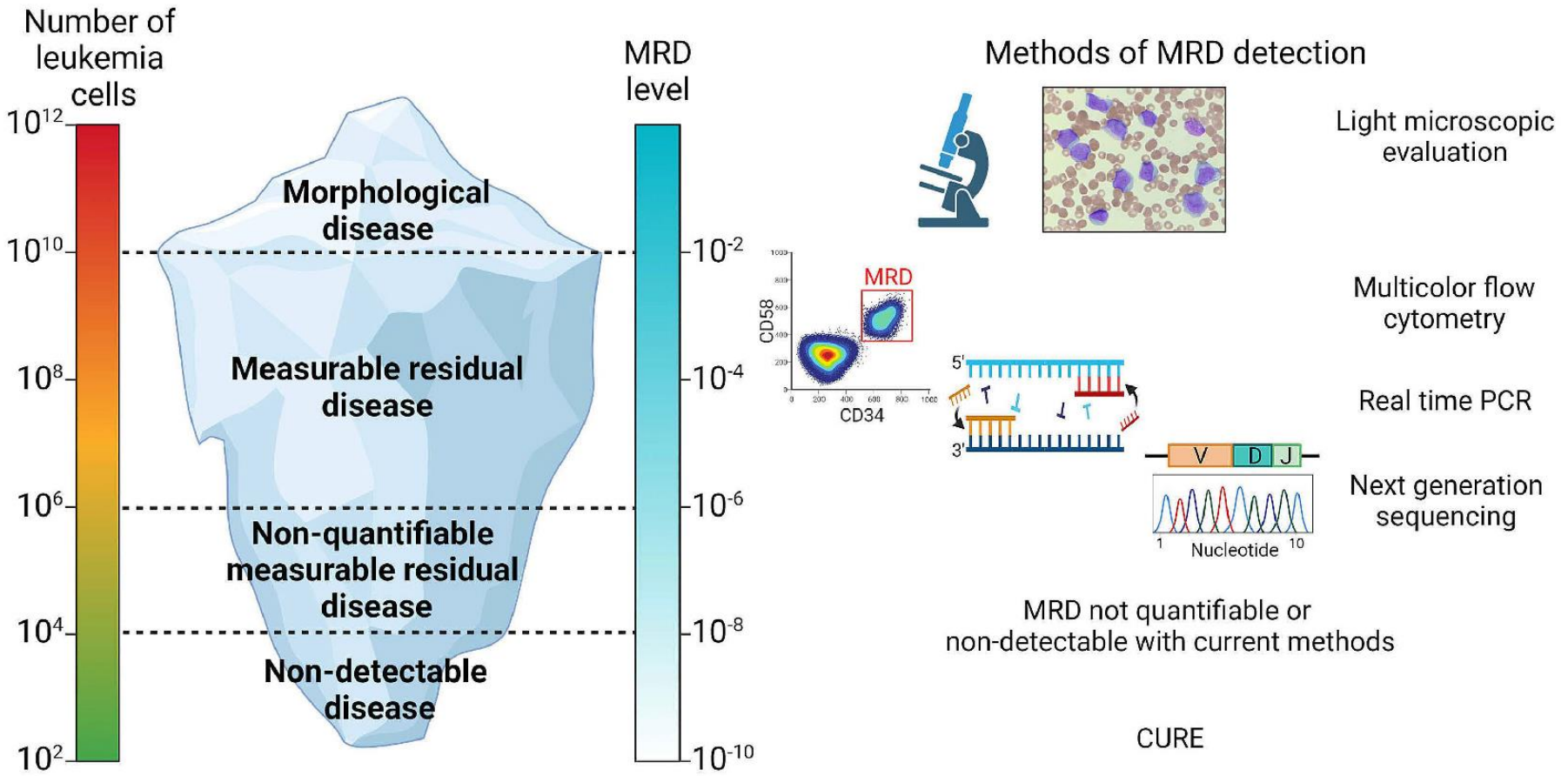
These residual cells can persist locally, circulate in the bloodstream as circulating tumor cells (CTCs), or reside in distant organs as disseminated tumor cells (DTCs) or micrometastases

MRD detection after completion of local therapy could identify which patients will recur and allow personalization of adjuvant therapy



Pantel et al. – Nature Reviews Clin Onc 2025

MRD in Hematologic Malignancies

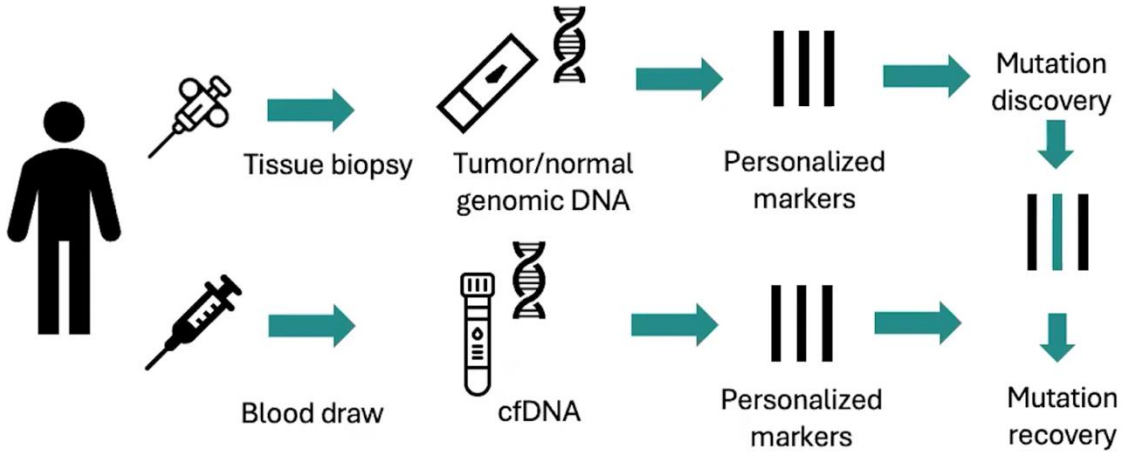


Saygin et al – Haematologica 2022

ctDNA MRD: Tumor-Informed vs. Tumor-Naive

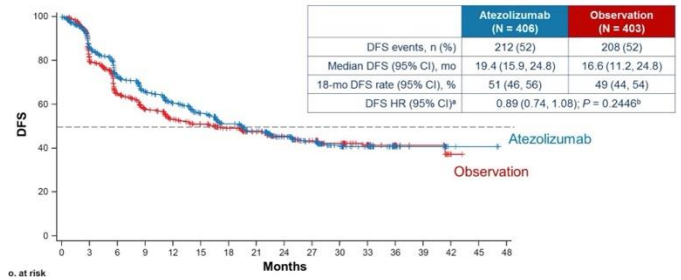
@alantanmd

	Tumor Informed	Tumor Naïve
Adequate specimen	Limitation – UTUC, bone mets, no Nx	Not required
Sensitivity	Better LOD (.01 to <1ppm)	Less sensitive
Specificity	Very good Screens out CHIP	Very good CHIP needs filter algorithm, epigenomics and fragmentomics improve
Emerging Variants/biomarkers	No	Yes
Turnaround time	Slower ~ 4-6 weeks for baseline, subsequent 1 week	7-10 days
Key Applications	• MRD • Assess treatment response • Serial monitoring	• MRD • Assess heterogeneity, actionable alterations, resistance • Serial monitoring

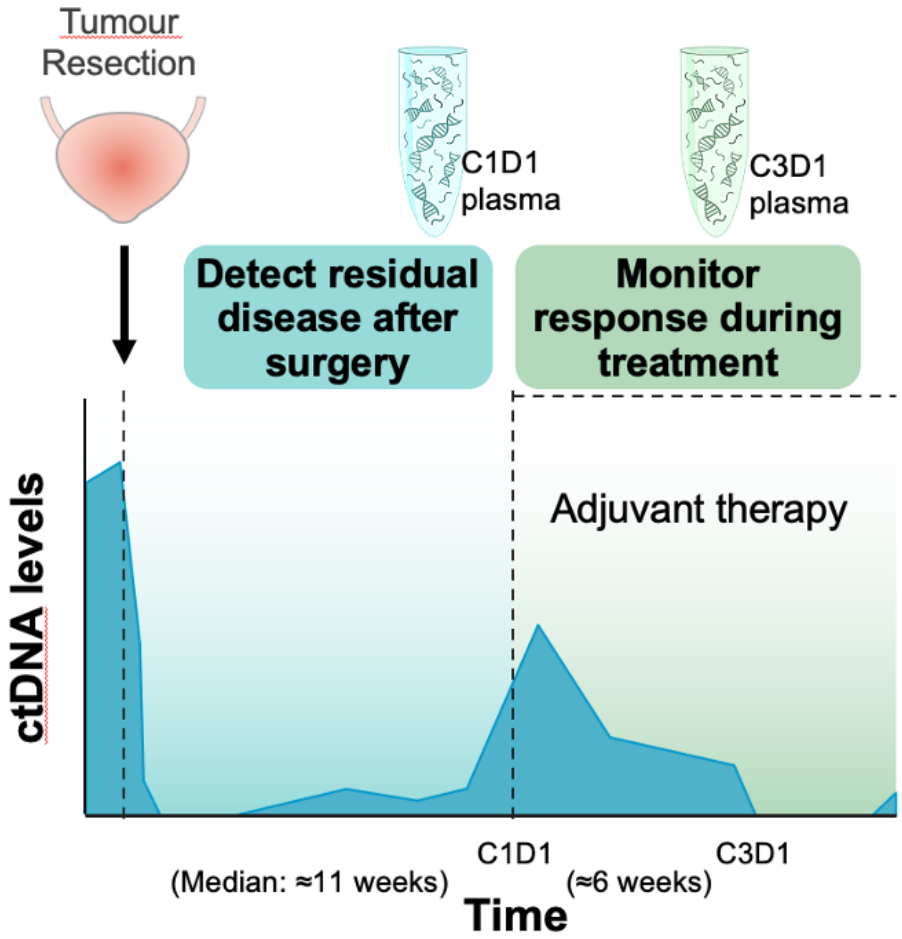


Tumor-Informed ctDNA

Evaluation of ctDNA in IMvigor010



	Atezolizumab (N = 406)	Observation (N = 403)
DFS events, n (%)	212 (52)	208 (52)
Median DFS (95% CI), mo	19.4 (15.9, 24.8)	16.6 (11.2, 24.8)
18-mo DFS rate (95% CI), %	51 (46, 56)	49 (44, 54)
DFS HR (95% CI)[a]	0.89 (0.74, 1.08); *P* = 0.2446[b]	

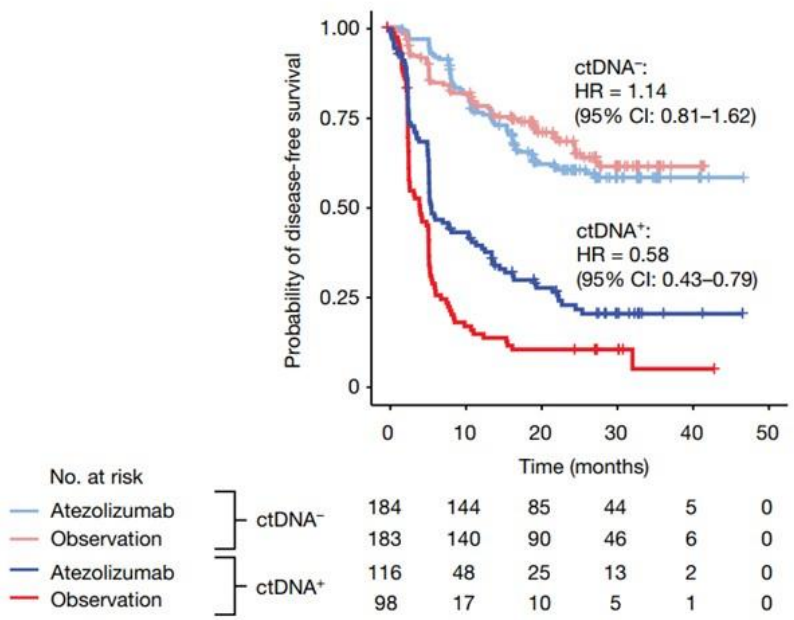


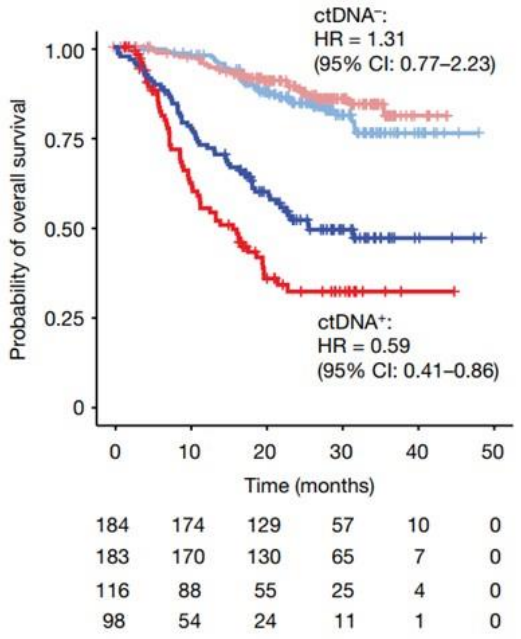
1. Tumour tissue and germline material were sequenced (whole exome sequencing)
2. Up to 16 mutations for personalised mPCR ctDNA assay were identified for each patient
3. Plasma samples were sequenced to ≈100,000×
4. If ≥2 mutations were detected, sample was defined as ctDNA(+)
5. MRD sample timepoint before adjuvant treatment (C1D1) was collected
6. On-treatment sample (C3D1; week 6) was also collected

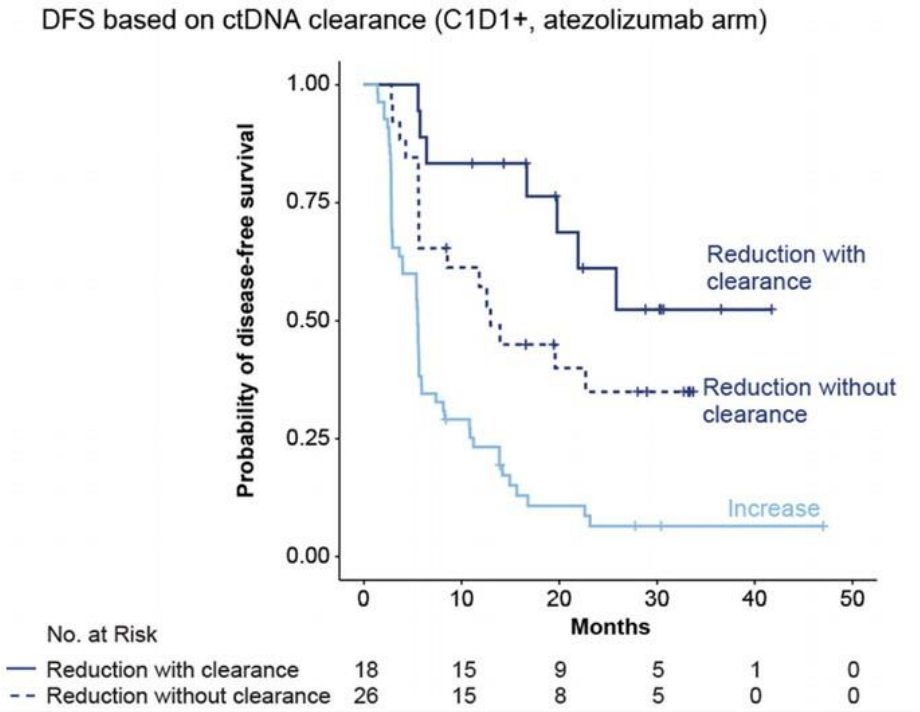
C, cycle; D, day; mPCR, multiplex polymerase chain reaction; MRD, molecular residual disease.

Powles et al. IMvigor010 ctDNA
https://bit.ly/2IxYIlE

ctDNA dynamics in the adjuvant setting







Powles et al., Nature. 2021

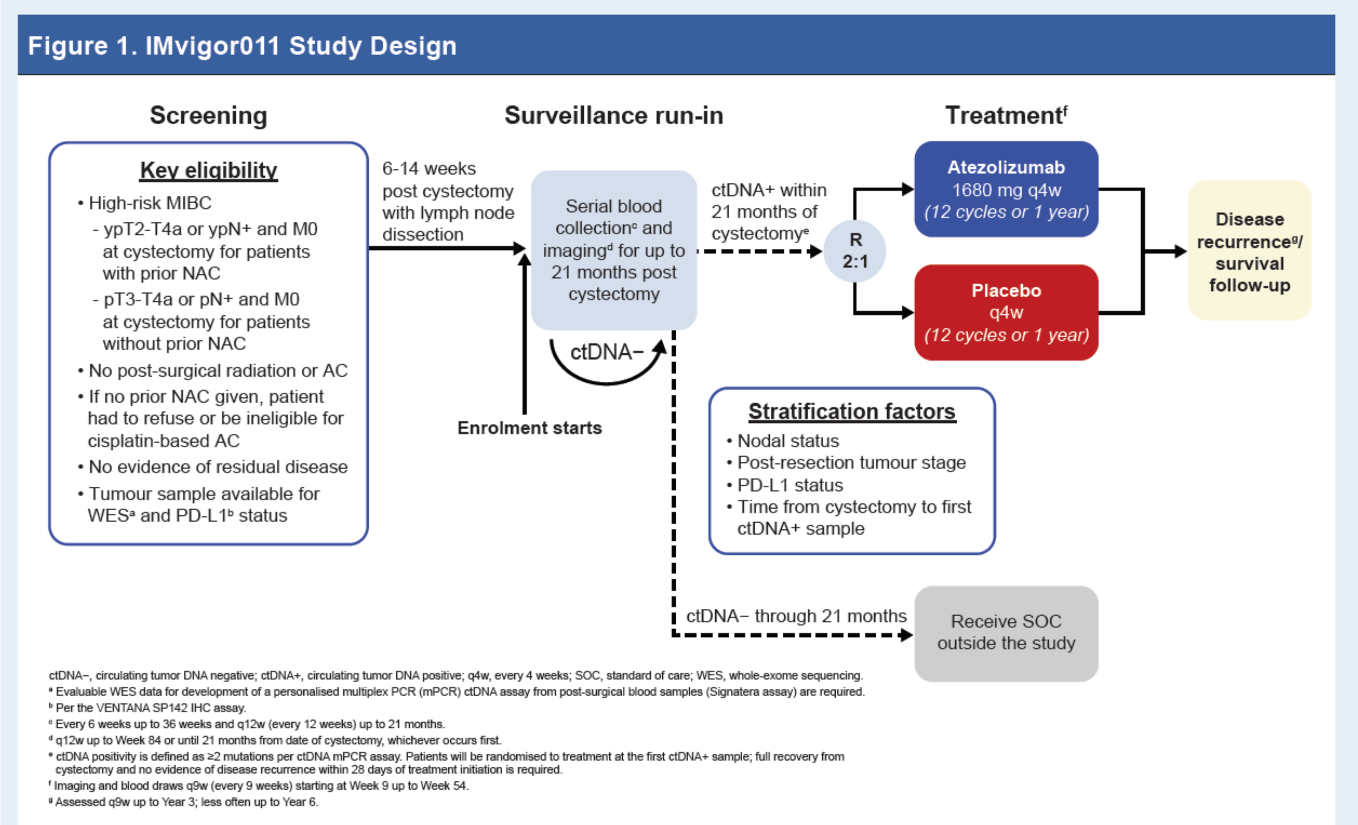
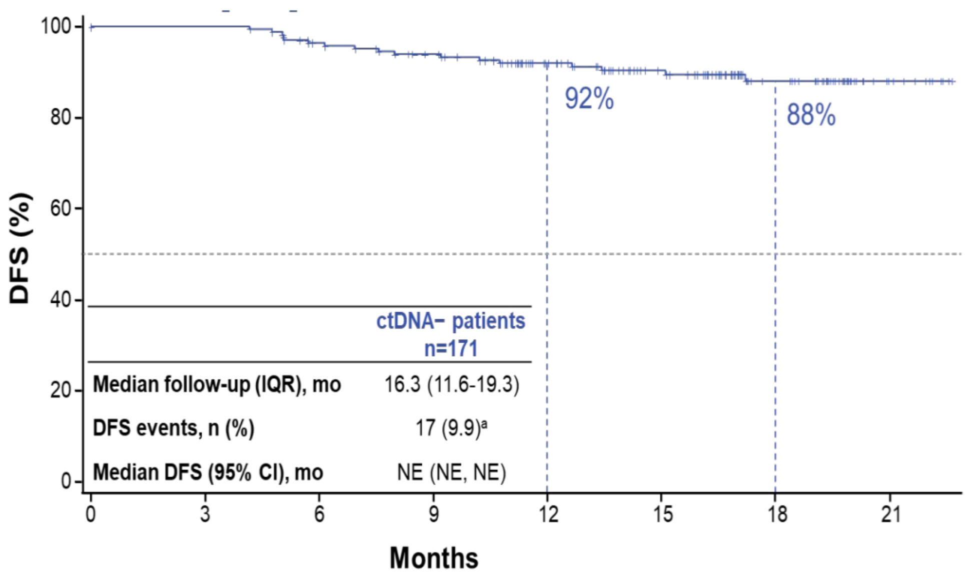


Figure 1. IMvigor011 Study Design
Screening
Surveillance run-in
Treatment[f]
Key eligibility
• High-risk MIBC
- ypT2-T4a or ypN+ and M0 at cystectomy for patients with prior NAC
- pT3-T4a or pN+ and M0 at cystectomy for patients without prior NAC
• No post-surgical radiation or AC
• If no prior NAC given, patient had to refuse or be ineligible for cisplatin-based AC
• No evidence of residual disease
• Tumour sample available for WES[a] and PD-L1[b] status
6-14 weeks post cystectomy with lymph node dissection
Serial blood collection[c] and imaging[d] for up to 21 months post cystectomy
ctDNA−
Enrolment starts
ctDNA+ within 21 months of cystectomy[e]
R 2:1
Atezolizumab 1680 mg q4w (12 cycles or 1 year)
Placebo q4w (12 cycles or 1 year)
Disease recurrence[g]/ survival follow-up
Stratification factors
• Nodal status
• Post-resection tumour stage
• PD-L1 status
• Time from cystectomy to first ctDNA+ sample
ctDNA− through 21 months
Receive SOC outside the study
ctDNA−, circulating tumor DNA negative; ctDNA+, circulating tumor DNA positive; q4w, every 4 weeks; SOC, standard of care; WES, whole-exome sequencing.
[a] Evaluable WES data for development of a personalised multiplex PCR (mPCR) ctDNA assay from post-surgical blood samples (Signatera assay) are required.
[b] Per the VENTANA SP142 IHC assay.
[c] Every 6 weeks up to 36 weeks and q12w (every 12 weeks) up to 21 months.
[d] q12w up to Week 84 or until 21 months from date of cystectomy, whichever occurs first.
[e] ctDNA positivity is defined as ≥2 mutations per ctDNA mPCR assay. Patients will be randomised to treatment at the first ctDNA+ sample; full recovery from cystectomy and no evidence of disease recurrence within 28 days of treatment initiation is required.
[f] Imaging and blood draws q9w (every 9 weeks) starting at Week 9 up to Week 54.
[g] Assessed q9w up to Year 3; less often up to Year 6.

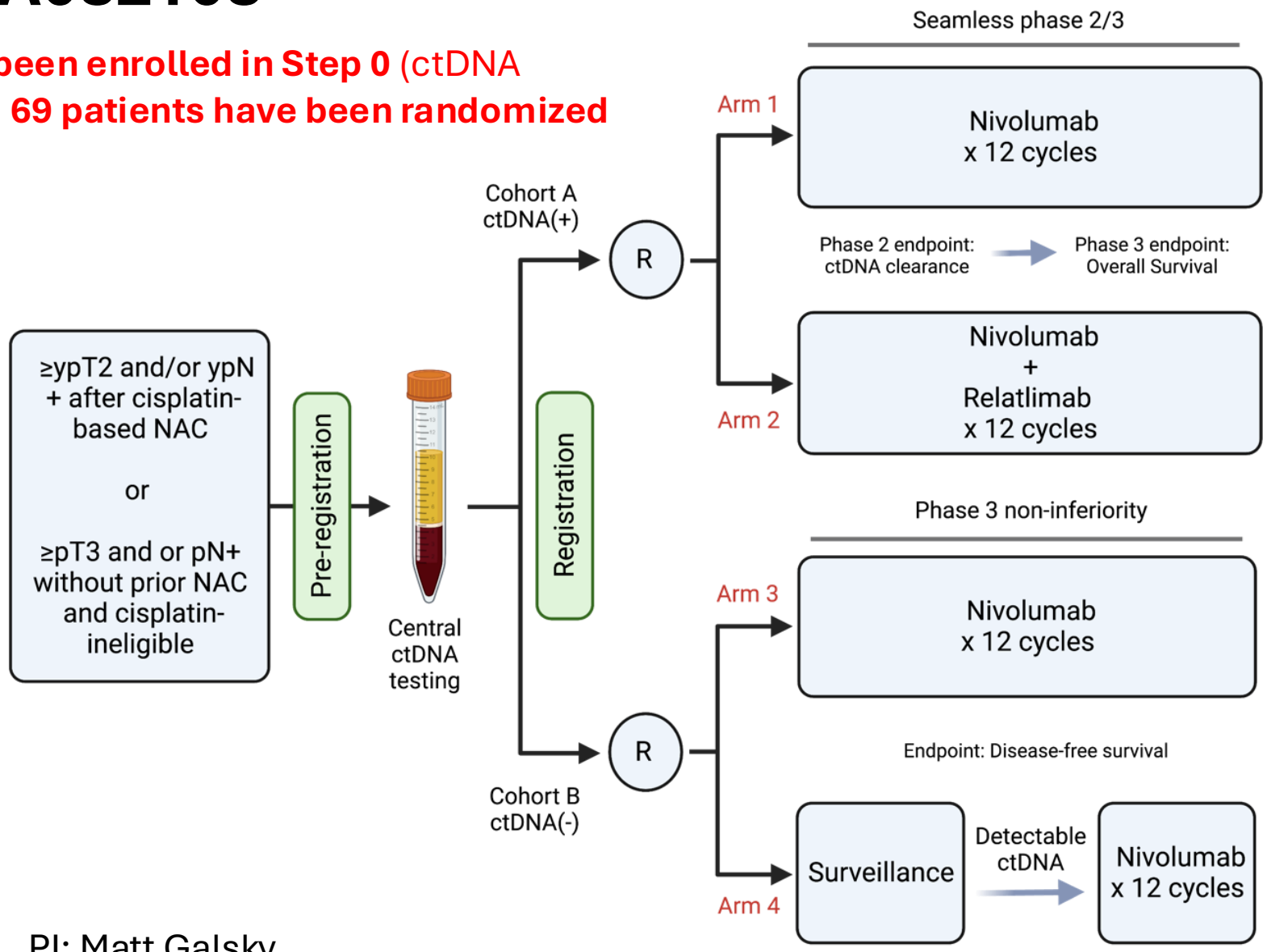
Relapse in the persistently ctDNA-ve surveillance population from IM011



Powles, Thomas, Zoe June Assaf, Nicole Davarpanah, Romain Banchereau, Bernadett E. Szabados, Kobe C. Yuen, Petros Grivas, et al. "CtDNA Guiding Adjuvant Immunotherapy in Urothelial Carcinoma." *Nature* 595, no. 7867 (July 2021): 432–37. https://doi.org/10.1038/s41586-021-03642-9.

MODERN A032103

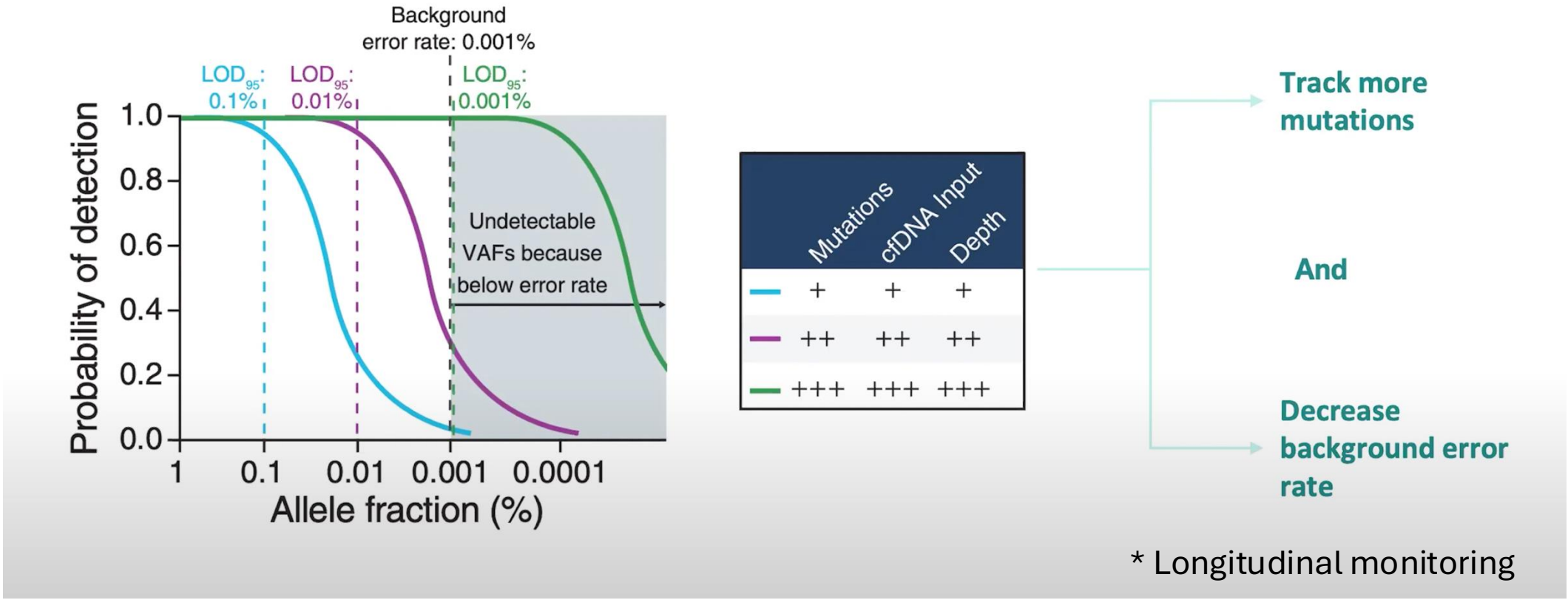
117 patients have been enrolled in Step 0 (ctDNA screening step) and **69 patients have been randomized**



Alliance for Clinical Trials in Oncology

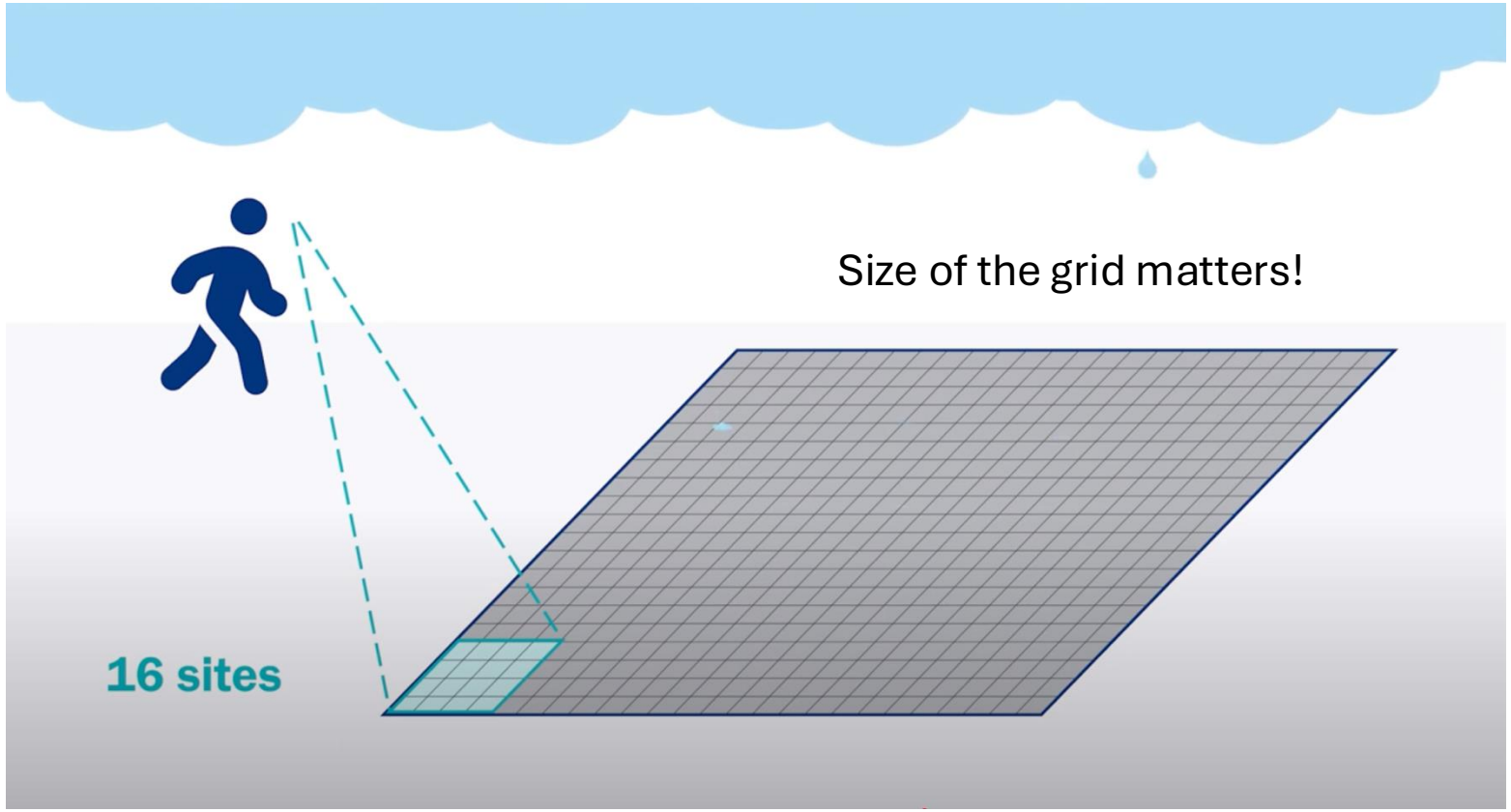
PI: Matt Galsky

How to increase sensitivity of ctDNA MRD



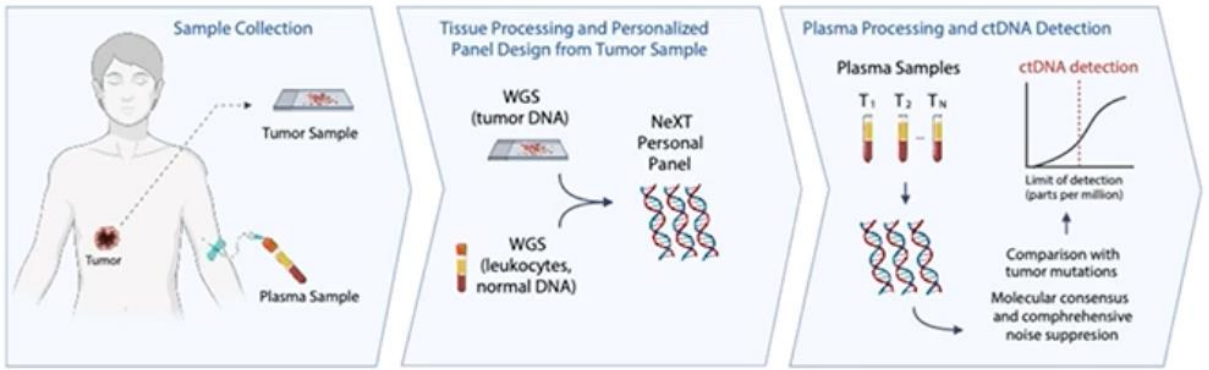
Moding et al. Cancer Discov. 2021

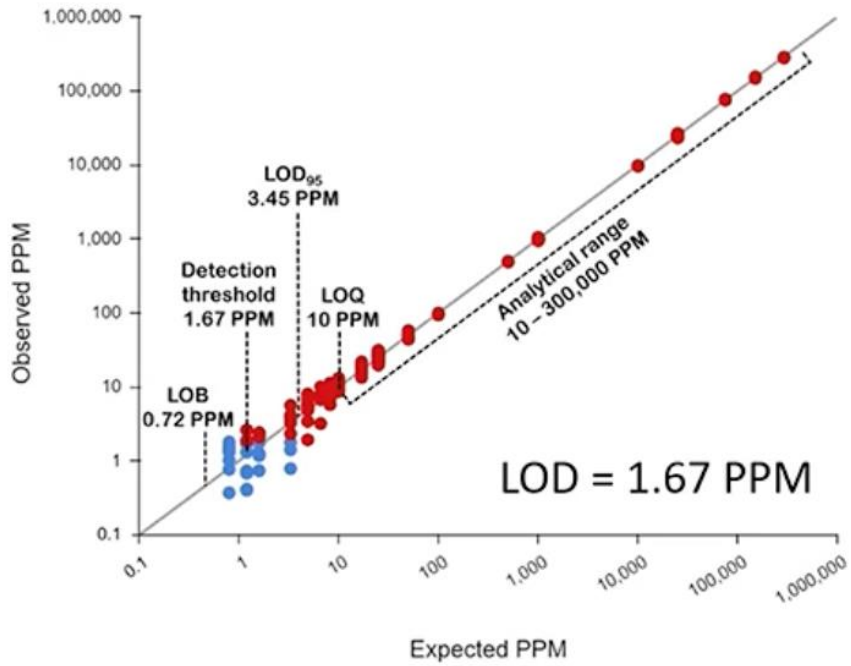
Is it Raining ctDNA?



- Better quantification (more raindrops detected)
- More precise with smaller tumors

Pushing the limit of detection: Increasing # of variants



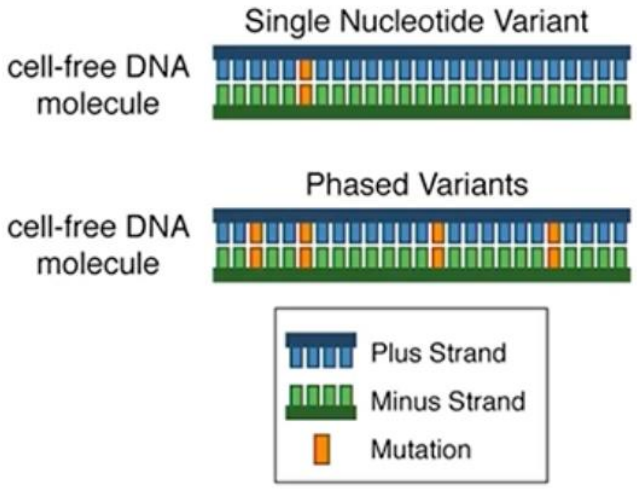


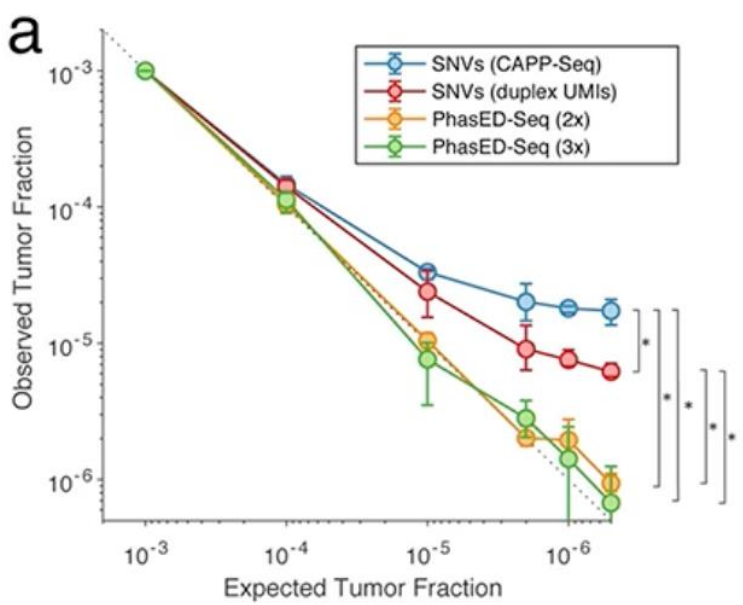
Whole Genome Sequencing up to ~1800 Variants

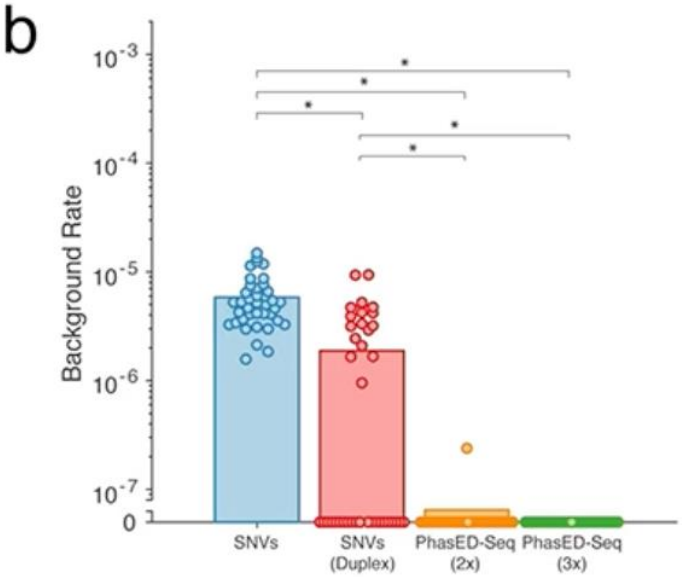
Northcott J et al, *Oncotarget* 2024

Pushing the limit of detection: Reducing background

Phased Variant Enrichment and Detection Sequencing to assess MRD
(**PhasED-Seq)**



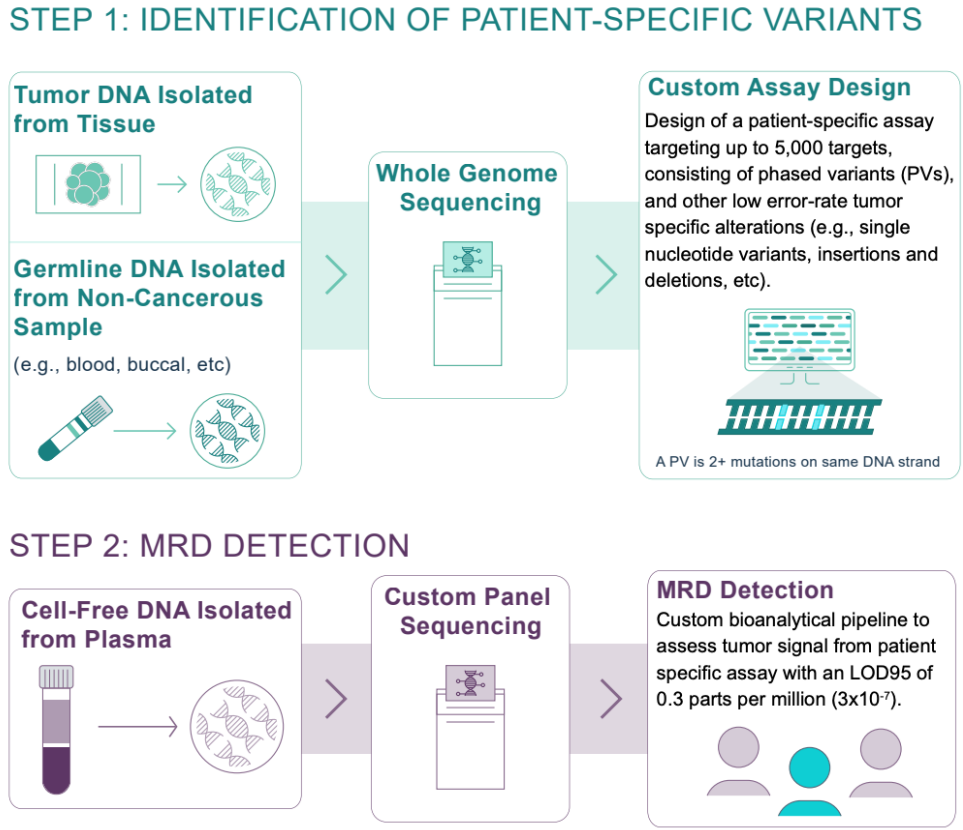




Kurtz DM et al, *Nature Biotechnology* 2021

Phased Variant Enrichment and Detection Sequencing to assess MRD (**PhasED-Seq**)

Figure 1. Overview of MRD Testing Process

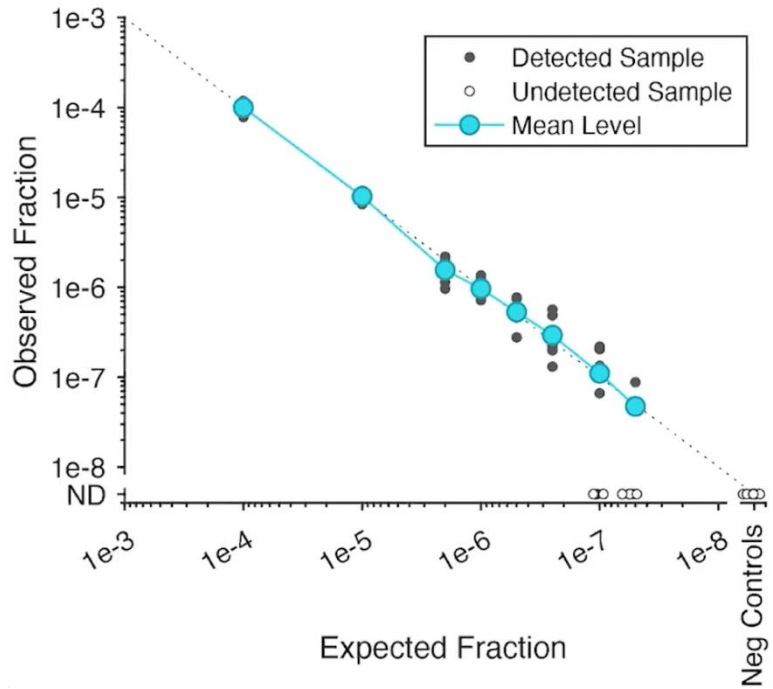


- PhasEd-Seq reduces background errors and enhancing detection sensitivity.
- Studies have demonstrated that PhasED-Seq can detect ctDNA at levels below 1 part per million, outperforming other methods such as CAPP-Seq and duplex sequencing

Cabel et al, ESMO 2024

Foresight CLARITY™ LOD95 in lung and breast cancer, presented at ASCO and ESMO 2024

LOD95 = **0.3 parts per million** (3×10^{-7})

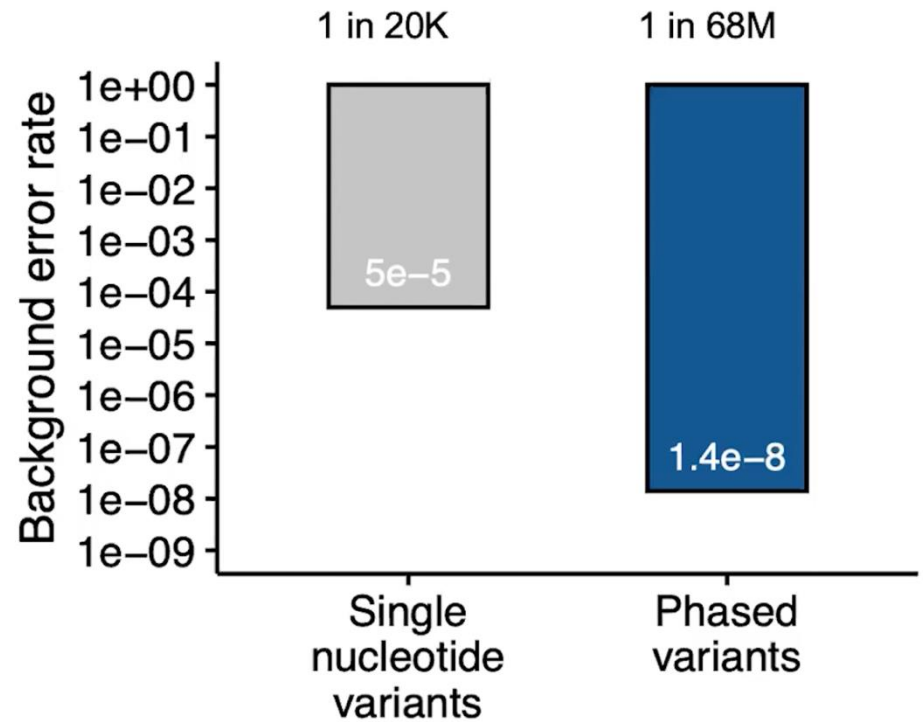


Includes 5,000 patient-specific loci

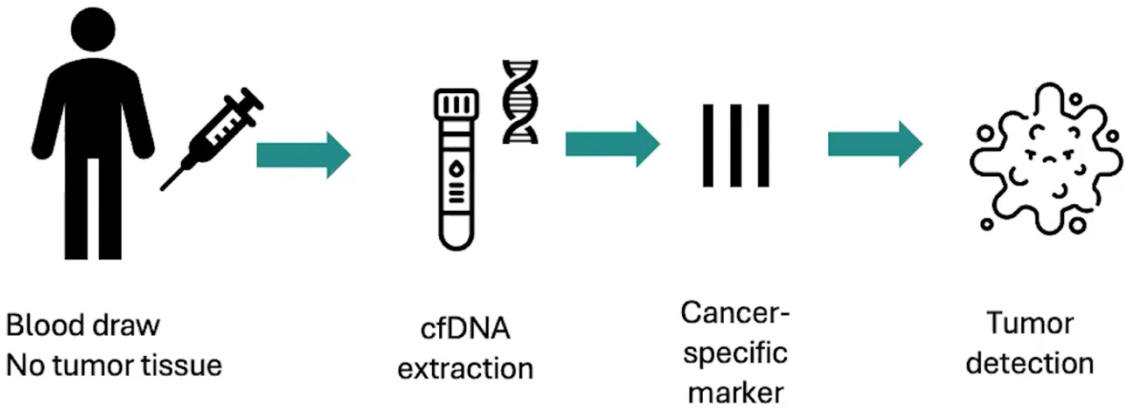
- <u>All</u> phased variants
- Selected additional alterations with very low error rates (e.g., subsets of SNVs, indels, etc) to bring total to 5,000

Cabel et al. *ESMO* 2024

100x improvement in sensitivity vs. SNV assays

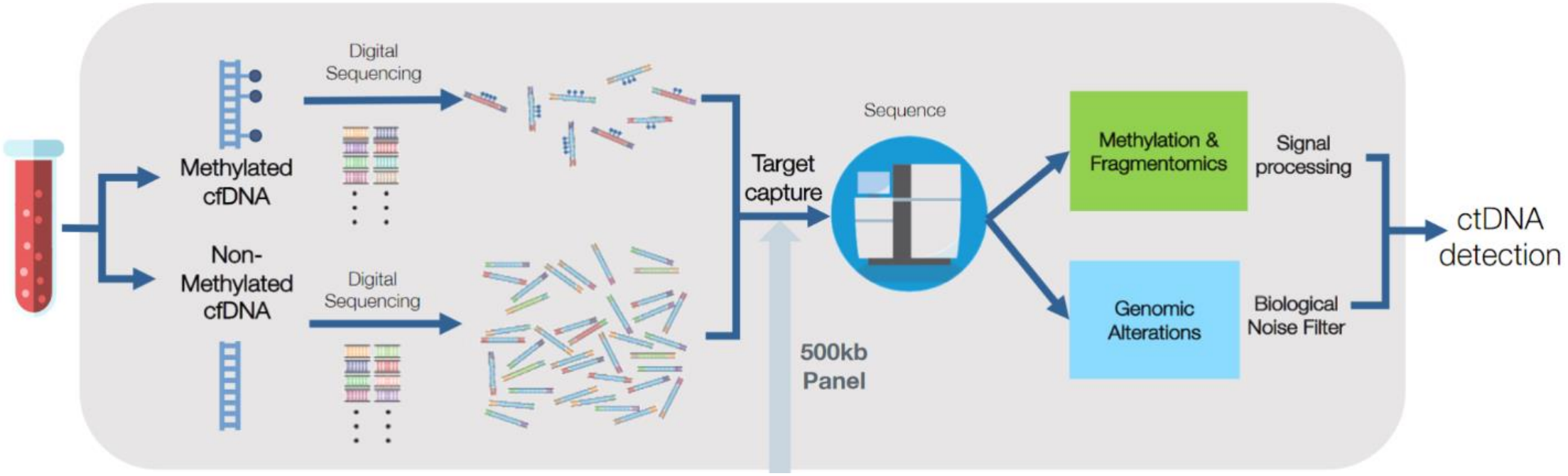


Isbell, et al. AACR 2023



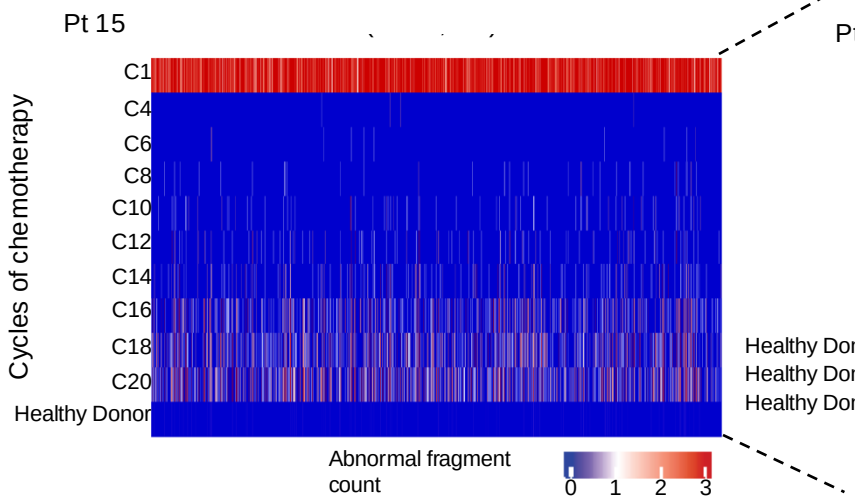
Tumor-Naive ctDNA

Tumor Uninformed (REVEAL)

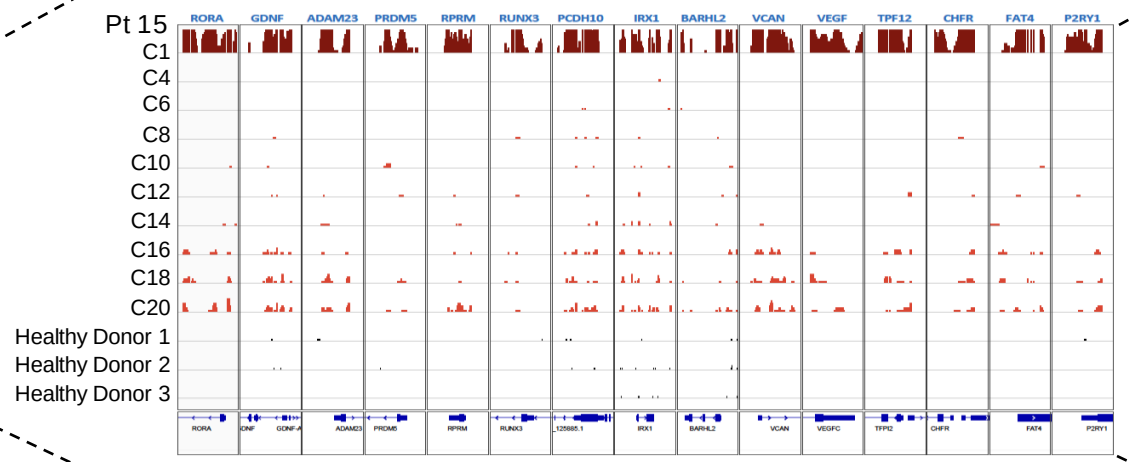


PredicineALERT™ | Methylation-based monitoring in gastric cancer

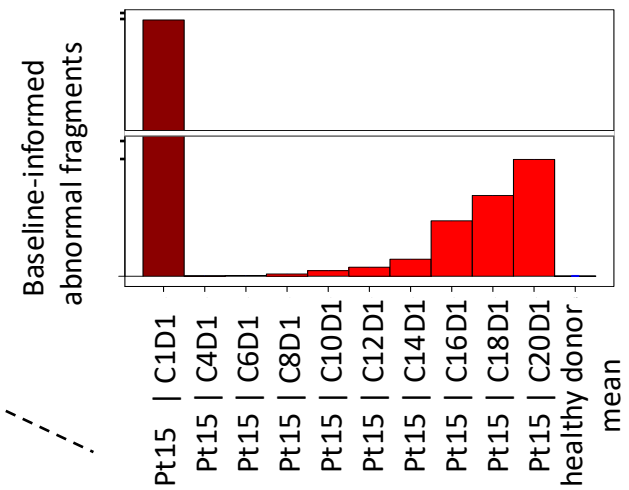
Methylated fragments by count



Methylated fragments by gene



Methylated fragments over time



More focused analysis on regions known to be hypermethylated in gastric cancer (Fig. D) confirmed robust methylation present in ctDNA at those loci at treatment naïve timepoints, their disappearance with therapy and re-emergence prior to radiographic progression consistent with evolution of disease burden with therapy. Representative data is shown for patient 15 (Fig. C and D).

Study Title: Clinical outcomes and ctDNA correlates for CAPOX BETR: A phase II trial of capecitabine, oxaliplatin, bevacizumab, trastuzumab in previously untreated advanced HER2+ gastroesophageal adenocarcinoma

In collaboration with Dana-Farber Cancer Institute

Nature Communications, 2024

BARCELONA 2024 ESMO congress

Circulating Tumour DNA (ctDNA) Clearance With Neoadjuvant Durvalumab (D) + Tremelimumab (T) + Enfortumab Vedotin (EV) for Cisplatin-Ineligible Muscle-Invasive Bladder Cancer (MIBC) From the Safety Run-in Cohort of the Phase 3 VOLGA Trial

Alexandra Drakaki,[1] Thomas Powles,[2] Ying Wang,[3] Manojkumar Bupathi,[4] Monika Joshi,[5] Mark Fleming,[6] Alfonso Gómez de Liaño,[7] Rafael Morales-Barrera,[8] Roberto Pili,[9] Suliman Boulos,[10] Yashaswi Shrestha[11]

[1]Division of Hematology/Oncology, David Geffen School of Medicine, Los Angeles, CA, USA; [2]Genitourinary Oncology, Barts Cancer Institute, Queen Mary University of London, London, UK; [3]Oncology Data Science, AstraZeneca, Waltham, MA, USA; [4]Medical Oncology, Rocky Mountain Cancer Centers, Littleton, CO, USA; [5]Department of Medicine/Division of Hematology and Oncology, Penn State Cancer Institute, Hershey, PA, USA; [6]US Oncology Research, Virginia Oncology Associates, Norfolk, VA, USA; [7]Medical Oncology, Complejo Hospitalario Universitario Insular-Materno Infantil, Las Palmas, Spain; [8]Medical Oncology Department, Vall d'Hebron Institute of Oncology and University Hospital, Universitat Autonoma de Barcelona, Barcelona, Spain; [9]Division of Hematology/Oncology, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY, USA; [10]Oncology, AstraZeneca, Cambridge, UK; [11]Translational Medicine, AstraZeneca USA, Gaithersburg, MD, USA

1970MO

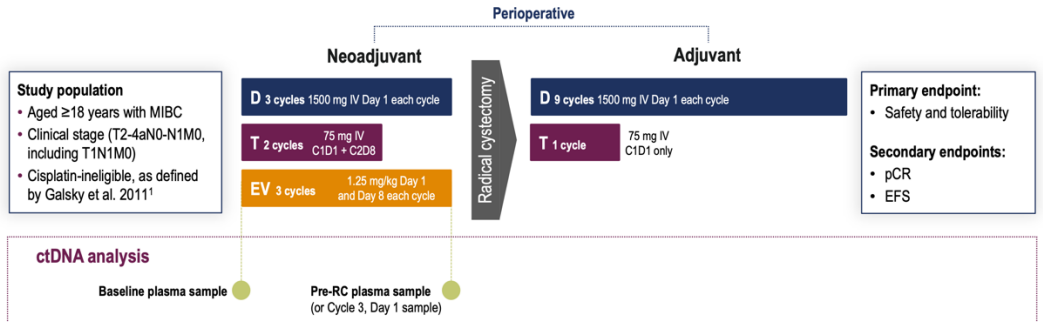
Presenter: Alexandra Drakaki, MD, PhD, UCLA, USA

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Cystectomy	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N
Clinical stage at baseline	T2	**>T2**	T2	T2	T2	T2	T2	T2	T2	T2	T2	**>T2**	T2	**>T2**	T2	T2	**>T2**
Pathological assessment at RC			**pCR**			**Downstaged**				**No change**		**Upstaged**			NA	NA	NA
Baseline ctDNA status	+	+	+	+	+	–	–	–	–	+	+	+	+	+	–	–	NS
Pre-RC ctDNA status	–	–	–	–	–	–	NS	–	–	–	–	+	+	+	–	NS	NS

ctDNA clearance

- At baseline, the overall ctDNA-positive rate was 62.5% (10/16 patients) and the overall ctDNA-negative rate was 37.5% (6/16 patients)
- After neoadjuvant treatment, the pre-RC ctDNA-negative rate was 78.6% (11/14 patients)
- A total of **7 out of 10 patients had ctDNA clearance** (baseline ctDNA positive, then pre-RC ctDNA negative)

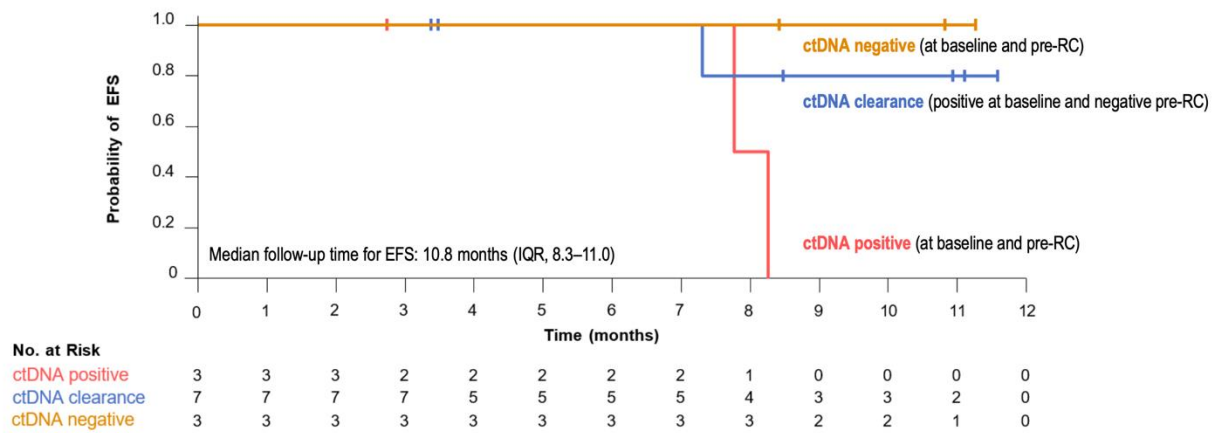
VOLGA safety run-in design and ctDNA analysis



- Cell-free DNA from plasma samples was assessed using GRAIL's Research Use Only (RUO) technology solution which detects methylation indicative of tumour DNA (GRAIL, Inc.)[2]
  - Cancer score cutoff: the exact choice of threshold is governed by the specificity parameter of GRAIL's algorithm; for this analysis, a specificity threshold of 98% was chosen, meaning 98% of non-cancer samples in a held-out calibration set would be correctly identified as negative for ctDNA[2]
- ctDNA detection at baseline and pre-RC were evaluated for association with pathologic assessment at RC and EFS

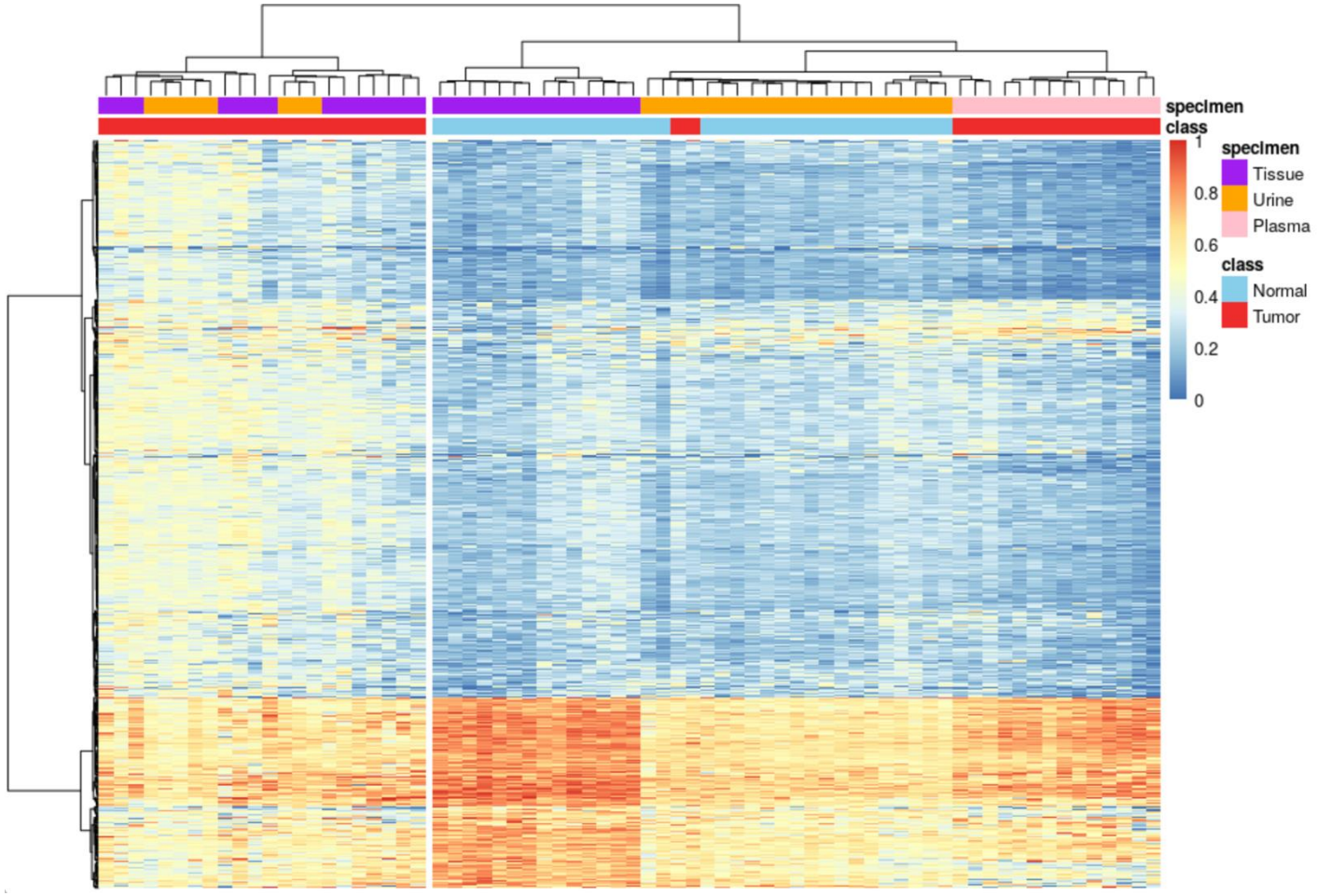
ClinicalTrials.gov, NCT04960709.
ctDNA, circulating tumour DNA; C1D1, Cycle 1 Day 1; C2D8, Cycle 2 Day 8; D, durvalumab; EFS, event-free survival; EV, enfortumab vedotin; IV, intravenous; MIBC, muscle-invasive bladder cancer; pCR, pathologic complete response; RC, radical cystectomy; T, tremelimumab.
1. Galsky MD, et al. Lancet Oncol. 2011;12:211–214. 2 Desai M, et al. American Association for Cancer Research (AACR). 2023: Poster LB297.

ctDNA clearance and its association with EFS

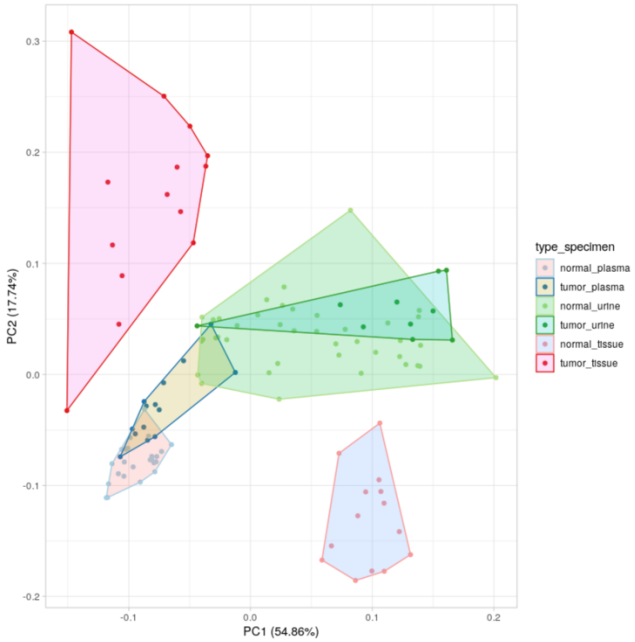


- EFS was assessed in 13 patients who completed RC; 10 were ctDNA-positive at baseline, and 3 were ctDNA-negative at baseline
- **Longer EFS was observed in the ctDNA clearance and ctDNA negative groups compared with the ctDNA positive group**

PredicineEPIC™ | Urine-based DNA methylation profiling for RCC



Tissue-Based DMRs distinguish urine samples from RCC patients and non-malignant donors



Pathological Diagnosis

Urine-based Prediction	Malignancy	non-Malignancy
Malignancy	8	0
non-Malignancy	2	19

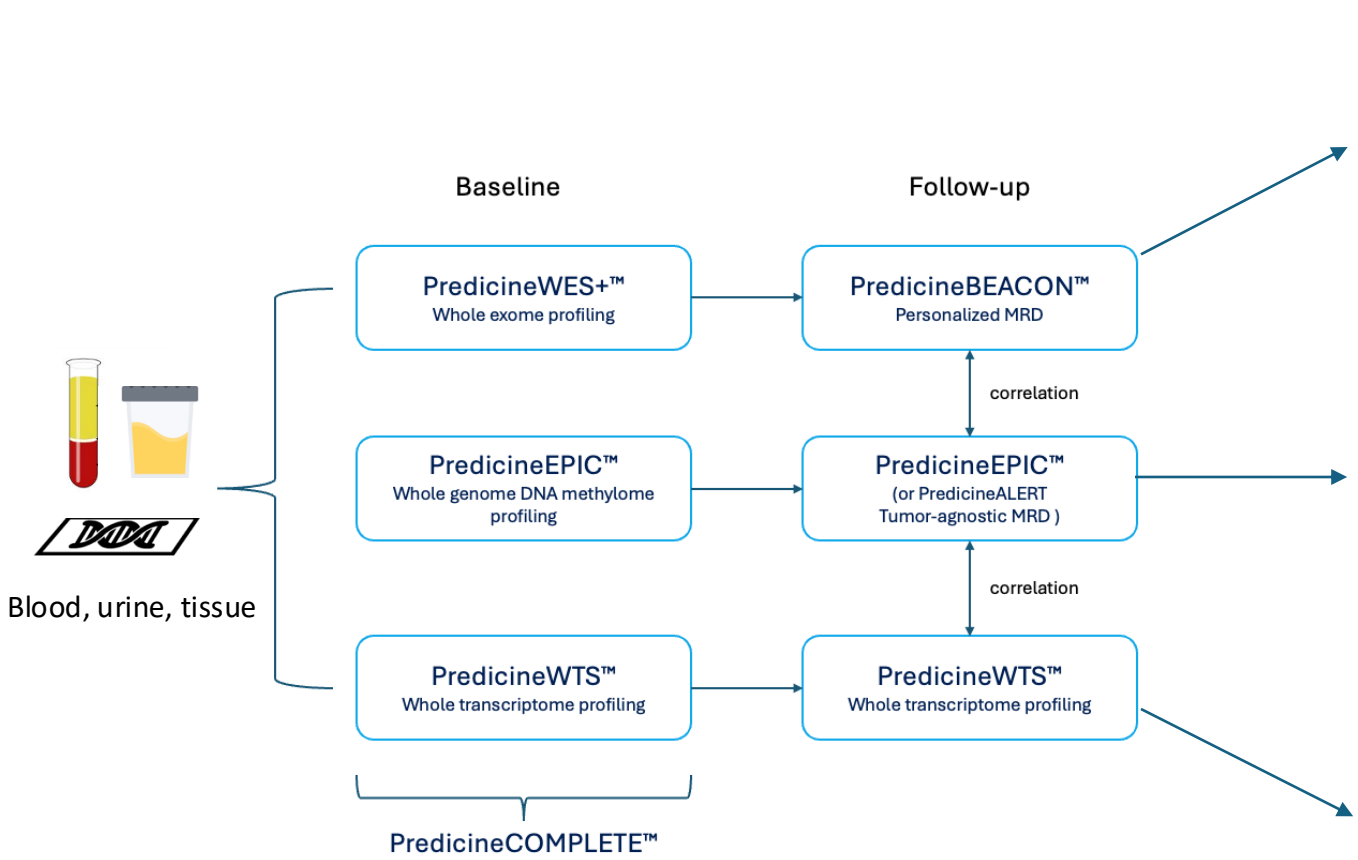
***Sensitivity**: 80%*
***Specificity**: 100%*

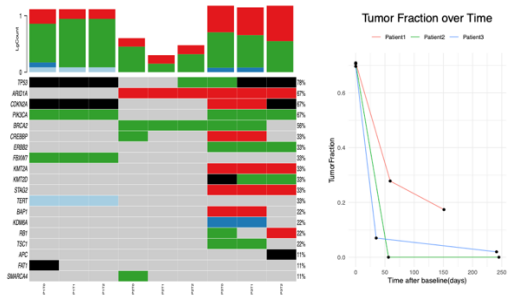
In collaboration with Renji Hospital

PredicineEPIC: genome-wide methylation assay

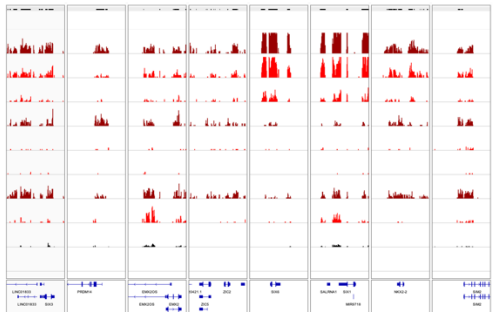
Zhang J et al, AACR 2024

Bladder | Urine-based WES, WTS, methylation: PredicineCOMPLETE

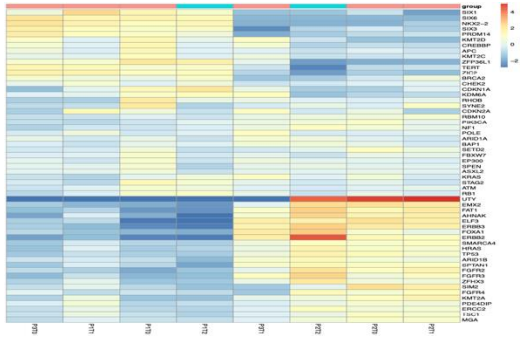




Tumor fraction
ctDNA dynamics



DNA methylation

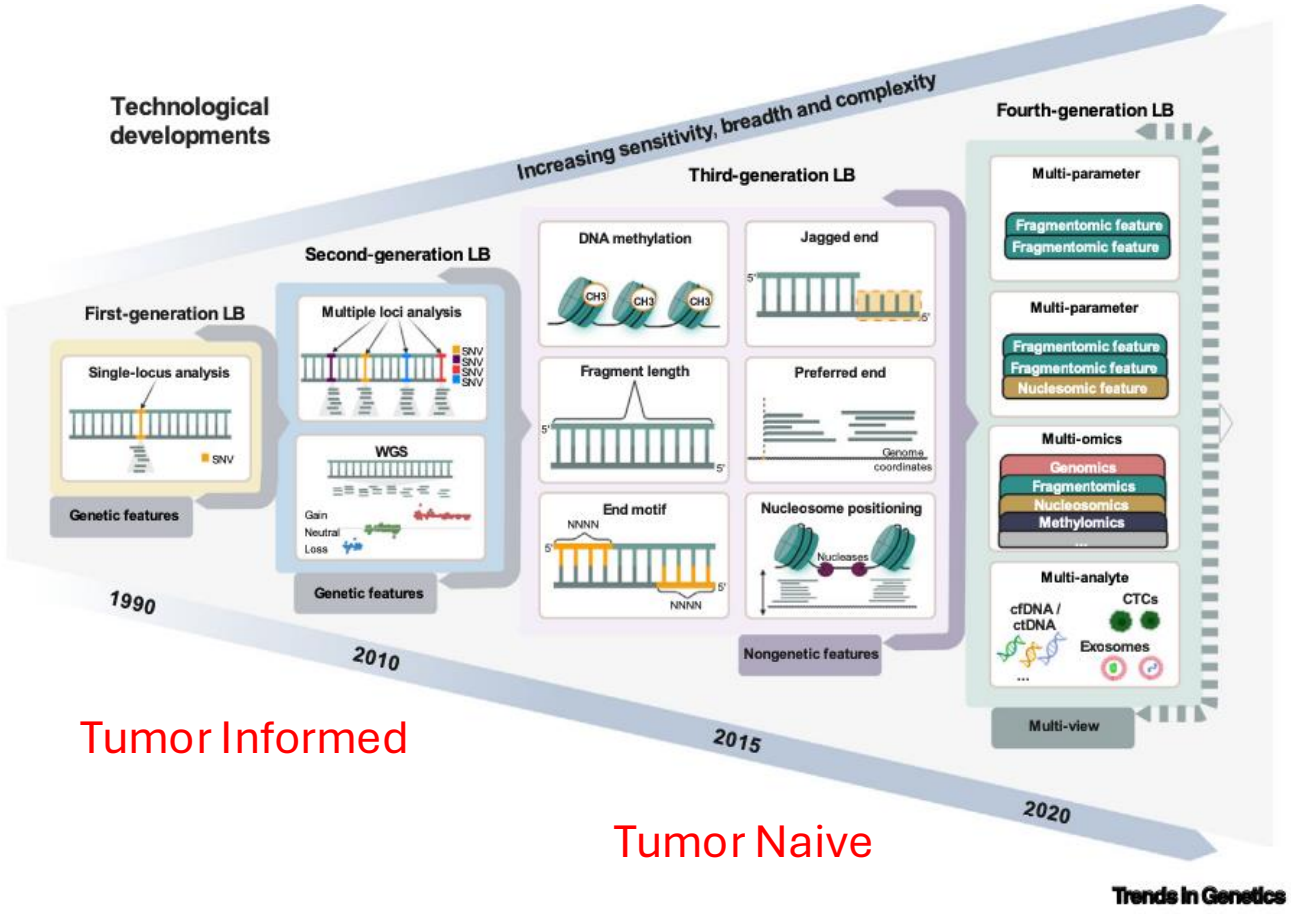


Gene expression

PredicineCOMPLETE: WES+, WTS, Methylation

Tan A, et al. **ASCO-GU**, 2025

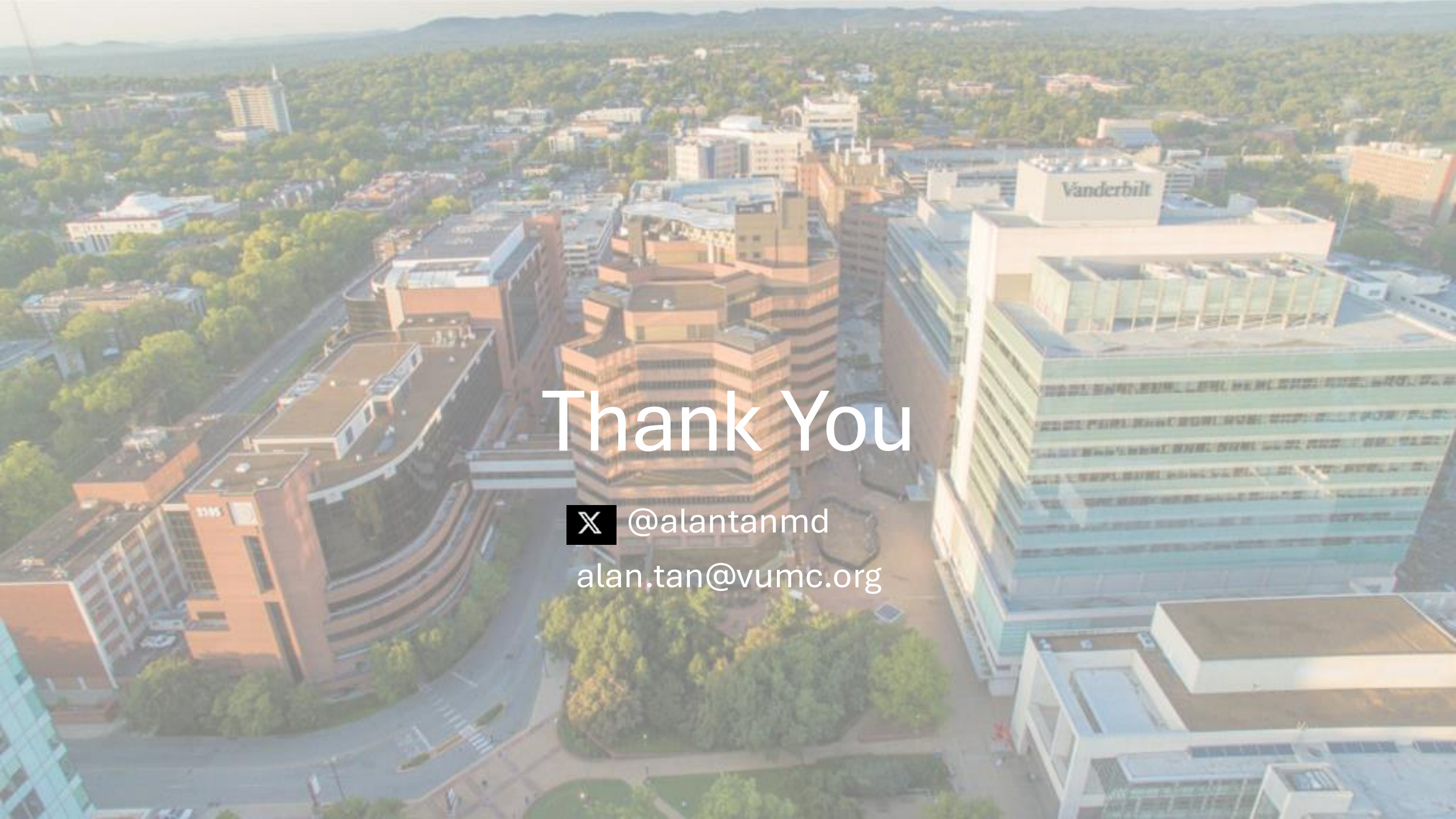
Next Generation MRD with Machine Learning



T. Moseer et al., Trends in Genetics, 2023

Conclusions

- Tumor informed have best sensitivity and optimal for detecting MRD at very low levels, < 1 PPM
- Next generation tumor informed tests increase sensitivity by tracking more variants (up to 5000) and filtering background
- Tumor naïve assays have rapid turnaround and capture tumor evolution, but are currently less sensitive. Methylation, Fragmentomics, and scanning entire genome poised to improve sensitivity and specificity.



Thank You

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