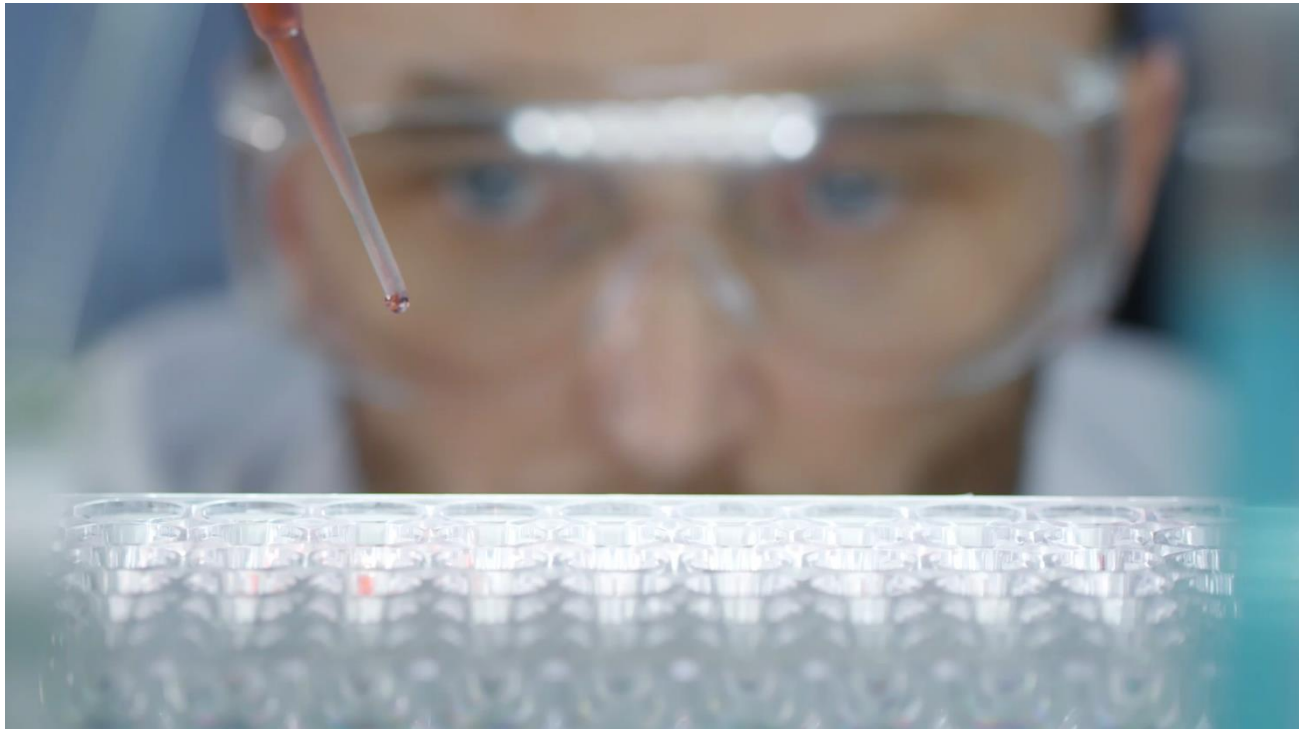
NGS Molecular Testing in Blood vs Tissue?

How About in Other Biospecimens?



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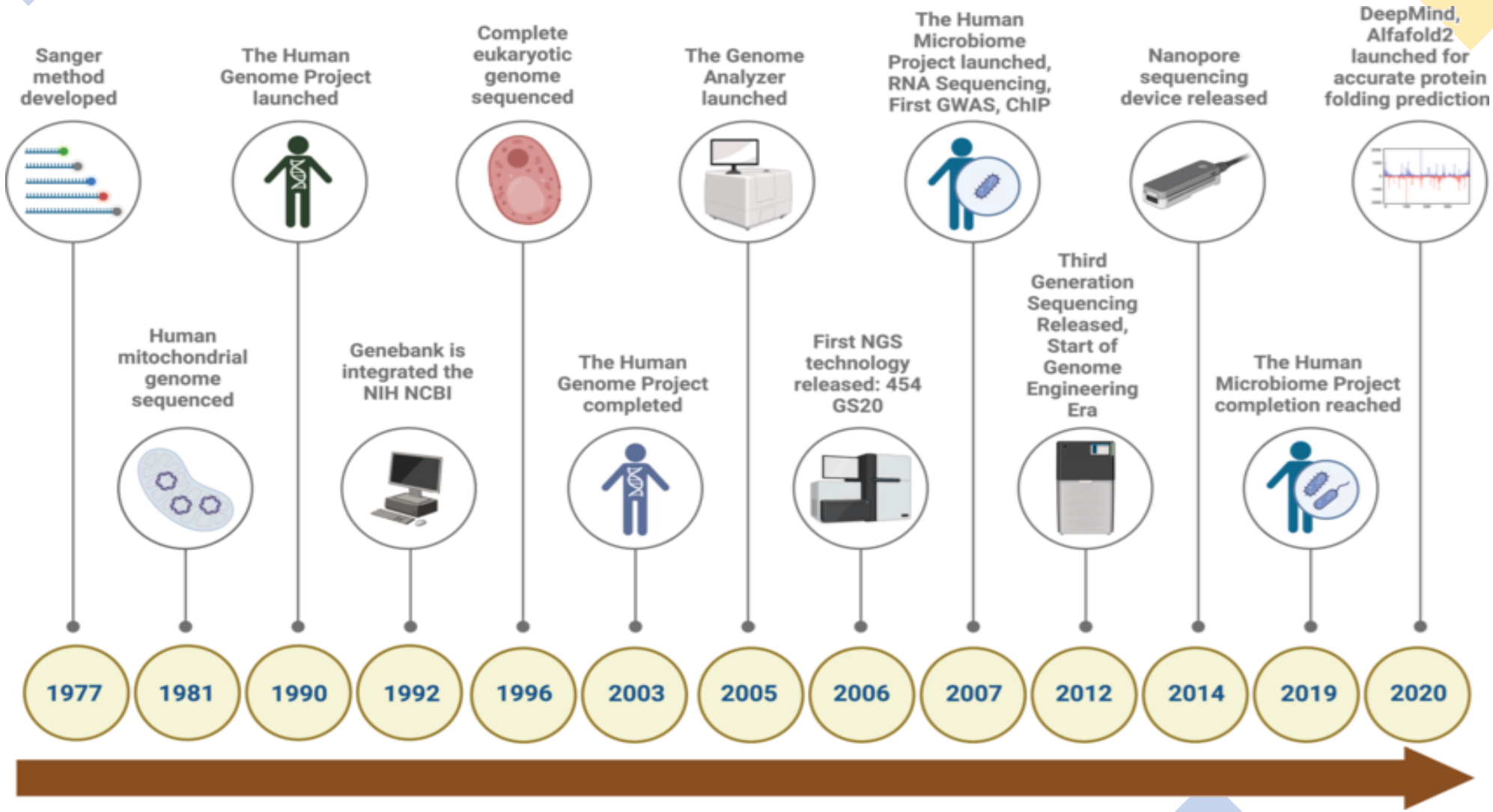
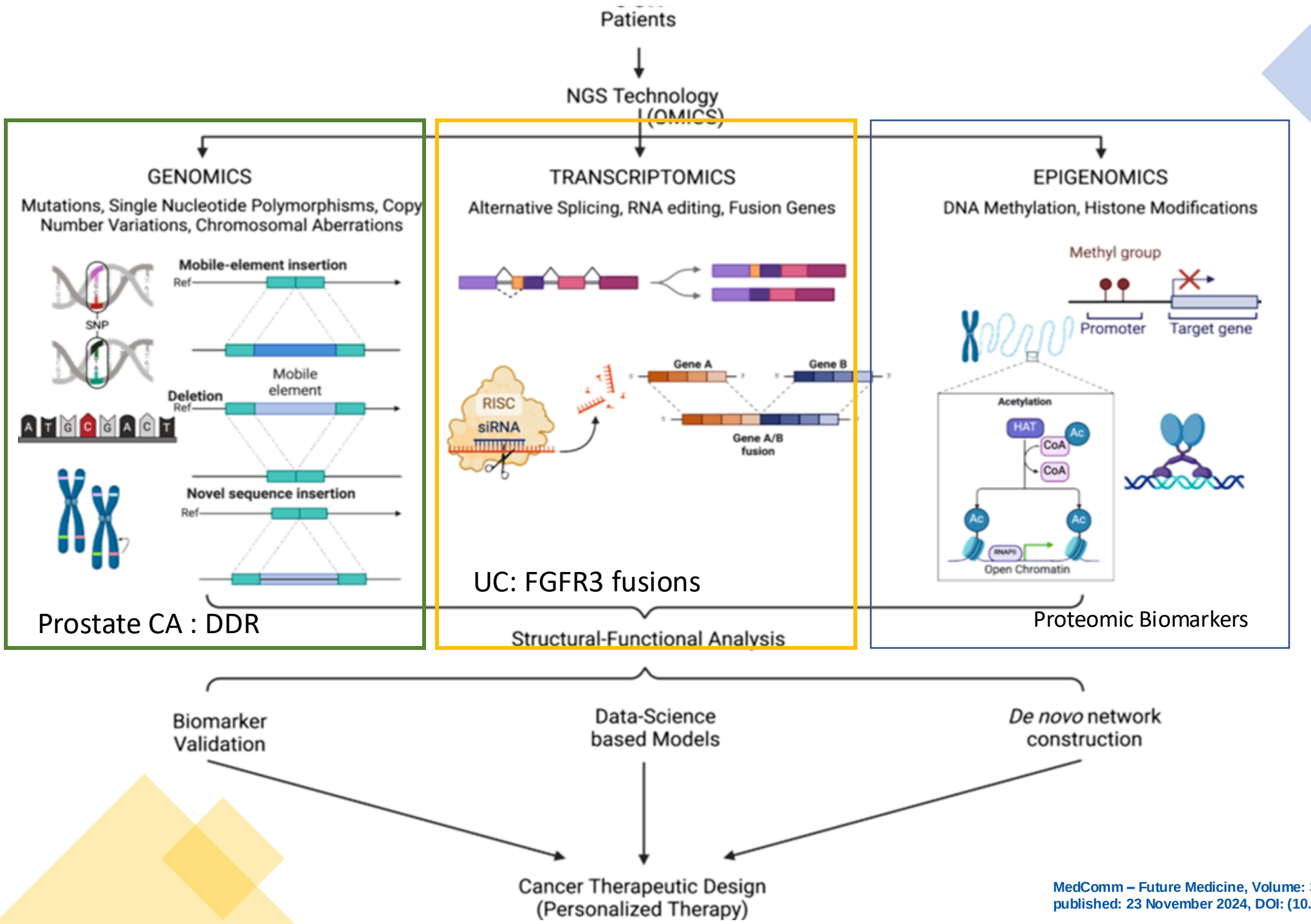
Technological Path – Genomic Sequencing





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Homologous Recombination Repair (HRR) mutation concordance between liquid biopsy (LB) and tumor tissue by NGS in a real-world prostate cancer (PC) database

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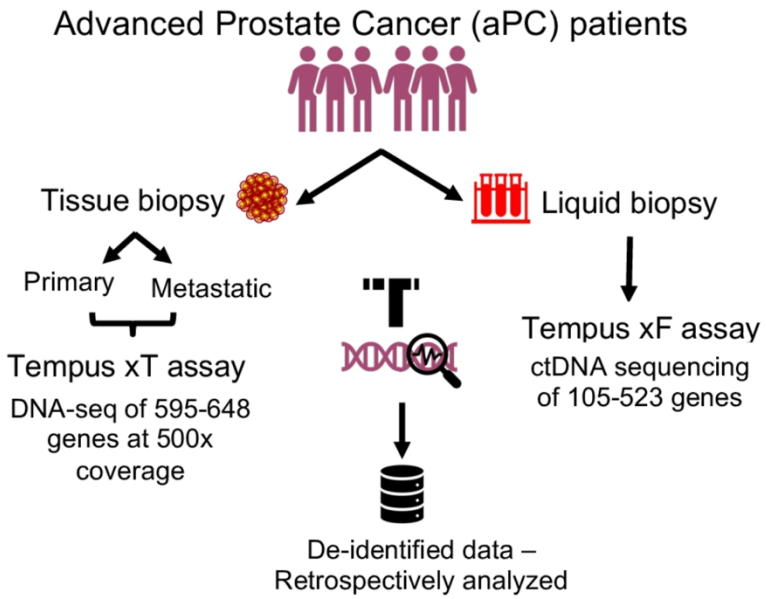
ASCO Genitourinary Cancers Symposium



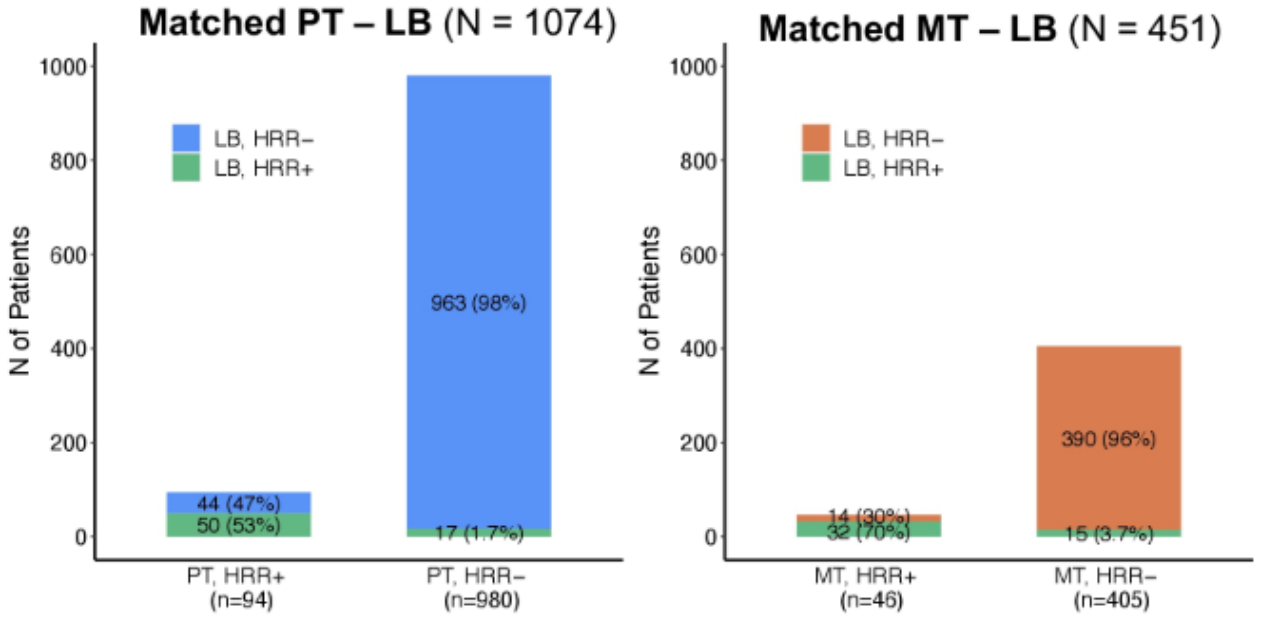


Figure and Table 2 – Agreement of HRR detection between tissue and liquid

- >1000 pt RW cohort examined by the Tempus platform (xT and xF)
- HRR+ was identified in 8.8% of primary tissue and 10% of metastatic tissue samples (in Tissue).
- Liquid biopsy detected **53% of HRR defects** identified via tissue analysis in **primary** tumors
- Liquid biopsy detected **70% of HRR defects** identified via tissue analysis in **metastatic** tumors

SCRUM-MONSTAR-Screen

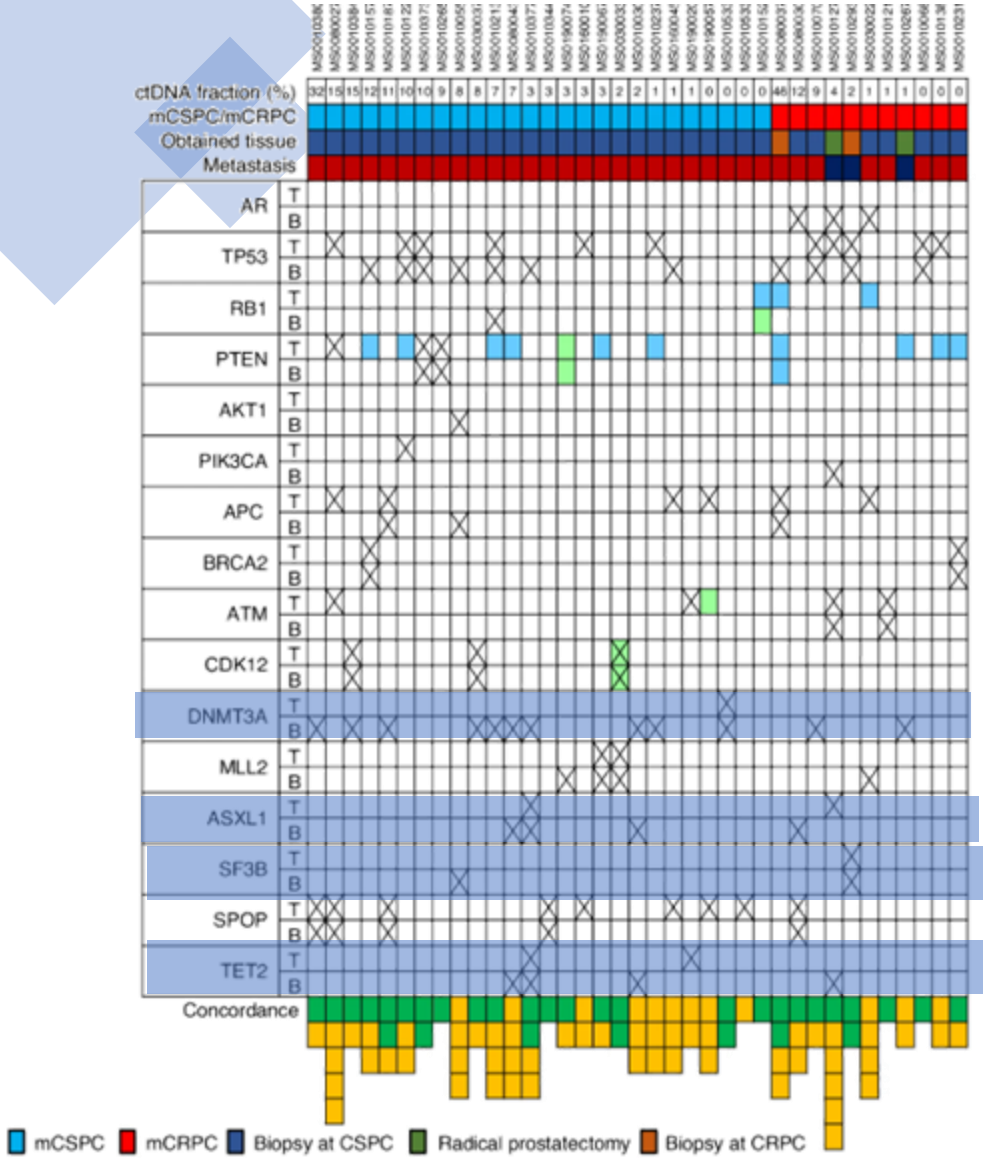
- Nationwide study involving core cancer institutions in Japan investigating ctDNA genomic profiling and gut microbiome

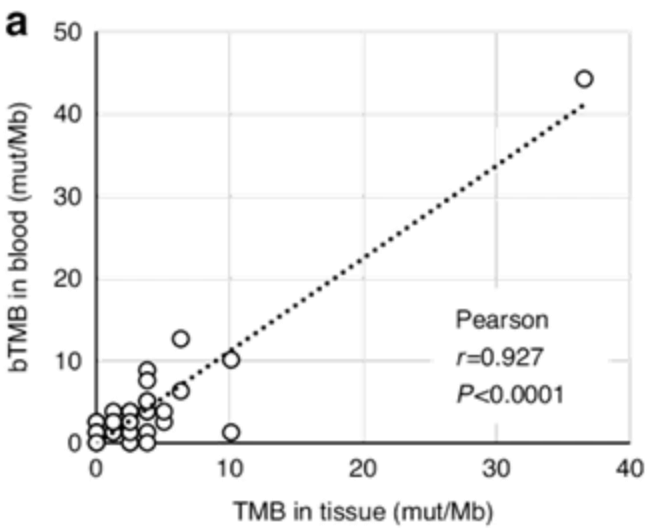
- Institutions involved were MONSTAR-Urology subgroup
  - National Cancer Center Hospital East, Osaka University Hospital, Kyushu University Hospital, Hokkaido University Hospital, Keio University Hospital, Saitama Medical University International Medical Center

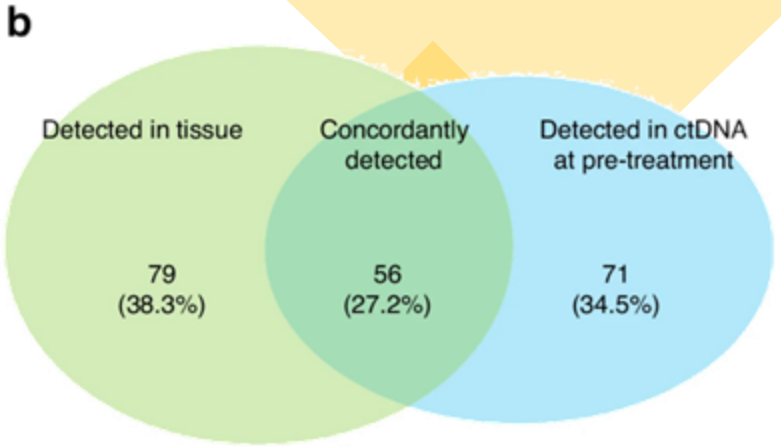
- Key Inclusion criteria
  - Histopathologically confirmed unresectable or metastatic solid cancer,
  - Receipt of or planned following systemic therapy
    - (cohort A) 1st line treatment
    - (cohort B) treatment after pre-defined genomic alterations were identified
    - (cohort C) immune checkpoint inhibitors,
    - **(cohort D) pre-defined androgen receptor pathway inhibitors (ARPI) including abiraterone and enzalutamide**

- **Testing used :** Blood sampling was performed before corresponding treatment including ADT in mCSPC (pre-treatment) and after progression (post-treatment) NGS analysis of ctDNA was performed using FoundationOne Liquid®CDx (F1LCDx®), tissue testing used FoundationOne®CDx

Shiota et al, BJC reports 2024







TMB in tissue and bTMB in ctDNA showed high correlation ($r = 0.927$, $P < 0.0001$)

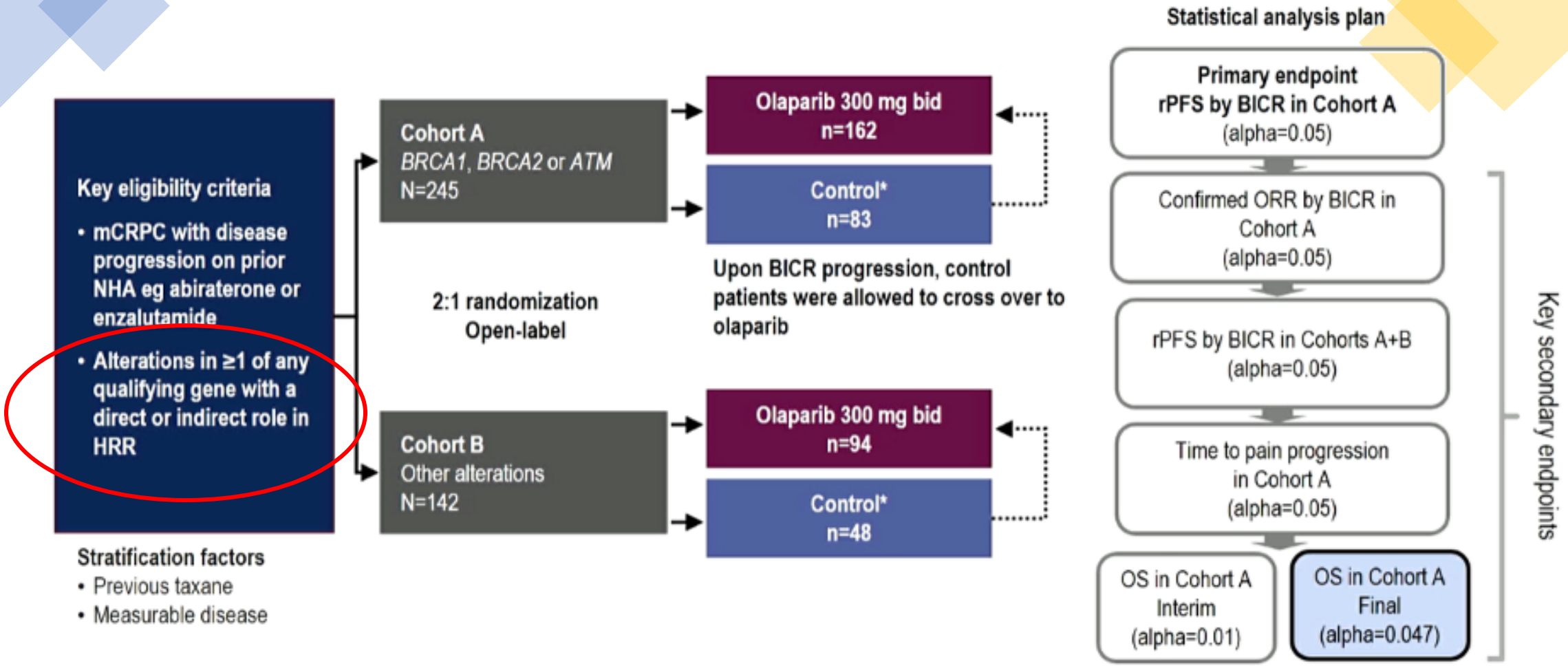
56 (**27.2%) of gene alterations were concordantly observed** between tissue and ctDNA.

***AR* alterations were detected only in ctDNA from patients with mCRPC,** whereas there was no *AR* alteration in tissue, which was mostly obtained before hormonal therapy.

But concordance doesn't tell the whole story. **Sensitivities** of ctDNA testing in mCSPC and mCRPC **were 43.8% and 35.9%,** respectively

Shiota et al, BJC reports 2024

PROfound- Olaparib in mCRPC



Patients randomized between April 2017 and November 2018; DCO for final OS: 20 March 2020

*Control either enzalutamide (160 mg qd) or abiraterone (1000 mg qd + prednisone [5 mg bid]).
BICR, blinded independent central review; bid, twice daily; DCO, data cut-off; ORR, objective response rate; qd, once daily; RECIST, Response Evaluation Criteria In Solid Tumours.

Hussain et al, SUO 2020

PROfound: Screening

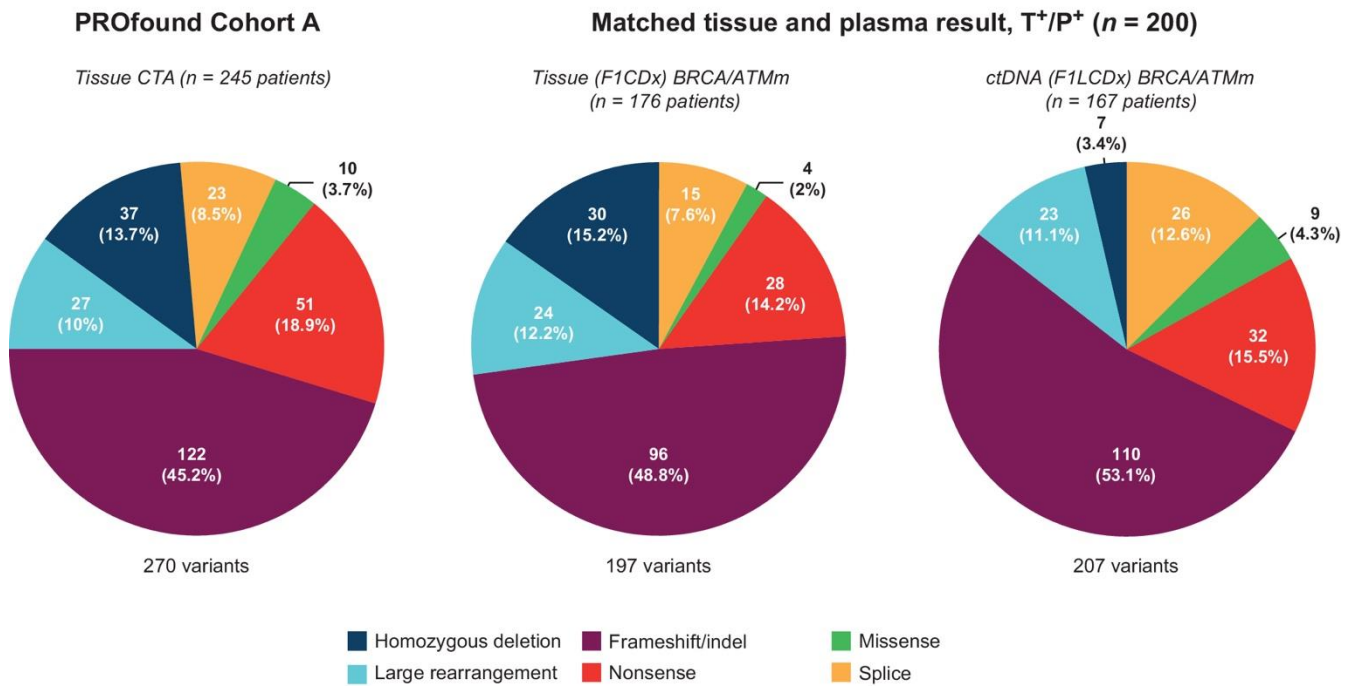
- Allocation to Cohort A or B based on prospective tumor tissue molecular profiling at by FMI CTA or FoundationOneCDx
- At screening, matched plasma samples were also collected for plasma-derived ctDNA and retrospectively sequenced -> FoundationOne®Liquid CDx assay
- The subset population of patients screened in PROfound with ctDNA testing performed -> 619 patients in total: 229 patients + and 390 -ve

Chi et al Clin Cancer Res 2023

Chi et al Clin Cancer Res 2023

Tissue BRCA/ATM mutation detected (T^+)	Tissue BRCA/ATM mutation not detected (T^-)	Total	
Plasma (ctDNA) BRCA/ATM mut detected (P^+)	143 (**81%;** T^+/P^+)	24 (8%; T^-/P^+)	167
Plasma (ctDNA) BRCA/ATM mut not detected (P^-)	33 (**19%;** T^+/P^-)	291 (92%; T^-/P^-)	324
Total	176	315	491
	T^+/P^+: 81% (95% CI, 75–87)	T^-/P^-: 92% (95% CI, 89–95)	PPV = 0.68 NPV = 0.96

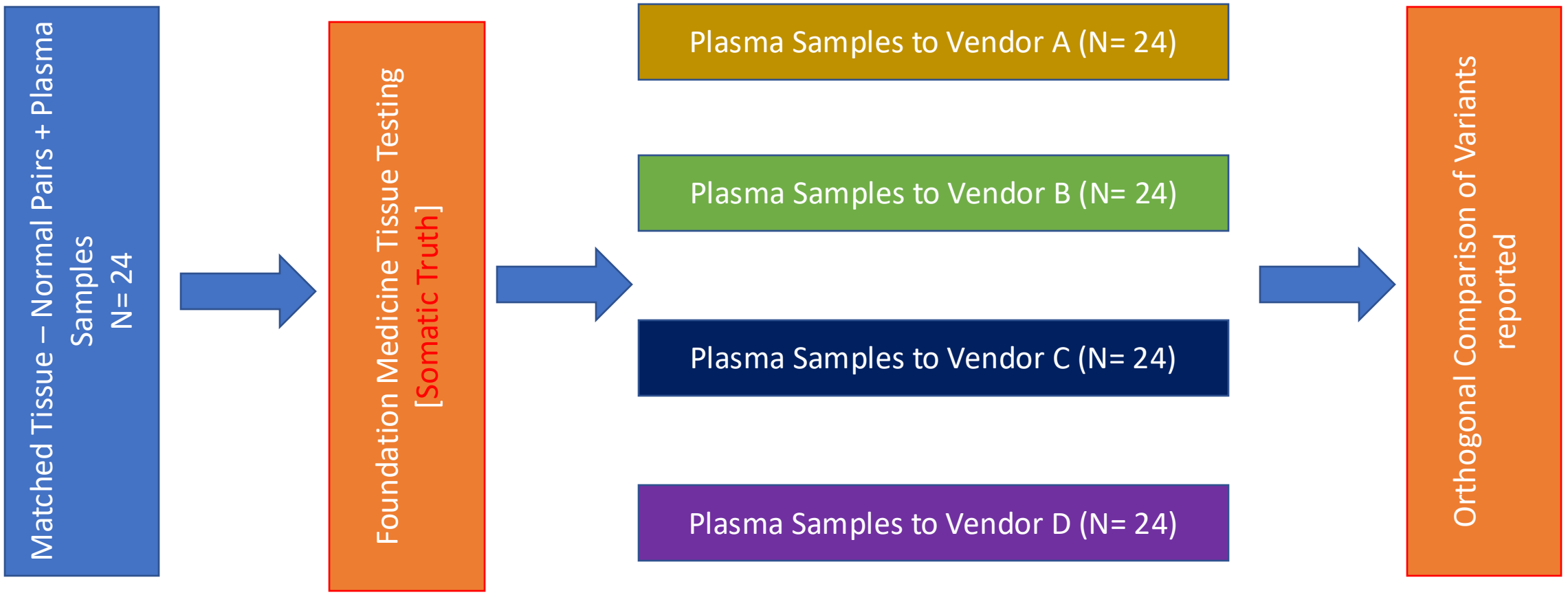
503 primary tumor samples, 93 soft tissue metastasis, 19 bone metastasis.



81% of BRCA/ATM mutations in tumor tissue were detected by liquid biopsy. While 8 % of Liquid biopsy mutations were not present in tumor tissue.

Homozygous deletions were considerably underrepresented in liquid biopsies, but other alteration types appeared balanced.

Does the assay matter?



Stetson et al . JCO PO, 2023

Orthogonal Comparison of Four Plasma NGS Tests With Tumor Suggests Technical Factors are a Major Source of Assay Discordance

Daniel Stetson, MS[1]; Ambar Ahmed, MS[1]; Xing Xu, PhD[2]; Barrett R.B. Nuttall, MS[1]; Tristan J. Lubinski, PhD[1]; Justin H. Johnson[1]; J. Carl Barrett, PhD[1]; and Brian A. Dougherty, PhD[1]

- variant calls were grouped into true positive (TP), FN, and FP calls on the basis of the comparative analysis
- Manual inspection of raw data was performed for the union of all plasma report variant calls
- Each variant call was also compared with germline and tumor variant databases (eg, Exome Aggregation Consortium, Catalogue of Somatic Mutations in Cancer, The Cancer Genome Atlas)

FIG 1. Variant concordance plot. Concordant true-positive variants (light blue) and discordant variants, both false positive (red) and false negative (teal), are plotted by the log of the allele fraction (AF). The red dotted line represents 1% AF. AF is approximated for false-negative calls by averaging the corresponding true-positive calls for that variant.

Stetson et al . JCO PO, 2023

TABLE 2. Sensitivity and Positive Predictive Value of All Variants

Vendor	TP	FP	FN	Sensitivity* (%)	PPV† (%)
A	6	5	10	38	55
B	8	2	3	73	80
C	17	10	2	89	63
D	13	23	6	68	36

Abbreviations: FN, false negative; FP, false positive; PPV, positive predictive value; TP, true positive.

*Sensitivity was calculated by dividing TP calls by the sum of TP and FN calls.

†Positive predictive value was calculated by dividing TP calls by the sum of TP and FP calls.

FN variant calls, were examined by comparing raw aligned data from tumor, normal, and plasma samples. Factors that caused this included high signal to noise ratio and bioinformatic calling thresholds for the various assays.

FP were due to nonspecific variant calling and sequencing noise at nonreference bases. Mutational biases were present in specific vendors (e.g C>T & G>A). Several FP variants were completely novel alterations not identified in established databases.

Majority of variation due to technical factors, but included variation due to CHIP and tumor heterogeneity

Stetson et al . JCO PO, 2023

Concordance Analysis of Tissue and ctDNA in RCC: Insights from a Multimodal Real-World Database

Chinmay Jani, MD
Clinical Fellow, Jackson Health System / University of Miami, Sylvester Comprehensive Cancer Center

- Retrospective analysis of NGS data from patients with both tissue and ctDNA testing.
- Tempus multimodal database was used (xF, xT)
- Concordance analysis restricted to 105 genes (xF), pathogenic somatic short variants and CNVs.

Results

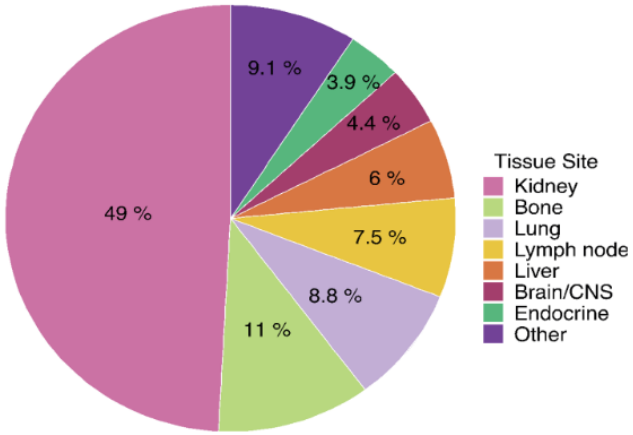


Figure 1: Breakdown of tissue biopsy site

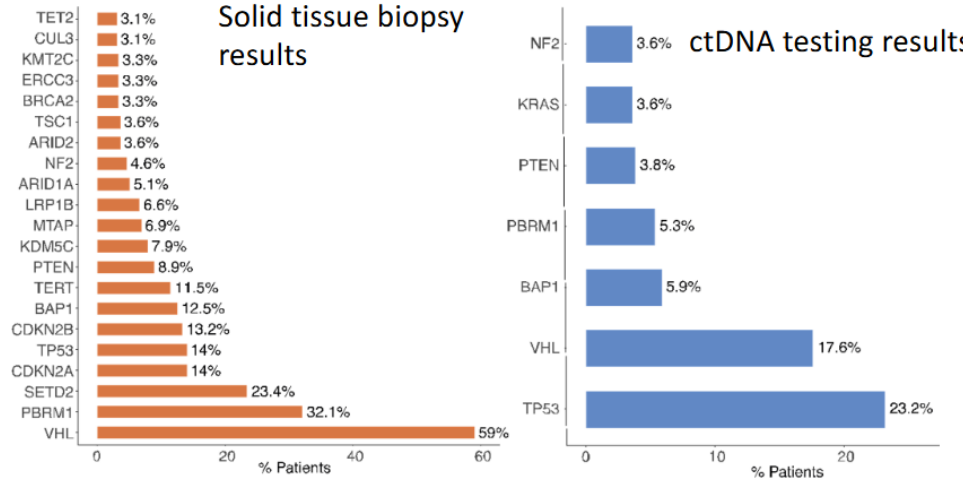


Figure 2: Prevalence of molecular alterations detected according to solid-tissue and ctDNA testing.

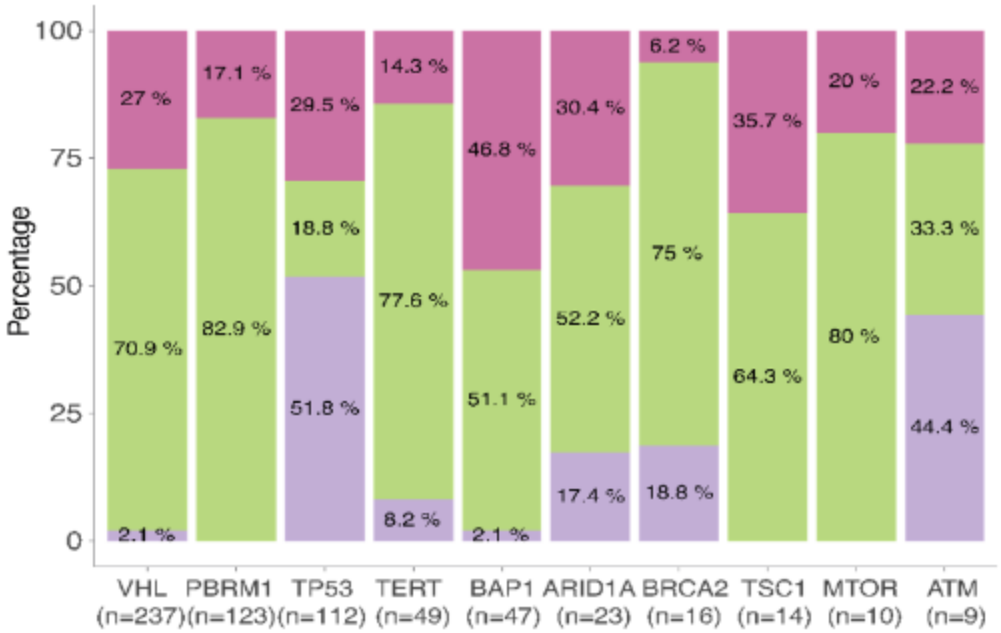


Figure 3: Breakdown of alterations according to assay detection type, including assay unique alterations and those detected by both assays.

Solid tissue and cfDNA
Solid tissue only
cfDNA only

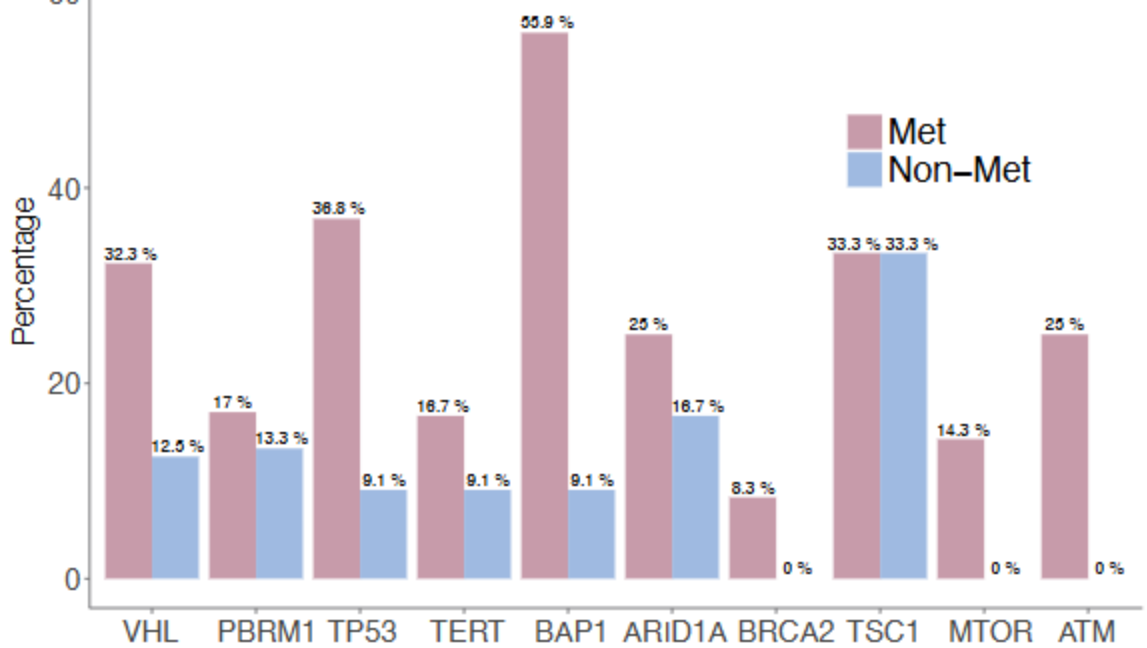


Figure 4: Concordant alterations identified in solid tissue and ctDNA (stratified based on metastasis)

Variations were noted in sensitivity by gene and primary vs met tumor. A high number of TP53 mutations were in cfDNA only, while a high number of VHL/PBRM1 were tissue only.

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-020-20493-6 OPEN

Plasma ctDNA is a tumor tissue surrogate and enables clinical-genomic stratification of metastatic bladder cancer

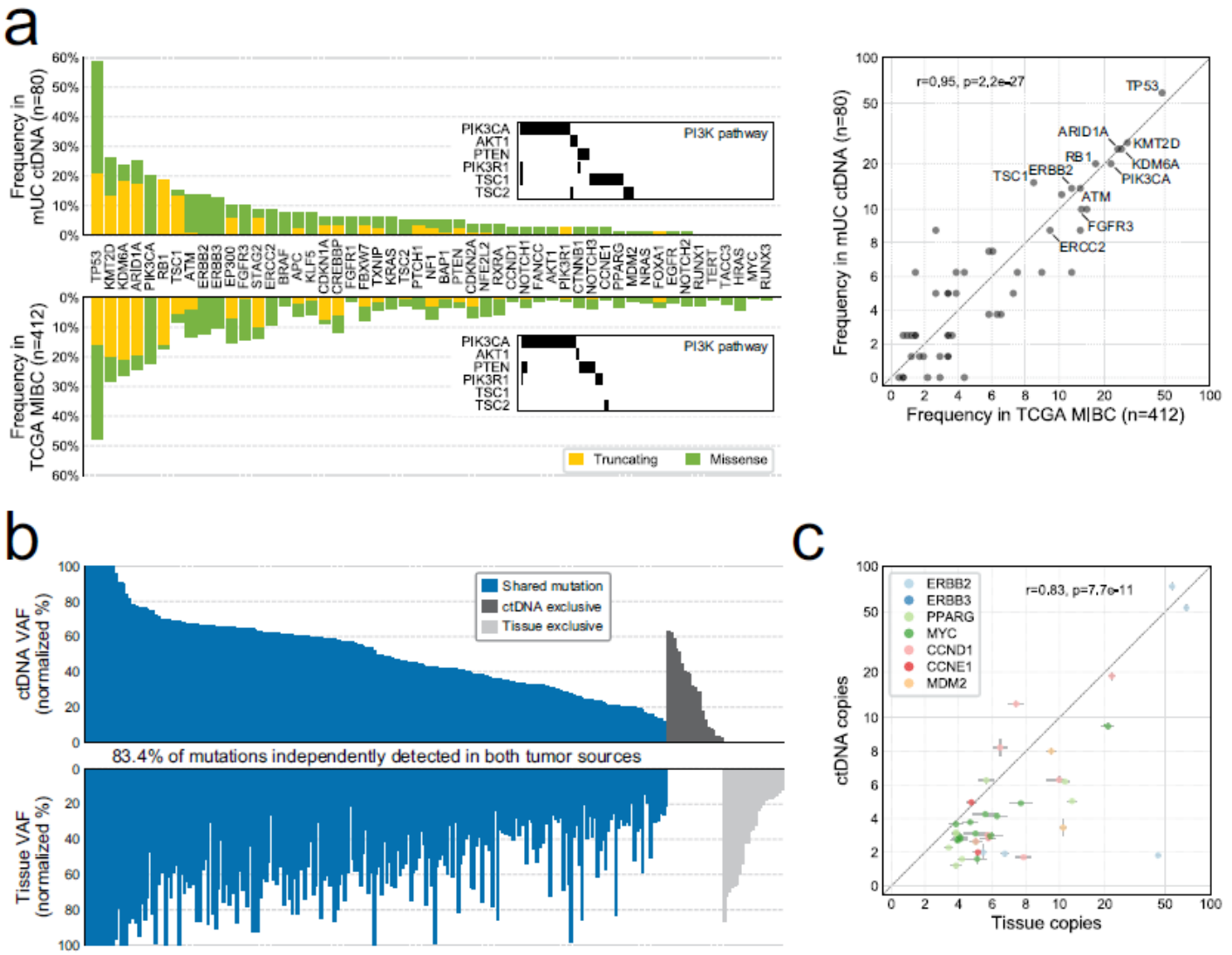
Gillian Vandekerkhove[1], Jean-Michel Lavoie[2], Matti Annala[1,3], Andrew J. Murtha[1], Nora Sundahl[4], Simon Walz[5], Takeshi Sano[1], Sinja Taavitsainen[3], Elie Ritch[1], Ladan Fazli[1], Antonio Hurtado-Coll[1], Gang Wang[6], Matti Nykter[3], Peter C. Black[1], Tilman Todenhöfer[7,8], Piet Ost[4], Ewan A. Gibb[9], Kim N. Chi[1,2], Bernhard J. Eigl[2,10✉] & Alexander W. Wyatt[1,10✉]

N=104 patients during treatment for mUC , 63 pts had cfDNA as well as tissue.

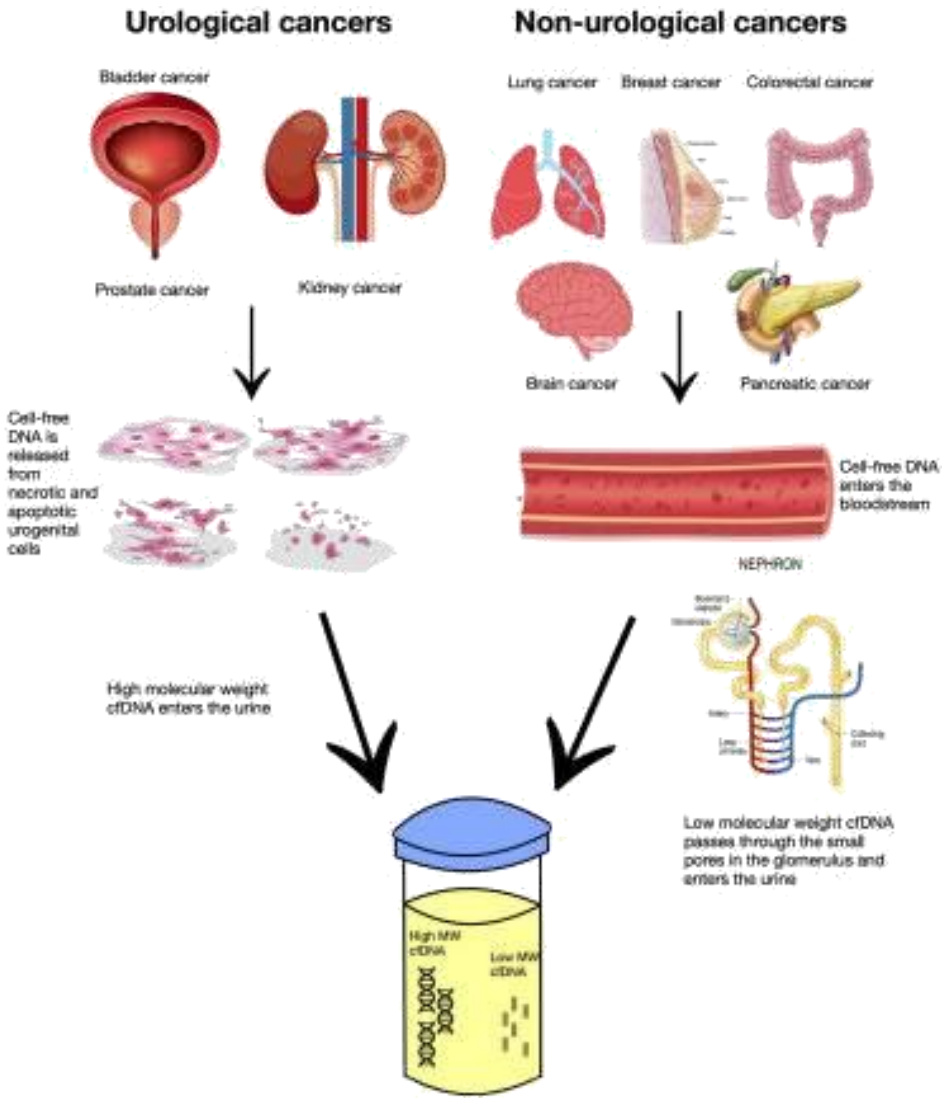
Plasma cfDNA subjected to targeted sequencing with custom gene panel (~60 genes)

85% of patients had VAF >1% in atleast 1 sample

cfDNA mutation patterns mimicked TCGA data (a) and (b) tissue based VAFs correlated strongly (84% overlap) and across most mutation types (c)

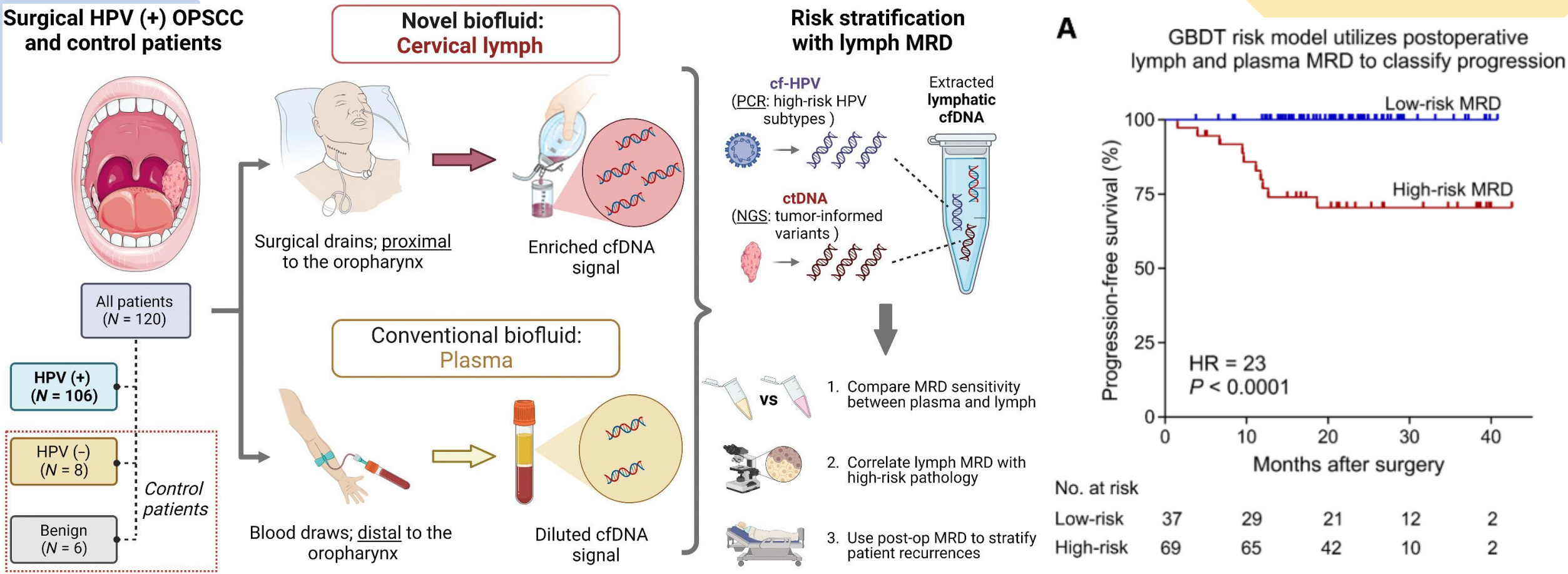


ucfDNA – a novel source?



Salfer et al, Clin Chem, 2023

- Urine contains many compounds such as cells, salts, and cell-free nucleic acids like DNA, mRNA, micro-RNAs (miRNAs), and long non-coding RNAs (lncRNAs)
- HMW cfDNA in urine is likely from urogenital tract, while LMW (<100 bp) cfDNA may accumulate from distal to nephron
- Urine also has higher concentrations of nucleases
- Ideal substrate (supernatant vs pellets) are to be established.
- Emerging data suggests ucfDNA may correlate with response/resistance to therapy for MIBC (Christensen et al)
- UroMark® – a targeted bisulfite NGS assay for urinary supernatant had 98% sensitivity and 97% specificity in MIBC and is being used in a trial (DETECT II) for NMIBC and MIBC recurrence.



Lymphatic fluid represents the preferred metastatic mechanism in a majority of solid tumors. ctDNA from surgical fluid can be used to detect MRD , recurrence risk , and for NGS for hotspot mutations.

Studies planned and ongoing in urothelial , prostate and RCC in addition to other tumor types.

Earland et al, CCR 2023

Summary

NGS techniques are improving and are being leveraged across tissue/liquid platforms.

ctDNA based assays may offer a quicker first pass solution at uncovering actionable mutations.

ctDNA assays still suffer from variability across platforms, susceptibility to errors at low VAFs and challenges with differentiating CHIPs

Urinary and Lymphatic ctDNA are showing promise for the future.