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Epigenomic features of circulating tumour DNA in metastatic prostate cancer

Dr. Alexander W Wyatt, D.Phil
Associate Professor, University of British Columbia
Senior Research Scientist, Vancouver Prostate Centre
Scientist, Canada's Michael Smith Genome Sciences Centre, BC Cancer
Twitter @ResearchWyatt

a place of mind
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Disclosures

- I have served on advisory boards and/or received honoraria from AstraZeneca, Astellas, Bayer, EMD Serono, Janssen, Merck, Pfizer
- My laboratory has contract research agreements with ESSA Pharma

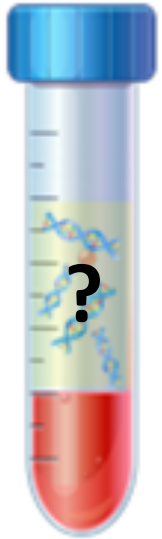
No relationships with genomic testing companies

Presentation overview

- **CtDNA fundamentals and constraints for precision oncology**
- Drivers of cancer evolution in serial ctDNA
- Phenotypic insights in mCRPC via ctDNA

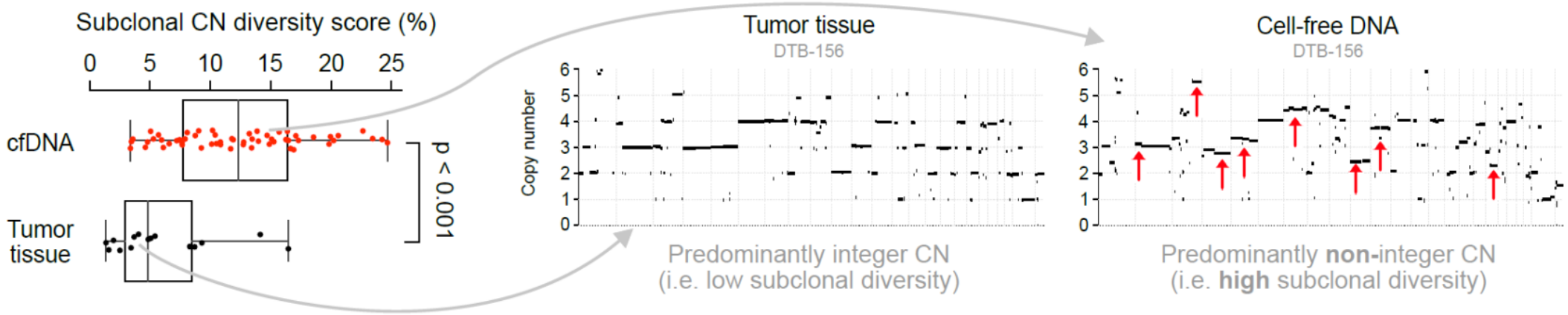
What is circulating tumor DNA (ctDNA)?

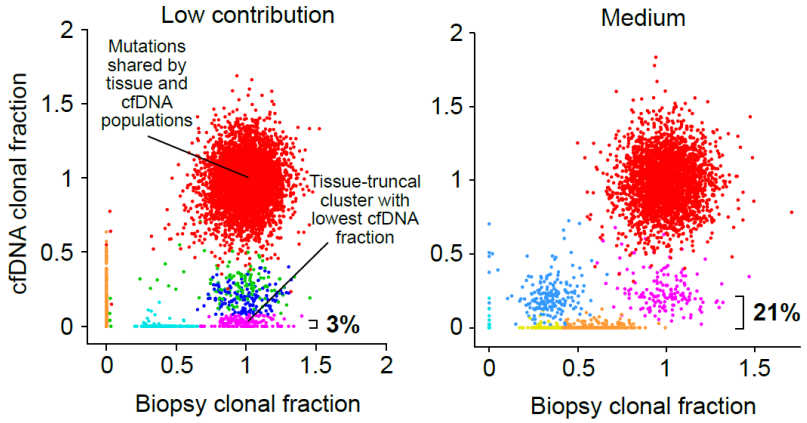
- Short fragments (post-apoptotic) of tumor-derived DNA in the blood
- Mixed with cell-free DNA from non-cancer cells (leukocytes)
- Half-life = hours
- CtDNA abundance is associated with proliferative tumor burden



Basic biology of cf/ctDNA shedding and clearance, including natural variability, remains largely unknown

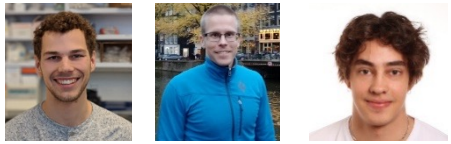
ctDNA is more diverse than a single metastasis





The contribution of individual metastatic foci to total ctDNA is often low

= ctDNA derived from multiple metastases

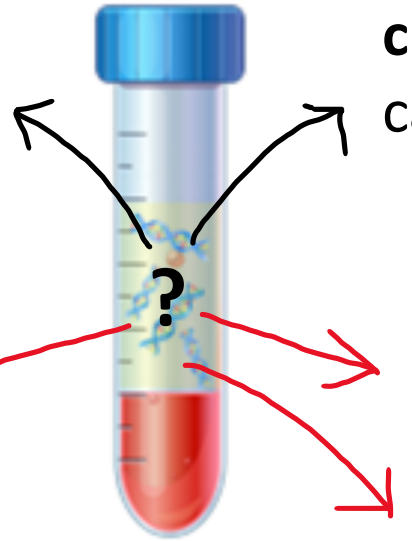


Herberts, Annala, Sipola, *et al*. Nature 2022

What contributes to plasma cell-free DNA in an individual with cancer?

normal (germline) cell-free DNA
from hematopoietic populations

circulating tumour DNA (ctDNA)
cancer populations; multiple sites



normal (germline) cell-free DNA
from non-hematopoietic tissues
-comorbidities
-treatment side effects?
-TME?

clonally-expanded hematopoietic populations with mutations (*e.g.* CHIP)

other somatic expansions (including independent cancers)

Profile matched white blood cells in all patients
- to reduce false positives and negatives

How can ctDNA tests help?

Trends in Cancer

CellPress

Review

Technical and biological constraints on ctDNA-based genotyping

Cameron Herberts[1] and Alexander W. Wyatt[1,2,*]

Cameron Herberts
@UBC PhD

Detection of ctDNA

Improve cancer screening in at-risk populations

Detect residual disease after surgery / radiation

Monitor for response / resistance to therapy

Characterization of ctDNA

Estimate cancer aggression, *i.e.* prognosis

Predict treatment efficacy

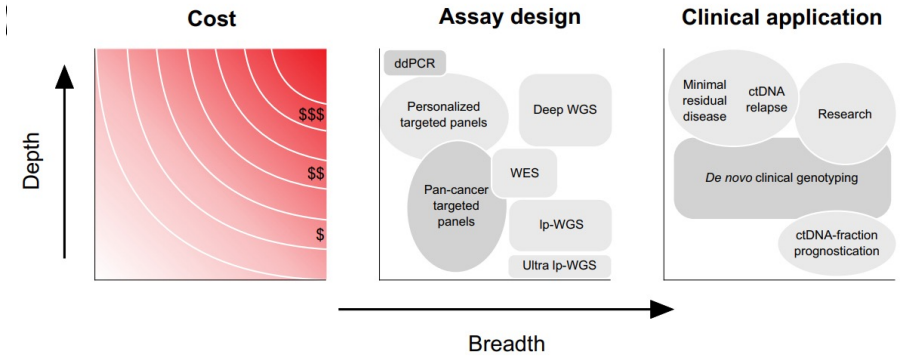
Define new metastatic biology & resistance mechanisms

Lack of recognition for the distinction between detection and characterization of ctDNA is contributing to unrealistic expectations for sensitivity in some settings

General methods for detection of ctDNA

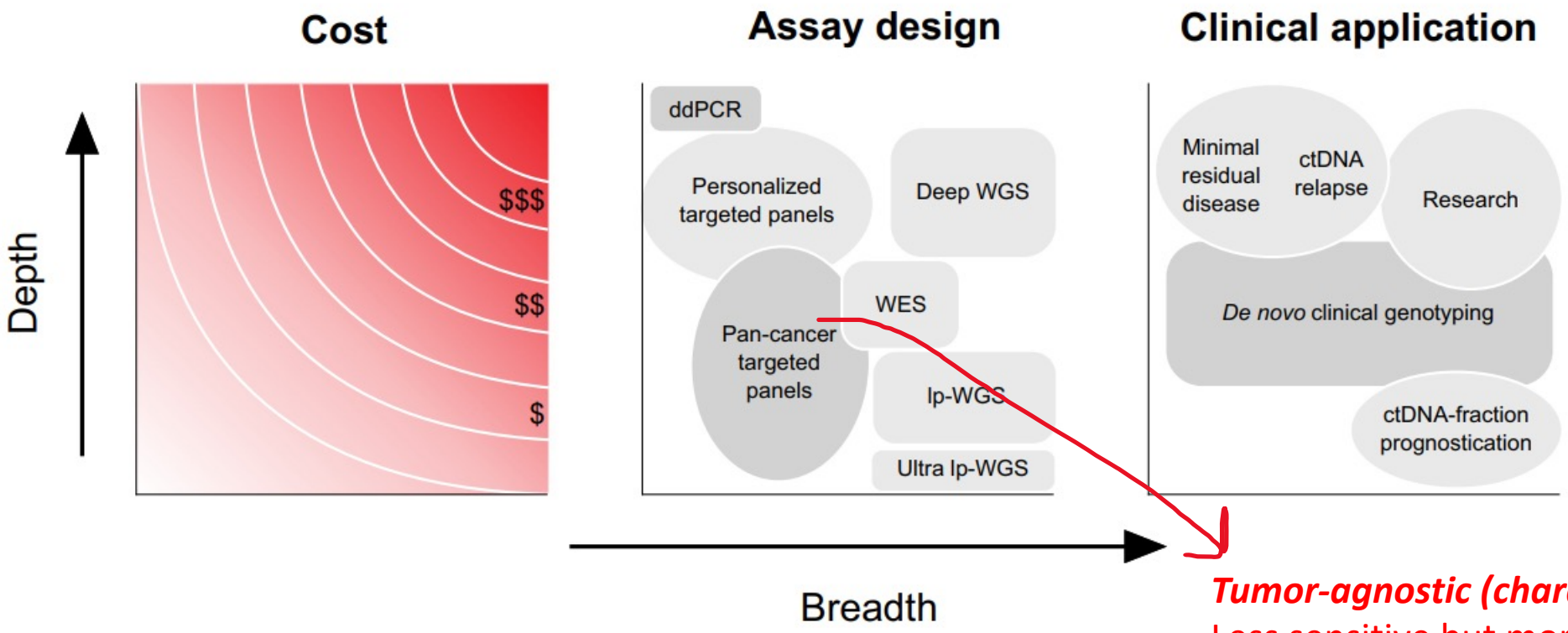
- Tumor-informed personalized mutation assays[1-2]
- Methylation profiling[3]
- Cell-free DNA fragment feature analysis[4]

Tumor-agnostic somatic genotyping assays used for later disease are not appropriate when sole goal is sensitive <u>detection</u> of ctDNA



[1] Christensen *et al*., J Clin Oncol 2019; [2] Powles *et al*., Nature 2021; [3] Klein et al., Annals of Oncology 2021; [4] Cristiano et al., Nature 2019

General methods for characterisation of ctDNA



Tumor-agnostic (characterization)
Less sensitive but more inclusive
Need to cover large genomic territory

Clinically-pragmatic assay design is trade-off between depth and breadth

Herberts and Wyatt, Trends in Cancer 2021

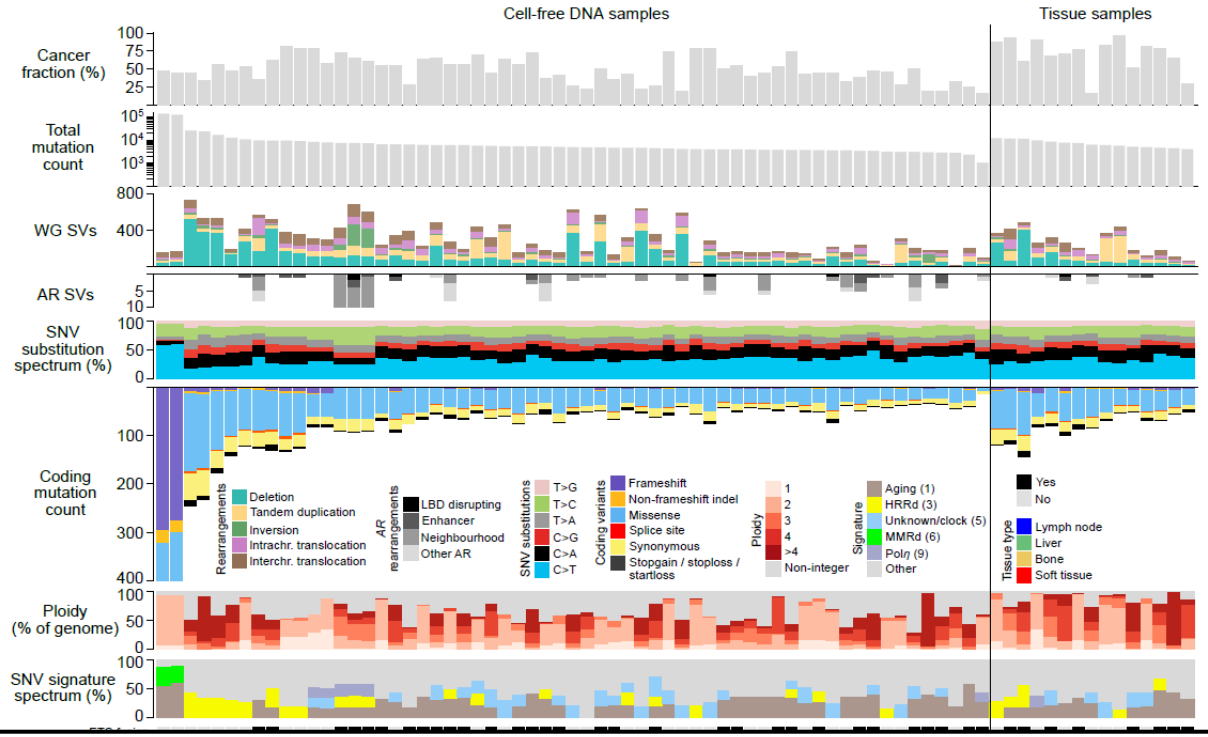
High-resolution insight possible from tissue or ctDNA deep whole-genome sequencing

Information

- Mutations
- → Germline
- → Somatic
- → Signatures
- Structural rearrangements
- Copy number
- → Ploidy
- Nucleosome positioning

Biological / clinical ***insight***

- Subclonal composition / heterogeneity
- Tumor phylogeny
- Treatment-resistance mechanisms
- Mitochondrial cfDNA
- ? Phenotypic profiling (transcription factor activity, gene transcription)
- ? *… and more*

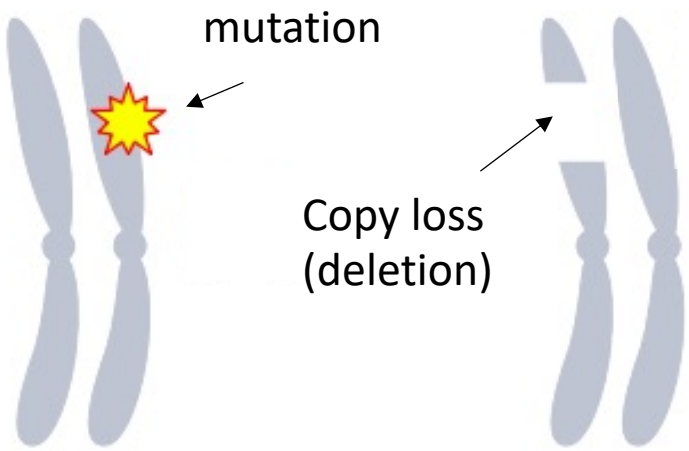


Current commercial panels are not <u>yet</u> *comprehensive* – and can constrain precision oncology

Herberts, Annala, Sipola, *et al*. *Nature 2022*

Mutations are just one mechanism of genomic alteration

- Some tests will report a tumor suppressor gene mutation but not a heterozygous (monoallelic) deletion

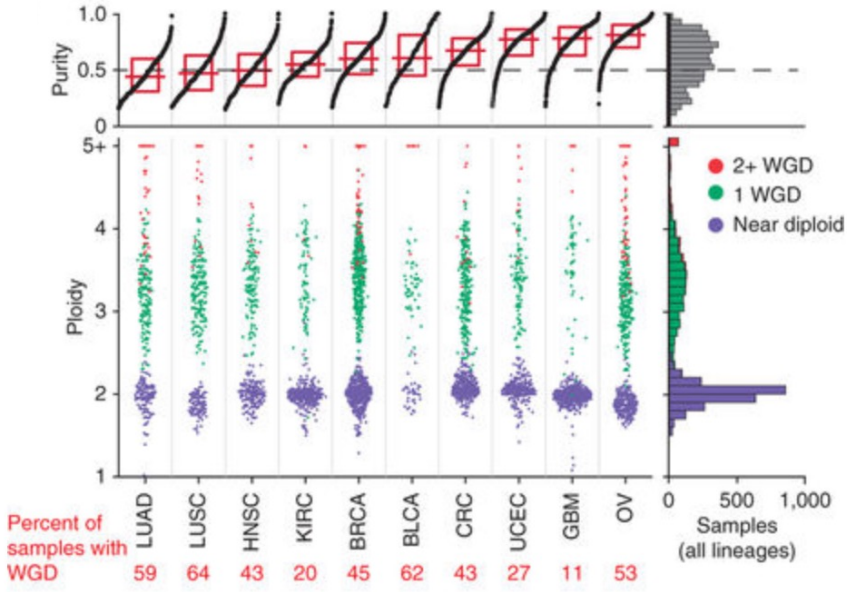


- Key cancer tumor suppressor genes *RB1*, *TP53*, *CDKN2A*, *PTEN* can be disrupted by <u>single copy losses</u> or complex <u>structural rearrangements</u>

Herberts and Wyatt, Trends in Cancer 2021

Whole-genome doubling is common

Zack et al. Nature Genetics **45:**1134–1140 (2013)



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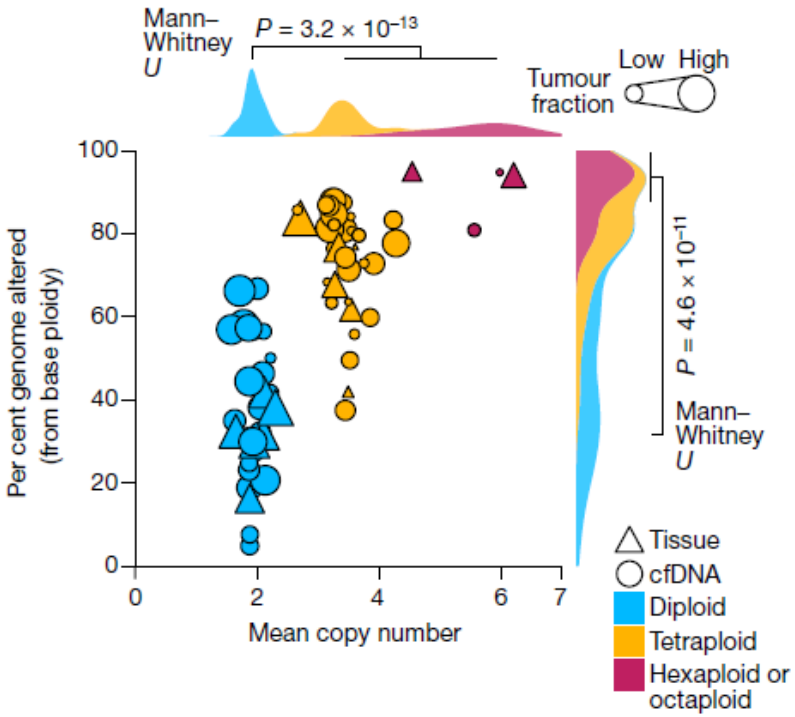
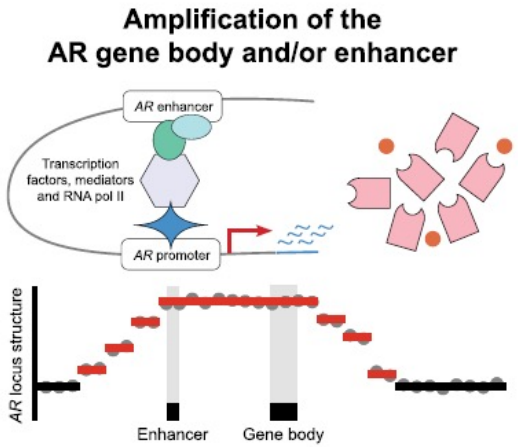
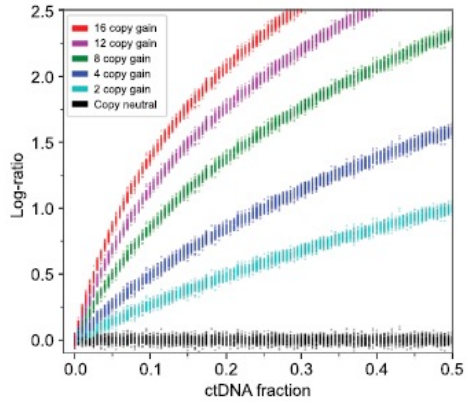


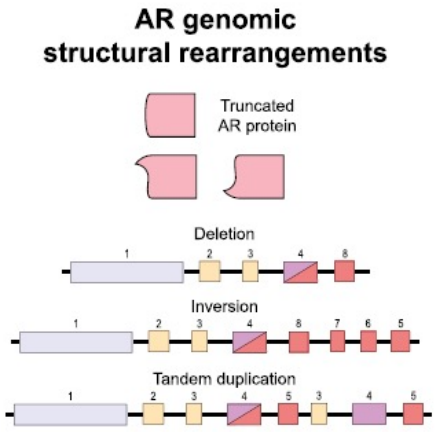
Biological relevance: genome instability, tolerance for TS mutation

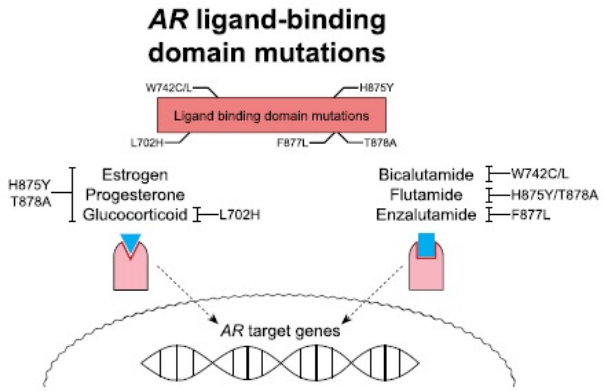
Technical relevance: can result in false positives/negatives in copy number analysis

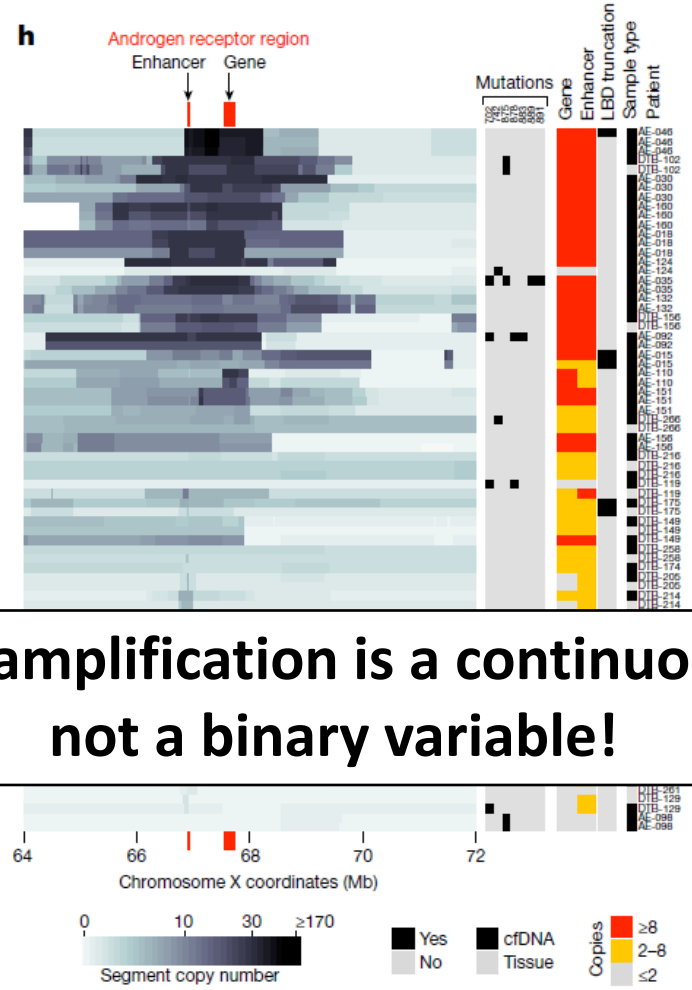
AR genotype is hugely variable in mCRPC











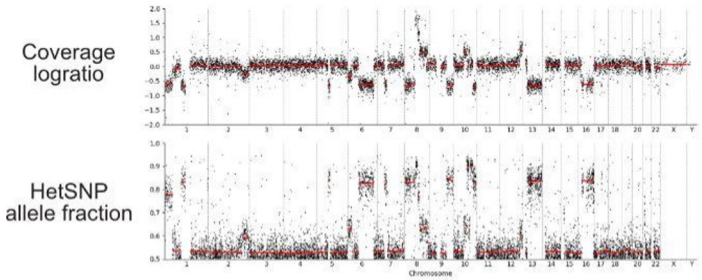
AR amplification is a continuous, not a binary variable!

Kwan E, The Prostate 2022

Herberts, Annala, Sipola, *et al*. *Nature 2022*

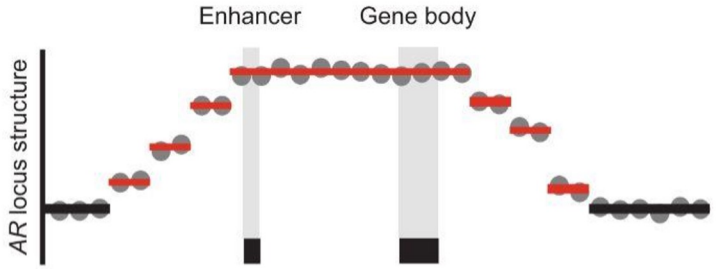
Our research panel for prostate cancer

** Always applied to paired patient-matched cfDNA <u>and</u> leukocyte DNA*



Incorporation of genome-wide SNP backbone

Expanded panel in use since 2020



Greater coverage across *AR* locus

Addition of unique dual indexes + unique molecular identifiers

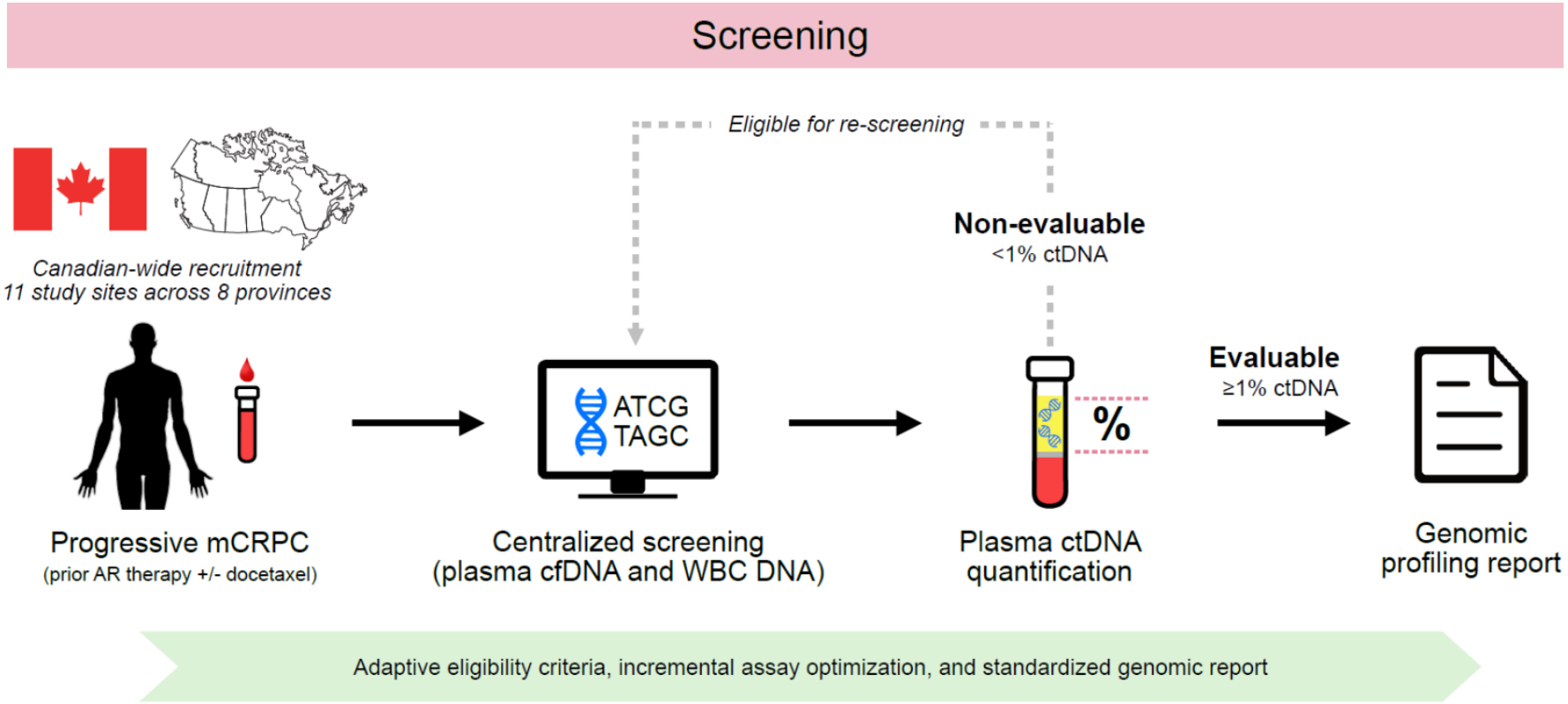
Expansion to introns and flanking regions of selected genes

Limitations

- with 1500-2000x unique read depth, minimum ctDNA% via mutations of ~0.5%
- Chromosomal copy number changes not resolvable below 5%, focal changes not below 10%
- Not always enough mutations to have accurate ctDNA% estimate in cases where no copy number changes detected

Prostate cancer biomarker enrichment and treatment selection (PC-BETS) study

Open-label multicentre 2-stage biomarker-driven phase II umbrella trial



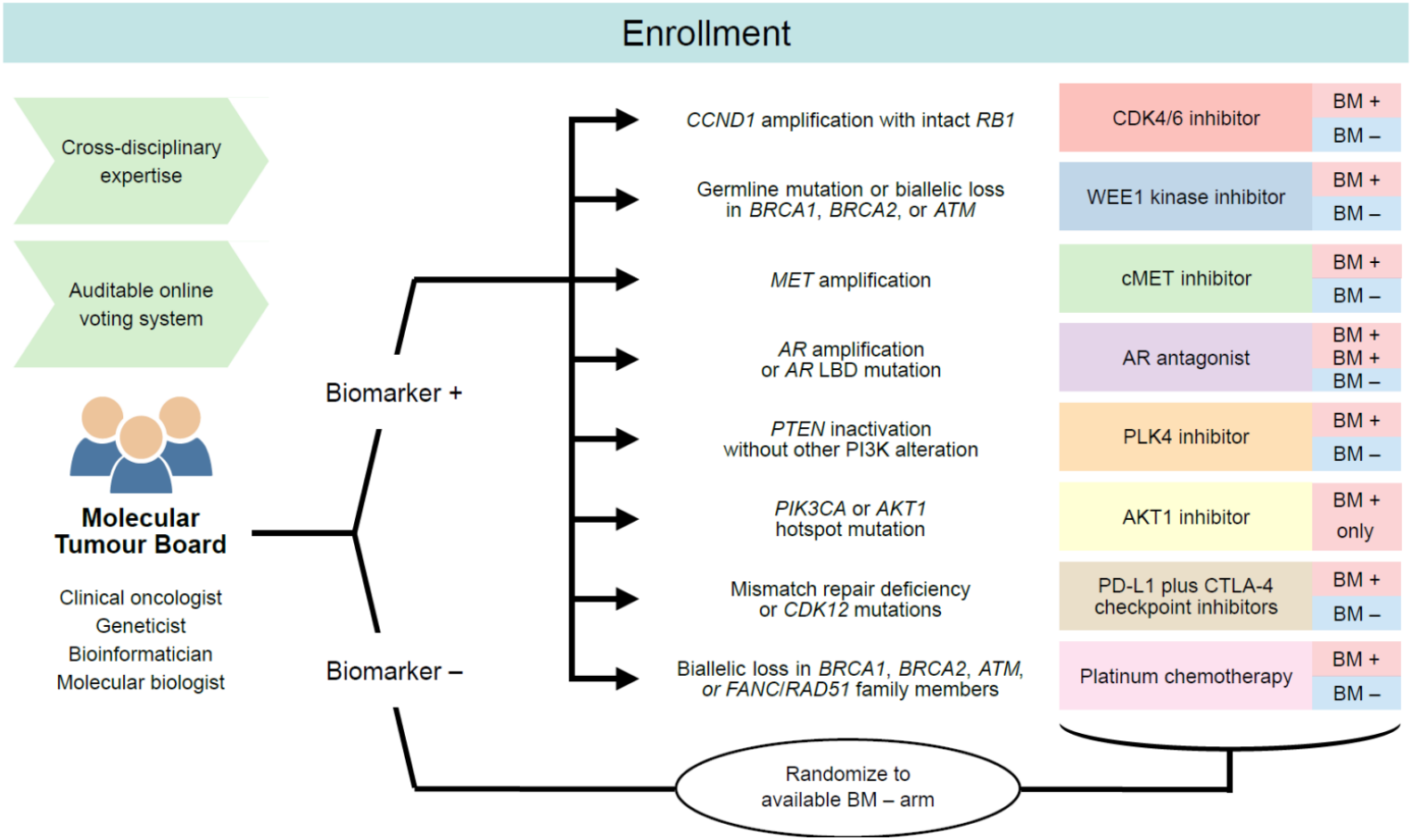
ClinicalTrials.gov: NCT03385655 (“IND234”)

Canadian Cancer Trials Group
A national program of the Canadian Cancer Society

Canadian Cancer Society Société canadienne du cancer

Chairs: **Kim Chi, Lesley Seymour**
Sub-study PIs & entire CCTG team

Prostate cancer biomarker enrichment and treatment selection (PC-BETS) study



~600 mCRPC patients screened to date

ClinicalTrials.gov: NCT03385655 (“IND234”)

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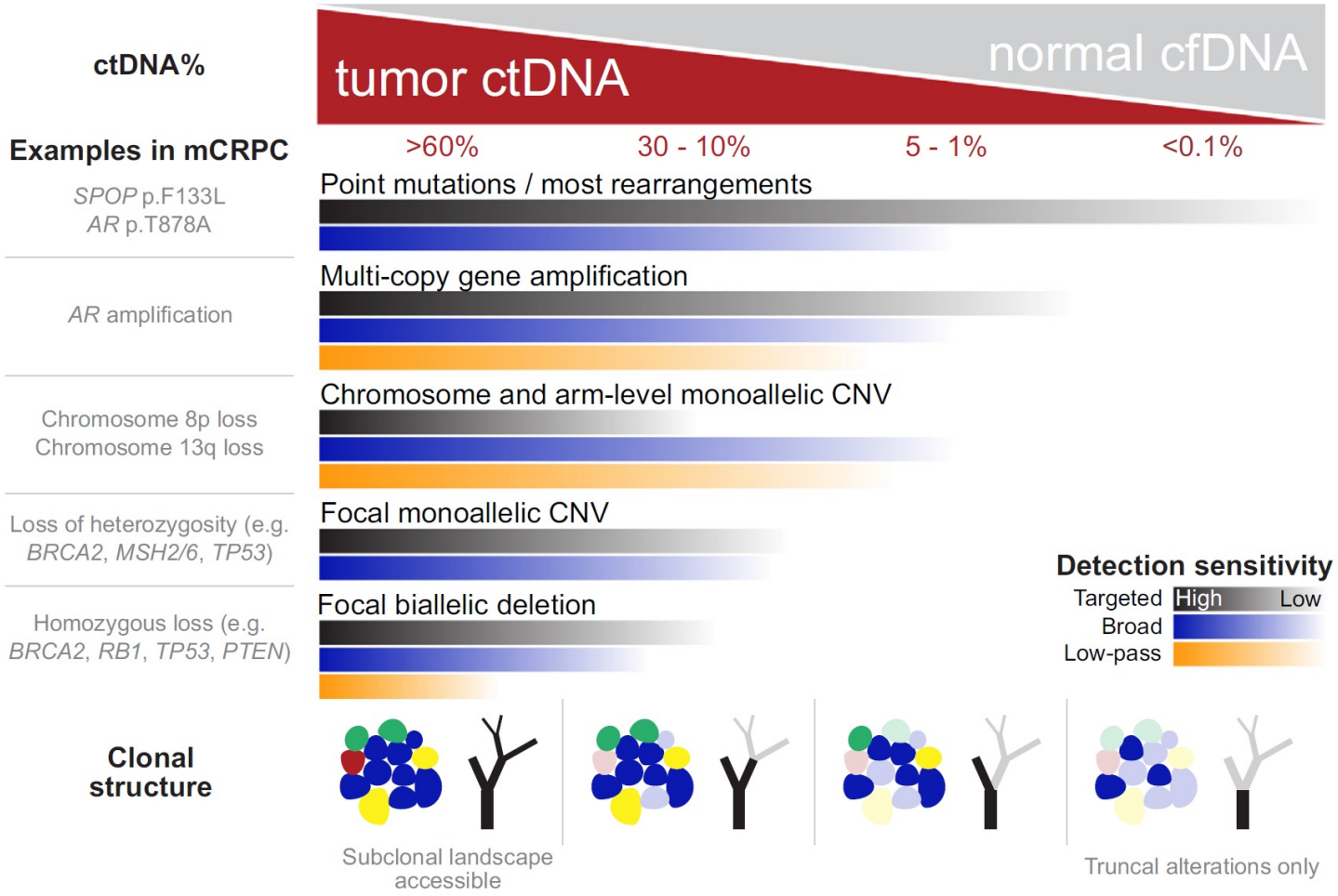
ctDNA fraction constrains the alterations that can be identified; independent of panel design



Herberts and Wyatt, Trends in Cancer 2021

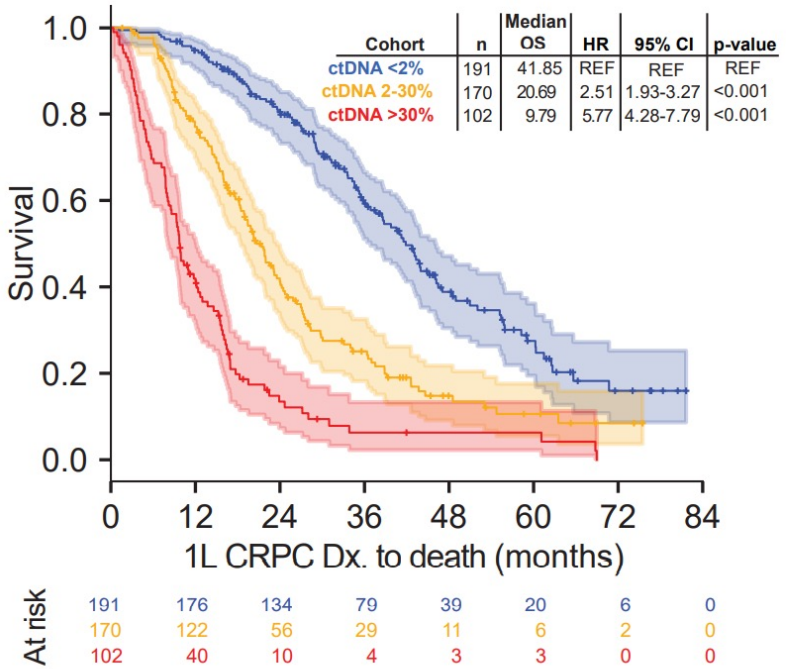
Concordance for ctDNA vs matched tissue is relatively high for truncal driver events, but ctDNA% dependent

Wyatt*, Annala*, *et al.*, J Natl Cancer Institute 2017
Vandekerkhove *et al.*, Eur Urol 2019

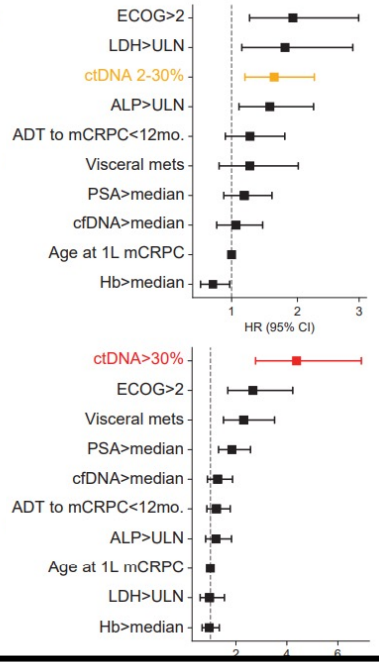
ctDNA% is an independent prognostic marker

Newly progressing mCRPC (n = 463)

Blood collection 1 – 30 days prior to 1L tx

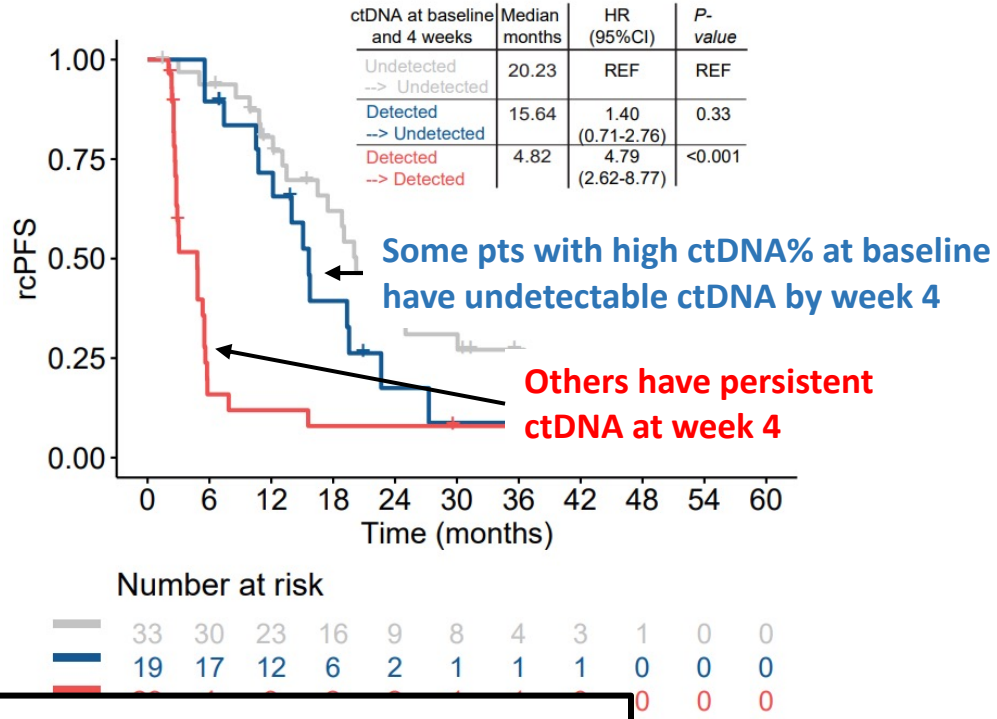


Cohort	n	Median OS	HR	95% CI	p-value
ctDNA <2%	191	41.85	REF	REF	REF
ctDNA 2-30%	170	20.69	2.51	1.93-3.27	<0.001
ctDNA >30%	102	9.79	5.77	4.28-7.79	<0.001



New mCRPC initiating 1L ARPI (n = 81)

Blood collections: baseline and 4-weeks



ctDNA at baseline and 4 weeks	Median months	HR (95%CI)	*P-value*
Undetected --> Undetected	20.23	REF	REF
Detected --> Undetected	15.64	1.40 (0.71-2.76)	0.33
Detected --> Detected	4.82	4.79 (2.62-8.77)	<0.001

If we select patients based on alterations in ctDNA, *we enrich our trials with poor prognosis disease*

Dr. Nicolette Fonseca, Dr. Corinne Maurice-Dror, Cameron Herberts

Unpublished

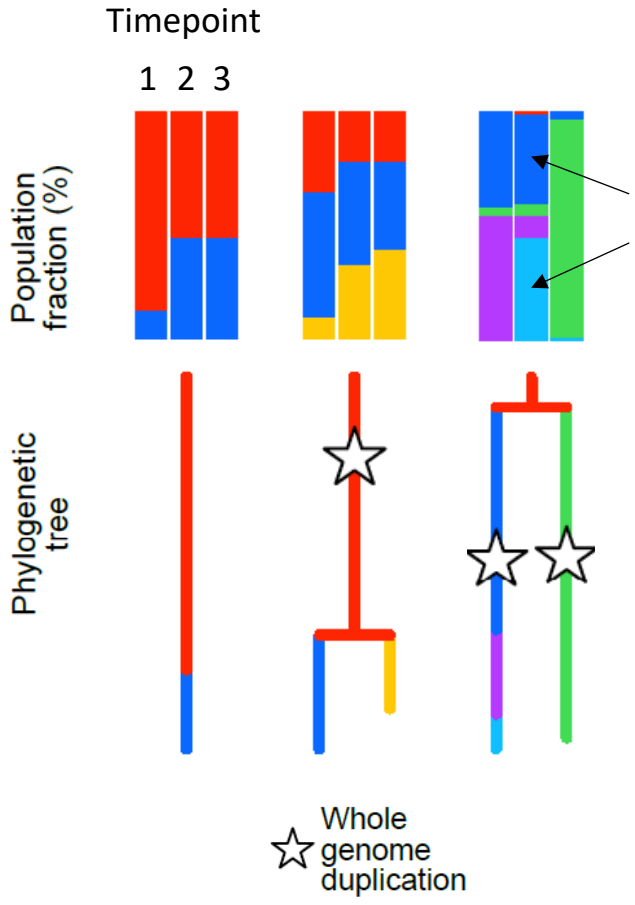
Emmy Boerrigter & Sofie Tolmeijer @RadboudUMC

Presentation overview

- CtDNA fundamentals and constraints for precision oncology
- **Drivers of cancer evolution in serial ctDNA**
- Phenotypic insights in mCRPC via ctDNA

Distinct tumor populations can be resolved in ctDNA

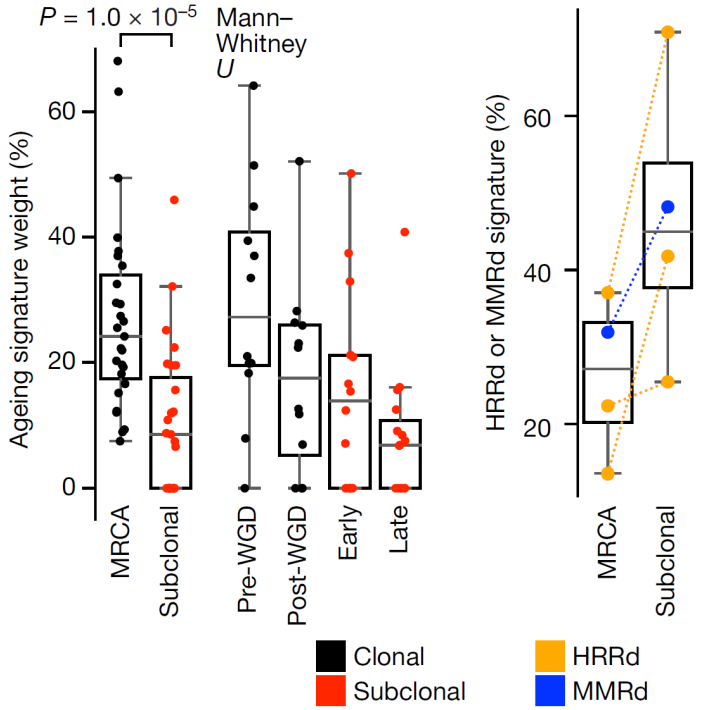
Three representative patients



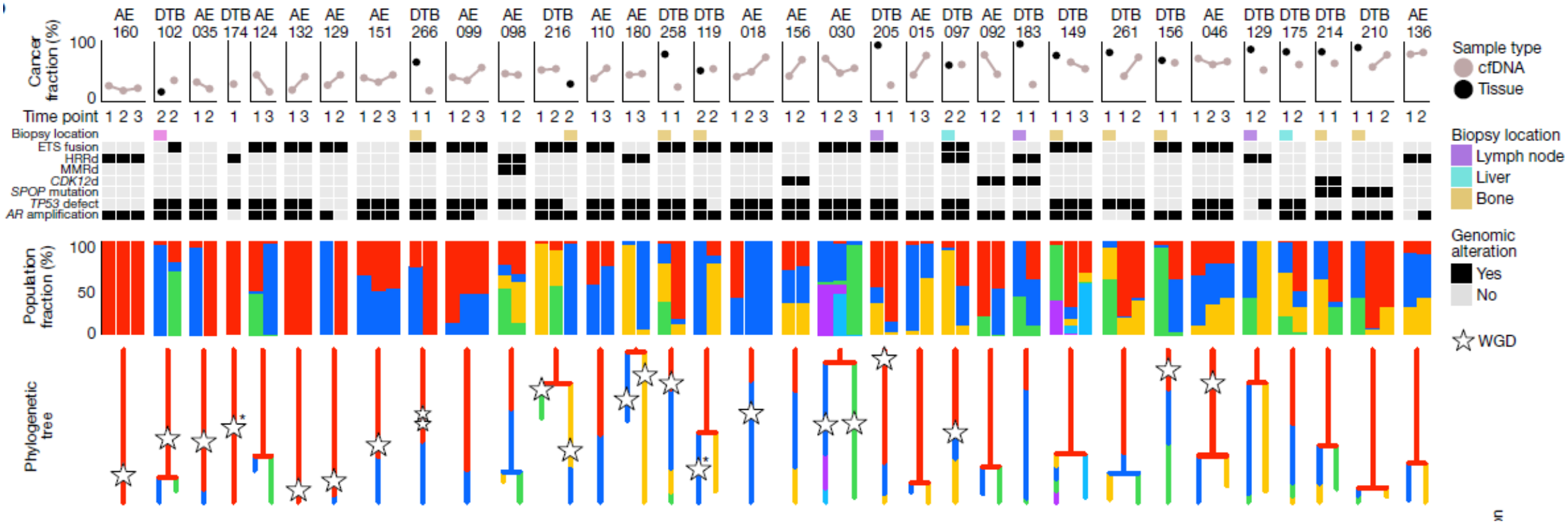
Different populations represented in ctDNA, <u>changing over time</u>

Represents the highest level of evidence for continued evolution of (sub)clonal populations in patients with mCRPC

Different distribution of mutational signatures in subclones



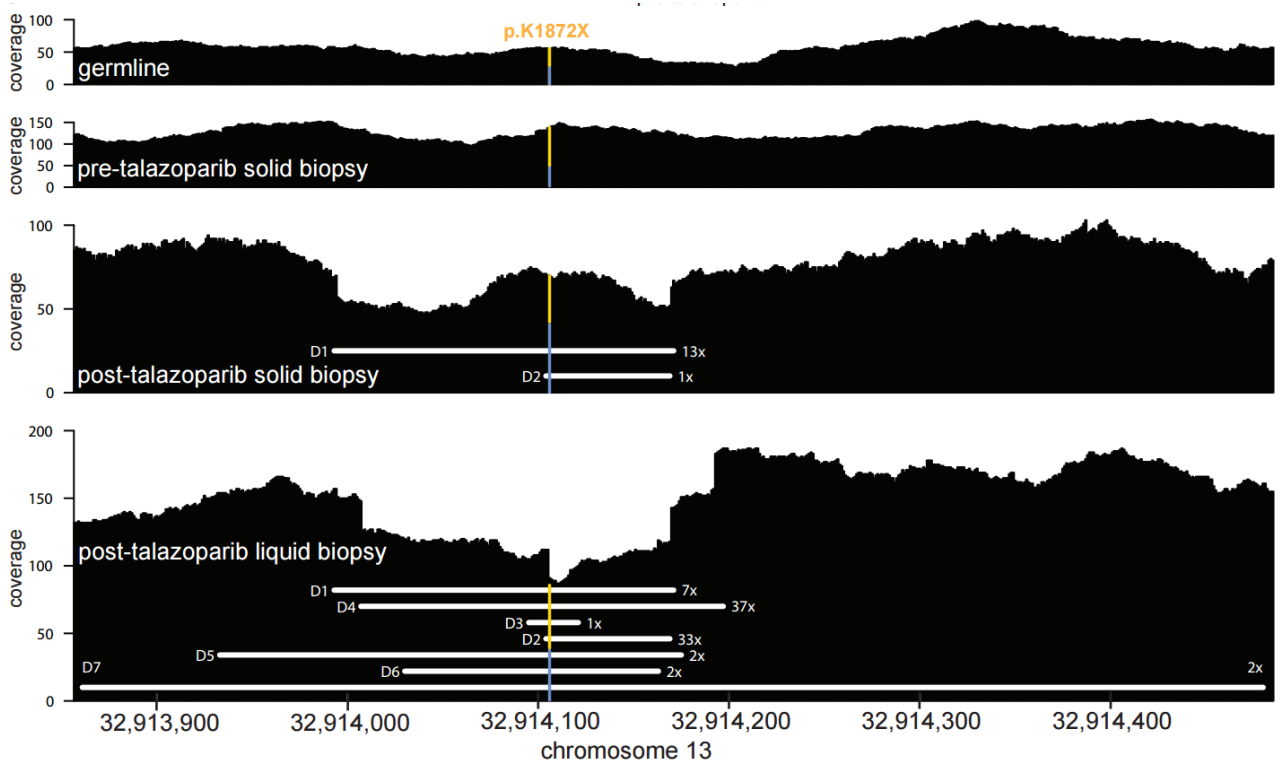
Herberts, Annala, Sipola, *et al. Nature 2022*



Herberts, Annala, Sipola, *et al*. *Nature 2022*

Targeted therapy selects for resistant clones

Example of reversion mutations post-PARP inhibition



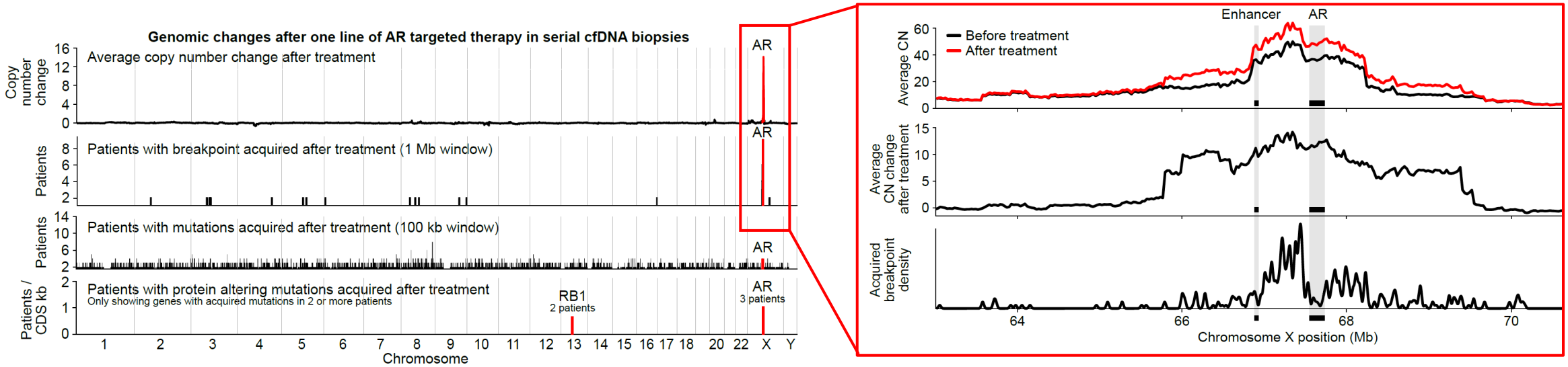
mCRPC, germline BRCA2 mutation (that prevents BRCA2 from functioning)

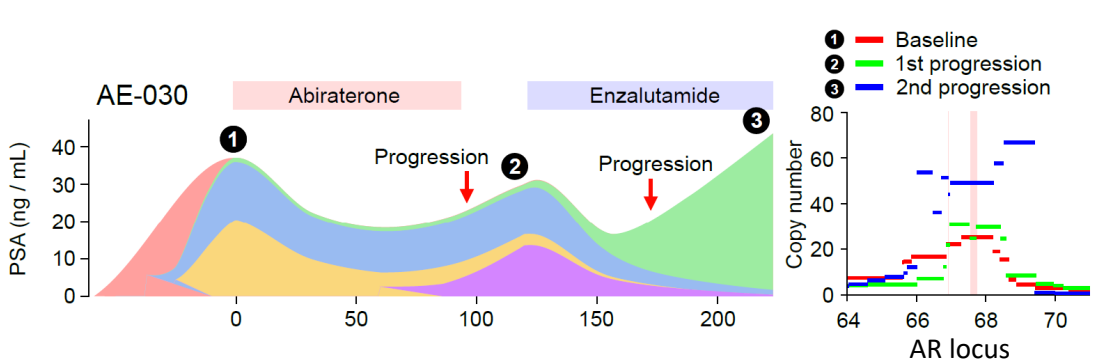
- Clinical response to PARPi
- Developed progressive disease after 6 months
- Serial ctDNA samples revealed development of 'reversion mutations'
- The cancer cells restore function to BRCA2

Quigley D, *et al. Cancer Discovery* 2017;7(9):999-1005.

Changes in clonal composition favor *AR* augmentation after ARPI

the only highly recurrent changes in copy number, mutations, or structural rearrangements were at the AR locus





Selected patient with drastic population and AR genotype changes

Herberts, Annala, Sipola, *et al*. *Nature 2022*

Presentation overview

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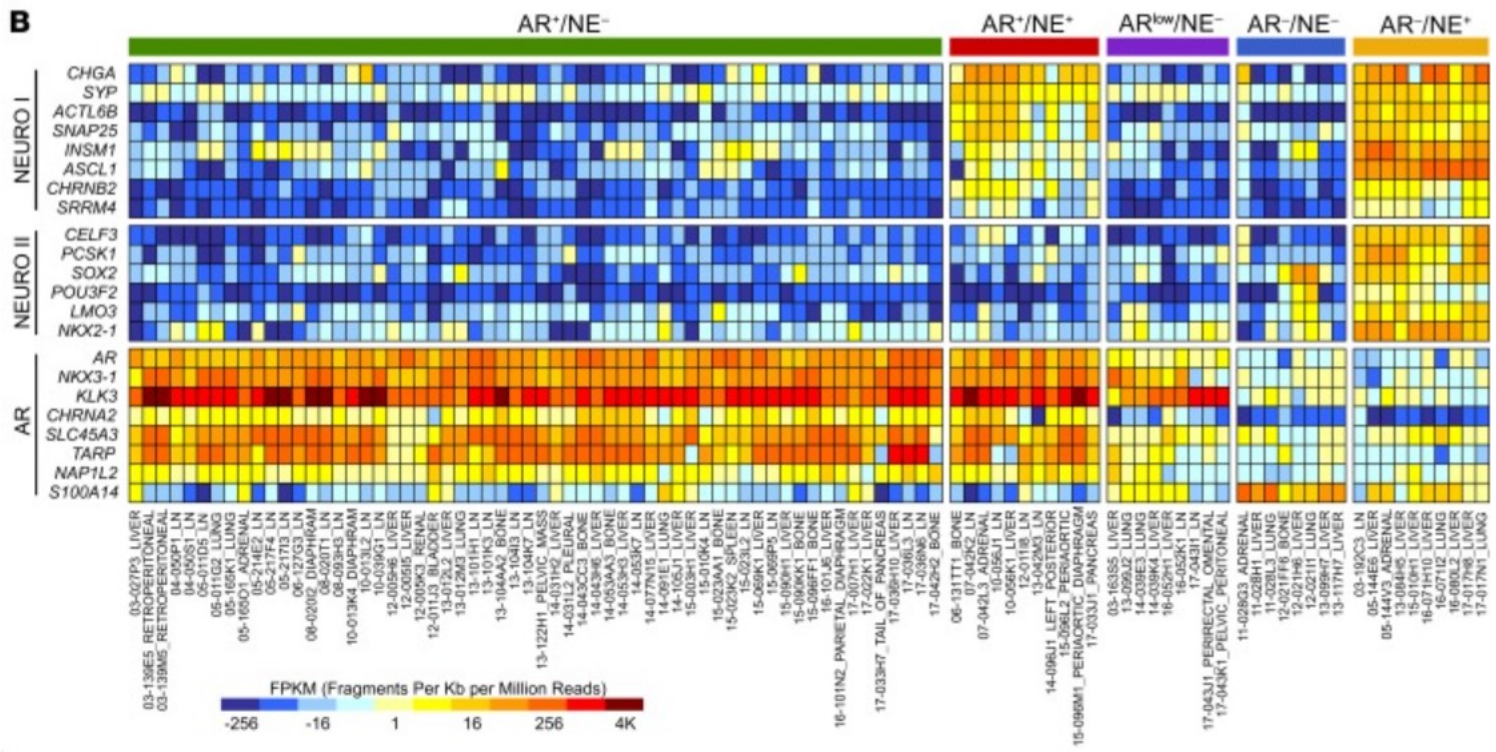
- Drivers of cancer evolution in serial ctDNA
- **Phenotypic insights in mCRPC via ctDNA**

mCRPC exhibits distinct transcriptomic subtypes across a spectrum of AR activity



1. AR-high
2. AR-low
3. amphicrine (AR+/NE+)
4. double-negative (AR–/NE–)
5. NEPC (NE+/AR–)

Labreque *et al*., J Clin Invest 2019

Methylation profiling of cell-free DNA

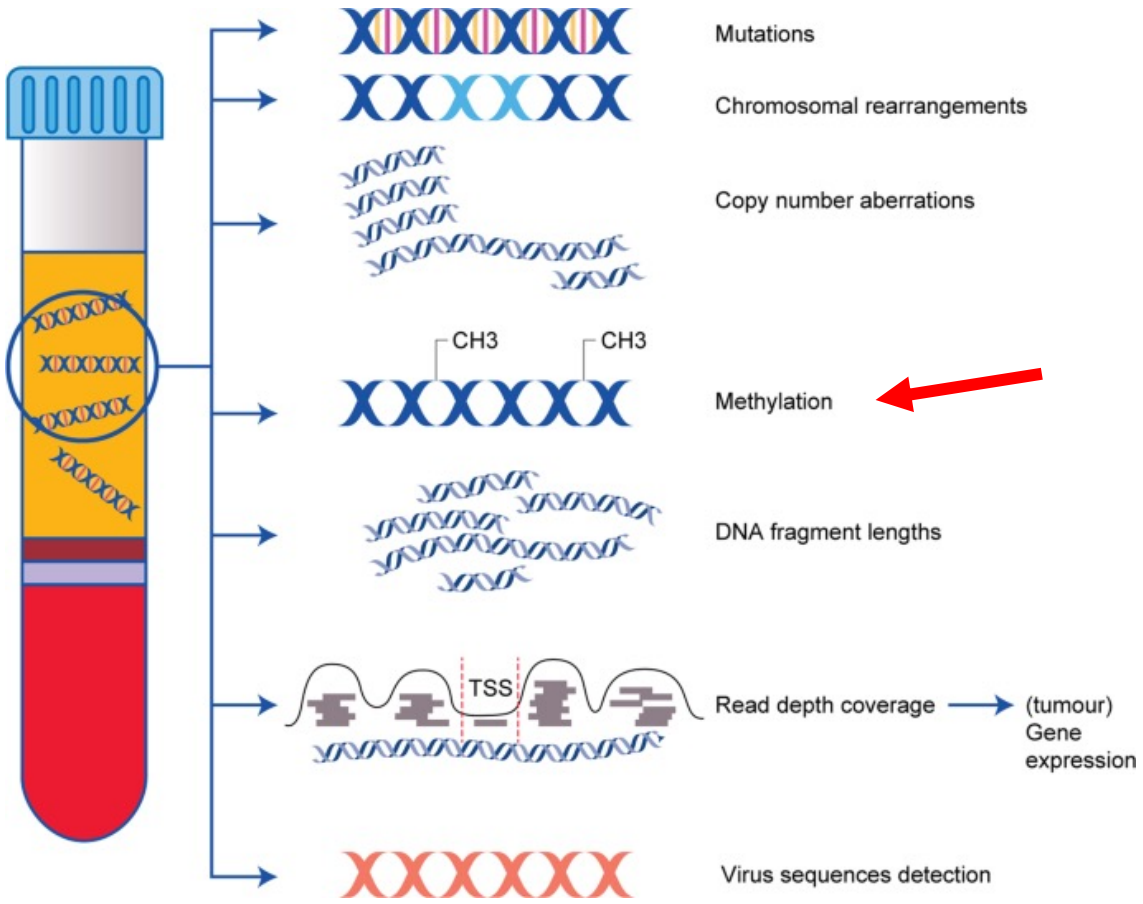


Figure from Keller *et al.*, British Journal of Cancer 2020

nature communications

Article

https://doi.org/10.1038/s41467-022-34012-2

The cell-free DNA methylome captures distinctions between localized and metastatic prostate tumors

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Check for updates

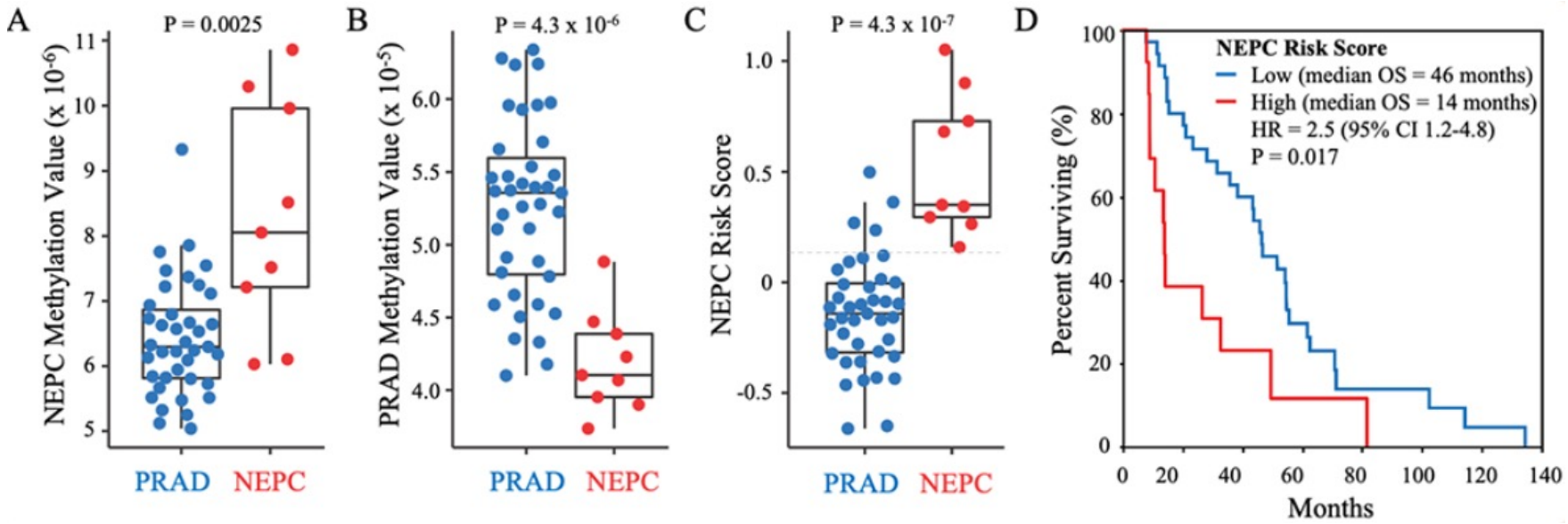
Sujun Chen[1,2,3,18], Jessica Petricca[1,2,18], Wenbin Ye[1,4,5,18], Jiansheng Guan[1,6], Yong Zeng[1], Nicholas Cheng[2], Linsey Gong[1,2], Shu Yi Shen[1], Junjie T. Hua[7,8], Megan Crumbaker[9], Michael Fraser[1], Stanley Liu[2,10,11], Scott V. Bratman[1,2], Theodorus van der Kwast[2,12], Trevor Pugh[1,2], Anthony M. Joshua[12], Daniel D. De Carvalho[1,2], Kim N. Chi[13], Philip Awadalla[14], Guoli Ji[4,5], Felix Feng[7,8,15,16], Alexander W. Wyatt[17] & Housheng Hansen He[1,2]

CANCER RESEARCH | GENOME AND EPIGENOME

The 5-Hydroxymethylcytosine Landscape of Prostate Cancer

Martin Sjöström[1,2,3], Shuang G. Zhao[4,5], Samuel Levy[6], Meng Zhang[1,2], Yuhong Ning[6], Raunak Shrestha[1,2], Arian Lundberg[1,2], Cameron Herberts[7], Adam Foye[1,8], Rahul Aggarwal[1,8], Junjie T. Hua[1,2], Haolong Li[1,2], Anna Bergamaschi[6], Corinne Maurice-Dror[7,9], Ashutosh Maheshwari[1,2], Sujun Chen[10,11], Sarah W.S. Ng[7], Wenbin Ye[10,11,12], Jessica Petricca[10,11], Michael Fraser[11,13], Lisa Chesner[1,2], Marc D. Perry[1,2], Thaidy Moreno-Rodriguez[1,2], William S. Chen[1,2], Joshi J. Alumkal[14], Jonathan Chou[1,8], Alicia K. Morgans[15], Tomasz M. Beer[16], George V. Thomas[16,17], Martin Gleave[7], Paul Lloyd[6], Tierney Phillips[6], Erin McCarthy[6], Michael C. Haffner[18,19], Amina Zoubeidi[7], Matti Annala[7,20], Robert E. Reiter[21,22], Matthew B. Rettig[21,22,23], Owen N. Witte[24], Lawrence Fong[1,8], Rohit Bose[1,8,25,26], Franklin W. Huang[1,8], Jianhua Luo[27], Anders Bjartell[28,29], Joshua M. Lang[30], Nupam P. Mahajan[31], Primo N. Lara[32,33], Christopher P. Evans[33,34], Phuoc T. Tran[35], Edwin M. Posadas[36], Chuan He[37,38], Xiao-Long Cui[37,38], Jiaoti Huang[39], Wilbert Zwart[40], Luke A. Gilbert[1,25,41], Christopher A. Maher[31,42,43,44], Paul C. Boutros[10,45,46], Kim N. Chi[7], Alan Ashworth[1,8], Eric J. Small[1,8], Housheng H. He[10,11], Alexander W. Wyatt[7,47], David A. Quigley[1,25,48], and Felix Y. Feng[1,2,8,25]

Methylation profiling of cell-free DNA: opportunity to identify NEPC



Plasma cfDNA profiled with cfMeDIP-seq
Berchuck *et al*., Clin Cancer Res. 2022

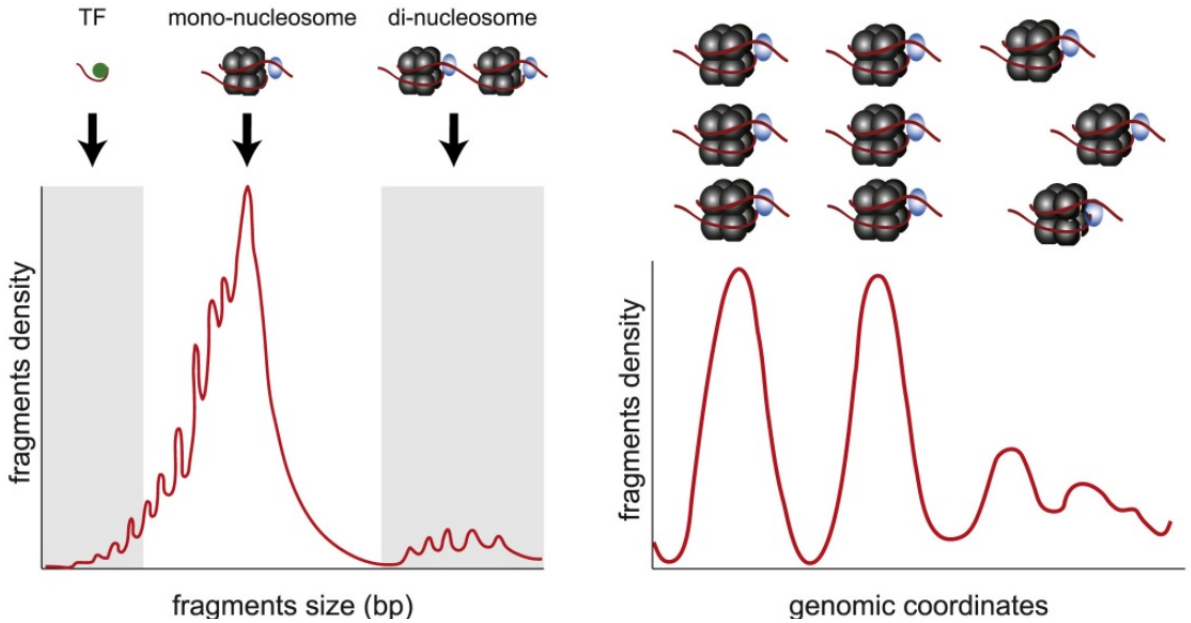
See also prior work from Beltran, Demichelis et al., J Clin Invest. 2020

Methylation profiling of ctDNA: challenges

- Requires a separate assay
- Unclear clinical utility beyond NEPC classification
  - Can pathway activity differences be identified?
- Sensitivity for characterization must be established (across ctDNA%)
- Remains unknown how methylation reflects rapid adaptive changes in gene expression
  - Correlative studies in tissues and models are required

ctDNA fragment features and cancer phenotype

cfDNA fragmentation patterns are biased by nucleosomes

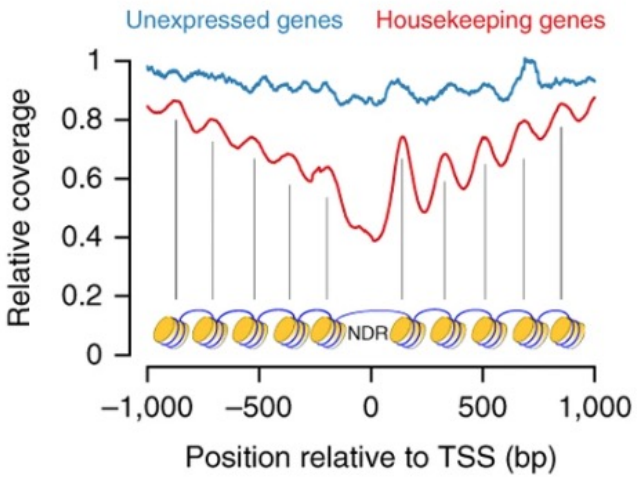


Van der Pol and Mouliere, Canc Cell Rev, 2019; Murtaza and Caldas, Nat Gen, 2016

Nucleosome position reflects important cellular processes: **gene transcription** and **transcription factor binding**
→ **can be inferred from ctDNA**

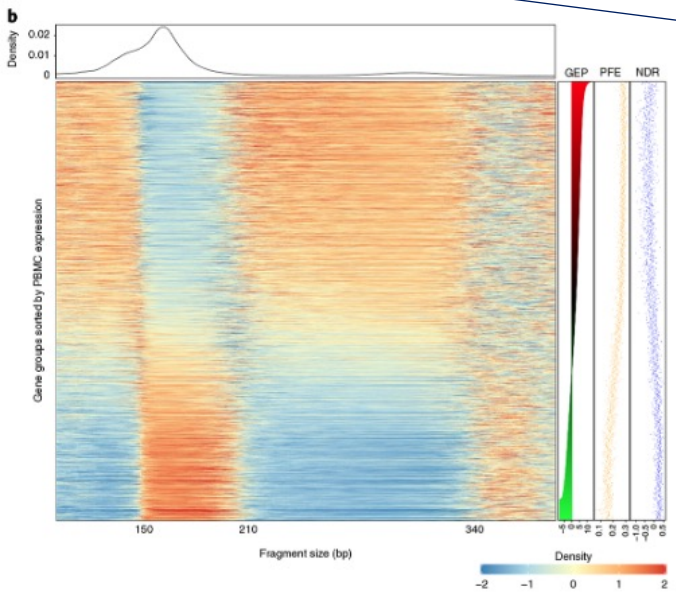
cfDNA fragments reflect chromatin organization and gene regulation

cfDNA fragment **depth**, **size**, and fragment-**end motifs** communicate information about nucleosome occupancy



Ulz et al. *Nat Genetics* (2016)

Ulz et al. *Nat Comm* (2019)



Esfahani et al. *Nat Biotech* (2022)

Jiang et al. *Cancer Discovery* (2020)

Can we combine this information for enhanced detection + characterization?

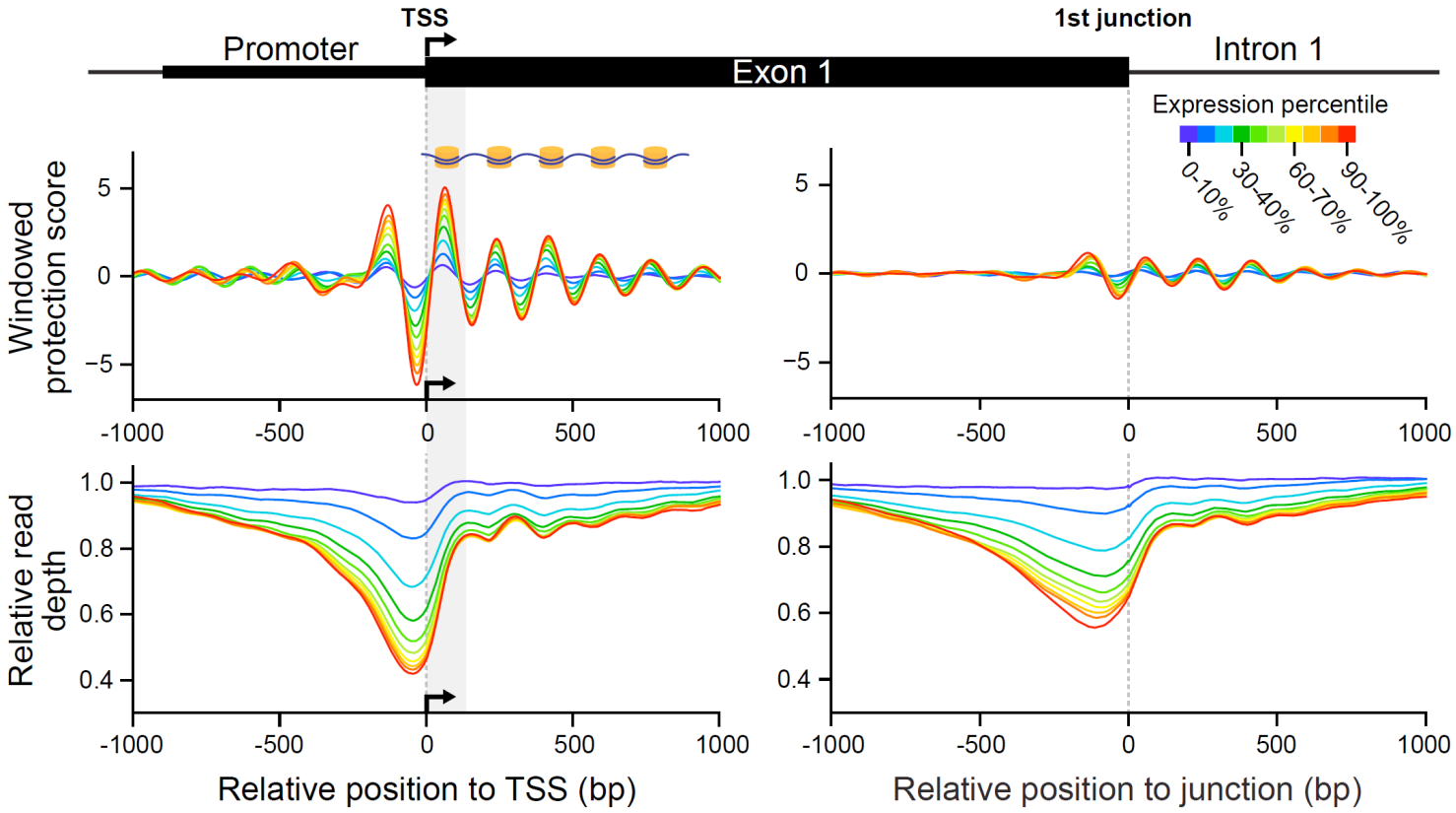
ctDNA nucleosome patterns recapitulate tissue RNA abundance

Highly expressed genes in tissue → strong nucleosome depletion at transcription start site (TSS)

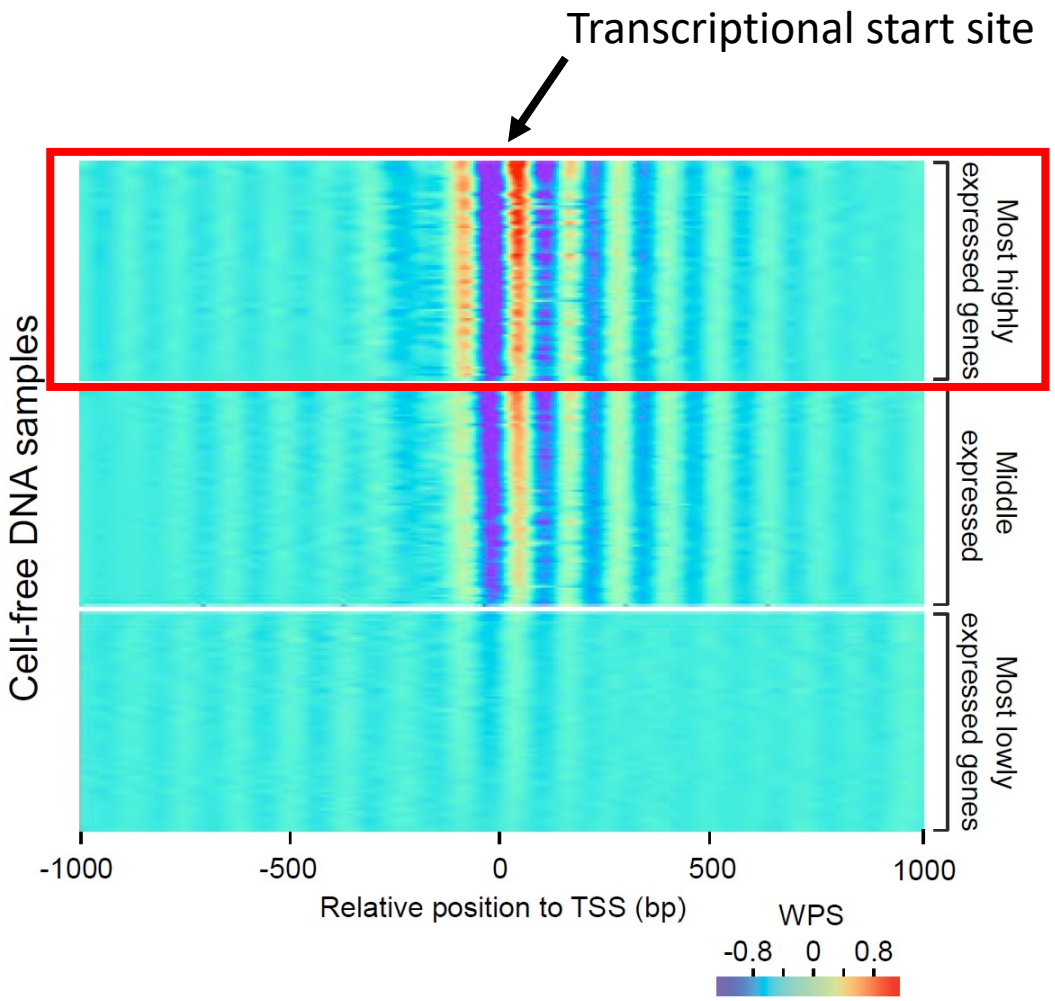
cfDNA TSS signals match RNA-Seq abundance from same-day metastatic tissue biopsy

Emilia Chen

Sarah Ng



ctDNA nucleosome occupancy patterns recapitulate tissue mRNA abundance

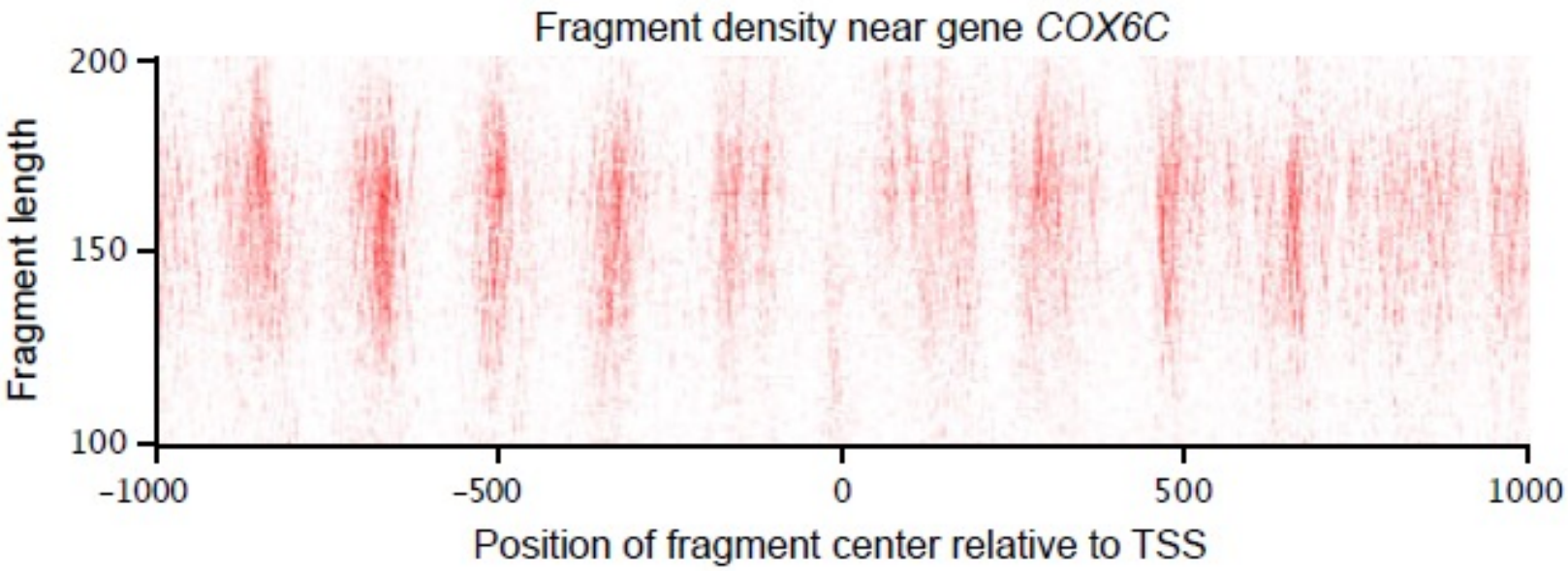


!
Relatively low resolution data, not suitable for single genes/transcripts

Many remaining unknowns

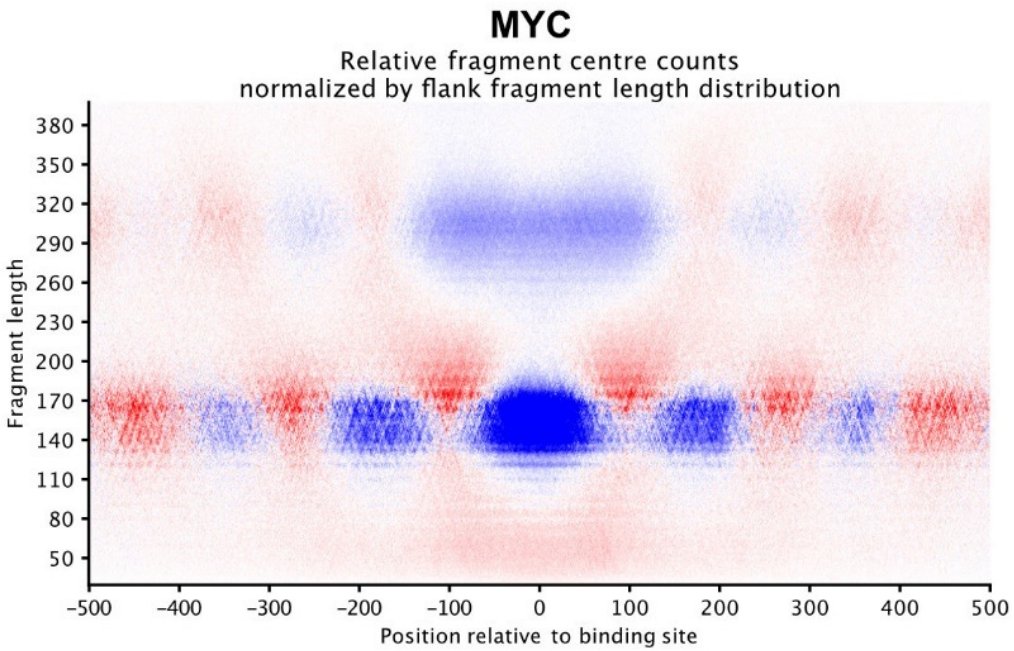
Herberts, Annala, Sipola, *et al*. *Nature 2022*

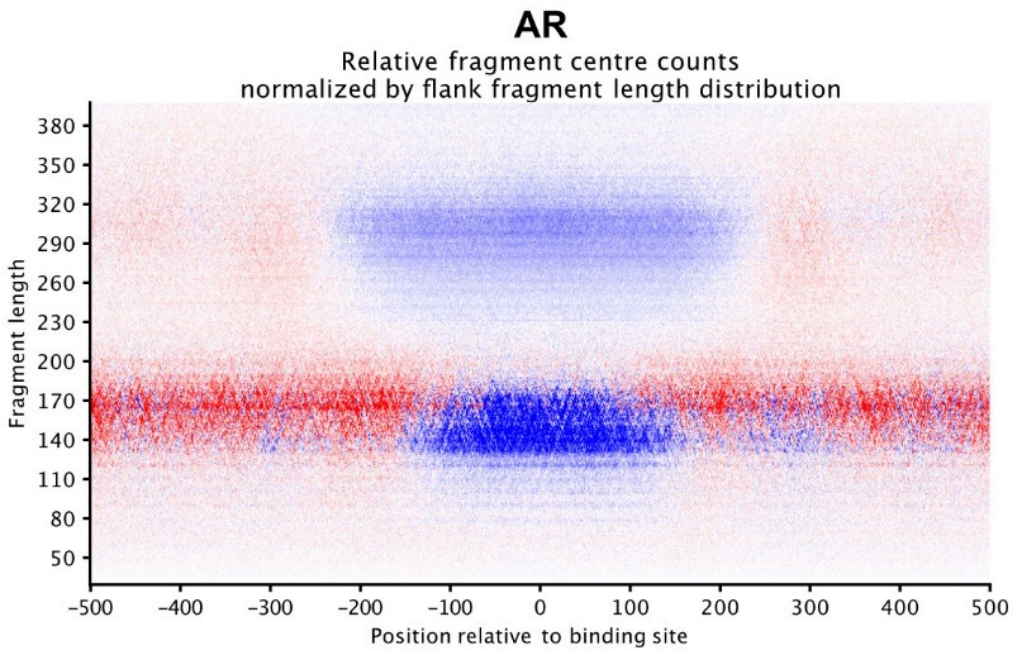
'Nucleogram': tool for cfDNA fragment-based phenotyping



cfDNA fragments reflect chromatin organization and gene regulation

'*Nucleograms*' enable interrogation of TF binding activity and trans-acting protein-DNA engagement patterns.





See also elegant work by Navonil De Sarkar… Peter Nelson, **Gavin Ha**. Cancer Discovery 2022

AR binding inferred from ctDNA WGS

Measured nucleosome occupancy at 3224 prostate-cancer AR-binding sites

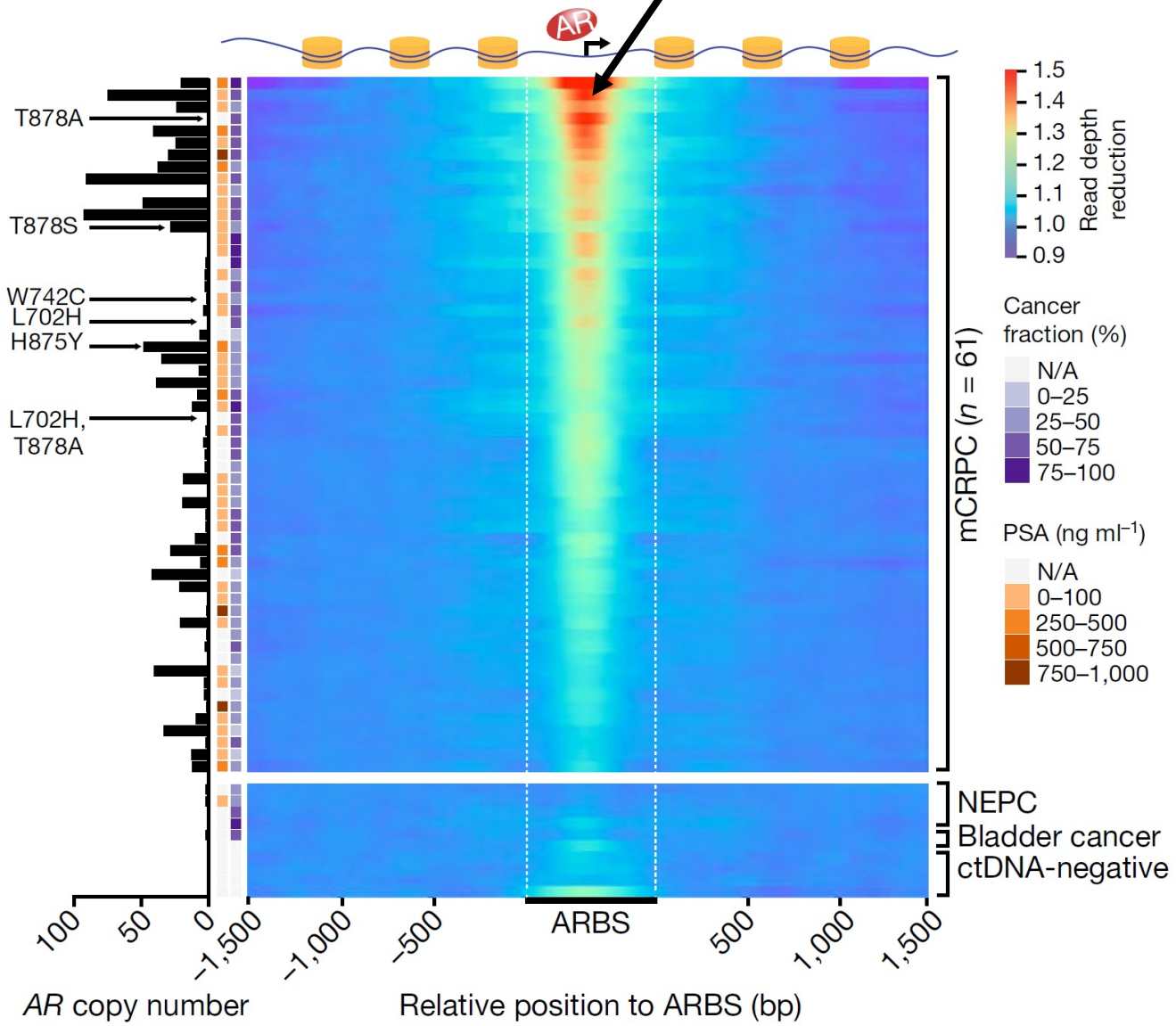
Most samples exhibited strong ARBS nucleosome depletion = **persistent AR signalling?**

This signal was largely absent from ctDNA negative samples, bladder cancer, and NEPC

ARBS nucleosome depletion correlated with average *AR* gene and enhancer copy-number (but not PSA)

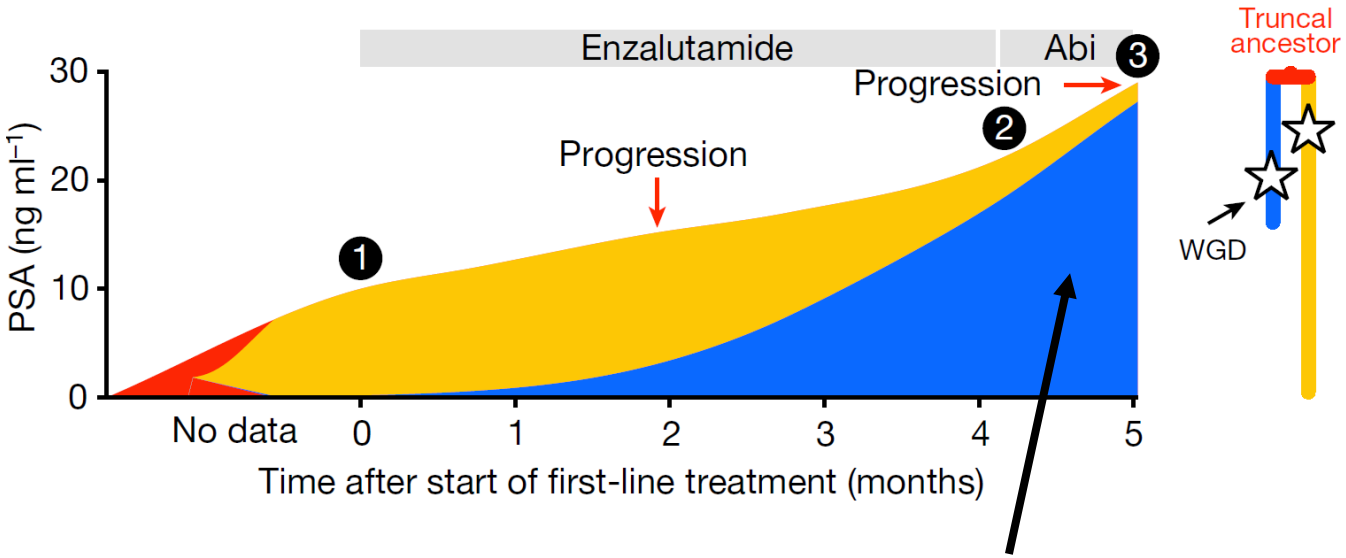
Cameron Herberts, Emilia Chen, Sarah Ng (UBC students)

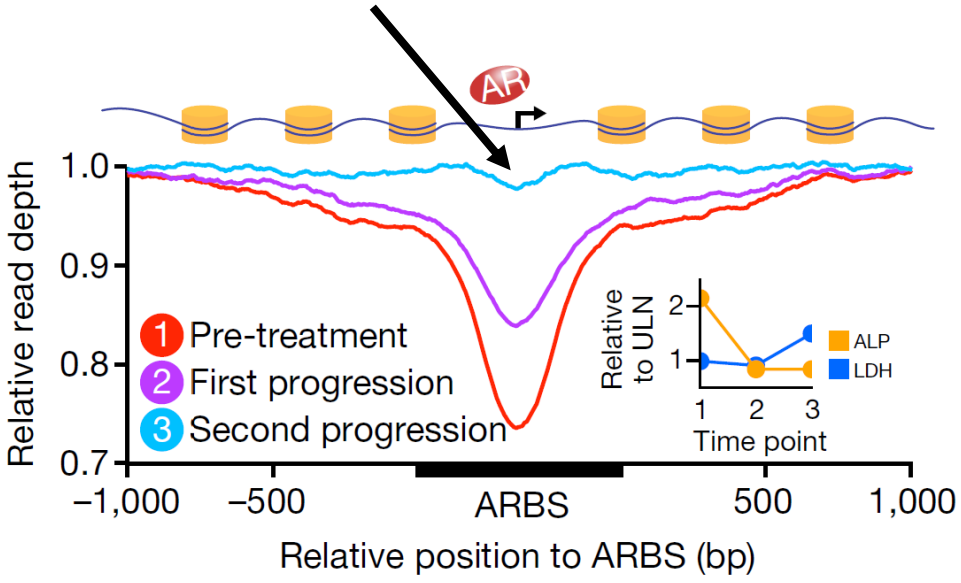
Read depth depletion at AR binding sites = AR engaged with DNA



Identifying emerging lineage plasticity through ctDNA phenotyping

Complete loss of ARBS nucleosome depletion signal in the new population





Rapid expansion of a new population with an *RB1* mutation

ctDNA fragmentomics: nascent field with unique challenges

1. **Low signal:noise ratio** (sampling stochasticity, GC bias, intrinsic properties of genome, other sources)

   Need prior knowledge of TFBS locations

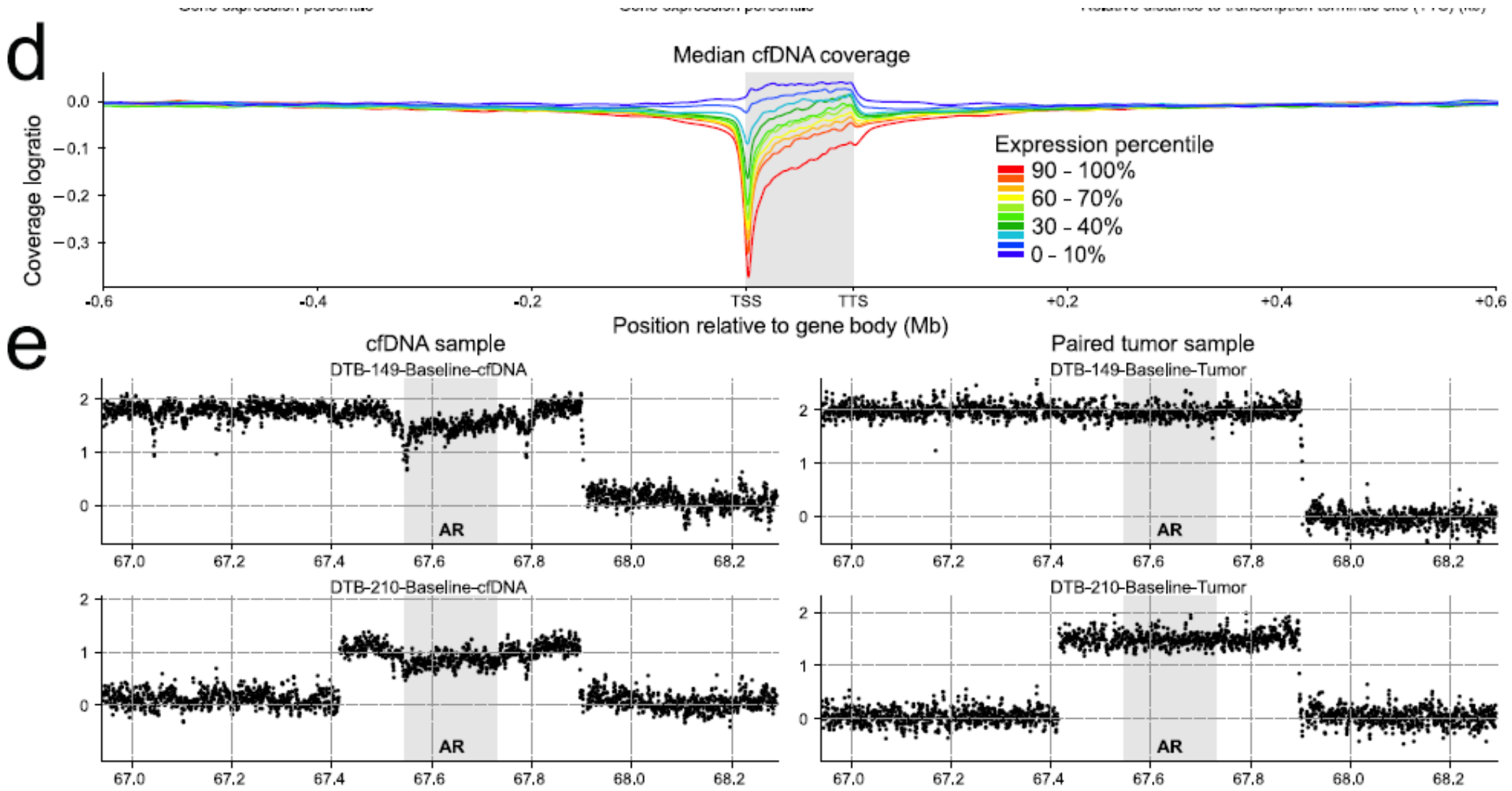
   Interrogating individual sites (i.e. single gene TSS or TFBS) usually not possible → **must examine many sites in aggregate**

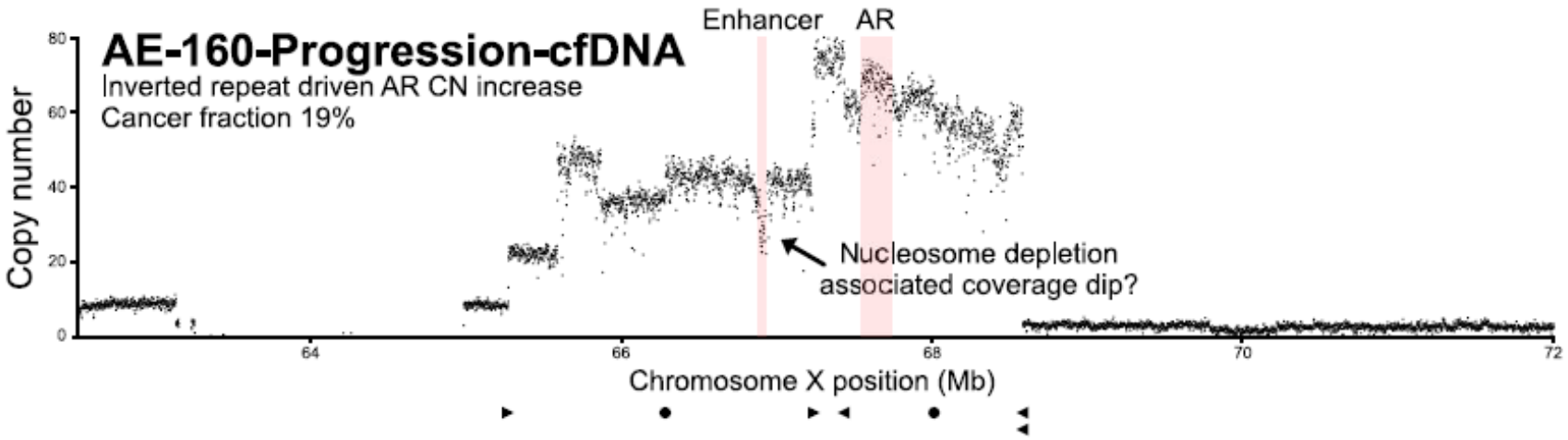
2. **Sensitivity and lower limits of detection** depend on sequencing depth, ctDNA-fraction, and patient-specific biology (heterogenous / unpredictable)

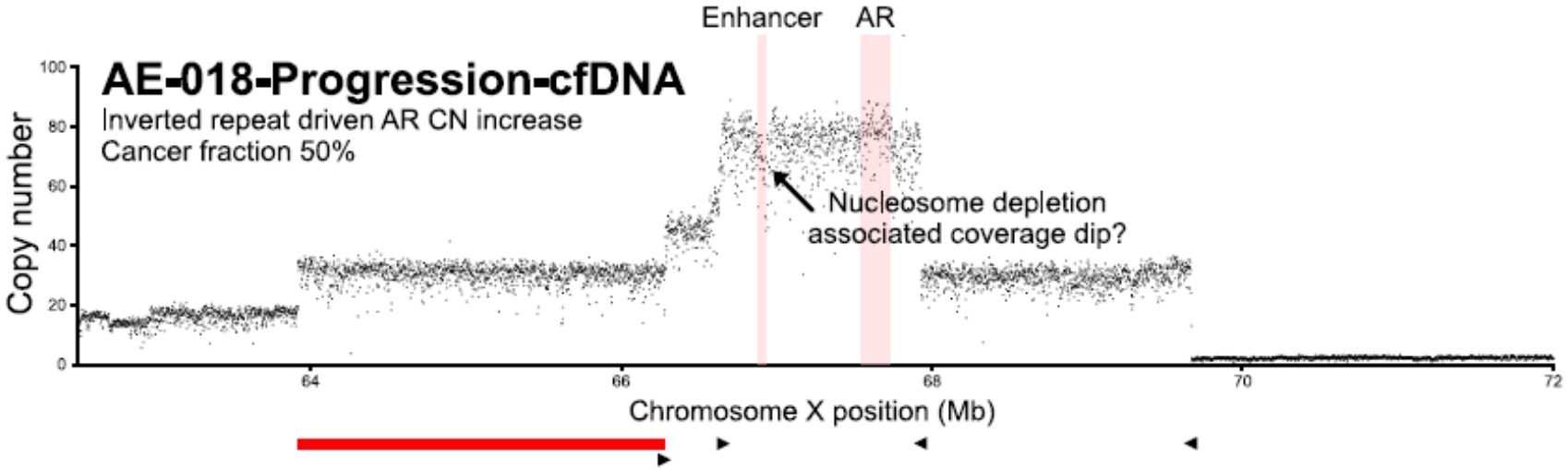
3. ***Implications for ctDNA-genotyping?***

   Reduced variant detection sensitivity at depth-depleted sites (e.g. TSS)

   Nucleosome-mediated variation in depth → source of noise for CNV calling?


d
Median cfDNA coverage
Coverage logratio
0.0
−0.1
−0.2
−0.3
Expression percentile
90 - 100%
60 - 70%
30 - 40%
0 - 10%
-0.6
-0.4
-0.2
TSS
TTS
+0.2
+0.4
+0.6
Position relative to gene body (Mb)
e
cfDNA sample
Paired tumor sample
DTB-149-Baseline-cfDNA
DTB-149-Baseline-Tumor
DTB-210-Baseline-cfDNA
DTB-210-Baseline-Tumor
AR
67.0
67.2
67.4
67.6
67.8
68.0
68.2


AE-160-Progression-cfDNA
Inverted repeat driven AR CN increase
Cancer fraction 19%
Enhancer
AR
Nucleosome depletion
associated coverage dip?
Copy number
Chromosome X position (Mb)
0
20
40
60
80
64
66
68
70
72


AE-018-Progression-cfDNA
Inverted repeat driven AR CN increase
Cancer fraction 50%
Enhancer
AR
Nucleosome depletion
associated coverage dip?
Copy number
Chromosome X position (Mb)
0
20
40
60
80
100
64
66
68
70
72

Article

Deep whole-genome ctDNA chronology of treatment-resistant prostate cancer

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Check for updates

Cameron Herberts[1,12], Matti Annala[1,2,12], Joonatan Sipola[2,12], Sarah W. S. Ng[1], Xinyi E. Chen[1], Anssi Nurminen[2], Olga V. Korhonen[2], Aslı D. Munzur[1], Kevin Beja[1], Elena Schönlau[1], Cecily Q. Bernales[1], Elie Ritch[1], Jack V. W. Bacon[1], Nathan A. Lack[1,3,4], Matti Nykter[2], Rahul Aggarwal[5,6], Eric J. Small[5,6], Martin E. Gleave[1], SU2C/PCF West Coast Prostate Cancer Dream Team*, David A. Quigley[5,7,8], Felix Y. Feng[5,6,7,9,13], Kim N. Chi[1,10,13] & Alexander W. Wyatt[1,11,13]

Circulating tumour DNA (ctDNA) in blood plasma is an emerging tool for clinical cancer genotyping and longitudinal disease monitoring[1]. However, owing to past emphasis on targeted and low-resolution profiling approaches, our understanding of the distinct populations that comprise bulk ctDNA is incomplete[2–12]. Here we perform deep whole-genome sequencing of serial plasma and synchronous metastases in patients with aggressive prostate cancer. We comprehensively assess all classes of genomic alterations and show that ctDNA contains multiple dominant populations, the evolutionary histories of which frequently indicate whole-genome doubling and shifts in mutational processes. Although tissue and ctDNA showed concordant clonally expanded cancer driver alterations, most individual metastases contributed only a minor share of total ctDNA. By comparing serial ctDNA before and after clinical progression on potent inhibitors of the androgen receptor (AR) pathway, we reveal population restructuring converging solely on *AR* augmentation as the dominant genomic driver of acquired treatment resistance. Finally, we leverage nucleosome footprints in ctDNA to infer mRNA expression in synchronously biopsied metastases, including treatment-induced changes in AR transcription factor signalling activity. Our results provide insights into cancer biology and show that liquid biopsy can be used as a tool for comprehensive multi-omic discovery.

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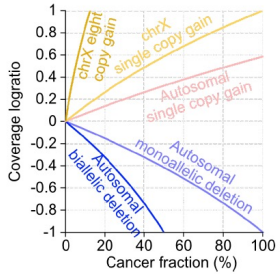
250 page supplementary methods with walkthroughs

Coverage logratios contain information about the relative copy number of different genomic regions, but do not directly measure absolute copy number, as they are confounded by other factors, such as the total number of sample DNA fragments sequenced, the fraction of normal cell DNA in a sample, and the presence of whole genome duplication (which can uniformly increase the copy number of all genomic regions). In samples composed of a homogeneous cancer cell population and a normal cell population at fractions *F* and 1 - *F*, the coverage logratio of a genomic segment can be modeled as

$$LR = \log_2 \frac{FC + (1-F)\,P}{P} + D \quad \text{where}$$

F = cancer fraction in sample
C = copy number in cancer cells
P = copy number in normal cells
D = diploid level (coverage logratio at C=2)

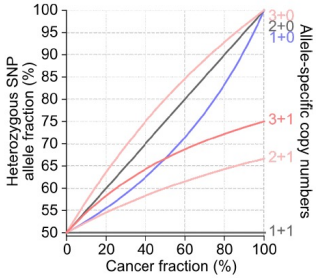
D=0



For a heterozygous SNP with allele-specific copy numbers A and B (such that A+B=C) in a cfDNA/tumor sample, the B allele fraction can be modeled as

$$HSAF = \frac{BF + (1-F)\cdot 1}{F(A+B) + (1-F)\cdot 2} \quad \text{where}$$

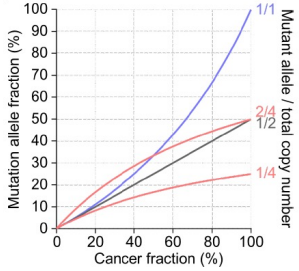
F = cancer fraction in sample
A = copy number of allele A in cancer cells
B = copy number of allele B in cancer cells



For a somatic mutation present in a sample's cancer cells, the mutation allele fraction can be modeled as

$$MAF = \frac{MF}{F(M+N) + (1-F)\cdot P} \quad \text{where}$$

F = cancer fraction in sample
M = mutant allele copy number in cancer cells
N = wildtype allele copy number in cancer cells
P = wildtype allele copy number in normal cells



To infer the allele-specific copy number of genomic segments, we searched for a copy number model providing the best fit to the observed coverage logratios, HSAFs, and somatic mutation allele fractions (MAF) in autosomal regions. Each model had only two parameters: the cancer fraction *F* and diploid level *D*. There is