Combinatorial genomic and epigenomic analysis of plasma cell-free DNA identifies stemness features associated with worse prognosis in high-risk metastatic castration resistant prostate cancer

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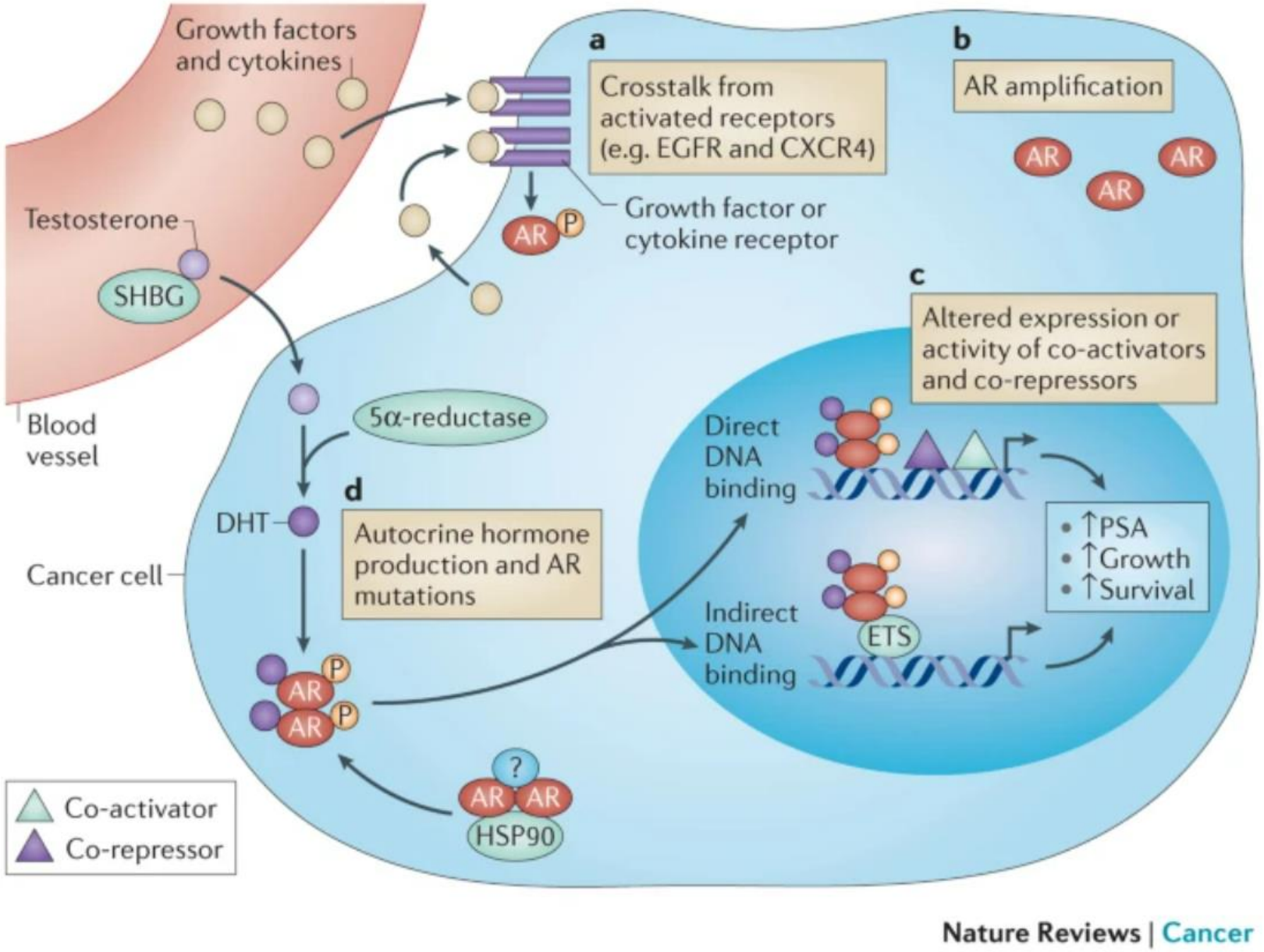
Outline

- General Background Information
  - Prior Research
- Current project
  - Methods
  - Results and Discussion
    - Methylation
    - Nucleosome profiling
    - Stemness Analysis

Prostate Cancer

- How common is prostate cancer?
  - Prostate cancer is the most common cancer among American men other than skin cancer.
  - About 288,300 new cases of prostate cancer
- Risk of Prostate Cancer
  - About 1 man in 9 will be diagnosed with prostate cancer during lifetime
- Death from Prostate Cancer
  - Prostate cancer is the second leading cause of cancer death among men in USA behind lung cancer
  - About 34,700 deaths from prostate cancer

Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023

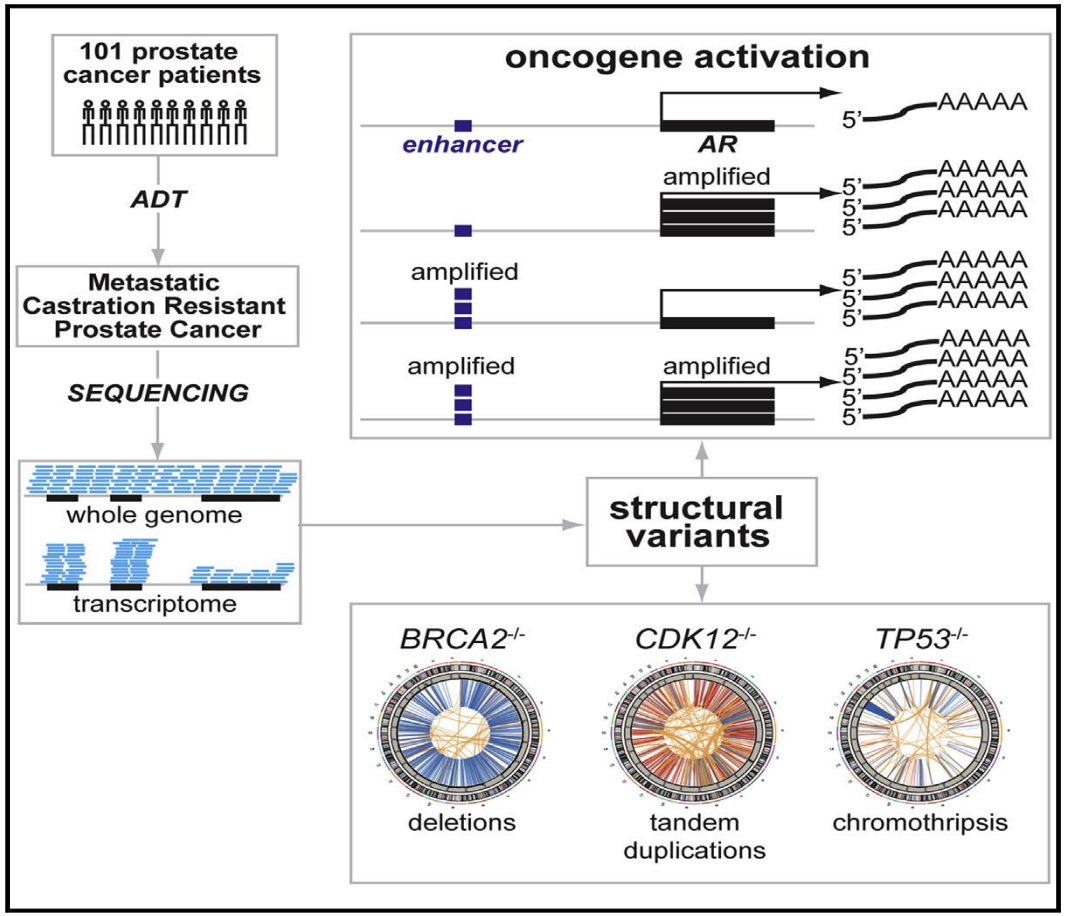


Mills, Nature, 2014

Cell

Genomic Hallmarks and Structural Variation in Metastatic Prostate Cancer

Graphical Abstract



Authors

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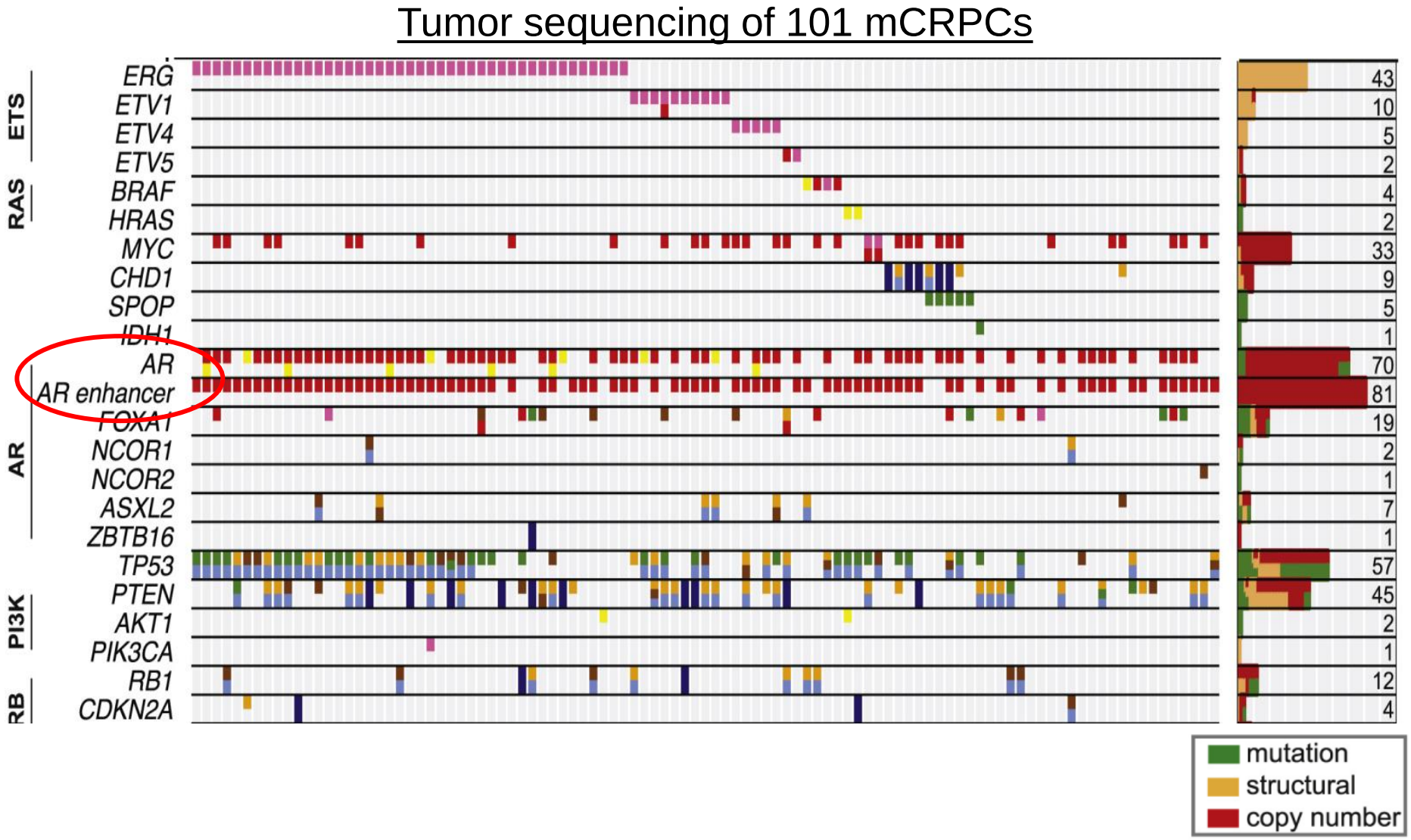
In Brief

Integrative whole-genome and -transcriptome sequencing provides a comprehensive view of structural variations that affect major regulators in prostate cancer and would escape detection by exome-based approaches.

Quigley & Dang et al, Cell, 2018

AR gene body and enhancer are commonly altered in mCRPC

81% of tx-resistant patients had amplification of an enhancer region 624 kb upstream of AR, 11% more than had gene body alterations.



Quigley & Dang et al, Cell, 2018

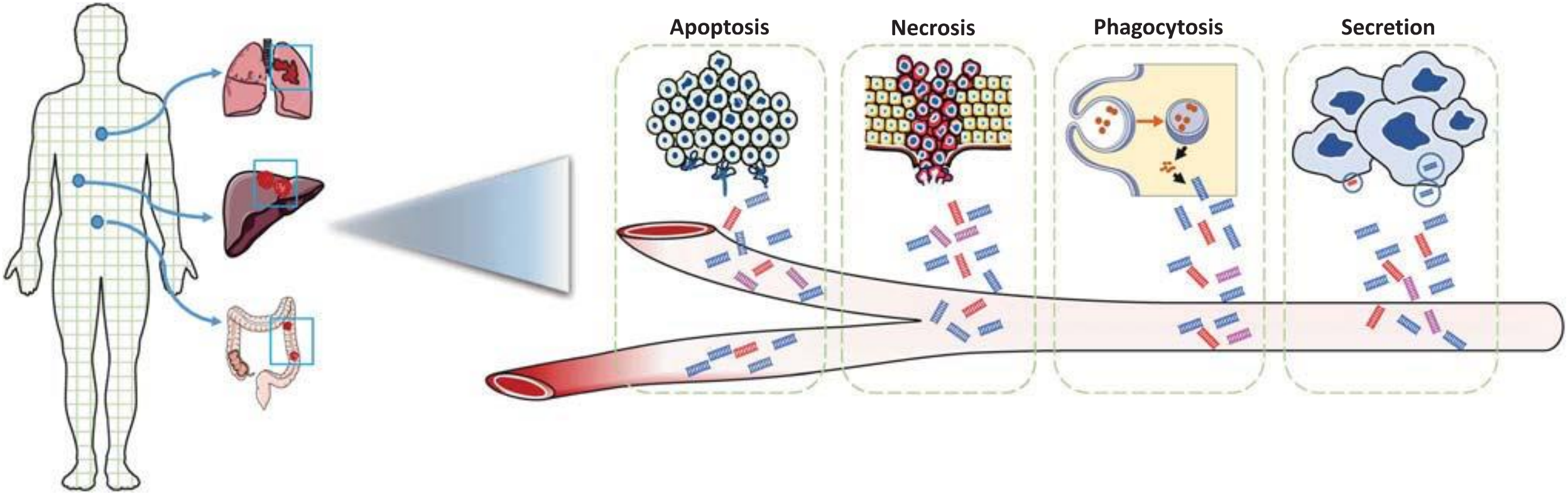
Highlights

- Deep whole-genome and -transcriptome sequencing of 101 prostate cancer metastases
- Tandem duplication affects intergenic regulatory loci upstream of AR and MYC
- Inactivation of CDK12, TP53, and BRCA2 affect distinct classes of structural variants
- Androgen receptor is affected by mutation or structural variation in 85% of mCRPC

Questions

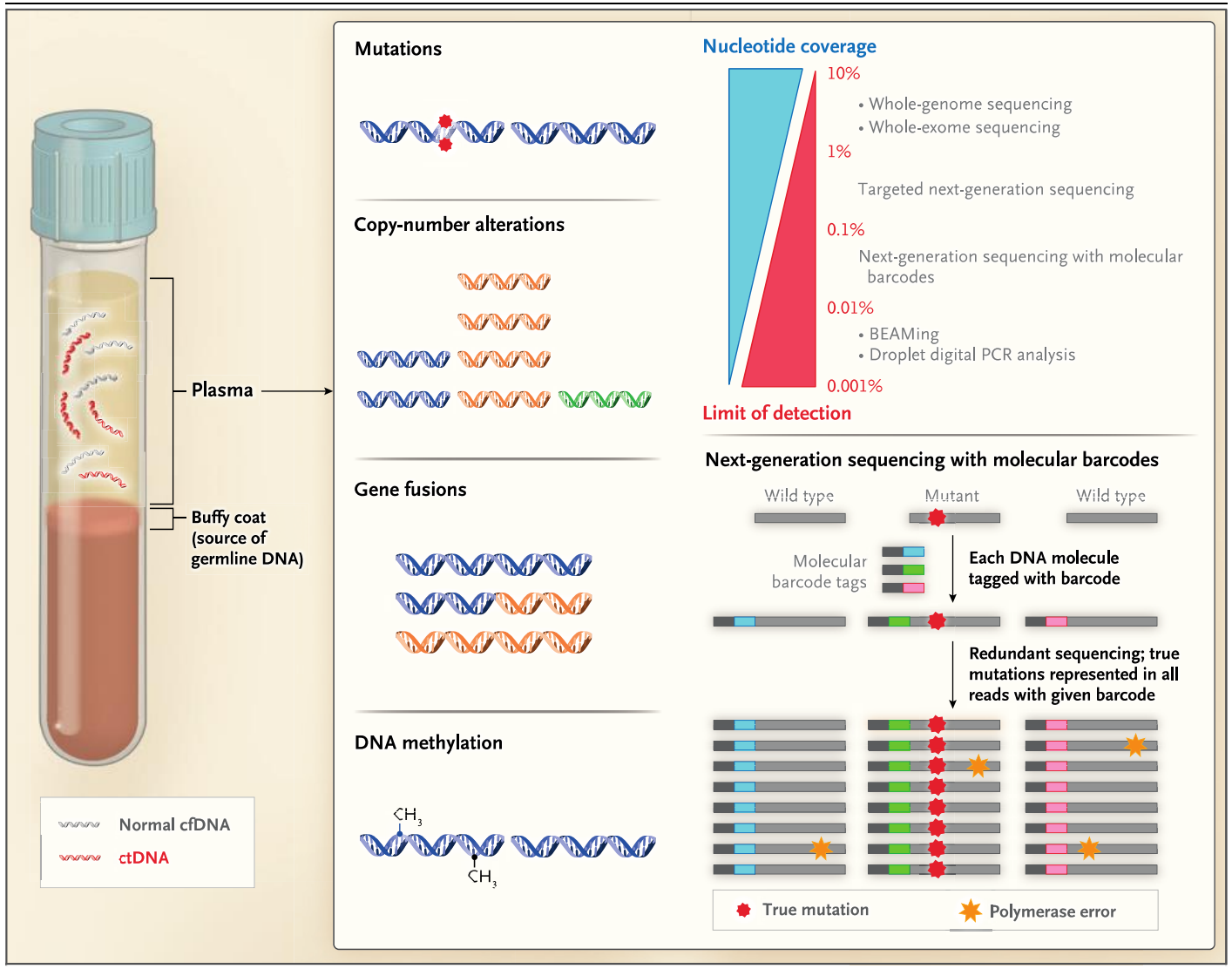
- Can we detect somatic alterations in cell-free DNA from metastatic prostate cancer patients?
  - Copy number alterations (particularly of AR and AR enhancer)
  - Gene rearrangements (i.e. TMPRSS2-ERG)
  - Single nucleotide variants & indels

Mechanisms of ctDNA release



Chin et al., Molecular Diagnosis & Therapy, 2019

Liquid biopsy



Corcoran & Chabner, NEJM, 2018

original reports

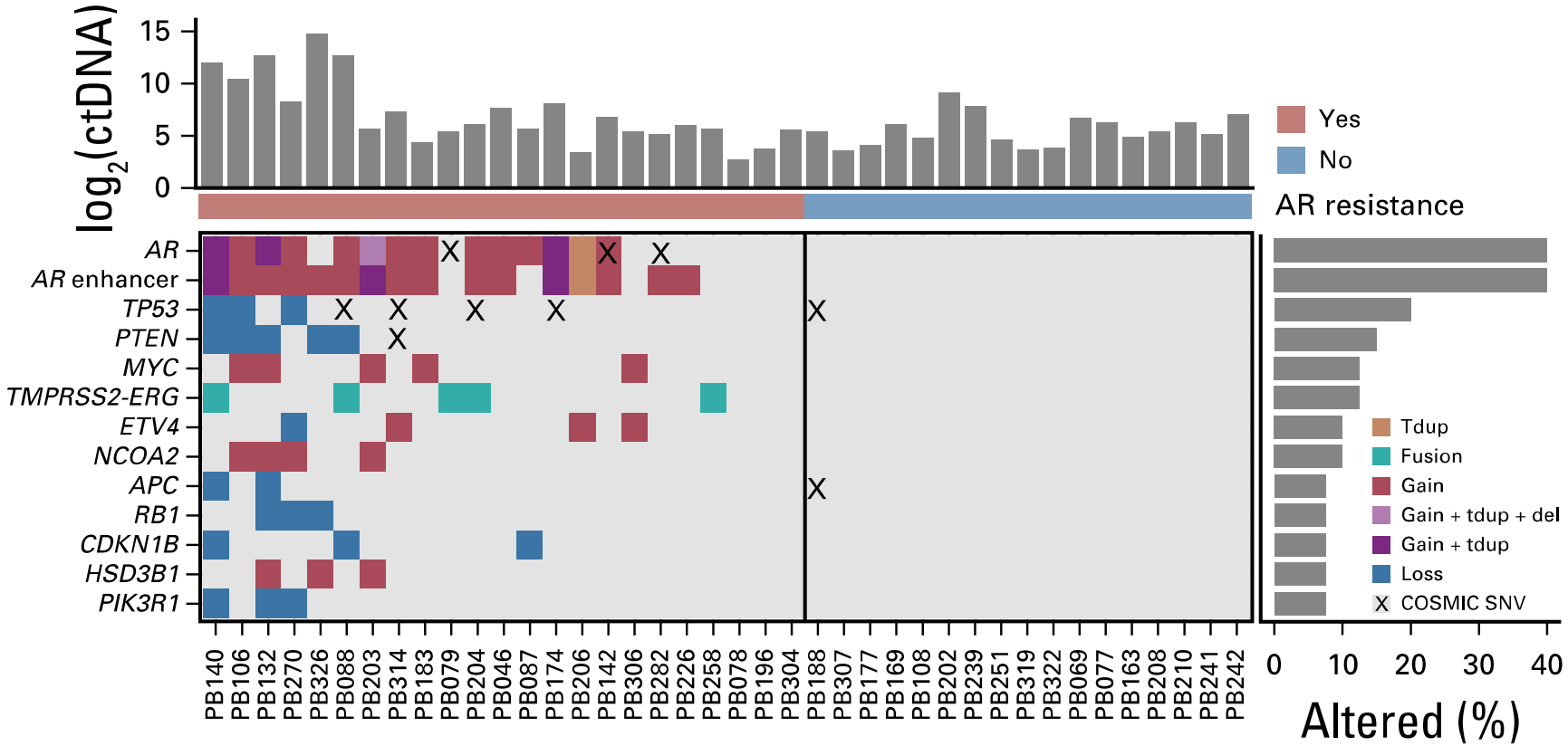
Cell-Free DNA Alterations in the *AR* Enhancer and Locus Predict Resistance to AR-Directed Therapy in Patients With Metastatic Prostate Cancer

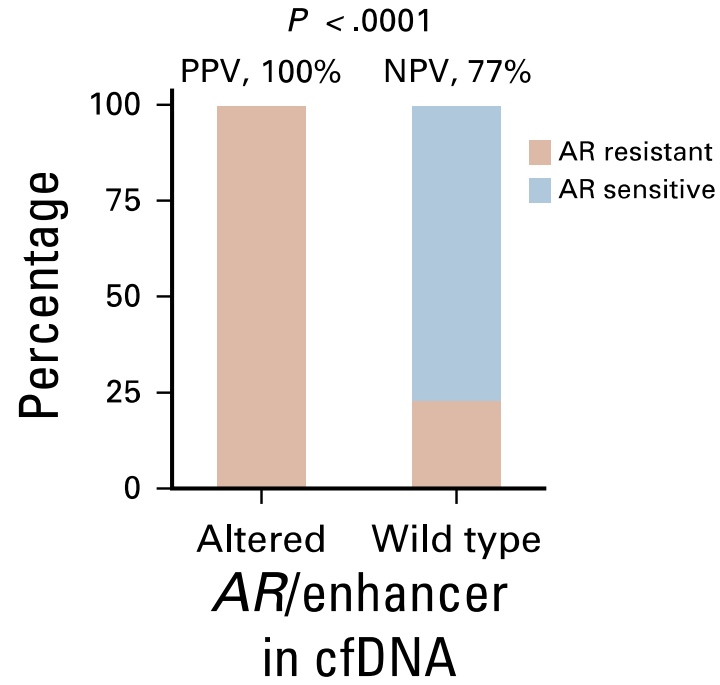
Ha X. Dang, PhD[1,2,3]; Pradeep S. Chauhan, PhD[4]; Haley Ellis, MD[1,4]; Wenjia Feng, MS[4]; Peter K. Harris, PhD[4]; Grace Smith, BS[4]; Mark Qiao, BS[4]; Katherine Dienstbach, MPH[1,3]; Rachel Beck, PhD[1,3]; Andrew Atkocius, BS[1,3]; Faridi Qaium, BS[4]; Jingqin Luo, PhD[5]; Jeff M. Michalski, MBA, MD[3,4]; Joel Picus, MD[1,3]; Russell K. Pachynski, MD[1,3]; Christopher A. Maher, PhD[1,2,3,6]; and Aadel A. Chaudhuri, MD, PhD[3,4,6,7,8]

JCO Precision Oncology 2020

Landscape of Somatic and Structural Alterations in Metastatic Prostate Cancer

cell-free DNA



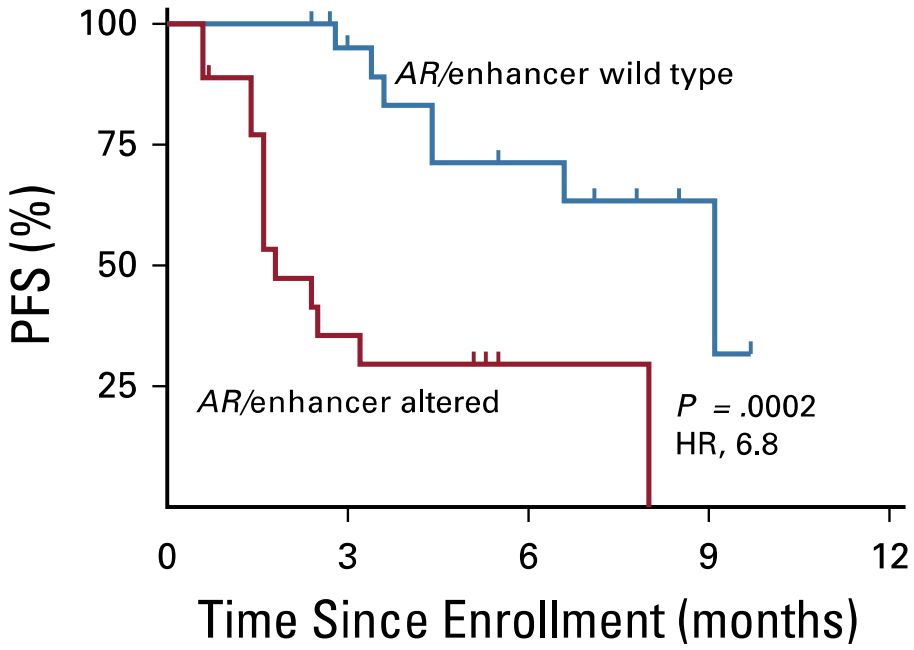


AR locus alterations apparent in cell-free DNA in 45% of AR-directed treatment-resistant cases.

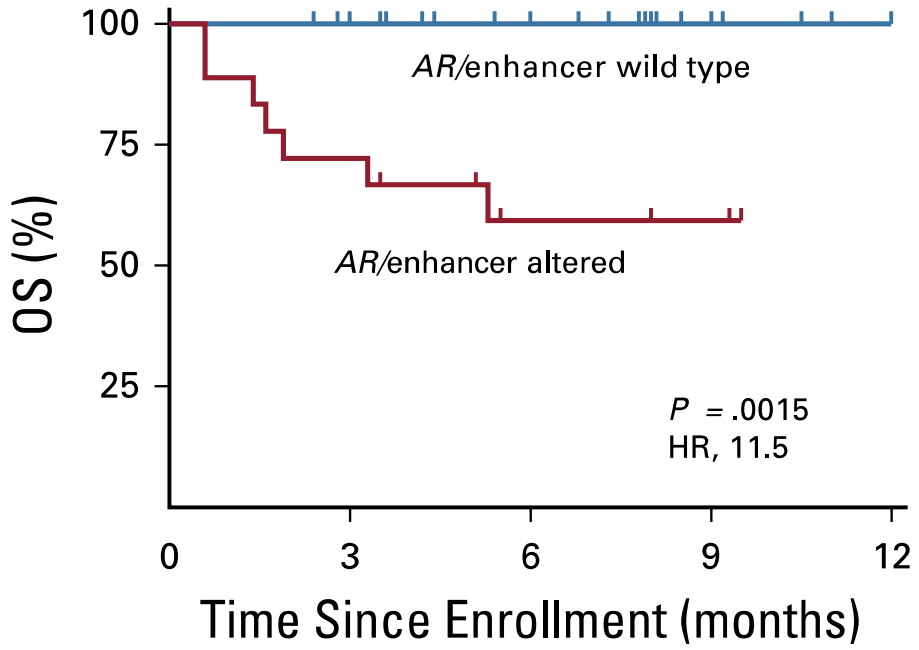
AR locus alterations portended resistance with high sensitivity and specificity.

Dang, Chauhan, Ellis,…, Chaudhuri, JCO PO, 2020

AR locus alterations predict primary resistance to AR-directed therapy



No. at risk:					
AR/enhancer altered	18	6	1	0	0
AR/enhancer wild type	22	17	9	2	0



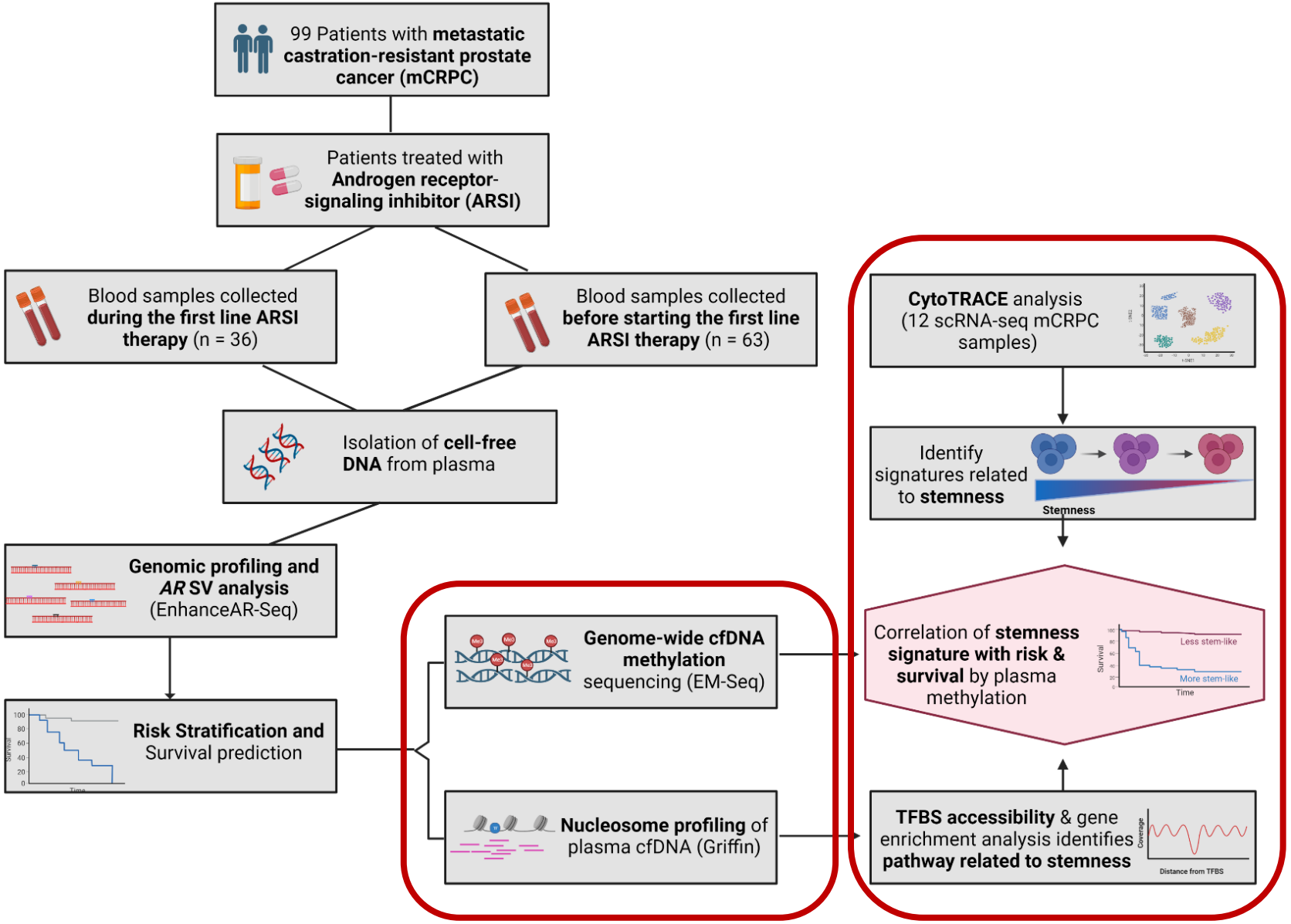
No. at risk:					
AR/enhancer altered	18	12	3	2	0
AR/enhancer wild type	22	20	14	5	0

Dang, Chauhan, Ellis,…, Chaudhuri, JCO PO, 2020

Can we learn more about the underlying biology of high-risk mCRPC by studying cfDNA epigenomics?

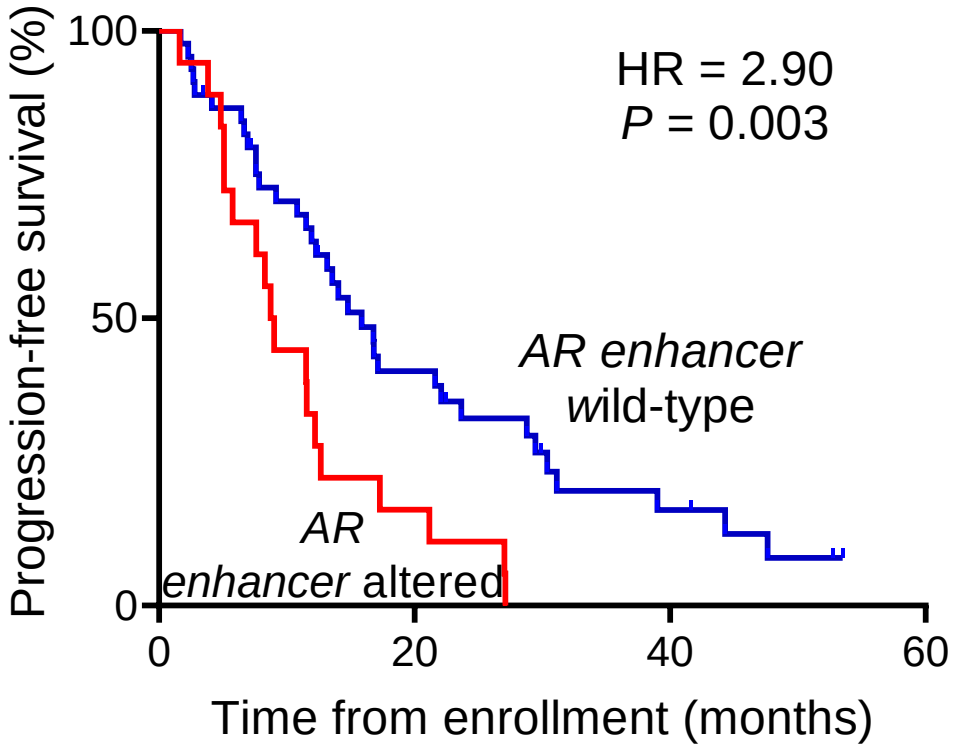
Current Project

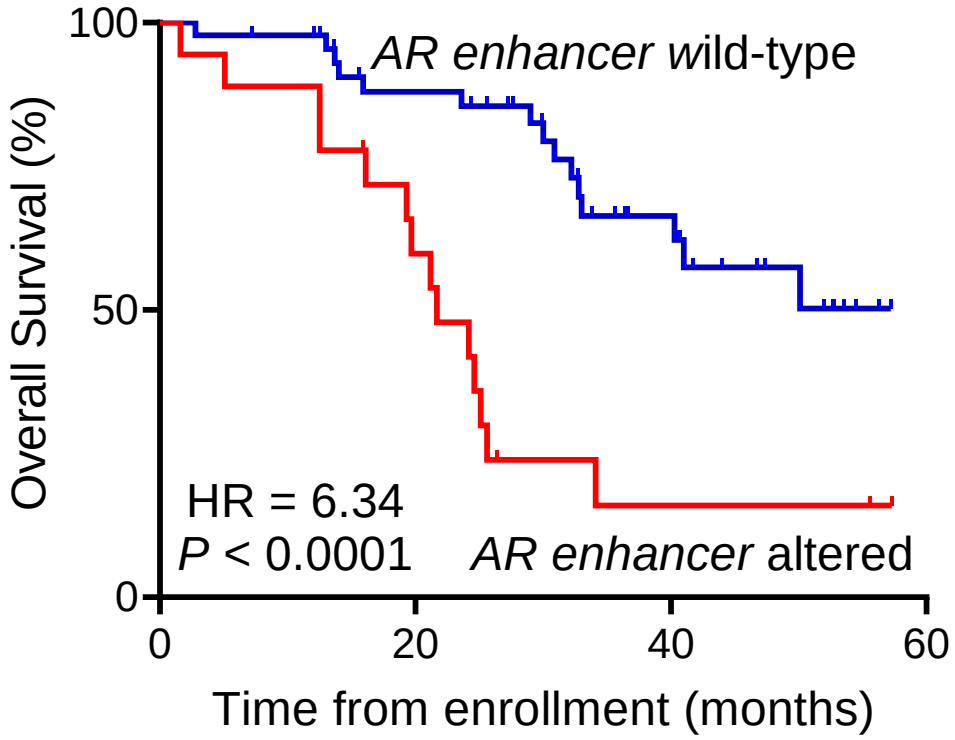
Combinatorial genomic and epigenomic cfDNA analysis of high-risk mCRPC



Kaplan-Meier analysis in plasma cfDNA samples analyzed prior to first-line ARSI treatment for *AR* enhancer region

Pre-treatment samples (63)

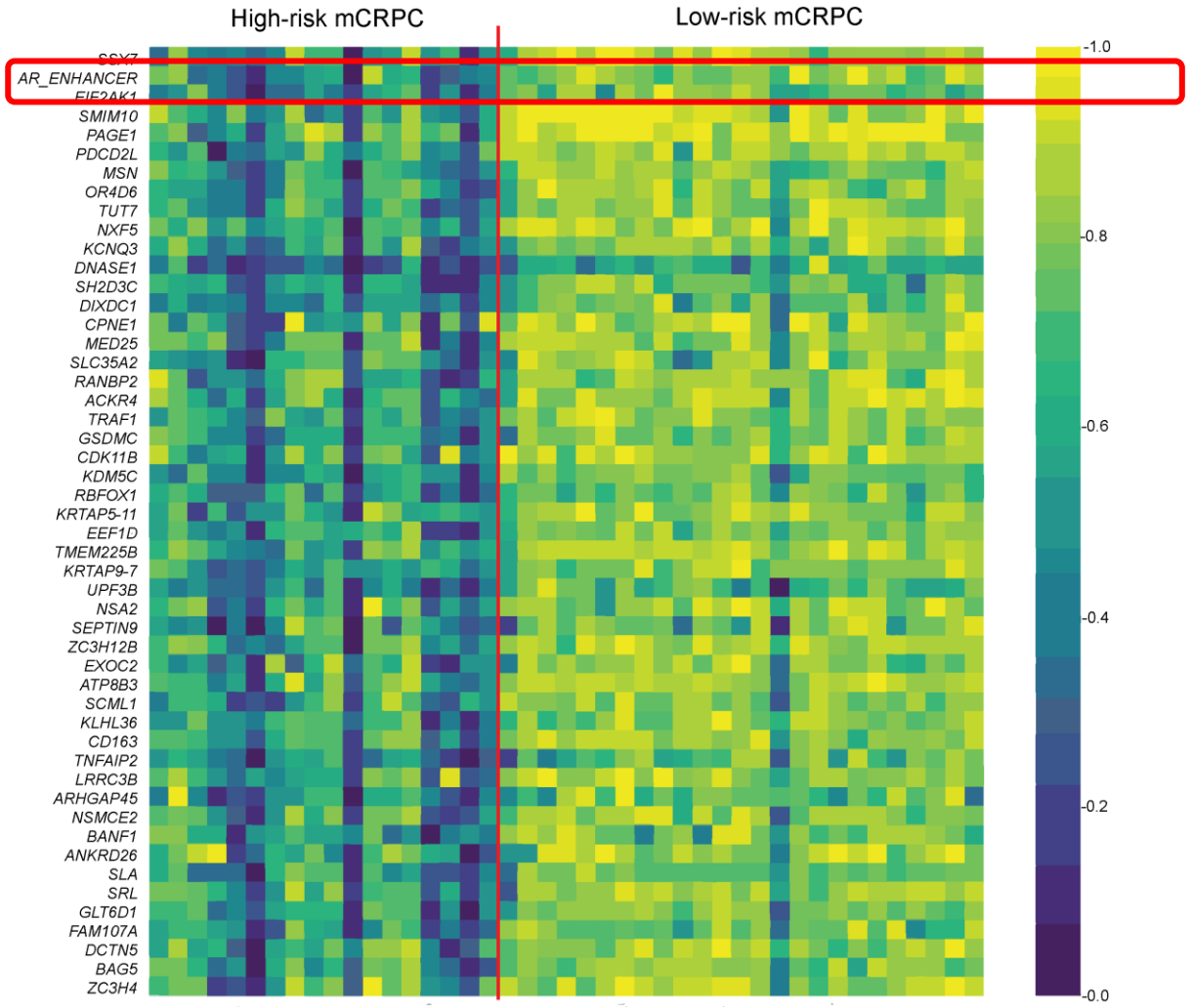


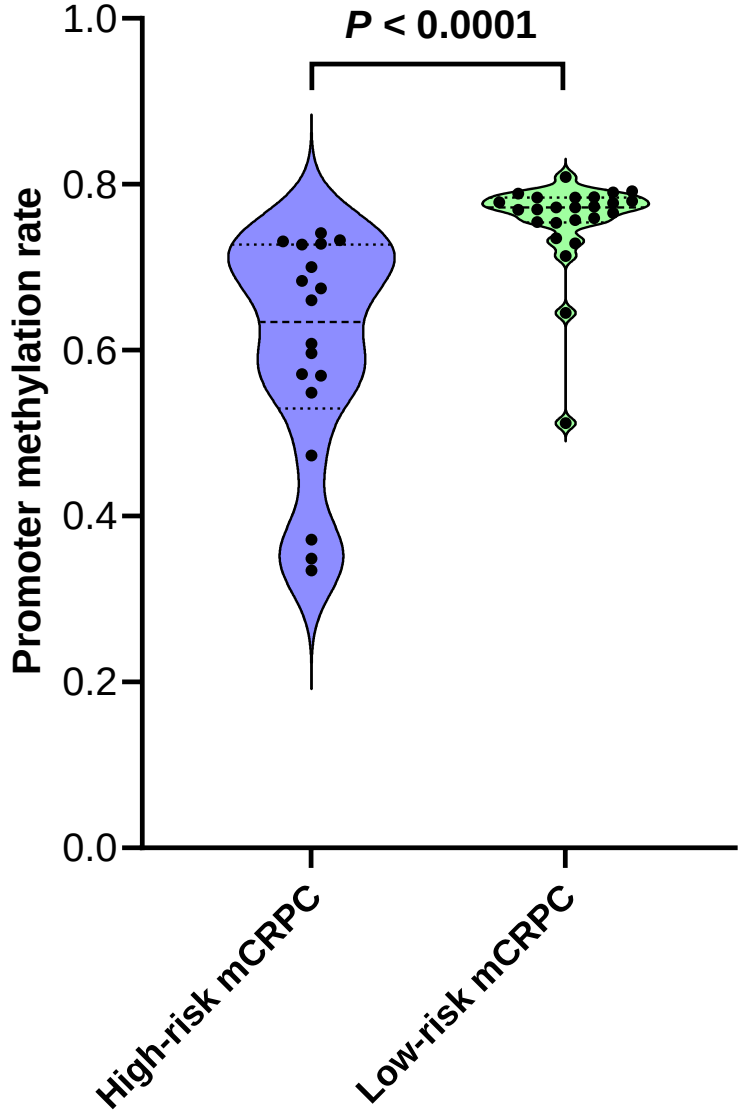


AR enhancer region associate with worse clinical outcomes in mCRPC patients treated with first-line *AR*-directed therapy

cfDNA methylation : Genome-wide distribution of significantly hypomethylated DMRs in pre-treatment plasma

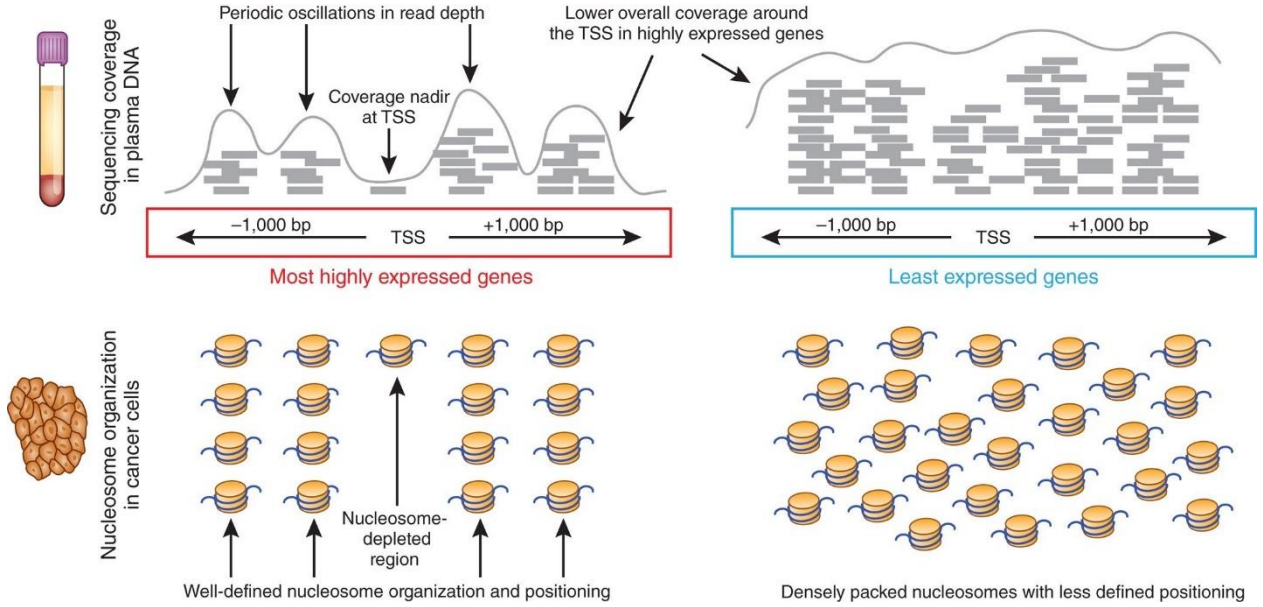
Top 50 hypomethylated DMRs in pre-treatment cfDNA



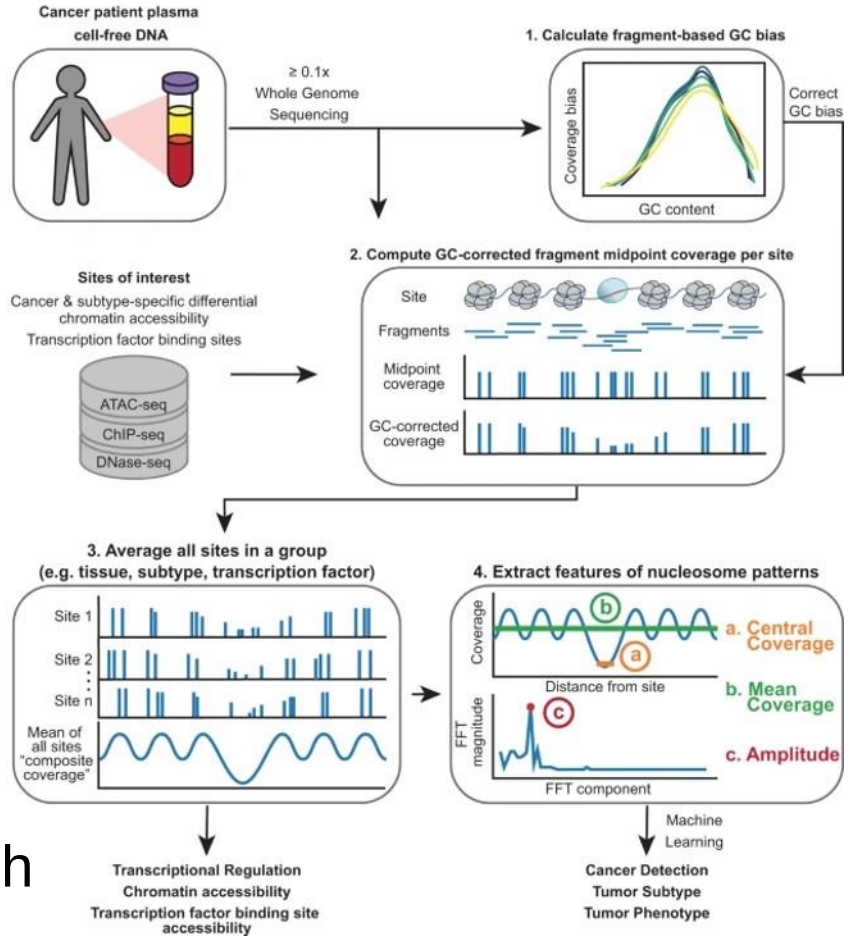


Can we infer epigenomic or transcriptomic features from cfDNA fragmentomics profiling

Griffin – Nucleosome profiling of cell-free DNA



Murtaza & Caldas. Nature Genetics, 2016

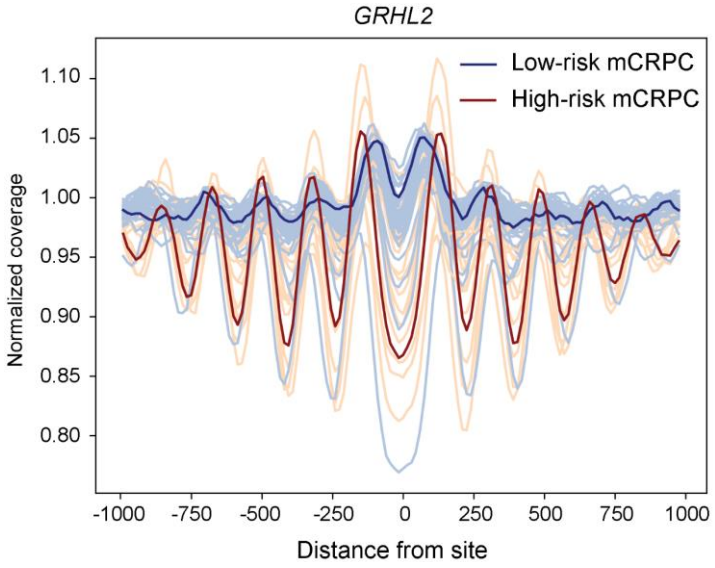


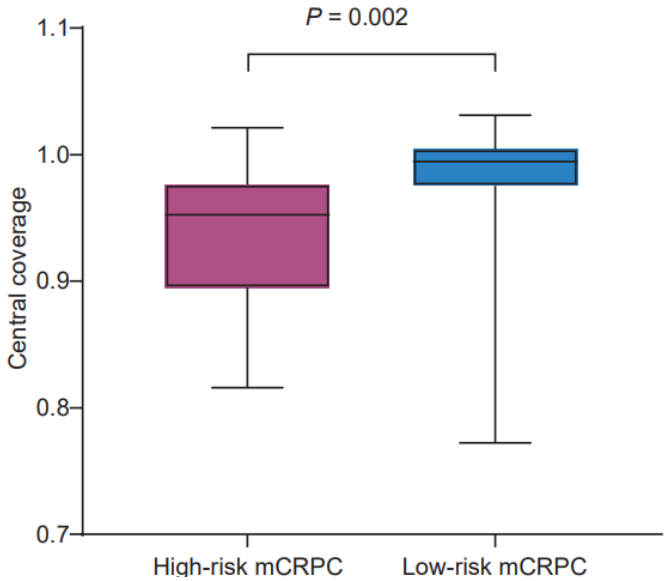
377 TFs with ~10K TFBS each

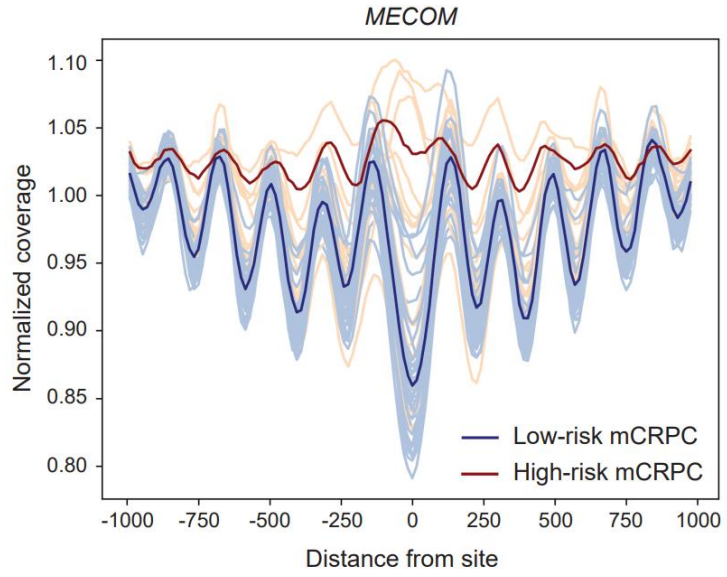
Nucleosome position reflects important cellular process: Transcriptional regulation, Transcription factor binding

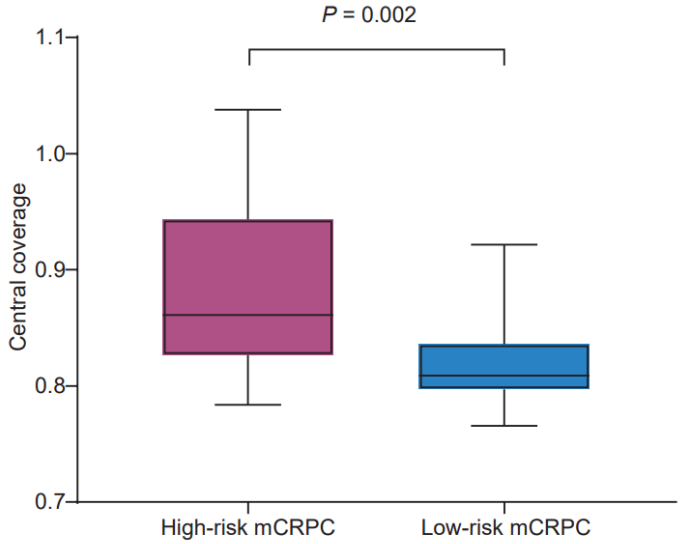
Doebley & Ha et al. Nature Communication, 2022

Central coverage profiles for TFBS sites corresponding to transcription factors, *GRHL2* and *MECOM* in high-risk mCRPC patients



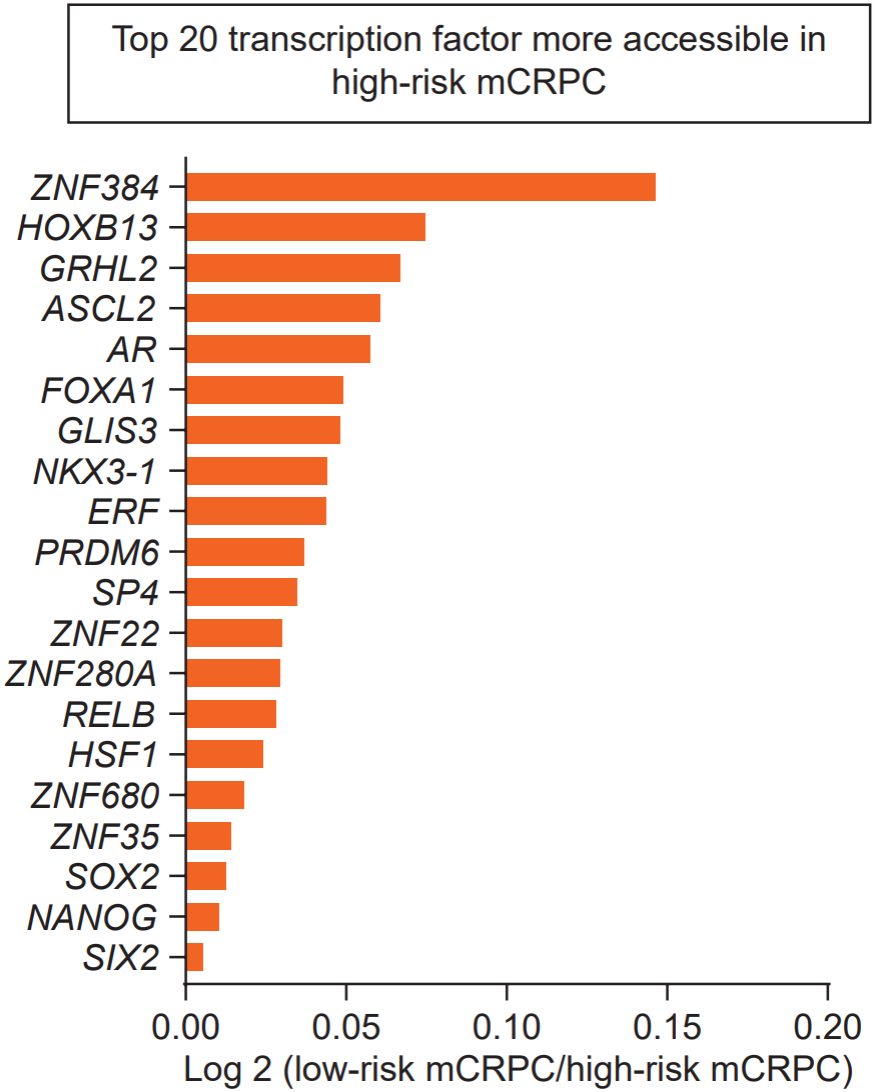


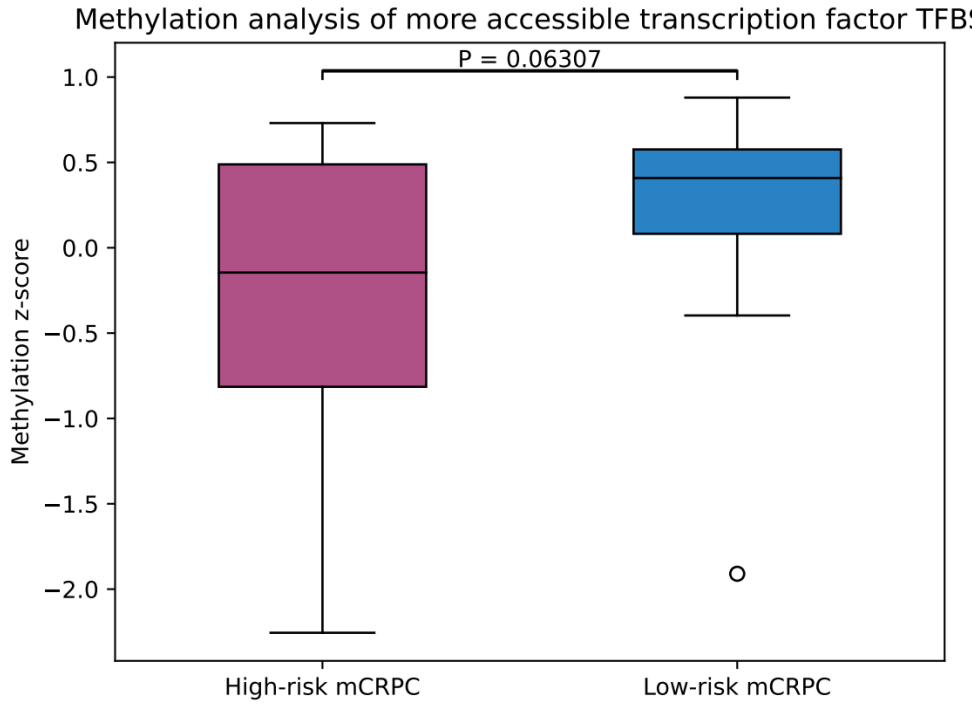




Top 20 most accessible TFs in high-risk patients

TFBS cfDNA methylation analysis



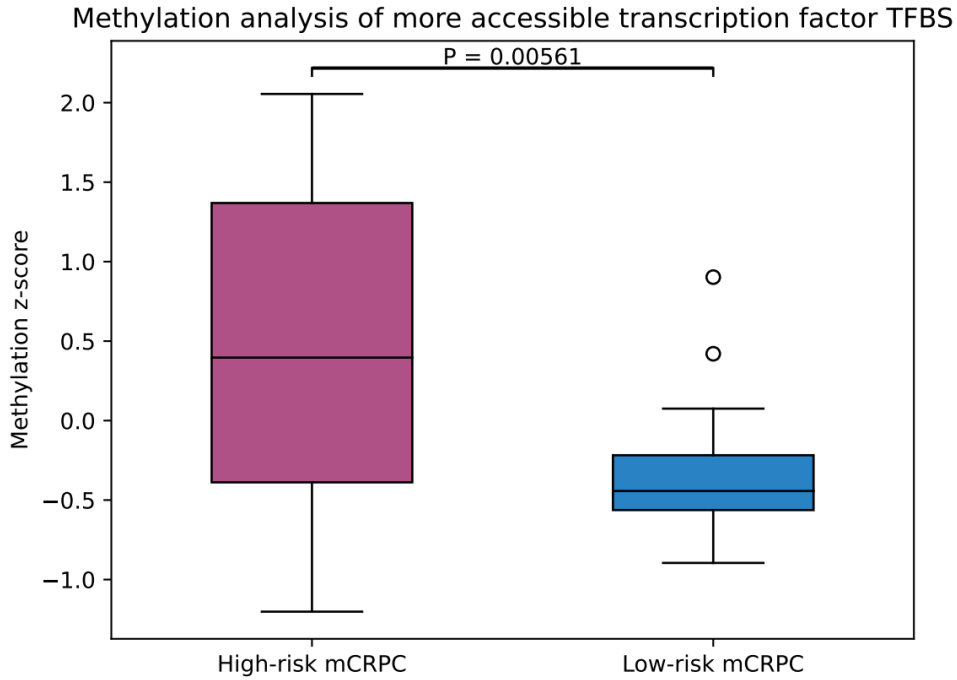


Top 20 least accessible TFs in high-risk patients

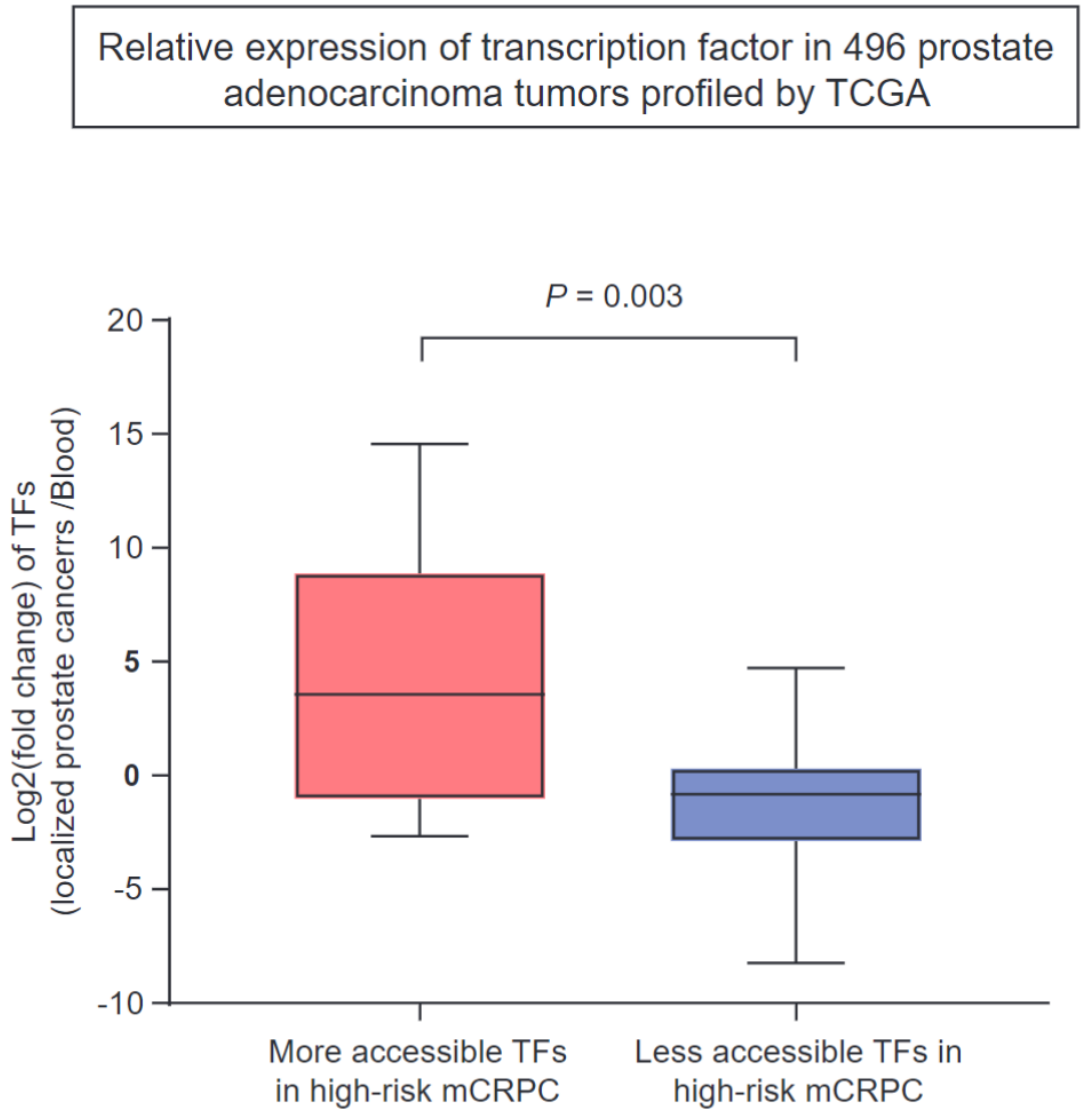
TFBS cfDNA methylation analysis

Top 20 transcription factor less accessible in high-risk mCRPC

LYL1
MECOM
MAFF
CEBPA
FOXO1
MAFK
HIC1
SPIB
MEF2A
GFI1
NR4A1
MEF2C
BCL11A
SPI1
ZBTB16
BACH2
ZFX
RUNX3
TCF3
SMAD5



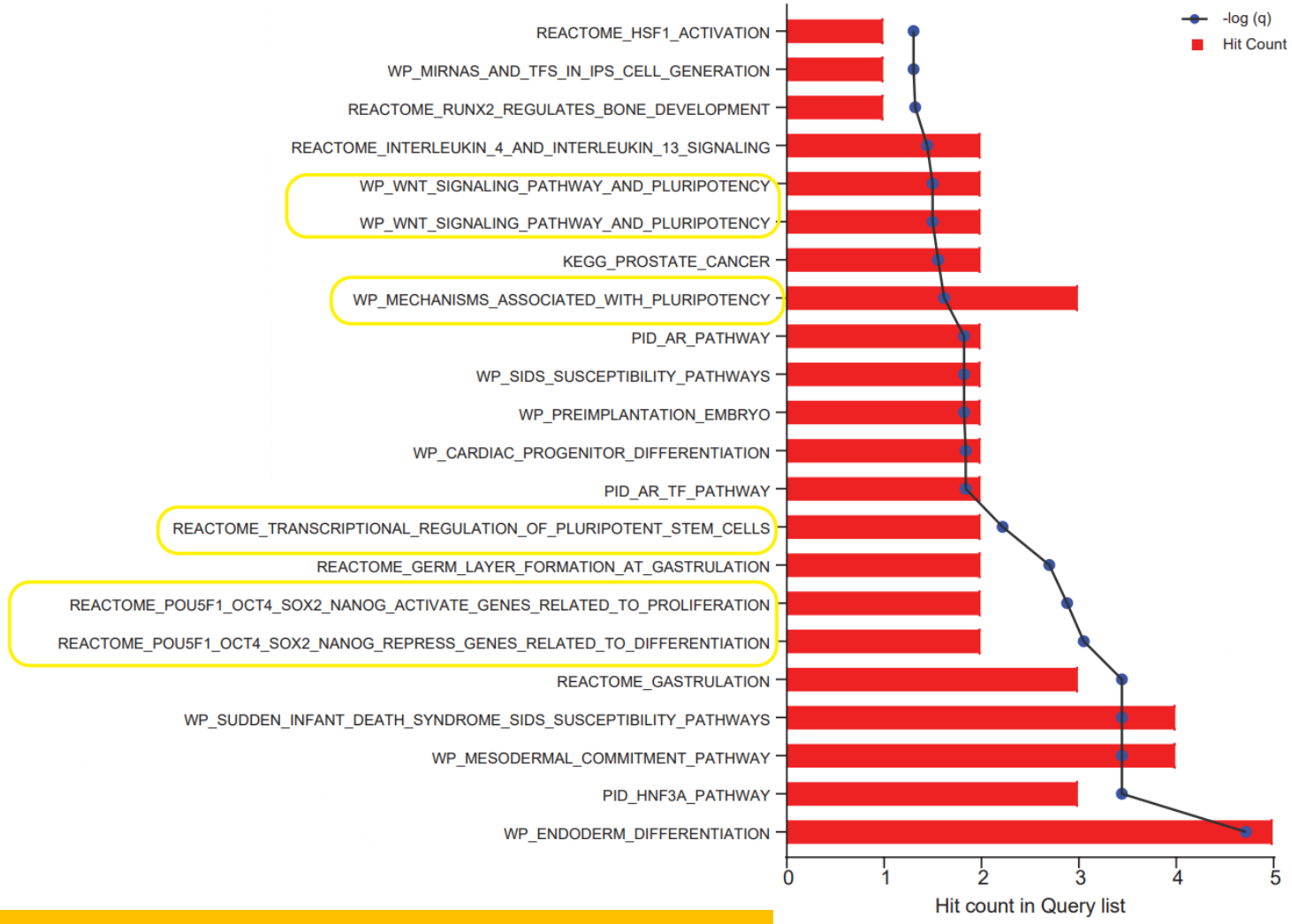
Most and least accessible TFs fold change comparison



Gene enrichment analysis of top 20 transcription factor accessible in high-risk mCRPC

Top 20 transcription factor more accessible in high-risk mCRPC

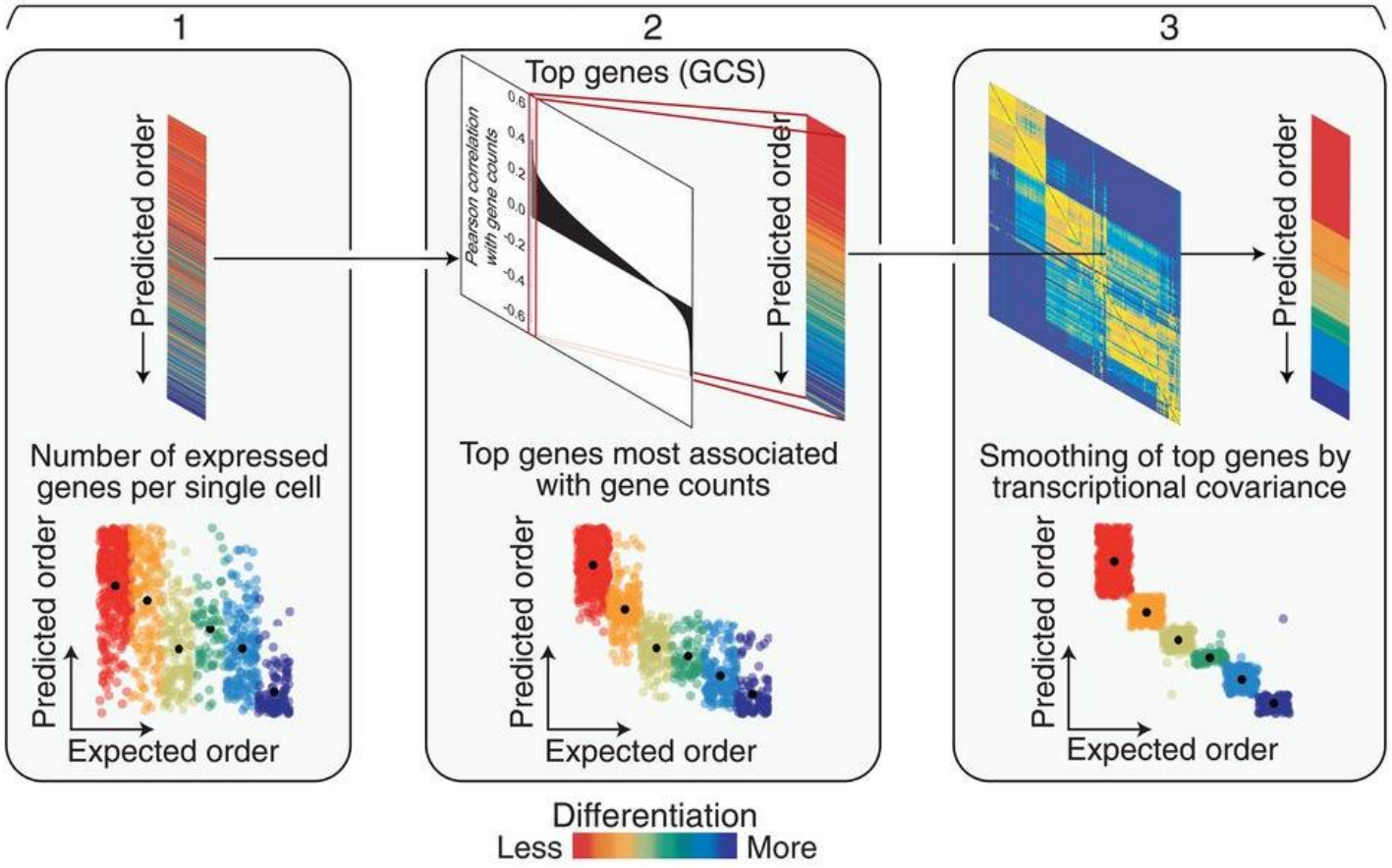
ZNF384
HOXB13
GRHL2
AR
ASCL2
FOXA1
GLIS3
NKX3-1
ERF
PRDM6
HMGA1
ZNF22
ZNF280A
SP1
RELB
SP4
NFKB2
HNF4G
HSF1



Stem-cell signatures were significantly enriched in high-risk mCRPC

Stemness analysis

CytoTRACE : Identify the stem-cell signatures



CytoTRACE cell score

Each cell is given a stemness score

- 1: More stem-like
- 0: Less stem-like

CytoTRACE Gene score

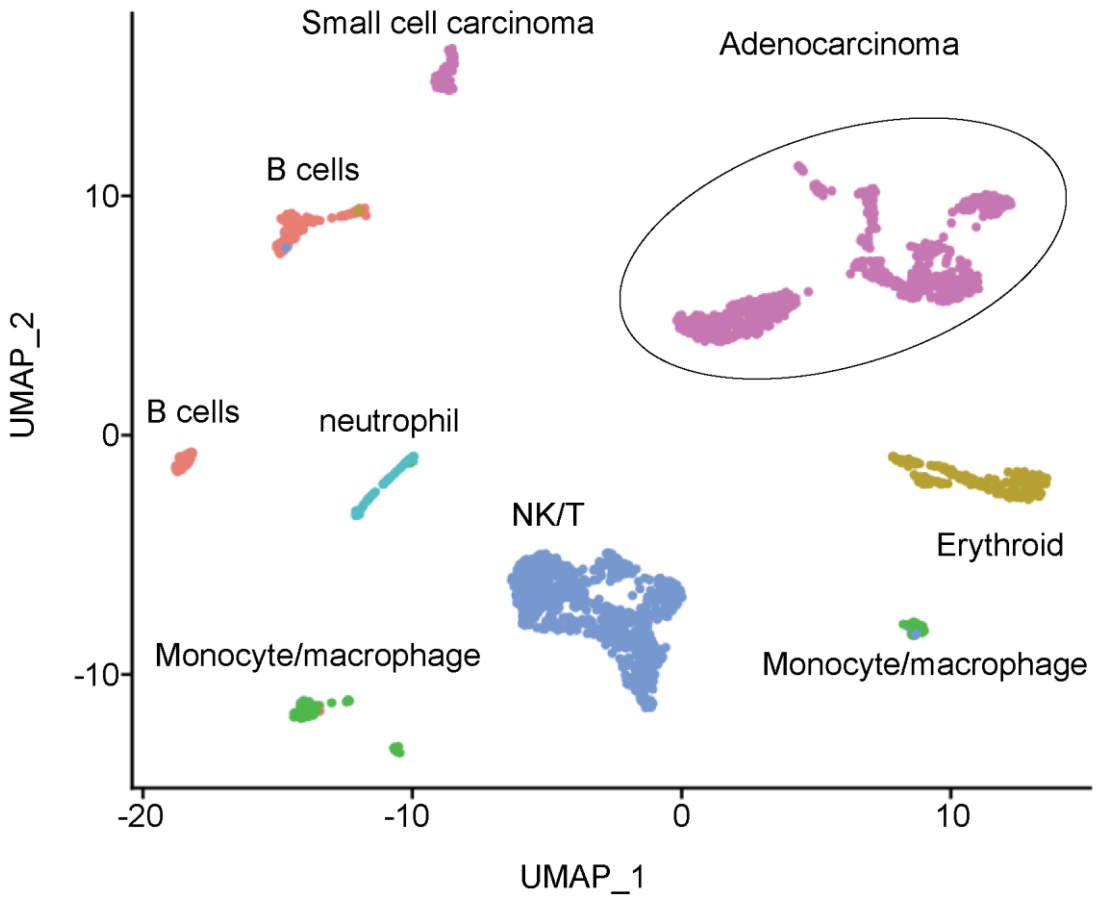
Each cell state-specific gene is given a score

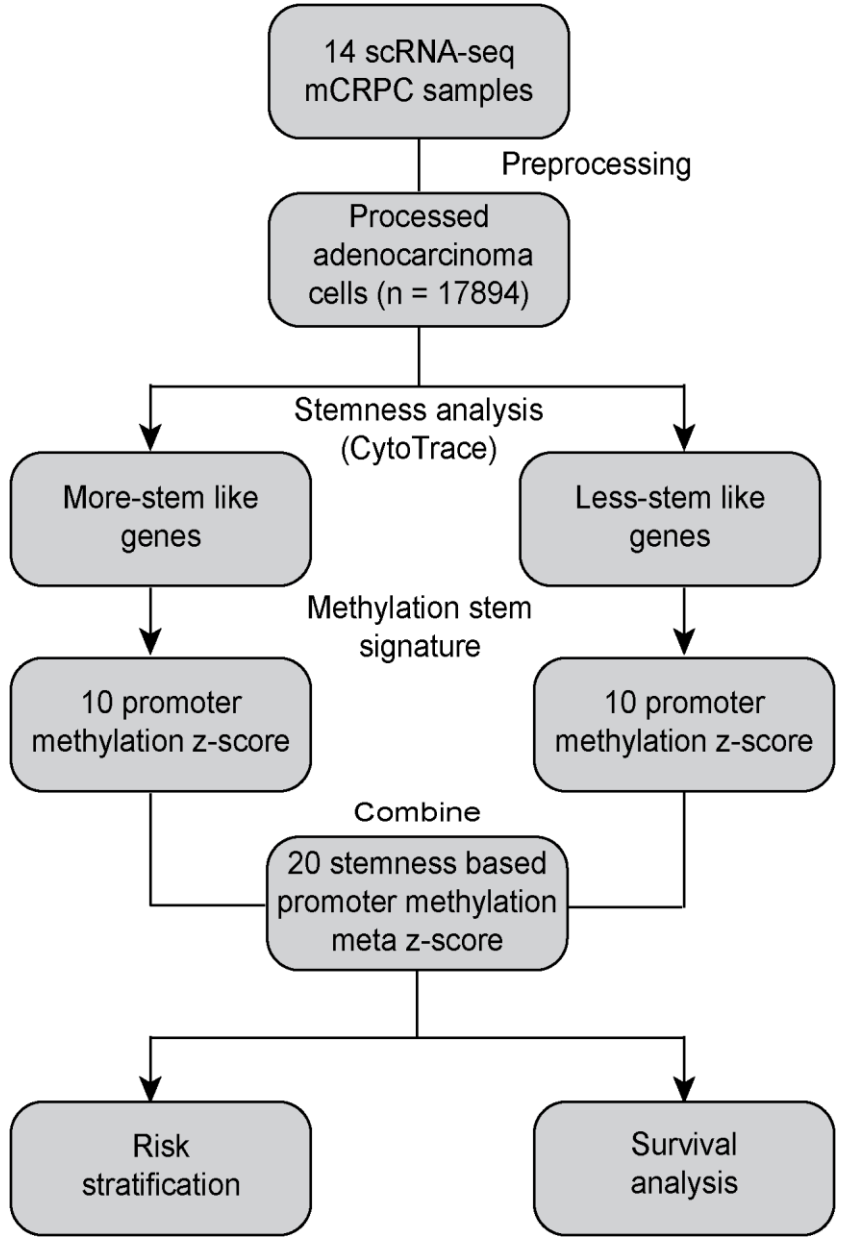
- 1: Genes correlated with more more stem-like features
- 0: Genes correlated with less stem-like features

Gulati & Newman et al. Science, 2020

CytoTRACE on mCRPC scRNA-Seq cohort

Single Cell RNA-Seq (mCRPC = 14)

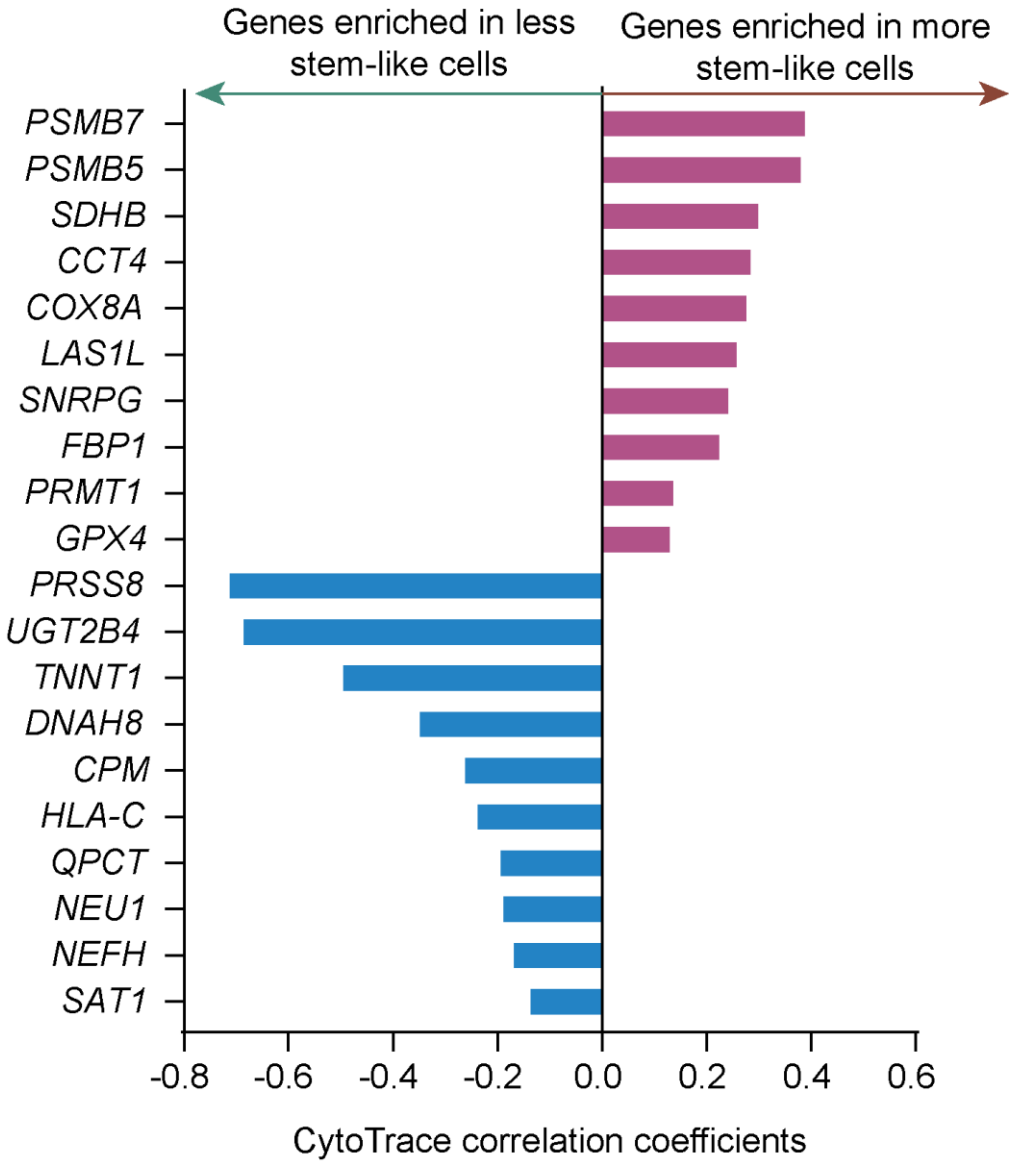


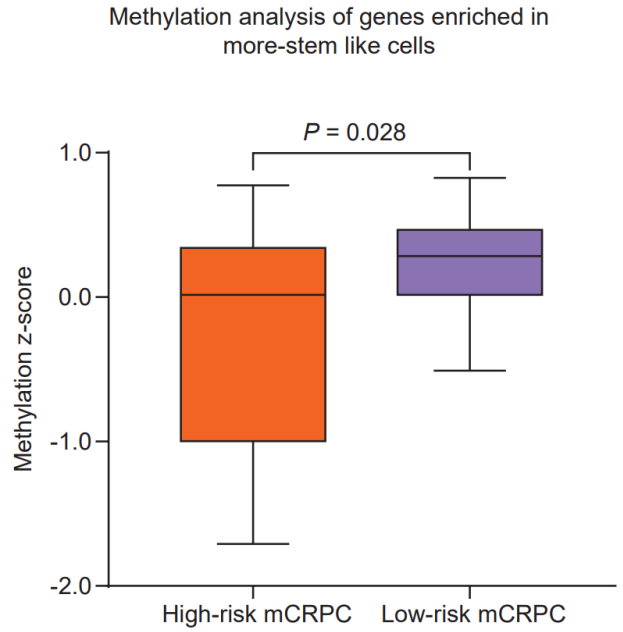


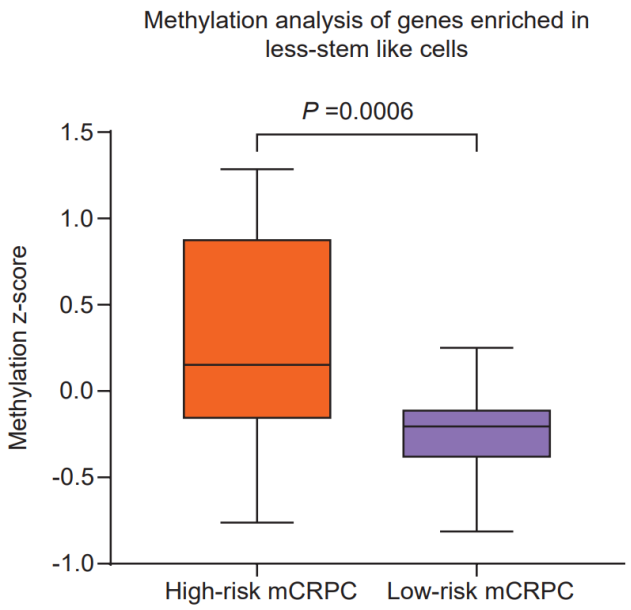
He & Allen et al. Nature medicine, 2021

Enrichment of a stemness signature in cell-free DNA in high-risk patients

Promoter-level cfDNA methylation analysis

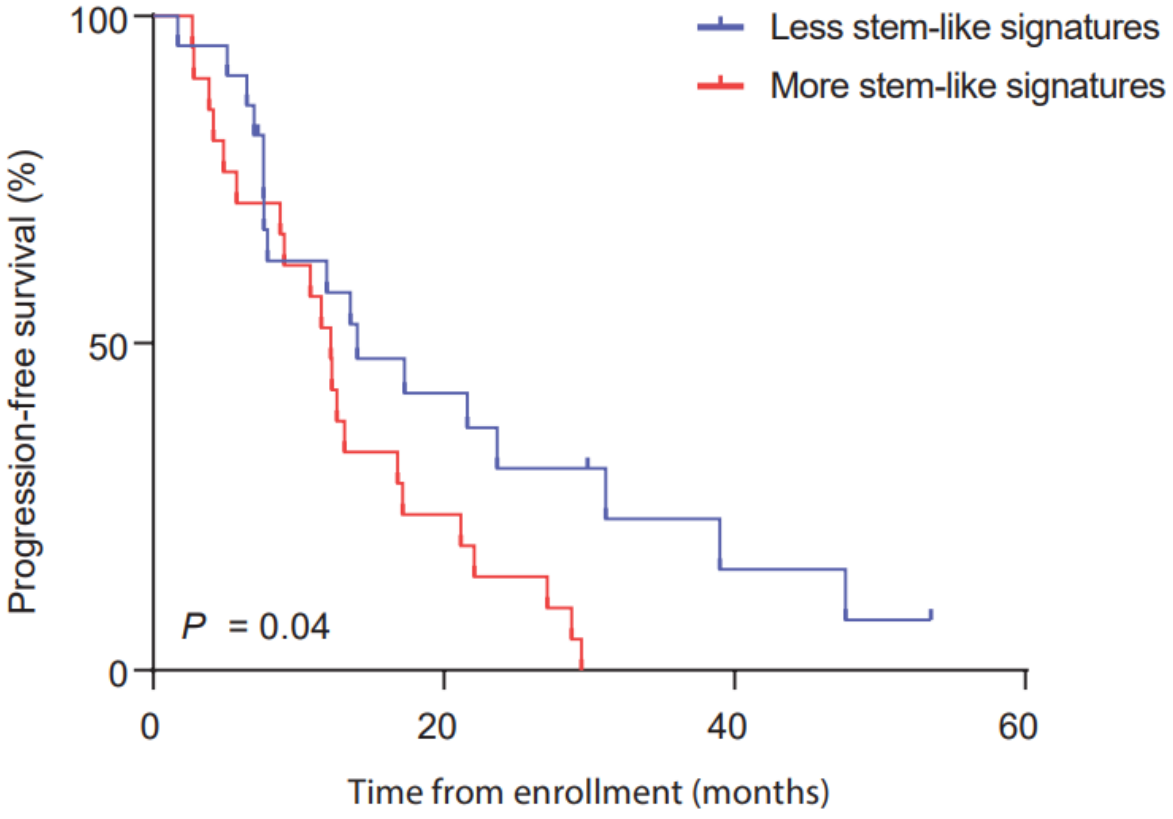


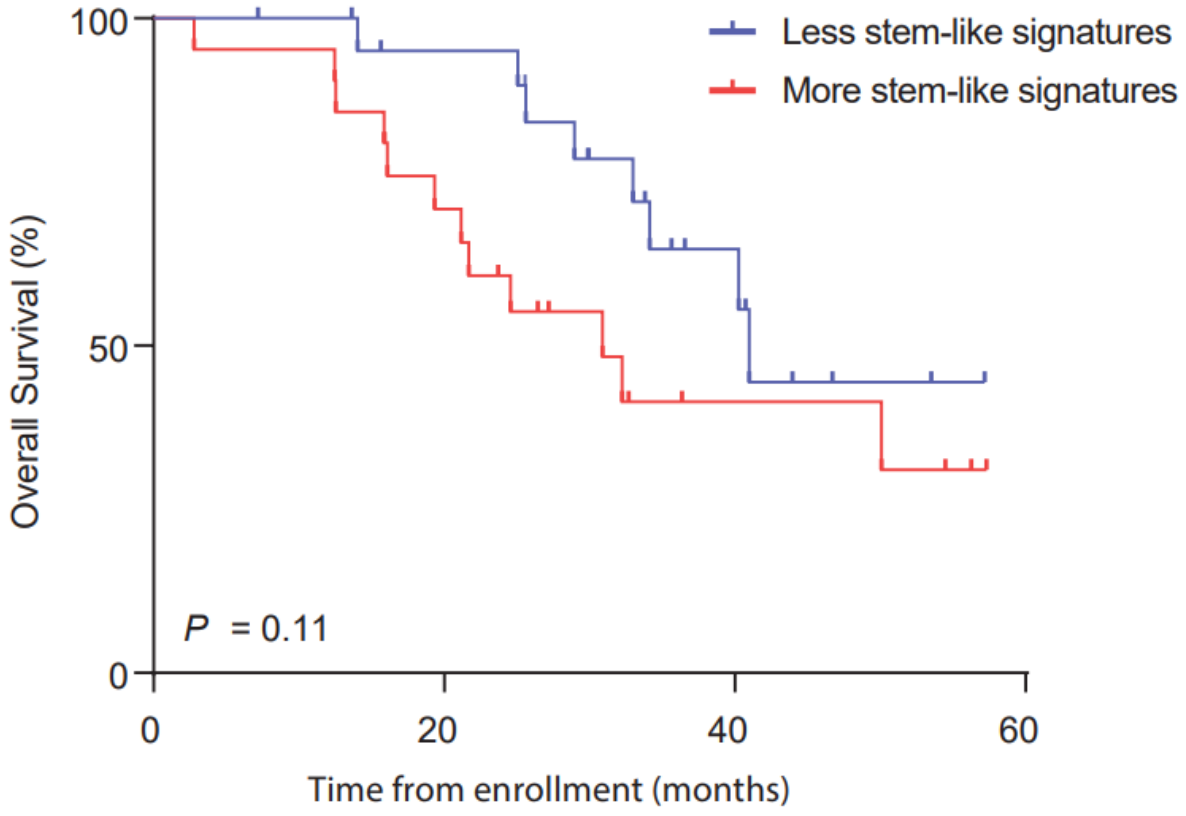




Enrichment of a stemness signature in cell-free DNA in high-risk patients

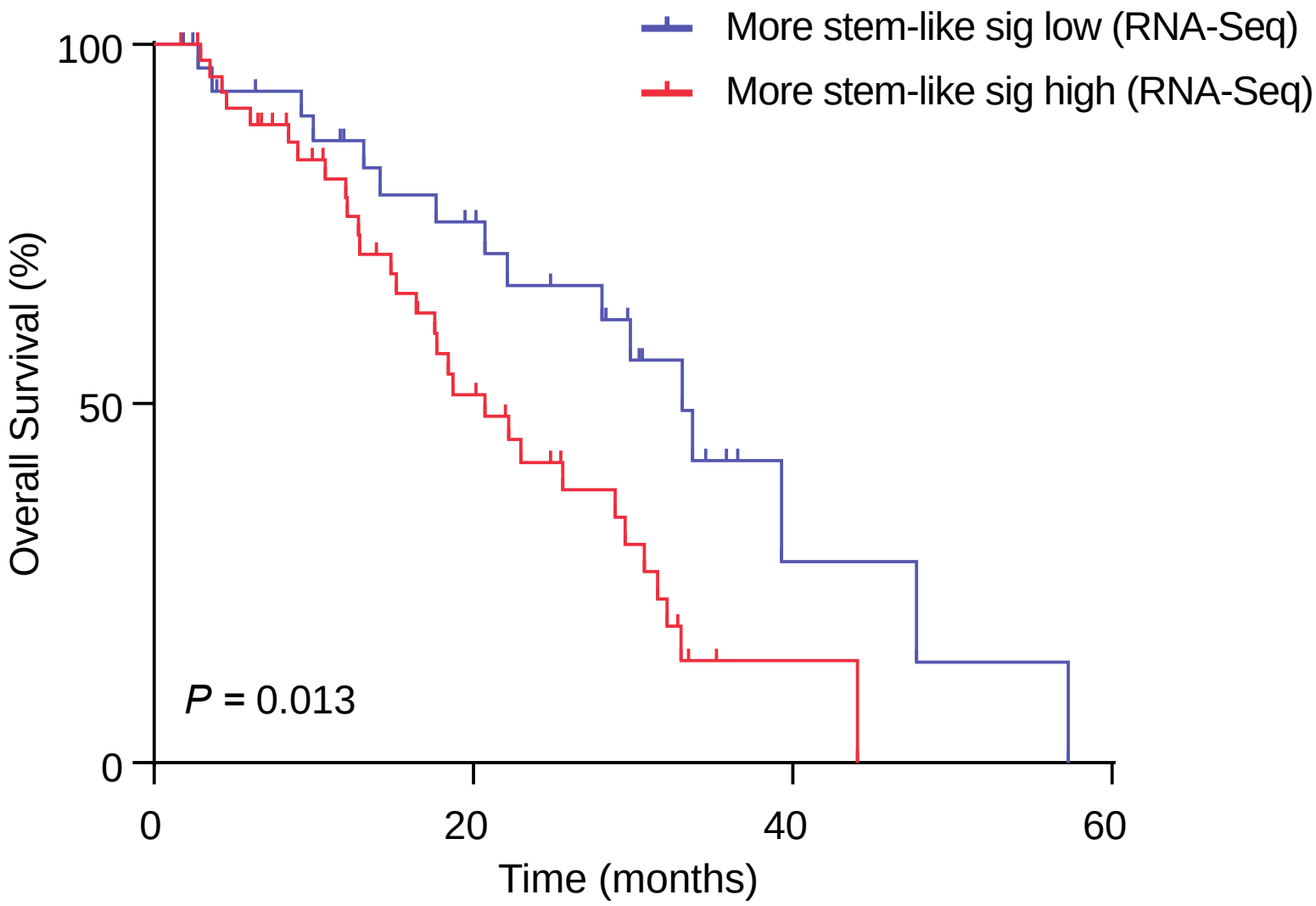
cfDNA stemness metagene Kaplan-Meier analysis





Stem-like signature also predicts survival in bulk RNA-seq data

(External cohort of 80 mCRPC patients from Abida et al. *PNAS*, 2019)



Summary & Future Directions

- Plasma cell-free DNA alterations in the AR/enhancer locus correlate with significantly worse outcomes in mCRPC patients
- Transcriptional profiles of mCRPC can be predicted from cell-free DNA epigenomics (methylation and fragmentomics)
- Higher-risk mCRPC patients have a more stem-like signature profile as inferred from plasma cell-free DNA epigenomics, which correlates with worse survival outcomes
- It will be important to independently validate these findings with outside cohorts, and perform further cfDNA-tumor cross-correlative analyses

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Acknowledgements

Chaudhuri Lab

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Chloe Sachs
Lilli Greiner
Andrew Chen
Breanna Yang

http://chaudhurilab.wustl.edu

Patients & their families

Chaudhuri Lab Alumni

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Haley Ellis – Dana Farber (Fellow)
Rummi Babbra – Rochester (Fellow)
Grace Smith – Harvard (MSTP)
Re-I Chin – WashU (RO Chief Res)
Kevin Chen – WashU (RO Resident)
Wenjia Feng – WashU (PhD student)
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Melanoma Research Alliance
V Foundation V Scholar
Alvin Siteman Foundation
Cancer Research Foundation
Children's Discovery Institute
Children's Tumor Foundation
Emerson Collective
Rabushka Fund