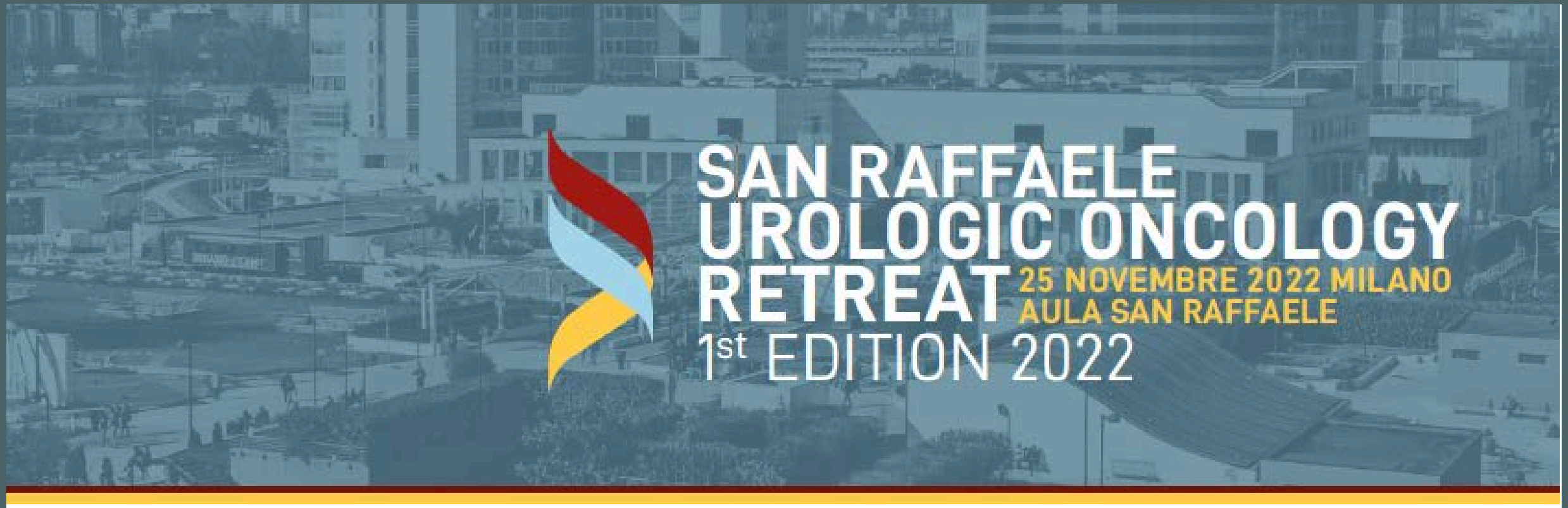



# Addressing tumor heterogeneity in prostate cancer

Marzia Del Re

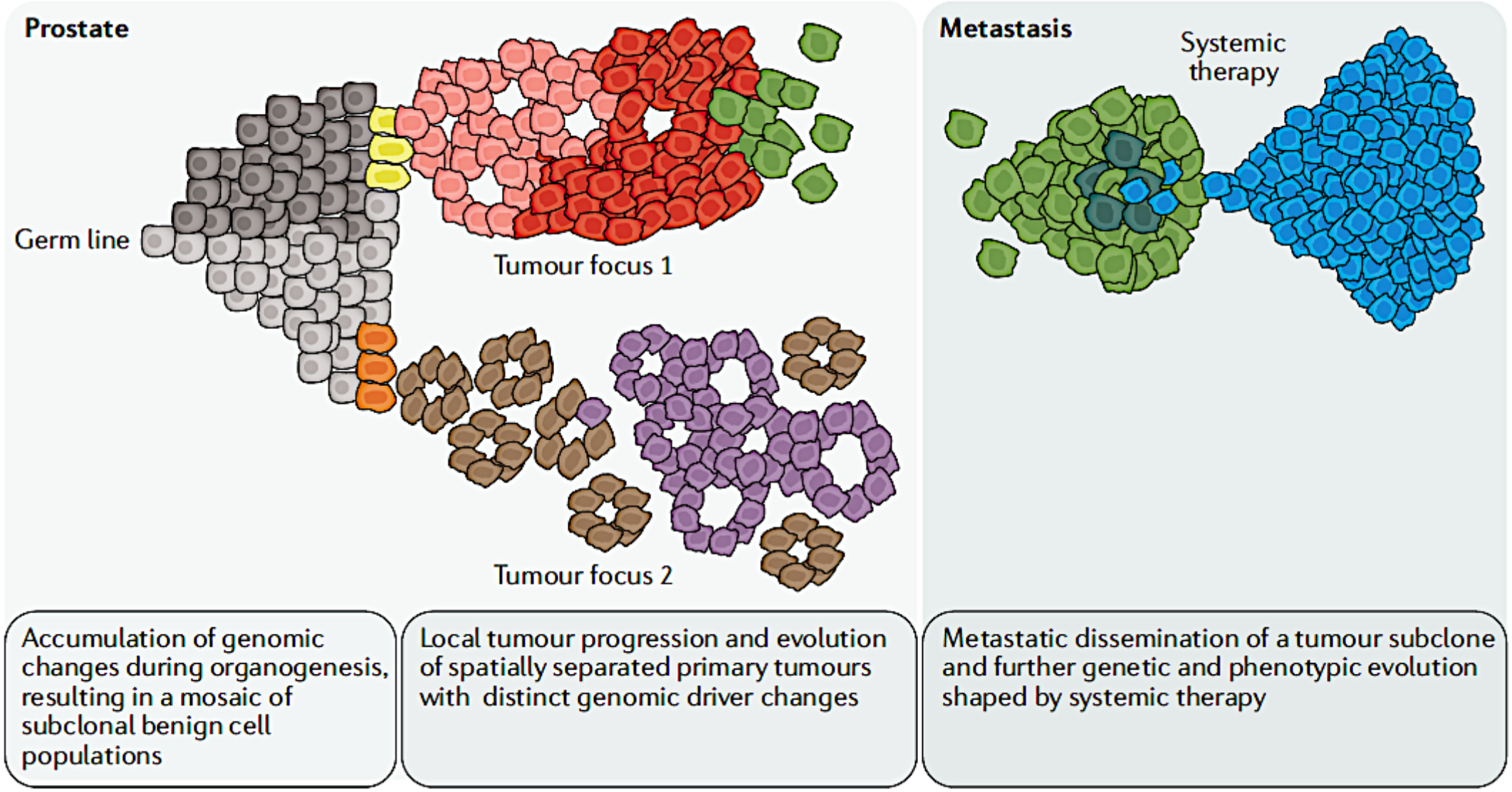
Unit of Clinical Pharmacology and Pharmacogenetics

University of Pisa

# What do we know about tumor heterogeneity?

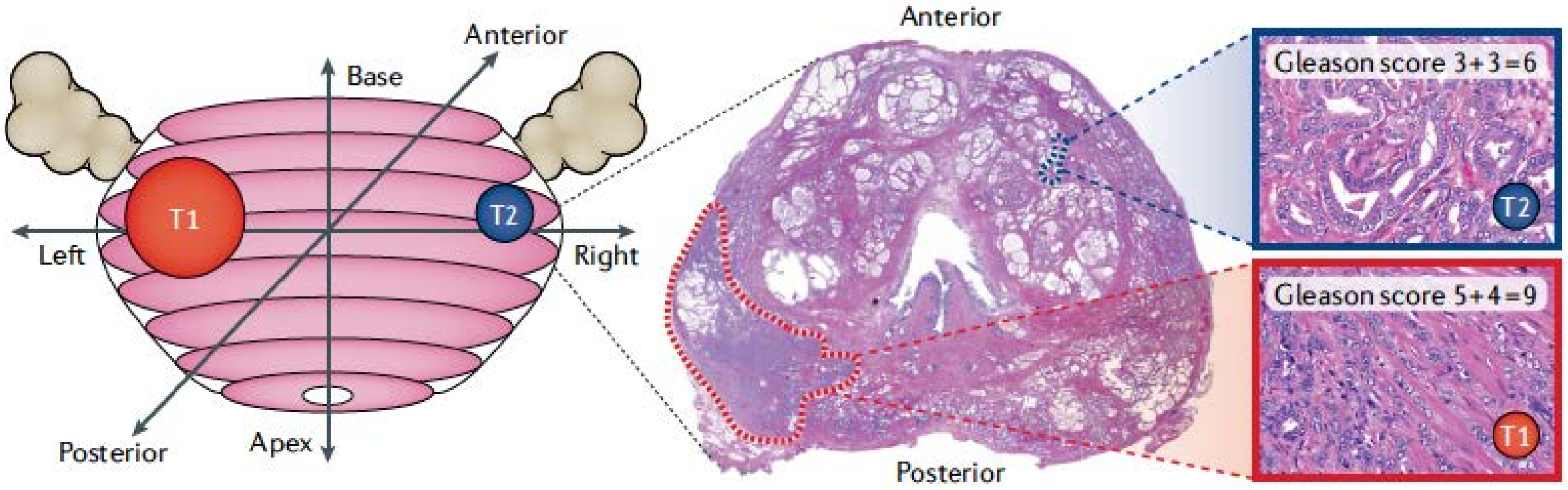
- From a clinical, morphological and molecular perspective, prostate cancer is a heterogeneous disease, determined by heritable genetic and epigenetic alterations.
- Technological improvements demonstrated a high level of genomic diversity between different patients (*inter- patient het.*) but also within a given primary tumour (*intra-tumoural het.*) as well as its distinct tumour foci and different metastatic sites (*inter-tumoural het.*).
- Epigenetic, expression, post- translational, morphological and phenotypic heterogeneities, which all probably contribute to disease progression and clinical manifestations, exist within solid tumours.

# Model of clonal progression of prostate cancer




Haffner MC, et al. Nat Rev Urol 18, 79–92 (2021)

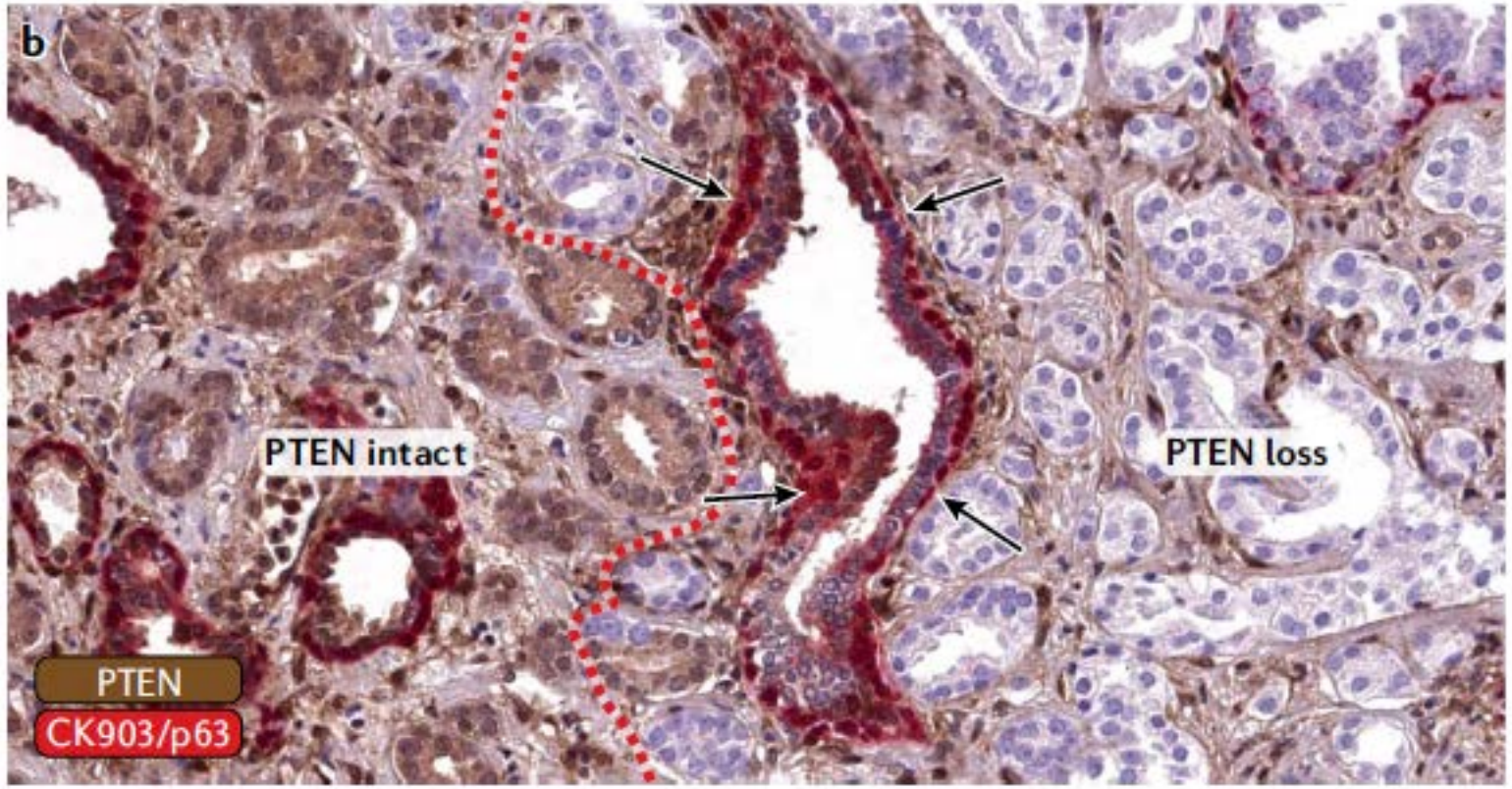
# Multifocal prostate cancer




Reconstruction and whole- mount cross- section of a radical prostatectomy specimen with two distinct tumour foci. The larger tumour focus located in the left posterior prostate shows high- grade morphology and extra-prostatic extension (Gleason score 5+4=9), whereas a smaller anterior tumour appears well differentiated (Gleason score 3+3=6).

Haffner MC, et al. Nat Rev Urol 18, 79–92 (2021)

# Visualizing clonal and subclonal heterogeneity in tumour tissues

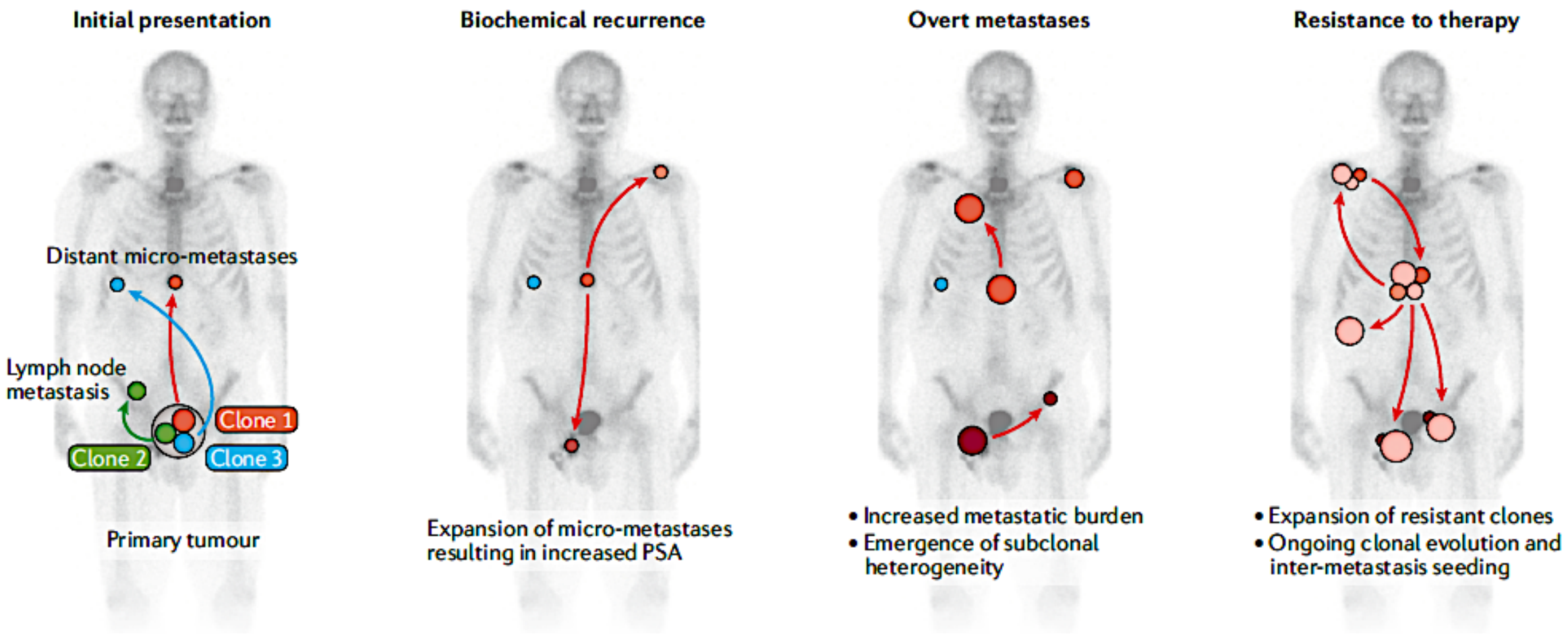



The tumour shows sub-clonal loss of PTEN (loss of cytoplasmic staining) in a subset of cancer glands (separated by the red dotted line).

Intact basal cells are shown in red. (Arrows show a benign gland.)

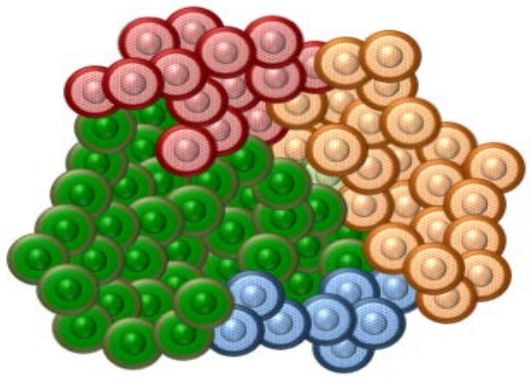
Haffner MC, et al. Nat Rev Urol 18, 79–92 (2021)

# Schematic of scenarios of clonal evolution of metastatic prostate cancer




Haffner MC, et al. Nat Rev Urol 18, 79–92 (2021)

# Treatment clonal selection

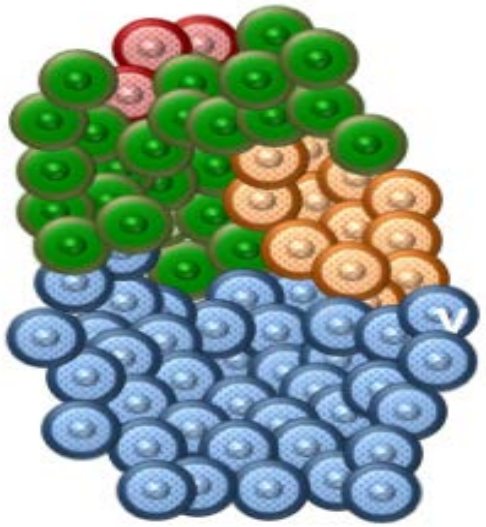


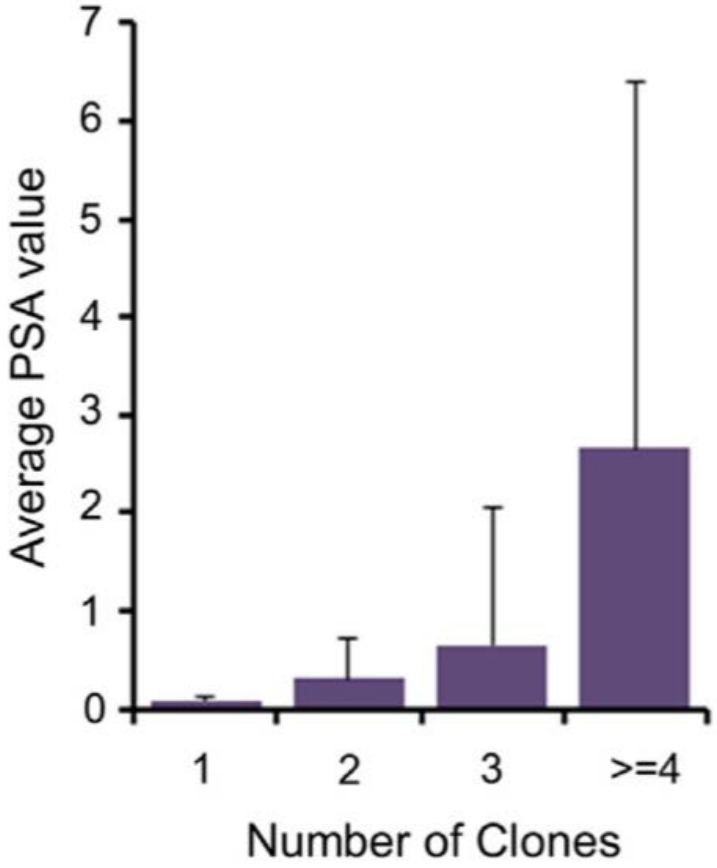
Coexisting of heterogeneous cancer cell clones

Pharmacological treatment

Killing of sensitive cancer cells

Proliferation and differentiation of resistant cells






Won Yun J, et al. Transl Oncol. 2019 Jan; 12(1): 43–48

# Common Genomic Alterations in CRPC

- *AR* alterations (50% to 60%) → Tumor driver and predictive biomarker
- *PTEN* deletion (40% to 50%) → Tumor driver and predictive biomarker
- *TP53* mutation or deletion (40% to 50%)
- *RB1* deletion (20%)

  → Predictive biomarker of resistance
- DNA repair genes (10% to 20%)—*BRCA1/2, ATM* → Tumor driver and predictive biomarker

Beltran. 2016 ASCO Educ Book. 2016;35:131. Hosoya. Cancer Sci. 2014;105:370
Won Yun J, et al. Transl Oncol. 2019 Jan; 12(1): 43–48
Tomlins SA, et al. Science 2005;310:644–8
Li Q, et al. Nat Commun 2018; 9, 3600
Beltran H, et al. J Clin Invest. 2020;130(4):1653–1668

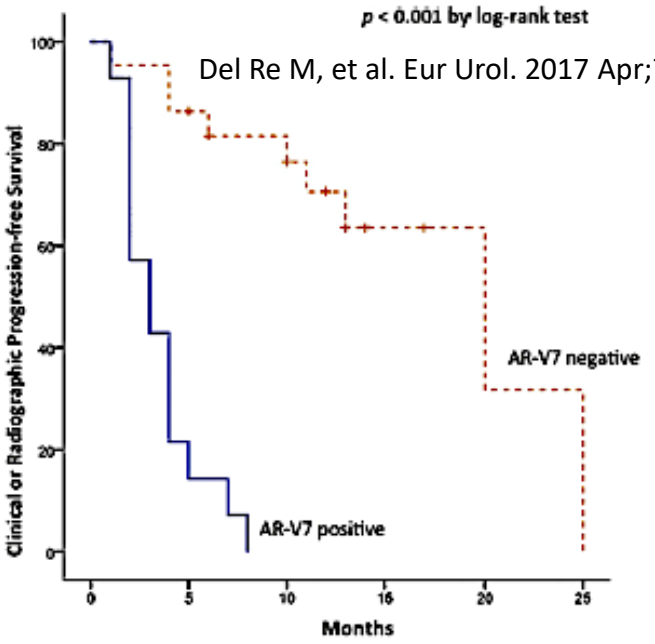
# The strange story of the AR-V7

N ENGL J MED 371;11 NEJM.ORG SEPTEMBER 11, 2014
The NEW ENGLAND JOURNAL of MEDICINE

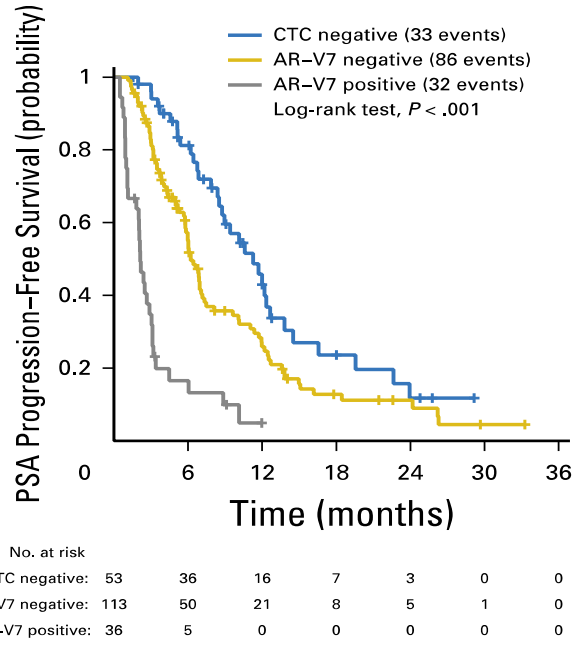
ORIGINAL ARTICLE

## AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer

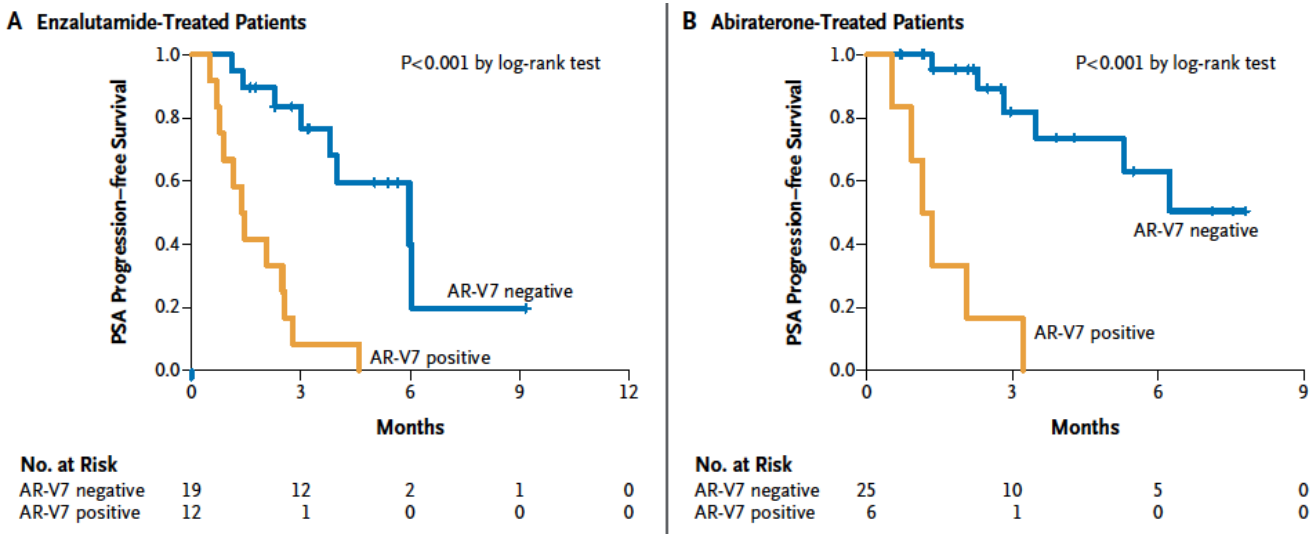
Emmanuel S. Antonarakis, M.D., Changxue Lu, Ph.D., Hao Wang, Ph.D., Brandon Luber, Sc.M., Mary Nakazawa, M.H.S., Jeffrey C. Roeser, B.S., Yan Chen, Ph.D., Tabrez A. Mohammad, Ph.D., Yidong Chen, Ph.D., Helen L. Fedor, B.S., Tamara L. Lotan, M.D., Qizhi Zheng, M.D., Angelo M. De Marzo, M.D., Ph.D., John T. Isaacs, Ph.D., William B. Isaacs, Ph.D., Rosa Nadal, M.D., Channing J. Paller, M.D., Samuel R. Denmeade, M.D., Michael A. Carducci, M.D., Mario A. Eisenberger, M.D., and Jun Luo, Ph.D.

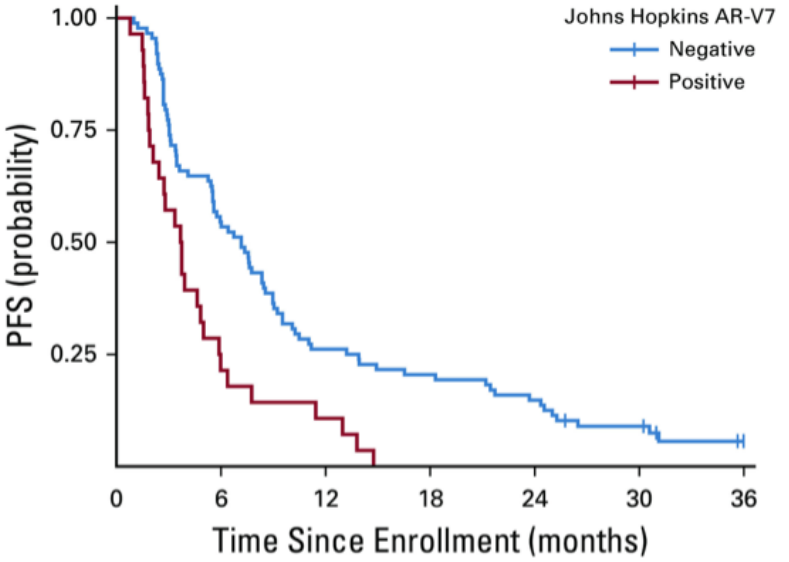



Del Re M, et al. Eur Urol. 2017 Apr;71(4):680-687




Antonarakis ES, et al. J Clin Oncol. 2017:JCO2016701961



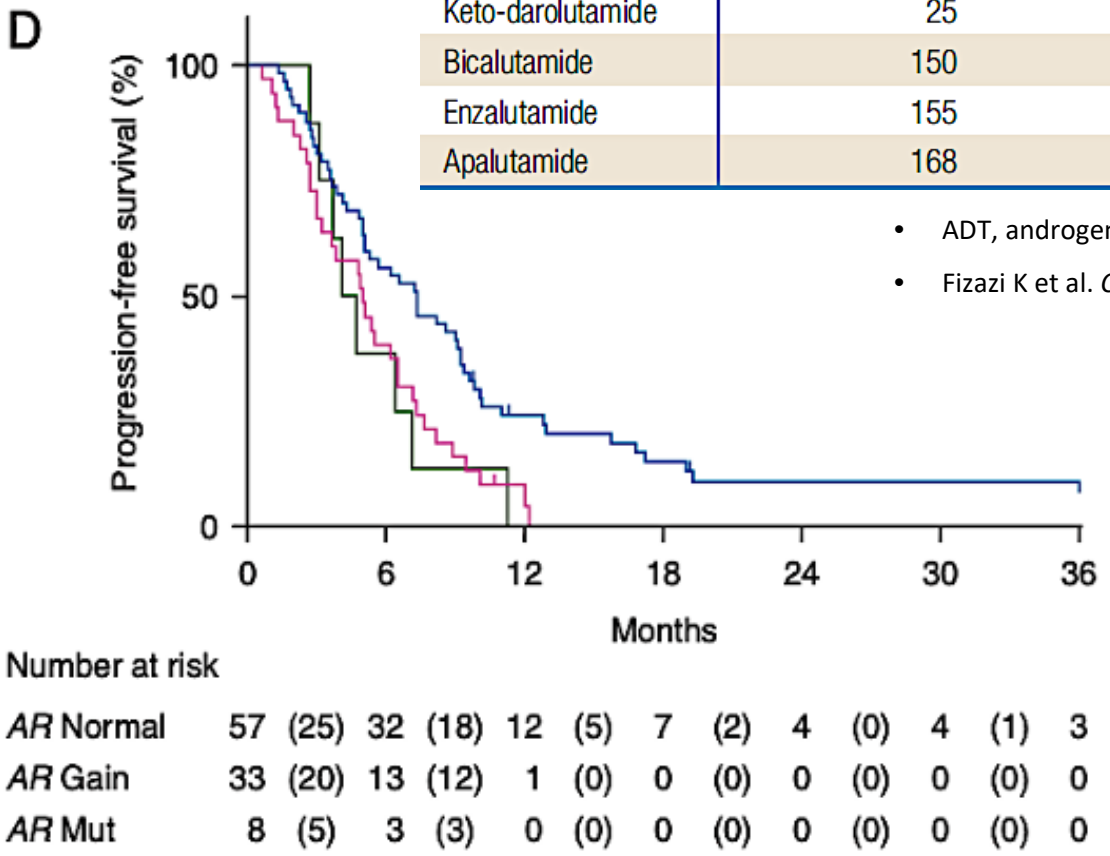

Armstrong AJ, et al JCO Precis Oncol. 2020;4:PO.20.00200

# AR affinity of antagonist drugs

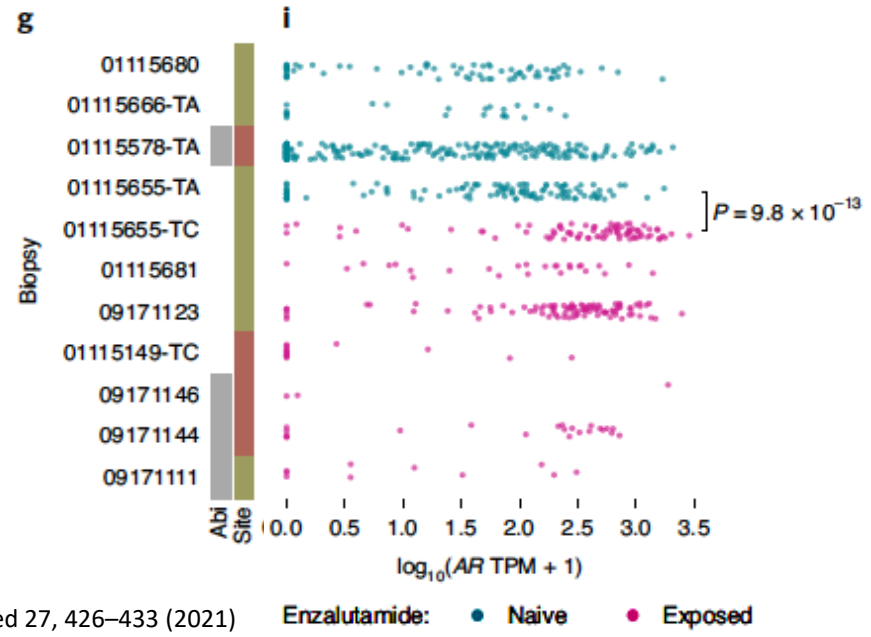
## Antagonism of mutant ARs linked to resistance to ADT[1]

| Compound | Antagonism wtAR, nM | Antagonism AR (F877L) IC50, nM | Antagonism AR (T878A) IC50, nM | Antagonism AR (W742L) IC50, nM |
|---|---|---|---|---|
| Darolutamide | 65 | 66 | 1782 | 1500 |
| Keto-darolutamide | 25 | 51 | 700 | 1160 |
| Bicalutamide | 150 | 218 | 957 | Agonist |
| Enzalutamide | 155 | Agonist | 296 | > 10,000 |
| Apalutamide | 168 | Agonist | 1130 | > 10,000 |

- ADT, androgen-deprivation therapy; AR, androgen receptor; IC50, half maximal inhibitory concentration; wtAR, wild type AR
- Fizazi K et al. *Clin Genitourin Cancer*. 2018;16:332–340.




Conteduca V, et al. Annals of Oncology 28: 1508–1516, 2017




He, et al. Nat Med 27, 426–433 (2021)

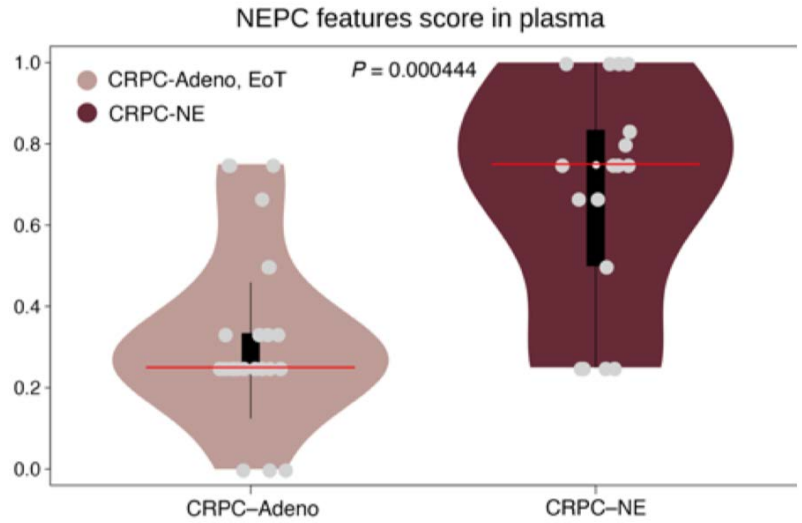
J Clin Invest. 2020;130(4):1653–1668. https://doi.org/10.1172/JCI131041.

The Journal of Clinical Investigation

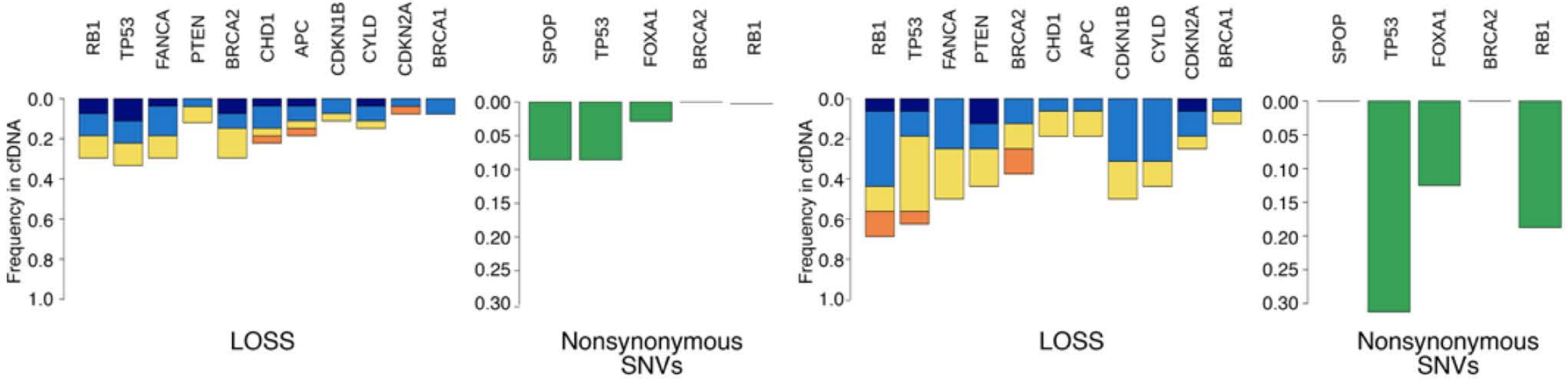
RESEARCH ARTICLE

# Circulating tumor DNA profile recognizes transformation to castration-resistant neuroendocrine prostate cancer

Himisha Beltran,[1,2] Alessandro Romanel,[3] Vincenza Conteduca,[1,4] Nicola Casiraghi,[3] Michael Sigouros,[2] Gian Marco Franceschini,[3] Francesco Orlando,[3] Tarcisio Fedrizzi,[3] Sheng-Yu Ku,[1] Emma Dann,[3] Alicia Alonso,[5] Juan Miguel Mosquera,[5,6] Andrea Sboner,[5,7] Jenny Xiang,[5] Olivier Elemento,[5,7] David M. Nanus,[2,5] Scott T. Tagawa,[2,5] Matteo Benelli,[3,8] and Francesca Demichelis[3,5,7]



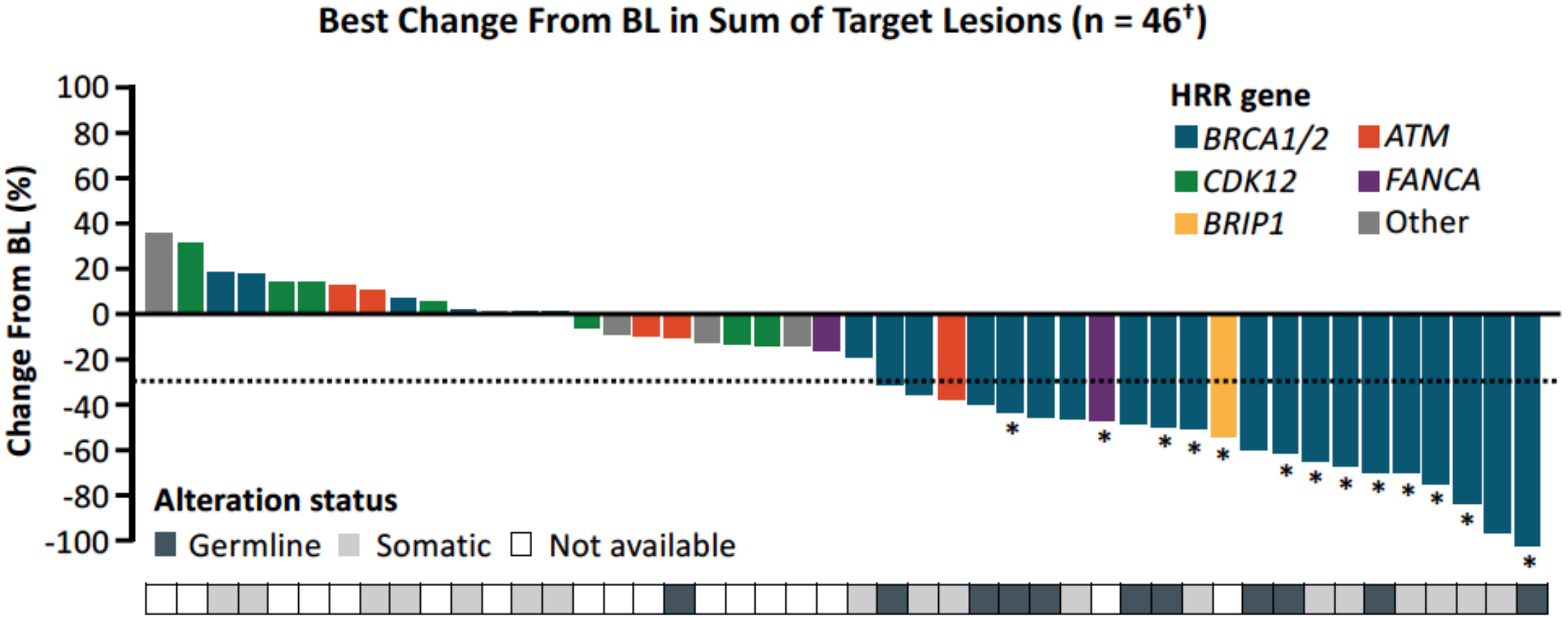

TP53, RB1, CYLD, lack of mutation or focal gain of AR, and aggregated hypo- and hyper-methylation of 20 differential sites




CRPC-ADENO

CRPC-NE

# TRITON2: Radiographic Responses in Evaluable Patients With HRR Gene Alterations

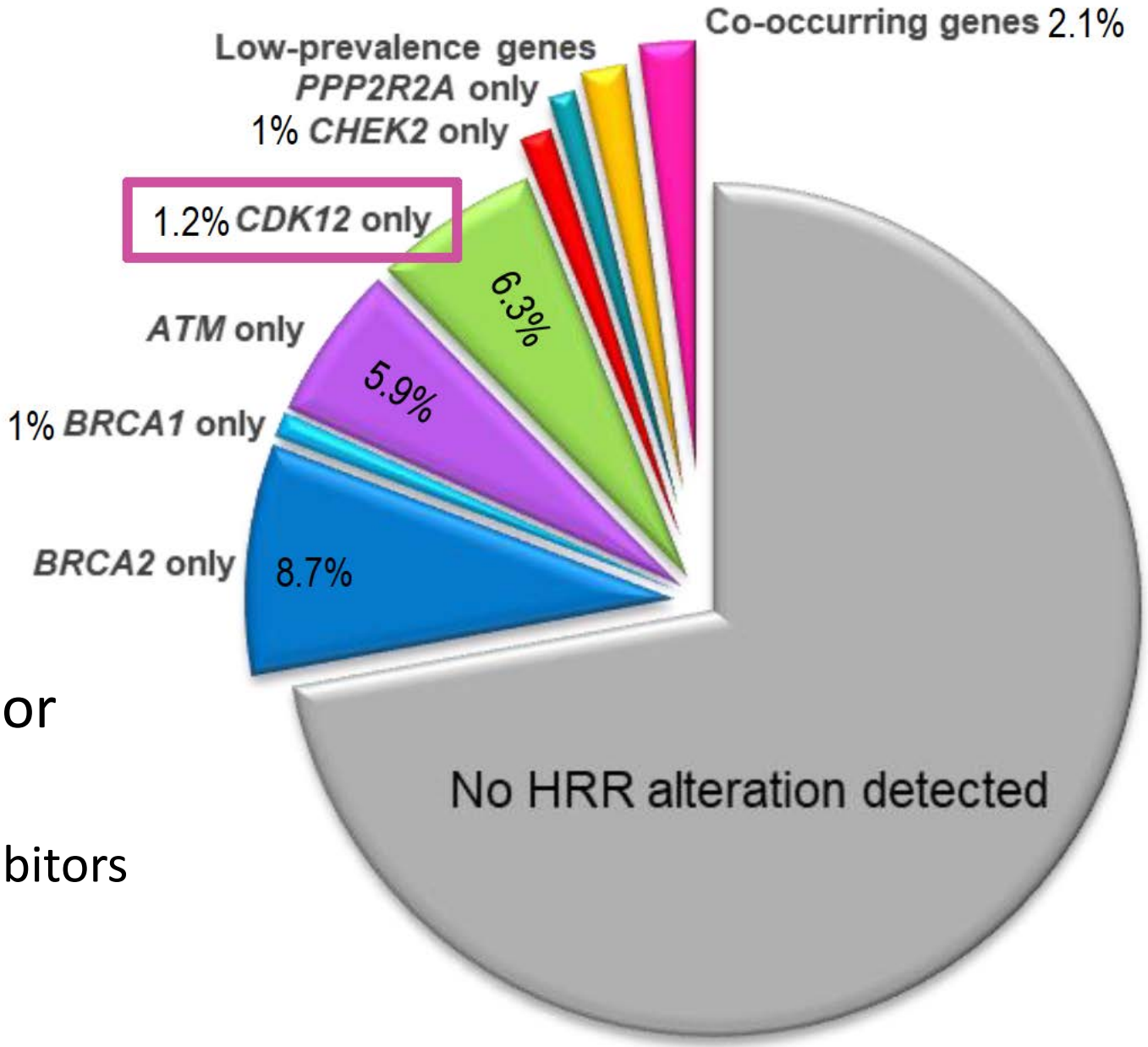



Each bar represents a single patient; patients with no change from BL are shown as 0.5% for clarity. Threshold for PR (30% decrease from BL) indicated by dotted line. *Confirmed RECIST/PCWG3 response. †Includes patients with measurable disease at BL and ≥ 1 post-BL scan.

Abida. ESMO 2018. Abstr 793PD.

# Focus on CDK12 loss

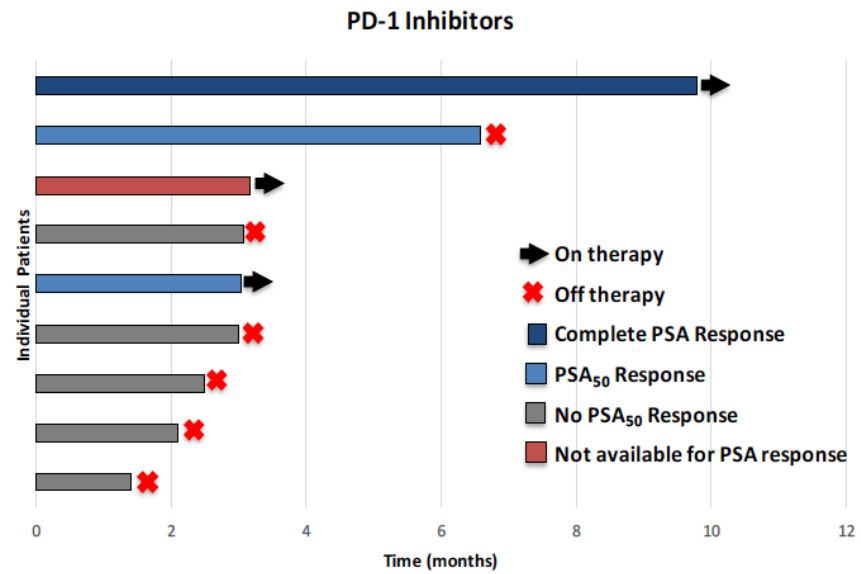
- Biallelic *CDK12* mutations
- Lead to genomic instability
  - Extensive tandem duplications
- Lead to gene fusions
  - Increased neoantigen expression
- Immune cell infiltration into tumor tissue
  - Target for immune checkpoint inhibitors

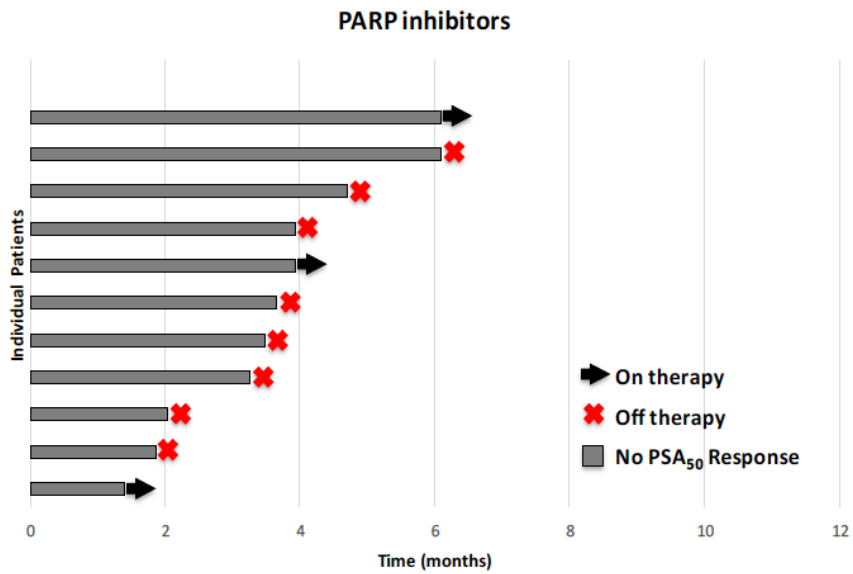



Wu. Cell. 2018;173:1770

# CDK12 patients: $PSA_{50}$ response to various systemic therapies

| Agent | N | $PSA_{50}$ response rate |
|---|---|---|
| First-line ADT | 54 | 85.1% (46/54) |
| Abiraterone / Enzalutamide | 34 | 47.1% (16/34) |
| Taxane | 20 | 35.0% (7/20) |
| PARP inhibitor | 11 | 0% (0/11) |
| PD-1 inhibitors | 9<br>(8 available for PSA response) | 37.5% (3/8) |



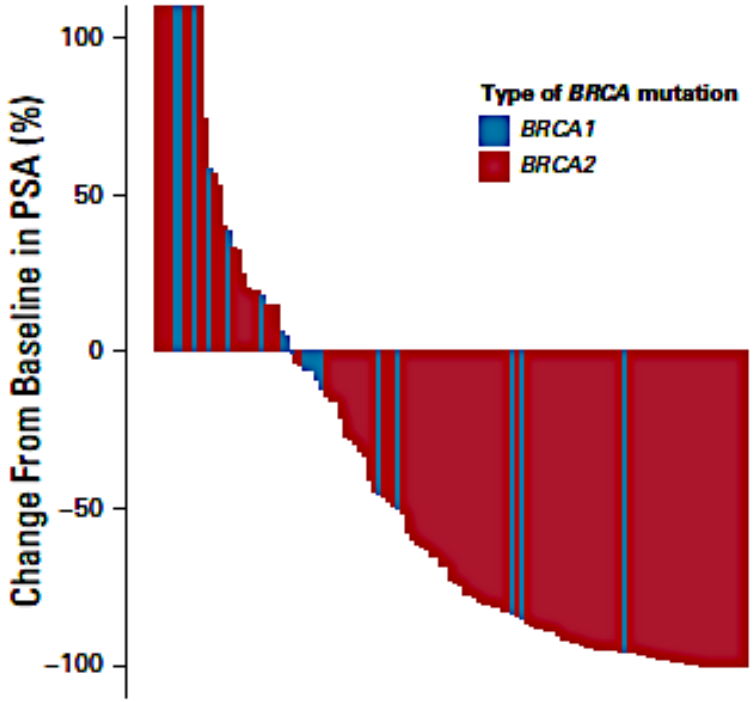
original reports

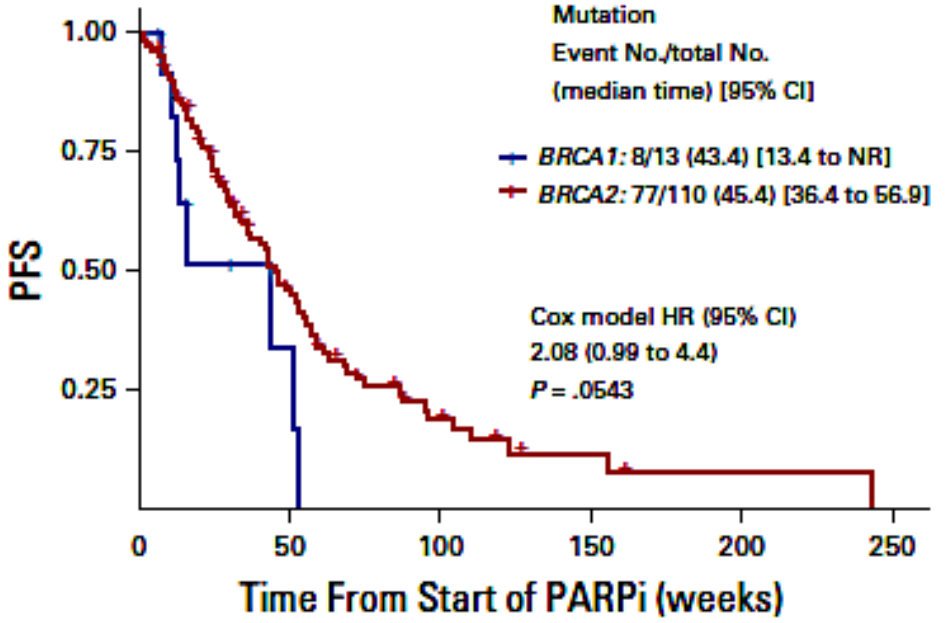
PRECISION MEDICINE

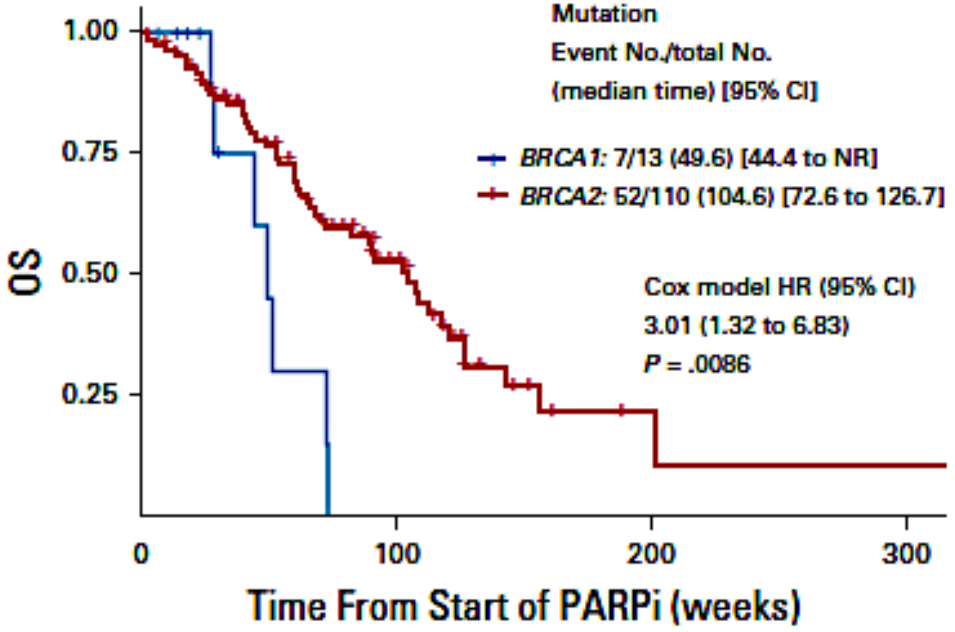
# Differential Activity of PARP Inhibitors in *BRCA1*- Versus *BRCA2*-Altered Metastatic Castration-Resistant Prostate Cancer

Fadi Taza, MD[1,2]; Albert E. Holler, BA[1]; Wei Fu, PhD[1]; Hao Wang, PhD[1]; Nabil Adra, MD[3]; Costantine Albany, MD[3]; Ryan Ashkar, MD[3]; Heather H. Cheng, MD[4]; Alexandra O. Sokolova, MD[4]; Neeraj Agarwal, MD[5]; Adam Kessel, MD[5]; Alan Bryce, MD[6]; Nellie Nafissi, MD[6]; Pedro Barata, MD[7]; A. Oliver Sartor, MD[7]; Diogo Bastos, MD[8]; Oren Smaletz, MD[9]; Jacob E. Berchuck, MD[10]; Mary-Ellen Taplin, MD[10]; Rahul Aggarwal, MD[11]; Cora N. Sternberg, MD[12]; Panagiotis J. Vlachostergios, MD[12]; Ajjai S. Alva, MD[13]; Christopher Su, MD[13]; Catherine H. Marshall, MD[1]; and Emmanuel S. Antonarakis, MD[1]

There were significantly fewer PSA50 responses in patients with BRCA1-altered versus BRCA2-altered mCRPC (23% vs 63% respectively; OR, 0.18; 95% CI, 0.04 to 0.62; p=0.01).



original reports

PRECISION MEDICINE

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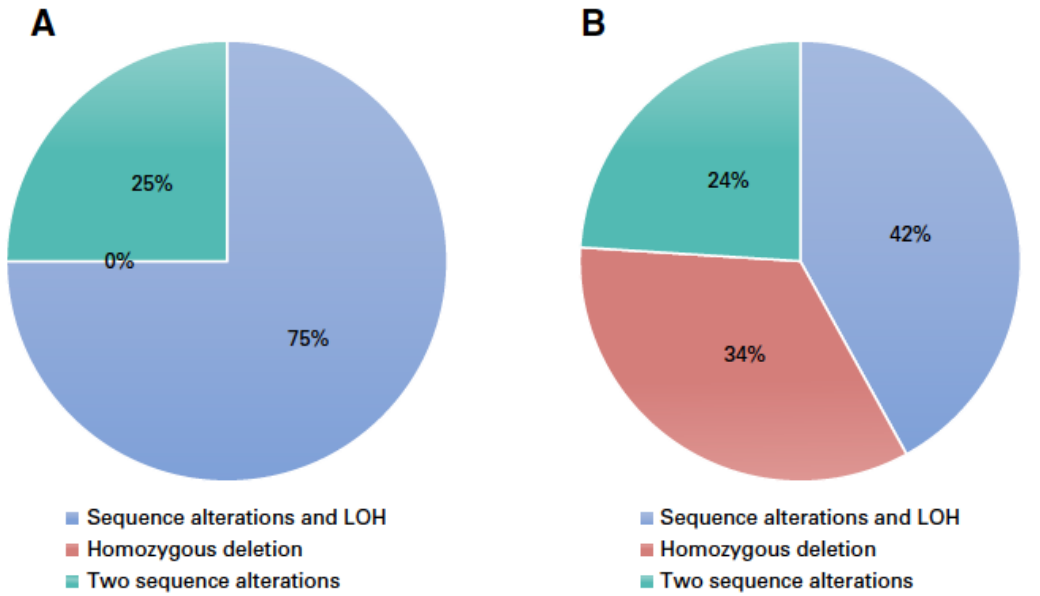
Fadi Taza, MD[1,2]; Albert E. Holler, BA[1]; Wei Fu, PhD[1]; Hao Wang, PhD[1]; Nabil Adra, MD[3]; Costantine Albany, MD[3]; Ryan Ashkar, MD[3]; Heather H. Cheng, MD[4]; Alexandra O. Sokolova, MD[4]; Neeraj Agarwal, MD[5]; Adam Kessel, MD[5]; Alan Bryce, MD[6]; Nellie Nafissi, MD[6]; Pedro Barata, MD[7]; A. Oliver Sartor, MD[7]; Diogo Bastos, MD[8]; Oren Smaletz, MD[9]; Jacob E. Berchuck, MD[10]; Mary-Ellen Taplin, MD[10]; Rahul Aggarwal, MD[11]; Cora N. Sternberg, MD[12]; Panagiotis J. Vlachostergios, MD[12]; Ajjai S. Alva, MD[13]; Christopher Su, MD[13]; Catherine H. Marshall, MD[1]; and Emmanuel S. Antonarakis, MD[1]




Pie charts of the biallelic inactivation mechanisms by BRCA mutation type: (A) BRCA1 and (B) BRCA2

## CONTEXT

### Key Objective

We conducted a multicenter retrospective study to determine whether the efficacy of poly (ADP-ribose) polymerase (PARP) inhibitors differs between cancers with *BRCA1* and *BRCA2* mutations and to examine differences in other genomic alterations that coexist with *BRCA1/2* mutations.

### Knowledge Generated

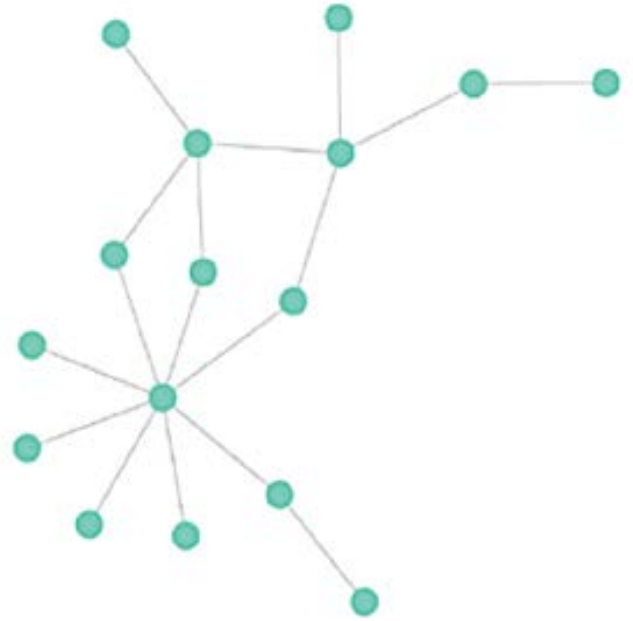
We show that PARP inhibitor efficacy is diminished in *BRCA1*- versus *BRCA2*-altered metastatic castration-resistant prostate cancer. This is not due to an imbalance in germline mutations but might be related to more monoallelic mutations and/or concurrent *TP53* alterations in the *BRCA1* group.

### Relevance

Additional therapeutic approaches are needed for patients with *BRCA1*-altered prostate cancer. These findings may have broad implications for other *BRCA1/2*-associated malignancies (breast, ovarian, and pancreatic cancers) where PARP inhibitors are used.

# NETWORK BIOLOGY: UNDERSTANDING THE CELL'S FUNCTIONAL ORGANIZATION

*Albert-László Barabási* & Zoltán N. Oltvai‡*



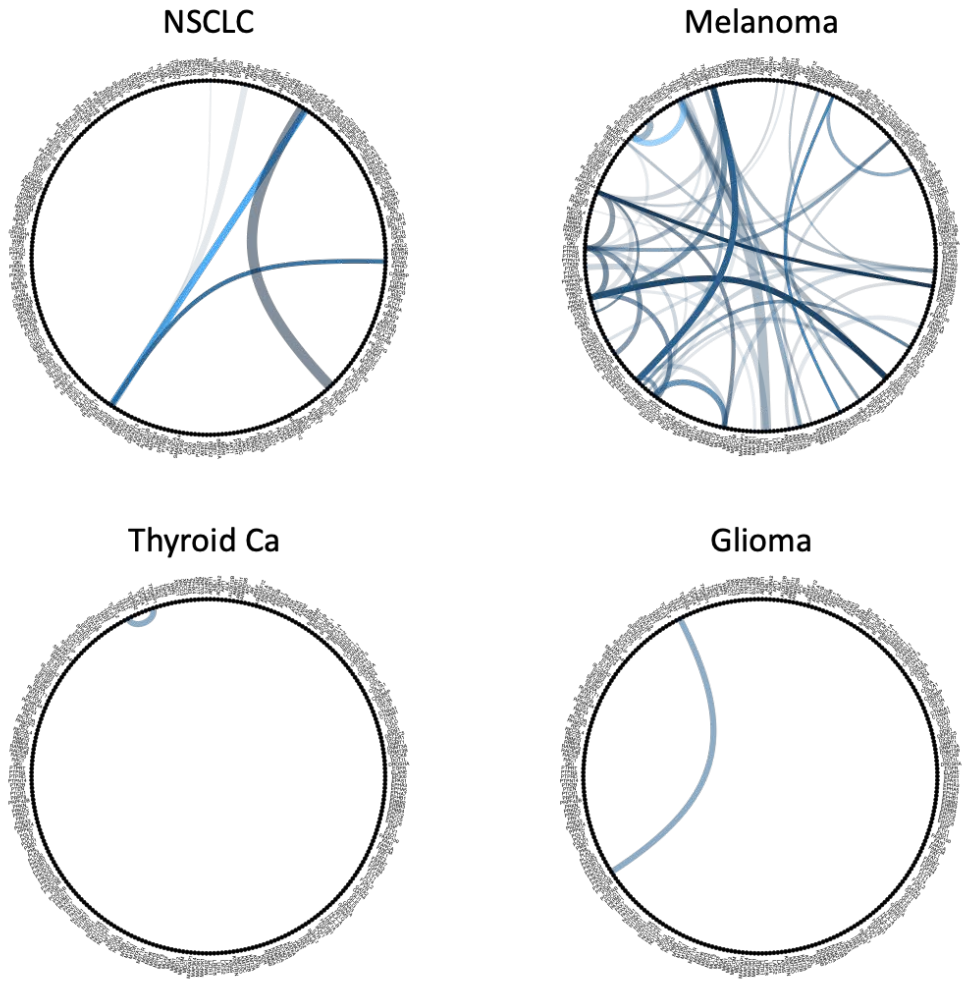
17 Genes

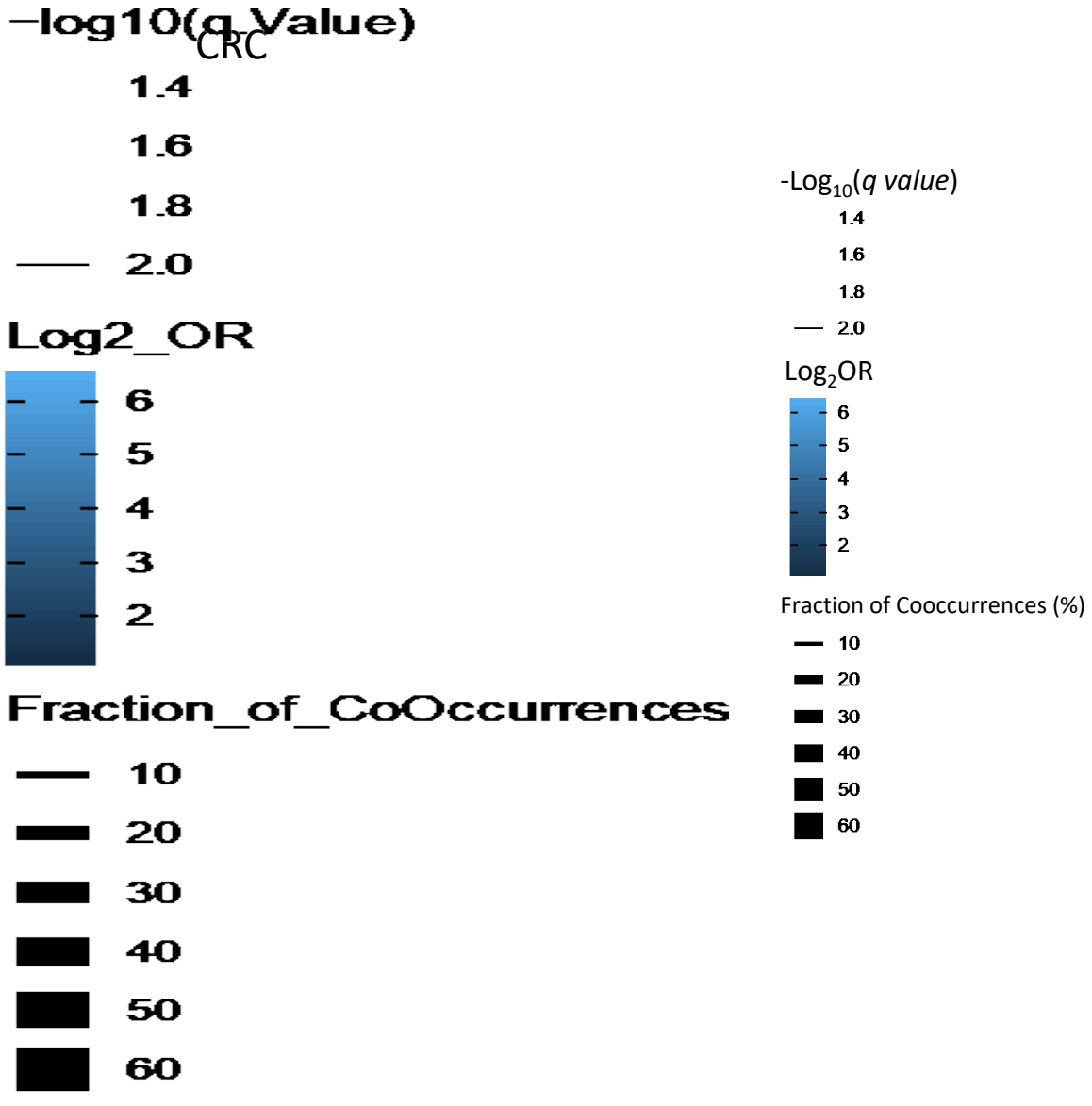
- Most biological characteristics arise from complex **interactions** between the cell's constituents, including proteins, DNA, RNA and small molecules.

- A key challenge for biology is to understand the structure and the dynamics of the complex intercellular web of interactions that contribute to the structure and function of a living cell (**gene regulatory network**) [***degree*** - or connectivity; ***betweenness*** - centrality of a mutant gene within the nearby network].

Barabási, et al. Nat Rev Genet 5, (2004) 101–113
Zhang et al. BMC Bioinformatics (2019) 20:12

# Gene networks - BRAF




Cucchiara F, Del Re M, et al. 2022 unpublished

# Take home messages

- Primary prostate cancers are often multifocal with spatial and morphologically distinct tumour foci, which may show non- overlapping truncal genomic alterations, suggesting that multiple clonally distinct cancers can arise in a given patient.
- Intra- tumoural and inter- tumoural heterogeneity present within the prostate gland poses diagnostic and therapeutic challenges.
- Despite the multiclonality of primary cancer, therapeutic interventions seem to select for a single dominant clone.
- The development of novel technologies will allow us to navigate these challenges, refine approaches for translational research and ultimately improve patient care.

# Thank you for your attention!