

Clinical Utility of Molecular Biomarkers for Advanced Prostate Cancer

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Thank you to all our funders

Disclosures

- ***All biases in my interpretations are mine.***
- My institution has a commercial interest in abiraterone, PARP inhibition in DNA repair defective cancers and PI3K/AKT pathway inhibitors (no personal income)
- I have served on advisory boards for many companies including Astra Zeneca, Astellas, Bayer, Boehringer Ingelheim, Genentech/Roche, Genmab, GSK, Janssen, Merck Serono, Merck Sharp & Dohme, Menarini/Silicon Biosystems, Orion, Pfizer, Sanofi Aventis, Taiho.
- My institution has received funding or other support for my research work from AZ, Astellas, Bayer, Genentech, GSK, Janssen, Merck Serono, MSD, Menarini/Silicon Biosystems, Orion, Sanofi Aventis, Taiho.
- I have been the CI/PI of many industry sponsored clinical trials

Overview

- **Introduction**
- Predictive biomarkers
- Conclusions

Many biomarker classes

I will focus on predictive biomarkers

Pre-diagnosis

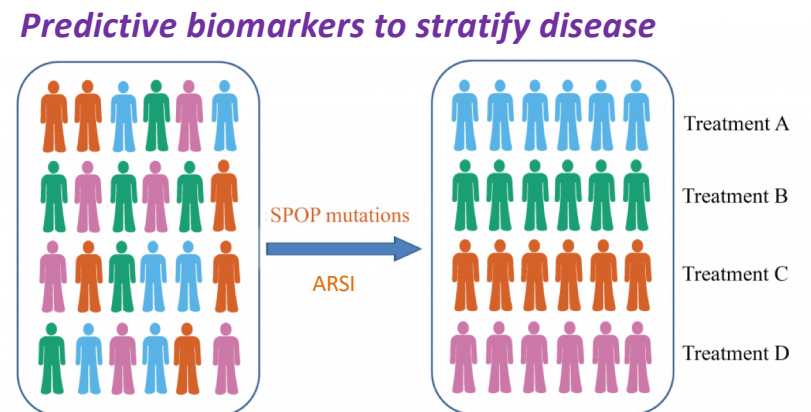
- Risk biomarkers (prevention; germline DNA repair defects)
- Diagnostic biomarkers (mpMRI, urinary tumour DNA, cfDNA)

Pre-treatment

- Prognostic biomarkers
- **Predictive**

Post-treatment

- Pharmacodynamic
- Response
- True surrogate



Boysen et al, 2018

We need to keep everything in perspective 1

Validation and qualification

- **Analytic validation matters**
 - **False positives:** *All that glitters is not gold*
 - Positive and negative controls key
 - **False negatives:** *Absence of evidence is not evidence of absence:* LLQ? (PSMA PET)
 - **Quantitation:** Binary variables suboptimal. (AR-V7+: 1 CTC positive?)
- **Clinical qualification matters too**
 - Prospective bespoke trials required
 - Qualifying predictive biomarkers require anticancer drugs targeting that subset



*Non-specific antibodies bind proteins other than target and result in false positives; eg some AR-V7 antibodies
Welti et al, European Urology 2016*

We need to keep everything in perspective 2

Multiplicity

- **Multi-purpose: Biomarkers frequently serve multiple purposes** EG: Prognostic & predictive (can be difficult to disentangle)
- **Multiple orthogonal assays:** One assay (NGS) may not be enough (for a test result)
 - MMR defects: NGS & IHC required?
 - ATM: NGS and IHC required?
- **Multiple alterations in one tumour:** Predictive biomarker hierarchy needed
 - MMRd causes mutations such as *subclonal* ATM or BRCA2 mutations



We need to keep everything in perspective 3

Regarding next generation sequencing

- **NGS calls: SNVs vs SNAs vs mutations**
 - Is it a germline SNP ($\geq 1\%$) or SNV (less common)?
 - Mutation? Deleterious truncating/frameshift?
 - Does it impact AA sequence?
 - Does AA sequence \rightarrow loss of function (LOF)?
- **NGS calls: Copy number calls**
 - Corrections needed for:
 - Tumor purity ($100\% \times \text{tumour/tumour+stroma}$)
 - Tumour ploidy (eg triploid)
- **Alterations and impact on function**
 - Alteration detected \neq loss of function
 - Need for complete protein loss?
 - Haploinsufficiency/gene dose effect (FISH?)
 - Dominant negative effect?



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Predictive biomarkers for advanced prostate cancer in 2019

- **AR biomarkers**
- PI3K/AKT and PTEN
- 'Transformation' biomarkers
- DNA repair defects
- Others

AR biomarkers

Alterations usually not present at diagnosis. Detected in blood.

- **AR mutations:** Largely in AR ligand binding domain
 - AR promiscuity; promiscuous AR driven sub-clone generated (at some point)
 - May indicate continued AR dependence actionable by AR targeting
 - Iatrogenic partial agonist (glucocorticoids to be avoided?)
- **AR amplification**
 - AR driven sub-clone generated (at some point); +ve blood assay indicates ↑ tumor burden
 - May indicate continued AR dependence actionable by AR targeting
- **AR rearrangements**
 - May impact AR splicing and signaling
- **AR splice variants** (pertains mainly to **AR-V7**)
 - Intra-patient, intra- and inter-lesion heterogeneity; difficult to disentangle prognostic vs predictive; +ve assay indicates ↑ tumour burden. May indicate disease drive by constitutively active AR. We need drugs targeting this.
- **AR driven transcripts** (including PSA in blood)
 - Indicate continued AR signaling
 - Low PSA despite disease burden may indicate low AR signaling

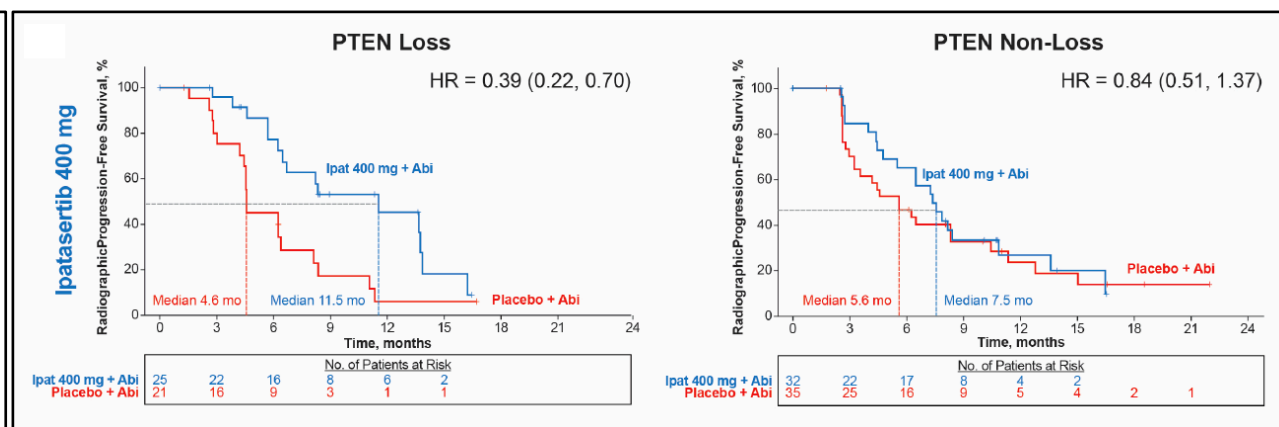
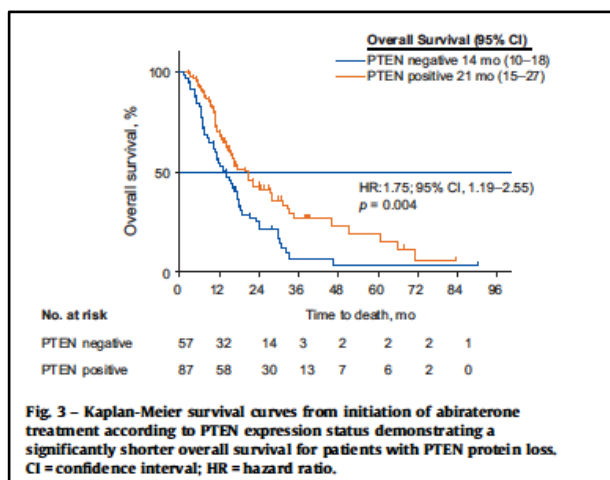
*Definitive studies needed with novel agents with antitumour activity against continued AR signaling
These biomarkers may have less utility as next generation ARSIs move into first-line space*

Predictive biomarkers for advanced prostate cancer in 2019

- AR biomarkers
- **PI3K/AKT and PTEN**
- 'Transformation' biomarkers
- DNA repair defects
- Others

PTEN and PI3K/AKT inhibitors

- PTEN loss (and activating pathway mutations) associates with poor prognosis
- PTEN loss associates with less benefit from abiraterone
- PI3K/AKT aberrations emerge with endocrine resistance
- Abiraterone and AKTi appears superior to abiraterone in PTEN loss disease
 - Awaiting Phase III data



Randomised Phase 2: Abiraterone +/- AKTi ipatasertib; Phase III ongoing

Ferraldeschi et al, European Urology 2015; de Bono et al Clinical Cancer Research 2018

Predictive biomarkers for advanced prostate cancer in 2019

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'Tumor Transformation': Lineage plasticity

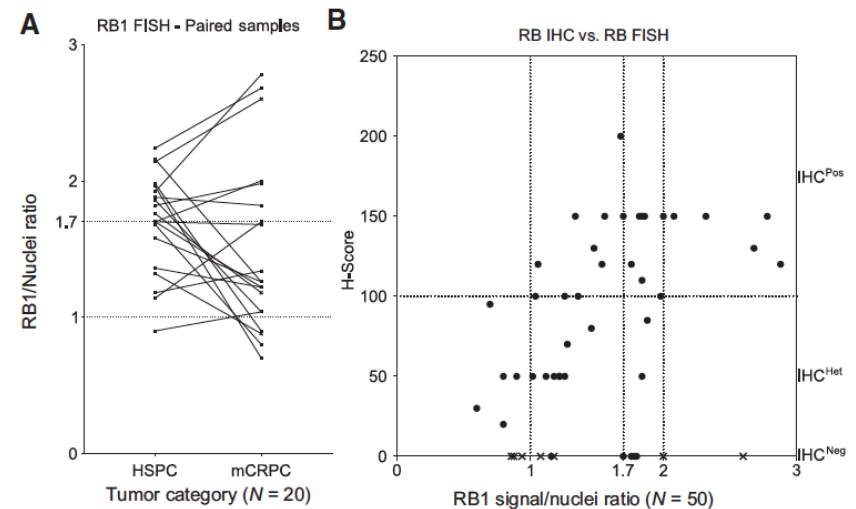


We need to settle on a best descriptor for what is termed: Neuroendocrine; small round blue cells; luminal-to-basal; AR independent clones; RB1/TP53 lost; SOX2 high.....?

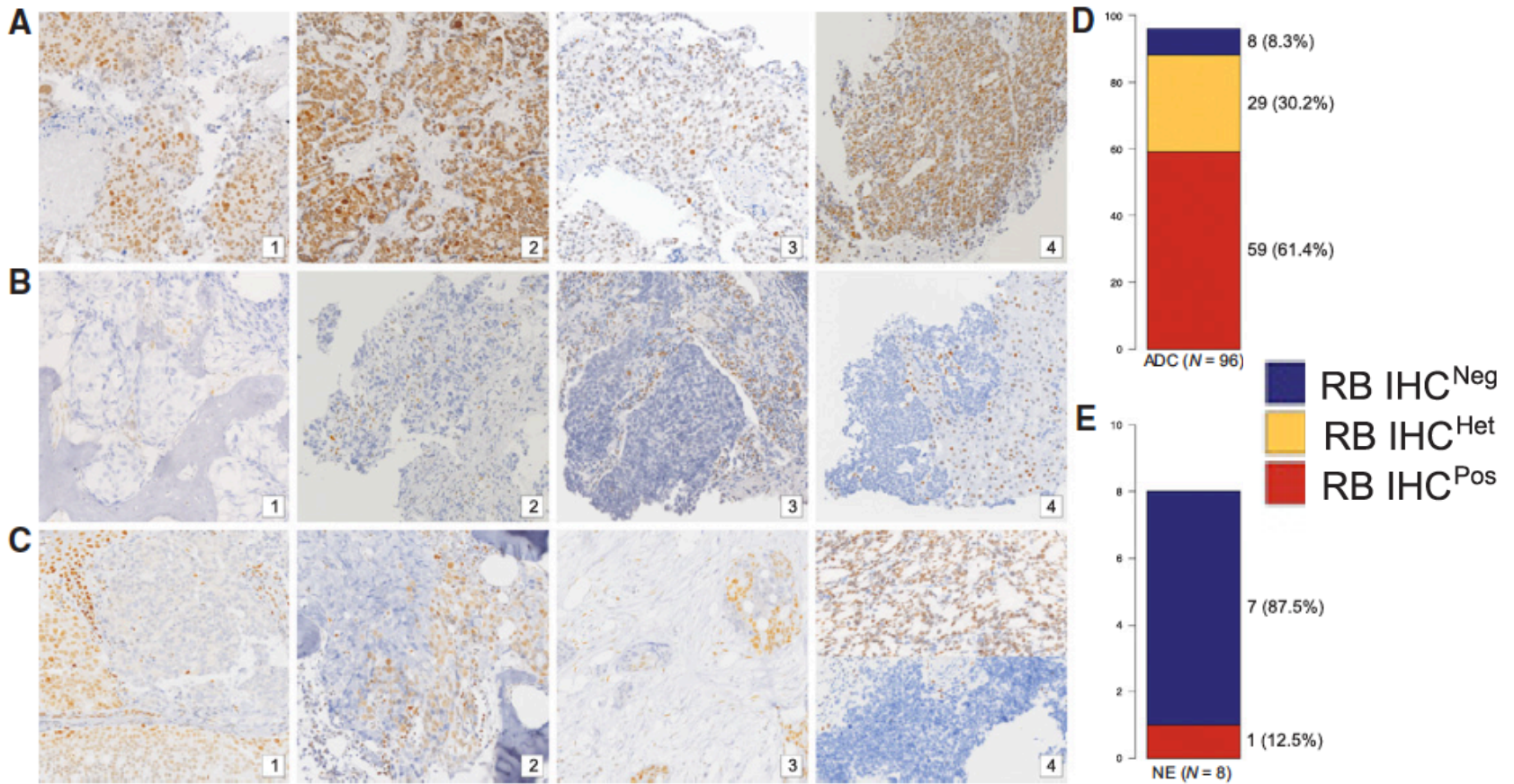
Identifying 'transformation' (may) matter

- The cancer tries to find ways to evade treatment
 - 'Transformation' commonly sub-clonal
 - RB1 FISH deletion is detectable at diagnosis in adenocarcinoma
- Lineage **plasticity** following ARSI pressure (eg abiraterone)
 - **Lineage plasticity**
 - Can lose "AR signaling" dependency ('stem cell' like)
 - Can result in 'neuroendocrine phenotype'
 - Can be PSMA negative

Rodrigues et al, CCR 2018

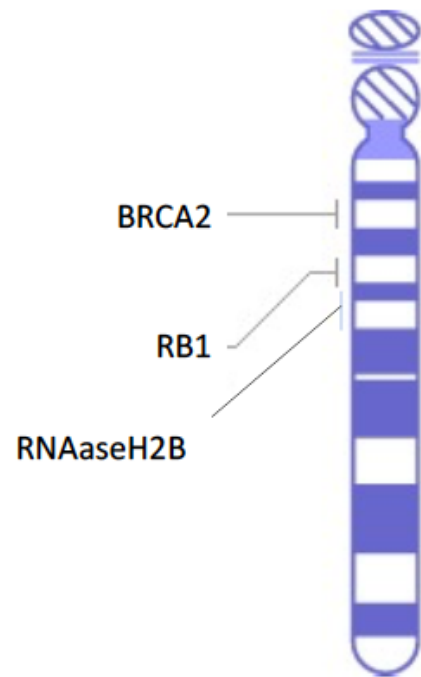


RB1 immunohistochemistry in mCRPC biopsies



Rodrigues et al, CCR 2018. Please note this transformation can happen in other cancers (lung, breast)

RB1 subclonal loss: Basal/Neuroendocrine phenotype
Concurrent loss of RNASEH2B and/or BRCA2 likely



Chromosome 13

Both RNASEH2B loss and BRCA2 loss sensitize to PARP inhibition/platinum

Seed G et al, CCR 2017; Zimmermann et al, Nature 2018

Predictive biomarkers for advanced prostate cancer in 2019

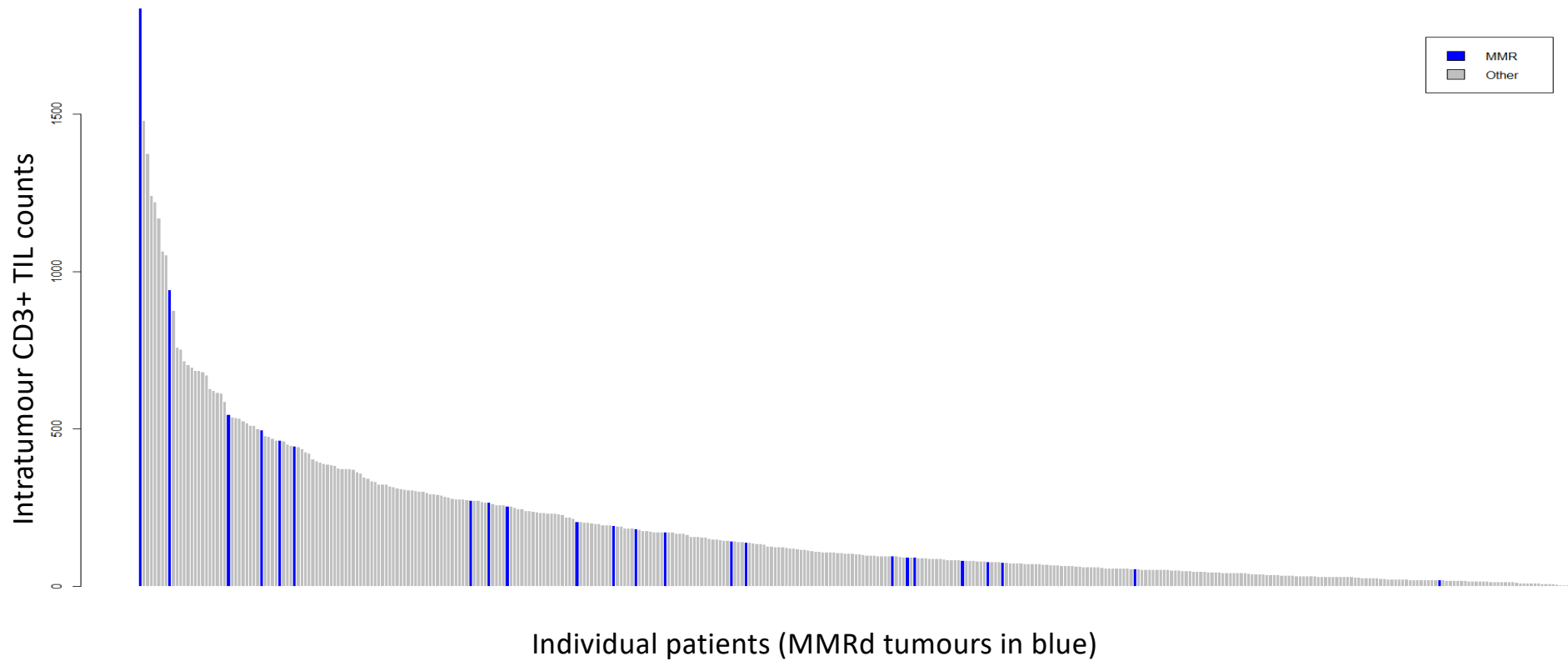
- AR biomarkers
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- **DNA repair defects**
- Others

Mismatch repair defective prostate cancer

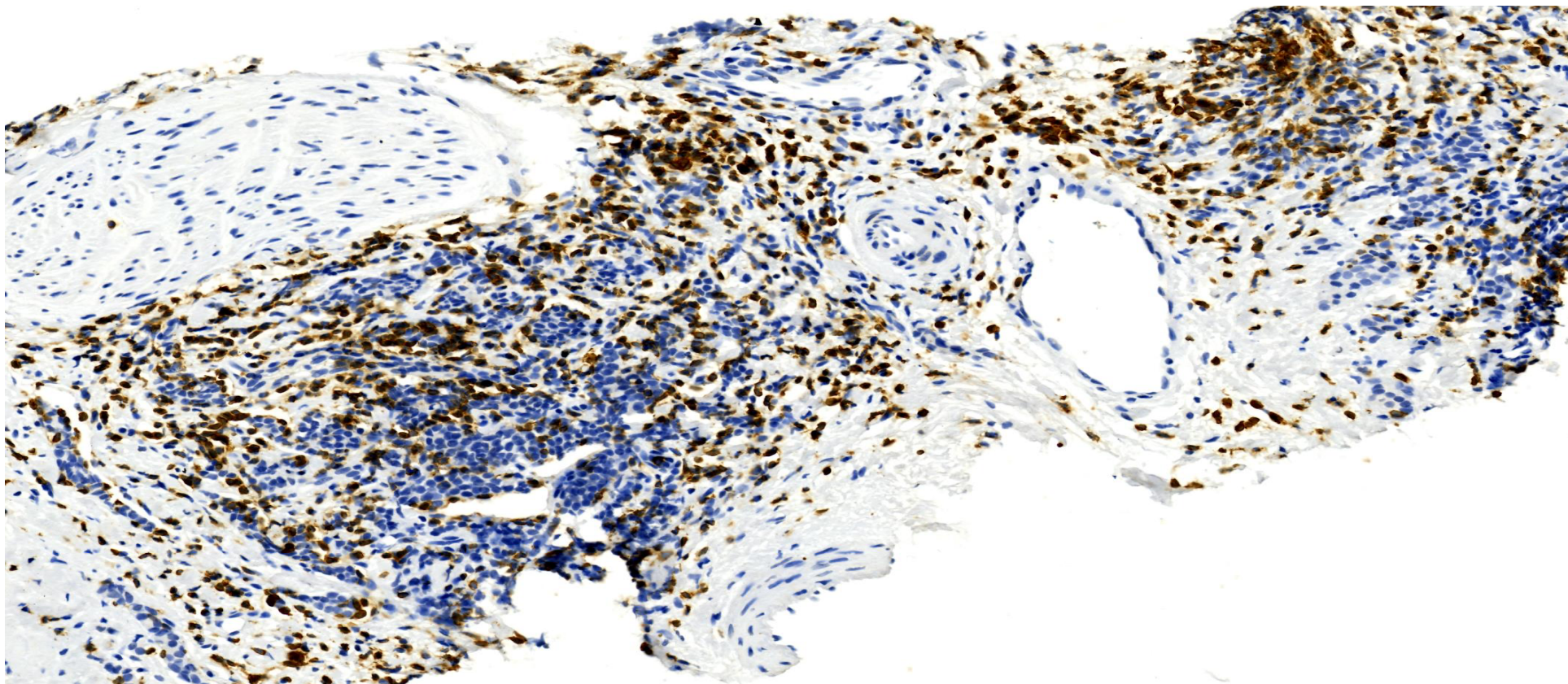
- Found in a small percentage ($\leq 5\%$) of mCRPC
 - Often but not always associated with high TILs
 - Sometimes but not always respond to PD-1/PD-L1 checkpoint inhibitors
- Orthogonal assays needed to ensure we detect them all
 - Promega MSI PCR assay works poorly on FFPE
 - NGS can miss many deleterious aberrations (eg rearrangements)
 - IHC can miss deleterious aberrations (non-functional protein can still be stained)
 - Can be detected by cfDNA analyses but precision of such assays needs confirmed.

Introducing complexity: CD3 T-cell mCRPC biopsy density

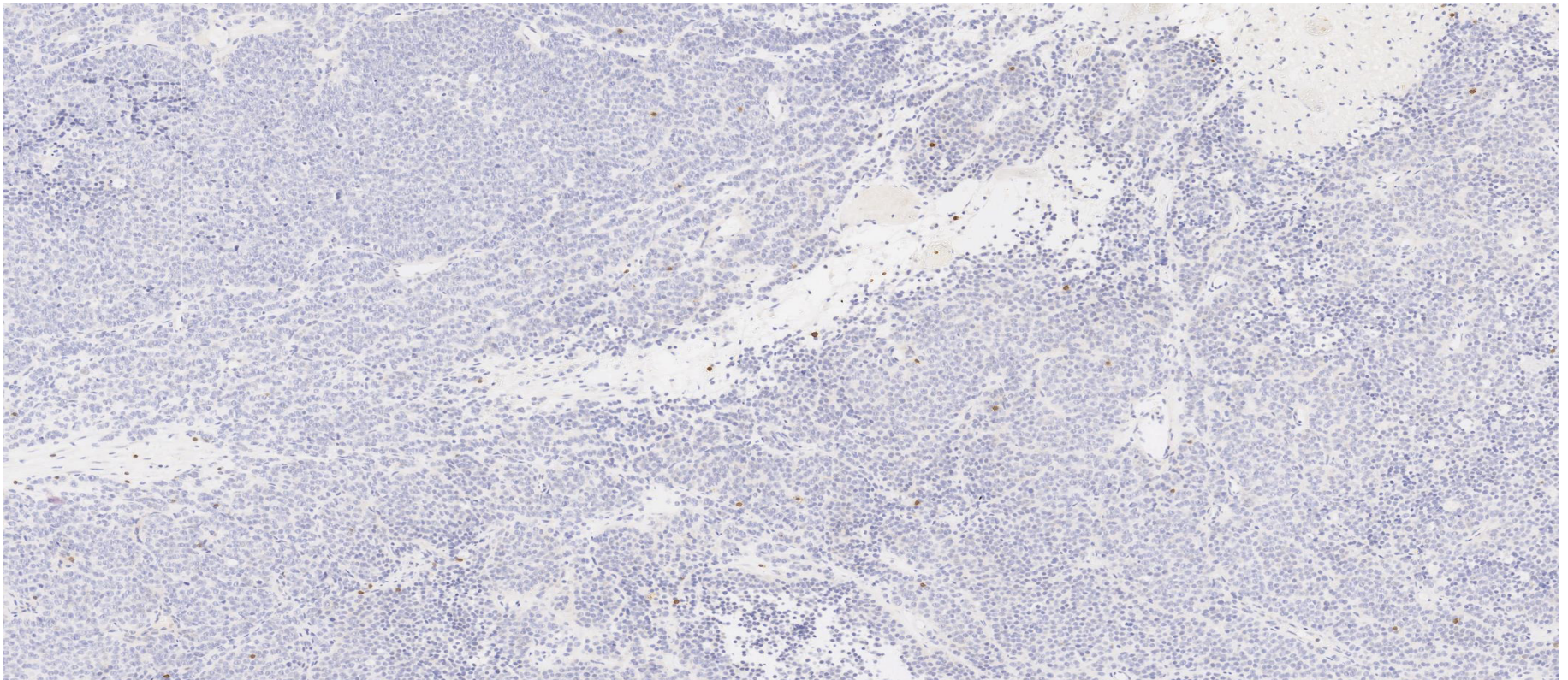
MMRd tumours highlighted in blue bars



CD3 cell high MMRd mCRPC

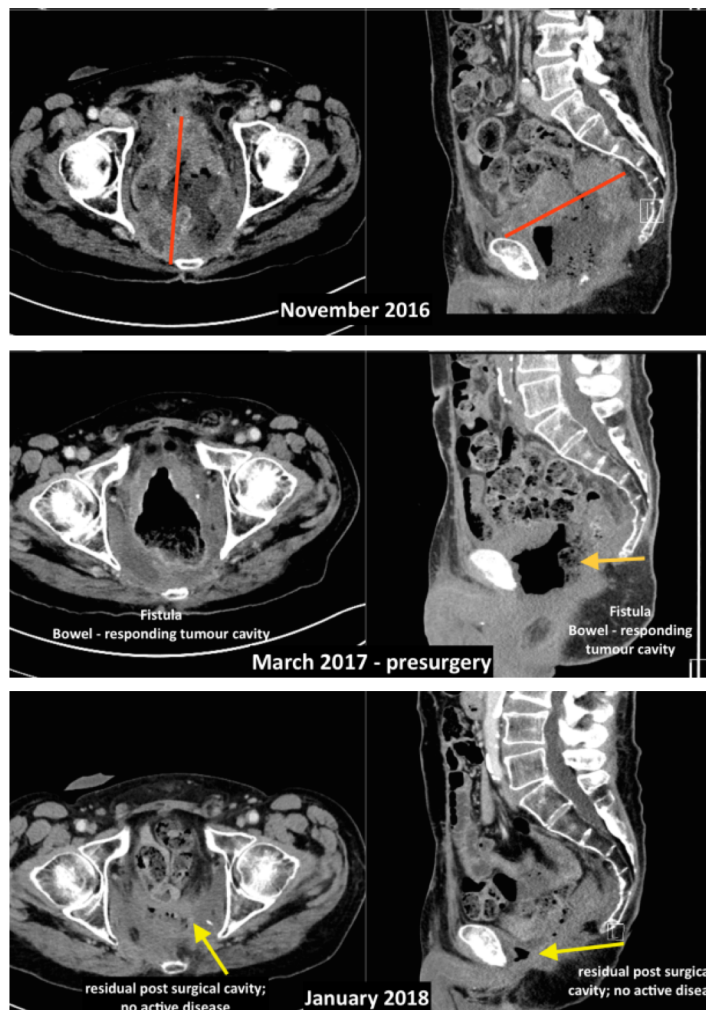


CD3 cell low MMRd mCRPC



MMRd responder

- Medical history: rectovesical fistula (2012)
- Disease course
 - Initial diagnosis: T4N0M0 GS10 (5 + 5) (Sep 2005)
 - Metastasized to lymph nodes (Nov 2009)
- Prior systemic therapy
 - Gosrelin (Sep 2005) + bicalutamide (Jan 2006)
 - Abiraterone (Nov 2009-Dec 2014)
 - Docetaxel (Mar 2015-Nov 2015)
 - Enzalutamide (Jan 2016-Jul 2016)
- Enrolled in KEYNOTE-199 cohort 1
 - Age 70 years
 - **MMRD by IHC^a**
 - First pembro dose: Nov 25, 2016
 - Last dose (cycle 11): Jun 13, 2017
 - Last survival follow-up: Summer 2020
 - Still in remission



**Nov 2016 (Baseline):
Bowel fistula**

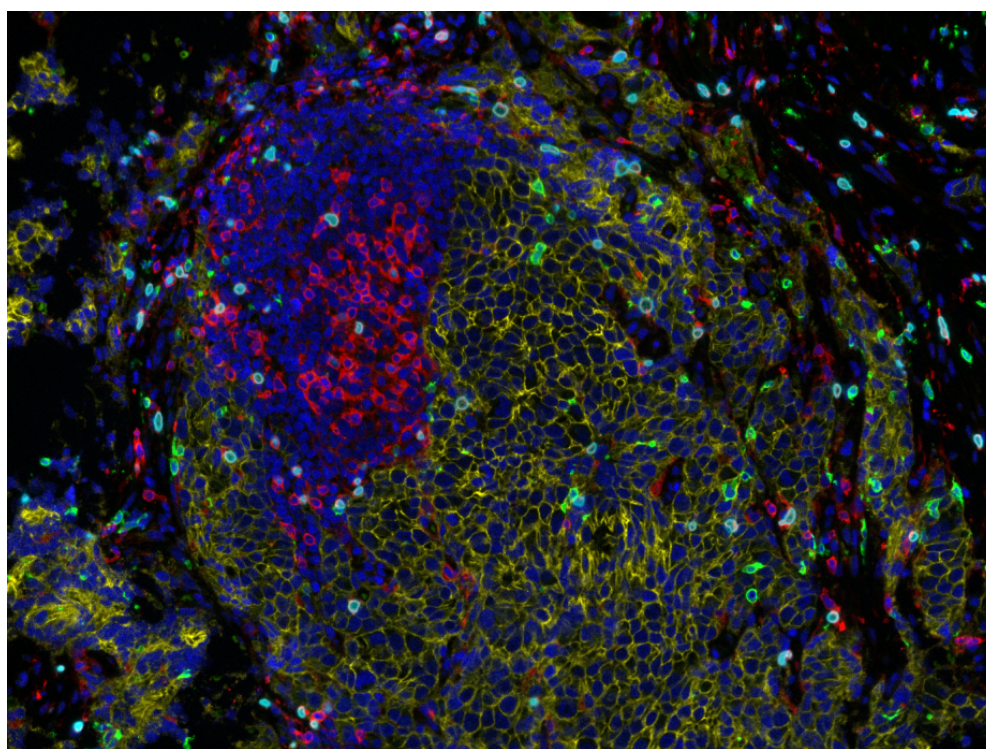
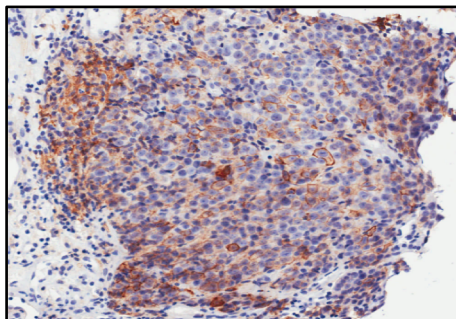
**Mar 2017
(Presurgery):
Bowel fistula shows
responding tumor
cavity**

**Jan 2018:
Residual post-surgical
cavity, no active
disease on whole body
MRI**

^a RM Patient. Analysis performed in the de Bono lab at The Institute of Cancer Research in London. This work was funded by Movember and the PCF

TILs and PD-L1 IHC in the mCRPC biopsy of this responder

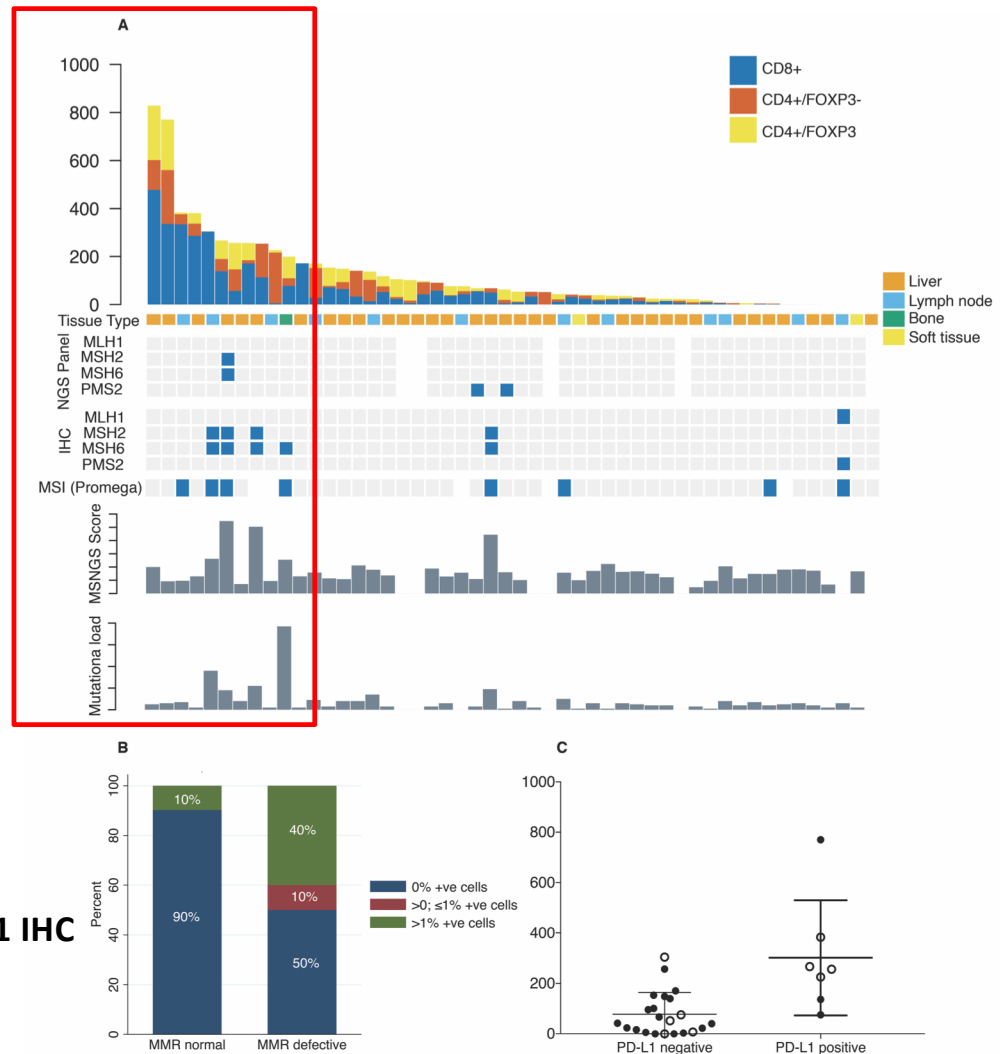
PD-L1 IHC



- CD4
- CD8
- CD4/FOXP3
- Tumor Mask
- Nuclear counterstain

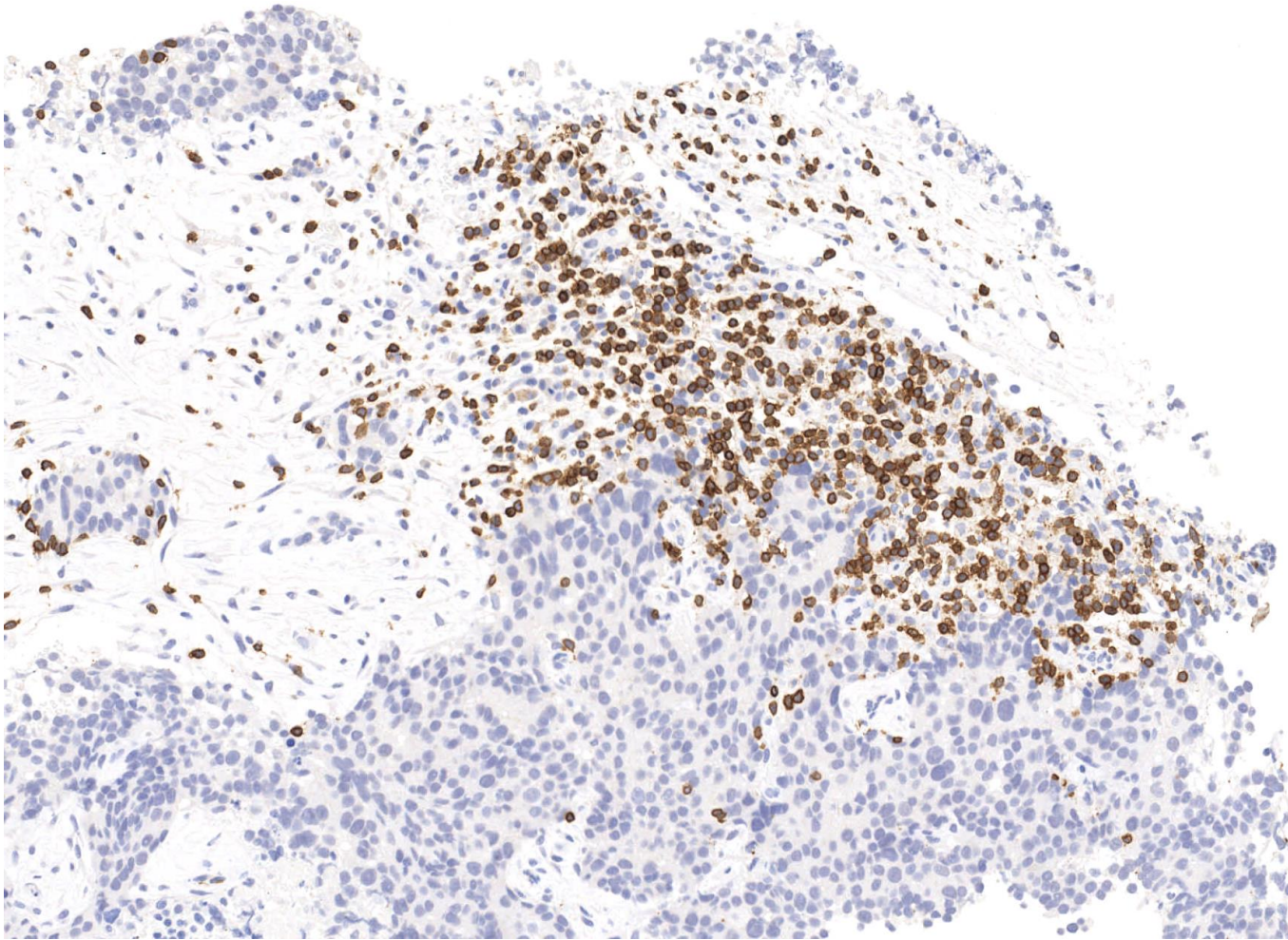
Analysis performed in the de Bono lab at The Institute of Cancer Research in London. This work was funded by Movember and the PCF

50 RM mCRPC patients Immunogenomic analyses

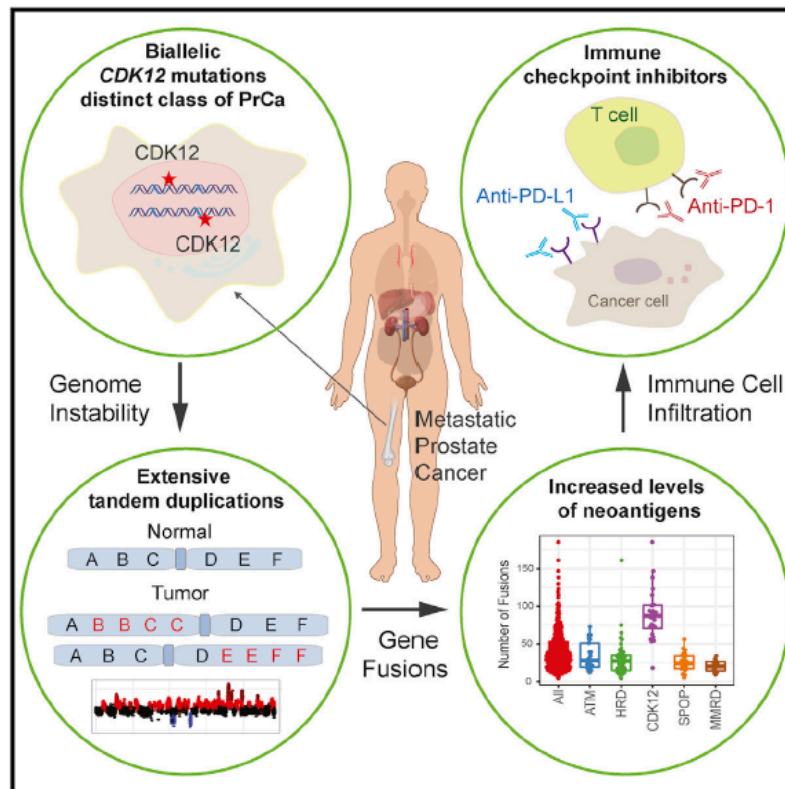


Rodrigues D et al, JCI 2018

CD3 cell high mCRPC: MMR normal but CDK12 bi-allelic mutations



Inactivation of *CDK12* Delineates a Distinct Immunogenic Class of Advanced Prostate Cancer



Highlights

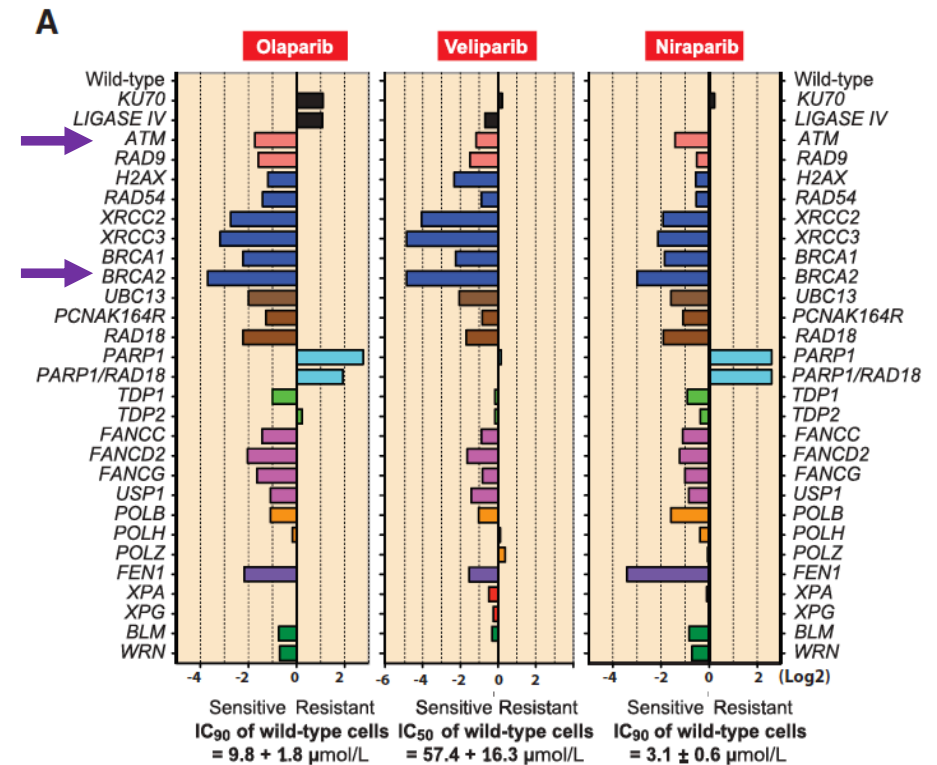
- *CDK12* biallelic inactivating mutations define a distinct subtype of prostate cancer
- *CDK12* loss is associated with genomic instability and focal tandem duplications
- *CDK12* loss leads to increased gene fusions, neoantigen burden, and T cell infiltration
- Patients with *CDK12* mutant tumors may benefit from immune checkpoint inhibition

CDK12 Aberrations (NGS): Royal Marsden cohort

- >400 samples analysed
- ~5-6% had CDK12 mutations
- CDK12 mutations not associated with worse prognosis
- **Bi-allelic CDK12 mutated cancers have significantly higher TILs**
 - Not seen in mono-allelic CDK12 mutated disease in this cohort

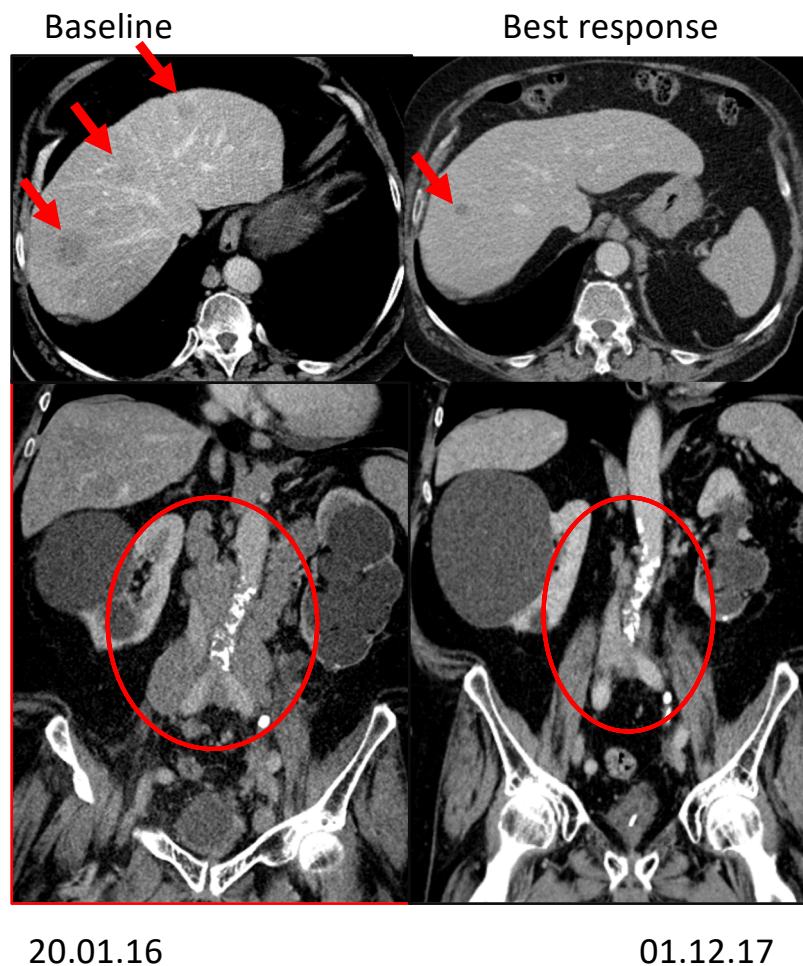
PARP inhibition, platinum, and DNA repair defects

- DNA damage repair (DDR) gene aberrations found in 20-25% of mCRPC
 - Germline and/or somatic
 - *BRCA2* is commonest altered DDR gene (10%)
- PARP inhibitors and platinum synthetically lethal with many DDR gene aberrations including *BRCA2*, *BRCA1*, *PALB2*, *ATM*
 - Different magnitudes of sensitization
 - Bi-allelic loss usually required to sensitize
- PARP inhibitors already approved for treating ovarian and breast cancers

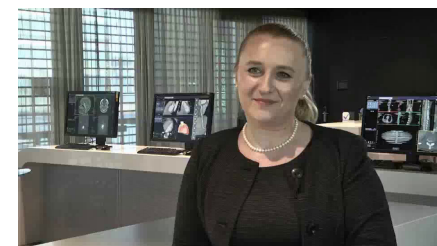


Murai et al, Cancer Res 2012

BRCA2 altered mCRPC responding patient on TOPARP-B



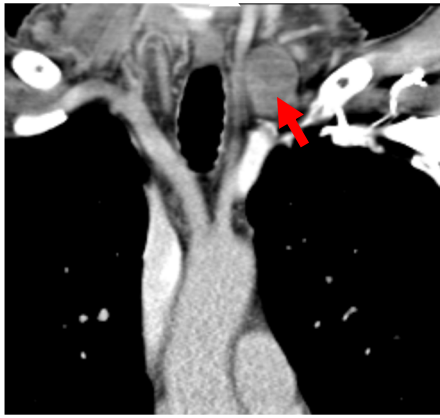
Axial and Coronal Contrast Enhanced CT images at baseline and at best response (23 months of treatment) showing maintained Partial Response with almost complete resolution of multiple liver metastases (arrows) and significant reduction in size of retroperitoneal lymphadenopathy (circles);



Courtesy of Dr Nina Tunariu

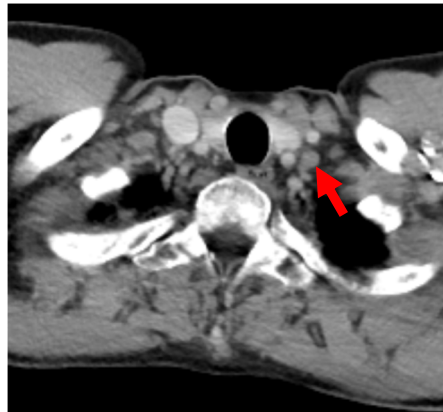
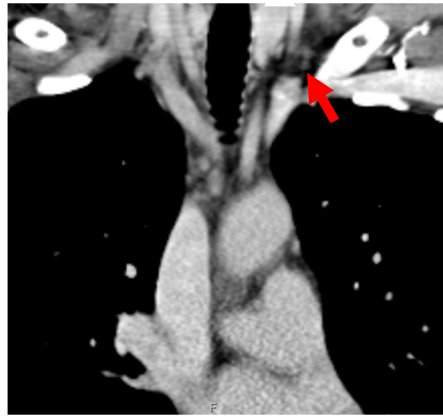
PALB2 altered mCRPC responding patient on TOPARP-B

Baseline



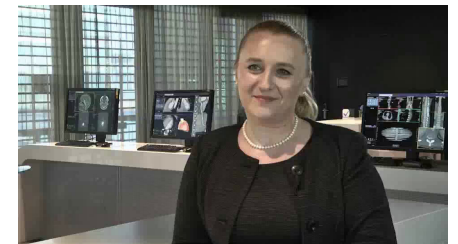
29.05.18

Best response



29.07.19

Coronal and Axial Contrast Enhanced CT images at baseline and present (24 months of treatment) complete response (CR) of left supraclavicular lymphadenopathy (arrows). Patient also had CR at the the sites of small volume lymphadenopathy.



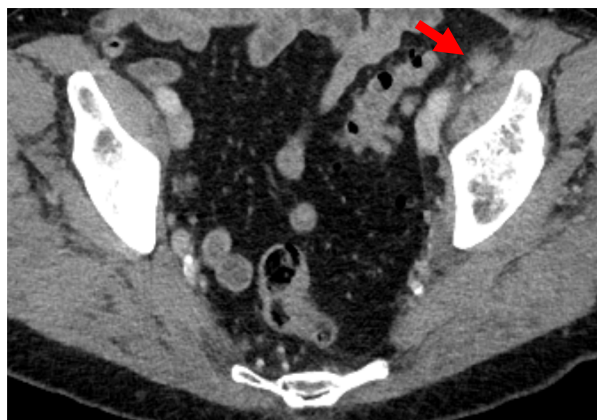
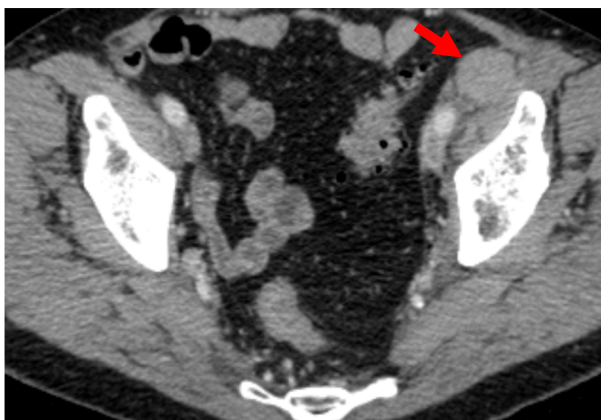
Courtesy of Dr Nina Tunariu

ATM altered mCRPC responding patient on TOPARP-B

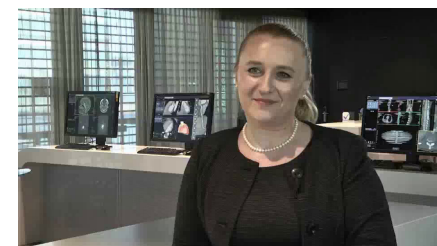
Baseline



Best response

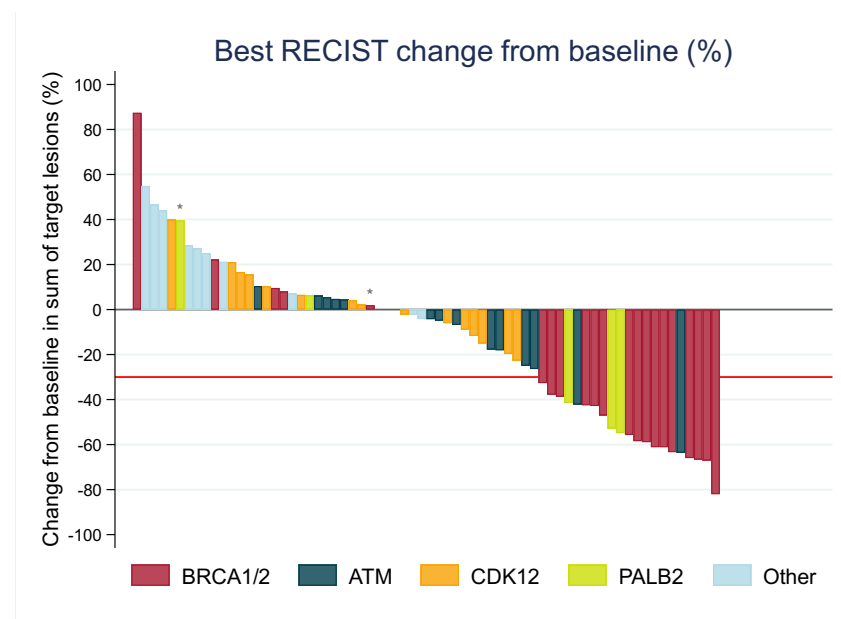
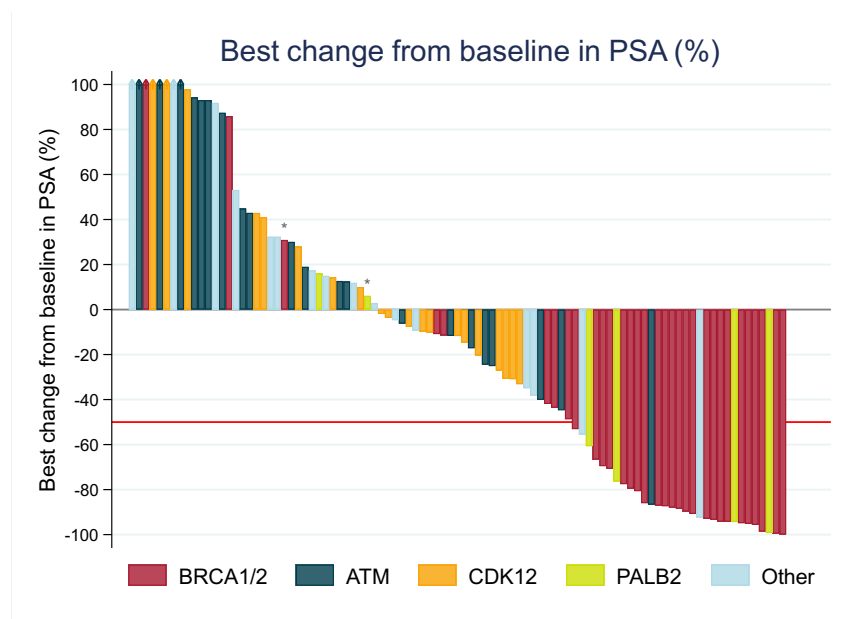


Axial Contrast Enhanced CT images at baseline and at best response (9 months of treatment) showing maintained PR with significant reduction in porto-caval and left external iliac nodes (arrows)



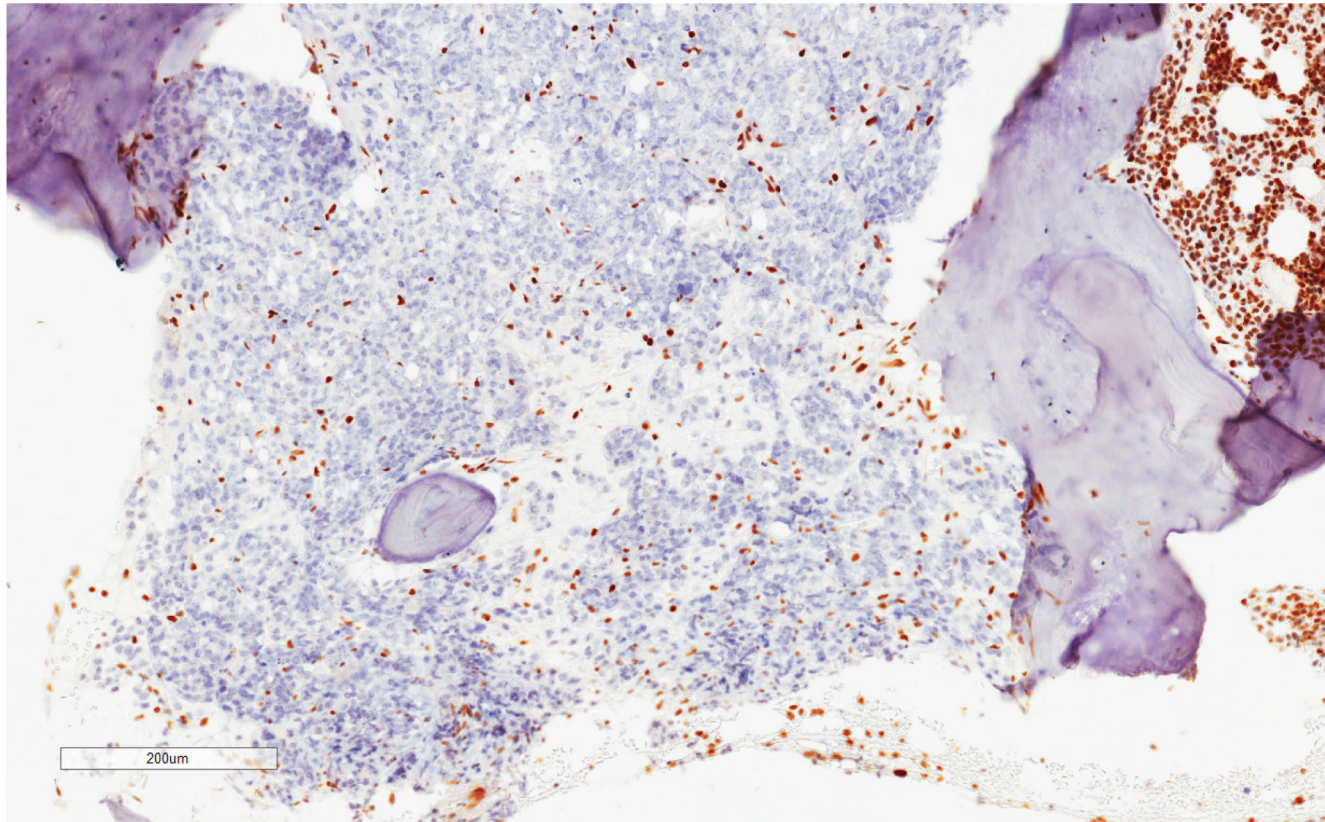
Courtesy of Dr Nina Tunariu

TOPARP-B antitumour activity by gene alteration*



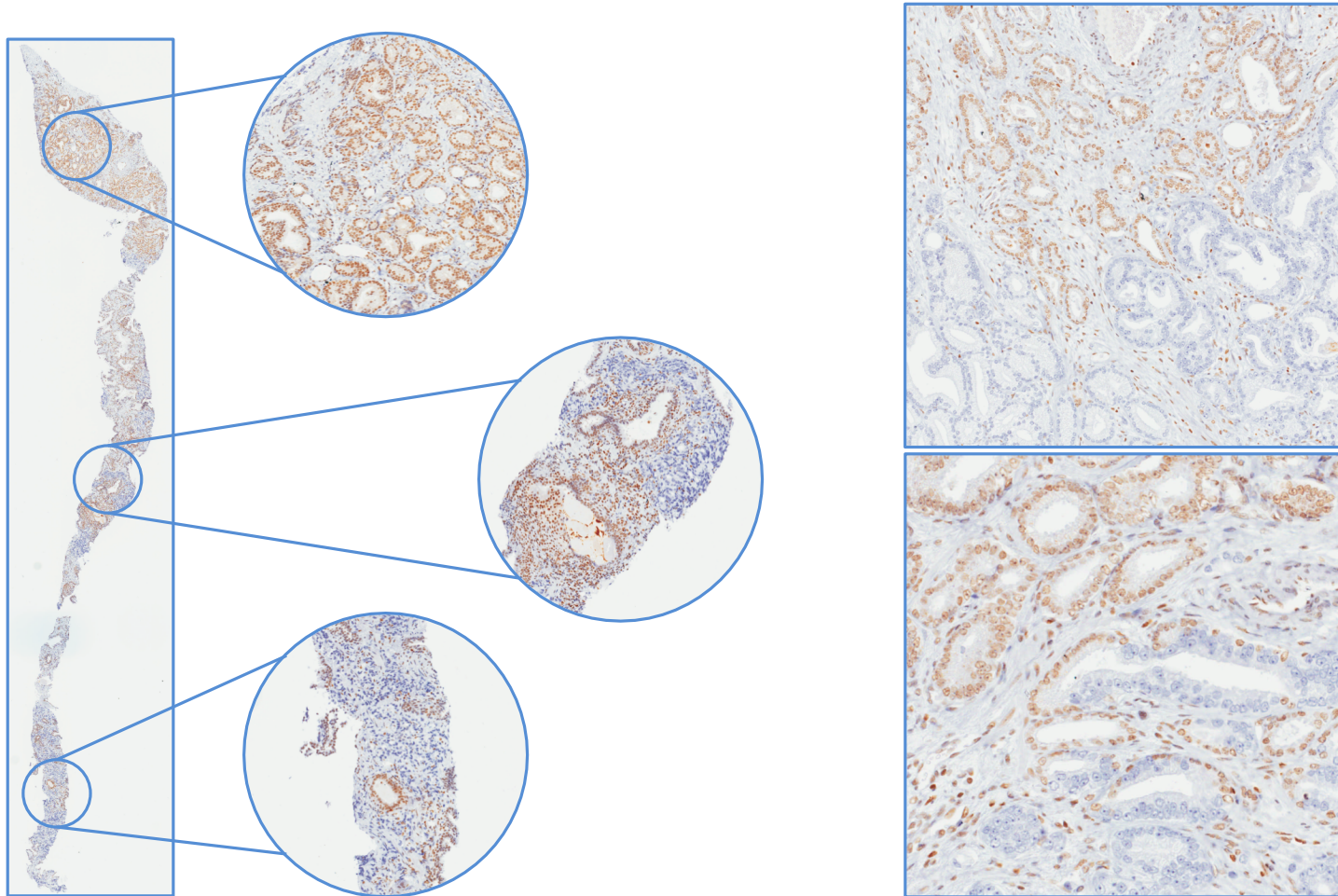
*Any alteration detected by NGS deemed possibly deleterious

ATM immunohistochemistry: Is this the best ATM assay?



Does biological context matter?

ATM loss can demonstrate intra-patient heterogeneity

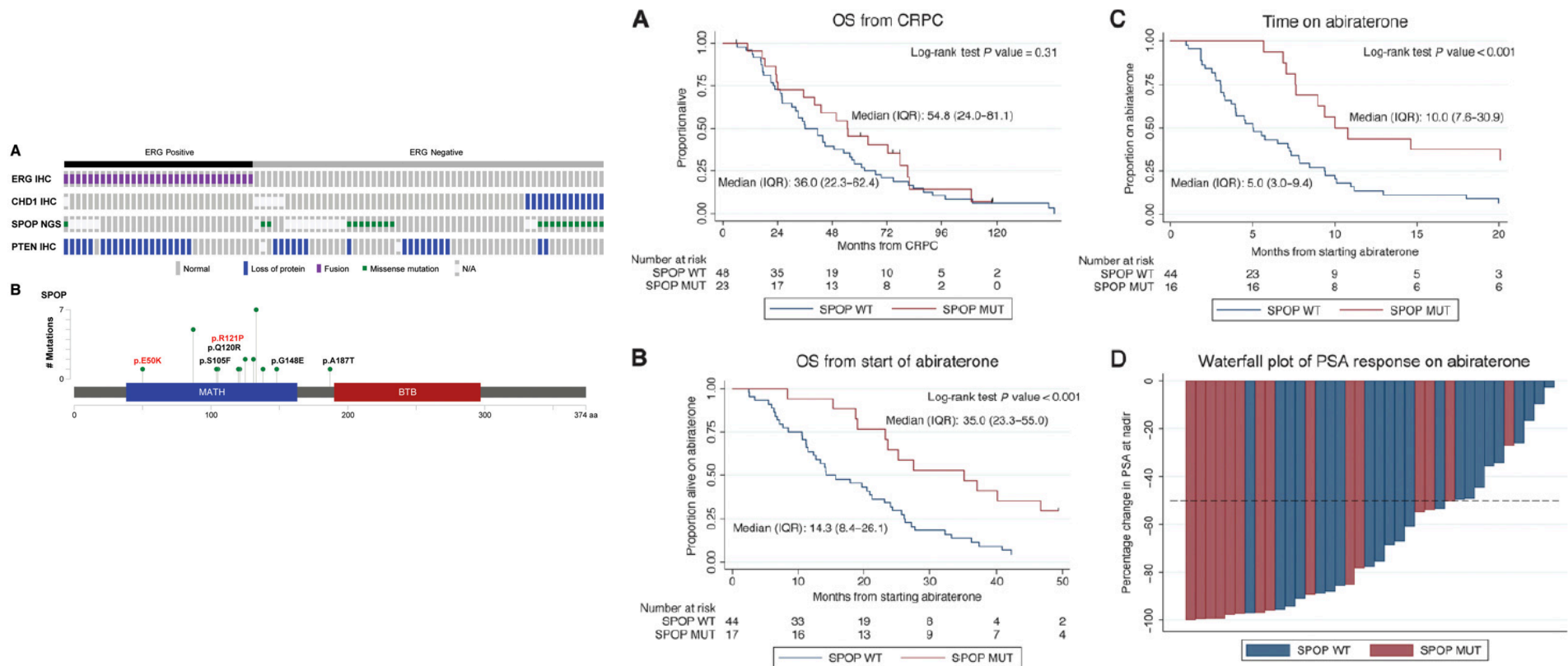


Predictive biomarkers for advanced prostate cancer in 2019

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- PI3K/AKT and PTEN
- 'Transformation' biomarkers
- DNA repair defects
- **Others**

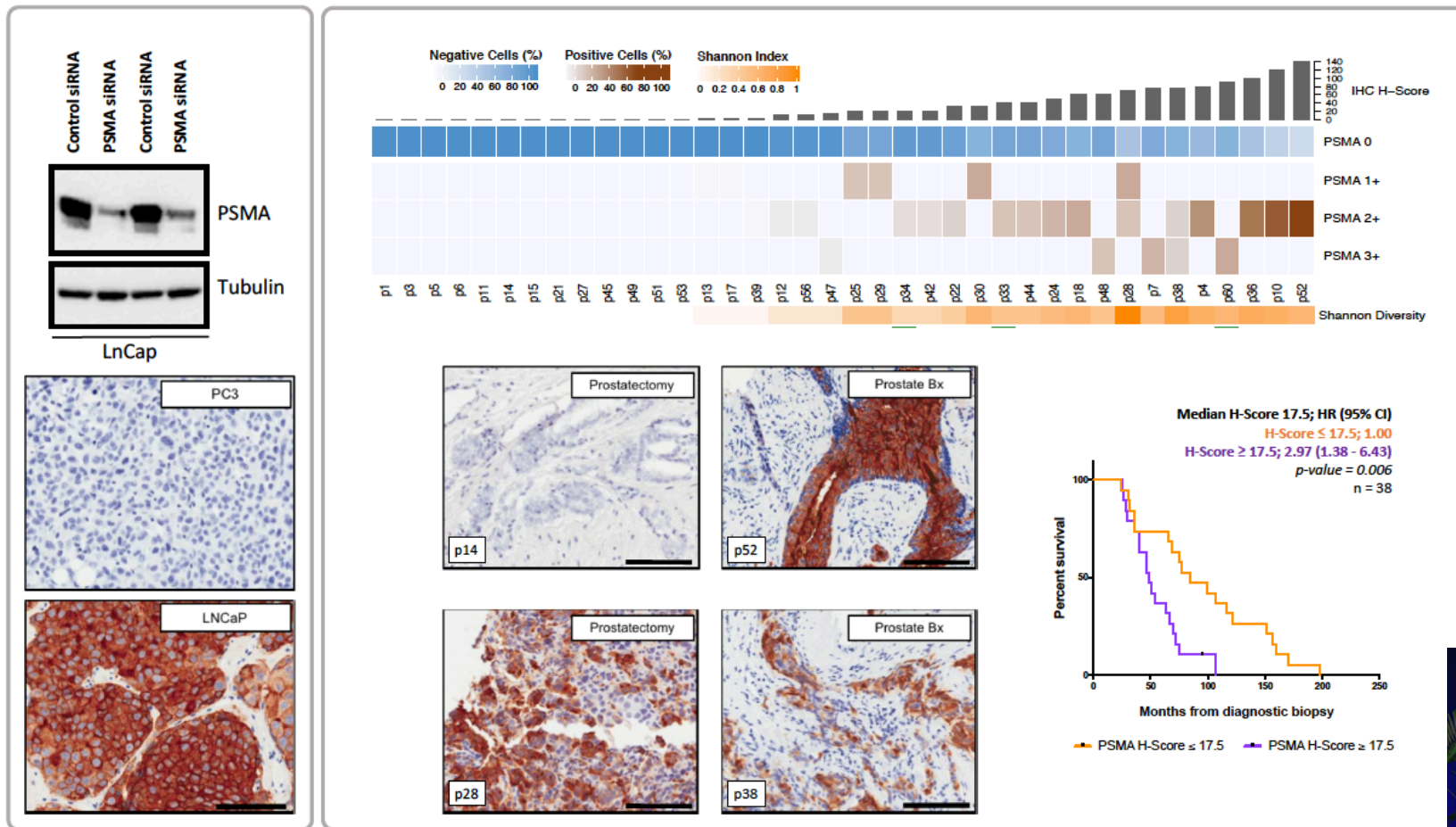
SPOP and abiraterone sensitivity

Most patients with SPOP mutated mCRPC respond



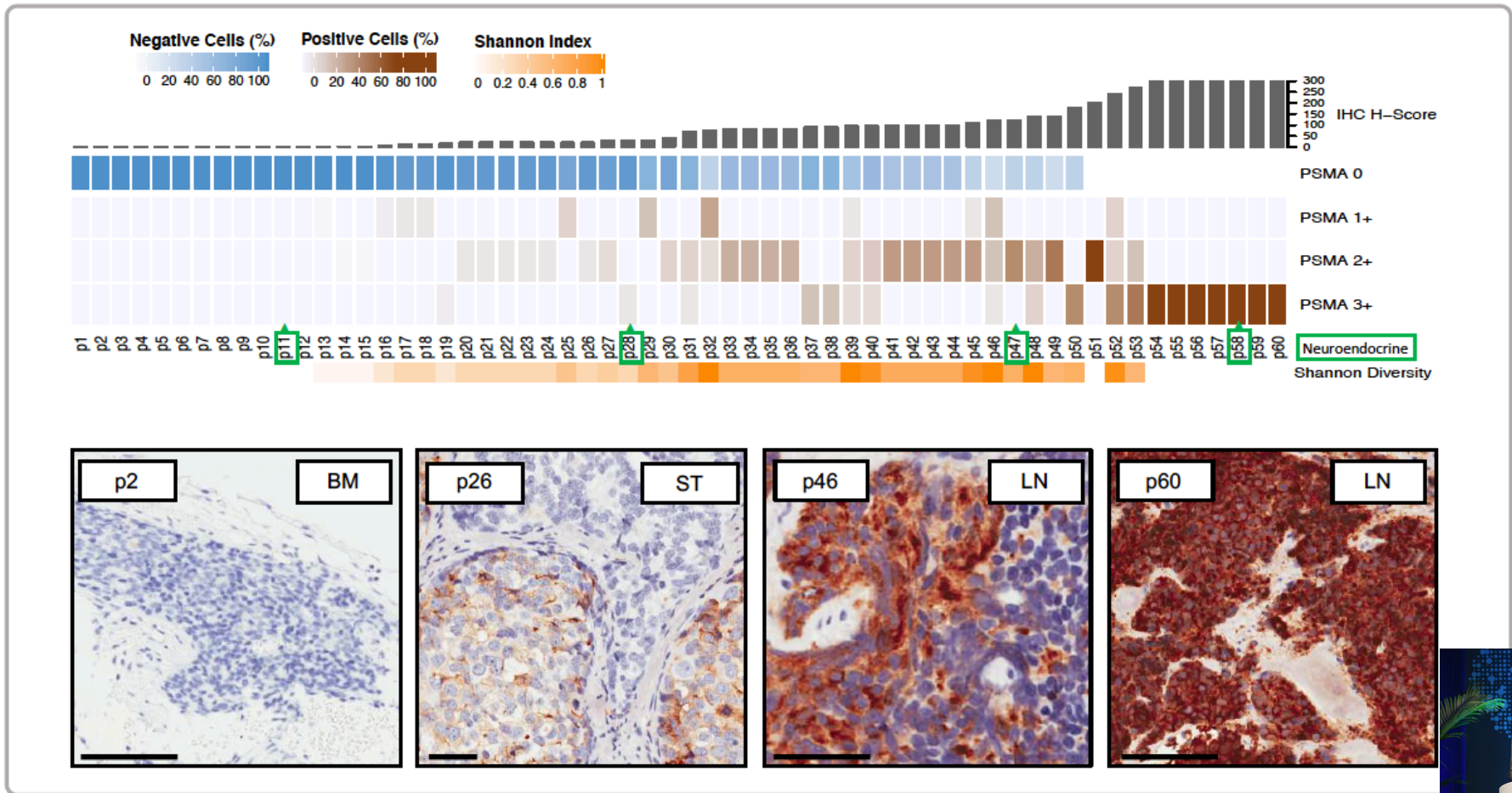
Boysen et al, CCR 2018

mPSMA: Intra- and inter-patient heterogeneity in HSPC



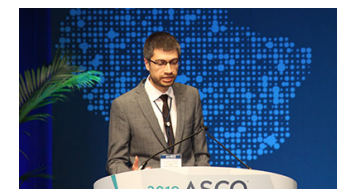
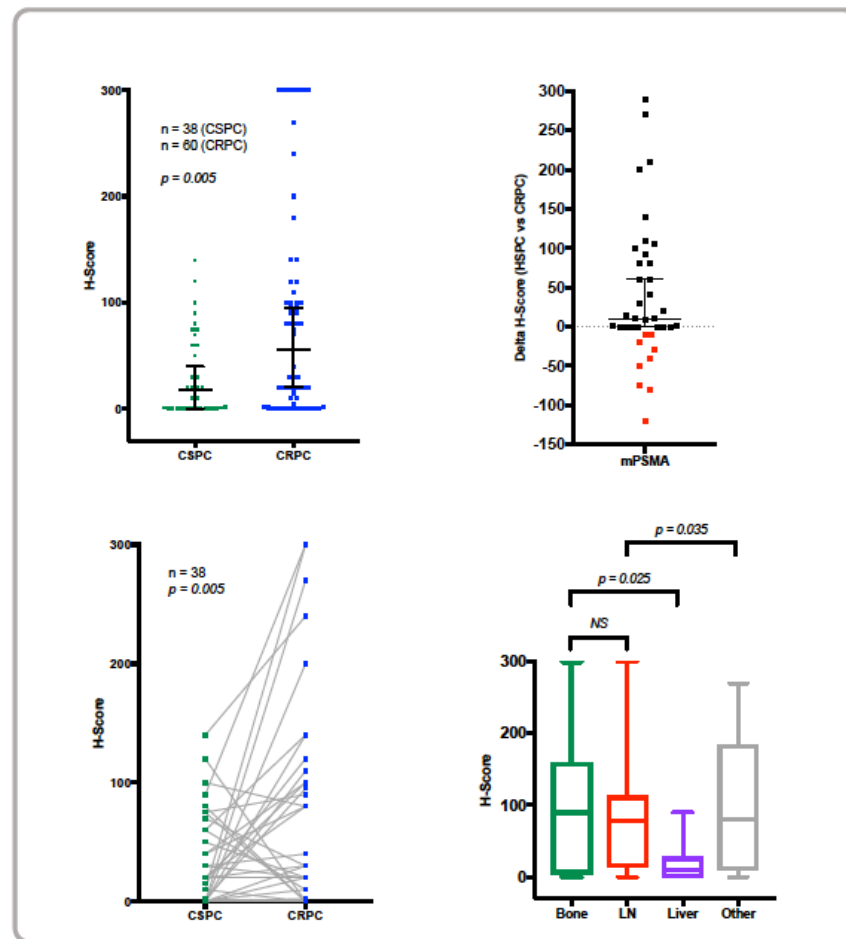
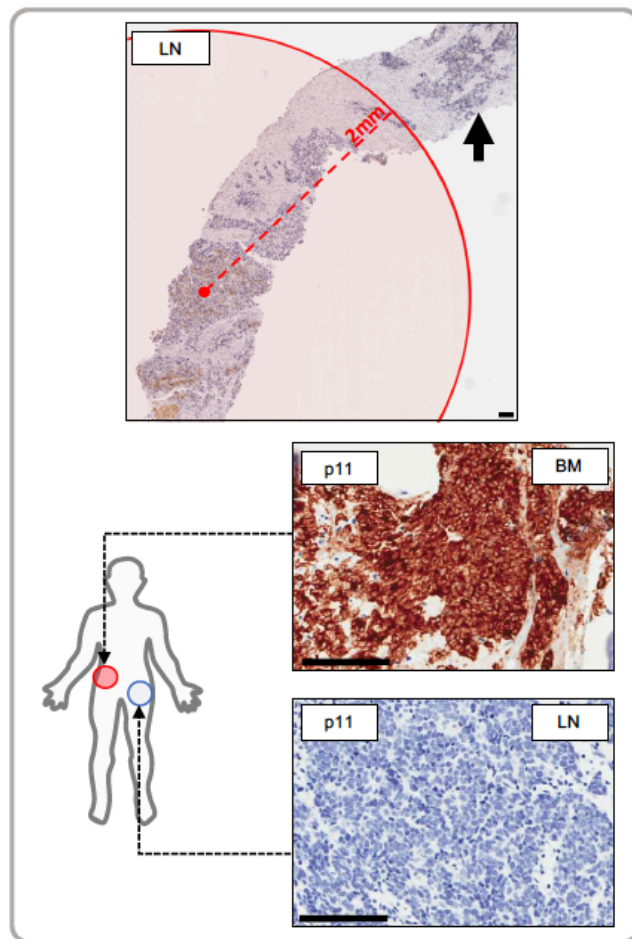
Paschalis et al, 2019

mPSMA : Intra- and inter-patient heterogeneity in mCRPC



Paschalis et al, 2019

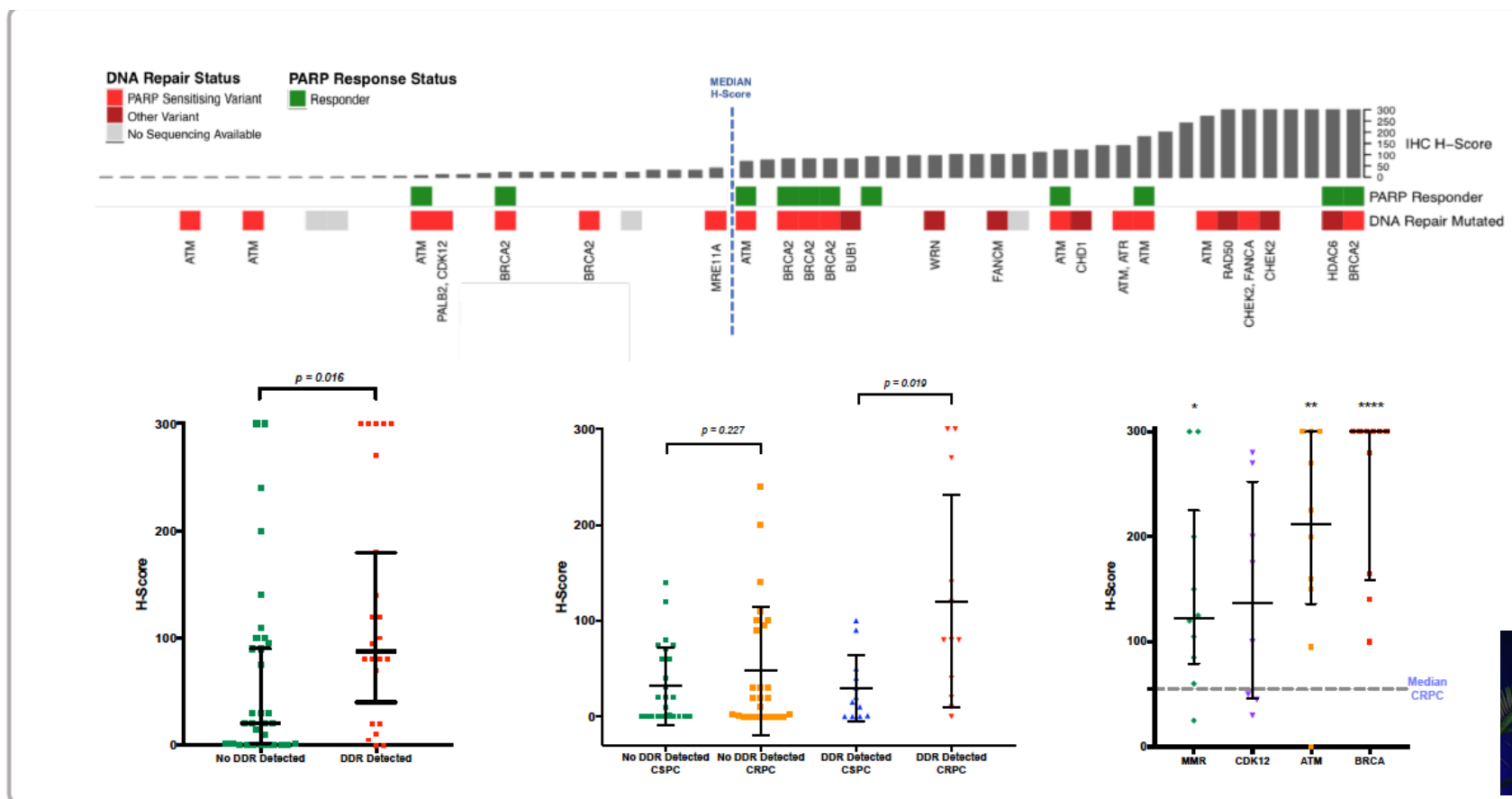
mPSMA and beta-particle penetration depth
mPSMA is low in mCRPC liver mets (in keeping with PSMA PET data)



Paschalis et al, 2019

mPSMA (folate hydrolase) and DNA repair defects

Test and validation sets



Paschalidis et al, s2019

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Conclusions

- Assay analytic validation and clinical qualification is an urgent need
 - An alteration/mutation \neq loss of function
 - Orthogonal assays may be needed for precision
 - Bespoke prospective trials needed to qualify validated biomarkers
- Molecular stratification for mCRPC is going to become a standard
 - Assays for MMRd and BRCA2, BRCA1, PALB2, ATM, FANCA, RAD51, ATM, CDK12
 - SPOP mutated cancers: Do very well on ARSI
- More data needed to prove utility of AR and PTEN/PI3K/AKT assays
 - AR alterations data needs active drugs eg AR degraders, AR-SV inhibitors
 - Phase III trials of ARSI and AKTi could make PI3K/AKT/PTEN assays standard of care
- PSMA targeting agents need to pursue patient selection
 - Function of PSMA needs to be elucidated to improve combination selection
- Many new therapeutic strategies emerging
 - Further major advances in prostate cancer care envisioned in the next 5-years

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