Genomic Markers and DNA Sequencing Testing

Future Directions in Urology Symposium
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Daniel W. Lin, MD
Professor and Chief of Urologic Oncology
Pritt Family Endowed Chair in Prostate Cancer Research
University of Washington, Department of Urology
Fred Hutchinson Cancer Research Center, Division of Public Health Sciences

The Challenge

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PRESS RELEASES

NCI Press Release

TAILORx trial finds most women with early breast cancer do not benefit from chemotherapy

Posted: June 3, 2018

Contact: NCI Press Office
240-760-6600

New findings from the groundbreaking Trial Assigning Individualized Options for Treatment (Rx), or TAILORx trial, show no benefit from chemotherapy for 70 percent of women with the most common type of breast cancer. The study found that for women with hormone receptor (HR)-positive, HER2-negative, axillary lymph

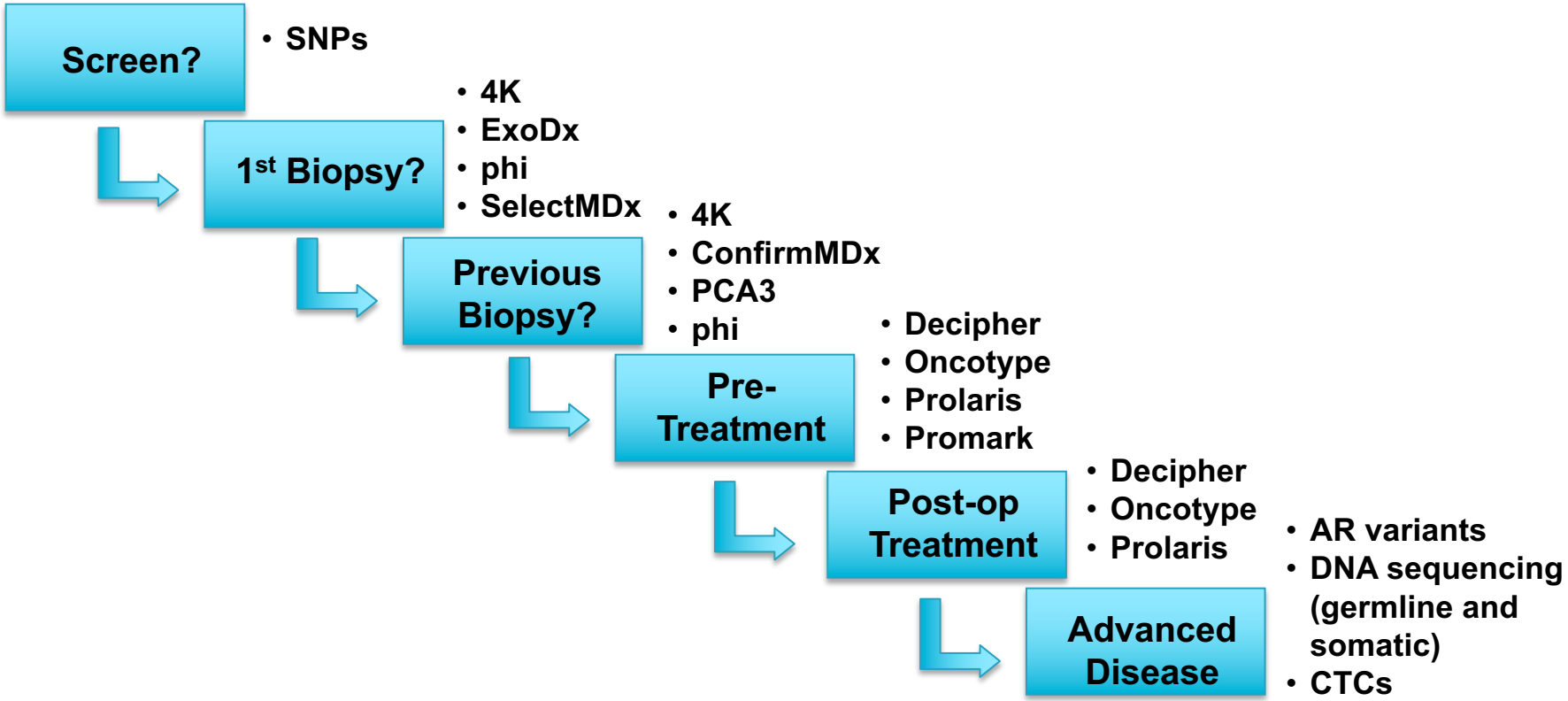
Important Biomarker Questions

- What is data to support the biomarker?
  - Biologic rationale, feasibility
  - Appropriate application and validation

- Does it add to established models that are based on readily available clinical and pathologic data?

- What is clinical impact of improved prediction?
  - Will it change pre-/post-therapy decision-making? (utility)
  - Impact on choice of therapy?
  - Impact on disease-specific outcomes? (OS, PFS, etc)

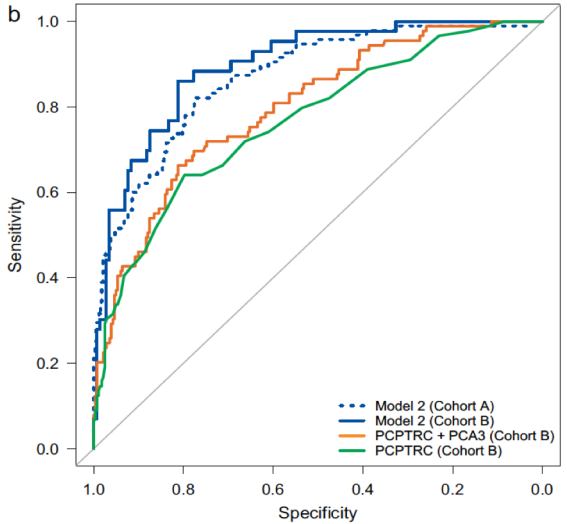
Disease State Biomarkers (partial list)



Detection of High-grade Prostate Cancer Using a Urinary Molecular Biomarker–Based Risk Score

Leander Van Neste[a,1], Rianne J. Hendriks[b,1], Siebren Dijkstra[b,1], Geert Trooskens[c], Erik B. Cornel[d], Sander A. Jannink[c], Hans de Jong[c], Daphne Hessels[c], Frank P. Smit[c], Willem J.G. Melchers[e], Gisèle H.J.M. Leyten[b,f], Theo M. de Reijke[f], Henk Vergunst[g], Paul Kil[h], Ben C. Knipscheer[i], Christina A. Hulsbergen-van de Kaa[j], Peter F.A. Mulders[b], Inge M. van Oort[b], Wim Van Criekinge[k], Jack A. Schalken[b,]*

- Two independent, prospective trials, n=905
  - mRNA levels of homeobox C6 (HOXC6), distal-less homeobox 1 (DLX1), and tudor domain containing 1 (TDRD1)
  - Source: post-DRE urine
  - Endpoint: High grade prostate cancer



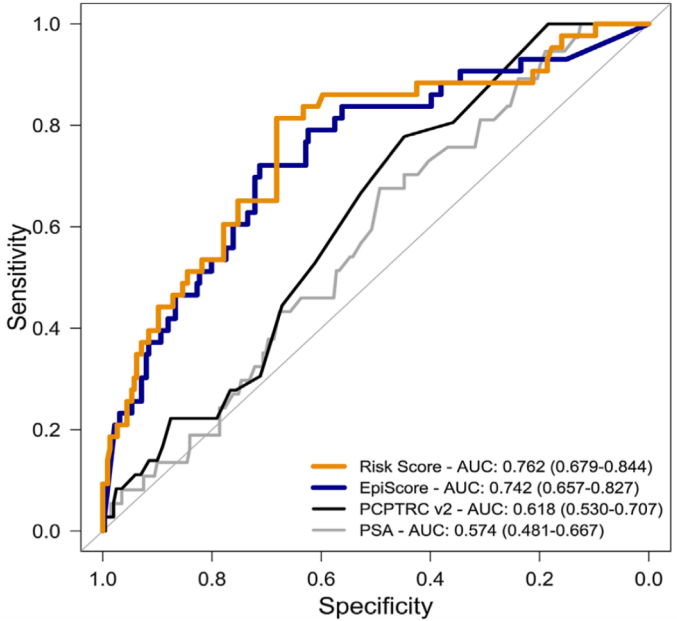
- Outperforms PCPT risk calculator
- Reduces biopsy rate
  - Estimated 43-58% reduction
- NPV 93-94% for high grade PCa

Van Neste et al, Eur Urol, 2016

Risk Score Predicts High-Grade Prostate Cancer in DNA-Methylation Positive, Histopathologically Negative Biopsies

Leander Van Neste,[1] Alan W. Partin,[2] Grant D. Stewart,[3] Jonathan I. Epstein,[2]
David J. Harrison,[4] and Wim Van Criekinge[5]*

- Examined association of degree of methylation and high grade prostate cancer
- Outperforms PCPT risk calculator
- Estimated 30% additional biopsy reduction
- NPV 96% for high grade PCa

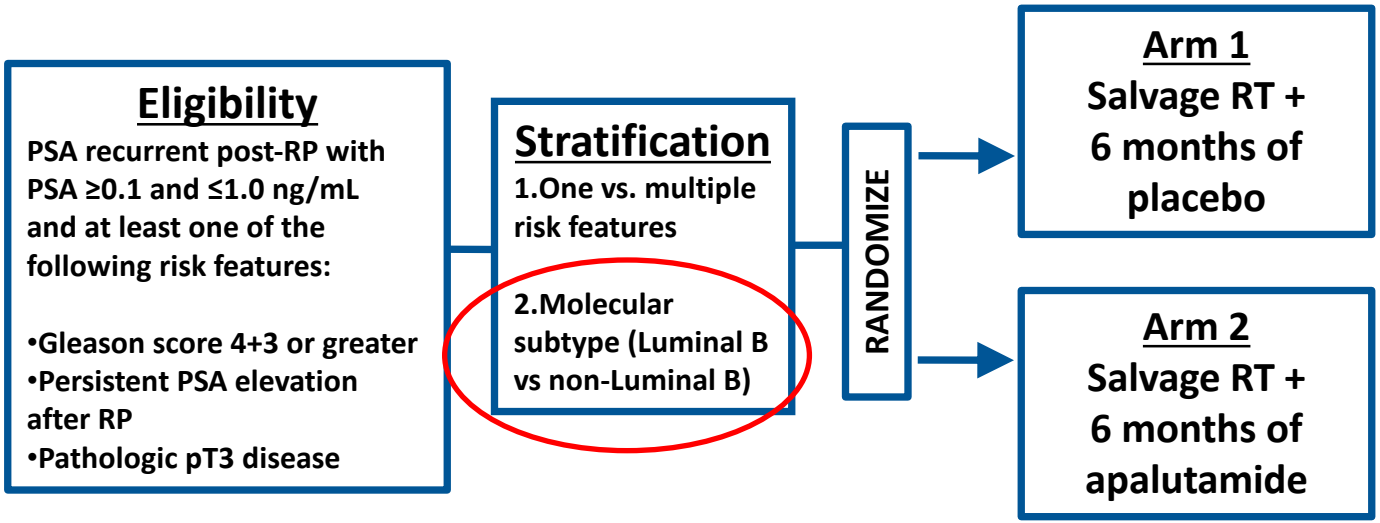


Van Neste et al, Prostate, 2016

Tissue-based Platforms for Prognosis

- Prolaris® (Myriad, Inc)
  - Cell cycle progression signature (31 genes)
  - Endpoint: PCSM, risk stratification
- Oncotype DX® Prostate (Genomic Health, Inc)
  - Multi-pathway signature (17 genes)
  - Endpoint: adverse pathology
- Decipher® (GenomeDx Biosciences, Inc)
  - Whole genome RNA-seq (22 genes)
  - Endpoint: metastasis-free survival, response to adjuvant RT
- ProMark® (Metamark Genetics, Inc)
  - Proteomic signature (8 biomarkers)
  - Endpoint: unfavorable pathology

NRG GU006: Phase II, Double-Blinded, Placebo Controlled Randomized Trial of Salvage Radiotherapy With or Without Enhanced Anti-Androgen Therapy With Apalutamide in Recurrent Prostate Cancer



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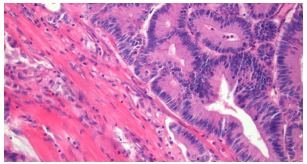
PIs: F Feng & D Spratt

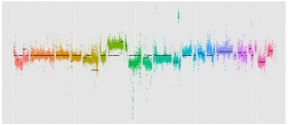
Emerging Model of Cancer Treatment

Tumor, plasma, and germline acquired for molecular diagnostics

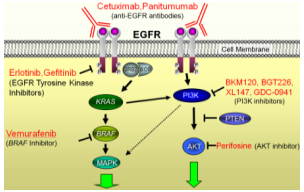




All/or all actionable mutations assessed

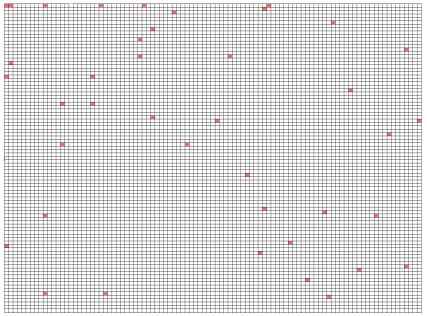



Therapy selected based on molecular characteristics

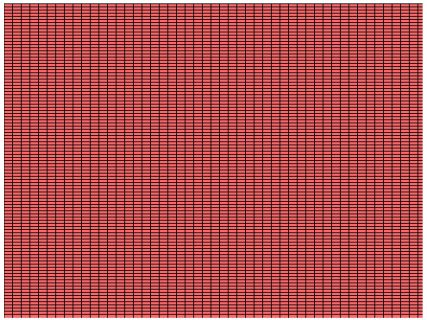



Somatic Mutation Panels

- **Hotspot Panels**
  - 1kb-200kb typical
  - Partial gene sequencing
  - Multiplex PCR-based enrichment
  - CNV/fusion detection uncommon



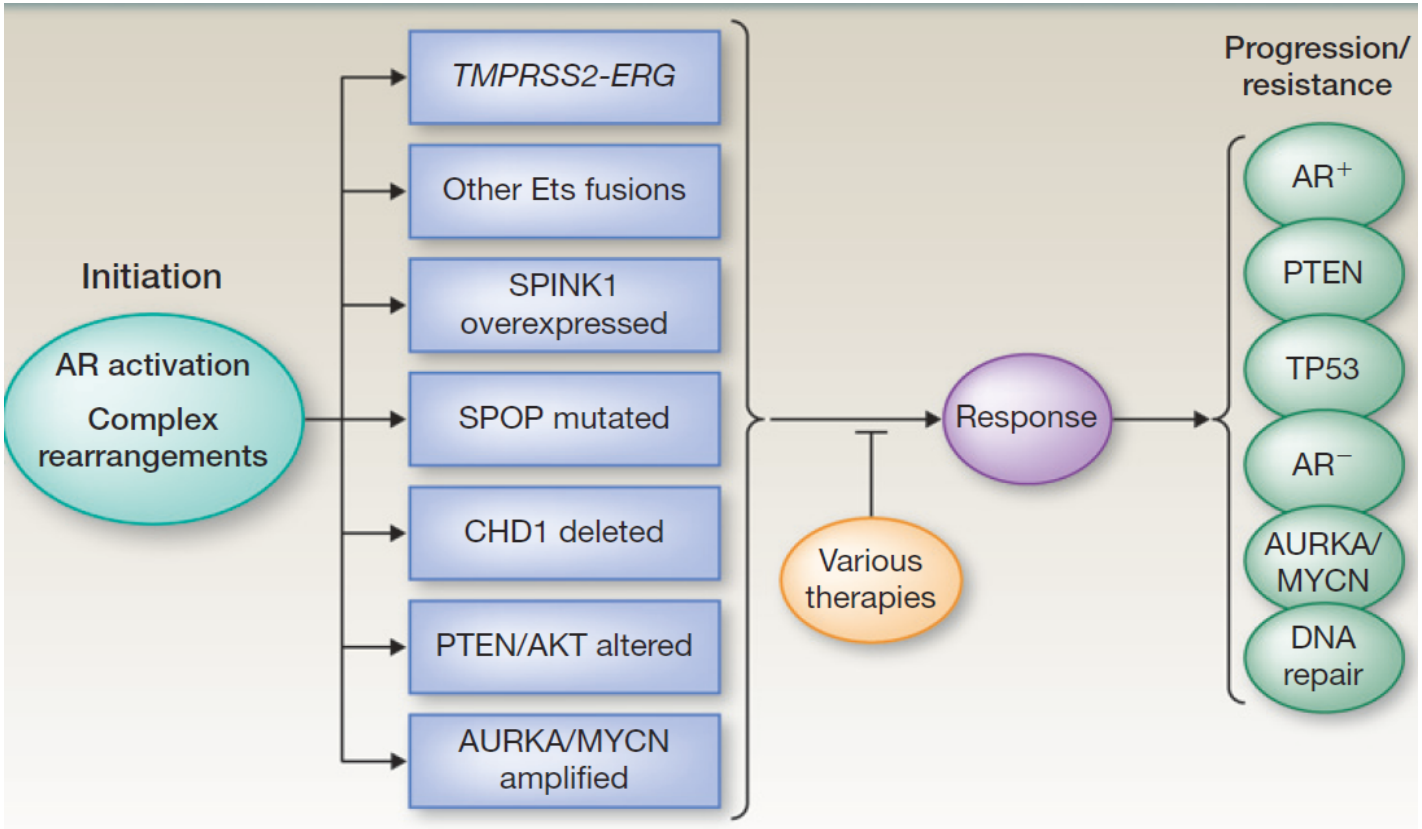
- **Comprehensive Sequencing Panels**
  - 200kb-2,000kb typical
  - Full gene sequencing
  - Capture-based enrichment
  - CNV/fusion detection common



Recently Identified Recurrently Mutated Genes in Prostate Cancer using NGS

Gene	Mechanism	Frequency	Representative Study	Tumors Tested
SPOP	Mutation	6-15%	Barbieri 2012 *Nat Genet*	Localized
FOXA1	Mutation	4%	Barbieri 2012 *Nat Genet*	Localized
MED12	Mutation	5%	Barbieri 2012 *Nat Genet*	Localized
CHD1	Deletion	8%	Grasso 2012 *Nature*	Advanced
MLL2	Mutation	9%	Grasso 2012 *Nature*	Advanced
AURKA/ MYCN	Co-Amplification	40% (NEPC)	Beltran 2011 *Cancer Discovery*	Neuroendocrine
CTNNB1	Mutation	4%	Robinson 2015 *Cell*	Advanced
CDK12	Mutation	13%	Robinson 2015 *Cell*	Advanced
ZBTB16	Deletion	10%	Robinson 2015 *Cell*	Advanced

Genomic Classification of Prostate Cancer



From Beltran and Rubin 2013

Comprehensive Somatic Panel Example

Tier 1: Currently actionable	*ABL1*	*AKT1*	*ALK*	*AR*	*ARAF*	*ASXL1*	*ATM*	*AURKA*	*BCR*	*BRAF*
	BRCA1	*BRCA2*	*CALR*	*CCND1*	*CEBPA*	*CSF3R*	*DDR2*	*DNAJB1*	*DNMT3A*	*EGFR*
	EML4	*ERBB2*	*ERCC2*	*ESR1*	*FGFR2*	*FGFR3*	*FGFR4*	*FLT3*	*HIF1A*	*IDH1*
	IDH2	*JAK2*	*KIT*	*KRAS*	*MAP2K1*	*MET*	*MLL*	*MPL*	*MTOR*	*MYD88*
	NOTCH1	*NOTCH2*	*NPM1*	*NRAS*	*NTRK1*	*NTRK2*	*NTRK3*	*PALB2*	*PDGFRA*	*PIK3CA*
	PML	*PTEN*	*RARA*	*RET*	*ROS1*	*SETBP1*	*SMO*	*STK11*	*TP53*	*VHL*
Tier 2: Actionable in the near future	*ABL2*	*AKT2*	*AKT3*	*ARID1A*	*ATRX*	*AURKB*	*AXL*	*BAP1*	*BARD1*	*BCL2L11*
	BCOR	*BCORL1*	*BRIP1*	*CBL*	*CBLB*	*CCNE1*	*CDK4*	*CDK6*	*CDK8*	*CHEK1*
	CHEK2	*DAXX*	*ERBB3*	*ERBB4*	*FAM175A*	*FANCA*	*FBXW7*	*FGFR1*	*FLT1*	*FLT4*
	GATA2	*GATA3*	*GLI1*	*GNA11*	*GNAQ*	*GRM3*	*H3F3A*	*HDAC4*	*HRAS*	*IGF1R*
	IKZF1	*JAK3*	*KDM6A*	*KDR*	*KIF5B*	*MAP2K2*	*MAPK1*	*MC1R*	*MCL1*	*MDM2*
	MDM4	*MEN1*	*MITF*	*MLH1*	*MRE11A*	*MSH2*	*MSH6*	*MYC*	*MYCN*	*NBN*
	NF1	*NF2*	*NKX2-1*	*PAX5*	*PDGFRB*	*PHF6*	*PIK3R1*	*PMS2*	*POLD1*	*POLE*
	RAD51C	*RAD51D*	*RAF1*	*RB1*	*RSPO2*	*RSPO3*	*RUNX1*	*SHH*	*SMAD4*	*SMARCA4*
	SRSF2	*SUFU*	*SUZ12*	*TACSTD2*	*TET2*	*TMPRSS2*	*TSC1*	*TSC2*	*WT1*	
Tier 3: Frequently mutated	*APC*	*BAK1*	*BCL2*	CBLC	*CBLC*	*CDH1*	*CDK12*	*CDK9*	*CDKN1A*	*CDKN2A*
	CHD1	*CREBBP*	*CRLF2*	*CSF1R*	CTNNB1	*CUX1*	*DEPDC5*	*DOCK7*	*EPHA3*	*EPHA5*
	EPHB2	*EPHB6*	*ETV6*	*EZH2*	*FKBP1A*	*FOXA1*	*GAB2*	*GATA1*	*GNAS*	*GRIN2A*
	HNF1A	*IL7R*	*JAK1*	*MAP2K4*	*MED12*	*MIOS*	*MLH3*	*MTAP*	*MUTYH*	*MYCL1*
	NPRL2	*NPRL3*	*PAK1*	*PBRM1*	*PLK1*	*PLK3*	*PLK4*	*PRPF40B*	*PTCH1*	*PTPN11*
	PTPRD	*RAC1*	*RAD21*	*RHEB*	*RICTOR*	*RPS14*	*RPTOR*	*SF1*	*SF3B1*	*SMAD2*
	SMAD3	*SMARCB1*	*SMC1A*	*SMC3*	*SPOP*	*SPRY4*	*SRC*	*TACC3*	*TET1*	*TET3*
	TFG	*TGFBR2*	*TRRAP*	*U2AF1*	*U2AF65*	*ZBTB16*	*ZRSR2*			
Germline pharmaco-genomics	*ABCB1*	*ABCC4*	*ABCG2*	*CYP1B1*	*CYP2C19*	*CYP2C8*	*CYP2D6*	*CYP3A4*	*CYP3A5*	*DPYD*
	EIF3A	*ESR2*	*FCGR1A*	*FCGR2A*	*FCGR3A*	*GSTP1*	*ITPA*	*LRP2*	*MAN1B1*	*MTHFR*
	NQO1	*NRP2*	*SLC19A1*	*SLC22A2*	*SLCO1B3*	*SOD2*	*SULT1A1*	*TPMT*	*TYMS*	*TYR*
	UGT1A1	*UMPS*								

Genes Targeted: 260

DNA Sequenced: >1,200,000 bp

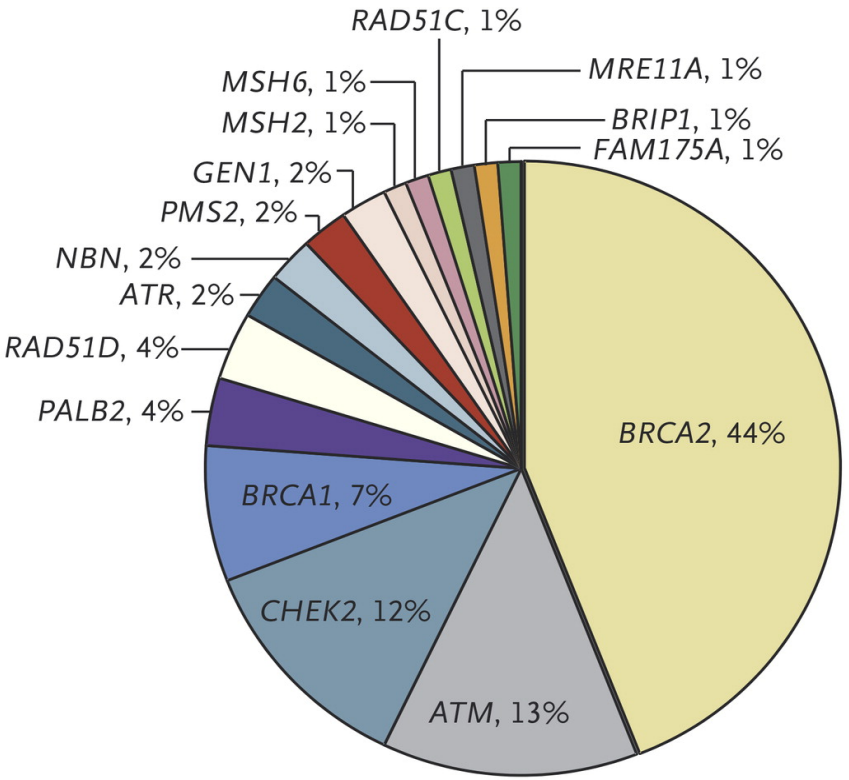
>500X Coverage

Pritchard et al. 2014 *JMD* 16:56-67.

Sample Issues

	Fresh Tumor Tissue 	Fixed Tumor Tissue 	Plasma ctDNA 
Quality	High	Moderate	Low
Quantity	High	Moderate	Very Low
Tumor Content	High (usually)	High (usually)	Low (usually)
False Negatives	Less common	Less common	More common
False Positives	Less common	Fixation artifact	Somatic clones in blood misinterpreted as cancer-derived

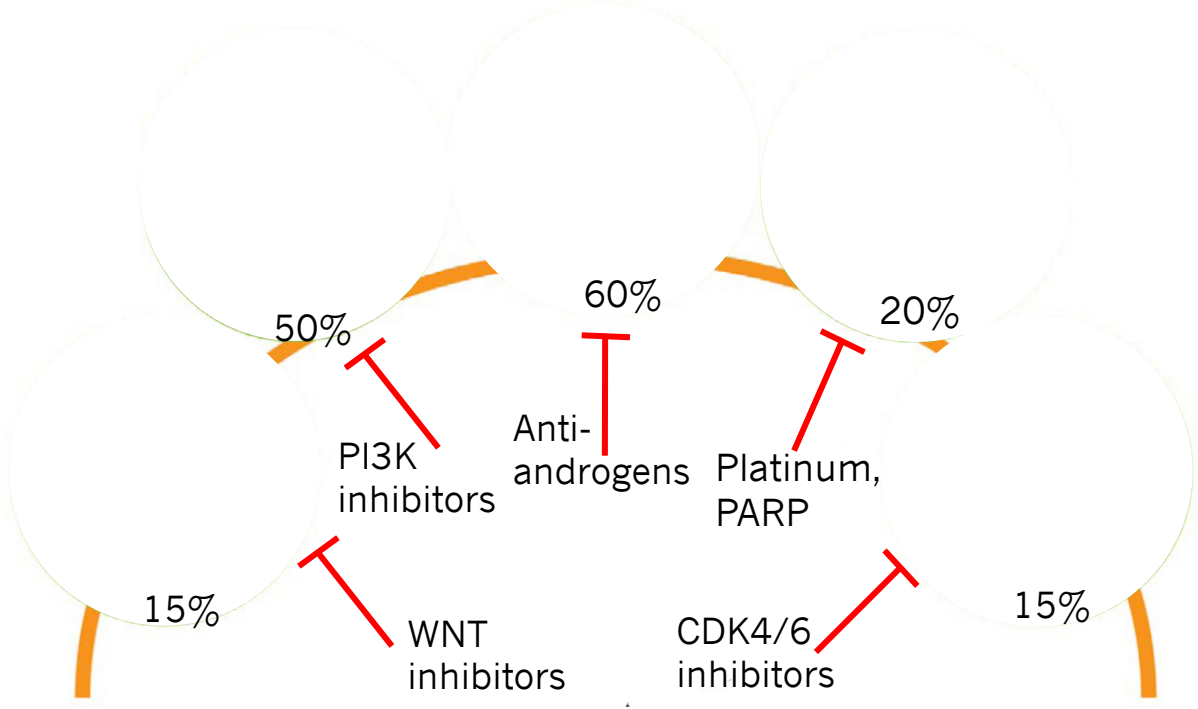
Germline DNA Repair Mutations Are Common in Metastatic Disease



11.8% (82/692) with deleterious germline mutations in 16 DNA repair genes

Pritchard CC et al. N Engl J Med 2016. DOI: 10.1056/NEJMoa1603144

Landscape of Metastatic Disease

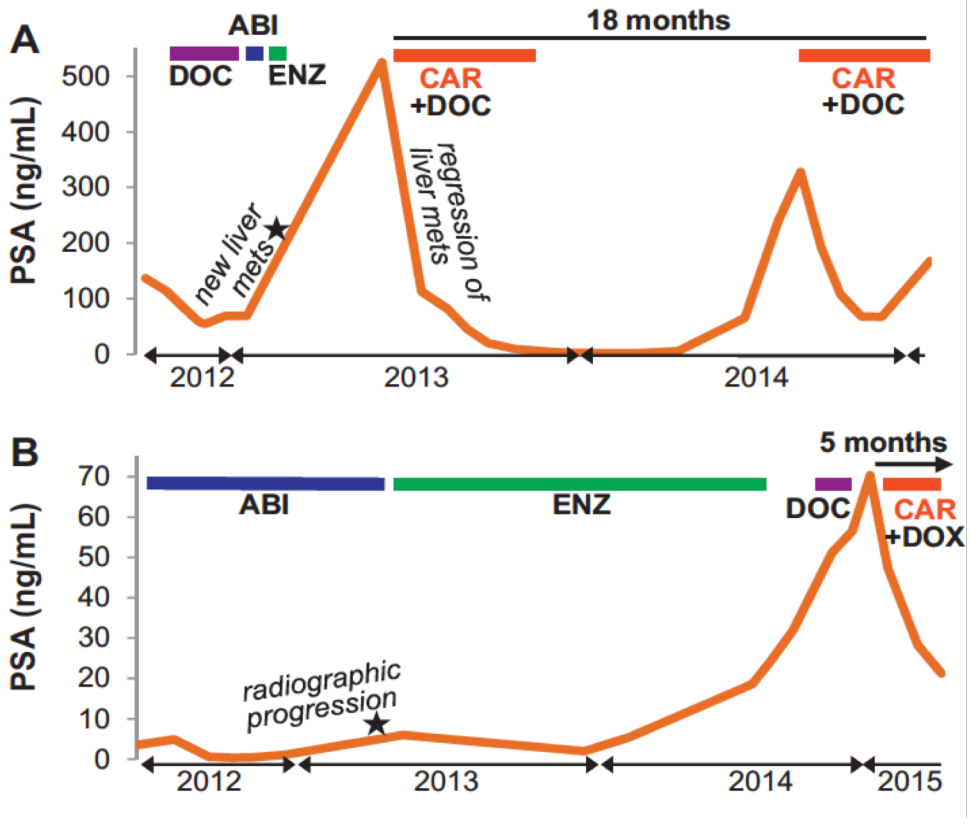


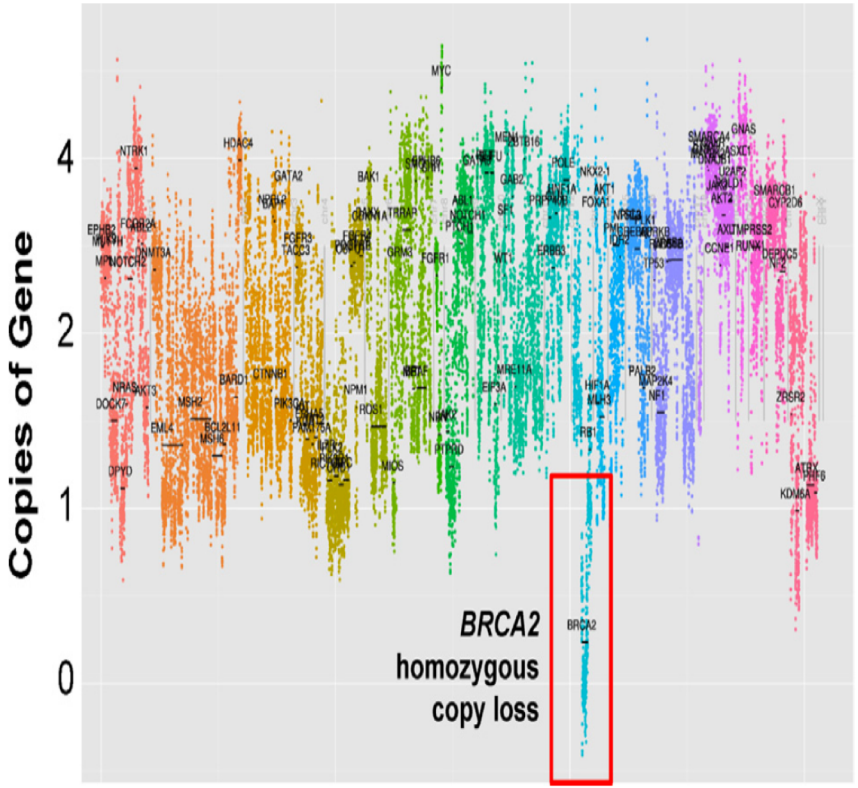
Adapted from Robinson 2015

Emerging Precision Targets

Mutation(s)	Metastatic Prostate Cancer Frequency	Potential Utility
HR DNA Repair *(e.g. BRCA1/2)*	**~20%**	**Platinum therapy, PARP inhibitors**
Mismatch DNA Repair (e.g. *MSH2*)	**~5%**	**Immunotherapy**
Androgen Receptor	40-60%	Anti-androgens
PI3K Pathway	30-60% (*PTEN*)	PI3K inhibitors
BRAF mutation/ rearrangement	~3%	BRAF or MEK inhibitors
RSPO2 fusions	~3%	WNT inhibitors

BRCA2-directed Chemotherapy



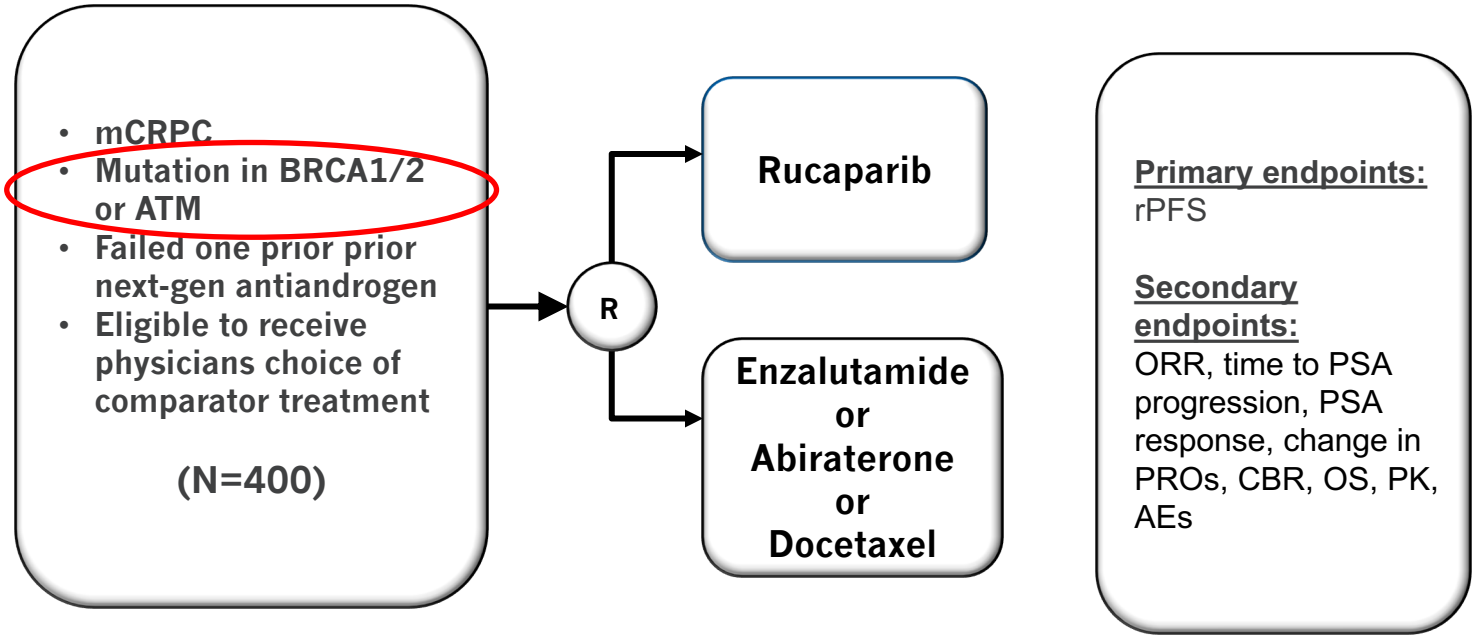


Cheng HH et al, *Eur Urol* 2016

Selected Trials for mCRPC with Relevance to DNA repair defects

Phase	Agent	Short Name	Clinicaltrials.gov
III	Rucaparib	TRITON3	NCT02975934
III	Olaparib	PROFOUND	NCT02987543
II	Niraparib	GALAHAD	NCT02854436
II	Talazoparib	TALAPRO	NCT03148795
II	Olaparib	BRCAaway	NCT03012321
II	Docetaxel and Carboplatin	(V) ABCD	NCT02598895 NCT02985021
II	Pembrolizumab	KEYNOTE-199	NCT02787005 NCT02312557
Ib/II	Pembrolizumab Combination Therapies	KEYNOTE-365	NCT02861573
II	Nivolumab Combination Therapies	CheckMate 9KD	NCT03338790

TRITON3: Rucaparib vs Enzalutamide, Abiraterone, or Docetaxel in mCRPC with HRD



ClinicalTrials.gov. NCT02975934

PROfound: Olaparib vs. Enzalutamide or Abiraterone in mCRPC with Prior Tx and HRD

- **mCRPC with somatic (tumor) HRD mutation**
- **Failed prior abiraterone and/or enzalutamide (prior taxane allowed, but no prior DNA-damaging chemo or PARPi)**

2:1

Olaparib

Enzalutamide or Abiraterone → **Optional post-PD switch: Olaparib**

Primary endpoints:
rPFS

Secondary endpoints:
ORR, time to pain progression, OS

N = 340

ClinicalTrials.gov NCT02987543

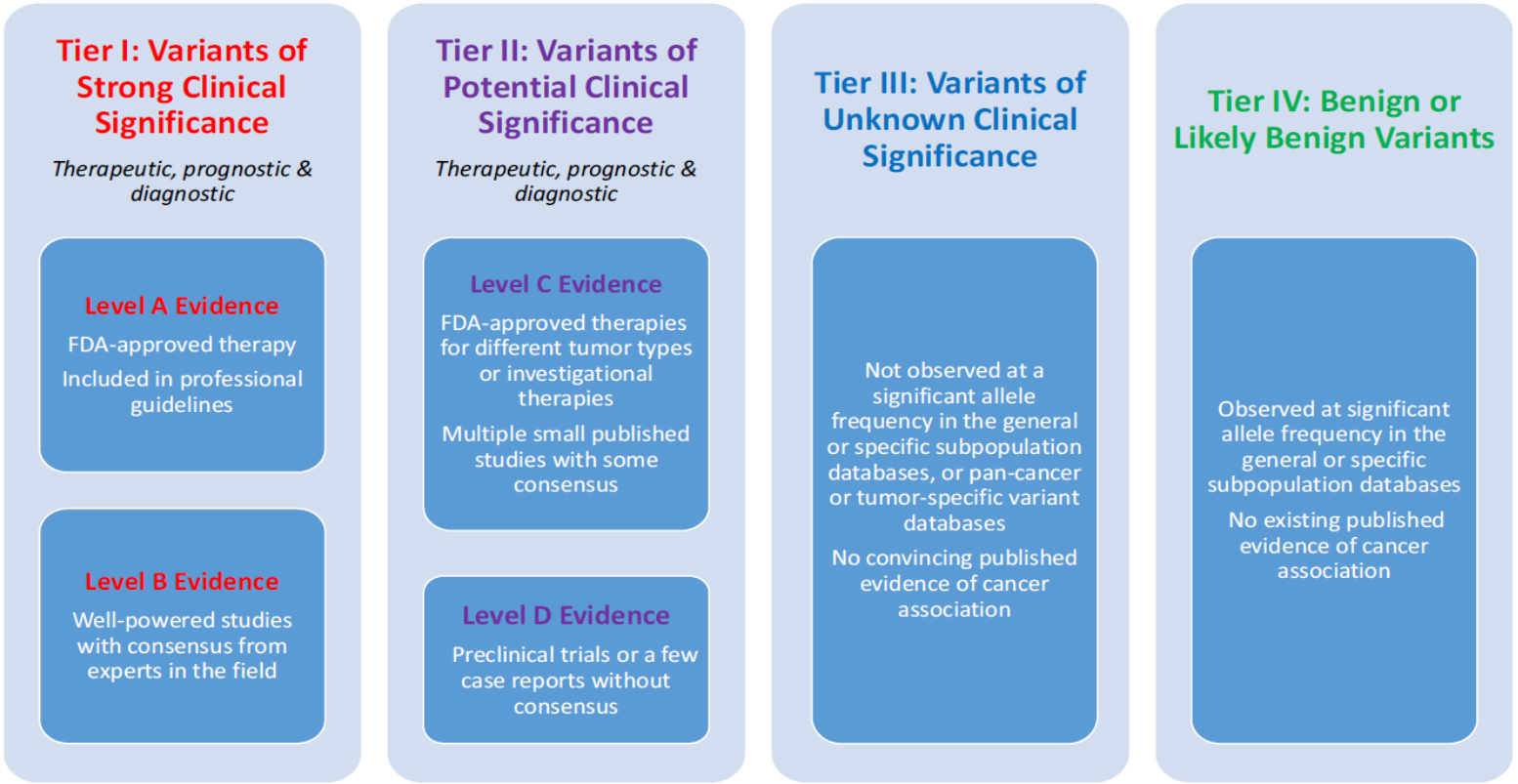
Summary

- Many novel and emerging biomarkers across the spectrum of prostate cancer

- Newer precision targets have been identified in prostate cancer through genomic sequencing efforts
  - Mutations may be actionable and have implications for both patient and unaffected relatives
  - Multiple issues in methodology, specimen considerations, and interpretations that may impact clinical decision making

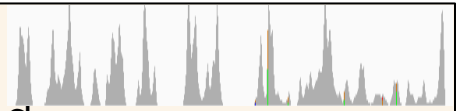
- Await clinical trials in select (and unselected) patient populations

Somatic Variant Interpretation: AMP/ASCO/CAP



Li et al. *JMD* (2017) PMID:27993330

Reporting Considerations



ANALYTICAL

1. Types of mutations validated
2. Limits of detection
3. Pseudogenes
4. Platform-specific considerations

CLINICAL

1. Clinical context (e.g. post Abi or Enza)
2. Strategy for poorly characterized variants
3. Specimen source
4. Net Benefit or Harm (e.g. reporting VUS)

VUS= variants of uncertain significance

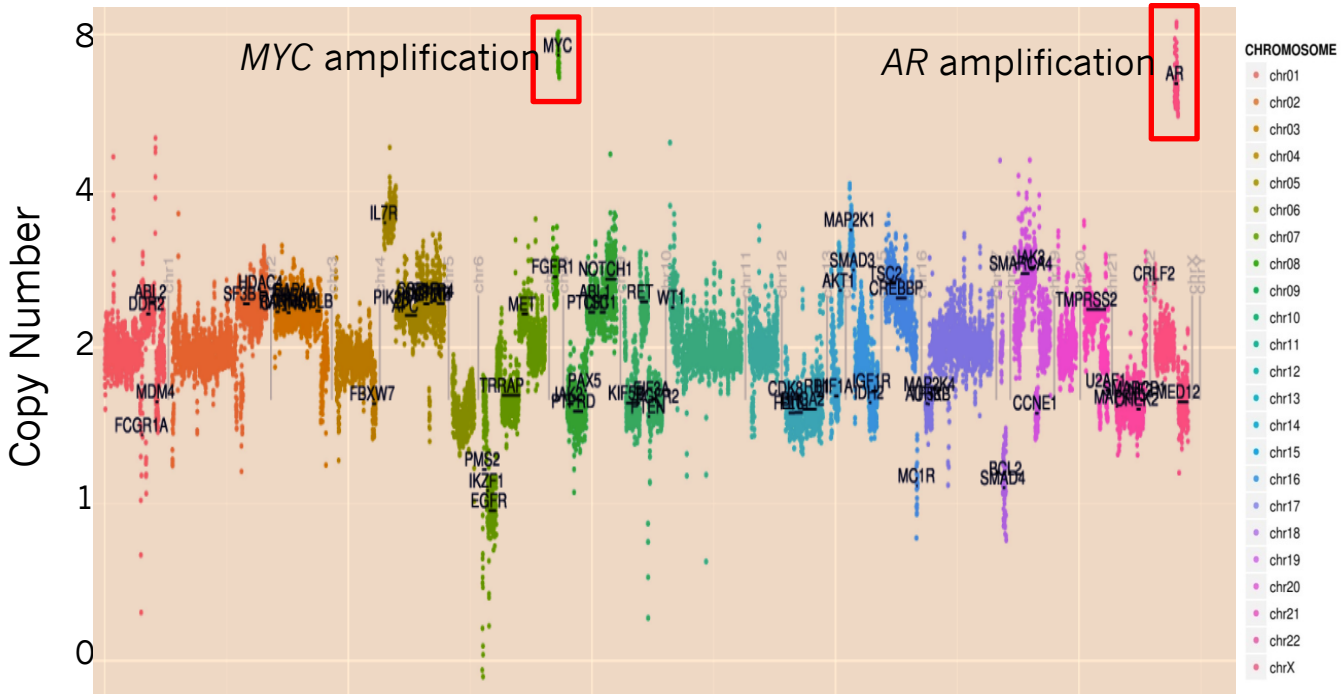
Issues with Plasma ctDNA

- Source of somatic mutations may NOT be from the cancer of interest
  - Age related clones
  - Somatic clones from other benign sources

- Sub-clonal passenger mutations misinterpreted as actionable
  - Beware mutations reported at low variant fraction

Zhang et al. *NEJM* 2017 376:1991-1992.

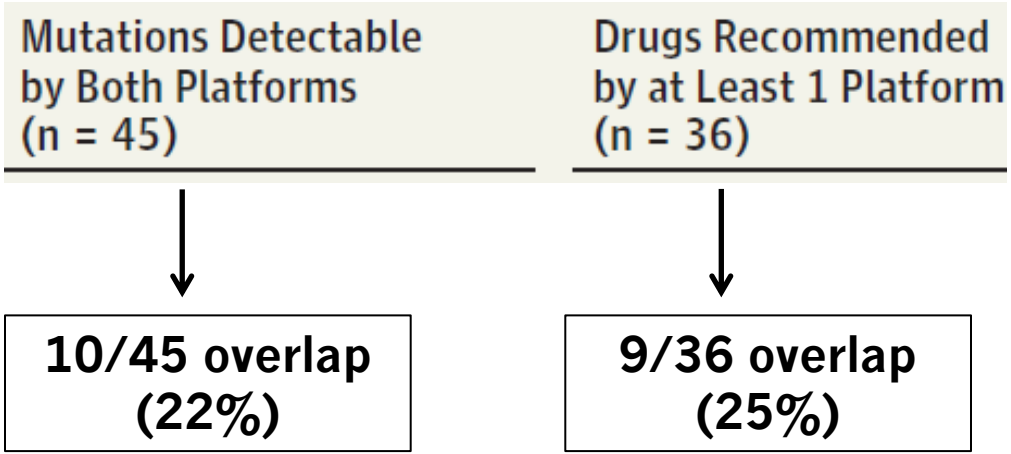
Gene Amplification/Deletions Detected by NGS



(example of copy number calling next-gen sequencing in an advanced prostate cancer tumor)

ctDNA vs. Tissue

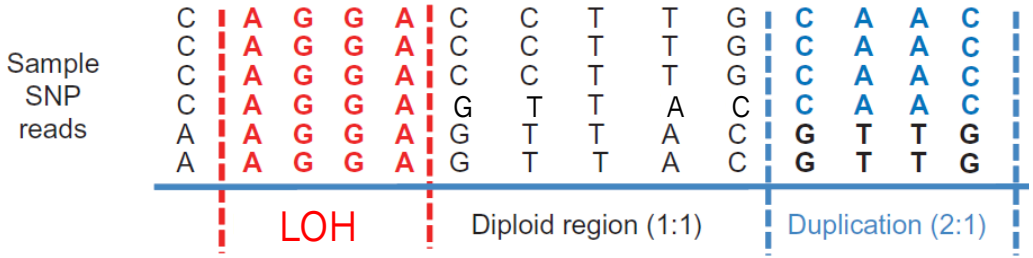
- n=9 cancer patients



Kuderer et al. *JAMA Oncol.* (2016) PMID:27978570

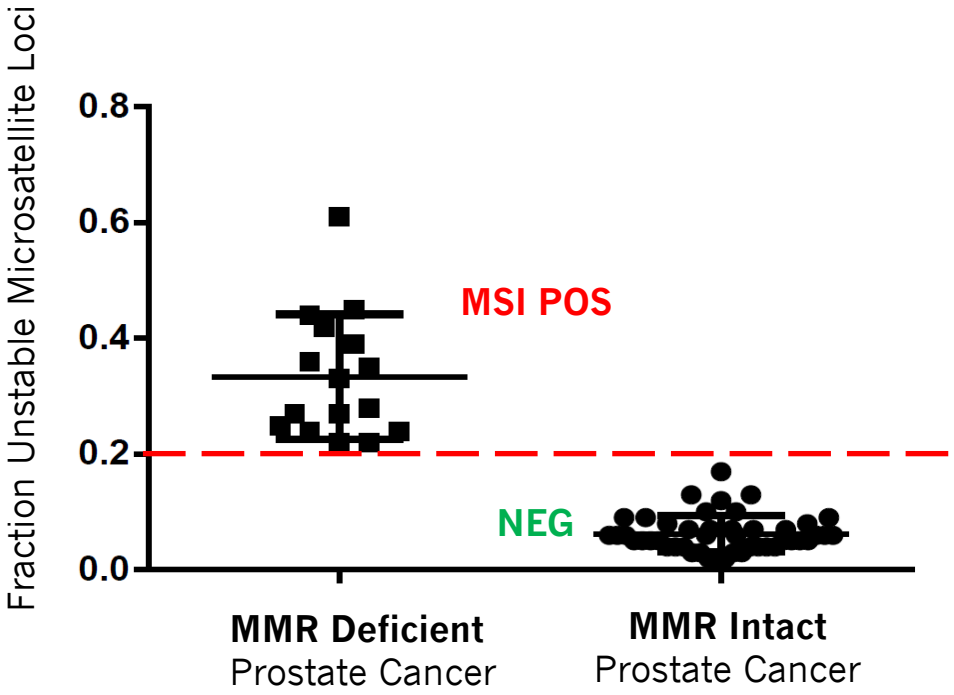
Loss of Heterozygosity (LOH)

- Can be determined by SNP variant allele fraction
- Useful as measure of “HRDness”
- Performance dependent on tumor content



Reference: Clinical Genomics, 2015, Pages 165–187
http://www.sciencedirect.com/science/article/pii/B9780124047488000113

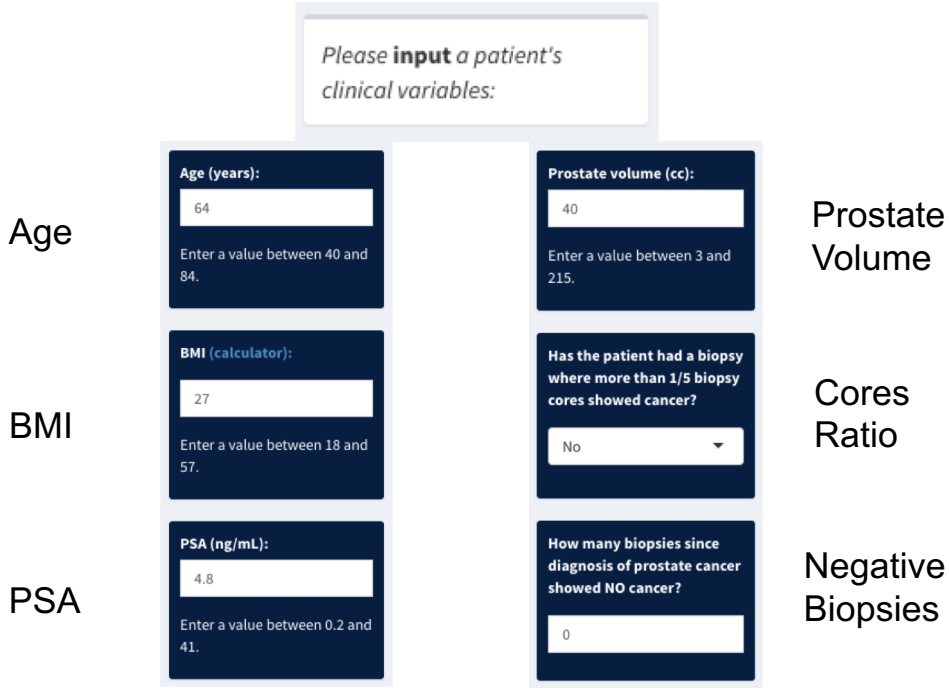
MSI Detection by NGS



MSI = microsatellite instability, MMR= Mismatch Repair

MSI-NGS methods (mSINGS): Salipante et al. *Clin. Chem.* 2014; Hempelmann et al. *JMD* 2015

PASS Risk Calculator Interface v2.0



Age

BMI

PSA

Prostate Volume

Cores Ratio

Negative Biopsies

*Pending variables: PSA kinetics, biomarkers

Kearns et al, unpublished work

https://canarypass.shinyapps.io/biopsy_nomogram/

PASS Risk Calculator v2.0

Please **input** *a patient's clinical variables:*

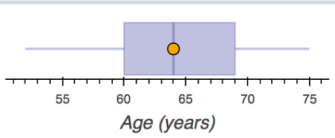
Age (years):
64
Enter a value between 40 and 84.

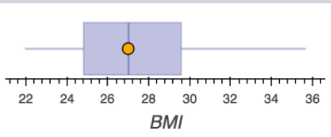
BMI (calculator):
27
Enter a value between 18 and 57.

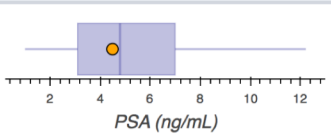
PSA (ng/mL):
4.5
Enter a value between 0.2 and 41.

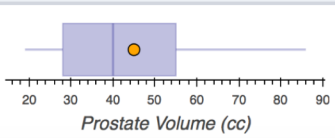
Prostate volume (cc):
45
Enter a value between 3 and 215.

The shaded box **shows the interquartile range** *of the clinical variable, and the lines extend to the 5th and 95th percentiles. The orange point shows the* **patient's value** *as input in the left column.*







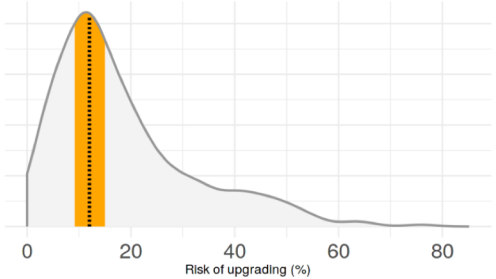


The figure below shows the distribution of risk in the PASS cohort. The black vertical line shows the **estimated personalized risk** *for the patient upgrading at the next biopsy. The bands around the point show 95% confidence intervals for the estimated risk.*

Risk of Gleason upgrade at next biopsy:

12%

95% confidence interval: (9% - 15%)



How does the estimated risk rank among the AS population?

40%

of the AS population have lower risk.

Kearns et al, unpublished work

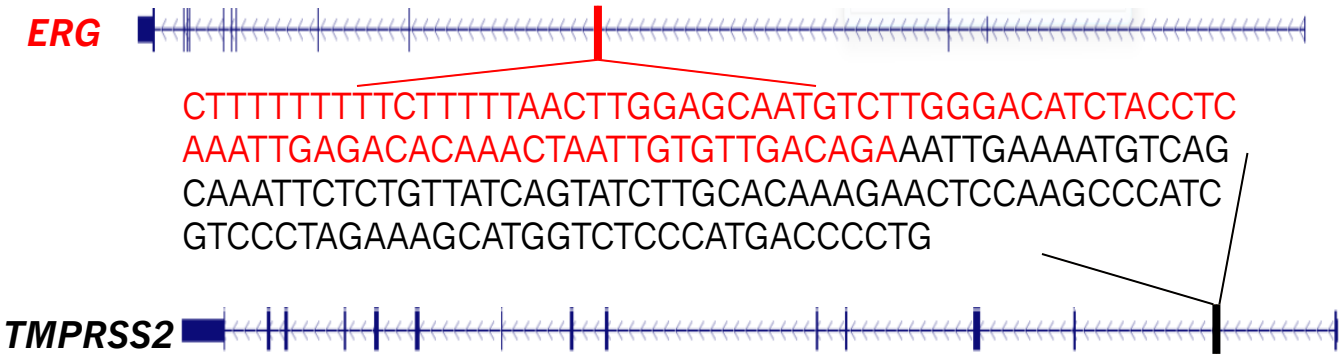
https://canarypass.shinyapps.io/biopsy_nomogram/

“Next-Generation” Sequencing (NGS)

- Sequence up to a whole tumor genome quickly
- Can accurately detect tumor mutations
- Now used routinely for cancer care
- Not widely used yet for prostate cancer

Gene Fusions Detected by NGS

TMPRSS2-ERG identified by Comprehensive NGS Panel



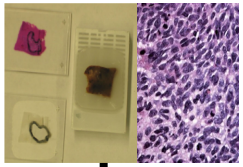
Fusions can be detected at DNA or RNA level

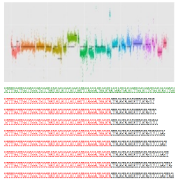
Biomarker Sources and Targets

Source	Targets	Examples
Tissue	RNA, DNA, Protein	Gene expression signatures, cytogenetic changes, various protein targets
Blood/BM	Circulating/secreted molecules, cells	CTCs, 4Kscore, PHI
PBMCs	DNA	genomics: CNV, SNPs
Urine	RNA, DNA, Protein	PCA3, T2-ERG fusion

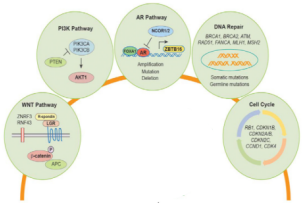
Emerging Model For Metastatic Prostate Cancer

Tumor, plasma, and germline tissue evaluated

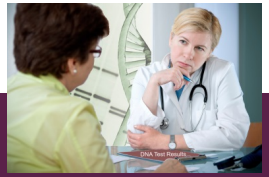


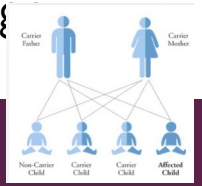


Therapy selected based on tumor and germline findi



Genetic counseling based on tumor and germline finding





Heterogeneity

- Specimen
- Tumor