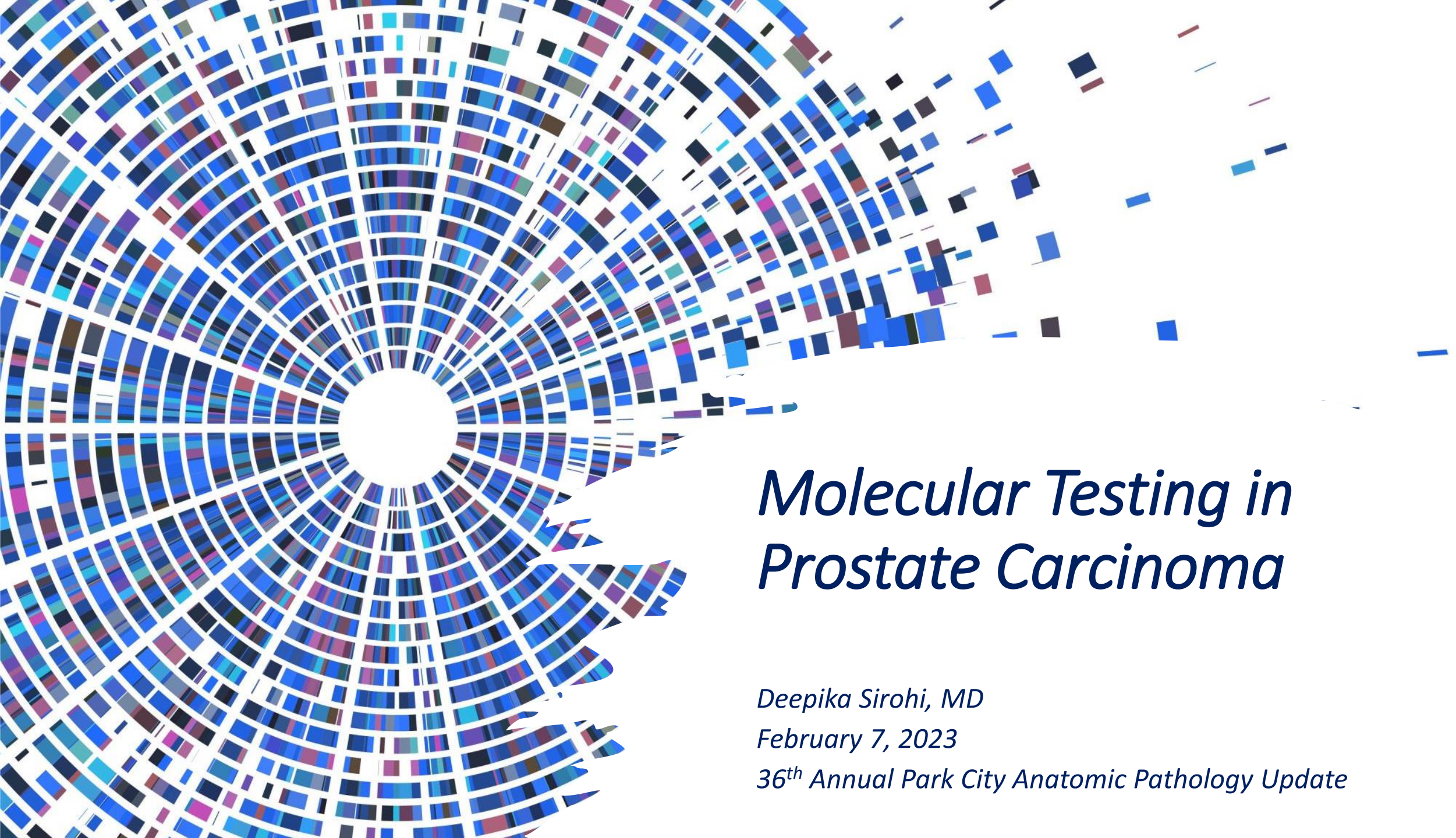

Molecular Testing in Prostate Carcinoma

Deepika Sirohi, MD

February 7, 2023

36th Annual Park City Anatomic Pathology Update

Outline

- Screening tests
- Diagnostic
- Germline and Somatic Testing
- Prognostic
- Biomarkers in Metastatic Setting
- NCCN Guideline Recommendations

Prostate Carcinoma

- Benign
- HGPIN
- Atypical Glands
- Carcinoma: Localized or metastatic

Private Information

Screening

Private Information

Conventional Screening

- PSA
  - High sensitivity, lacks specificity
  - Over diagnosis of low-risk prostate cancers
  - Increased number of biopsies
- 4K score: Total PSA, free PSA, intact PSA, human Kallikrein 2
- Prostate Health index: includes inactive precursor form of PSA
- Radiological imaging

World Journal of Urology (2019) 37:991–999

Private Information

Smarter Screening: Molecular Biomarkers

- Post DRE Urine PCA3: noncoding prostate specific RNA
  - FDA approved
  - Identifying patients over 50 years with prior negative biopsies that require repeat biopsy
- Urine 3 gene RNA levels: *HOXC6, DLX1, TDRD1*

NCCN Guidelines Version 1.2023, Urol 2013;190:64-69, J Urol 2010;184:1947-1952, Eur Urol 2016;70:45-53, Eur Urol 2018;74:731-738

Private Information

Smarter Screening: Multimodality

- SelectMDx (post DRE urine): 3 genes (*DLX1, HOXC6, KLK3*), clinical risk factors
  - NPV 94%
- MyProstateScore (urine): serum PSA, urine PCA3, *TMPRSS2::ERG*

NCCN Guidelines Version 1.2023, Urol 2013;190:64-69, J Urol 2010;184:1947-1952, Eur Urol 2016;70:45-53, Eur Urol 2018;74:731-738

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Smarter Screening: Alternative Biomarkers

- ExoDx Prostate® (IntelliScore) (EPI) (urine): exosomal RNA expression of 3 genes: *PCA3, ERG, SPDEF*
  - NPV 89%
  - Reduced biopsies by 20%
  - Missed 7% of GG2 cancers
- ConfirmMDx (tissue): methylation specific assay, 3 genes *GSTP1, APC, RASSF1*
  - Methylation field effect
  - NPV: 88-90%
  - Predictive of outcome on multivariate analysis Identify patients who should get repeat biopsy
- Sentinel PCa Test: small noncoding RNAs (sncRNA) from urinary exosomes

NCCN Guidelines Version 1.2023, J Urol 2019;202:256-263, J Urol 2013;189:1110- 1116, JAMA Oncol 2017;3:1085-1093.

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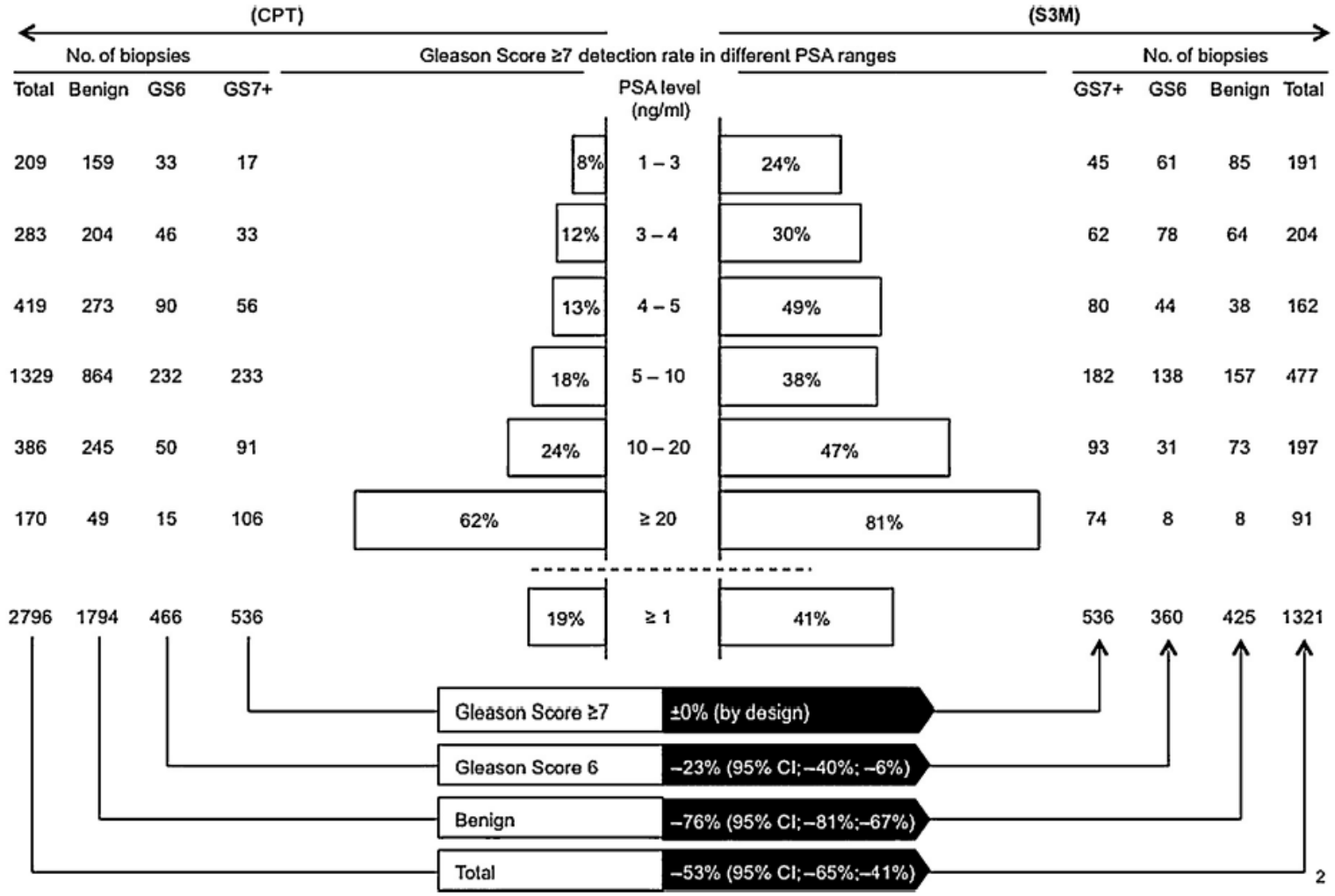
Combined Strategies

- Stockholm-3 Model
  - Plasma biomarkers: PSA< free PSA, intact PSA, hK2, microseminoprotein-beta, macrophage inhibitory cytokine 1
  - 254 SNPs
  - Clinical parameters: age, family history, prostate exam, previous prostate biopsies
- Outperforms PSA for predicting clinically significant Pca (GS≥7)
  - (AUC 0.74 vs 0.56)
- Reduced number of unnecessary biopsies by 32%
- Increased percentage of clinically significant biopsies from 42 to 65%
- Ability to detect aggressive Pca when PSA 1.5-3ng/mL
- Estimated healthcare costs reduced by 23-28% per person

World Journal of Urology (2019) 37:991–999
European Urology Focus 4(2018);707-710

Private Information

Stockholm3



European Urology Focus 4(2018);707-710

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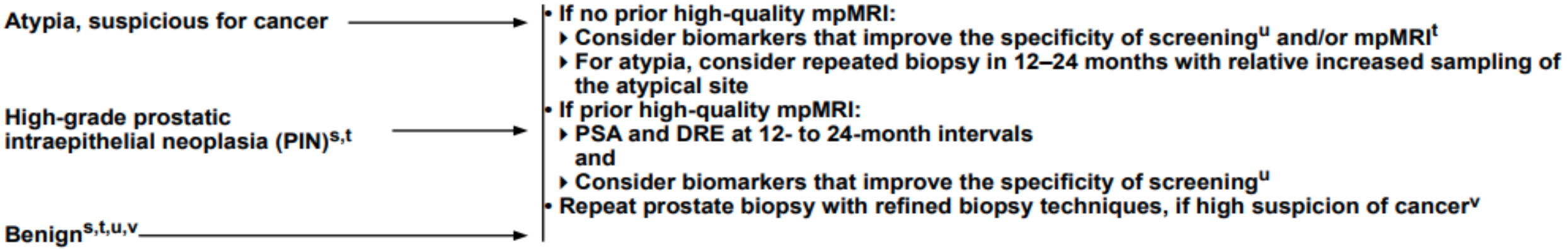
NCCN Guideline Recommendations



- Percent-free PSA, 4Kscore, PHI, PCA3, ConfirmMDx, ExoDx Prostate Test, MPS, and IsoPSA

NCCN Guidelines Version 1.2023

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Carcinoma: Localized/Metastatic

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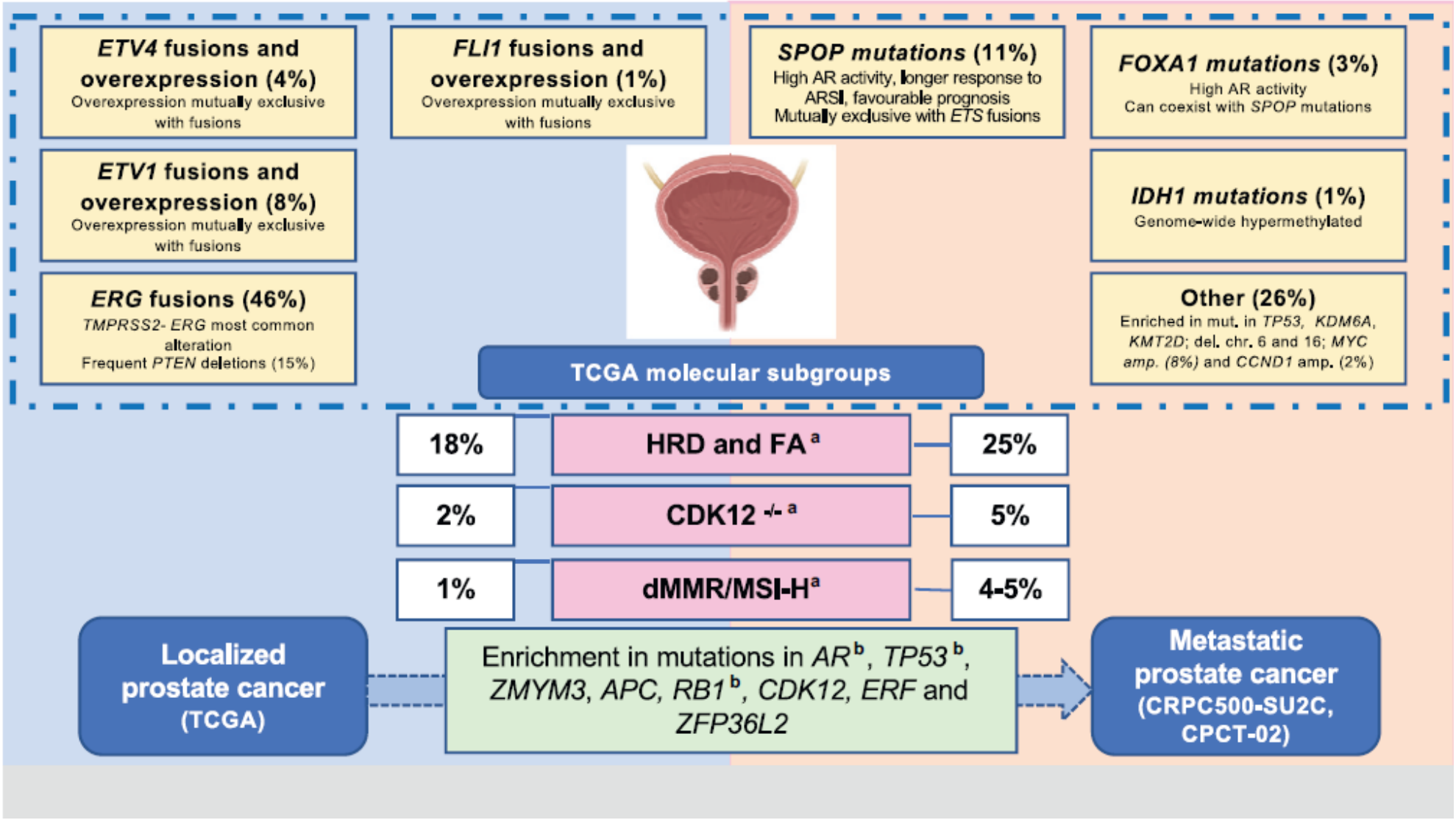
Molecular Landscape of Prostate Ca



Surgical Pathology 15 (2022) 617–628

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Diagnostic

- *TMPRSS2:ERG* fusions
  - In 50% prostate carcinomas
  - Early clonal event
  - Positive in HGPIN and PCa
  - IHC: high specificity
- Establishing prostatic origin
  - Metastatic tumors
  - Bladder vs prostate
- Differentiating atypical glands vs small focus of carcinoma (when HGPIN has been excluded)

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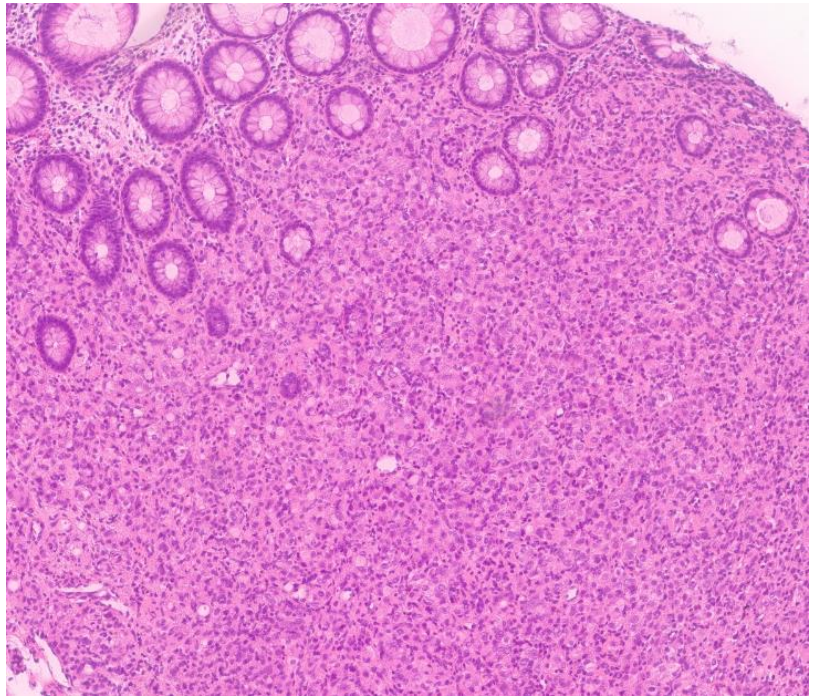

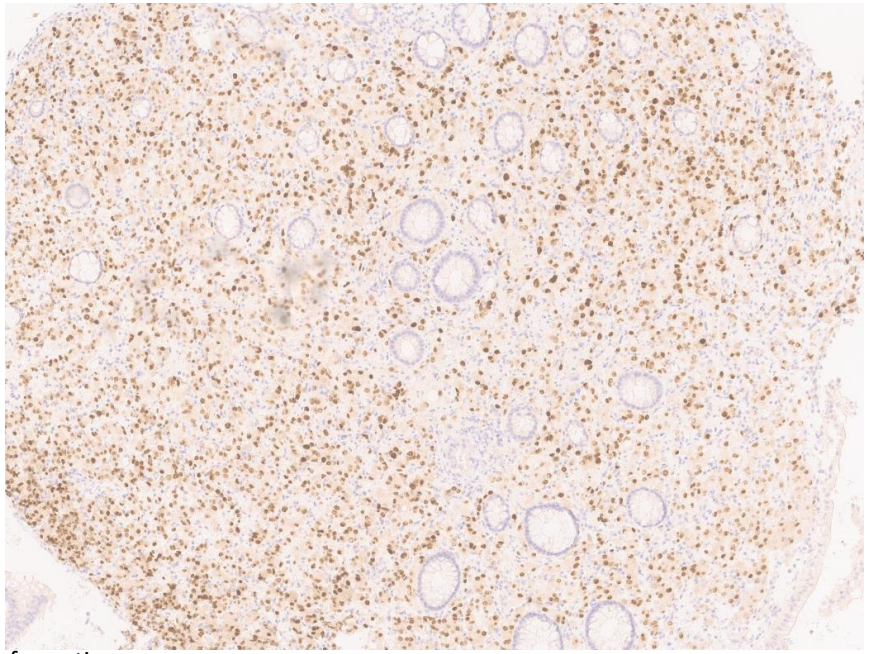

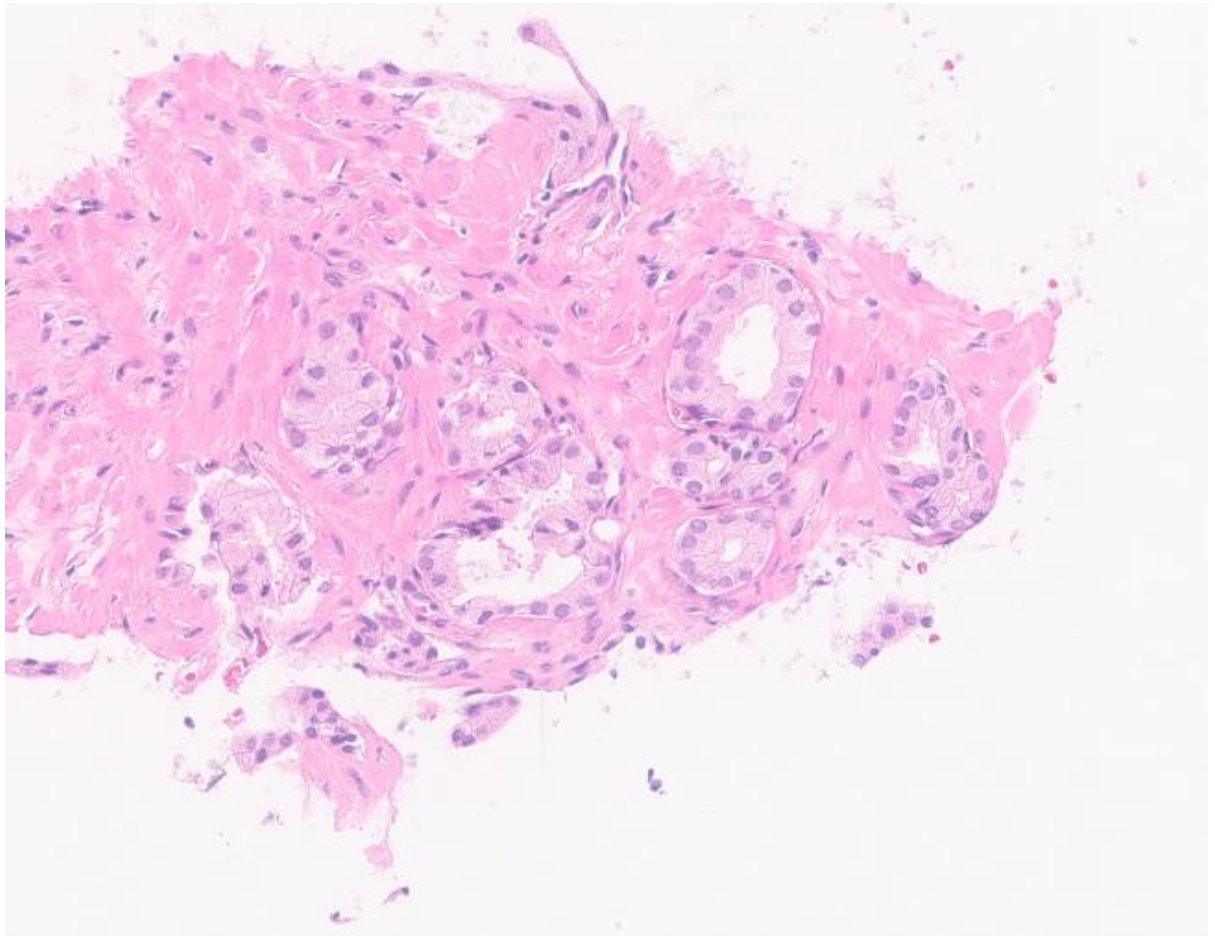

Private Information

Management

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Molecular Alterations in Prostate Carcinoma

- Germline
  - Implications for family members
  - Personal risk of other cancers
  - Prognostic impact: overall survival, response to treatment
  - Treatment implications
- Somatic
  - If tumor only testing: testing for germline
  - Treatment implications

Private Information

Germline Alterations: High Risk Population

- Black/African Americans
- First-degree relative with Pca (especially if <60 y)
- Family history of breast Ca

NCCN Guidelines Version 1.2023, Prostate 2008;68:1582-1591, Ann Oncol 2017;28:1098-1104, BMC Cancer 2019;19:871.

Private Information

Genetic Syndromes and Prostate Cancer

- Homologous Recombination Repair Defects
  - Hereditary breast and ovarian cancer syndrome
  - *BRCA2* (5%), *ATM* (2%), *CHEK2* (2%), *BRCA1* (1%), *RAD51D* (0.4%), *PALB2* (0.4%), *ATR* (0.3%), and *NBN*, *PMS2*, *GEN1*, *MSH2*, *MSH6*, *RAD51C*, *MRE11A*, *BRIP1*, or *FAM175A*

- DNA mismatch repair genes
  - Lynch Syndrome

NCCN Guidelines Version 1.2023, N Engl J Med 2016;375:443-453, J Clin Oncol 2013;31:1713- 1718, Cancer Epidemiol Biomarkers Prev 2014;23:437-449.

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Germline Mutations in Prostate Ca

- 8 to 18.5% of PCa
- Metastatic CRPC
  - 89% actionable somatic mutations
  - 9% germline

Germline DNA-Repair Gene Mutations in Seven Metastatic Prostate Cancer Case Series.

Case Series	Description	Patients	Patients with Mutations
		no.	*no.* (%)
1	Stand Up To Cancer–Prostate Cancer Foundation discovery series	150	15 (10.0)
2	Stand Up To Cancer–Prostate Cancer Foundation validation series	84	9 (10.7)
3	Royal Marsden Hospital	131	16 (12.2)
4	University of Washington	91	8 (8.8)
5	Weill Cornell Medical College	69	7 (10.1)
6	University of Michigan	43	4 (9.3)
7	Memorial Sloan Kettering Cancer Center	124	23 (18.5)
Total		692	82 (11.8)

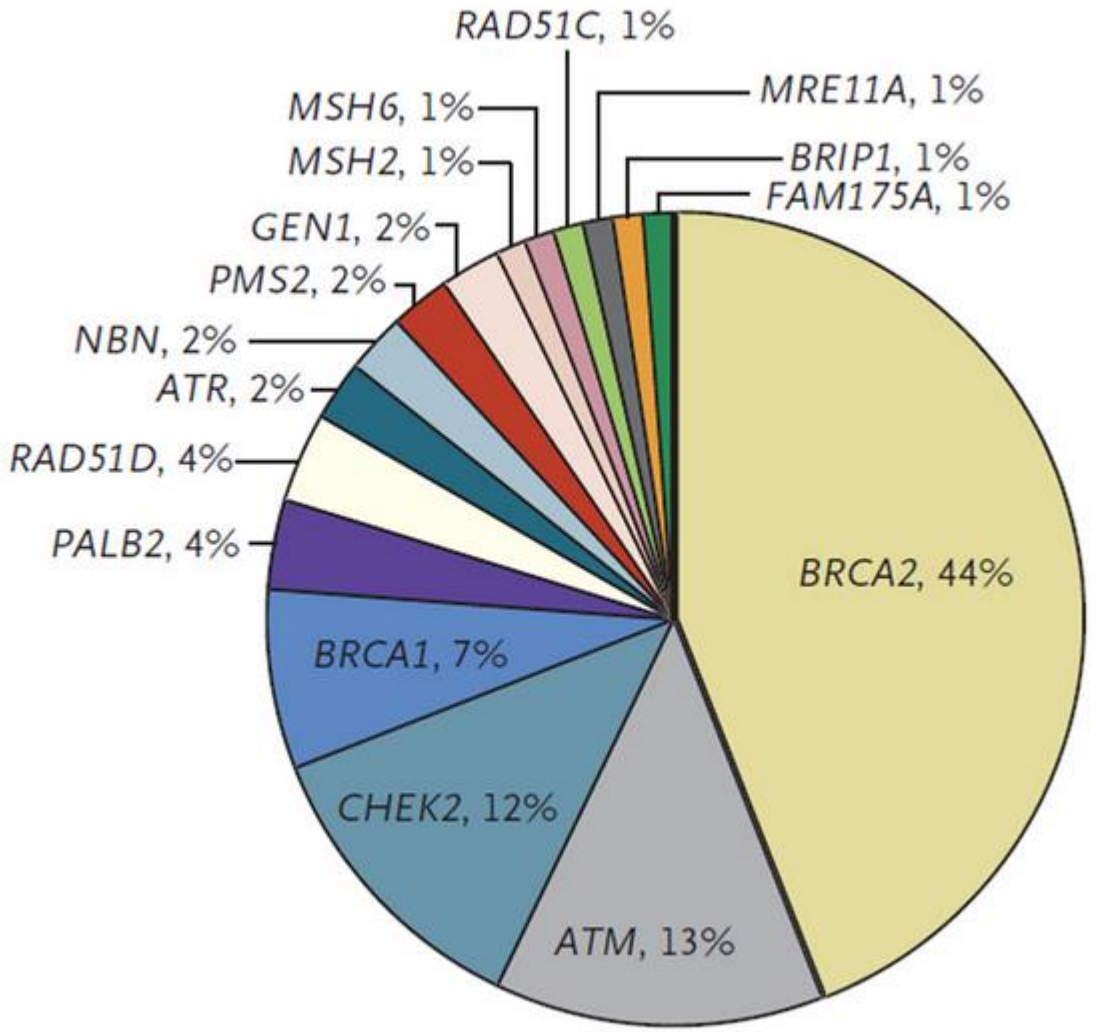
N. Engl. J. Med. 2016, 375, 443–453.

Private Information

Germline Mutations in Prostate Ca

Diverse genes

Complex interactions



N. Engl. J. Med. 2016, 375, 443–453.

Private Information

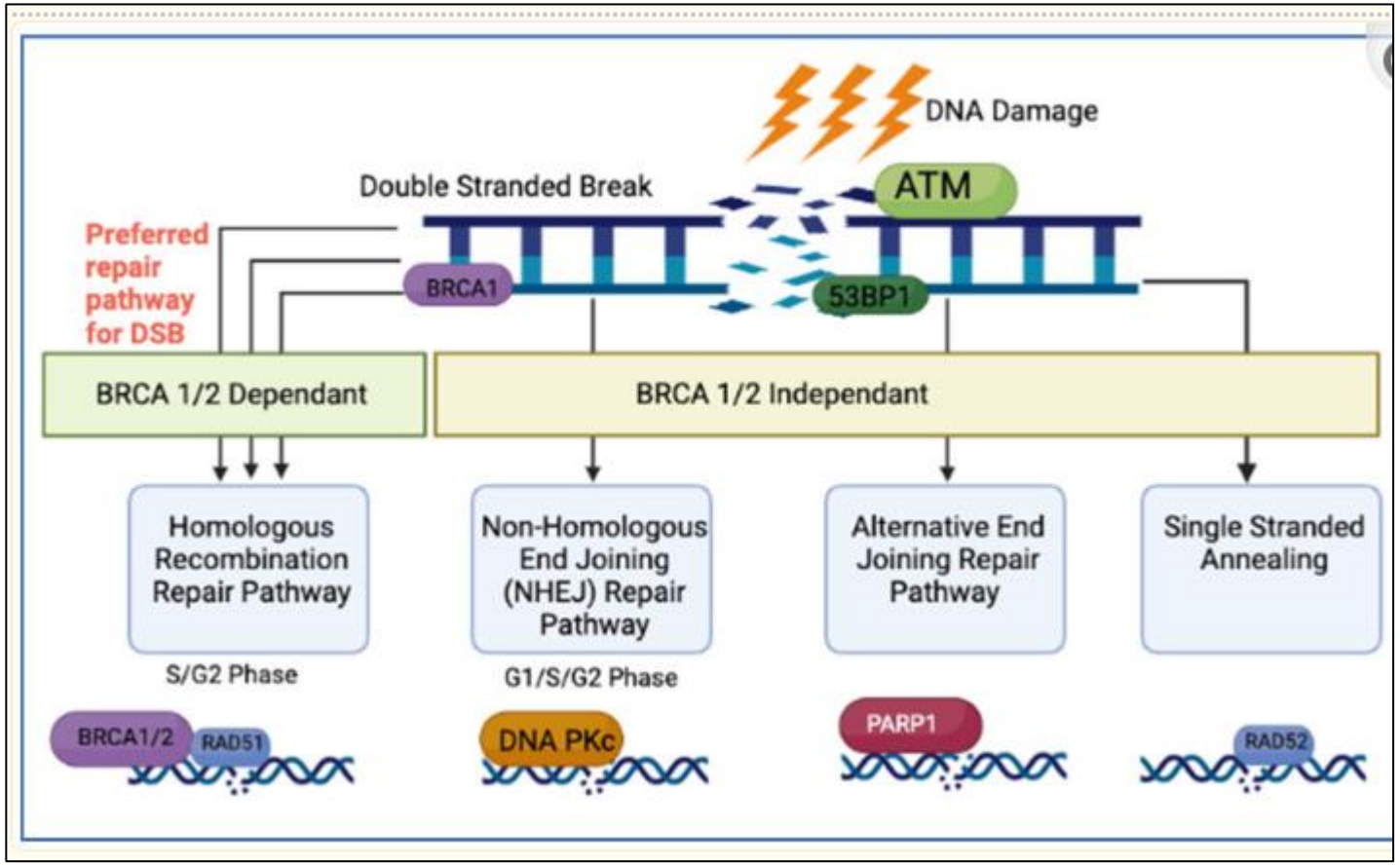
HRR Genes

- Detect and repair DNA damage
- Mutations: genomic instability, cell death, tumorigenesis
- Mutations: loss of function, epigenetic
- Genomic instability
  - Loss of heterozygosity, telomeric imbalance, large state transitions
  - Genomic scars
  - HRD mutational signatures
- Poly ADP-ribose polymerase (PARP):
  - Family of enzymes: single stranded breaks
- Targetable with PARP inhibitors
- Efficacy of response: BRCA mutant>non BRCA HRD mutant> non HRD mutant

Front Pharmacol. 2021; 12: 777663.

Private Information

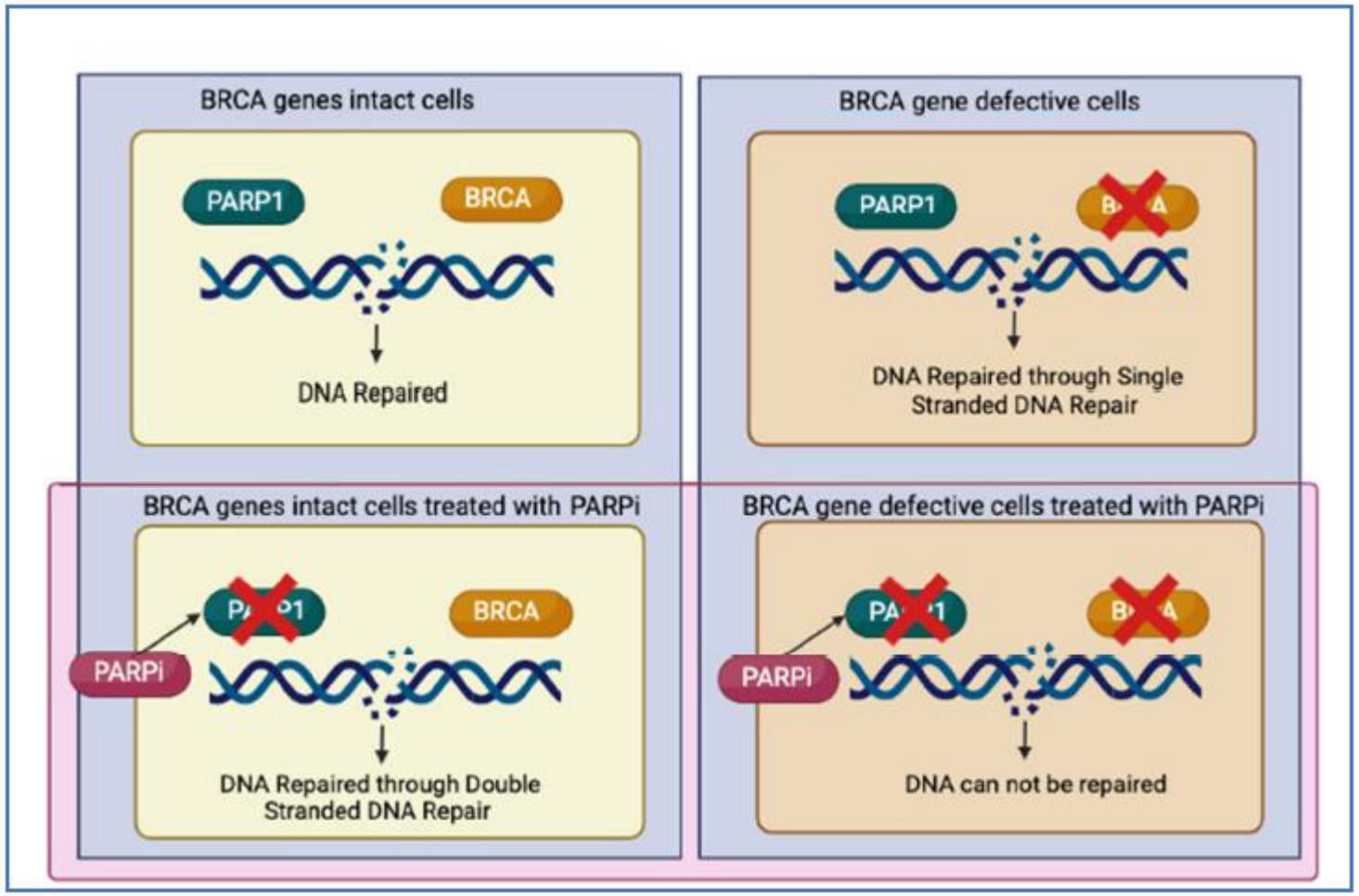
Cellular Repair Pathways



Cancers 2022, 14, 4751.

Private Information

Synthetic Lethality of PARPi in *BRCA* Deficient Cells



Cancers 2022, 14, 4751.

Private Information

HRR Gene Mutations in Prostate Ca

- HRR genes
  - Somatic or germline in 20% of aggressive primary and metastatic prostate carcinoma
  - Somatic
    - Localized: 19%
    - Metastatic: 23%
  - Frequency of mutations increases across Pca risk categories
- TCGA: 33% of primary Pca DDR mutated (n=333)
- SU2C-PCF: 23%
- Cohort of 3476 localized and metastatic Pca: 24%

NCCN Guidelines Version 1.2023, Clin Cancer Res 2009;15:1112-1120, Cancer 2015;121:269-275, J Clin Oncol 2013;31:1748-1757, Eur Urol 2017;71:740-747.

Private Information

HRR Gene Mutations

- Germline *BRCA1/2* (hereditary breast and/or ovarian cancer syndrome)
  - Increased risk of Pca
  - *BRCA2*: 2-6 fold risk, *BRCA1*: less consistent
  - Younger age
  - ***Aggressive phenotype***
  - Reduced overall survival

Private Information

HRR Gene Mutations

- Most commonly mutated genes: *BRCA2, ATM*
- Likely to respond to Poly ADP-ribose polymerase (PARP) inhibitors
- Confer sensitivity to Platinum
- FDA approved PARPi for HRD associated mCRPC in 2020 (somatic or germline)

NCCN Guidelines Version 1.2023, Clin Cancer Res 2009;15:1112-1120, Cancer 2015;121:269-275, J Clin Oncol 2013;31:1748-1757, Eur Urol 2017;71:740-747.

Private Information

Response to Treatment

- TOPRAP-B: Response dependent on specific gene mutated
  - BRCA1/2>PALB2>ATM>CDK12
- PROFOUND study
  - BRCA1/2, ATM mutated better PFS than other HRD gene mutations
- TRITON2:
  - Better response in BRCA1/2 mutated
  - No objective response in ATM and CDK12
- GALAHAD:
  - Better response in BRCA1/2 mutated

Genes Chromosomes Cancer. 2021;60:344–351

Private Information

HRD assessment

- Myriad Genetics MyChoice CDx: FDA approved for ovarian Ca
- Genomics Instability Score
  - Loss of heterozygosity
  - Telomeric imbalance
  - Large scale transitions
  - *BRCA1* and *BRCA2* variants
- Localized Pca
  - *BRCA2* mutated: higher scores
  - Longer OS on PARPi
- *ATM, CHEK2*: lower scores vs *BRCA2*

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Private Information

HRD Assays

Method	Assay/Analysis Method	Score and Threshold	Interpretation
HRD tumor testing			
Targeted NGS	Myriad Genetics MyChoice CDx	• LOH + LST + TAI (threshold ≥ 42) • Variants and large rearrangements in 15 genes (*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51 B, RAD51 C, RAD51D*, and *RAD54 L*).	• GIS • Pathogenicity of variants
	Myriad Genetics MyChoice CDx Plus	• LOH + LST + TAI (threshold ≥ 42) • Variants and large rearrangements in *BRCA1* and *BRCA2*	• GIS • Pathogenicity of variants
	TruSight Oncology 500 HRD	• SNV, indels, CNV in 523 genes, rearrangements in 55 genes • MSI and TMB • LOH + LST + TAI (threshold ≥ 42)	• Genomic alterations • MSI and TMB • GIS
	FoundationOneCDx	• SNV, indels, CNV in 324 genes, rearrangements in selected genes • MSI and TMB • gLOH ≥ 16	• Genomic alterations • MSI and TMB • gLOH low/high
Genome-wide NGS (WGS, WES)	CHORD	• Biallelic loss (deep deletion), presence of LOH, pathogenicity of variants • Threshold ≥ 0.5	• Probability of *BRCA1/2* deficiency • HRD
	HRDetect	• Mutational signatures analysis, HRD index score, analysis of variants in *BRCA1/2* and other HRR-related genes • Threshold > 0.7	• Probability of *BRCA1/2* deficiency • HRD

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Private Information

Lynch Syndrome

- 3-5% of prostate ca patients: MSI-H or MSI-indeterminate
  - 5% of these (0.29-0.68% of all Pca): Lynch syndrome
- 2-5.8 increased risk of Pca
  - No age difference vs. sporadic
- *MSH2* most frequently mutated
- DNA mismatch repair deficient cancers: respond to immune checkpoint inhibitors

Private Information

Microsatellites

- Short Tandem Repeats
  - Mononucleotides AAAAAAA
  - Dinucleotides CACACACACA
  - Trinucleotides CGGCGGCGGCGG
  - Tetranucleotides GATAGATAGATA
  - Pentanucleotides AGAAAAGAAA
  - Hexanucleotides AGTACAAGTACA
- Highly Polymorphic
- Prone to replication errors

Private Information

Microsatellite Instability

- Defective DNA repair system
  - Hypermutator
  - Deletions and/or insertions in microsatellites
  - Mutations- tumor suppressor genes and oncogenes

Private Information

FDA Approval

- FDA approval: 2017
- Pembrozulimab
- Unresectable/metastatic tumors that have progressed on treatment
- dMMR or MSI-H

Private Information

Microsatellite Instability Testing

- Conventional microsatellite testing
  - Indicated for screening for Lynch syndrome in colorectal carcinomas
- Testing expanded for eligibility determination for immunotherapy agnostic of tumor type
  - How do these tests on other tumor types?

Private Information

Microsatellite Instability Testing

- Immunohistochemistry
  - Mismatch repair proteins: MSH2, MSH6, MLH1, PMS2
  - Loss of expression
- MSI PCR
  - 5 mononucleotide repeats
  - Fragment analysis by Capillary Electrophoresis
- Next Generation Sequencing

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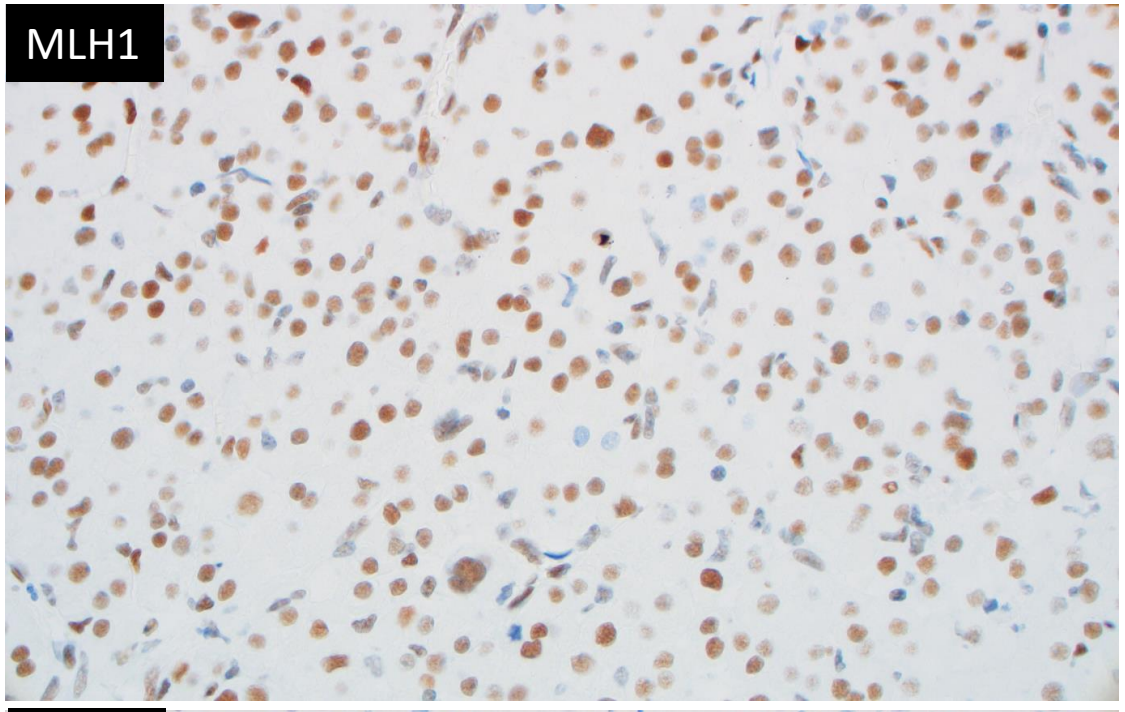

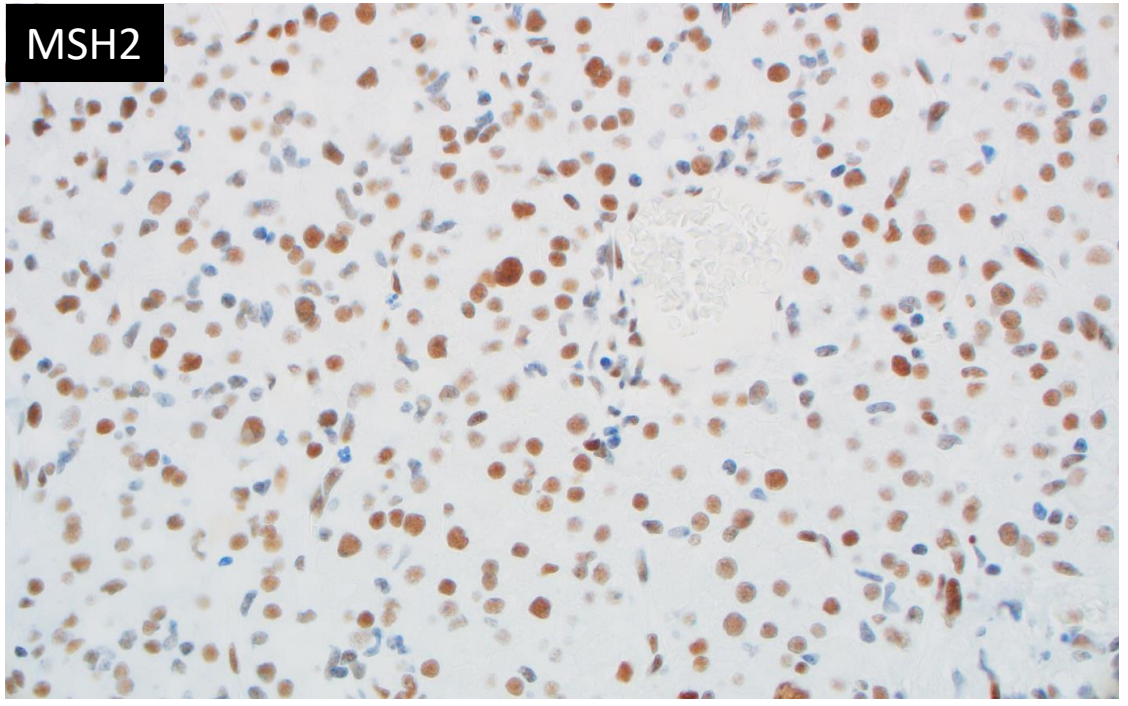

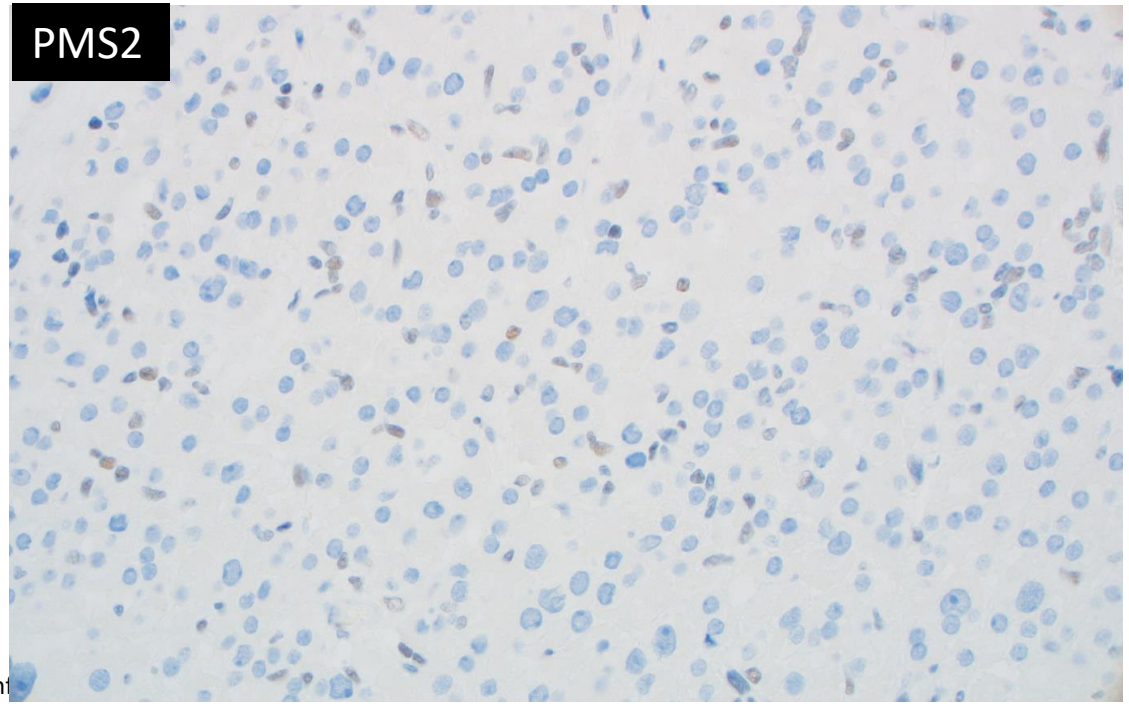

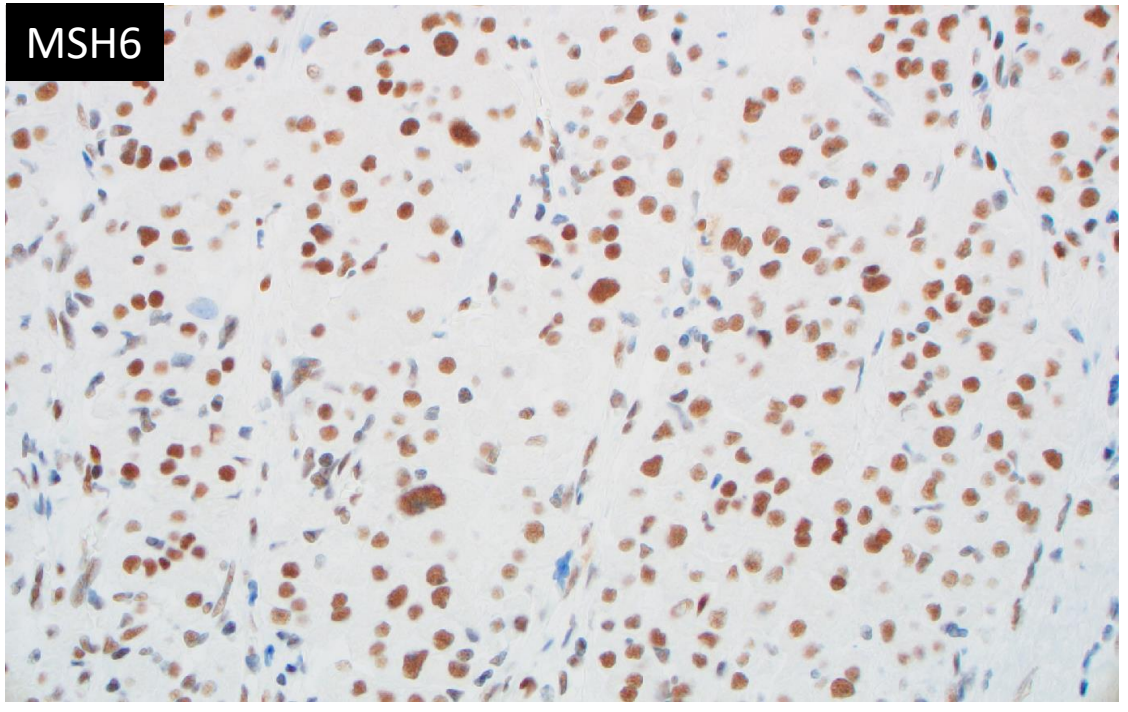

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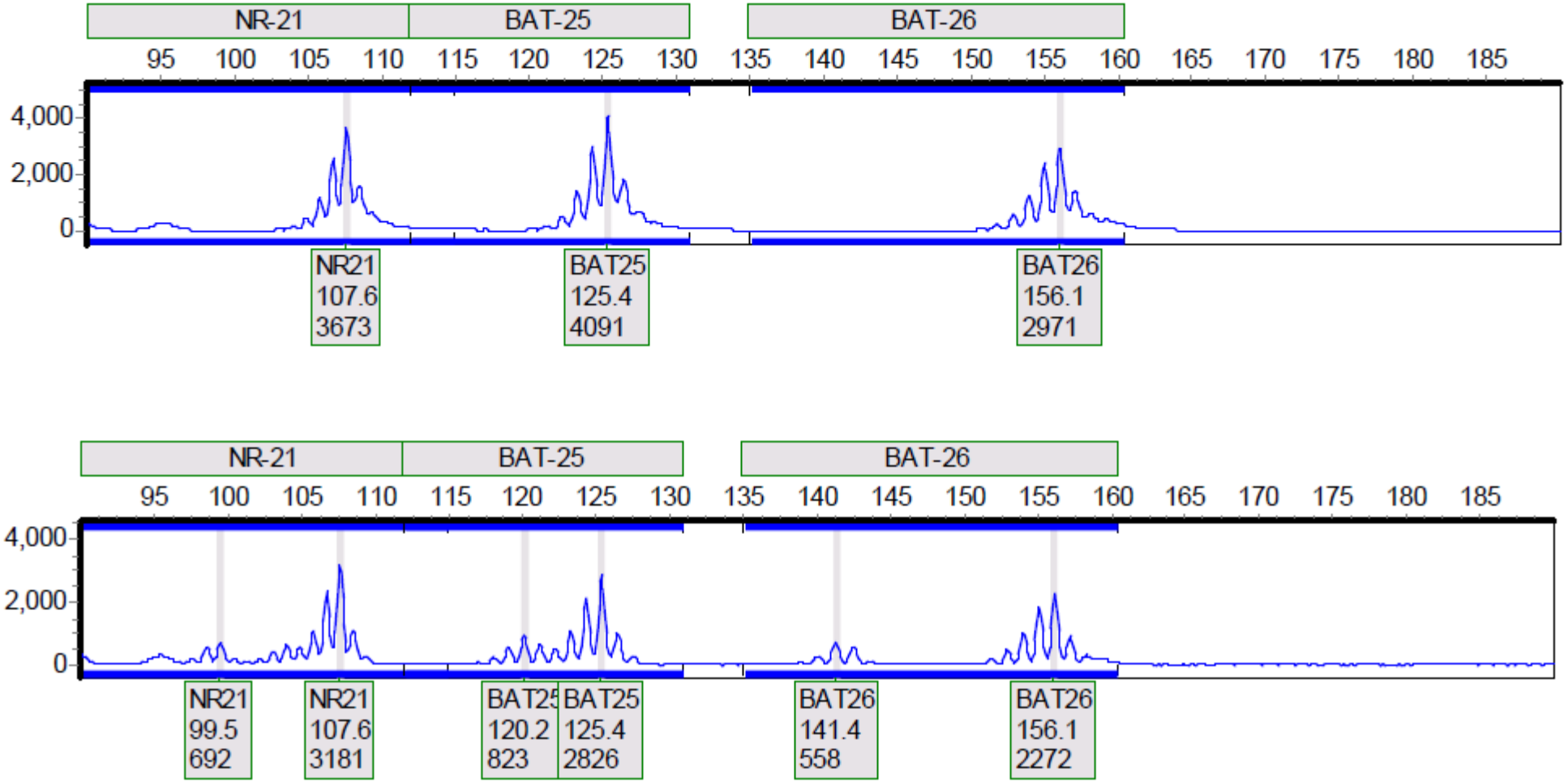
MSI PCR



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NGS

- Homopolymer regions can be evaluated using NGS
- Evaluates several STR regions (typically 80 or more)
- Sensitivity and specificity depends on size of the panel

- Concordance between IHC and PCR: 92%
- Concordance between IHC and NGS: 92%
- Concordance between PCR and NGS: 95%

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Which Test for Prostate Ca

- None validated for Pca
- NCCN:
  - MMR IHC
  - MSI by NGS validated for Prostate Ca

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HOXB13 Gene

- Prostate cancer risk
- Carriers of *HOXB13* G84E mutation
  - Early onset disease
  - Scandinavian descent
- No treatment implications

NCCN Guidelines Version 1.2023, Hum Genet 2013;132:5-14.

Private Information

Multigene Testing

- RNA expression analysis
- Localized disease
  - Risk of metastatic disease
  - Pca specific mortality
  - Active surveillance/intensification of treatment following RP
- Metastatic disease
  - Adjuvant therapy decisions

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Private Information

Multigene Testing

- Decipher, Prolaris and Oncotype Dx
  - Low or favorable risk Pca with life expectancy $\geq$ 10 years
  - Risk stratification
- Decipher, Prolaris
  - Unfavorable intermediate or high risk, life expectancy $\geq$ 10 years
- Decipher
  - Inform adjuvant therapy for adverse features after RP or on workup

Private Information

	Decipher	Oncotype Dx (GPS™)	Prolaris
Specimen type	Biopsy, radical prostatectomy	Biopsy	Biopsy, radical prostatectomy
Specimen requirement	10 sections for biopsy, 6 sections for radical prostatectomy, 3-5 µ. Tumor at least 0.5 mm in length.	15 sections, 5 µ	7 sections, 3-5 µ. Tumor at least 0.5 mm in length.
Assay gene coverage	22 genes (7 cancer pathways)	12 prostate cancer related genes and 5 reference genes	31 CCP genes, 15 reference genes
Scoring	0-.45 (Low), 0.45-0.60 (intermediate), and 0.60-0.80 (high) risk	Low, intermediate and high risk	Active surveillance, single-modal treatment, multi-modal treatment

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Decipher

- High scores
  - Active surveillance: shorter time to treatment
  - On treatment: shorter time to treatment failure
  - Post RP: biochemical failure, metastatic disease, Pca specific mortality, OS
  - Benefit from adjuvant therapy

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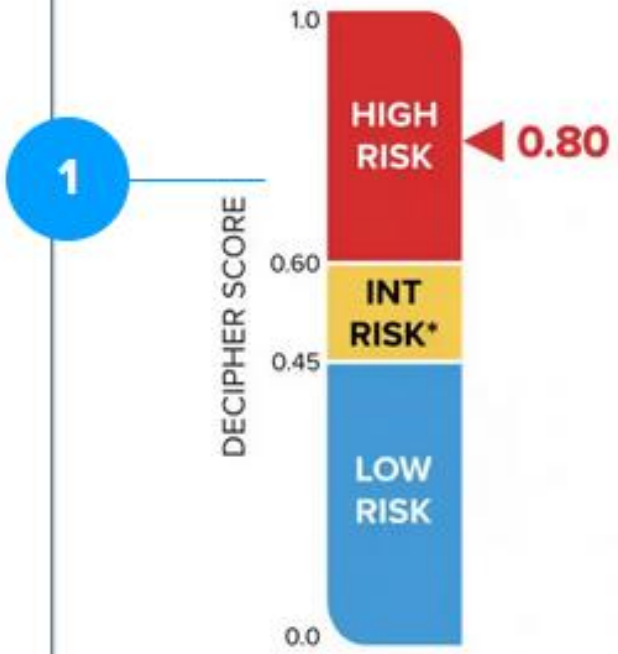
Decipher



GENOMIC RISK IS: HIGH			
2.6%	6.5%	8.8%	48.1%
5-year / *10-year* Risk of Metastasis with RT† or RP‡		*15-year* Risk of Prostate Cancer Mortality with RT or RP	*At RP* Risk of Adverse Pathology

2

Clinical studies have shown that Decipher high-risk patients have an unfavorable prognosis.

- These patients may benefit from treatment intensification with multimodal therapy.[2-5,9,10]
- These patients may not be ideal candidates for active surveillance.[1-3,8]

3

The Decipher score is determined solely by genomic characteristics of the tumor, independent of the NCCN risk category. No other clinical or pathologic parameters factor into the score.

Private Information

Oncotype Dx

- Scores correlate with
  - Adverse pathologic features
  - Biochemical recurrence
  - Metastatic disease
- Findings not validated in other cohorts

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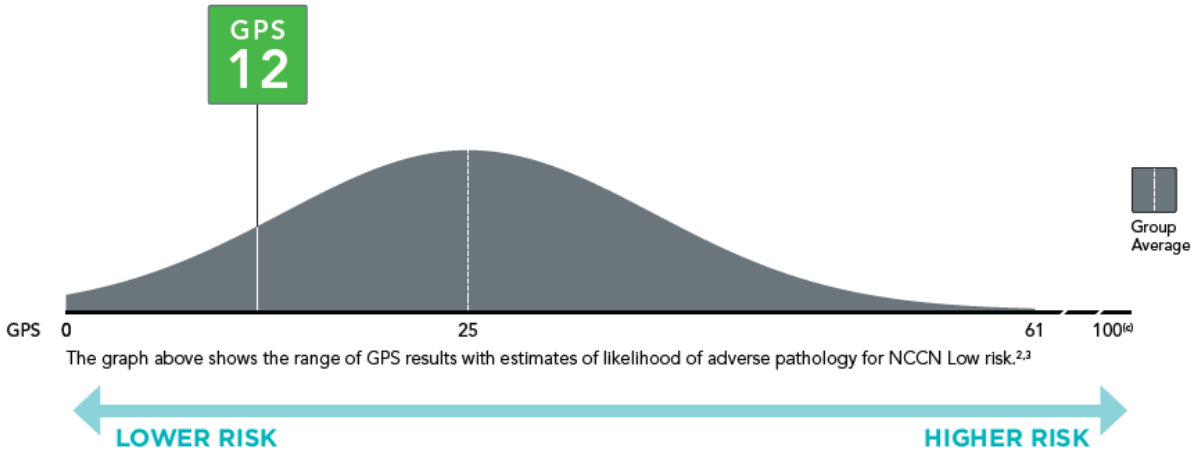
Oncotype Dx

Patient's GPS result is 12



The graph above shows the range of GPS results with estimates of likelihood of adverse pathology for NCCN Low risk.[2,3]

LOWER RISK — HIGHER RISK

	ADVERSE OUTCOME	LIKELIHOOD OF ADVERSE OUTCOME
1	Metastasis Within 10 Years after Radical Prostatectomy(g)	1% (95% CI: <1% - 6%)
2	Prostate Cancer Death Within 10 Years After Radical Prostatectomy	<1% (95% CI: <1% - <1%)

Private Information

Prolaris

- Predicts
  - 10-year metastatic risk
  - Pca specific mortality

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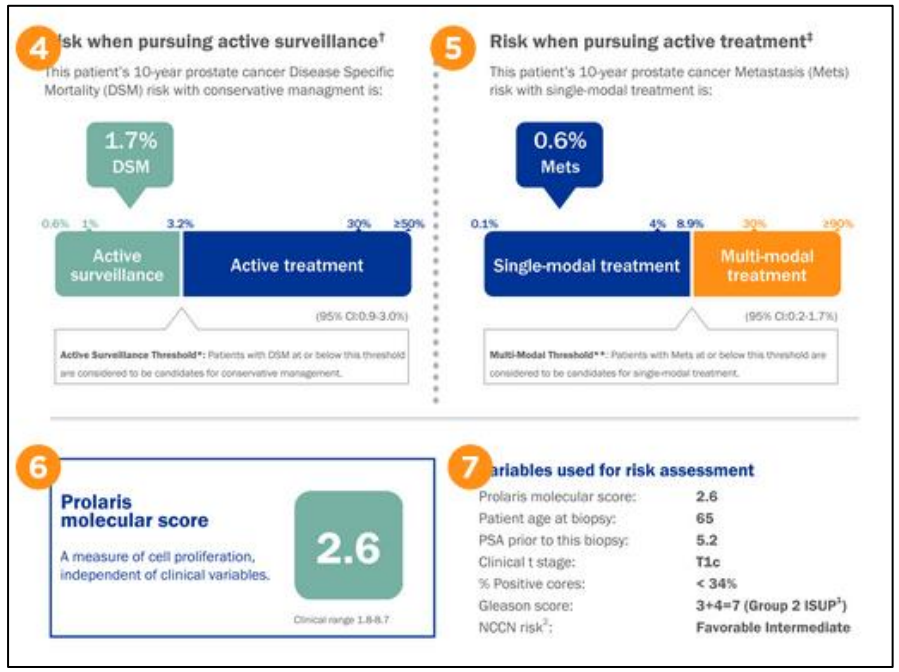
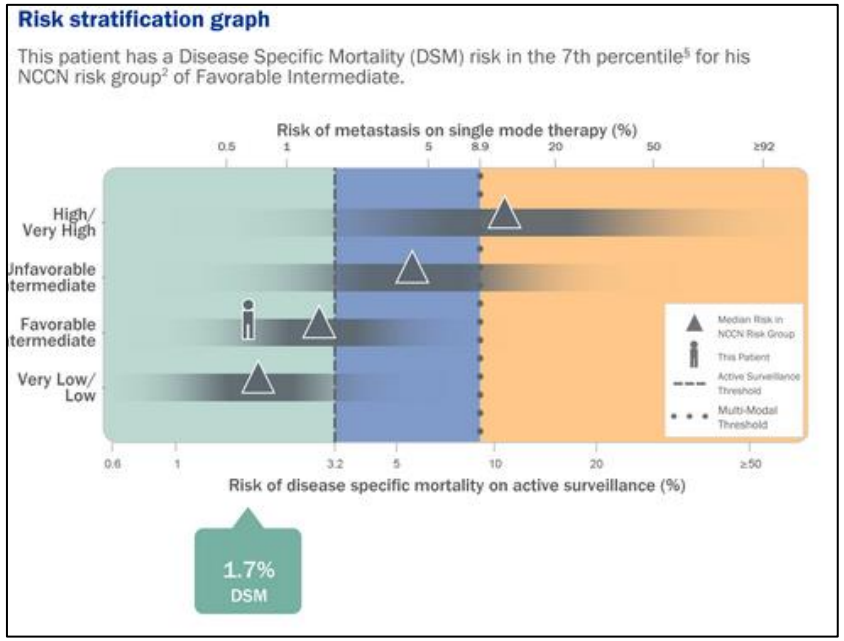
Prolaris

Prolaris test result summary

Based on a 10-year Disease Specific Mortality (DSM) risk of 1.7% with conservative management, this patient is a candidate for Active Surveillance.





Private Information

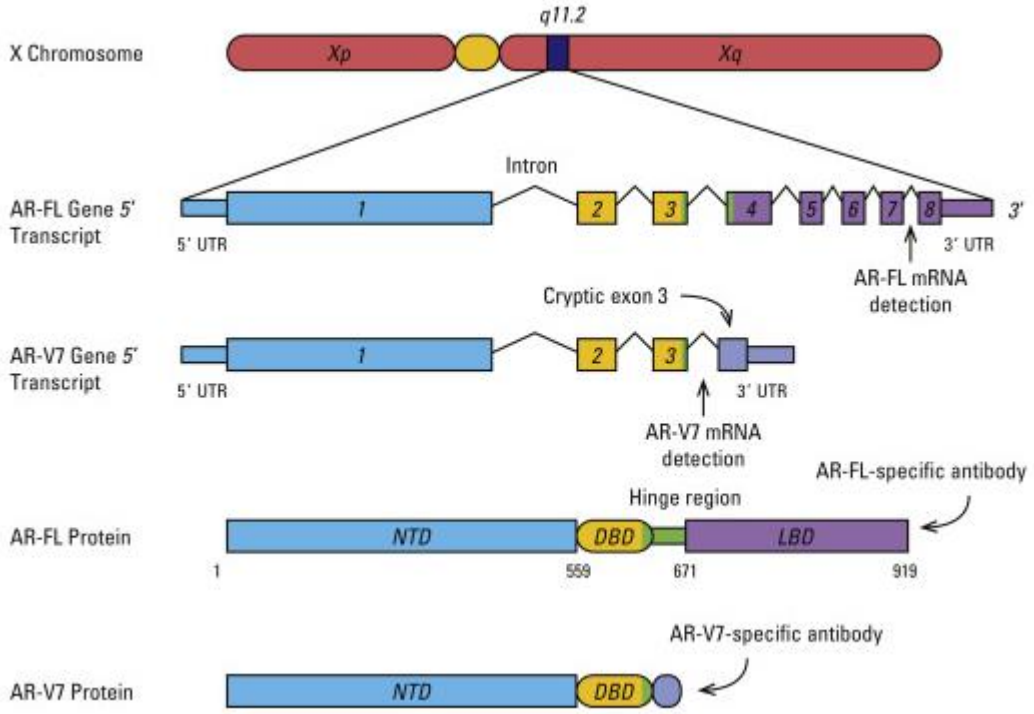
Androgen Receptor Splice Variant 7 (AR-V7)

Androgen Signaling: Androgen receptor agonists

AR: mutations, amplification, splice variants
AR-V7: 75% metastatic PCa
Shorter PFS and OS
Taxanes

Androgen independent growth: Castration resistance

Mutations in AR pathway genes
AR-V7 testing in circulating tumor cells



Cancers (Basel). 2021;13(21):5417.

Private Information

Mutations and Morphology

- Data is limited
- Increased genomic stability, likely to have MMR gene mutations
  - Invasive cribriform
  - Ductal
  - Intraductal
- Germline HRD gene mutations
  - Ductal/intraductal histologies
  - Intraductal: *BRCA2* mutations
- Recommendation for germline testing in patients with intraductal histology

BMC Cancer 2018;18:8.
JCO Precis Oncol 2019;3.

Private Information

Guideline Recommendations

Private Information

Germline Testing

- Pre-Test
  - Family history, known germline variants
  - Treatment impact
  - Ascertaining risk of other cancers
  - Risk to family members
- Testing (minimum)
  - *BRCA1, BRCA2, ATM, PALB2, CHEK2*
  - *MLH1, MSH2, MSH6, PMS2*
  - *HOXB13*
- Post-Test
  - Genetic counselling

NCCN Guidelines Prostate Version 1.2023

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Germline testing

PRINCIPLES OF GENETICS AND MOLECULAR/BIOMARKER ANALYSIS

Germline testing is recommended *in patients with a personal history of prostate cancer* in the following scenarios:

- By Prostate Cancer Stage or Risk Group (diagnosed at any age)
  - Metastatic, regional (node positive), very-high risk localized, high-risk localized prostate cancer

- By Family History[a] and/or Ancestry
  - ≥1 first-, second-, or third-degree relative with:
    - ◊ breast cancer at age ≤50 y
    - ◊ colorectal or endometrial cancer at age ≤50 y
    - ◊ male breast cancer at any age
    - ◊ ovarian cancer at any age
    - ◊ exocrine pancreatic cancer at any age
    - ◊ metastatic, regional, very-high-risk, high-risk prostate cancer at any age
  - ≥1 first-degree relative (father or brother) with:
    - ◊ prostate cancer[b] at age ≤60 y
  - ≥2 first-, second-, or third-degree relatives with:
    - ◊ breast cancer at any age
    - ◊ prostate cancer[b] at any age
  - ≥3 first- or second-degree relatives with:
    - ◊ Lynch syndrome-related cancers, especially if diagnosed <50 y: colorectal, endometrial, gastric, ovarian, exocrine pancreas, upper tract urothelial, glioblastoma, biliary tract, and small intestinal cancer
  - A known family history of familial cancer risk mutation (pathogenic/likely pathogenic variants), especially in: *BRCA1, BRCA2, ATM, PALB2, CHEK2, MLH1, MSH2, MSH6, PMS2, EPCAM*
  - Ashkenazi Jewish ancestry
- Personal history of breast cancer

Germline testing may be considered *in patients with a personal history of prostate cancer* in the following scenarios:

- By Prostate Cancer Tumor Characteristics (diagnosed at any age)
  - ◊ intermediate-risk prostate cancer with intraductal/cribriform histology[c]
- By prostate cancer[b] AND a prior personal history of any of the following cancers:
  - ◊ exocrine pancreatic, colorectal, gastric, melanoma, pancreatic, upper tract urothelial, glioblastoma, biliary tract, and small intestinal

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Somatic Testing

- Purpose
  - Treatment decisions
  - Genetic counseling
  - Clinical trial eligibility
- Testing recommendations
  - Metastatic/regional cancer
    - HRD genes: *BRCA1, BRCA2, ATM, PALB2,FANCA, RAD51D, CHEK2, CDK12*
  - Metastatic CRPC, castration naïve metastatic/regional cancer
    - Microsatellite instability
  - Metastatic CRPC
    - Tumor mutational burden
  - Multi gene testing
    - Low, intermediate or high risk with life expectancy ≥10 years
  - Decipher
    - Risk stratification post radical prostatectomy PSA resistance/recurrence

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Somatic Testing

- Specimen considerations
  - Metastatic sample preferred
  - Circulating tumor (ct) DNA: during biochemical and/or radiological progression
    - When biopsy is unavailable or not feasible
- Microsatellite instability
  - Eligibility for Pembrozulimab for CRPC patients
- Genetic counselling

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Summary

- Molecular testing-based tests:
  - Screening
  - Diagnostic
  - Therapeutic target eligibility determination
- Molecular Biomarkers
  - Germline and somatic alterations
  - Homologous recombinant repair defects (HRD)
  - Microsatellite
  - HOXB13: prostate ca risk
- AR gene alterations: follow up biomarker
- Molecular alterations and morphologic correlation: limited data

Private Information

Thank you

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