Multigene Testing in Prostate Cancer Risk Stratification

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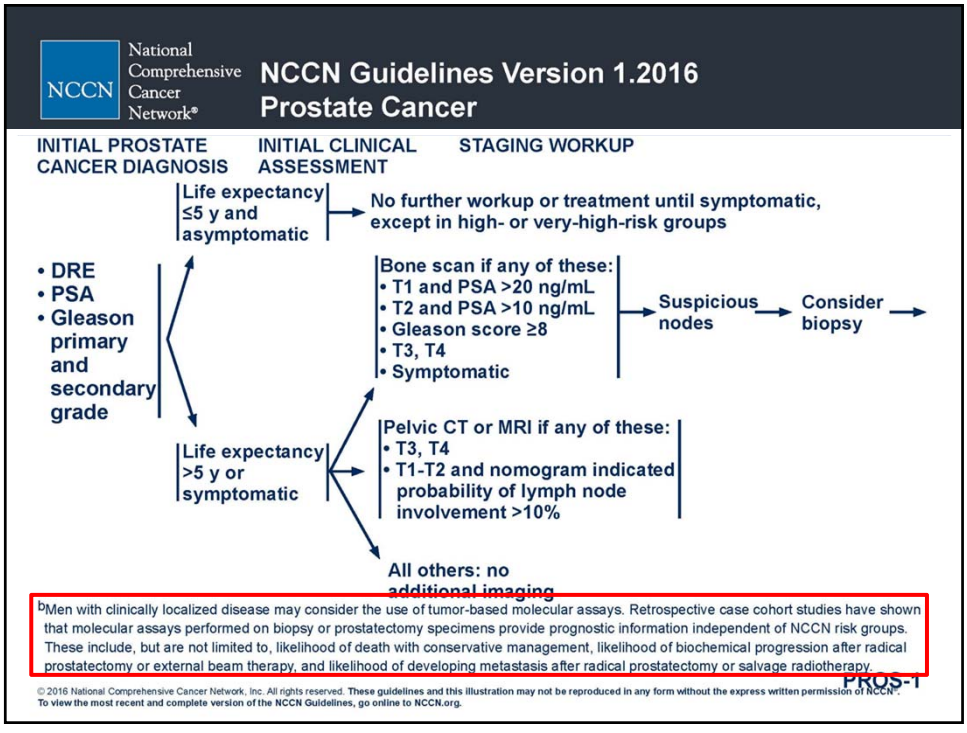
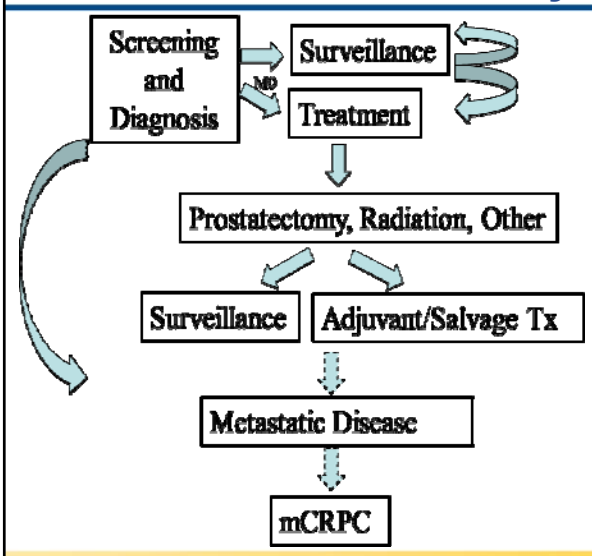


Objectives

- Briefly review relevant molecular biology of localized prostate cancer
- Review currently available molecular tests for prostate cancer and the potential risks and benefit of their use

Prostate Cancer – Landscape and Decision Uncertainty



Decision Making Complexity

- Broad Spectrum of Localized Disease
- Relatively Long Natural History (Competing Risks)
- Morbidity of Treatment

Advances

- Molecular understanding of localized prostate cancer has increased
- We have increased facility to obtain molecular information from routinely collected and stored formalin-fixed paraffin embedded tissue

Molecular Tissue Testing – Issues

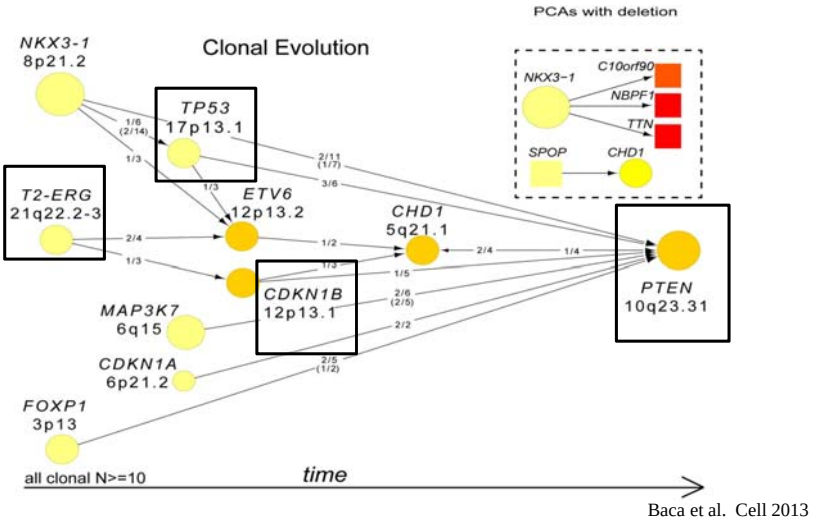
- Confusion among patients and providers
  - Tests assess similar molecular phenotypes
  - Aggressive marketing
  - Lack of head-to-head comparisons
- Existing risk stratification tools for localized disease perform well
- Cost

RELEVANT MOLECULAR BIOLOGY OF LOCALIZED PROSTATE CANCER

- "Prostate cancer is characterized by extraordinary genomic complexity"
  - Copy number alterations
    - Deletions and amplifications
  - Chromosomal rearrangements
  - Point mutations

Schoenborn et al Clin Cancer Res 2013

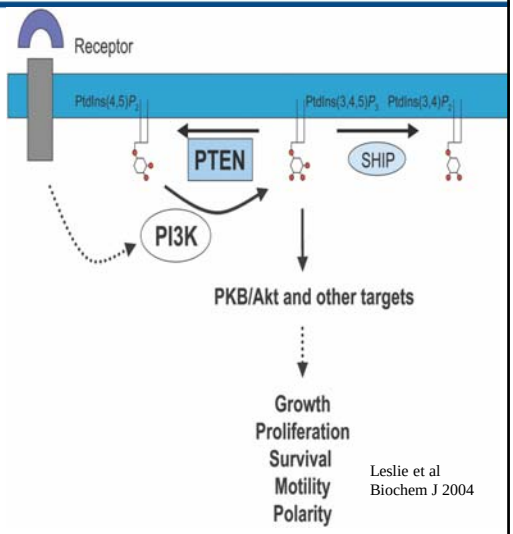
PTEN Loss, An Important Early Event



Baca et al. Cell 2013

PTEN Loss, An Important Early Event

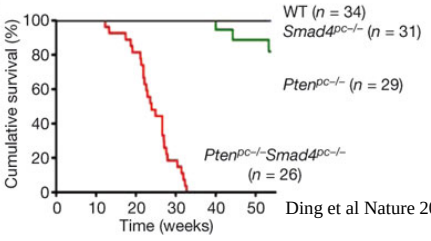
- PTEN is a tumor suppressor
  - Normally halts the PI3K/AKT pathway
  - PI3K pathway members are altered in up to 40% of all primary prostate cancer and 100% of metastasis



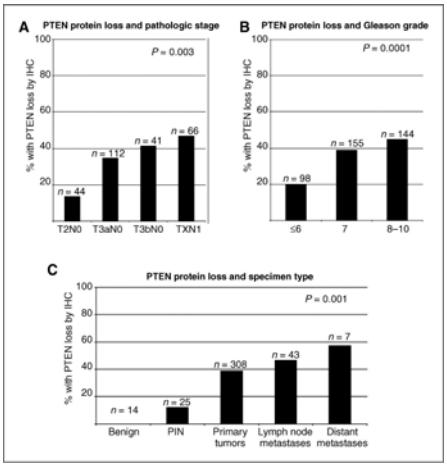
Leslie et al
Biochem J 2004

PTEN Loss, An Important Early Event

- PTEN loss is a key component of animal models for prostate cancer
- PTEN loss can be detected in ~15-40% of primary cases and ~50% of metastasis
- Loss correlates with stage and grade



Ding et al Nature 2011



Lotan et al Clin Cancer Res 2011

NCCN National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2016
Prostate Cancer

Table 1. Available Tissue-Based Tests for Prostate Cancer Prognosis

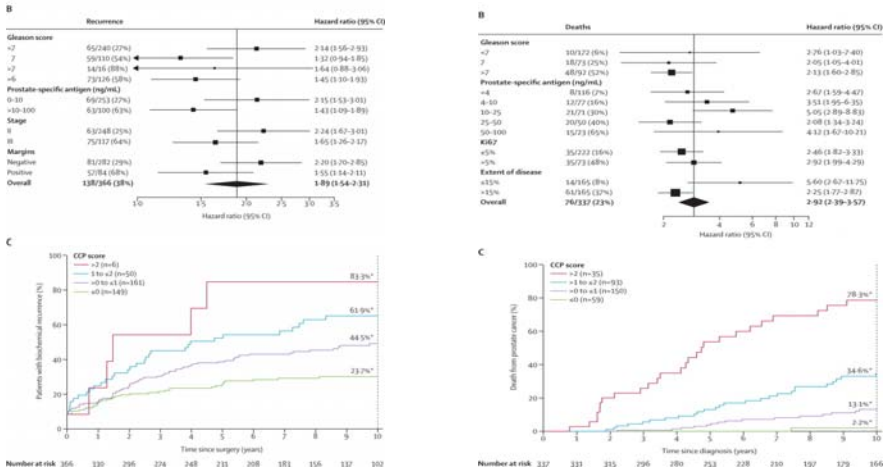
Test	Platform	Populations studied	Outcome Reported (Test independently predicts)	References	Molecular Diagnostic Services Program (MolDX) Recommendations
Decipher	Whole-transcriptome 1.4M RNA expression (44,000 genes) oligonucleotide microarray optimized for FFPE tissue	Post radical prostatectomy (RP), adverse pathology/high-risk features	Metastasis Prostate cancer-specific mortality	110,415-424	Cover post-RP for 1) pT2 with positive margins; 2) any pT3 disease; 3) rising PSA (above nadir)
		Post RP, biochemical recurrence	Metastasis Biochemical failure		
		Post RP, adjuvant or salvage radiotherapy	Metastasis		
Ki-67	IHC	Biopsy, intermediate- to high-risk treated with EBRT	Metastasis	425-428	Not recommended
		Biopsy, conservatively managed (active surveillance)	Prostate cancer-specific mortality		
Oncotype DX	Quantitative RT-PCR for 12 prostate cancer-related genes and 5 housekeeping controls	Biopsy, low- to intermediate-risk treated with RP	Non-organ-confined pT3 or Gleason grade 4 disease on RP	48,429	Cover post-biopsy for NCCN very-low- and low-risk prostate cancer at diagnosis with 10-20 years life expectancy
Prolaris	Quantitative RT-PCR for 31 cell cycle-related genes and 15 housekeeping controls	Transurethral resection of the prostate (TURP), conservatively managed (active surveillance)	Prostate cancer-specific mortality	44-47,430,431	Cover post-biopsy for NCCN very-low- and low-risk prostate cancer at diagnosis with at least 10 years life expectancy
		Biopsy, conservatively managed (active surveillance)	Prostate cancer-specific mortality		
		Biopsy, localized prostate cancer	Biochemical recurrence Metastasis		
		Biopsy, intermediate-risk treated with EBRT	Biochemical failure		
		RP, node-negative localized prostate cancer	Biochemical recurrence		
ProMark	Multiplex immunofluorescent staining of 8 proteins	Biopsy, Gleason grade 3+3 or 3+4	Non-organ-confined pT3 or Gleason pattern 4 disease on RP	432	Not reviewed
PTEN	Fluorescent in situ hybridization or IHC	Transurethral resection of the prostate (TURP), conservatively managed (active surveillance)	Prostate cancer-specific mortality	433-435	Not recommended
		Biopsy, Gleason grade 3+3	Upgrading to Gleason pattern 4 on RP		
		RP, high-risk localized disease	Biochemical recurrence		

MS-40

Tests Based on Cell Cycle Proliferation

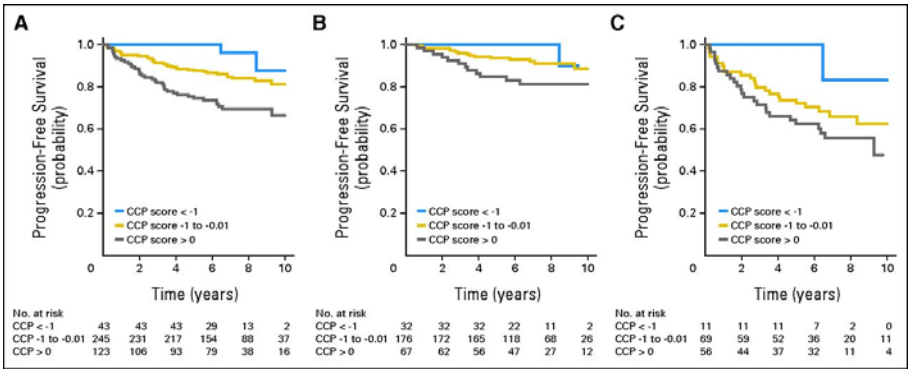
- Cell cycle abnormalities are common in localized prostate cancer
- Ki-67 IHC has independent prognostic significance after radiation (XRT) or radical prostatectomy (RP) (Khor et al JCO 2004, Tollefson et al Mayo Clin Proc 2014)
- Prolaris
  - qRT-PCR
  - 31 cell cycle genes normalized to 15 house keeping genes
  - Currently marketed for use in men diagnosed with very-low-risk and low-risk prostate cancer

Prolaris Scores Correlate with Clinical Outcomes Post RP / Watchful Waiting



Cuzick et al Lancet Onc 2011

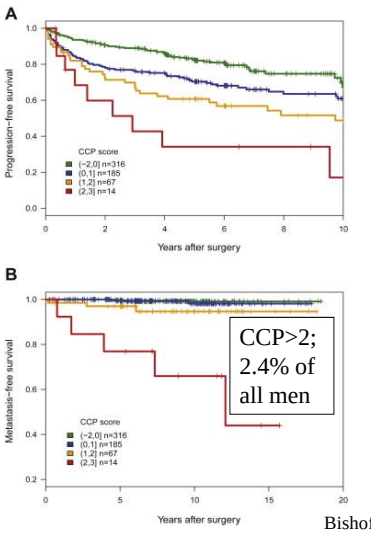
Prolaris Scores Correlate with Clinical Outcomes Post RP



Cooperberg et al JCO 2013

Prolaris / Cell Cycle Progression (CCP) Score – Needle Biopsy Cohorts (post RP)

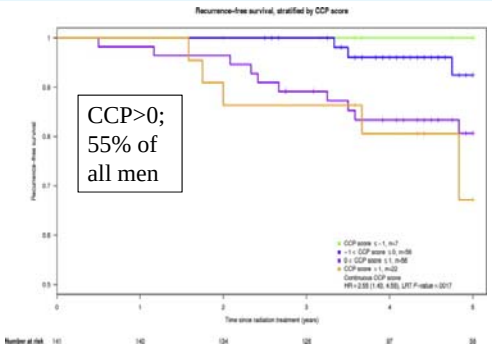
- 582 men undergoing RP, Needle Biopsy
- Primarily low- and intermediate-risk disease
- Relatively few metastatic events
- Hazard ratio (HR) per unit increase in score, 4.2 (2.1-8.5) on multi-variate analysis (MVA)



Bishoff J Urol 2014

Prolaris / CCP score – Needle Biopsy Cohorts (post radiation)

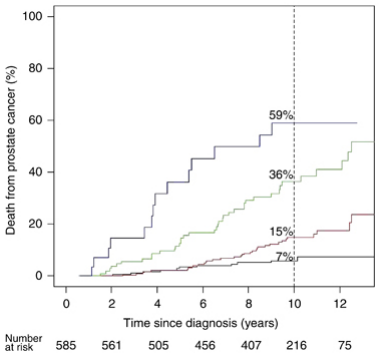
- 141 men undergoing XRT, Needle Biopsy
- 73% intermediate- or high-risk
- HR biochemical recurrence (BCR; Phoenix definition) per unit increase in score, 2.1 (1.05-4.25) MVA

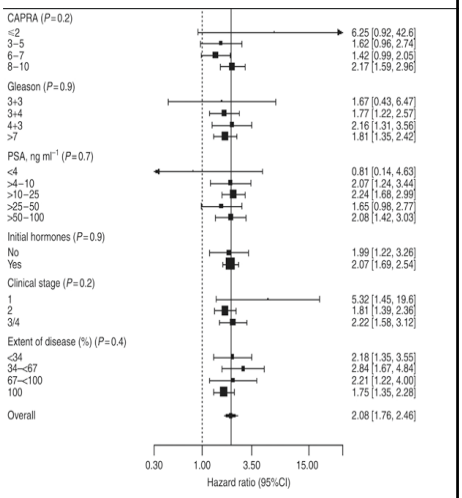


Freedland et al IJROBP 2013

Prolaris/CCP Score - Needle Biopsy Conservative Management

- Transatlantic Prostate Group
- HR death per unit CCP increase ~2 (bivariate analysis with CAPRA)





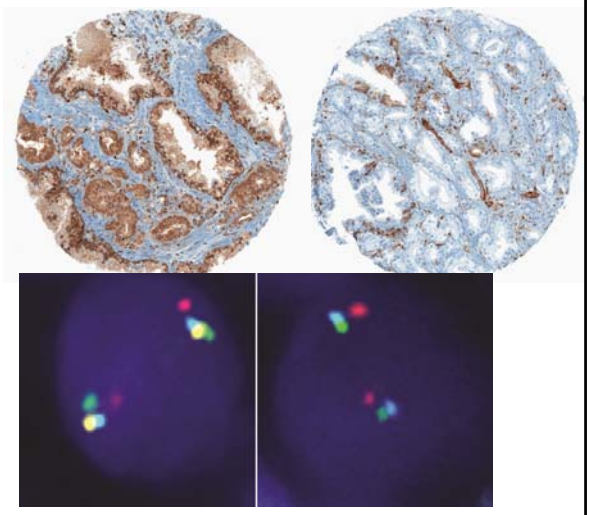
Cuzick et al Br J Cancer 2015

Tests Based on Molecular Features More Specific to Prostate Cancer

- PTEN/ERG
- Oncotype Dx Prostate
- ProMark
- Decipher

PTEN Testing

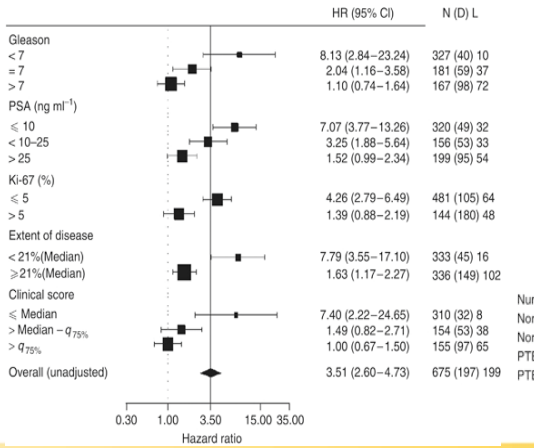
- IHC
- FISH

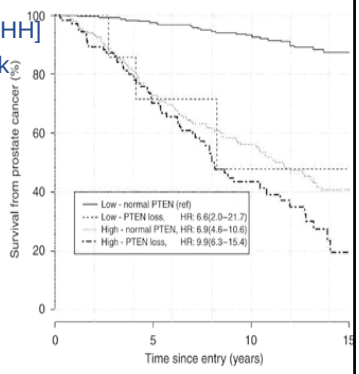


Shipitsin et al Proteome Science 2014

PTEN Testing – Watchful Waiting, Transatlantic Prostate Group

- 675 men on watchful waiting (WW)
- PTEN IHC & FISH (IHC better) [cheaper too, $95 at JHH]
- TURP specimens;18% PTEN loss; 3% among low risk





Number at risk:

Normal PTEN (low risk)	302	248	181	49
Normal PTEN (high risk)	206	118	64	19
PTEN loss (low risk)	8	5	2	1
PTEN loss (high risk)	103	63	23	2

Cuzick et al BJC 2013

PTEN Testing – Upgrading

- 174 men with Gleason score (GS) 6 disease undergoing RP
- PTEN IHC on biopsy tissue
- PTEN lost in 18% of upgraded cases with 7% of cases that did not upgrade
- OR 3 (1.1-8.6) for upgrading

Lotan et al Mod Path 2015

PTEN/ERG – Outcomes after Prostatectomy (Health Professionals Follow-Up Study and Physicians Health Study)

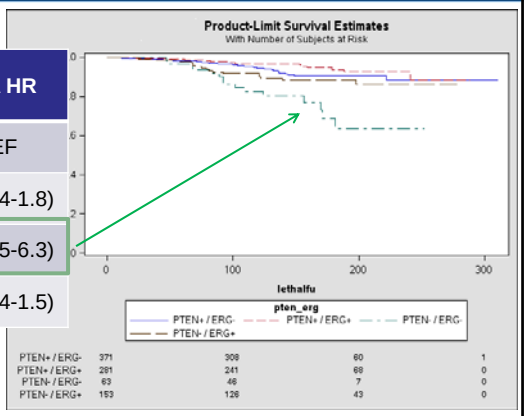
HPFS Cohort Multivariate Modeling

				Adjusted for age at diagnosis, BMI at diagnosis, Gleason grade, and cTNM
	N	Lethal		HR for lethal prostate cancer
PTEN positive	735	46		1
PTEN mixed	90	7		1.2 (0.6-3.0)
PTEN negative	139	28		1.9 (1.2-3.0)

Ahearn et al JNCI 2016

PTEN /ERG – Outcomes after RRP (Health Professionals Follow-Up Study and Physicians Health Study)

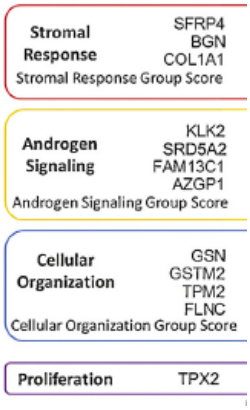
PTEN / ERG status	N	Lethal	MVA HR
PTEN+ and ERG-	306	20	REF
PTEN+ and ERG+	260	12	0.9 (0.4-1.8)
PTEN- and ERG-	48	13	3.1 (1.5-6.3)
PTEN- and ERG+	132	12	0.7 (0.4-1.5)



Ahearn et al JNCI 2016

Tests on Multiple Features of Prostate Cancer Oncotype DX Prostate

- qRT-PCR of 12 genes (derived from 732 genes which correlated with poor oncologic outcome) and 5 housekeeping genes

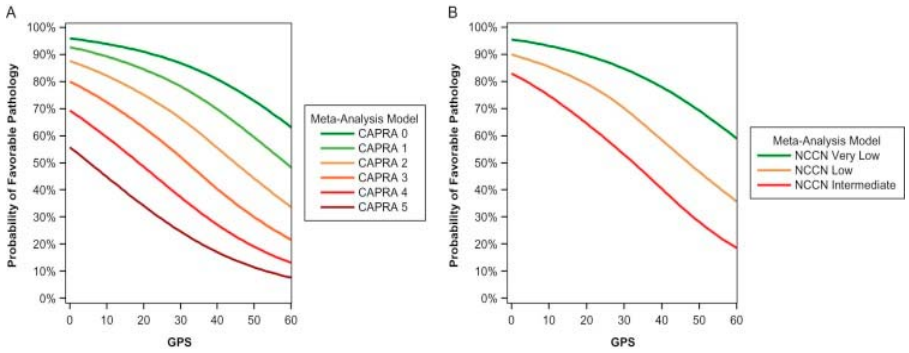


- Run on biopsies
- Each 20-point increase (~IQR) in Genomic Prostate Score (GPS) equals ~2 fold risk of ≥ 4+3 or pT3 disease at RP
- Marketed for men with low- to low intermediate-risk considering active surveillance
- Not reported in active surveillance populations

Klein et al Eur Urol 2014
Cullen et al Eur Urol 2014

Tests on Multiple Features of Prostate Cancer
Oncotype DX Prostate

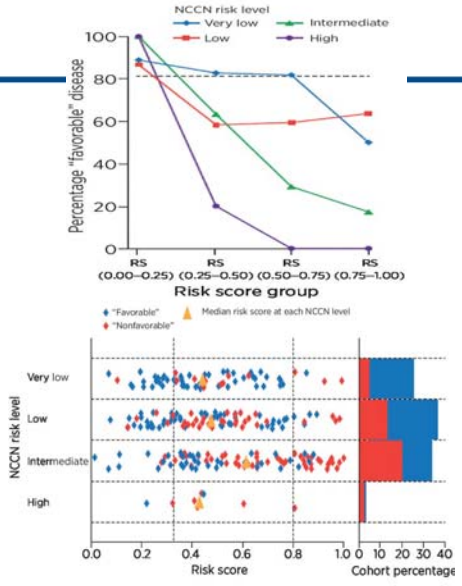
- Meta-analysis 732 patients (2 studies, UCSF, CPDR) for prediction of favorable pathology (pT2 and GS 3+4=7 or less)



Brand et al Urology 2016

Tests on Multiple Features of Prostate Cancer
ProMark

- Multiplexed proteomics assay
- Run on biopsies
- Score ranges form 0-1, each 0.25-point increase equals ~3 fold risk of unfavorable pathology
- Thresholds of <0.33 and >0.8 appear useful and include ~40% of patients
- Marketed for men with low- to low intermediate-risk considering AS
- Not yet tested in AS populations



Blume-Jensen Clin Can Res 2015

Tests on Multiple Features of Prostate Cancer Decipher

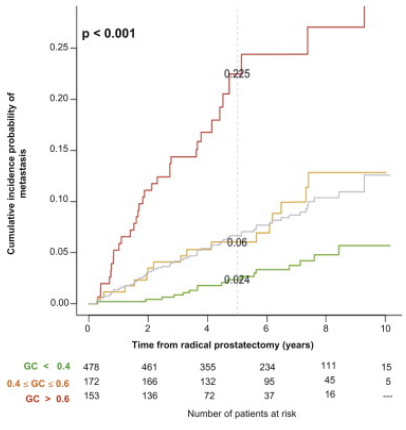
- High density array performed on FFPE prostate tissue
  - 1.4 million probes (coding and non-coding)
  - CLIA certified
- 22 genomic markers selected for ability to predict rapid mets after RP
  - Outputs a genomic classifier (GC) score (0-1)
  - Marketed for post RP testing and recently available for use on biopsy tissue

Cell Proliferation, Differentiation	Cell Structure, Adhesion, Motility	Immune System Modulation	Cell Cycle Progression	Androgen-Signaling
CAMK2N1	ANO7	GLYATL1P4	NFIB	ANO7
MYBPC1	EPPK1	S1PR4	NUSAP1	PCAT-32
PBX1	IQGAP3	TNFRSF19	ZWILCH	UBE2C
THBS2	LASP1	TSBP		
UBE2C	MYBPC1			
	PCDH7			
	RABGAP1			

Abdueva et al J Mol Diag 2010
Erho et al J Onc 2012

Tests on Multiple Features of Prostate Cancer Decipher – Post RP

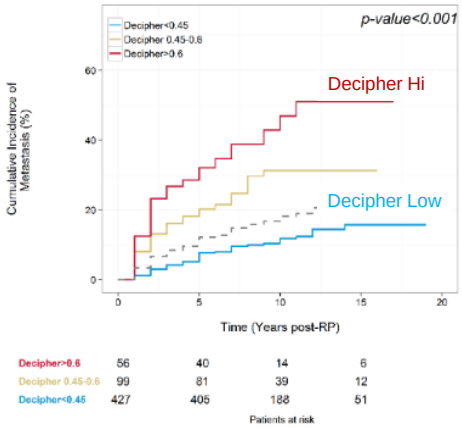
- Metastasis signature validation in 219 high-risk men after RRP at Mayo Clinic
- Categorical cut-offs at <0.4 (60%), 0.4-0.6 (20%) and >0.6 (20%) associated with HR for mets on MVA of 1, 2.4 (1.1-5.2) and 7.3 (3.5-15.1)
- Mayo cohort with high amount of adjuvant and salvage treatment post-RP



Karnes et al J Urol 2013

Tests on Multiple Features of Prostate Cancer Decipher – Post RP

- JHH cohort, 260 NCCN intermediate- or high-risk men, 99 with metastatic progression
  - Natural history (no treatment post-RP until mets)
- Median GC score 0.34 (IQR 0.22-0.52)
- HR met MVA 1.5 (1.3-1.7) per 0.1 increase in score
- Similarly predictive in RP and biopsy tissue from a Cleveland Clinic cohort managed without adjuvant radiation



Ross et al Eur Urol 2015
Klein et al Eur Urol 2015
Klein et al Urology 2015

Tests on Multiple Features of Prostate Cancer Decipher – Independent Predictor Cancer Specific Mortality

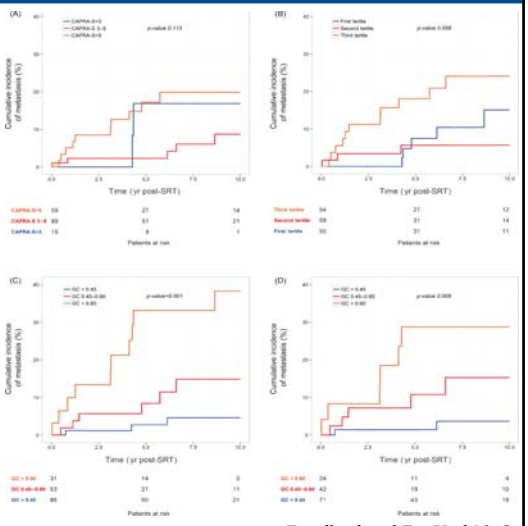
- HR 1.8 per 0.1 unit increase on MVA

		HR PCSM	P val
GC	0.4–0.6 (ref: <0.4)	1.09 (0.26–3.77)	0.9
	>0.6 (ref: <0.4)	11.26 (4.69–30.37)	<0.001
CAPRA-S	>5	2.36 (1.06–5.68)	0.04
Adjuvant therapy	Radiation	0.56 (0.11–1.80)	0.36
	Androgen deprivation	1.55 (0.72–3.36)	0.26

Cooperberg et al Eur Urol 2015

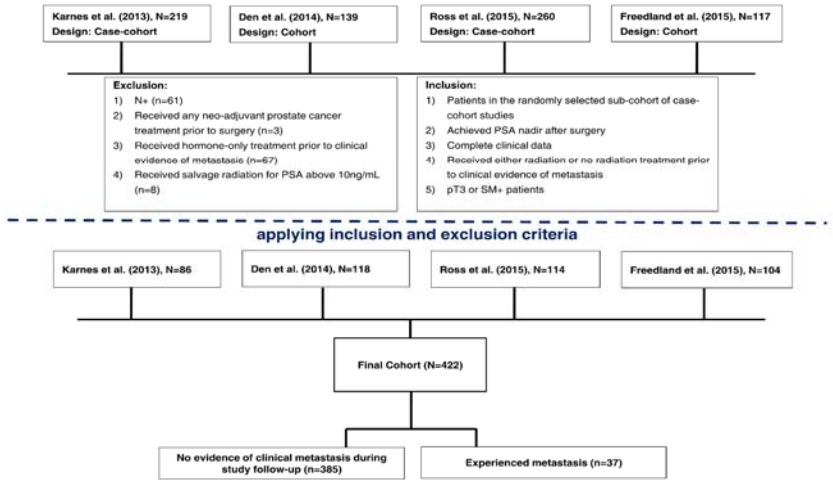
Tests on Multiple Features of Prostate Cancer Decipher – Post RP Adjuvant and Salvage Radiation

- 170 men with BCR undergoing salvage XRT
- MVA HR for metastasis 1.6 per 0.1 unit increase p=0.002
- 12% metastasis rate
- 39% patients in the upper 2 tertiles of Briganti Risk and 49% of CAPRA-S intermediate/high-risk patients reclassified as GC low risk (97% and 96% MFS)



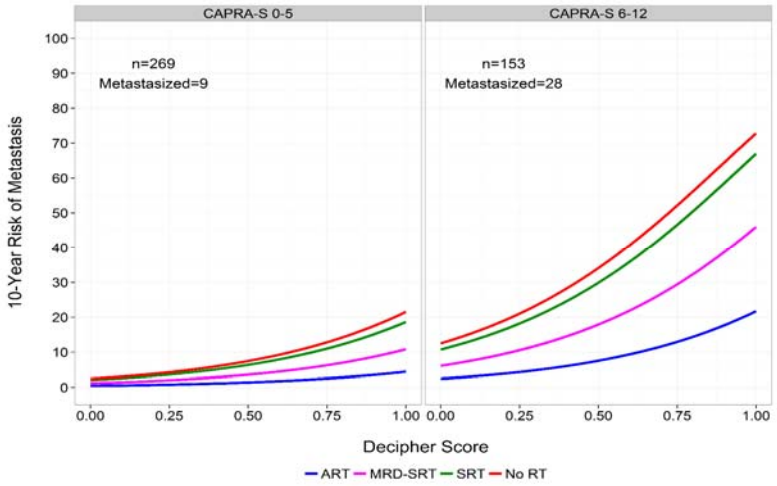
Freedland et al Eur Urol 2016

Decipher – Post RP Radiation Therapy



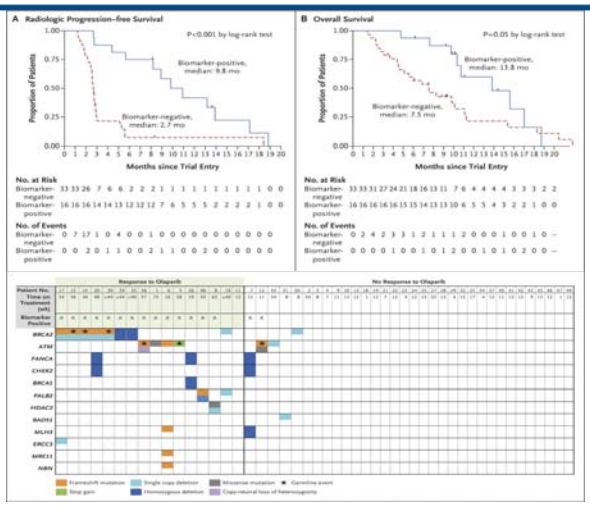
Ross and Den et al PCAN 2016 in press
Den et al JCO 2015
Den et al Int J Rad Onc Biol Phys 2015

Decipher – Post RP Radiation Therapy



Ross et al PCAN 2016 in press

Predictive of Treatment Response - Metastatic Castration-Resistant Prostate Cancer (mCRPC) Failing Multiple Agents



Mateo J et al. N Engl J Med 2015;373:1697-1708.

National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2016
Prostate Cancer

RISK GROUP

Clinically Localized:

Very low:
- T1c
- Gleason score ≤6
- PSA <10 ng/mL
- Fewer than 3 prostate biopsy cores positive, ≤50% cancer in each core
- PSA density <0.15 ng/mL/g

Low:
- T1-T2a
- Gleason score ≤6
- PSA <10 ng/mL

Intermediate:
- T2b-T2c or
- Gleason score 7 or
- PSA 10–20 ng/mL

High:
- T3a or
- Gleason score 8–10 or
- PSA >20 ng/mL

Locally Advanced:

Very high:
- T3b-T4 or
- Primary Gleason pattern 5 or
- >4 cores with Gleason score 8–10

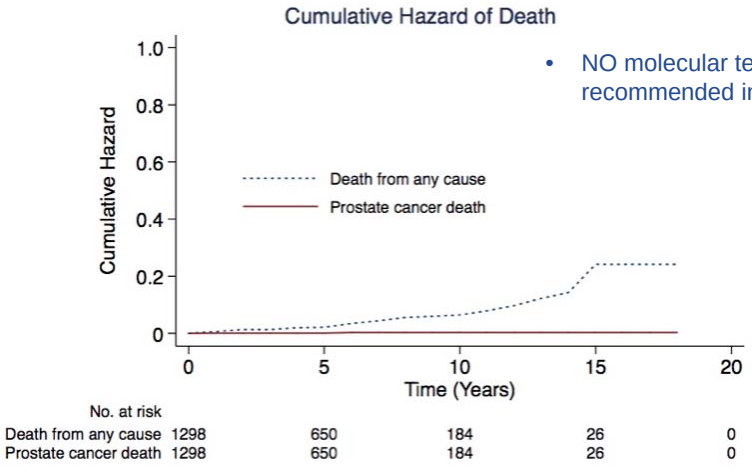
Metastatic:
Any T, N1 or
Any T, Any N, M1

Tissue Based Molecular Tests USE BY NCCN RISK CATEGORY

[e]Patients with multiple adverse factors may be shifted into the next highest risk group.

PROS-1

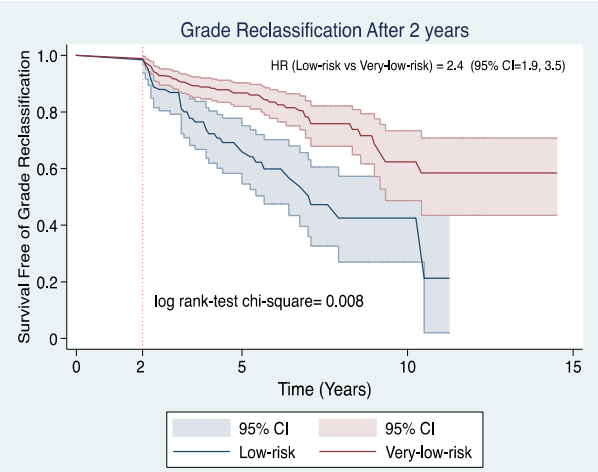
Surveillance of Very-Low-Risk (VLR) Men – Consider No Testing



- NO molecular testing recommended in VLR >65 years

Tosoian et al JCO 2016

Surveillance of Low-Risk Men – PTEN/ERG, Oncotype Dx, ProMark, Prolaris



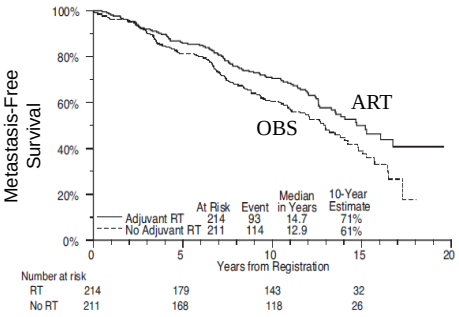
- Most testing will be confirmatory (non-informative)
  - Cost Considerations
- Limited published data from active surveillance cohorts

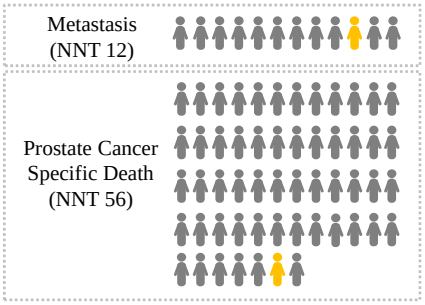
Alam et al J Urol 2015

Intensity/Use of Primary Radiation Therapy (RT) for all NCCN Risk Groups: ?

- Unlike RP, RT is biologic and modified based on predicted cancer aggression
- Androgen deprivation therapy (ADT) adds morbidity
- RTOG 0815: RT +/-ADT for intermediate-risk men
- Short term ADT (SADT) vs long-term ADT (LADT) for high-risk men
- Addition of chemotherapy for very-high-risk and locally advanced men
- Ongoing research

After RP – Adjuvant Radiation Example 2 Step Process – Nomogram First



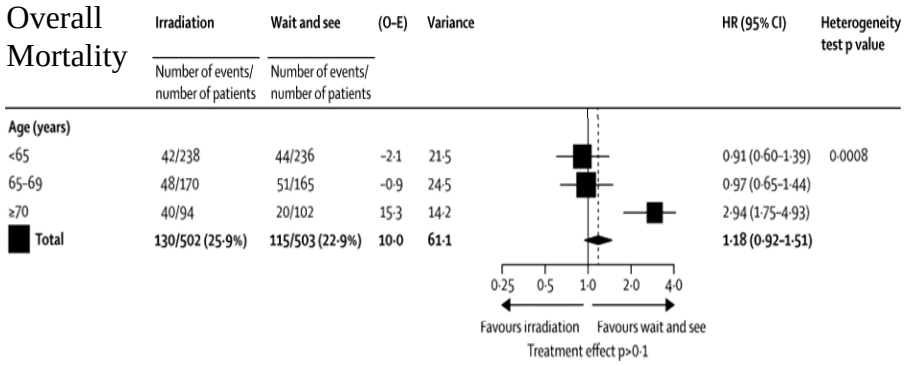


Thompson IM et al, JAMA 2006, J Urol, 2009 & 2013

- Most men with as adverse pathological features (APF) after RP do not benefit from adjuvant XRT

NNT, number needed to treat; ART, adjuvant radiation therapy; OBS, observation

After RP – Adjuvant Radiation Example 2 Step Process – Nomogram First

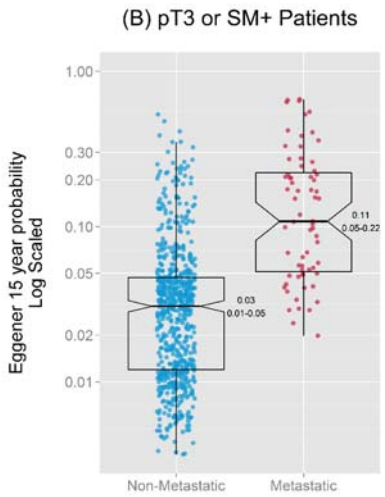


- Men with APF after RP may be harmed by adjuvant XRT (long term results EORTC 22911, Bolla et al. Lancet 2012)

After RP – Adjuvant Radiation Example 2 Step Process – Nomogram First

Nomogram	Cut-point	No. of APF Patients (%)	Cumulative Incidence of Metastasis APF Patients (at 10 yrs)
CAPRA-S	CAPRA-S <3	200 (26%)	3%
	CAPRA-S 3-5	435 (56%)	4%
	CAPRA-S >5	139 (18%)	30%
Eggener	Eggener <2.5%	302 (39%)	1%
	Eggener 2.5-5.0%	264 (34%)	5%
	Eggener 5-15%	133 (17%)	15%
	Eggener 15-25%	48 (6%)	31%
	Eggener >25%	27 (4%)	59%

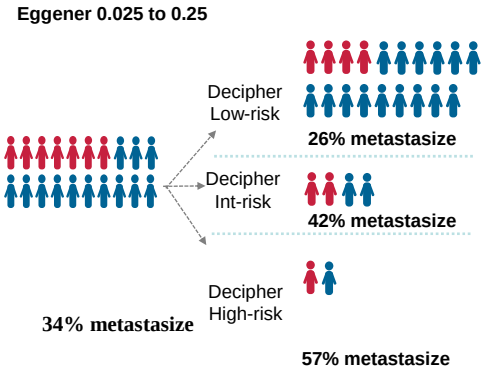
- Natural history cohort of men undergoing RP at JHH



Ross et al Eur Urol 2015

Adjuvant Radiation after RP – 2 Step Process Decipher if Eggener >2.5% <25%

- Strong consideration to treat with adjuvant XRT vs early salvage if Decipher >0.45, particularly if <70 years old



Summary

- Our molecular understanding of prostate cancer is increasing exponentially
  - PTEN loss appears to be an early and important event for the development of aggressive prostate cancer
- Tests have been developed that may help treatment decisions
- Current evidence suggests tests are prognostic and selective use of these tests may be beneficial
- Tests should be put into context of current practice
  - Multiparametric MRI (mpMRI) / fusion biopsy
  - PHI / PCA3 / 4Kscore
  - Use of nomograms for decision making
- Head-to-head comparisons are needed to inform providers
- Largest impact will come in areas of greatest decision uncertainty
  - RP vs RT
  - RT +/- ADT
  - Early use of docetaxel

Thanks and Questions