The future of Biomarkers in Prostate and Bladder Cancer

Prof Wim Van Criekinge, CSO

9th August 2015, Colorado Springs

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MDxHealth

Overview

Epigenetics

- Introduction
- DNA Methylation & Oncology

MDxHealth

- *NEXT-GEN*eration (Epi)genetic biomarkers
- Prostate Epigenetic Biomarkers
  - confirmMDx & Beyond
- Bladder Epigenetic Biomarkers

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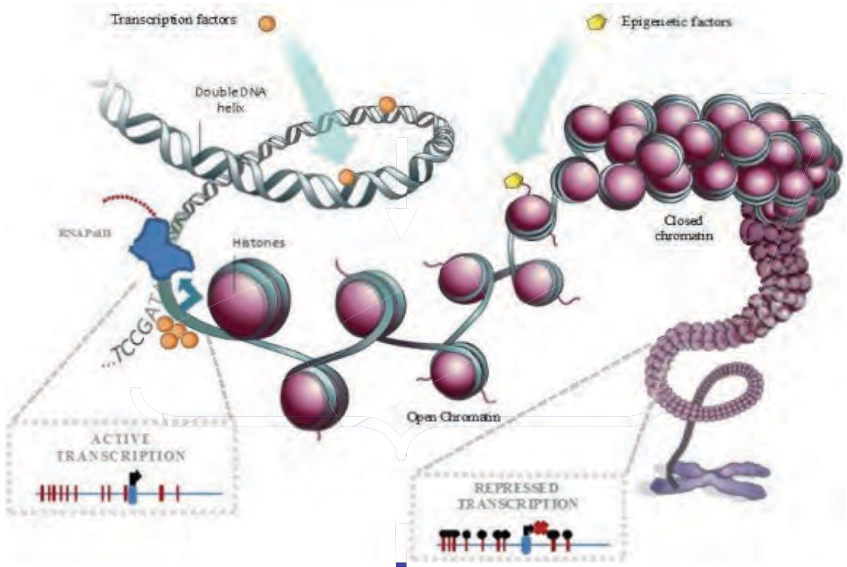
Defining Epigenetics

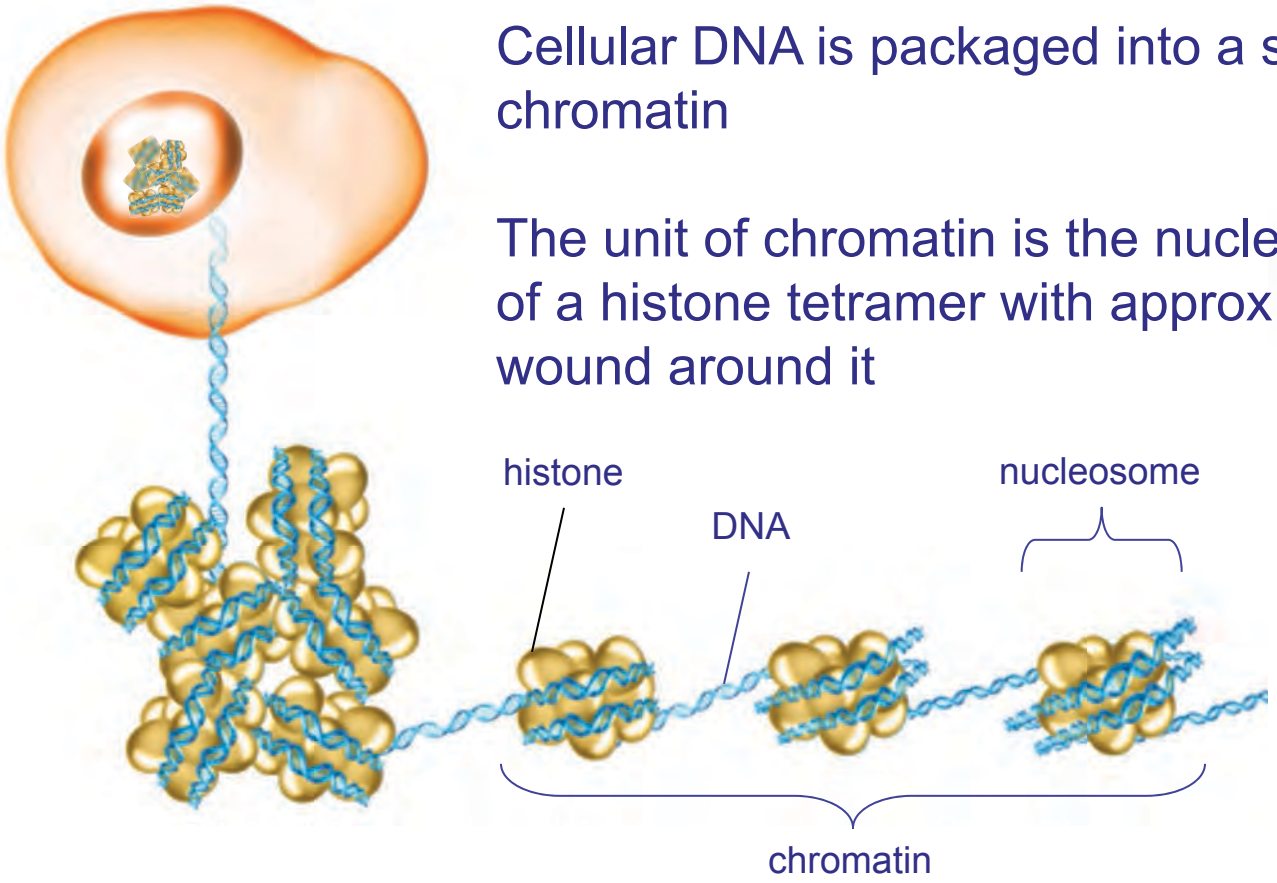


Gene Expression

Phenotype

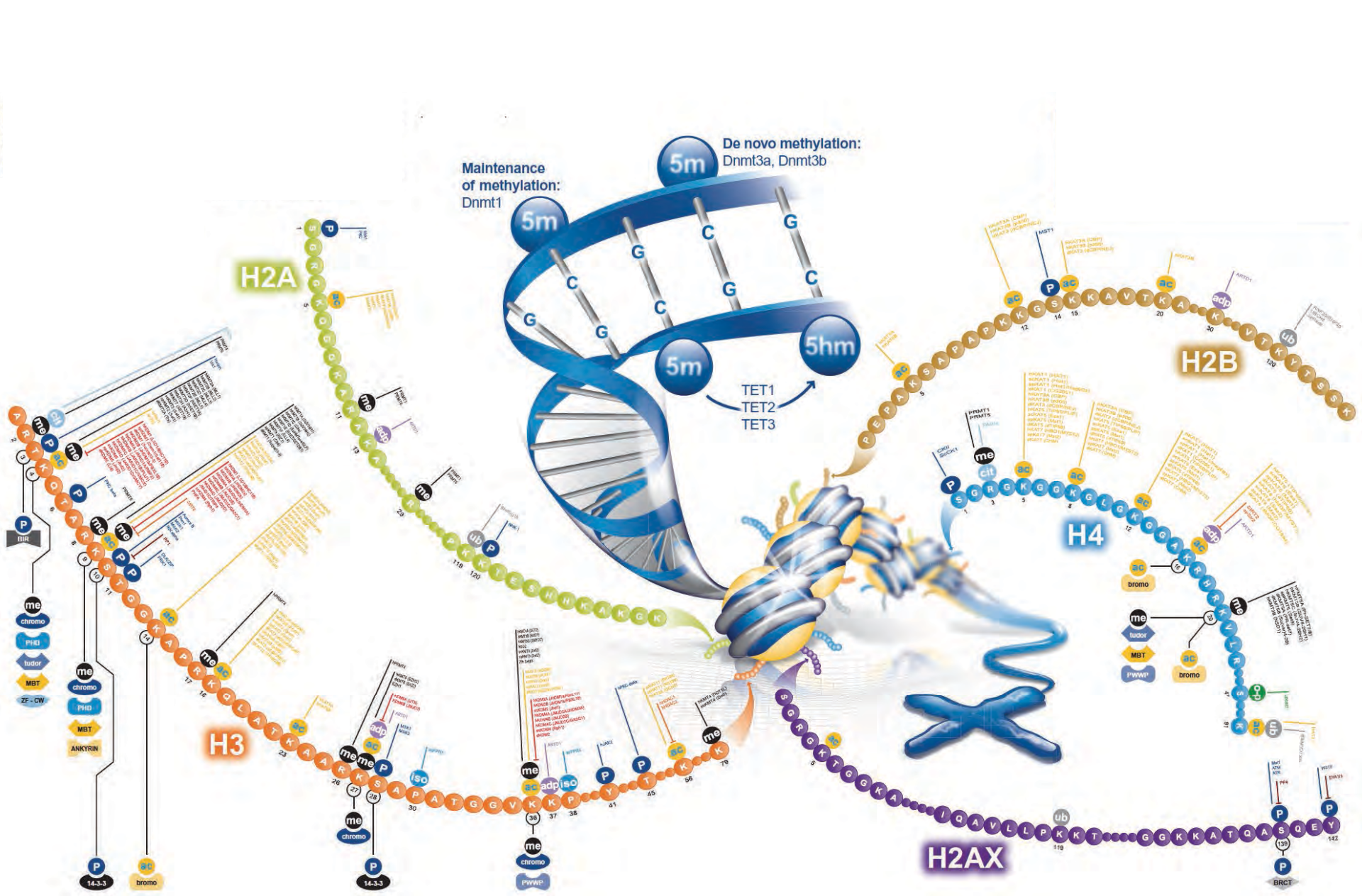
- Reversible changes in gene expression/function without changes in DNA sequence
- Can be inherited from precursor cells
- Allows to (re)use one genomes for different purposes
- Allows to integrate intrinsic with environmental signals

Chromatin,
a Key Component of Epigenetic Regulation



Cellular DNA is packaged into a structure called chromatin

The unit of chromatin is the nucleosome, a complex of a histone tetramer with approx. 147 bp of DNA wound around it


Maintenance of methylation: Dnmt1
De novo methylation: Dnmt3a, Dnmt3b
5m
5m
5m
5hm
TET1
TET2
TET3
H2A
H2B
H3
H4
H2AX

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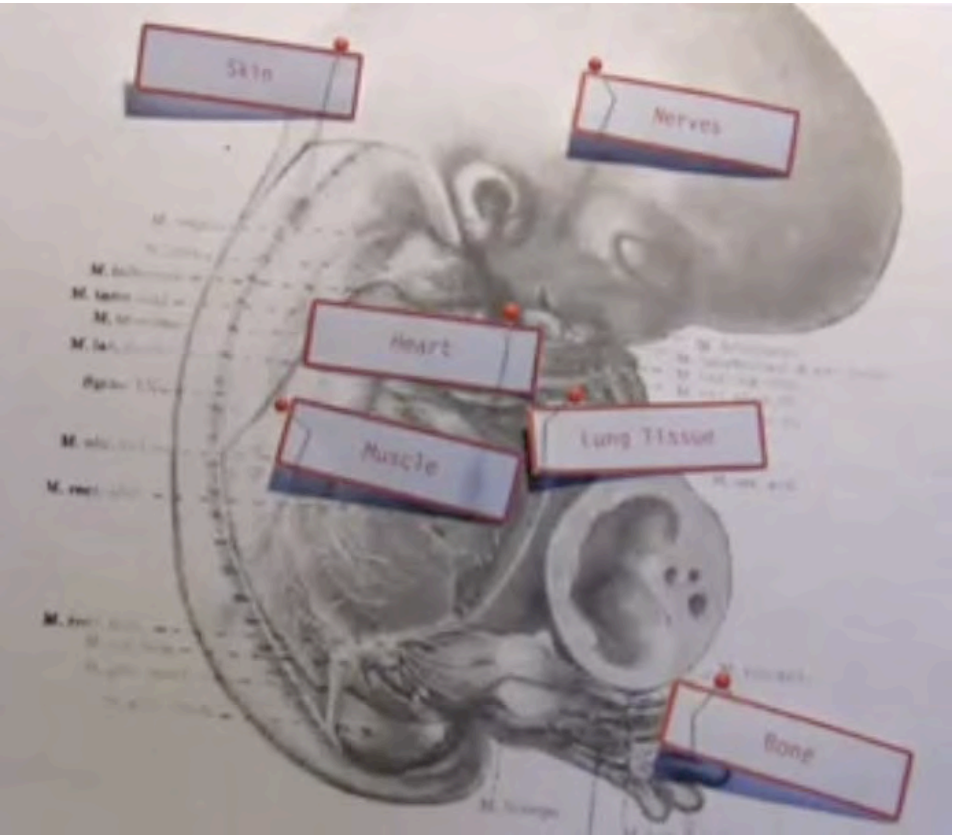
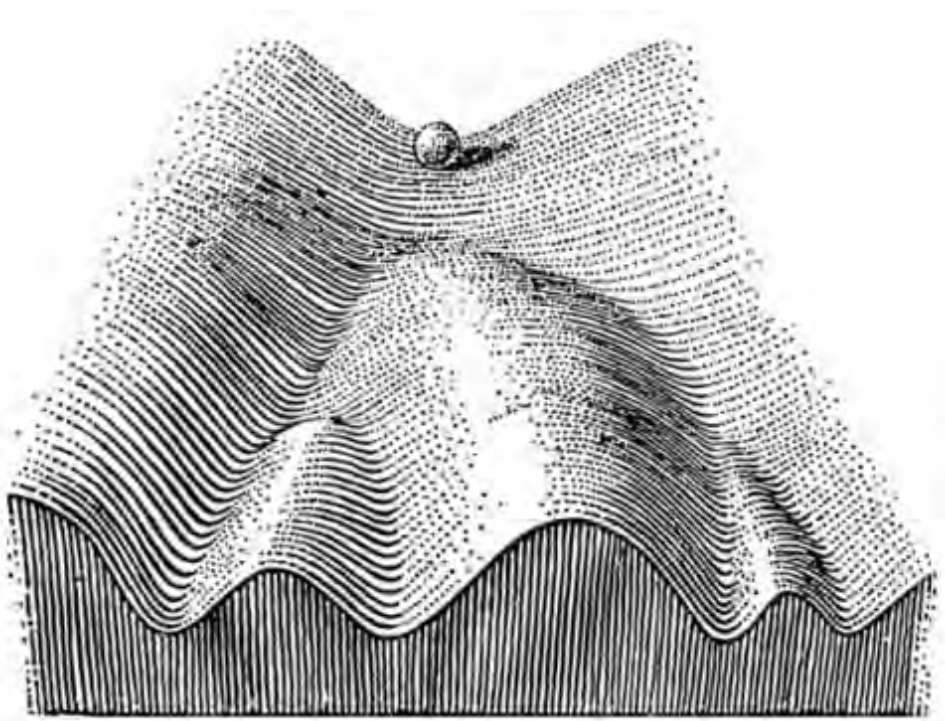
Evolutionary Perspective

epigenetic (meta)information = stem cells





Waddington's Epigenetic Landscape

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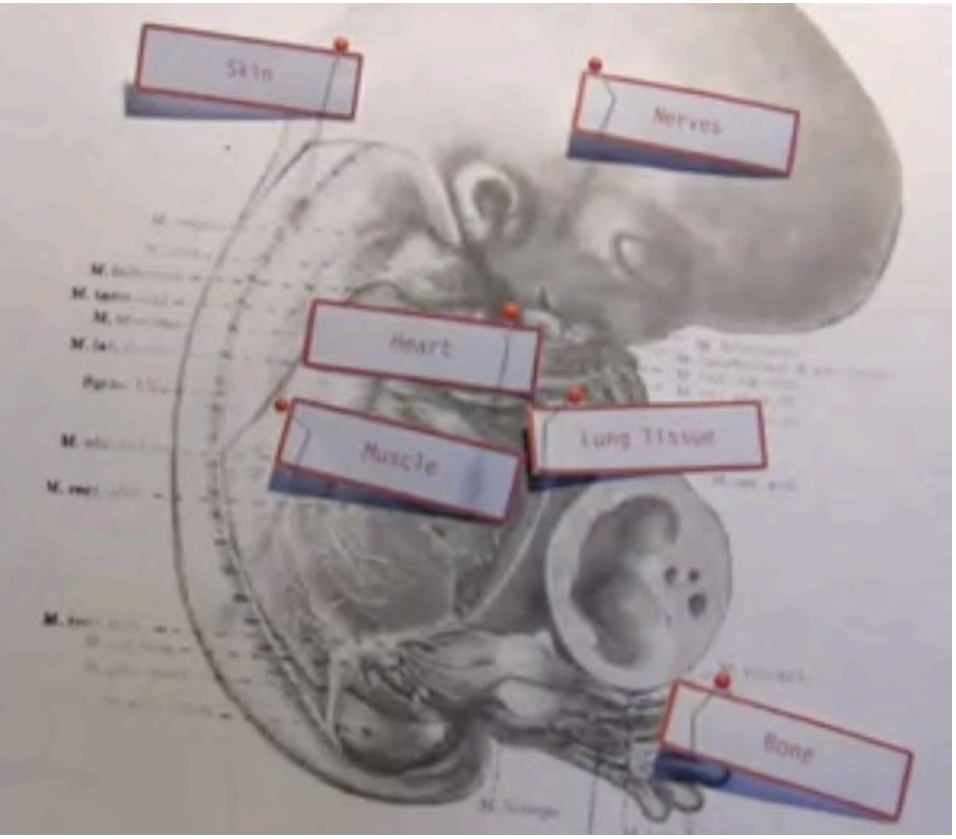
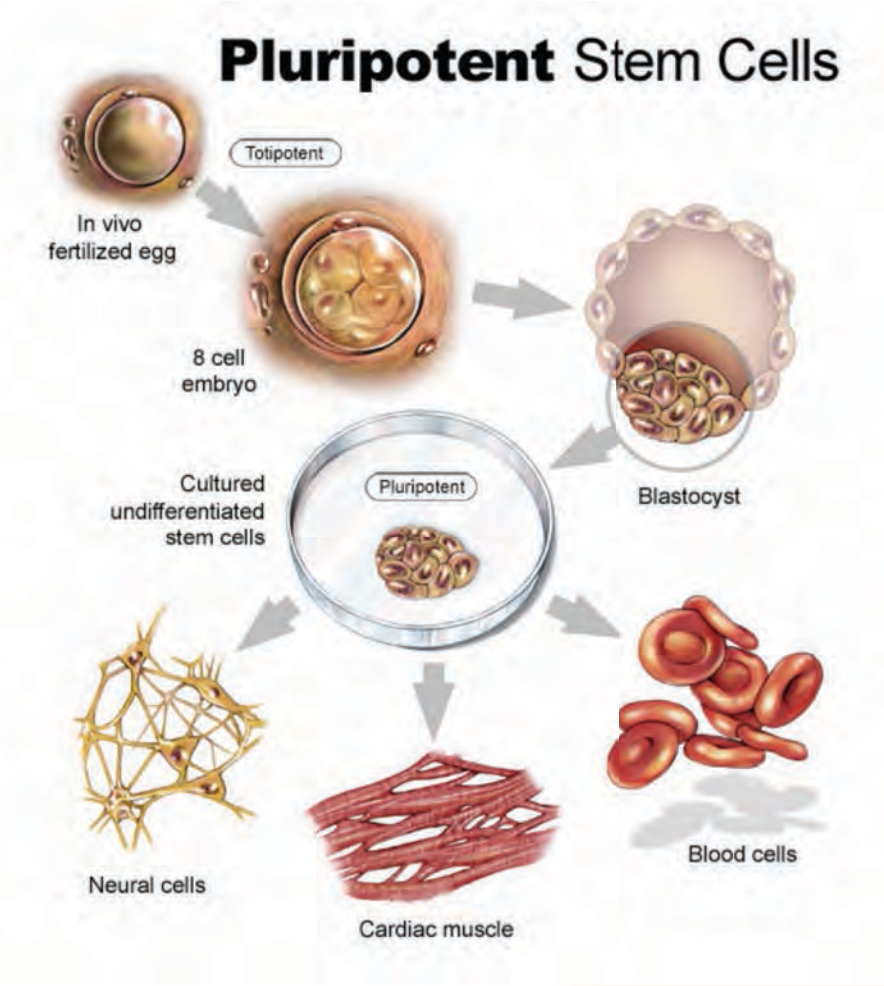
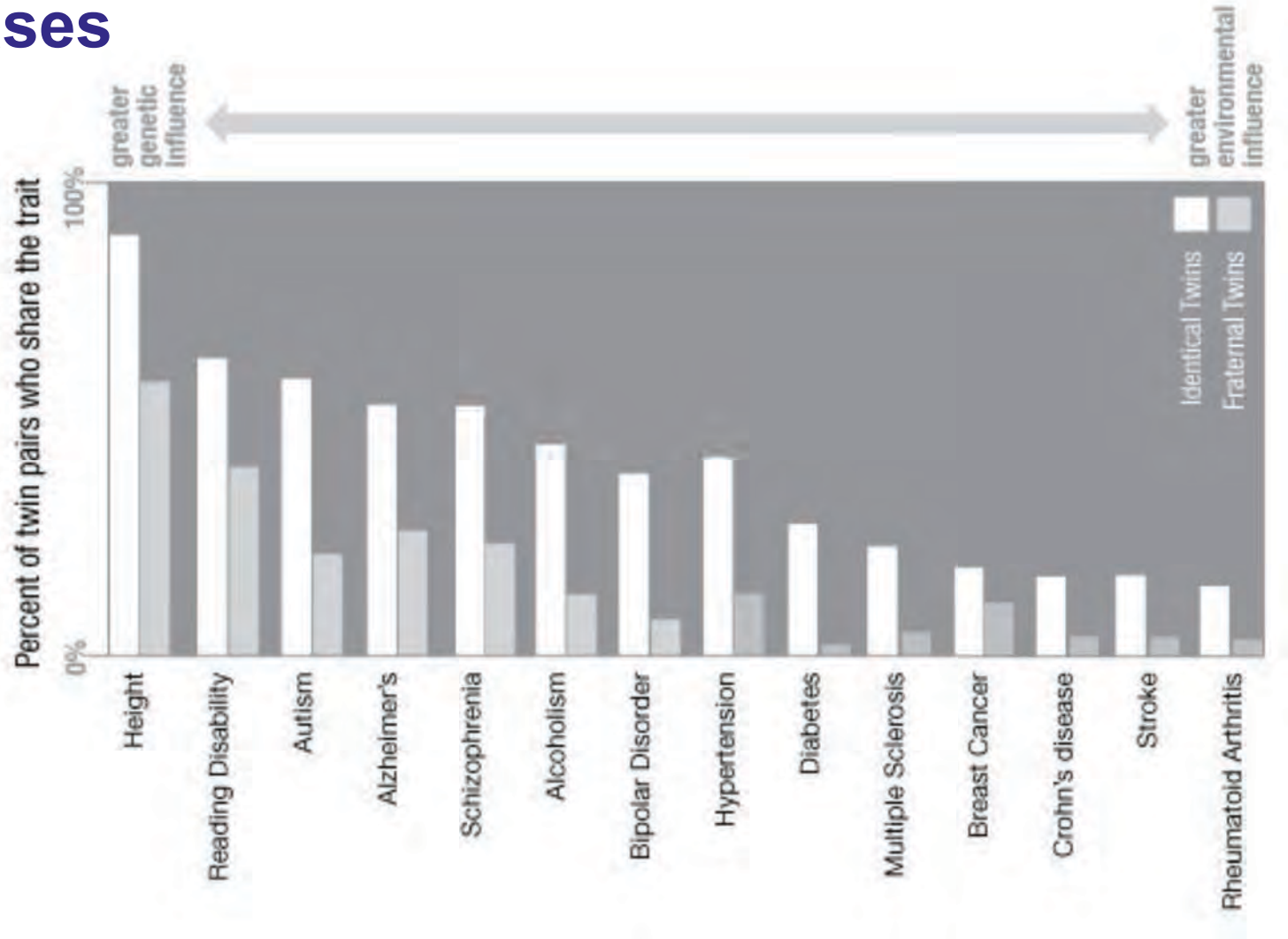
Evolutionary Perspective

epigenetic (meta)information = stem cells





Epigenetics driving etiology of many human diseases



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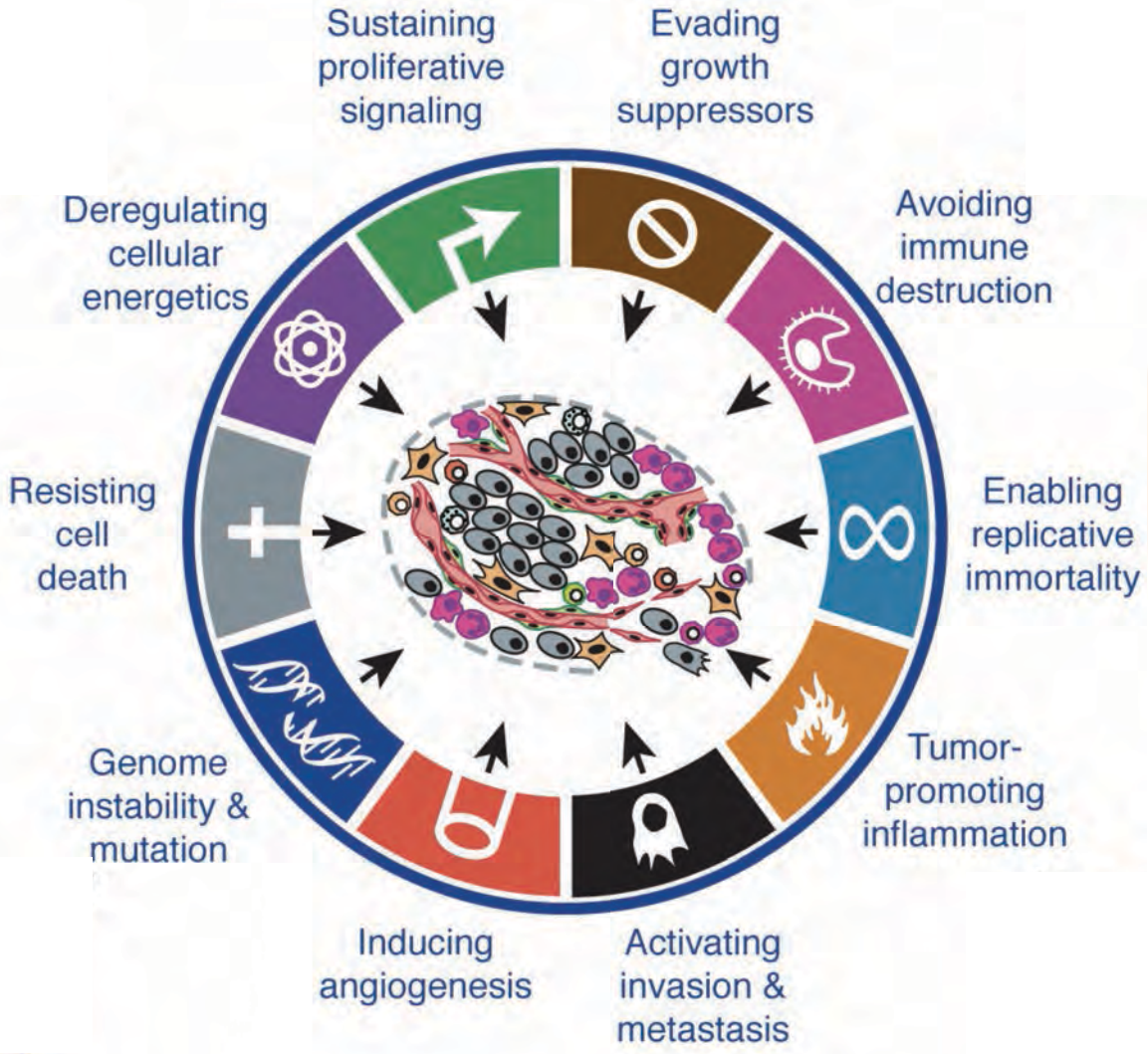
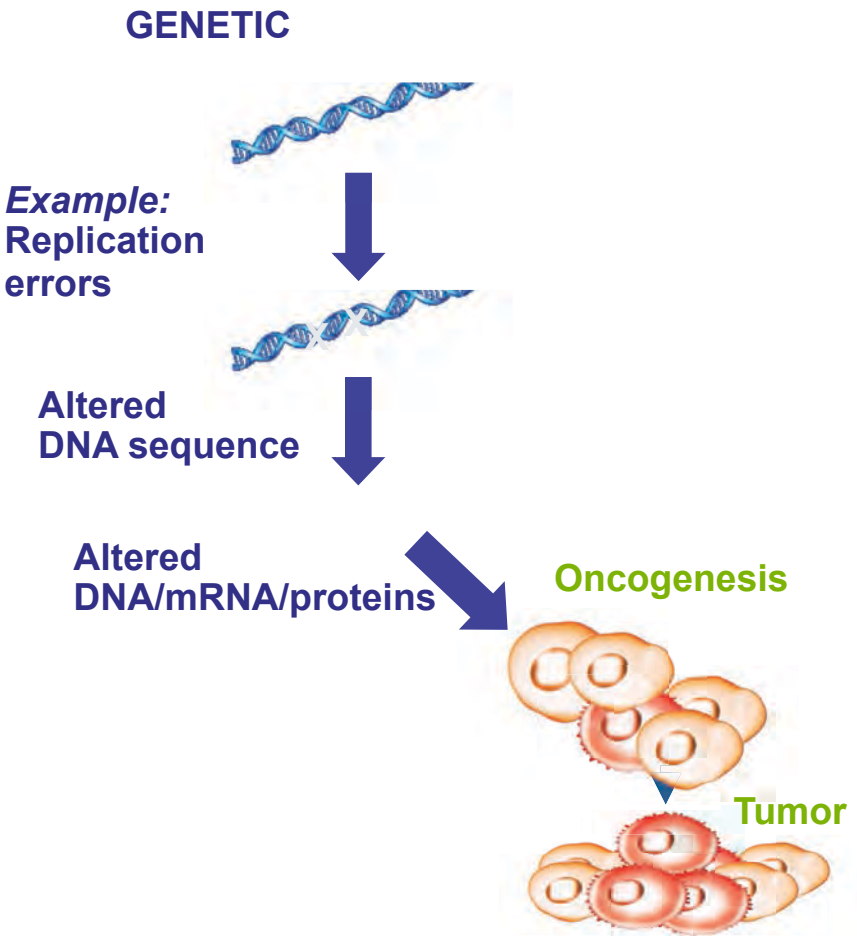
Cancer is impairing key pathways/modules/networks

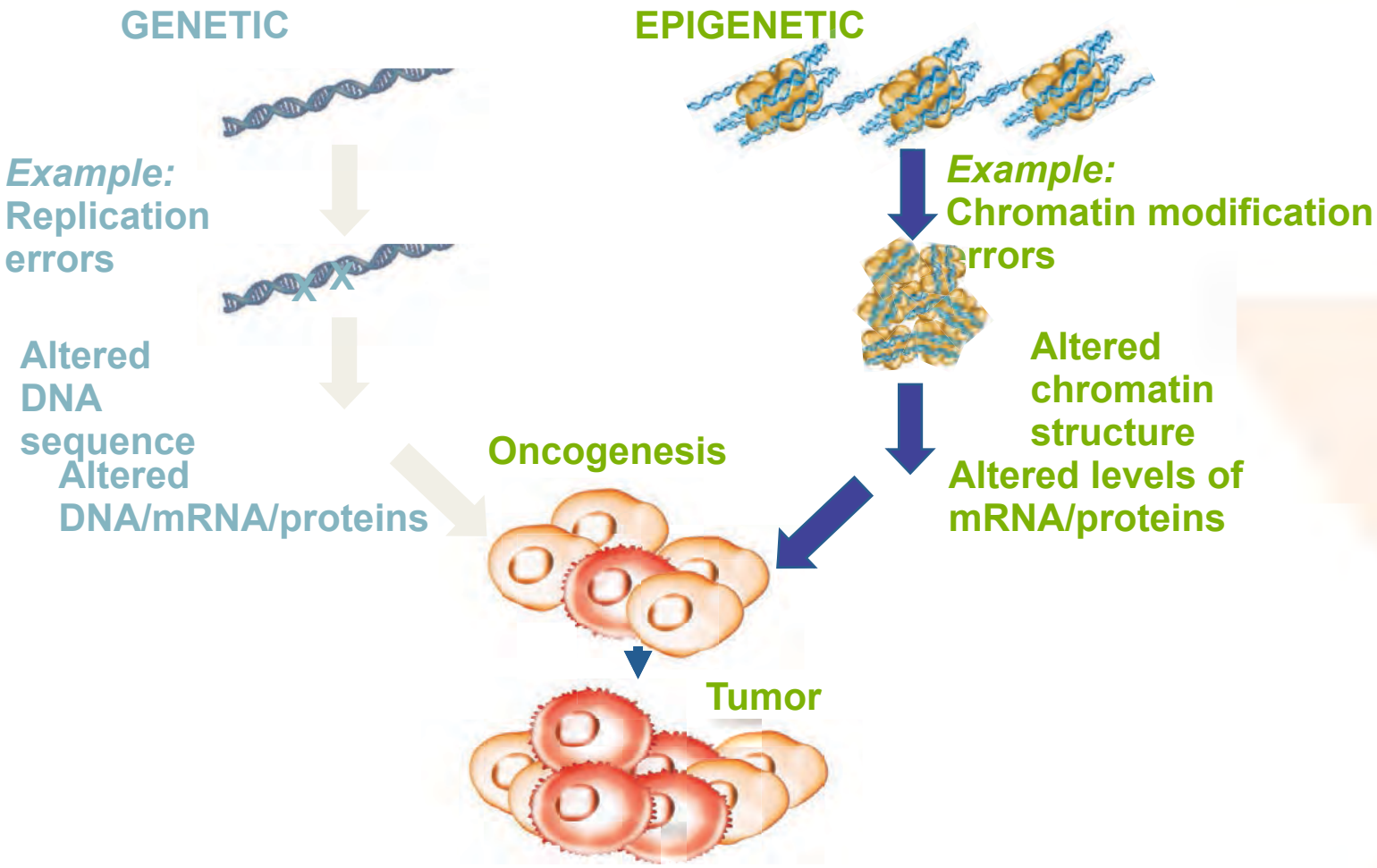


Historically, Cancer Was Considered to be Driven Mostly by Genetic Changes

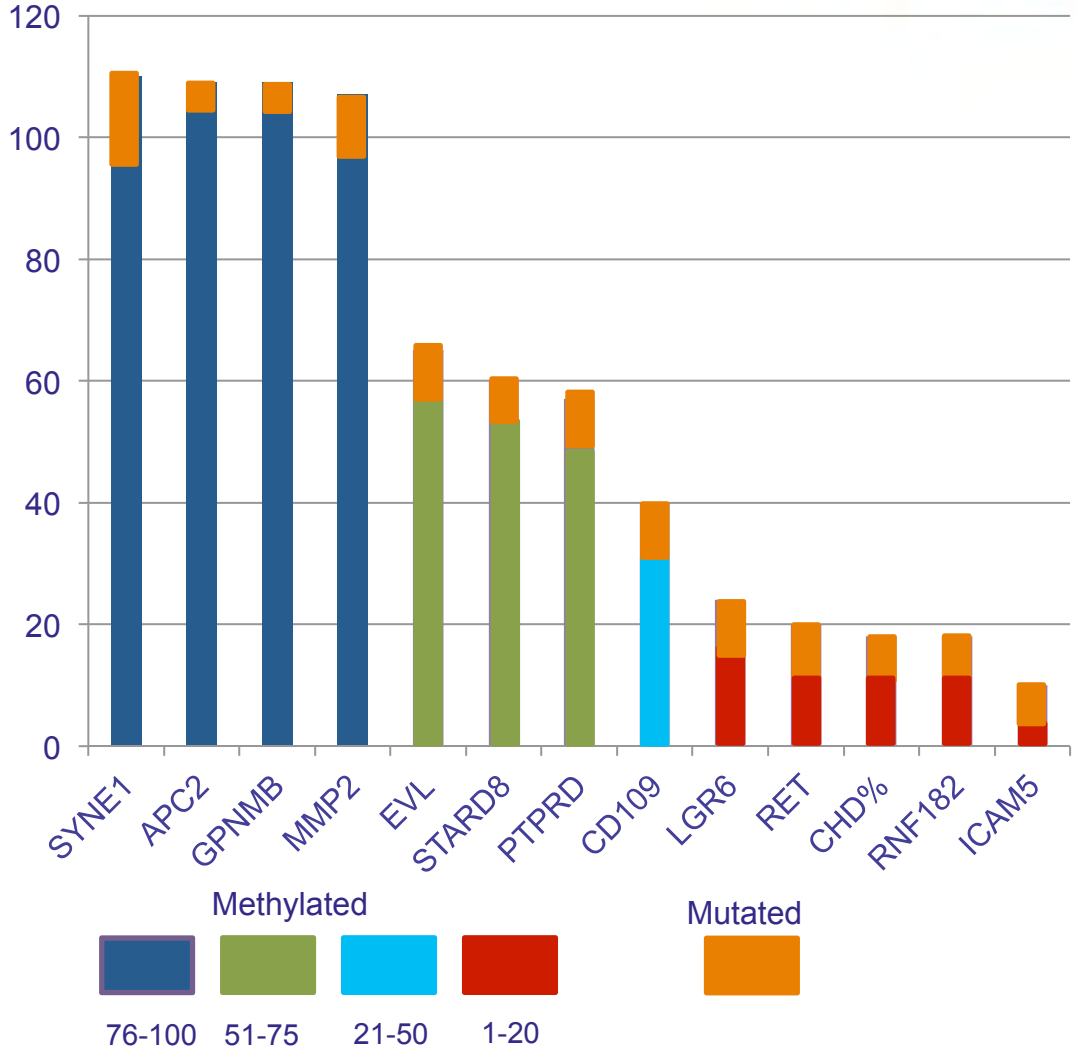


- Mutations in p53
- Activating mutations in RAS
- Mutations or amplifications of the HER-2 gene
- Chromosomal translocations in myeloid cells and the generation of the BCR-ABL fusion protein

Past decade has shown that Epigenetic Changes are Important in Causing Cancer



Example of Methylation vs Mutation: Colon & Breast Cancer



Source: Schuebel et al 2007

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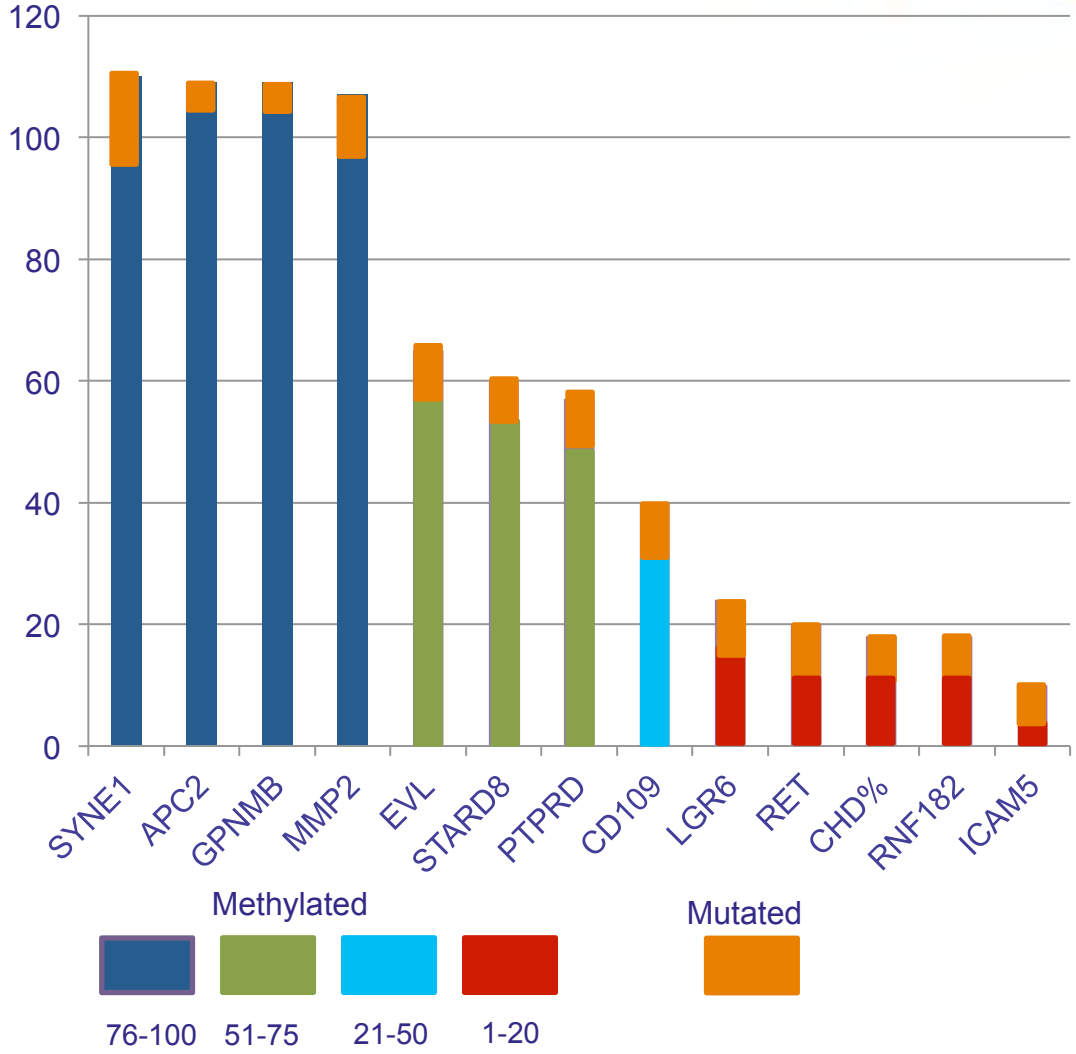

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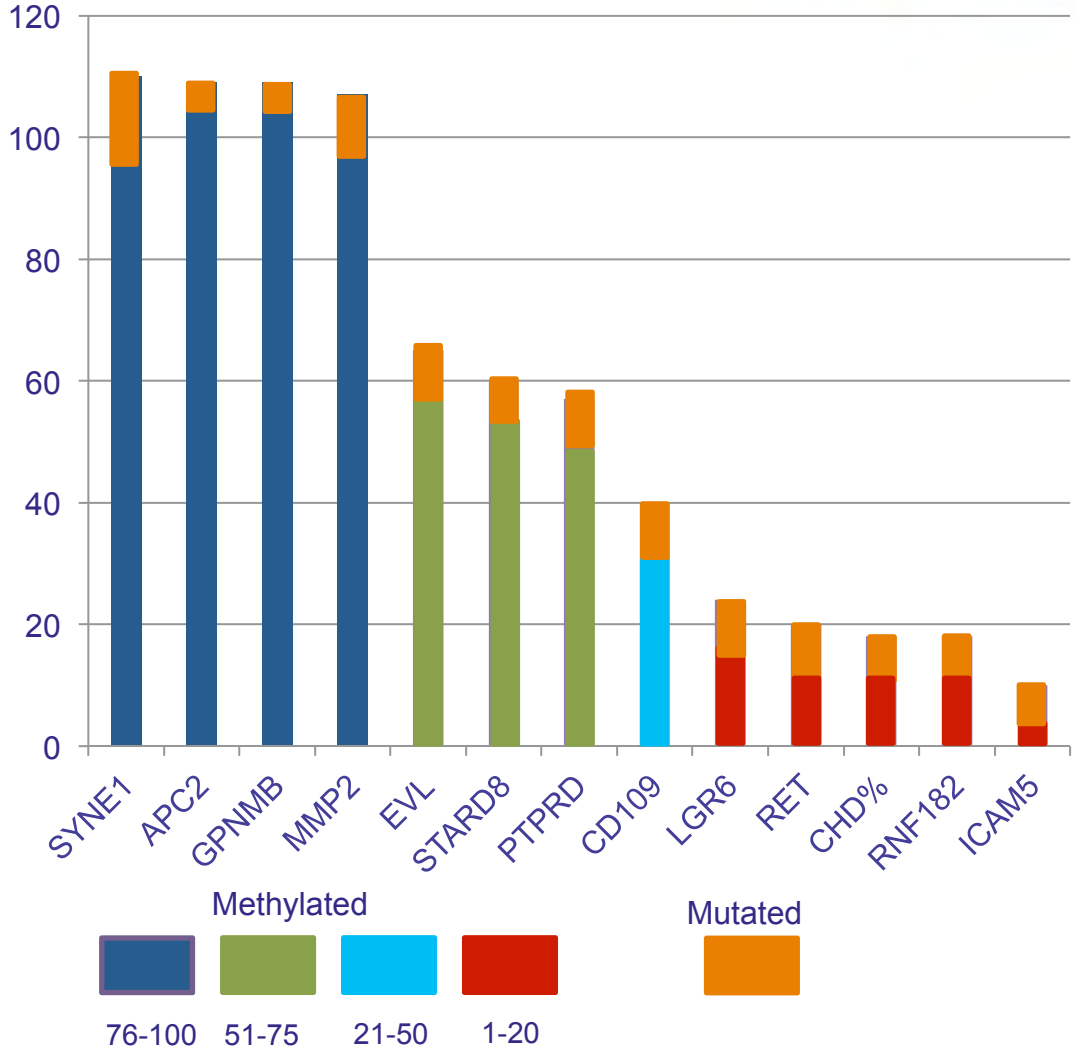

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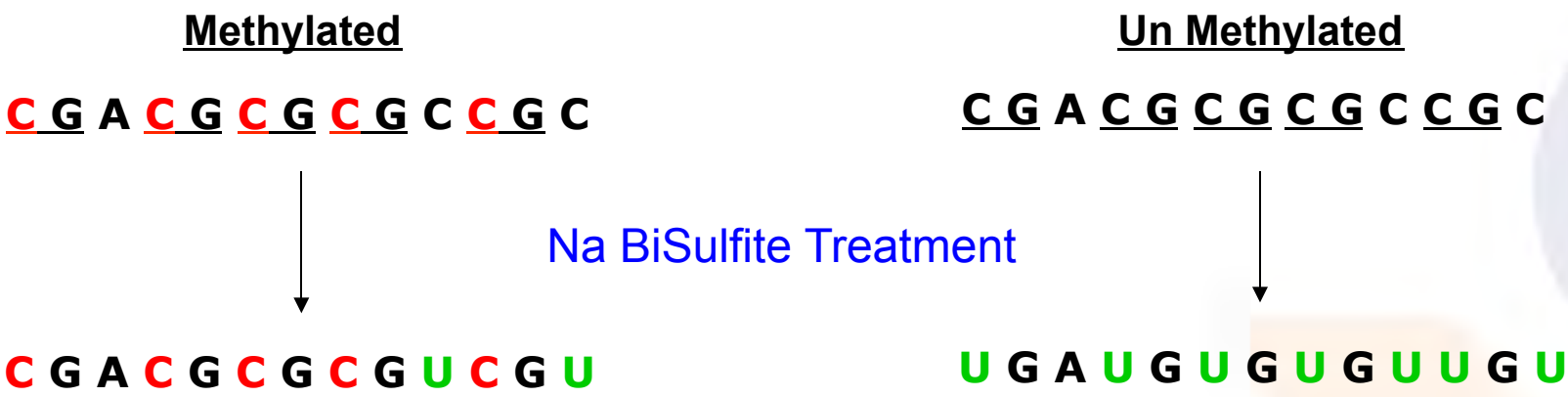
Methylation Specific PCR (MSP)

Step I: Bisulfite Treatment



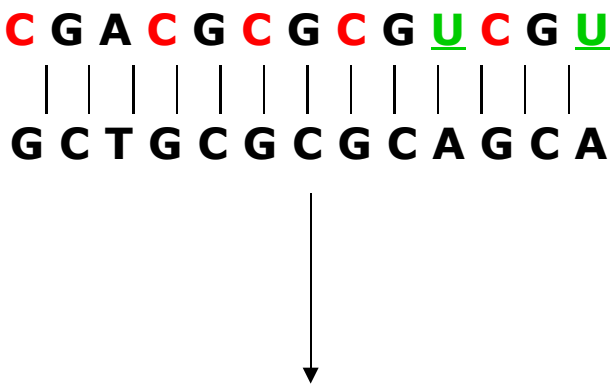
Methylation Specific PCR (MSP)

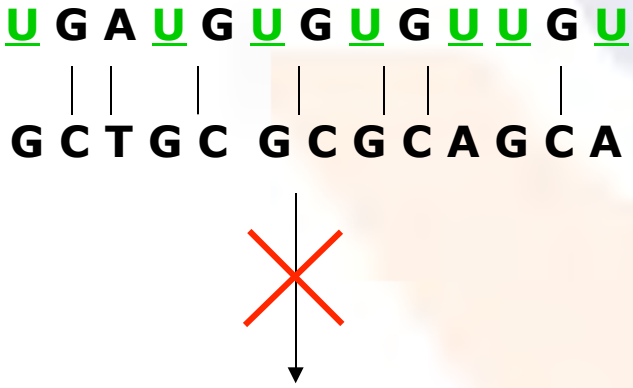
Step II: Amplification and Detection

Methylated

Un Methylated

PCR
Using methylation specific primers





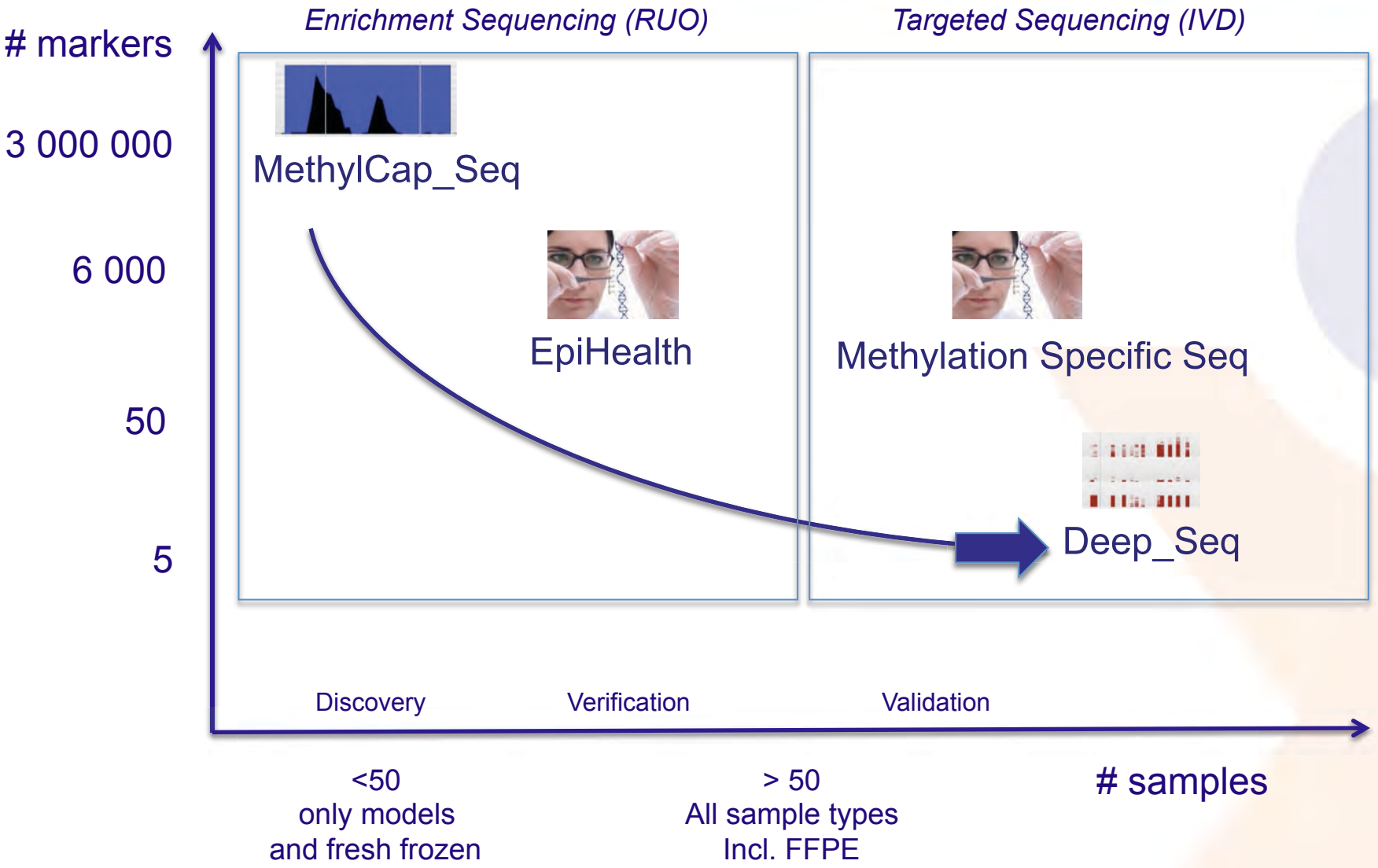
PCR Product

No PCR Product

DNA Methylation
compared to competing technologies

- Frequency of methylation in different cancer tissues is attractive
- Methylation is biologically the most efficient way to shutdown gene
- A small number of biomarkers provides clinically relevant information
- Methylation is highly stable especially relative to mRNA and proteins
- Tumor cell specific methylation patterns detectable in a background of normal cells (higher sensitivity)

Next Generation Epigenetic Profiling



Next Generation (Epi)genetic Profiling

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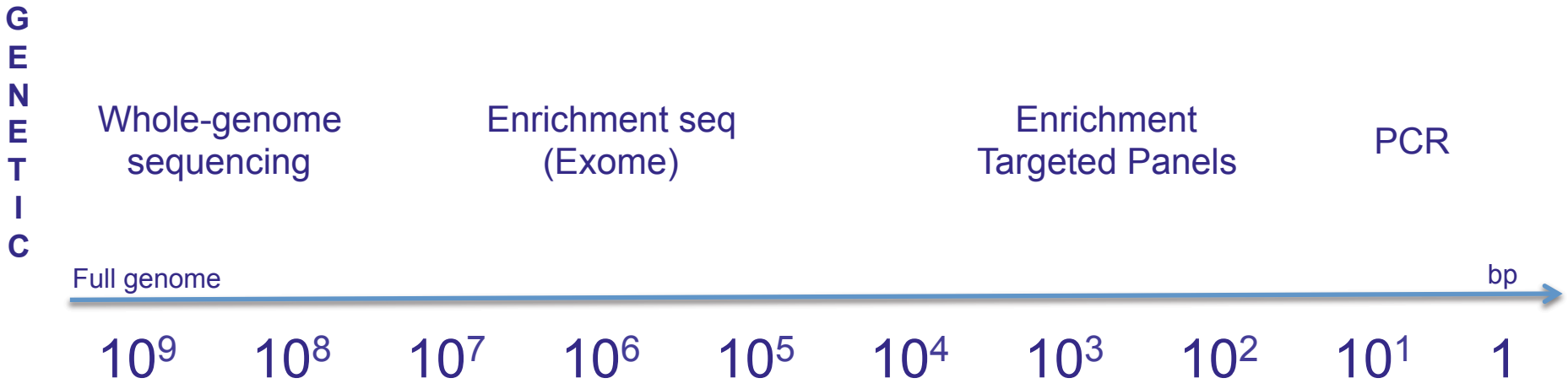
Next Generation (Epi)genetic Profiling



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Next Generation (Epi)genetic Profiling

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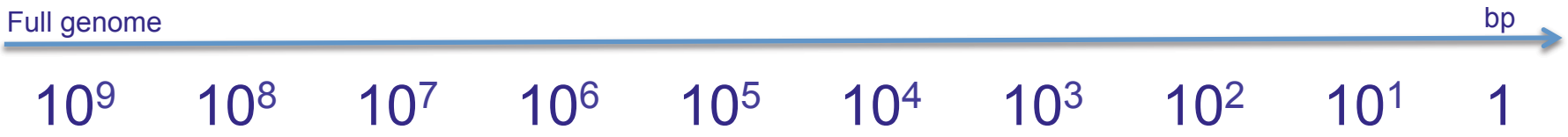
EPI	Whole-genome Bisulphite seq	Enrichment seq (MBD, RRBS)	Probes (450-27K)	Enrichment Targeted Panels	MSP
GENETIC	Whole-genome sequencing	Enrichment seq (Exome)		Enrichment Targeted Panels	PCR



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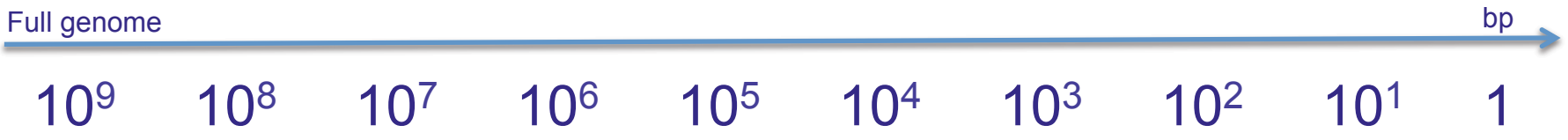

RUO sequencing

Clinical PCR

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Next Generation (Epi)genetic Profiling

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EPI	Whole-genome Bisulphite seq	Enrichment seq (MBD, RRBS)	Probes (450-27K)	Enrichment Targeted Panels	MSP
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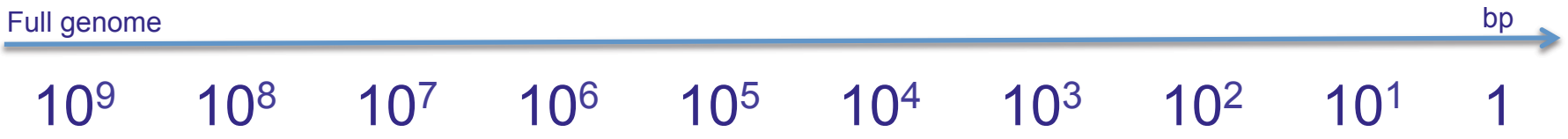

RUO sequencing

Clinical PCR

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Next Generation (Epi)genetic Profiling

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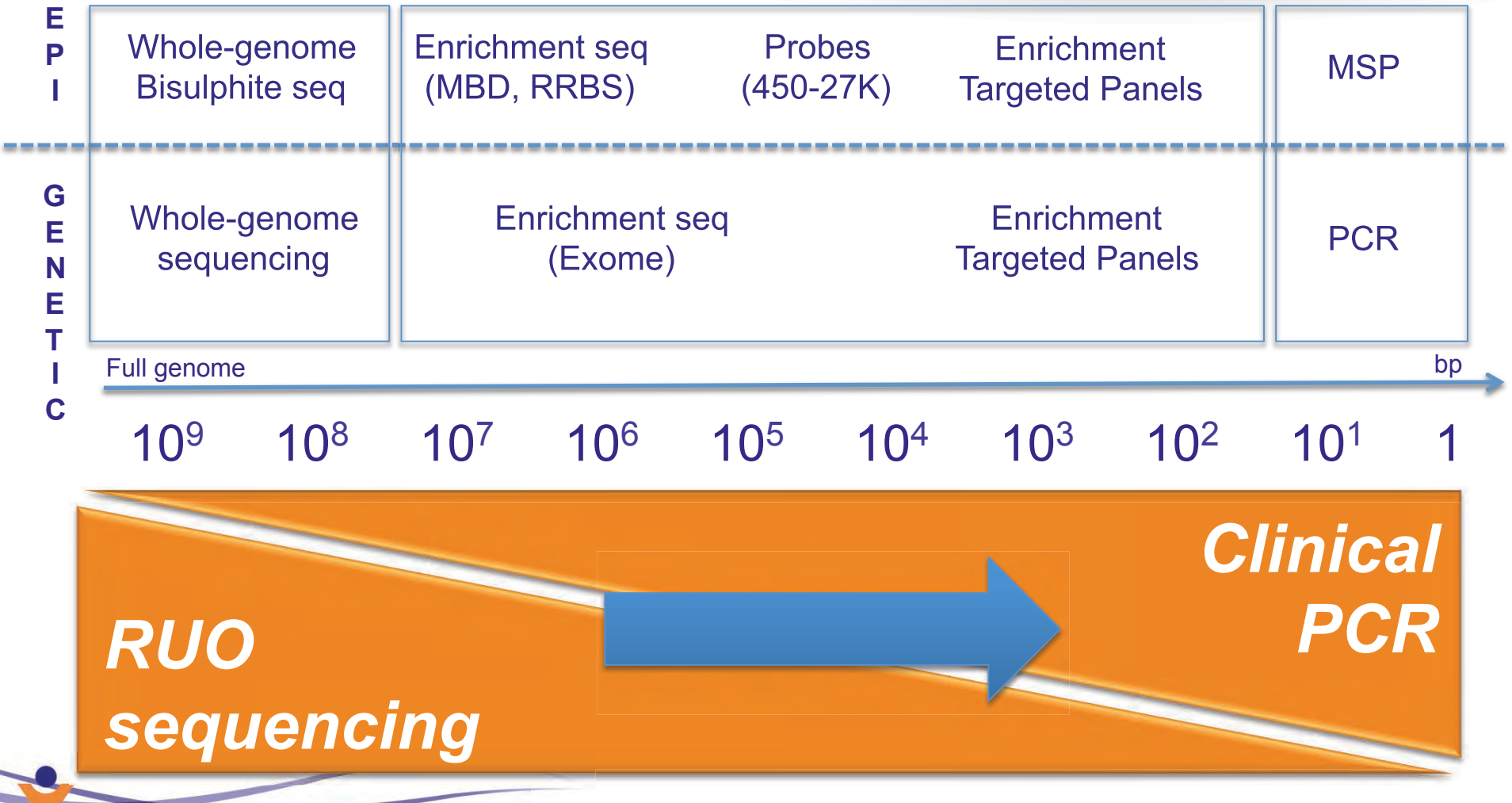

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ConfirmMDx Background

Performance of ConfirmMDx genes and methylation technology:

- Reported in over 45+ peer-reviewed scientific publications
- Over 5,000 subjects in studies
- Multinational, academic and community settings
- Prospective and retrospective multicenter, blinded studies

The Prostate 2011
Meta-Analysis Reports Clinical Utility of Epigenetic Assay

BJUI 2011
Assay shows 20% improvement in NPV over histopathology

American Health & Drug Benefits 2013
Reduces healthcare spending by >$588 per patient

Journal of Urology 2013
Multi-center clinical validation study demonstrates 90% NPV

American Health & Drug Benefits 2014
10-fold reduction in repeat biopsies in clinical utility study

Journal of Urology 2014
Multi-center, confirmatory validation study

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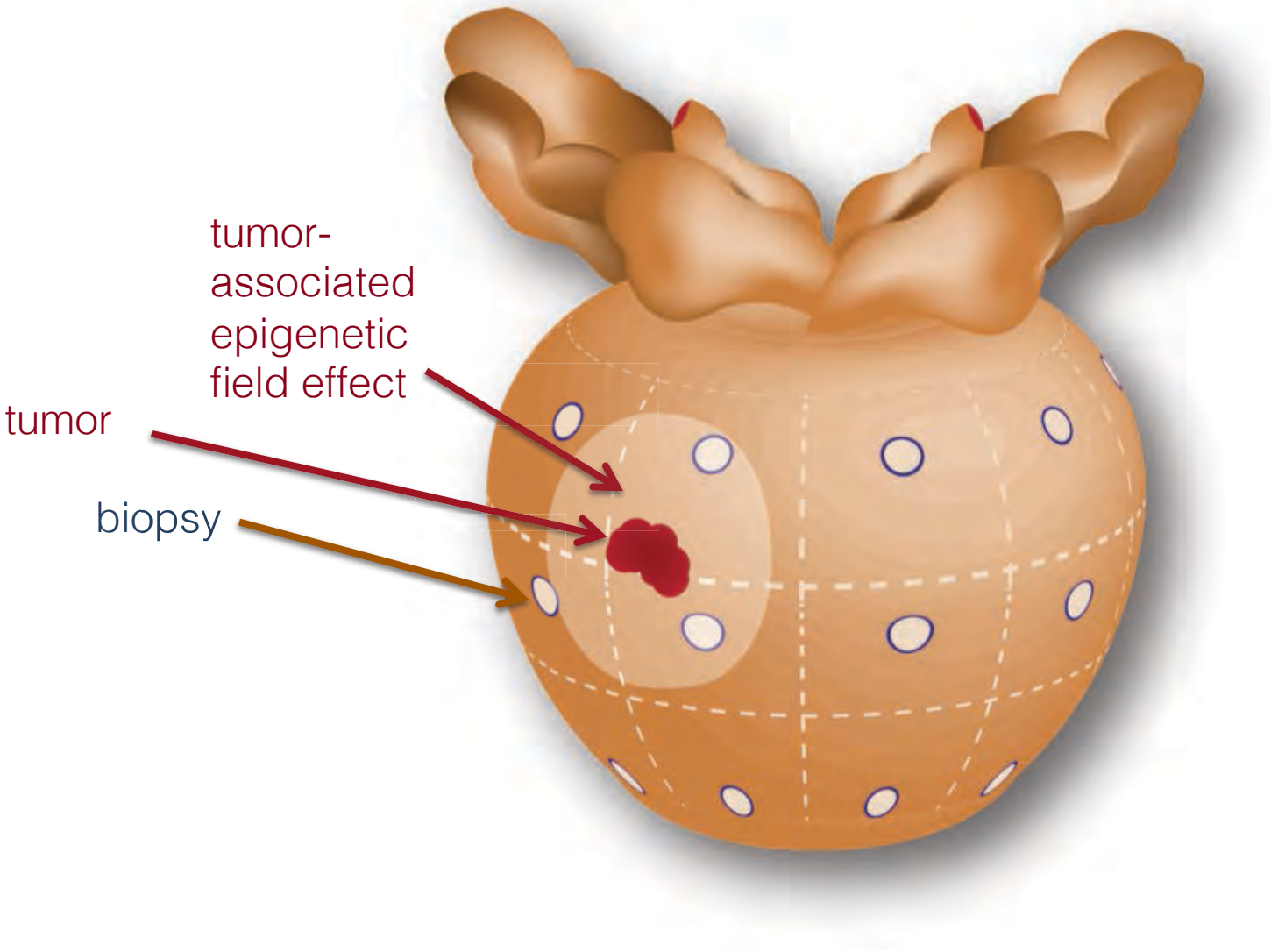

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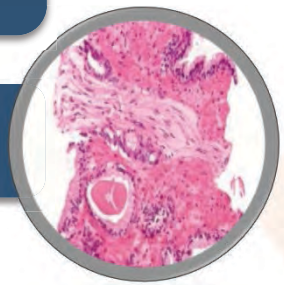
confirmMDx

Negative Biopsy
Persistent Risk Factors

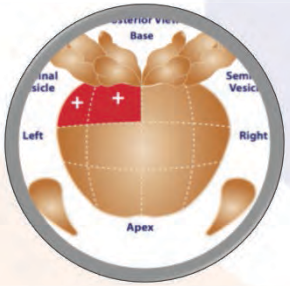
DNA Methylation

NPV ~96% Significant PCa
NPV ~90% All PCa

Avoid Repeat Biopsy

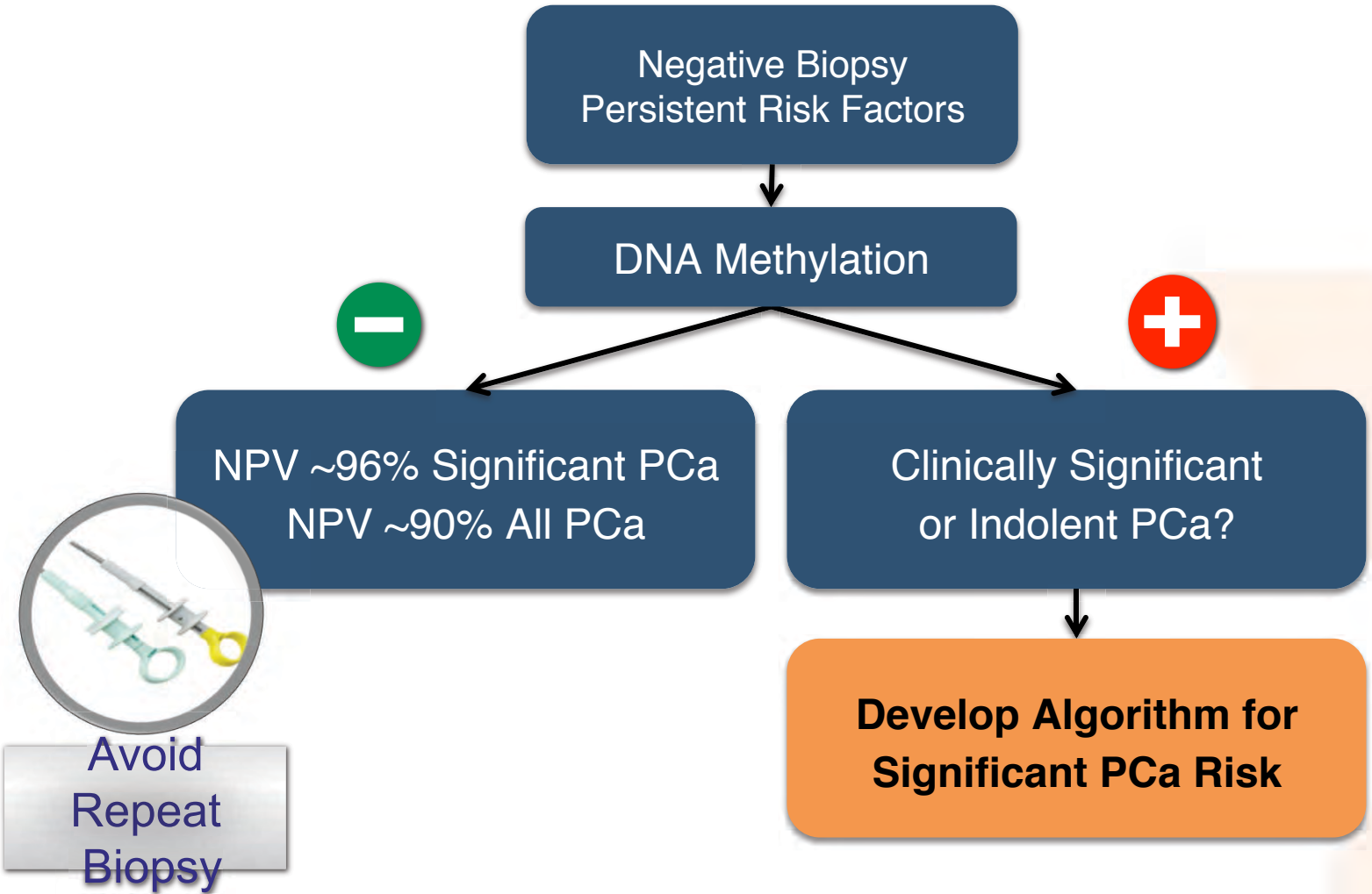


Residual Tissue



Prostate Mapping

… and beyond


Negative Biopsy
Persistent Risk Factors
DNA Methylation
NPV ~96% Significant PCa
NPV ~90% All PCa
Clinically Significant
or Indolent PCa?
Develop Algorithm for
Significant PCa Risk
Avoid
Repeat
Biopsy

confirmMDx Risk Score

	Weighed Methylation Intensity		
Core	*APC*	*GSTP1*	*RASSF1*
1	**0.73**	**46.95**	**1.15**
2	0.00	**16.05**	0.00
3	0.00	0.00	0.00
4	0.00	**15.04**	0.00
5	0.00	0.00	0.00
6	0.00	0.00	0.00
7	0.00	0.00	0.00
8	0.00	0.00	0.00
9	0.00	0.00	0.00

sum
48.83
16.05
0.00
15.04
0.00
0.00
0.00
0.00
0.00

average

Methylation Intensity
8.88

- Age: 71
- Caucasian
- 1st Biopsy
- HGPIN

3/9 cores positives

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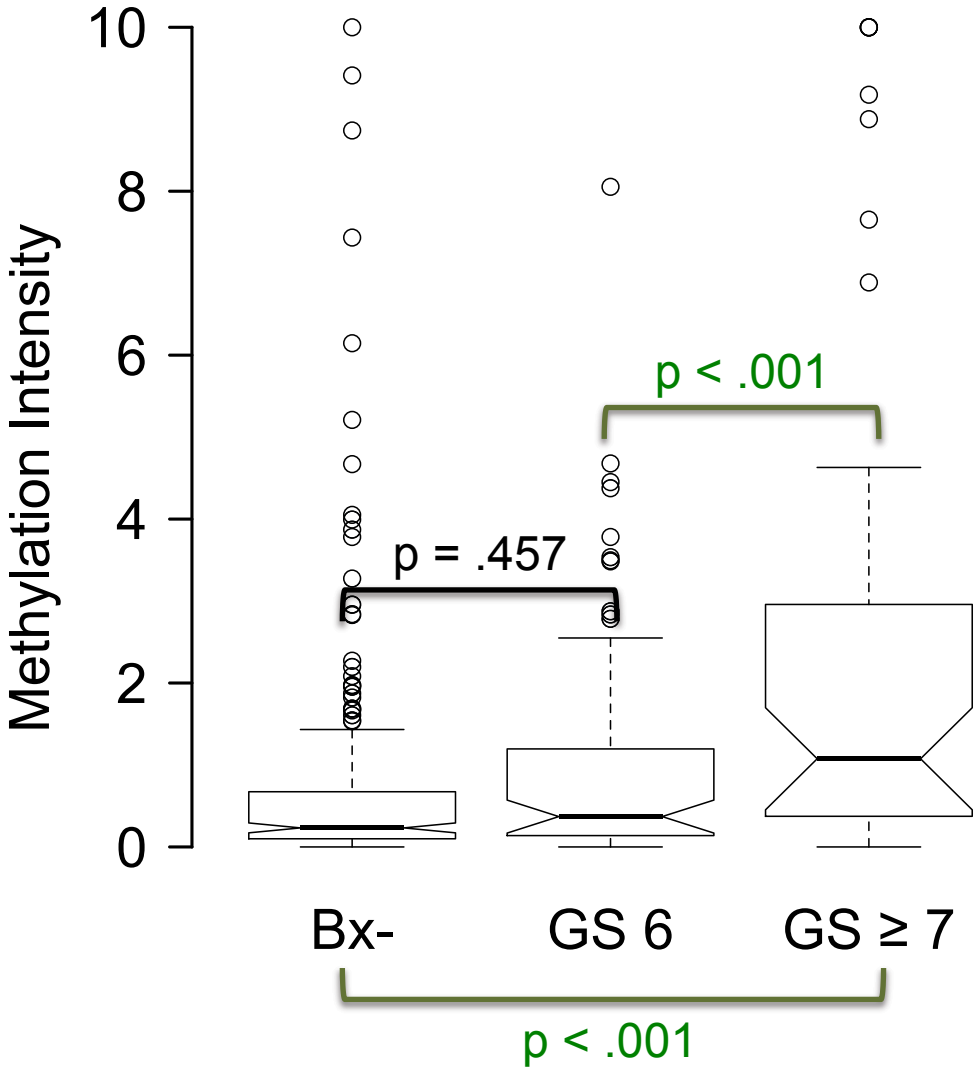
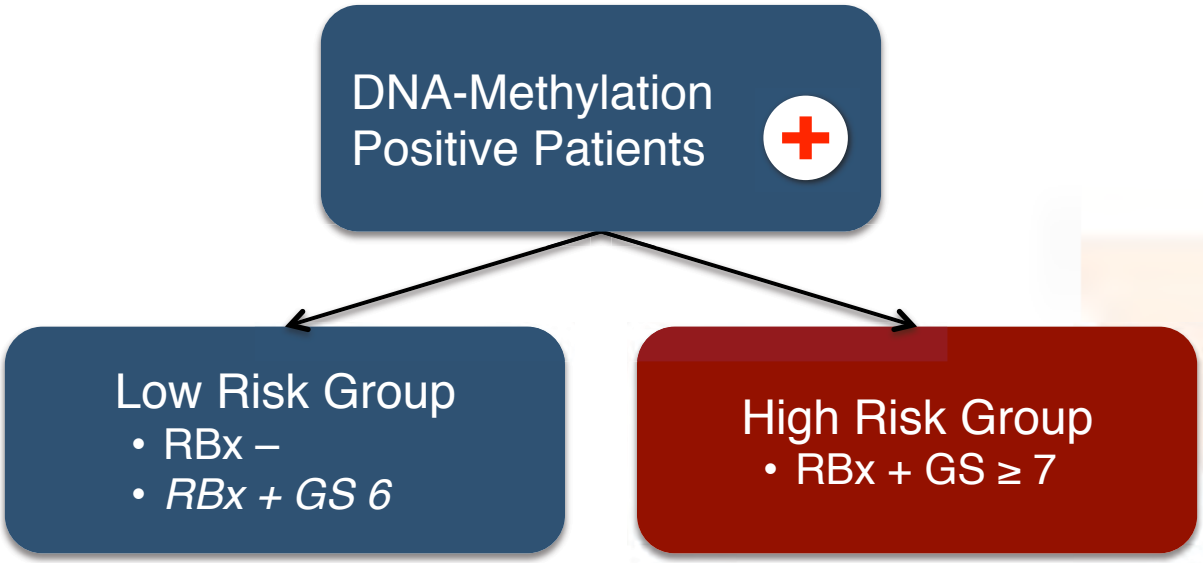
confirmMDx Risk Score



Epigenetic Health Index (EHI)
Risk Score to Further Stratify DNA-Methylation Positive Patients



Algorithm for Risk Stratification Using:

- Epigenetic Risk: Cores Positive and Methylation Intensity
- Clinical Risk Factors: Age, PSA, DRE, Histopathology

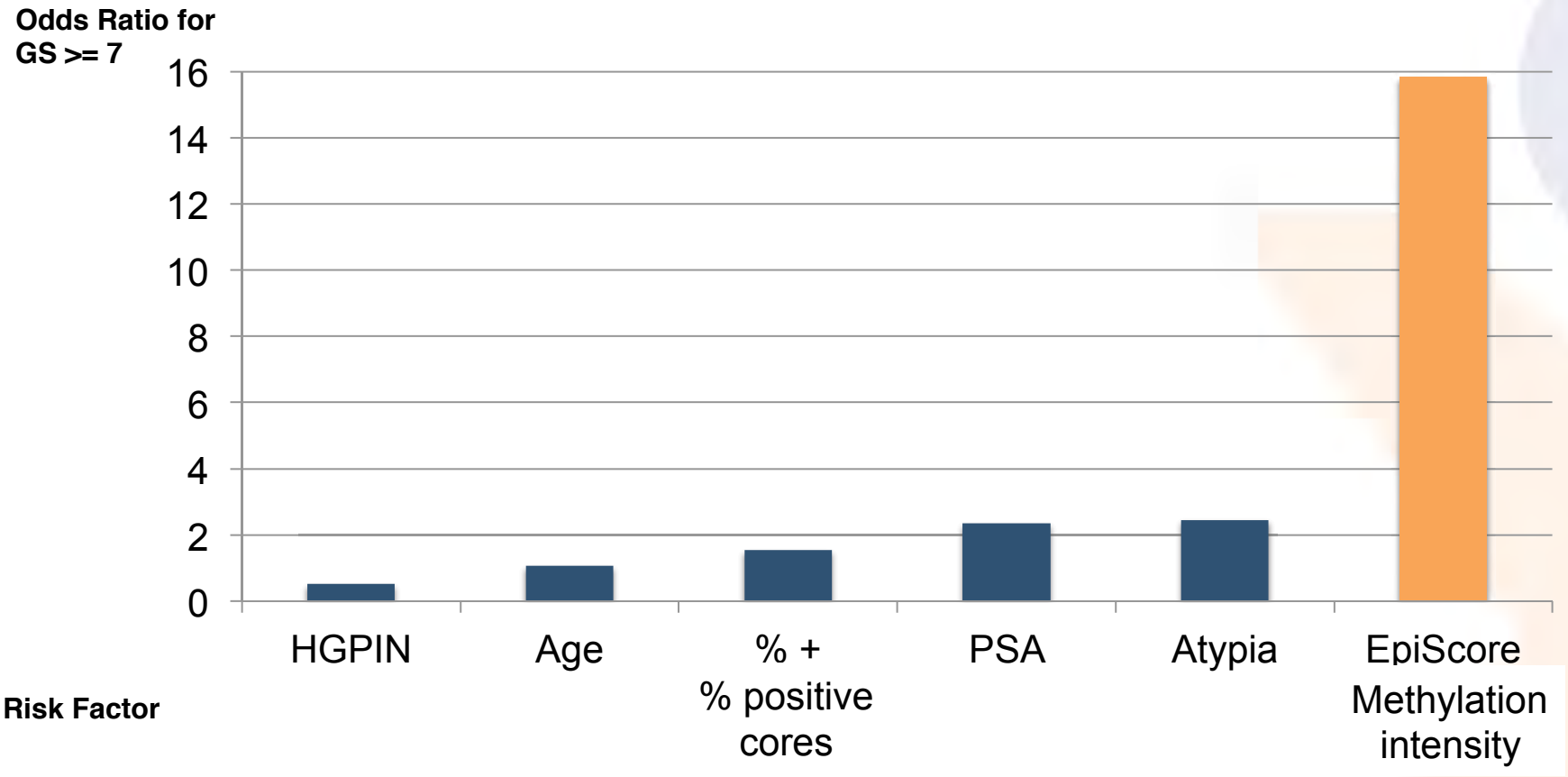
Epigenetic Health Index (EHI)

Risk Score Comparison

Parameters		NCCN	PCPTRC2	EHI
Demographic	Age			
	Race			
Clinical	Family history			
	Prior biopsy			
	DRE			
	Pathology of - index Bx			
	GS			
	PSA density			
Molecular	PSA			
	ConfirmMDx+ Bx Cores			
	Methylation Intensity			

http://deb.uthscsa.edu/URORiskCalc/

Epigenetic Health Index (EHI)
Multivariate Logistic Regression



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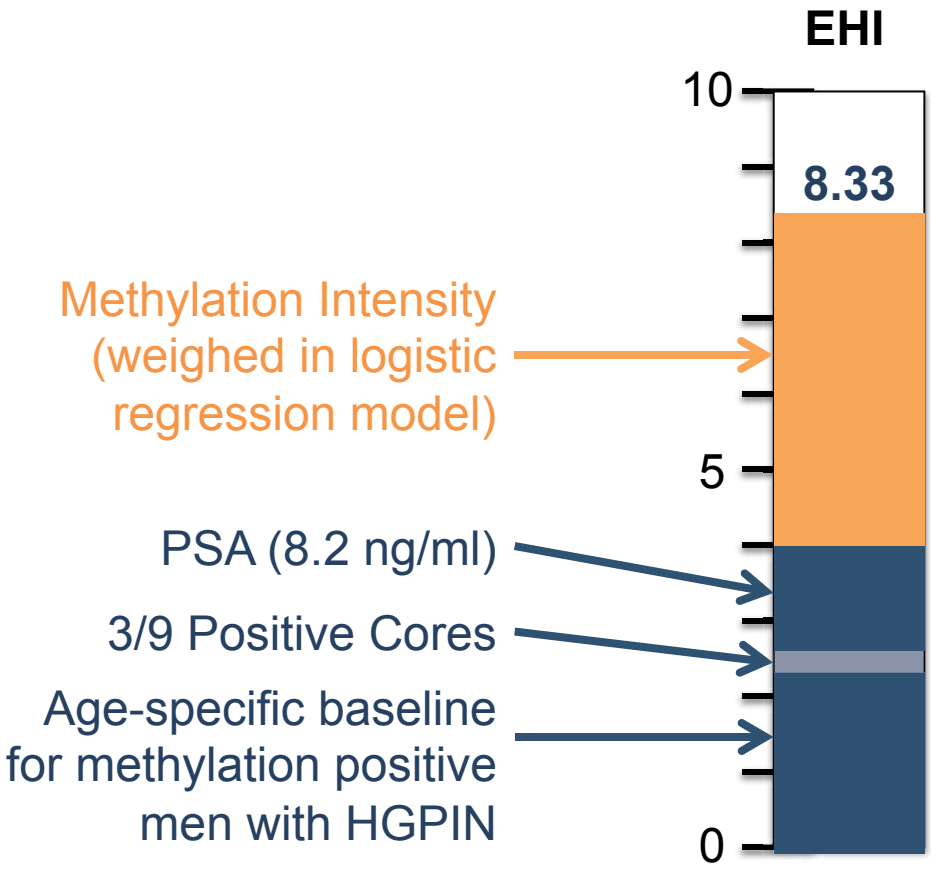
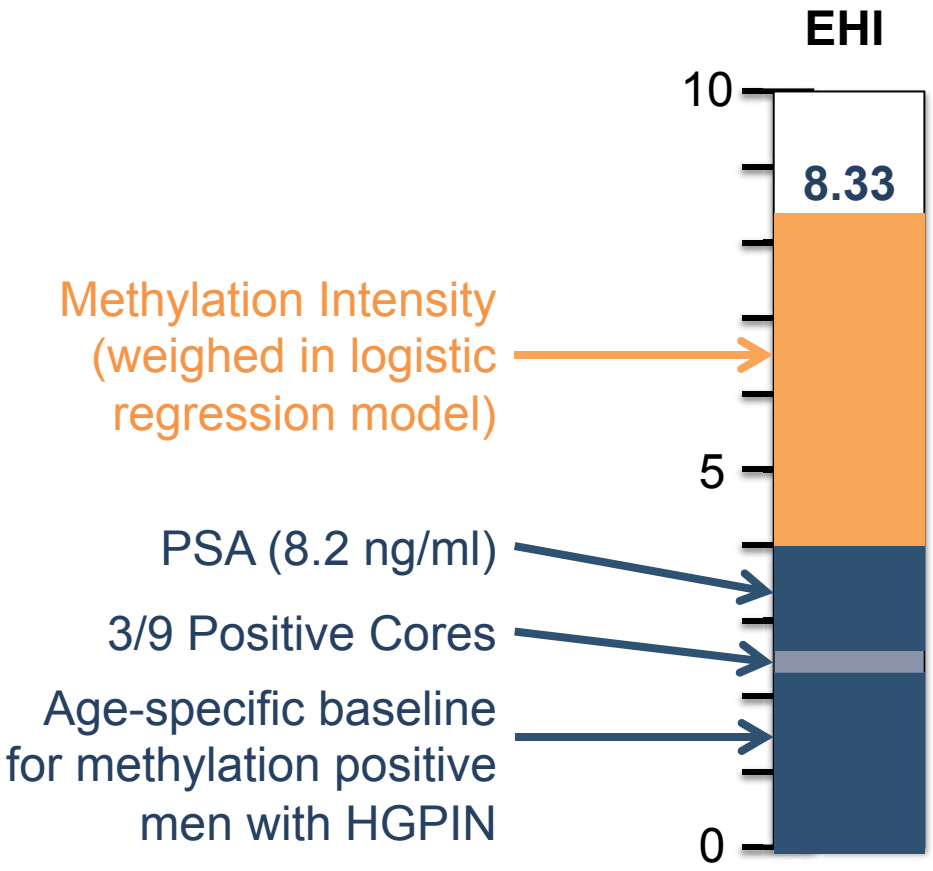


EHI on 1st Biopsy 8.33

Epigenetic Health Index (EHI)

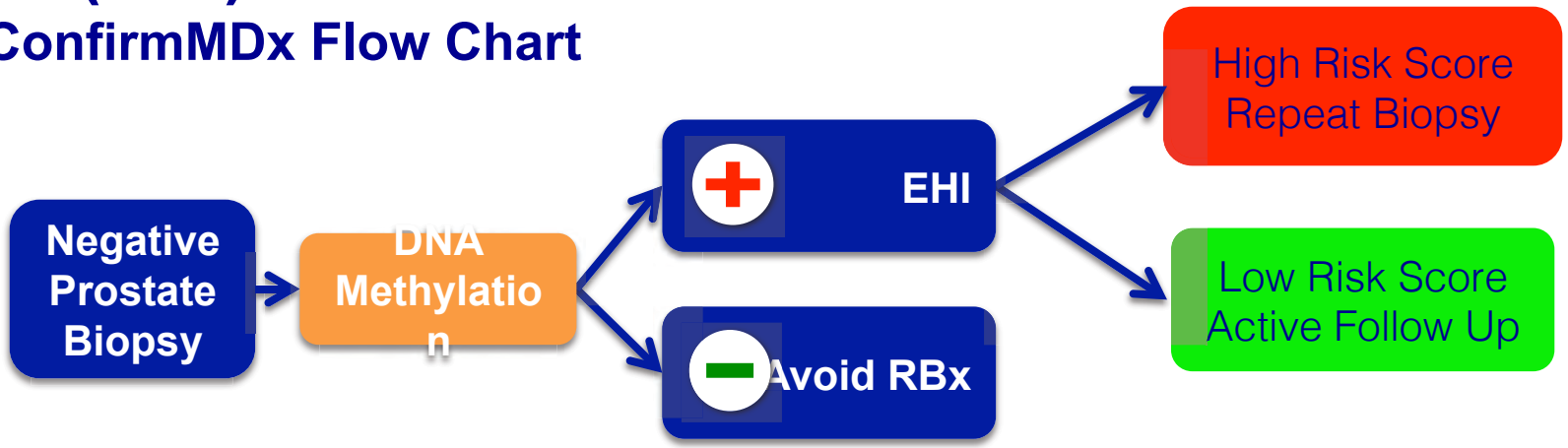


EHI on 1st Biopsy 8.33

Pathology on 2nd Biopsy GS 8

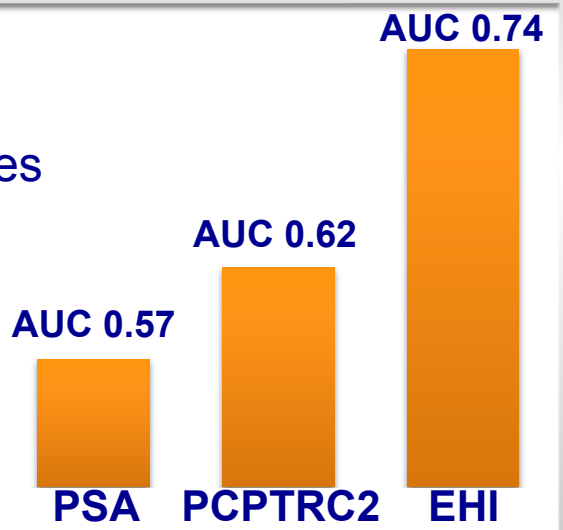
ConfirmMDx and Epigenetic Health Index (EHI)

ConfirmMDx Flow Chart



ConfirmMDx:

- Improves stratification on decision for repeat Biopsies
- Helps reduce unnecessary repeat Biopsies
- EHI identifies men with clinically significant PCa outperforming traditional risk score methods like PSA and PCPTRC2



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Bladder Confirm MDx (2010)

available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Bladder Cancer

Identification and Validation of the Methylated *TWIST1* and *NID2* Genes through Real-Time Methylation-Specific Polymerase Chain Reaction Assays for the Noninvasive Detection of Primary Bladder Cancer in Urine Samples

Isabelle Renard[a], *Steven Joniau*[b], *Ben van Cleynenbreugel*[b], *Catherine Collette*[a], *Christophe Naômé*[a], *Ilse Vlassenbroeck*[a], *Hubert Nicolas*[c], *Jean de Leval*[d], *Josef Straub*[a], *Wim Van Criekinge*[a], *Wissem Hamida*[d], *Majed Hellel*[d], *Alexandre Thomas*[d], *Laurence de Leval*[e,f], *Katja Bierau*[a], *David Waltregny*[d,f,*]

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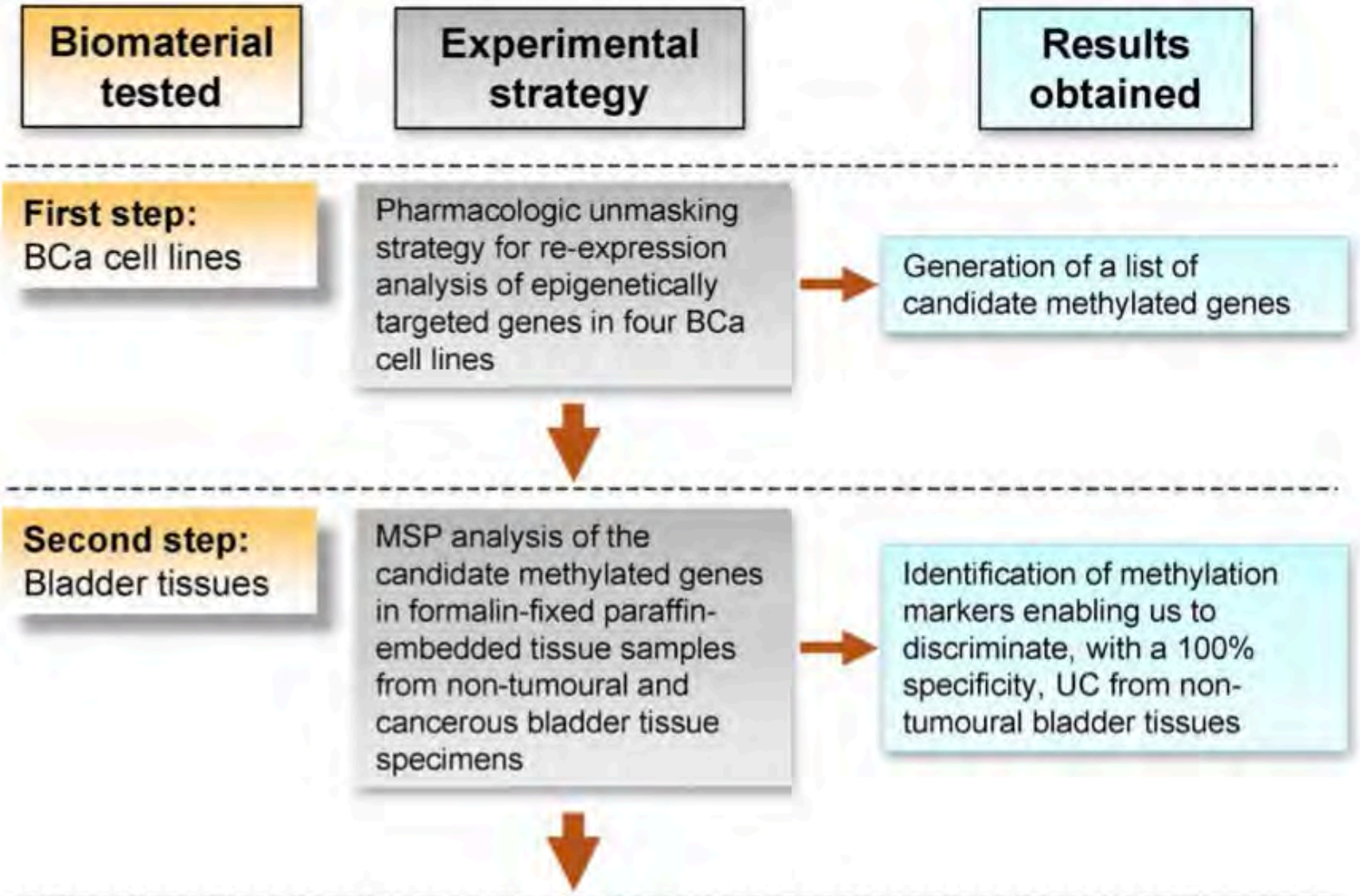
Bladder Confirm MDx



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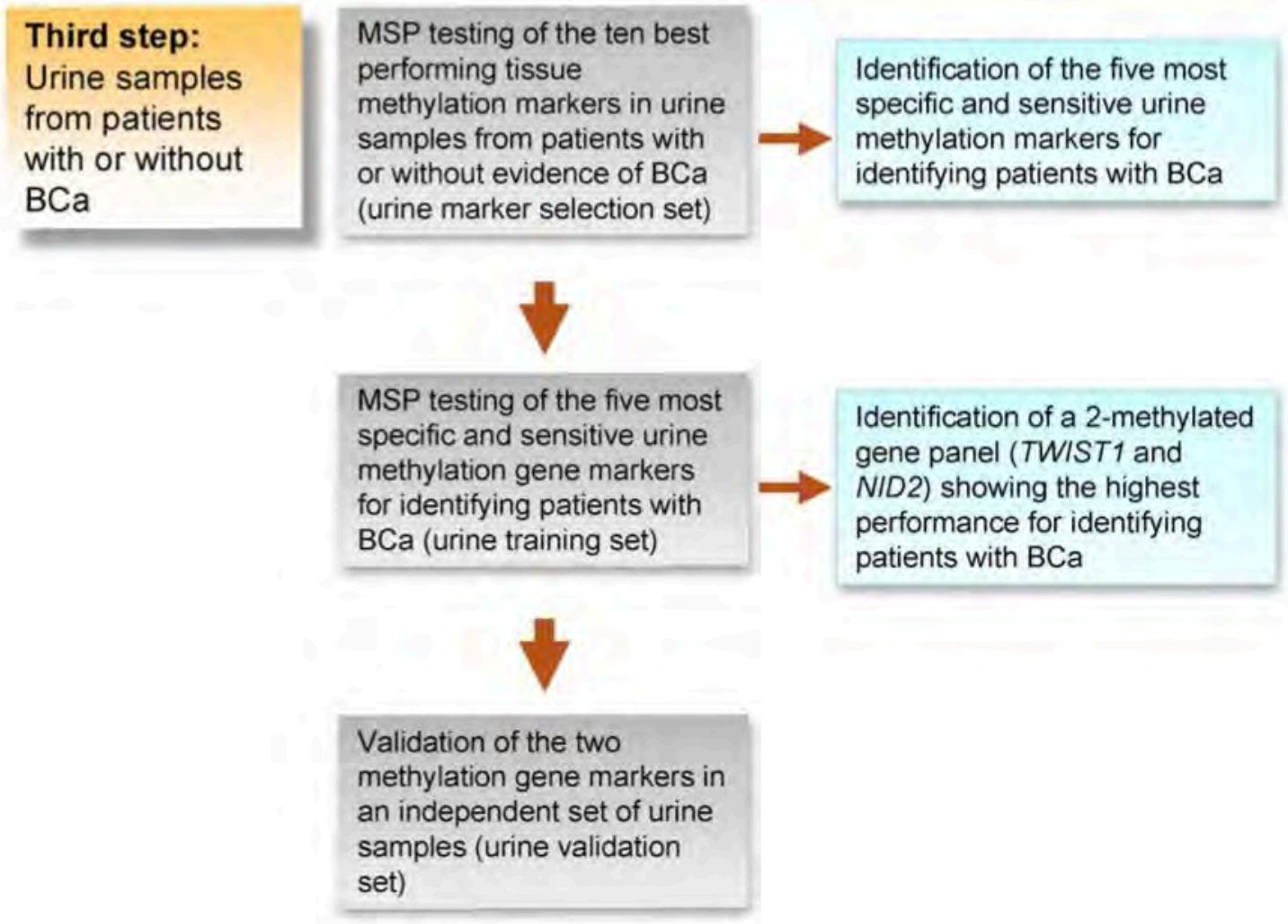
Bladder Confirm MDx



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Biomarkers validated by independent studies

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Hypermethylation of *TWIST1* and *NID2* in Tumor Tissues and Voided Urine in Urinary Bladder Cancer Patients

Zeynep Yegin,[1] Sezgin Gunes,[1] and Recep Buyukalpelli[2]

Bladder cancer like other cancers arises from the accumulation of many genetic and epigenetic changes that lead to the activation of proto-oncogenes or inactivation of tumor suppressor genes. We aimed to investigate the methylation patterns of *Twist homolog 1* (*TWIST1*) and *nidogen-2* (*NID2*) genes in bladder cancer. Fifty six histologically confirmed bladder tumor samples and paired 24 urine samples constituted the study group and was compared with 15 age- and gender-matched noncancerous individuals. DNA was purified from both tumor and urine samples. The methylation status of the two genes was analyzed by methylation-specific polymerase chain reaction (MSP) in both urinary bladder cell carcinoma samples and urine samples. Sensitivity and specificity values of the method were assessed and compared with the results of the cytology test. Me-

of the urine samples, respectively. The sensitivity of *TWIST1* and *NID2* genes (87.5% and 95.8% in urine samples, respectively), was higher compared with urine cytology (62.5%) for cancer detection. The sensitivity of any of the two genes was 88.8% (8/9) for low-grade cases. The sensitivity of urine cytology was 33.3% for the same low-

grade cases. To be used in the early noninvasive diagnosis of bladder cancer, the combined methylation analysis of *TWIST1* and *NID2* genes may be a simple, noninvasive, sensitive, and specific method for detecting cancer cells in urine.

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PLOS ONE

Diagnosis of Bladder Cancer Recurrence Based on Urinary Levels of *EOMES, HOXA9, POU4F2, TWIST1, VIM,* and *ZNF154* Hypermethylation

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Abstract

Background: Non muscle invasive bladder cancer (NMIBC) has the highest recurrence rate of any malignancy and as many as 70% of patients experience relapse. Aberrant DNA methylation is present in all bladder tumors and can be detected in urine specimens. Previous studies have identified DNA methylation markers that showed significant diagnostic value. We evaluated the significance of the biomarkers for early detection of tumor recurrence in urine.

Methodology/Principal Findings: The methylation levels of *EOMES, HOXA9, POU4F2, TWIST1, VIM,* and *ZNF154* in urine specimens were measured by real-time PCR (MethyLight). We analyzed 390 urine sediments from 184 patients diagnosed with NMIBC. Urine from 35 age-matched control individuals was used to determine the methylation baseline levels. Recurrence was diagnosed by cystoscopy and verified by histology. Initially, we compared urine from bladder cancer patients and healthy individuals and detected significant hypermethylation of all six markers ($P<0.0001$) achieving sensitivity in the range 82%–89% and specificity in the range 94%–100%. Following, we validated the urinary hypermethylation for use in recurrence surveillance and found sensitivities of 88–94% and specificities of 43–67%. *EOMES, POU4F2, VIM* and *ZNF154* were more frequently methylated in urine from patients with higher grade tumors ($P\leq0.08$). Univariate Cox regression analysis showed that five markers were significantly associated with disease recurrence; *HOXA9* (HR = 7.8, $P = 0.006$), *POU4F2* (HR = 8.5, $P = 0.001$), *TWIST1* (HR = 12.0, $P = 0.015$), *VIM* (HR = 8.0, $P = 0.001$), and *ZNF154* (HR = 13.9, $P<0.001$). Interestingly, for one group of patients ($n = 15$) we found that hypermethylation was consistently present in the urine samples despite the lack of tumor recurrences, indicating the presence of a field defect.

Conclusion/Significance: Methylation levels of *EOMES, HOXA9, POU4F2, TWIST1, VIM,* and *ZNF154* in urine specimens are promising diagnostic biomarkers for bladder cancer recurrence surveillance.

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MAYO CLINIC

ORIGINAL ARTICLE

A Noninvasive Multianalyte Urine-Based Diagnostic Assay for Urothelial Cancer of the Bladder in the Evaluation of Hematuria

R. Jeffrey Karnes, MD; Cecilia A. Fernandez, PhD; and Anthony P. Shuber, MS

Abstract

Objective: To test whether a noninvasive urine-based multianalyte diagnostic readout assay that uses protein and DNA biomarkers can risk stratify patients with hematuria into those who are or are not likely to have bladder cancer and those who should receive standard care.

Patients and Methods: This prospective, observational, multicenter, single-assessment study was conducted between June 12, 2009, and April 15, 2011. Eligible patients presented with hematuria and as part of their evaluation underwent cystoscopy. Urine samples were analyzed for the presence of mutant *FGFR3* and quantified matrix metalloproteinase 2 and the hypermethylation of *TWIST1* and *NID2*. A patient's chance of having (positive predictive value [PPV]) or not having (negative predictive value [NPV]) cancer was determined by *FGFR3* alone or by all 4 biomarkers, respectively.

Results: Cystoscopy/biopsy diagnosed 690 of 748 patients as negative and 58 as positive for bladder cancer. Of 21 patients identified by *FGFR3* as highly likely to have cancer, 20 were also positive by cystoscopy/biopsy, resulting in a PPV of 95.2% (20 of 21), with specificity of 99.9% (689 of 690). The 4-marker combination identified 395 patients as having a low likelihood of cancer. Of these, 56.2% (388 of 690) also had negative biopsy/cystoscopy findings, resulting in an NPV of 98.2% (388 of 395). In total, 416 of the 748 patients with hematuria (55.6%) were identified with extremely high NPV and PPV to have or not have bladder cancer.

Conclusion: This multianalyte assay accurately stratified patients with high confidence into those who likely do or do not have bladder cancer. This test was developed to enhance and not to eliminate referrals for urologic evaluation.

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MP-06.01

A 3-Gene DNA-Methylation Biomarker Panel Sensitively Detects Bladder Cancer and Discriminates Between High-grade and Low-grade Disease in Voided Urine

Hermanns, Thomas[1]; Olkhov-Mitsel, Ekaterina[2]; Savio, Andrea[2]; Zdravic, Darko[2]; Bhindi, Bimal[3]; Kuk, Cynthia[4]; Noon, Aidan[1]; Rendon, Ricardo[5]; Waltregny, David[6]; Lo, Kirk C.[4]; van der Kwast, Theodorus[7]; Finelli, Antonio[1]; Fleshner, Neil[1]; Bapat, Bharati[2]; Zlotta, Alexandre[4]

[1]Department of Surgical Oncology, Division of Urology, Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada; [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; [3]Department of Surgery, Division of Urology, University Health Network, University of Toronto, Toronto, ON, Canada; [4]Department of Urology, Mount Sinai Hospital, Toronto, ON, Canada; [5]Department of Urology, Dalhousie University, Halifax, NS, Canada; [6]Department of Urology, University of Liege, Liege, Belgium; [7]Department of Pathology, University Health Network, University of Toronto, Toronto, ON, Canada

Introduction and Objectives: Voided urine provides an excellent source of exfoliated bladder cells and is ideal to detect bladder cancer (BC) biomarkers. Using two different genome-wide methylation-array profiling platforms in Toronto, CA and Liège, BE, several differential
relevant genes (incl. TWIST1, NID2 and RUN
versus high grade (HG) BCs were commonly id
methylation of the 3 genes to identify BC in urine
LG and HG BC.

Methods: Voided urine from patients with LG (n
well as from BC-free controls (noBC, n=34) wa
status of the 3 genes was analyzed in the urinary
assay. Methylation levels (percent methylatio
obtained for each sample. Association between
versus LG disease vs. noBC was investigated usi
pairwise comparisons (BC versus no BC; HG ve
formed using Mann-Whitney U-test. Univariate
regression models were used to create ROC cur
and combined biomarker discrimination, respec
detecting BC (versus no BC) and HG (versus LG/
the area under the curve (AUC).

Results: Median PMRs for HG, LG and no BC v
for each gene (TWIST1: HG: 22, LG: 1, noBC:0;
noBC: 4.7; RunX3: HG:3.5, LG:0.01, noBC: 0; a
all genes were significantly higher in BC than in
and in HG BC compared to LG/noBC cases (a
predict BC was 0.83 (95%CI: 0.76-0.9) for TW
0.89) for NID2 and 0.73 (95%CI: 0.64-0.82) for R
HG BC was 0.86 (95%CI: 0.78-0.94) for TWIST
for NID2 and 0.77 (95%CI: 0.67-0.86) for RunX
genes, the AUC was 0.87 (95%CI: 0.8-0.94) to pr
0.79-0.95) to predict HG BC.

Conclusions: Combination of the 3 epigenetic m
tool for sensitive and specific detection of BC an
HG and LG BC in voided urine.

Results: Median PMRs for HG, LG and no BC were significantly different for each gene (TWIST1: HG: 22, LG: 1, noBC:0; NID2: HG: 27.4, LG: 7.5, noBC: 4.7; RunX3: HG:3.5, LG:0.01, noBC: 0; all $p<0.001$). The PMRs for all genes were significantly higher in BC than in noBC cases (all $p<0.001$) and in HG BC compared to LG/noBC cases (all $p<0.001$). The AUC to predict BC was 0.83 (95%CI: 0.76-0.9) for TWIST1, 0.81 (95%CI: 0.72-0.89) for NID2 and 0.73 (95%CI: 0.64-0.82) for RunX3. The AUC to predict HG BC was 0.86 (95%CI: 0.78-0.94) for TWIST1, 0.8 (95%CI: 0.71-0.9) for NID2 and 0.77 (95%CI: 0.67-0.86) for RunX3. When combining all 3 genes, the AUC was 0.87 (95%CI: 0.8-0.94) to predict BC and 0.87 (95%CI: 0.79-0.95) to predict HG BC.

Conclusions: Combination of the 3 epigenetic markers is a very promising tool for sensitive and specific detection of BC and discrimination between HG and LG BC in voided urine.

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Overview DNA based bladder cancer markers

detection

prognosis

TWIST1
NID2

epigenetic

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Overview DNA based bladder cancer markers

	detection	prognosis
epigenetic	TWIST1 NID2 VIM OSR1 ONECUT 2 OTX1	GSTP1 RASSF1 A APC RUNX3 TIMP3 SOX1 HIC1 LINE1 HOXA9 FHIT LAMC2 SFRP1 PMF1 ISL1 ALDH1A3 PCDH7 ITIH5 KLF4
	p16 CHH1 MLL2 ARID1A KDM6A	
genetic	FGFR3 P53 hTERT	

EP300, MLL3, MLL2, ARIDNA1, KDMA6A, ATM, ATRX, BRCA2, ATR, STAG2, ERCC2, PDGFRA, CDKNA2A, RB1, CDKN1A, NF1, PIK3CA, FBXW7, CDH1, NFE2L2, MALAT1, NCOR1, PRX

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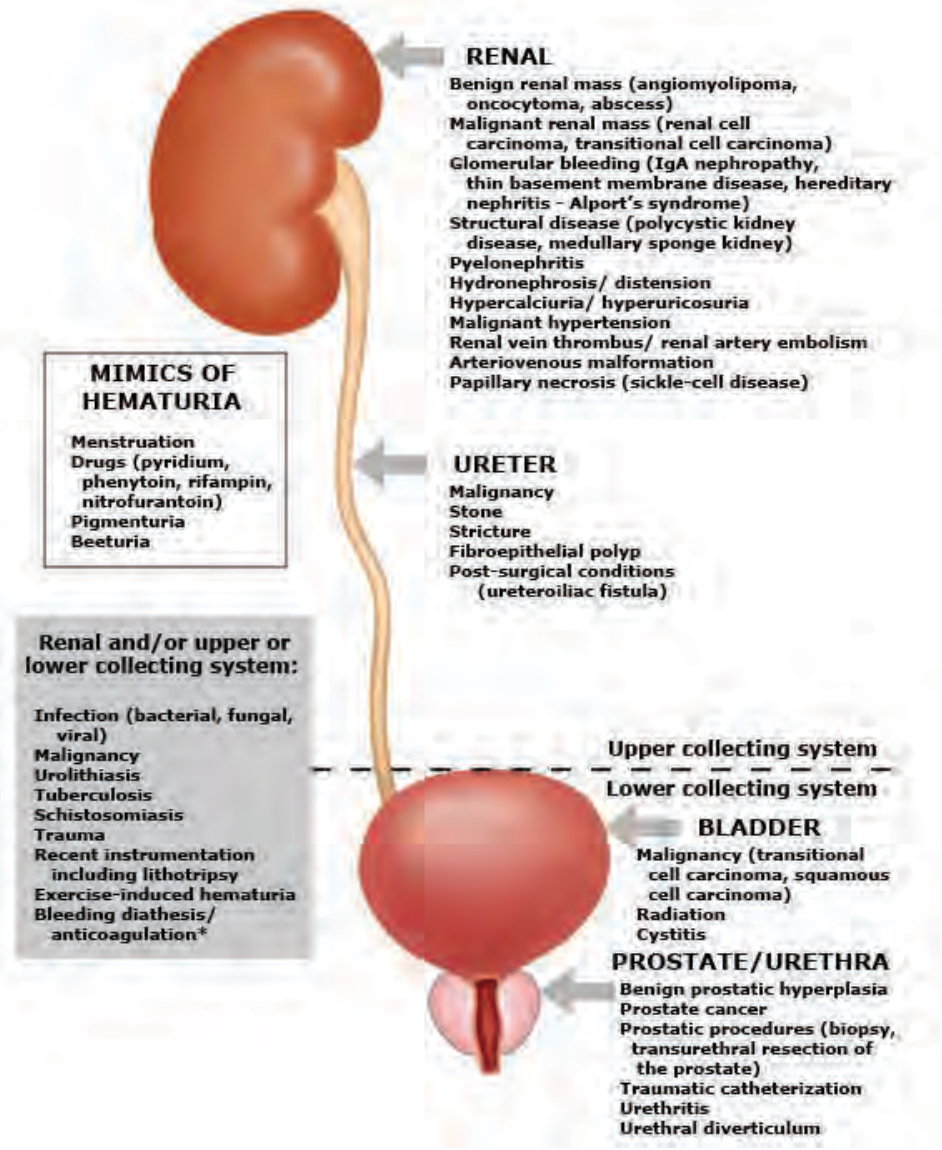
Select best biomarker panel for detecting BC in urine of patients with Hematuria

Hematuria

Bladder tumor 3-28%



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Validation Study / case-control / 160 Patients

MSP	SNaPshot	Mutation
TWIST1 NID2 (TWIST1 neutral)	ONECUT2 OTX1	FGFR3 TERT PIK3CA KRAS NRAS HRAS

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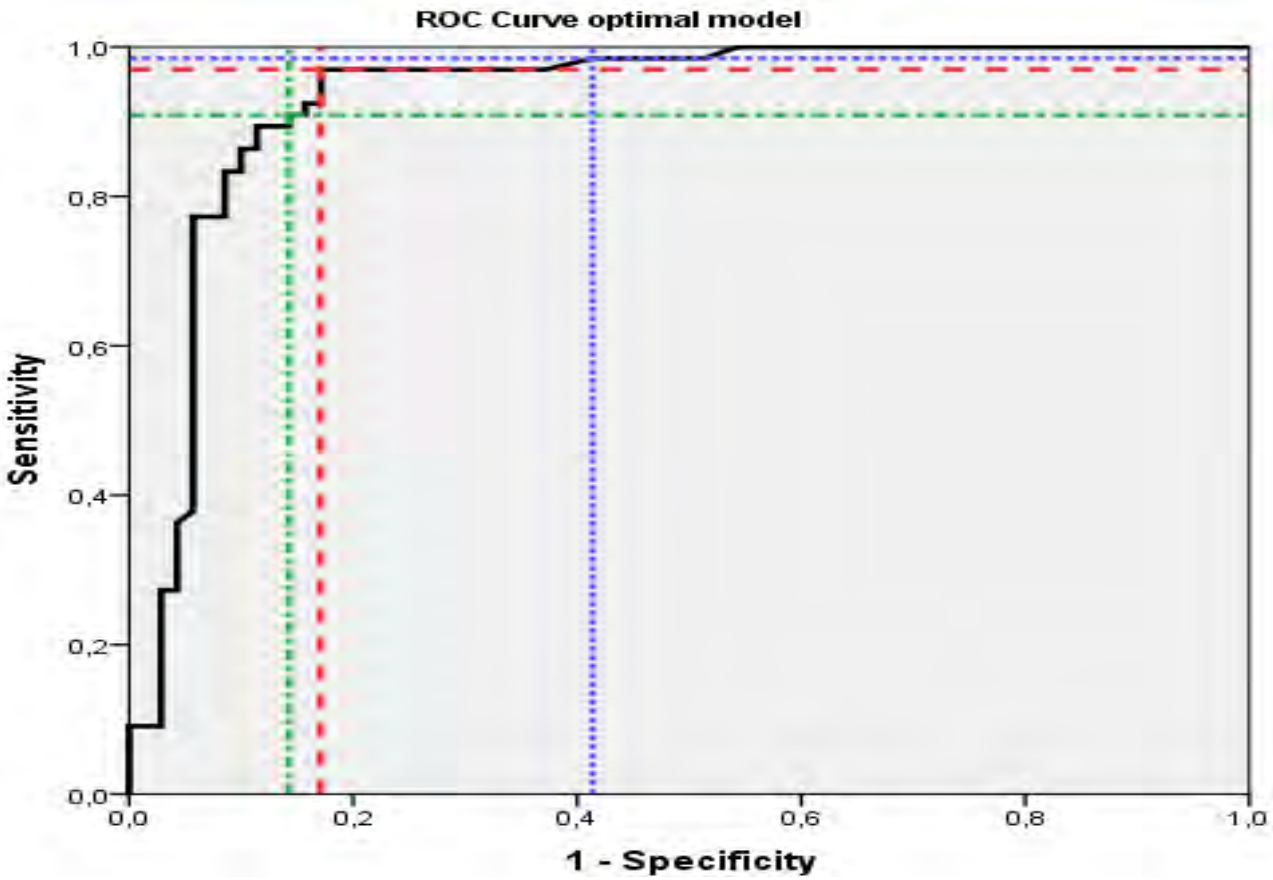
Optimal model: NPV > 99%, 97% sensitivity



Line	Cut-off	Sensitivity (%)	Specificity (%)	PPV 5% prev	PPV 10% prev	NPV 5% prev	NPV 10% prev
	0.1233907	98.5	58.6	11.3	21.0	1	99.8
	0.1965208	97.0	82.9	23.2	38.8	99.9	99.6
	0.3530504	90.9	85.7	25.4	41.6	99.5	98.8

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Conclusions

- A limited number of bladder cancer specific methylation markers can be measured in urine to accurately detect the presence of bladder cancer in hematuria patients

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Conclusions

- A limited number of bladder cancer specific methylation markers can be measured in urine to accurately detect the presence of bladder cancer in hematuria patients

- ConfirmMDx for Bladder can be used as a rule-in for cystoscopy (in case of hematuria) with a very high NPV and very high sensitivity thereby resulting in a significant reduction in the number of cystoscopies.

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Conclusions

- A limited number of bladder cancer specific methylation markers can be measured in urine to accurately detect the presence of bladder cancer in hematuria patients

- ConfirmMDx for Bladder can be used as a rule-in for cystoscopy (in case of hematuria) with a very high NPV and very high sensitivity thereby resulting in a significant reduction in the number of cystoscopies.

- It represents a significant improvement in PPV as compared to standard of care. Potential in the recurrence setting is actively investigated

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Q&A

Epigenetics

- Introduction
- DNA Methylation & Oncology

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- *NEXT-GEN*eration (Epi)genetic biomarkers
- Prostate Epigenetic Biomarkers
  - confirmMDx & Beyond
- Bladder Epigenetic Biomarkers