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The role of epigenetic modifications in diagnosis and treatment of bladder cancer

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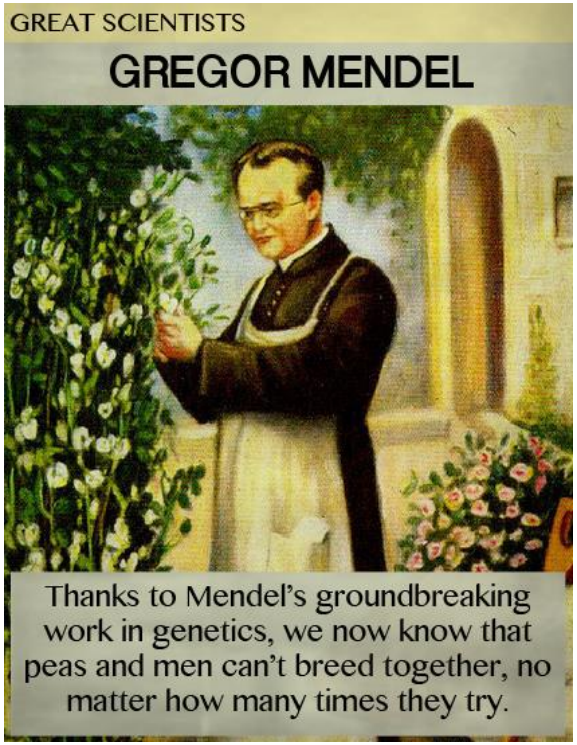
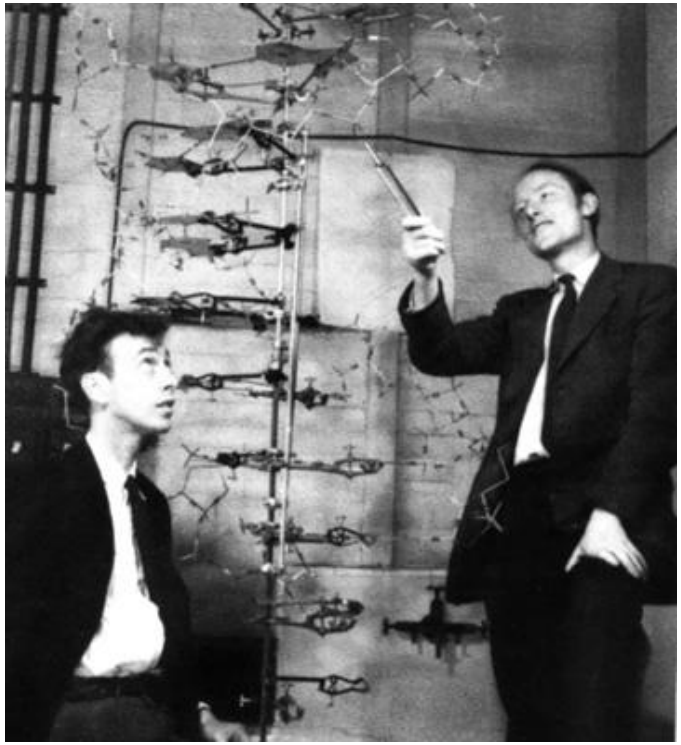
National Chung Cheng University
ChiaYi County
250KM from Taipei City (1.5 hrs HSR)
100KM from Kaohsiung (30 mins HSR)

From **Genetics** to **Epigenetics**

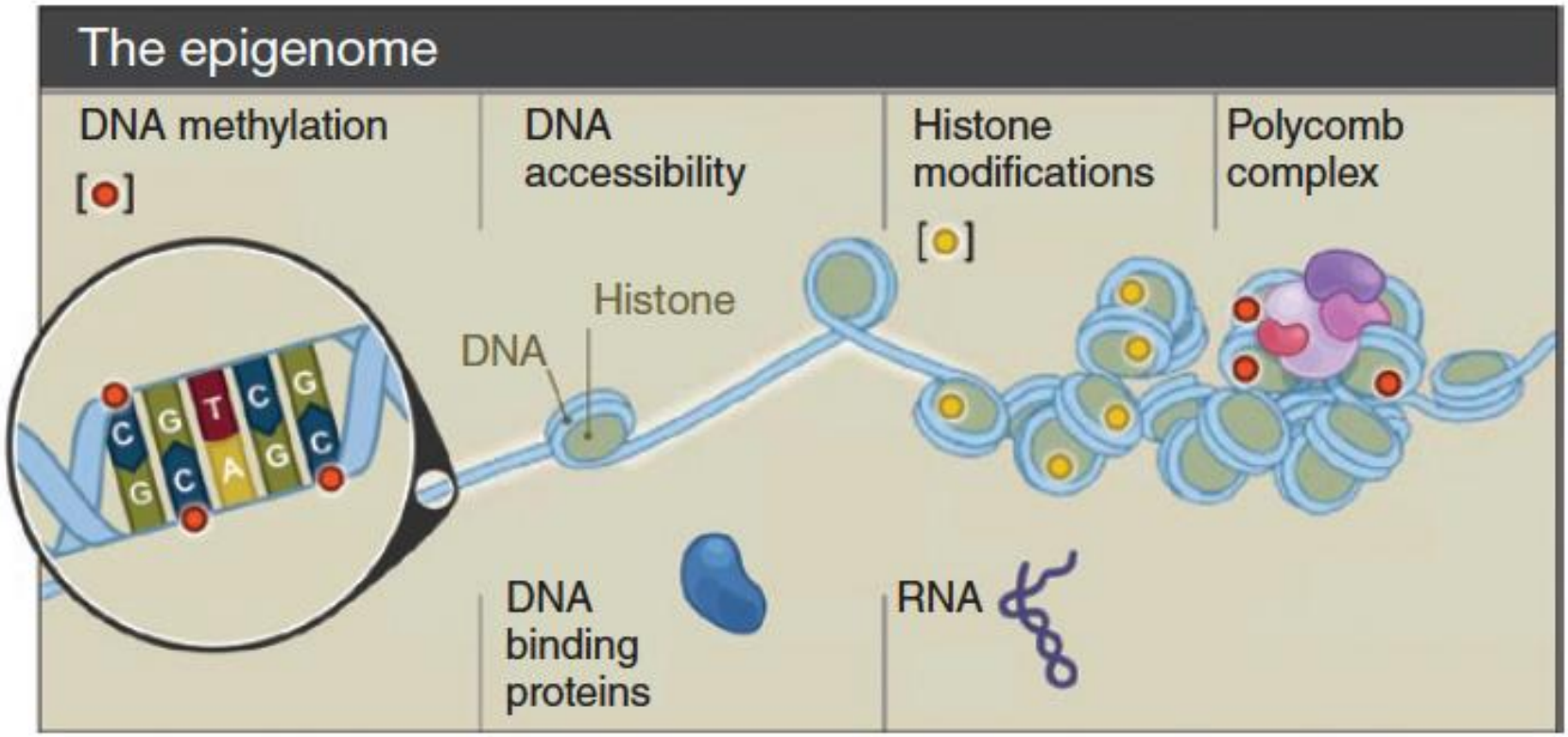




Time Magazine, 2010 Jan 18

Epigenetics

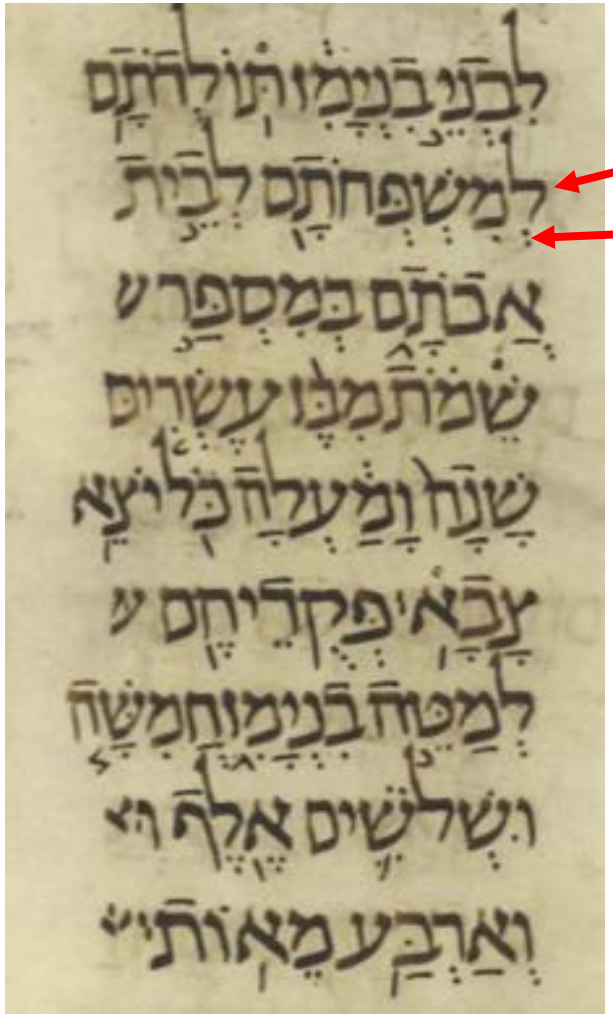
Heritable changes that modulate chromatin organization and gene expression without changes in DNA sequence (Riggs et al., 1996)



Bernstein *et al*., Nat Biotech 2010

Prof. Howard Cedar, Hebrew University
The father of DNA methylation

Ancient Bible

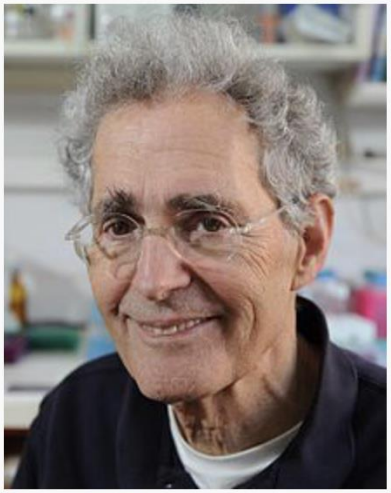


Character (Hebrew)

punctuation

Howard Chaim Cedar (Hebrew: חיים סידר; born January 12, 1943) is an Israeli American biochemist who works on DNA methylation, a mechanism that turns genes on and off.

Howard Cedar



Howard Cedar in 2016

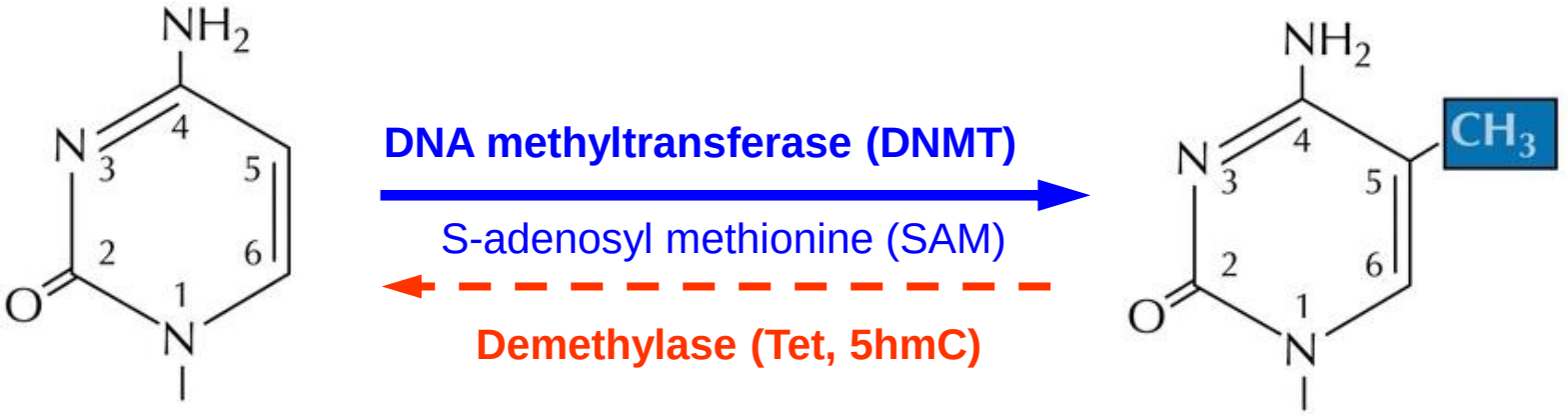
Born	Howard Chaim Cedar January 12, 1943 (age 79) New York City, U.S.

- THERAPIST
- THE RAPIST

- KIDS EXCHANGE
- KID SEX CHANGE

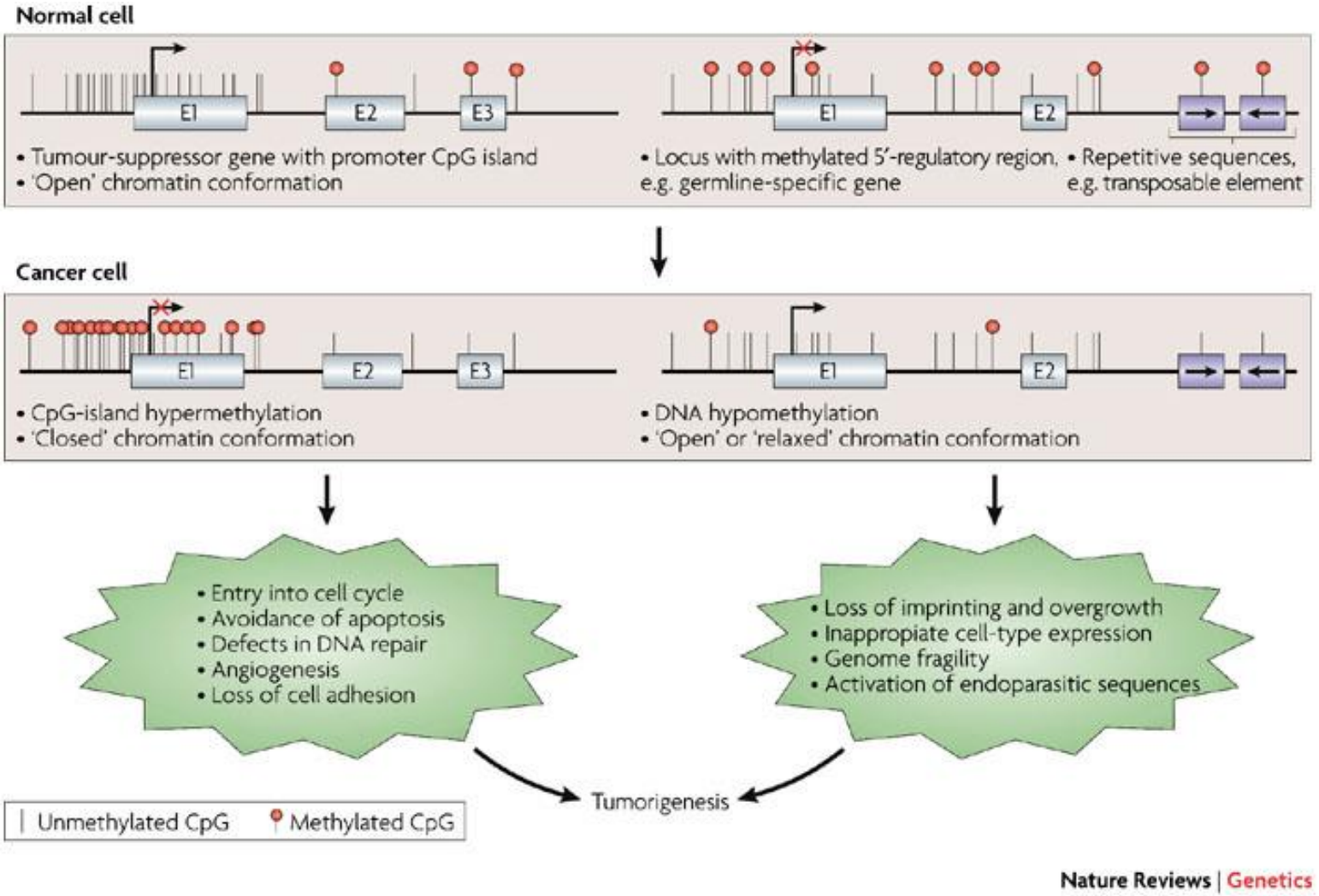
DNA Methylation

- Occurs in CG dinucleotide (CpG)
- CpG islands: CG rich region (500bp-2000bp) in the promoter region (of 50% human genes), normally unmethylated
- Methylation of CpG island is associated with transcriptional repression



cytosine **5-methylcytosine**

Altered DNA-methylation patterns in tumorigenesis



Esteller
Nat Rev Genet 2007

Methods for DNA methylation Analysis

Table 1 | **Main principles of DNA methylation analysis**

Pretreatment	Analytical step			
	Locus-specific analysis	**Gel-based analysis**	**Array-based analysis**	**NGS-based analysis**
Enzyme digestion	• *Hpa*II-PCR	• Southern blot • RLGS • MS-AP-PCR • AIMS	• DMH • MCAM • HELP • MethylScope • CHARM • MMASS	• Methyl–seq • MCA–seq • HELP–seq • MSCC
Affinity enrichment	• MeDIP-PCR		• MeDIP • mDIP • mCIP • MIRA	• MeDIP–seq • MIRA–seq
Sodium bisulphite	• MethyLight • EpiTYPER • Pyrosequencing	• Sanger BS • MSP • MS-SNuPE • COBRA	• BiMP • GoldenGate • Infinium	• RRBS • BC–seq • BSPP • WGSBS

Laird, Nat Rev Cancer 2010

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 9821–9826, September 1996
Medical Sciences

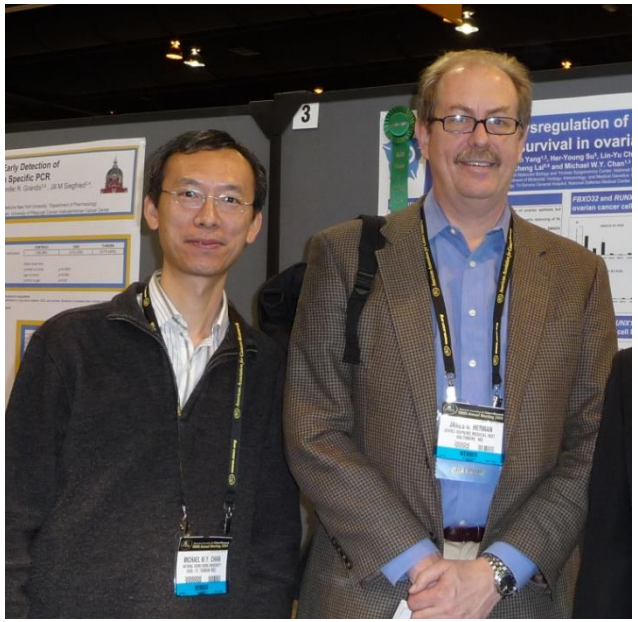
Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands

(DNA methylation/tumor suppressor genes/*p16*/*p15*)

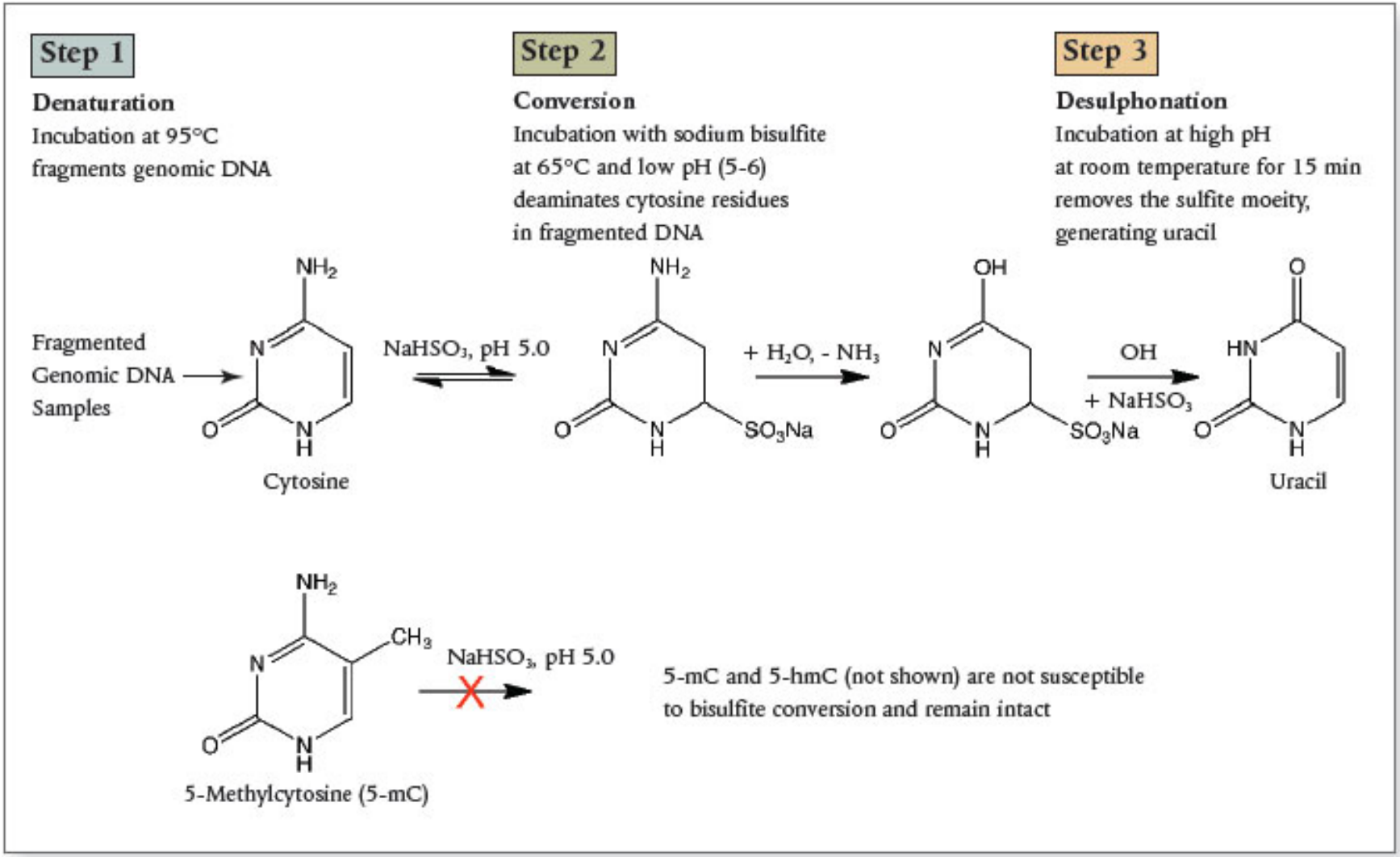
JAMES G. HERMAN*†, JEREMY R. GRAFF*, SANNA MYÖHÄNEN*, BARRY D. NELKIN*, AND STEPHEN B. BAYLIN*‡

*Oncology Center and ‡Department of Medicine, The Johns Hopkins Medical Institutions, 424 North Bond Street, Baltimore, MD 21231

Communicated by Victor A. McKusick, Johns Hopkins Hospital, Baltimore, MD, June 3, 1996 (received for review April 3, 1996)

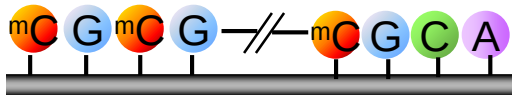


Sodium Bisulphite conversion

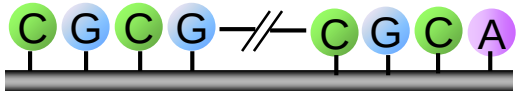


Bisulphite-based Methods

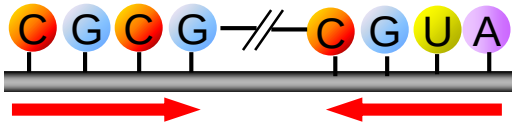
TUMOR DNA (METHYLATED)



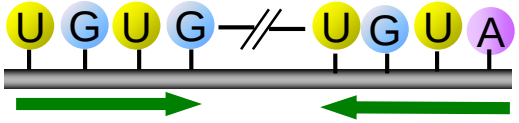
NORMAL DNA (UNMETHYLATED)



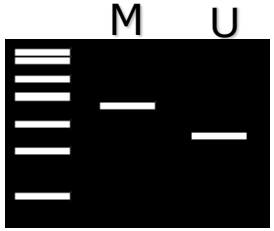
Sodium Bisulfite Modification



PCR with primers specific for methylated DNA



PCR with primers specific for unmethylated DNA

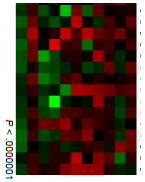


PAGE

or

qPCR

or



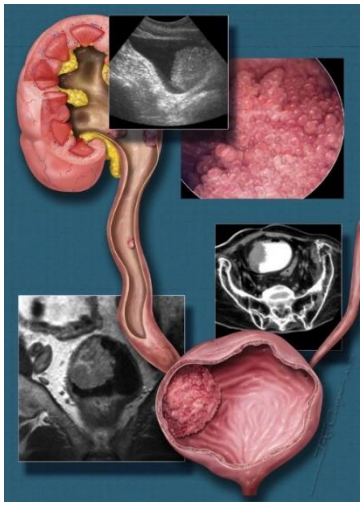
microarray

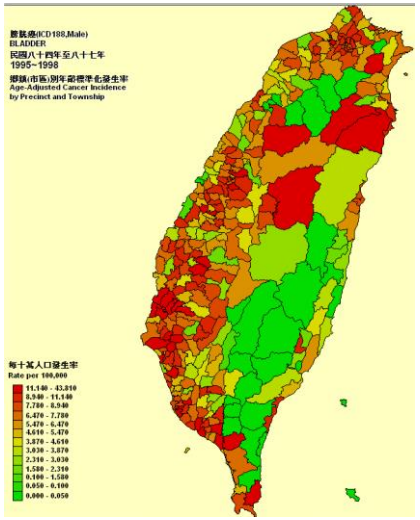
or

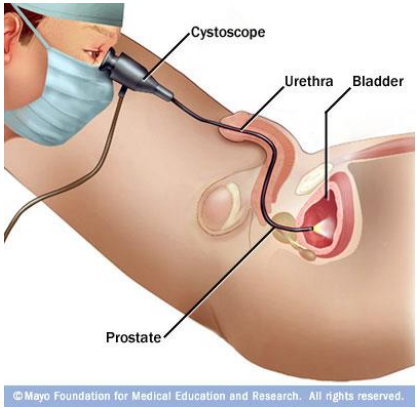
Sequencing/NGS

Urothelial Carcinoma (Bladder Cancer)

- one of the ten most prevalent malignancy in Taiwan (male)
- incidence particularly high in southwestern Taiwan. (Arsenic contamination)
- **Diagnosis :**
  - imaging (X-ray, CT, IVP)
  - urine cytology (sensitivity ~50%)
  - cystoscopy

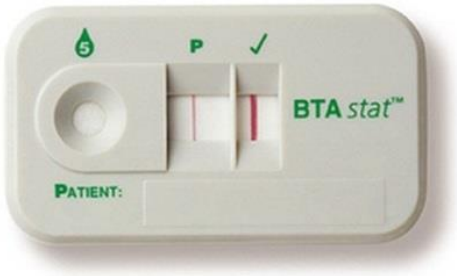






Cooper, Radiographics 2006;
http://www.drketandesai.com/kidney/things-to-know-about-urinary-bladder-tumor
http://www.svuhradiology.ie/case-study/obstructing-bladder-tcc/

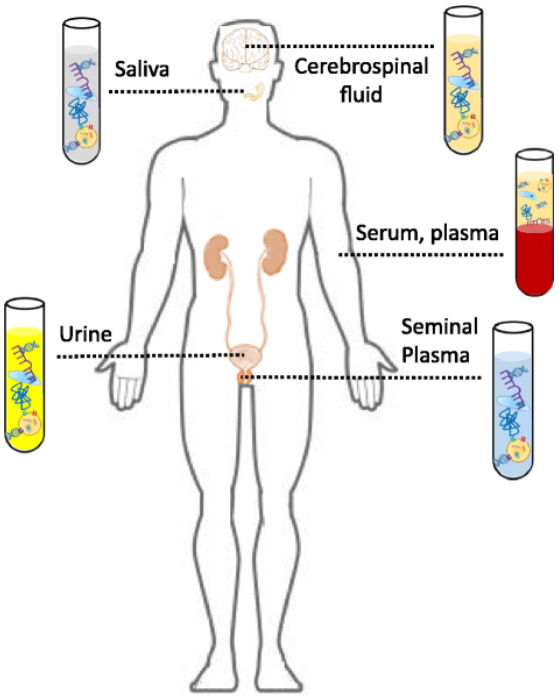
Current non-invasive molecular detection for bladder cancer



Test	Number of studies analyzed	Median sensitivity [%] (range)	Median specificity [%] (range)
BTA *stat*®	36	67 (34–91)	76 (38–96)
BTA TRAK®	12	63 (17–100)	76 (50–98)
NMP22® Bladder Cancer Test	41	72 (31–100)	80 (43–100)
NMP22® BladderChek®	6	57 (47–85)	86 (40–90)
UBC™-Rapid	13	67 (21–84)	80 (49–98)
HA–HAase	6	88 (83–91)	81 (61–93)
UroVysion®	19	73 (13–87)	90 (40–100)
ImmunoCyt®	16	81 (39–100)	75 (62–95)

Mitra and Cote, Nat Rev Urol 2010

Screening of urological cancer by DNA methylation



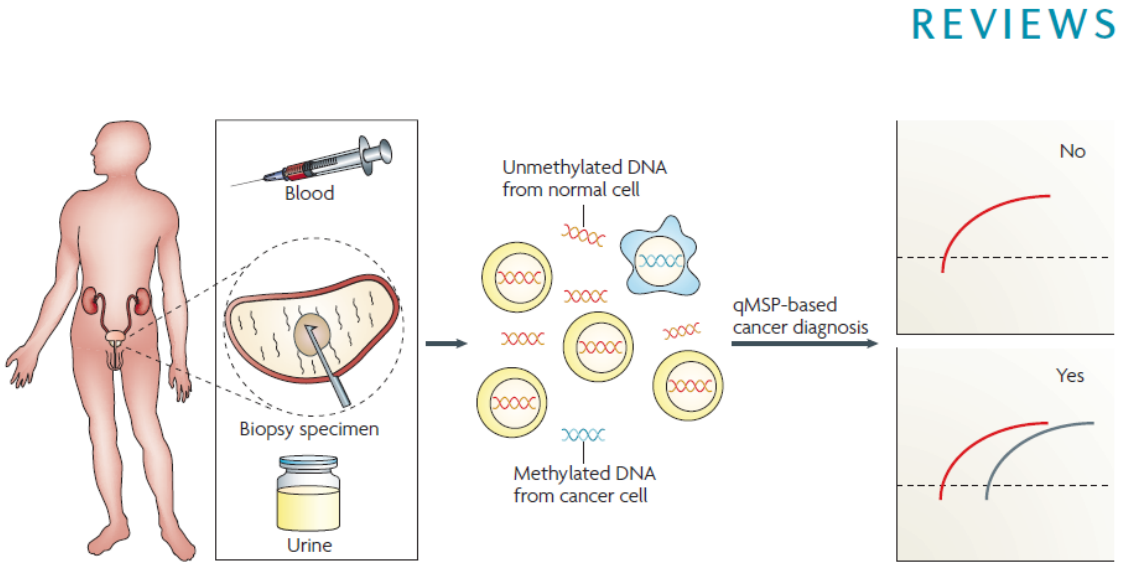


Figure 1 | **Screening for genitourinary cancer.** Tumour cells and free tumour DNA from dead cancer cells can access urine through secretion from the prostate into the urethra, and the proximity of urine to the transitional cell lining of the bladder and the renal system. Tumour cells that lack the capacity to metastasize and free tumour DNA can also access the circulatory system. Direct, but invasive, access by needle biopsy is performed for prostate cancer and, less commonly, renal cancer. DNA is isolated from the clinical specimen urine, blood or biopsy, and analysed for the presence of gene methylation by quantitative real-time methylation-specific PCR (qMSP).

Meo *et al*., Mol Cancer 2017
Cairns, Nature Rev Cancer 2007

464 Vol. 8, 464–470, February 2002 Clinical Cancer Research

Hypermethylation of Multiple Genes in Tumor Tissues and Voided Urine in Urinary Bladder Cancer Patients

Int. J. Cancer: **104,** 611–616 (2003)

UICC Publication of the International Union Against Cancer

FREQUENT HYPERMETHYLATION OF PROMOTER REGION OF *RASSF1A* IN TUMOR TISSUES AND VOIDED URINE OF URINARY BLADDER CANCER PATI

Michael W.Y. CHAN[1], L
Ho Y. CHEUNG[2], Wai S.
[1]*Department of Anatom*
[2]*Department of Surgery*
[3]*Department of Chemic*

Chen *et al. BMC Medical Genomics* 2011, **4**:45
http://www.biomedcentral.com/1755-8794/4/45

BMC Medical Genomics

RESEARCH ARTICLE Open Access

Distinct DNA methylation epigenotypes in bladder cancer from different Chinese sub-populations and its implication in cancer detection using voided urine

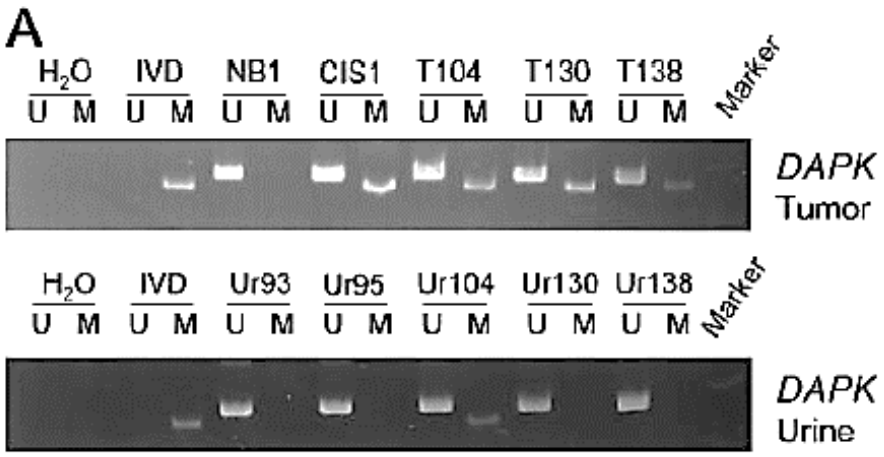
Pi-Che Chen[1], Min
Wei Huang[6], Chun
De-Ching Chang[2]

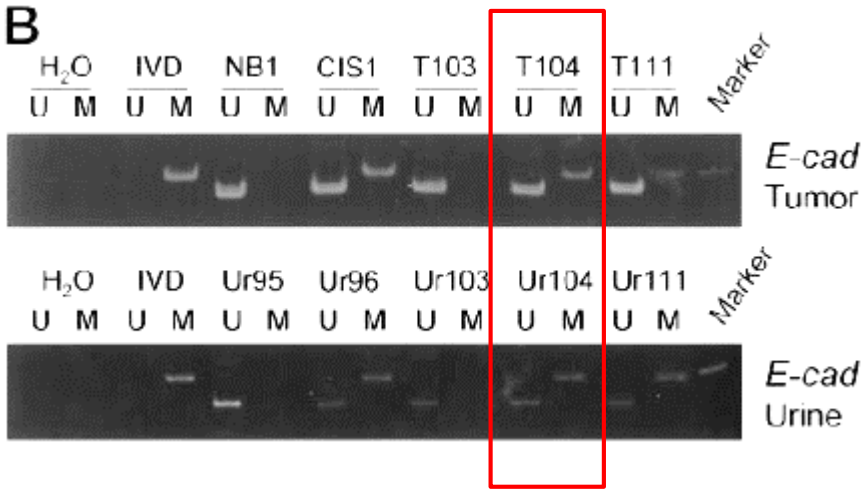
www.impactjournals.com/oncotarget/ Oncotarget, Vol. 6, No. 30

Methylomics analysis identifies ZNF671 as an epigenetically repressed novel tumor suppressor and a potential non-invasive biomarker for the detection of urothelial carcinoma

Chia-Ming Yeh[1,2,*], Pi-Che Chen[3,*], Hsiao-Yen Hsieh[2,5], Yeong-Chin Jou[3], Chang-Te Lin[3], Ming-Hsuan Tsai[1], Wen-Yu Huang[1,2], Yi-Ting Wang[1,2], Ru-Inn Lin[1,6], Szu-Shan Chen[1,2], Chun-Liang Tung[4], Shu-Fen Wu[1,2], De-Ching Chang[1,2], Cheng-Huang Shen[3], Cheng-Da Hsu[5] and Michael W.Y. Chan[1,2,*]

Methylation analysis in bladder cancer tissues and urines by MSP





Chan *et al*., Clin Cancer Res 2002

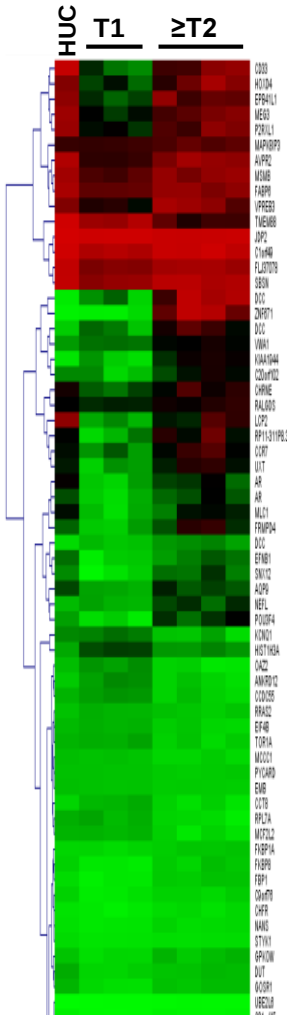
Table 8 Comparison of sensitivity and specificity between methylation markers and cytology

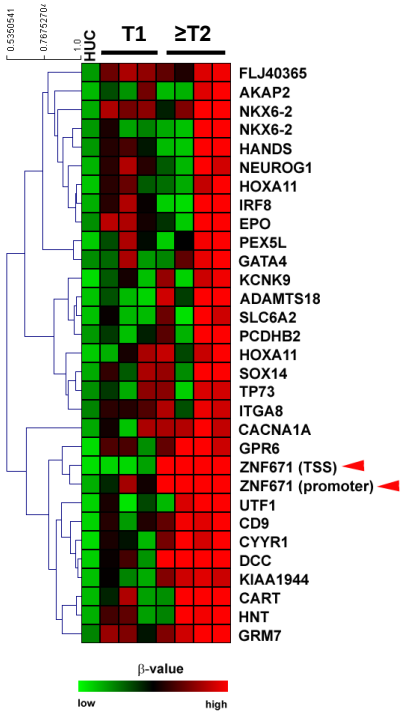
	Methylation markers[a]	Cytology	*DAPK*	*RAR*β	*E-cadherin*	*p16*
Sensitivity (%)						
All cases	90.9	45.5	45.5	68.2	59.1	13.6
Grade 1	100	11.1	55.5	66.7	66.7	22.2
Grade 2–3	84.6	69.2	38.4	69.2	53.8	7.6
Specificity (%)	76.4	100	100	76.4	100	100
Positive predictive value (%)						
All cases	83.3	100	100	78.9	100	100
Grade 1	69.2	100	100	60.0	100	100
Grade 2–3	73.3	100	100	69.2	100	100
Negative predictive value (%)						
All cases	86.6	58	58.6	65.0	65.3	47.2
Grade 1	100	68	80.9	81.3	85	70.8
Grade 2–3	86.6	80.9	68.0	76.4	73.9	58.6

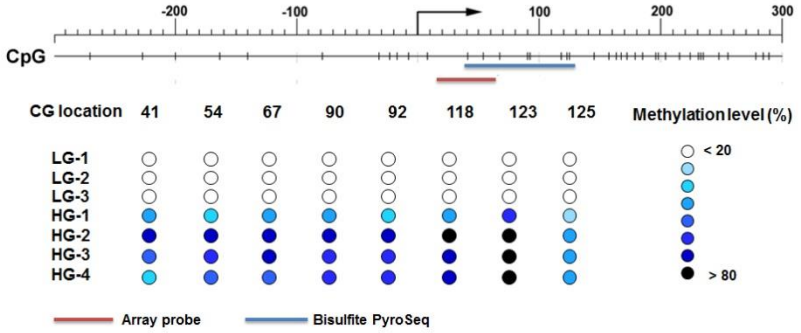
[a] Any one of the genes showed methylation in urine samples.
[b] Cases where either *DAPK* showed methylation or cytology diagnosed as cancer or suspicious.

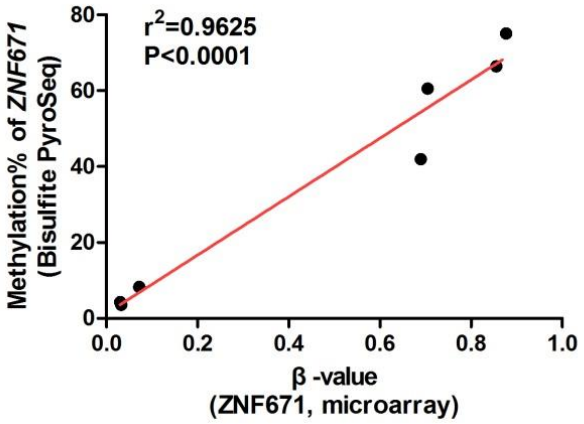
Chan *et al.*, Clin Cancer Res 2002

Methylomics analysis in bladder cancer tissue by Illumina 450K methylation microarray



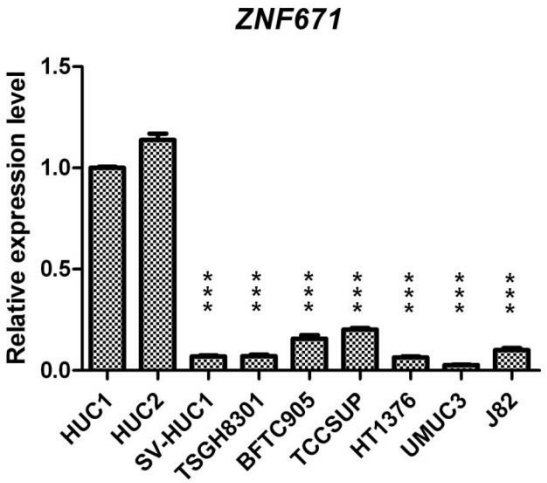


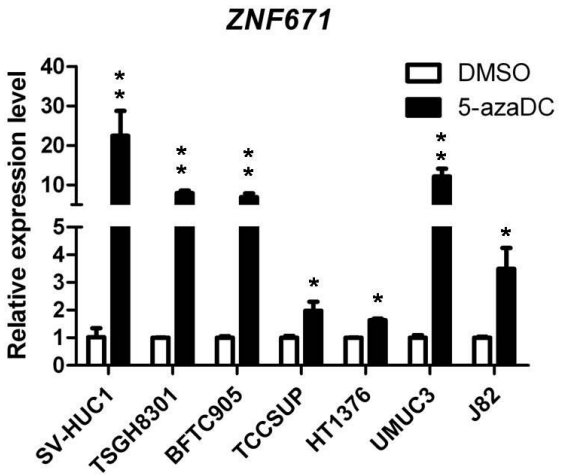


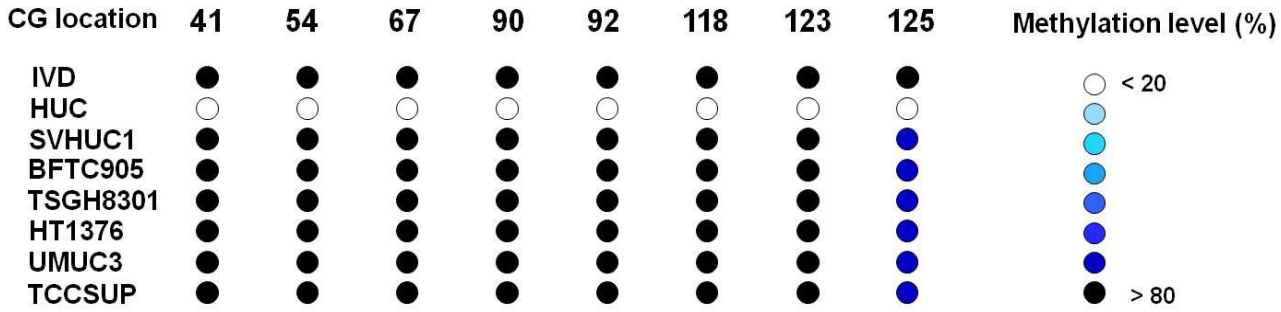


Yeh *et al,* Oncotarget 2015

ZNF671 is epigenetically silenced by DNA methylation in bladder cancer cell lines



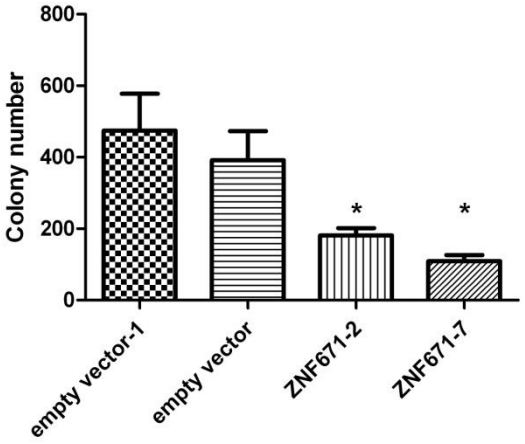




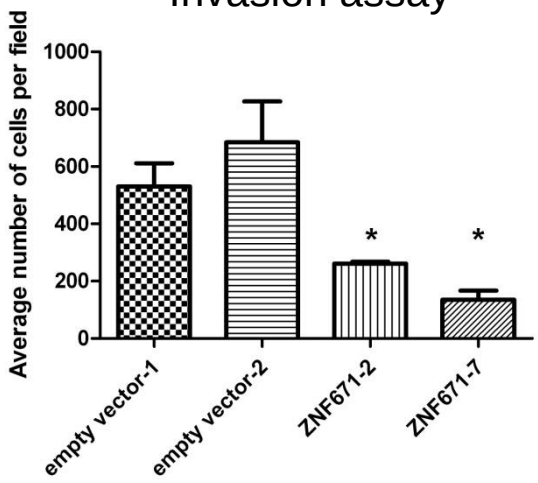
Yeh *et al,* Oncotarget 2015

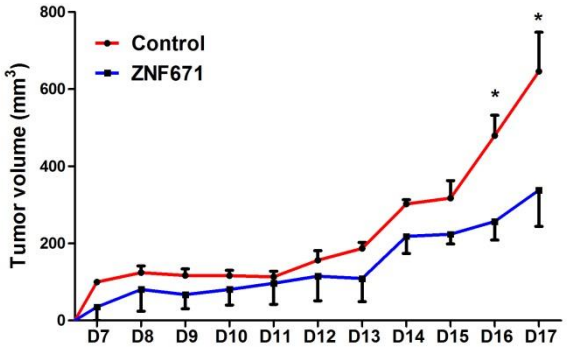
Ectopic expression of *ZNF671* suppress tumor growth *in vitro* and *in vivo*

Colony formation assay



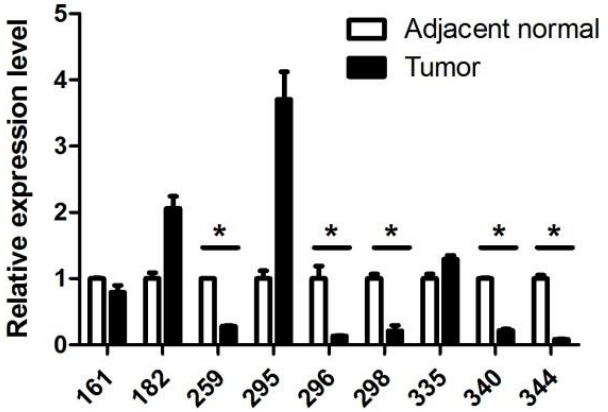
Invasion assay

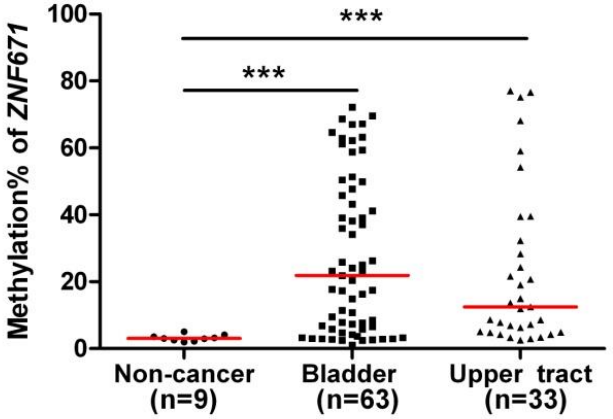


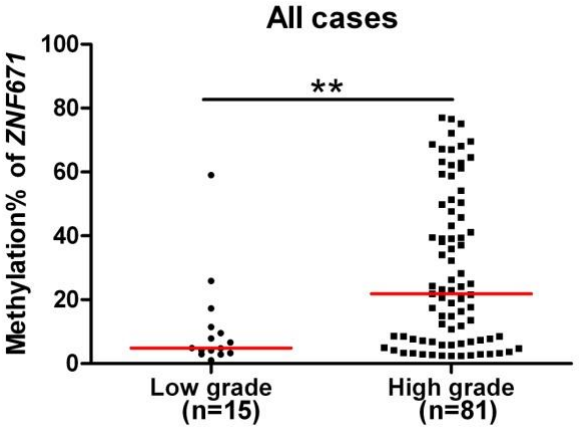


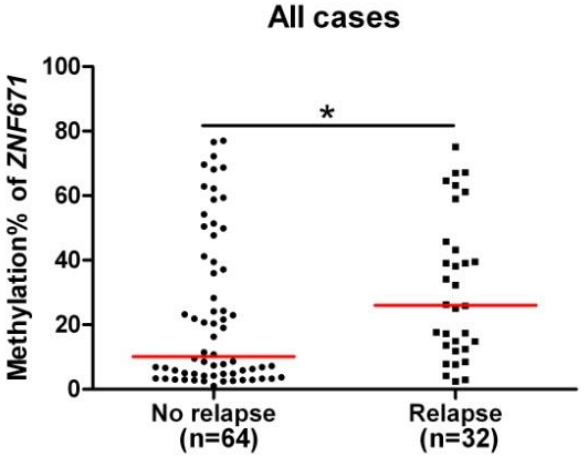
Yeh *et al,* Oncotarget 2015

ZNF671 is epigenetically silenced in urothelial carcinoma patient samples



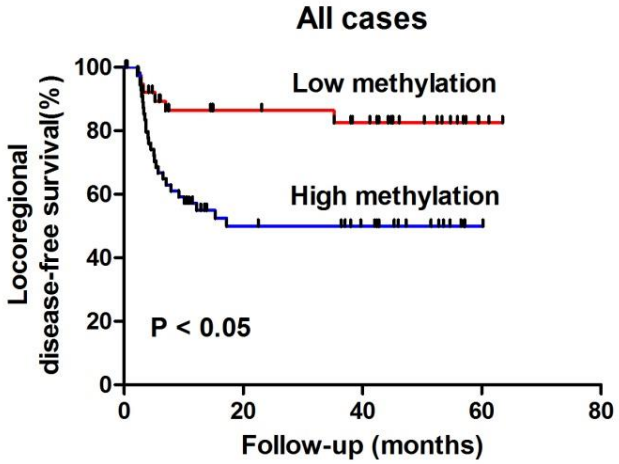


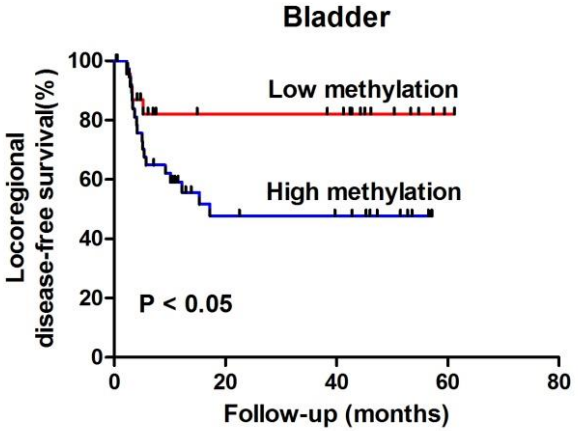


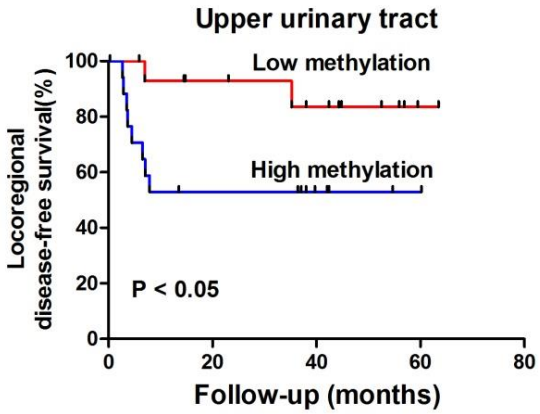


Yeh *et al,* Oncotarget 2015

Patients with higher ZNF671 methylation is associated with recurrence







Yeh *et al,* Oncotarget 2015

Table 5: Sensitivity and specificity of cancer detection using voided urine samples

	IRF8	*SFRP1*	*ZNF671*	*IRF8* or *SFRP1*	*ZNF671* or *IRF8*	*ZNF671* or *SFRP1*	[a]Marker panel
Sensitivity(%)							
All cases (n=26)	61.5%	50.0%	57.7%	88.4%	80.8%	84.6%	96.2%
Low grade (n=10)	50.0%	60.0%	40.0%	90.0%	60.0%	80.0%	90.0%
High grade (n=16)	68.8%	43.8%	68.8%	87.5%	94.1%	87.5%	100 %
Primary (n=22)	68.2%	45.4%	54.5%	86.3%	81.8%	81.8%	95.4%
Recurrent (n=4)	25.0%	75.0%	75.0%	100%	75.0%	100%	100%
Specificity(%)	94.7%	94.7%	89.5%	89.5%	84.2%	89.5%	84.2%
Positive predictive value (%)	94.1%	92.8%	88.2%	92.0%	87.5%	91.6%	92.6%
Negative predictive value(%)	64.2%	58.0%	60.0%	85.0%	76.2%	80.9%	94.4%

[a]Any one of these genes (*IRF8*, *SFRP1* or *ZNF671*) showed methylation in urine samples.

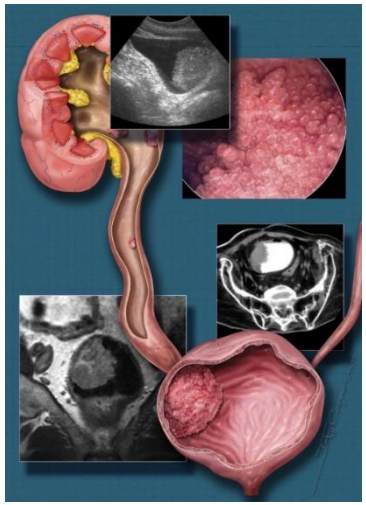
Yeh *et al*, Oncotarget 2015

Summary I

- Combination of DNA methylation biomarkers, including ZNF671, can be a sensitive non-invasive tool for bladder cancer detection and recurrent monitoring

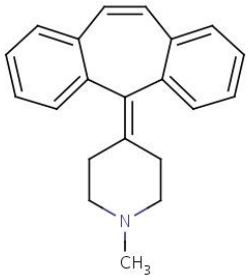
Treatment of Urothelial Carcinoma (Bladder Cancer)

- **Intravesical chemotherapy**
  - BCG
  - Mitomycin-C
  - Cisplatin
- **Radiotherapy**
- **Immune checkpoint inhibitors (only for advanced cancer with PD-L1 expression ≥ 5%)**



Cooper, Radiographics 2006;
http://www.drketandesai.com/kidney/things-to-know-about-urinary-bladder-tumor
http://www.svuhradiology.ie/case-study/obstructing-bladder-tcc/

Cyproheptadine (CPH, Periactin)



I. **The first-generation anti-histamine**
serotonin antagonist

II. **Treatment of allergic reactions**

- Hay Fever (1950s)
- atopic dermatitis
- appetite stimulant
- anorexia
- dyspeptic symptoms
- migraine prophylaxis
- antidepressant
- melatoninergic properties
- anti-cancer effect

III. **Common side effects**

- drowsiness

Novel treatment (new drug/intervention; established drug/procedure in new situation)

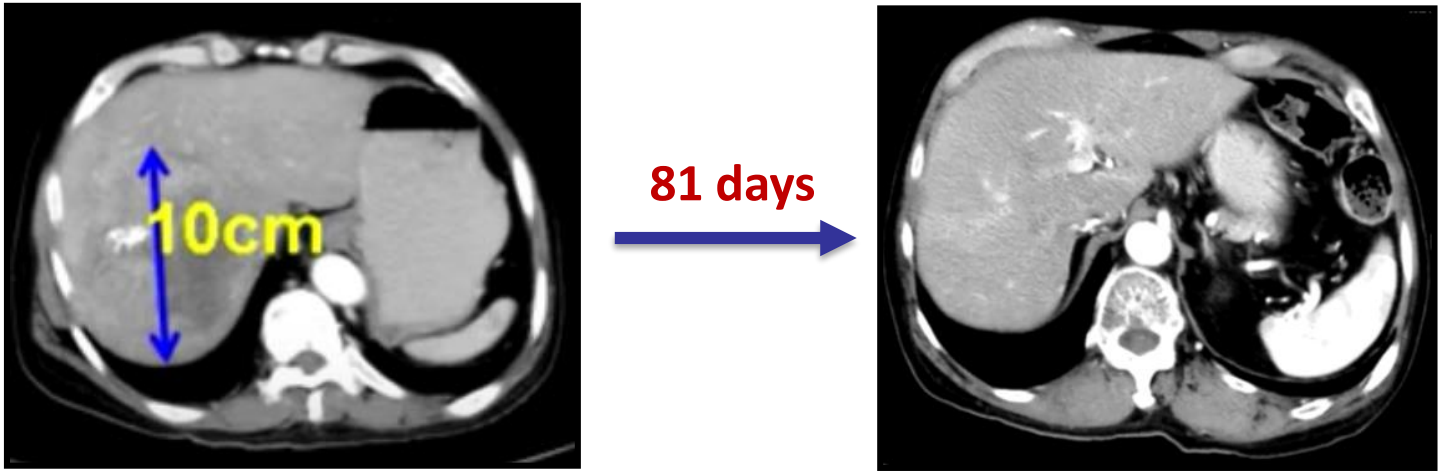
Unexpected remission of hepatocellular carcinoma (HCC) with lung metastasis to the combination therapy of thalidomide and cyproheptadine: report of two cases and a preliminary HCC cell line study

Yu-Min Feng,[1] Chin-Wen Feng,[2] Solomon Chih-Cheng Chen,[3] Cheng-Da Hsu[3]

BMJ Case Reports 2012

Case 1: Advanced HCC patient with lung metastasis, prescribed with Thalidomide (50mg BID) and **Cyproheptadine** (for skin itching, 4mg TID) for 6 months: complete remission of tumors in lung and liver.

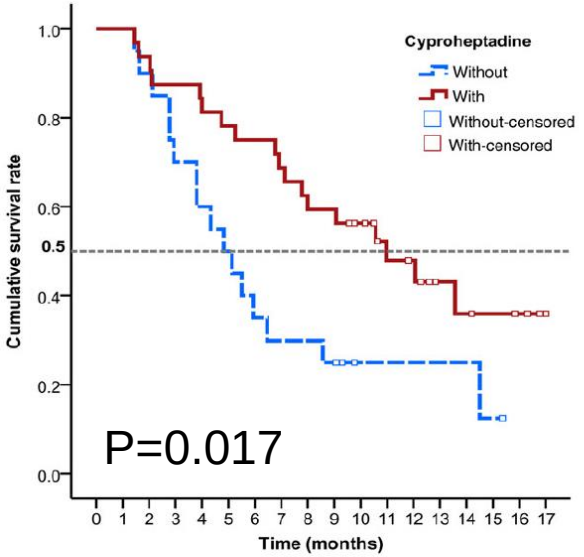
Before **After**



Cyproheptadine significantly improves the overall and progression-free survival of sorafenib-treated advanced HCC patients

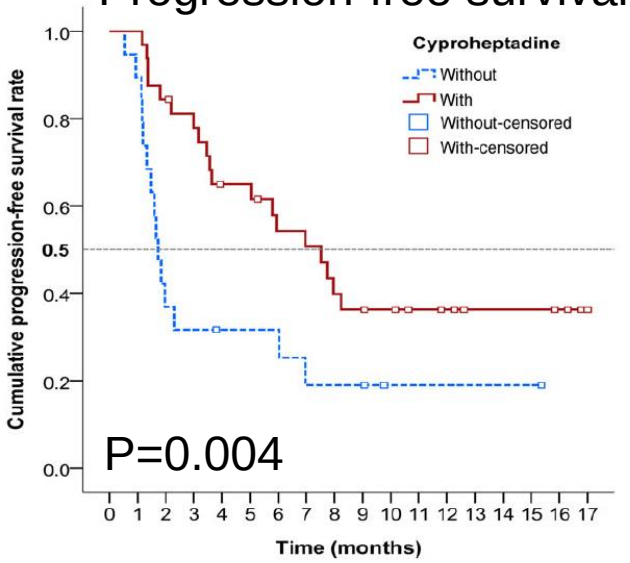
Yu-Min Feng[1], Chin-Wen Feng[2], Chin-Li Lu[3], Ming-Yang Lee[4], Chi-Yi Chen[1], and Solomon Chih-Cheng Chen[3,5,*]

Overall Survival



Cyproheptadine	0 months	3 months	6 months	9 months	12 months	15 months	17 months
Without	20	14	7	5	2	1	0
With	32	28	24	19	10	4	0

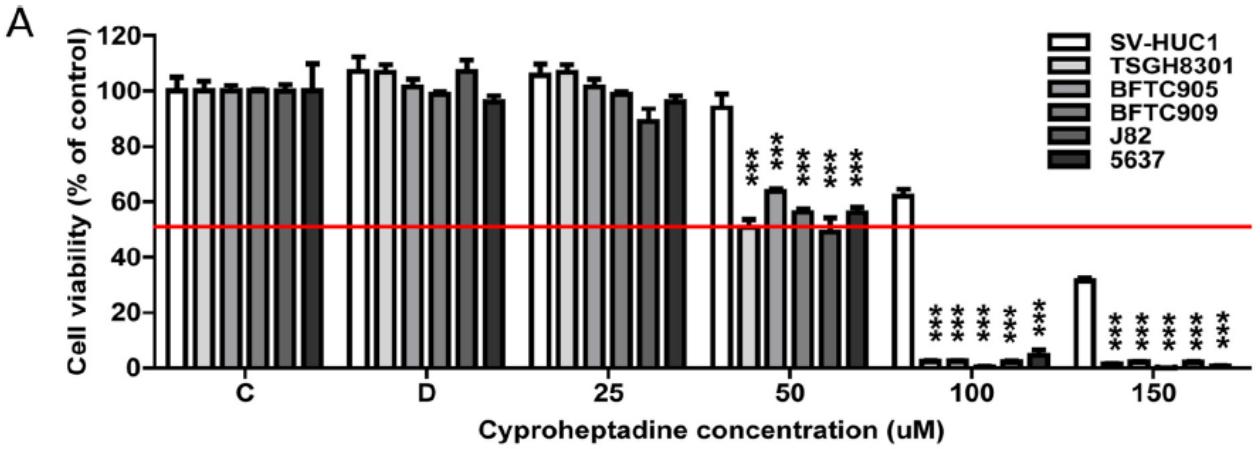
Progression-free survival

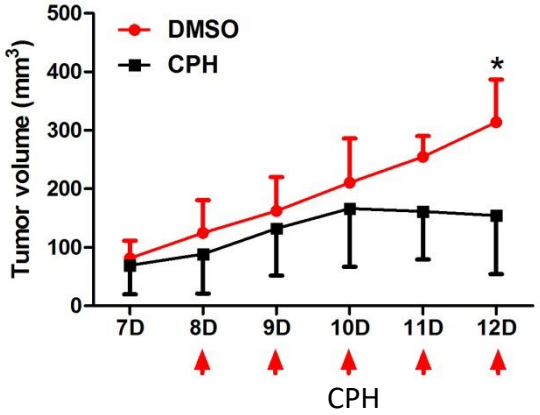


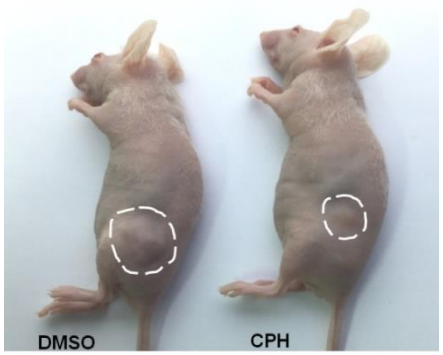
Cyproheptadine	0 months	3 months	6 months	9 months	12 months	15 months	17 months
Without	19	6	5	3	1	1	0
With	32	24	15	10	6	4	0

Feng *et al.*, Jpn J Clin Oncol 2015

Selective anti-cancer effect of CPH in bladder cancer *in vitro* and *in vivo*

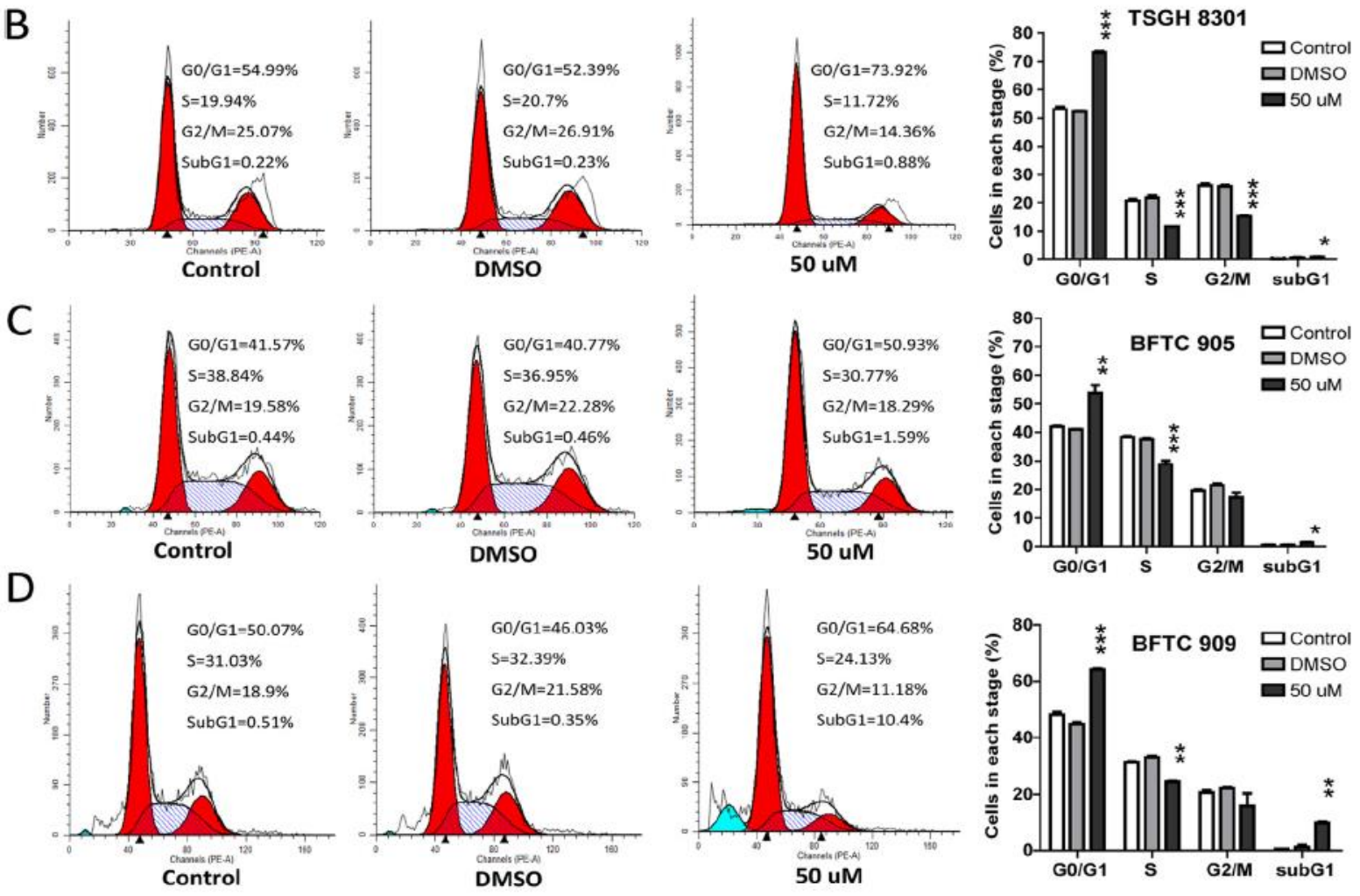






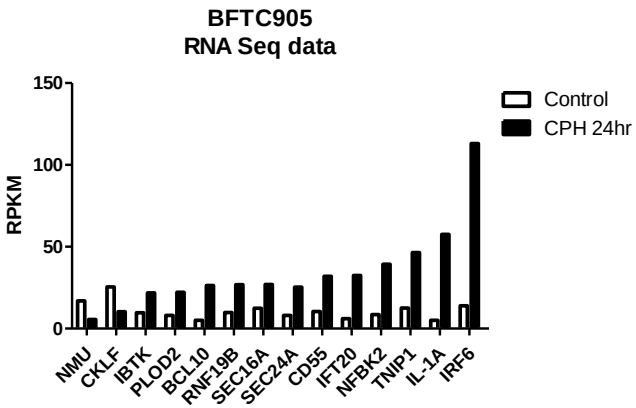
Hsieh *et al*., Cancer Lett 2016

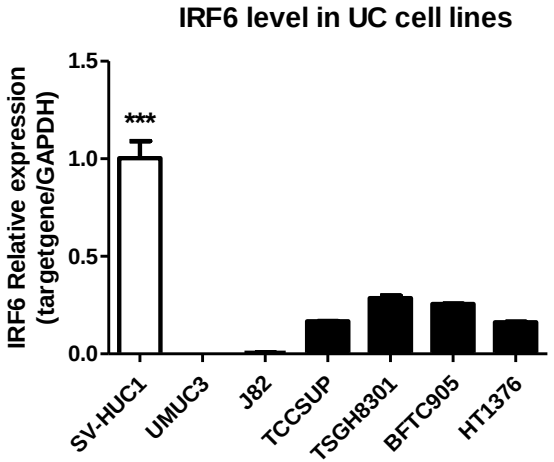
CPH induced G1 arrest in bladder cancer

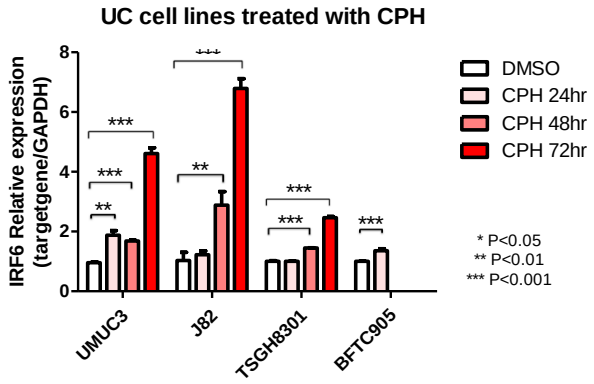


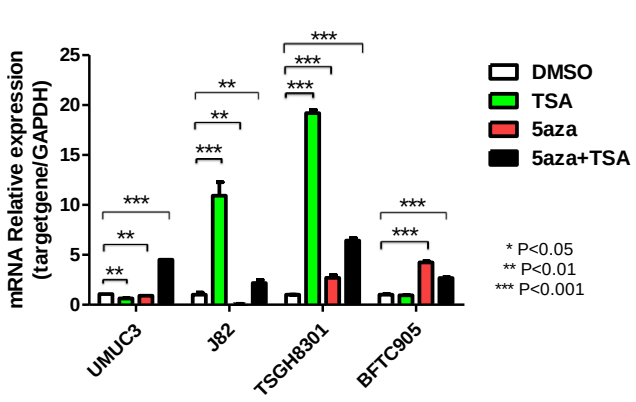
Hsieh *et al*., Cancer Lett 2016

IRF6 is overexpressed in bladder cancer cells treated with CPH or epi-drugs

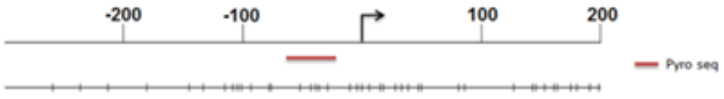


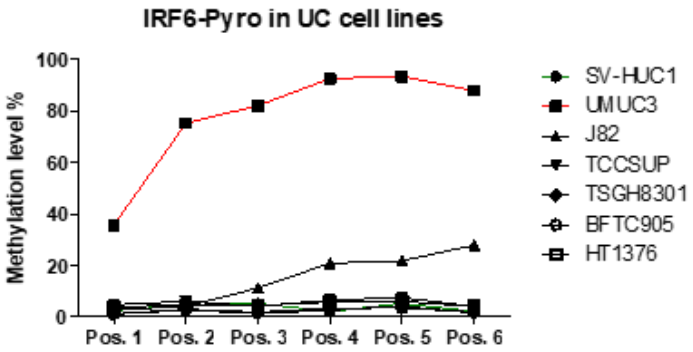


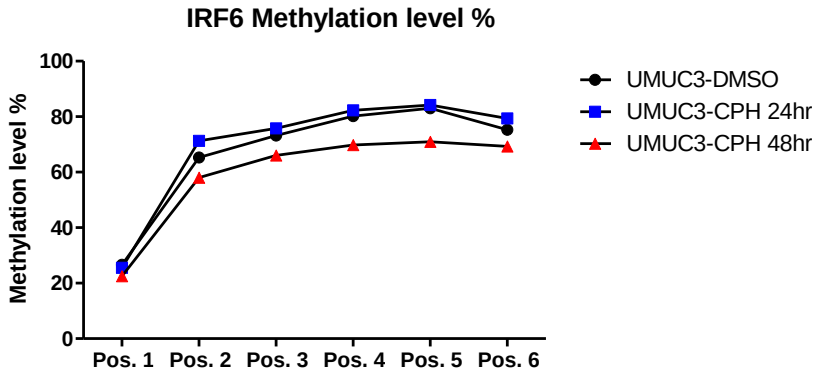


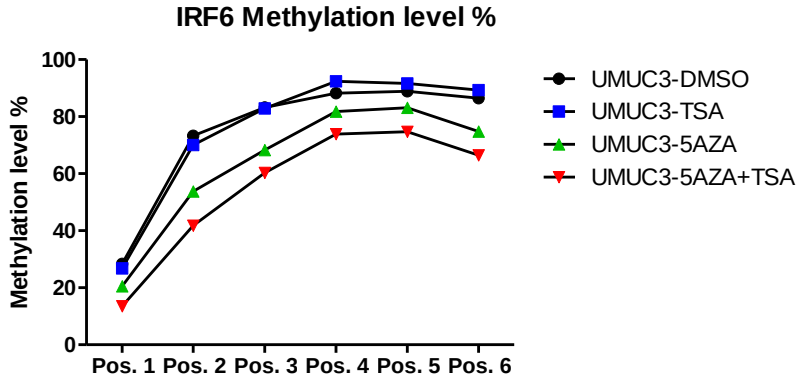


Treatment of CPH or epi-drugs induced DNA hypomethylation in IRF6 promoter

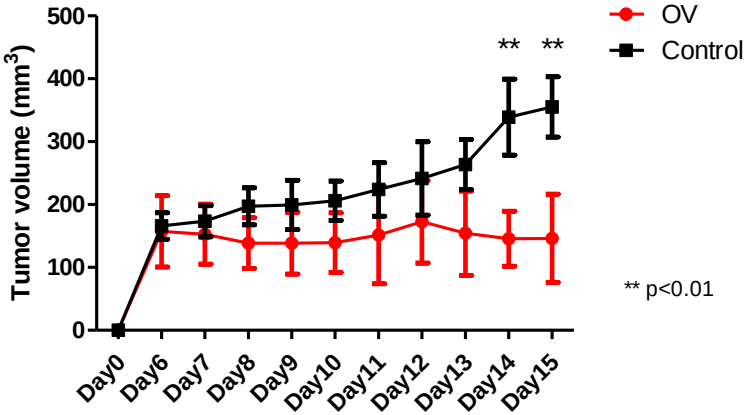


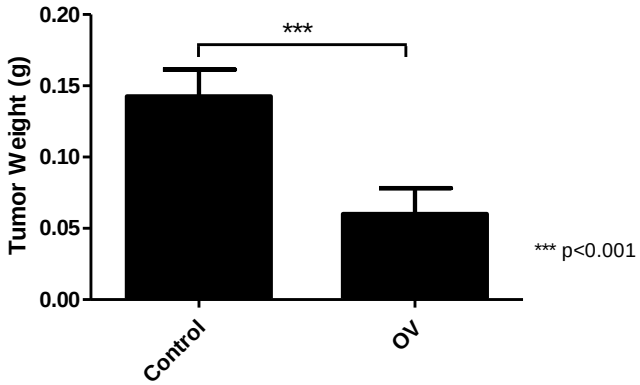




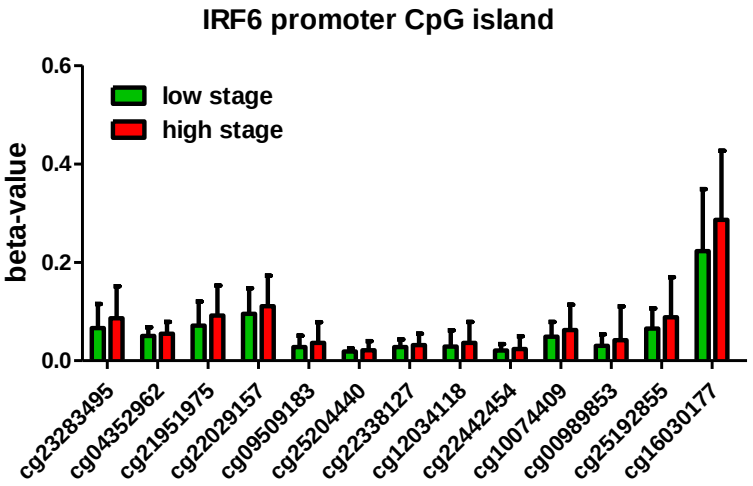


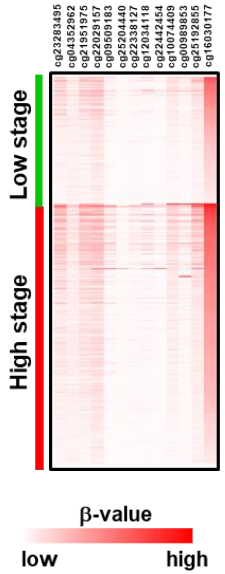
Overexpression of IRF6 inhibited tumor growth in vivo

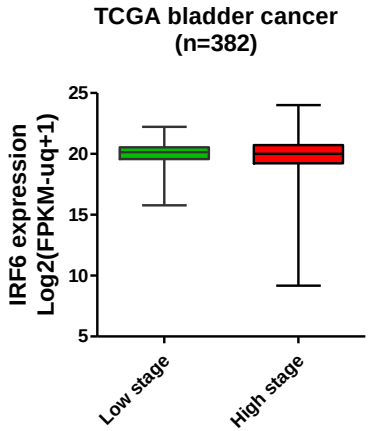


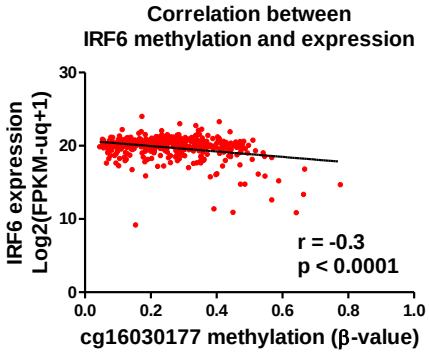


IRF6 is epigenetically silenced in high-staged bladder cancer patients







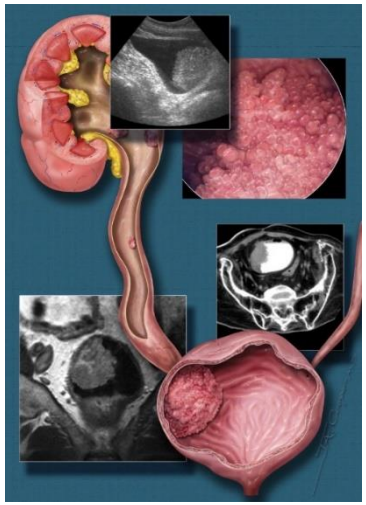


Summary II

- IRF6 is a potential tumor suppressor that is epigenetically silenced in bladder.
- CPH induced IRF6 promoter hypomethylation in bladder cancer cells

Treatment of Urothelial Carcinoma (Bladder Cancer)

- **Intravesical chemotherapy**
  - BCG
  - Mitomycin-C
  - Cisplatin
- **Radiotherapy**
- **Immune checkpoint inhibitors (only for advanced cancer with PD-L1 expression ≥ 5%)**



Cooper, Radiographics 2006;
http://www.drketandesai.com/kidney/things-to-know-about-urinary-bladder-tumor
http://www.svuhradiology.ie/case-study/obstructing-bladder-tcc/

The Nobel Prize in Physiology or Medicine 2018

James P. Allison
Tasuku Honjo

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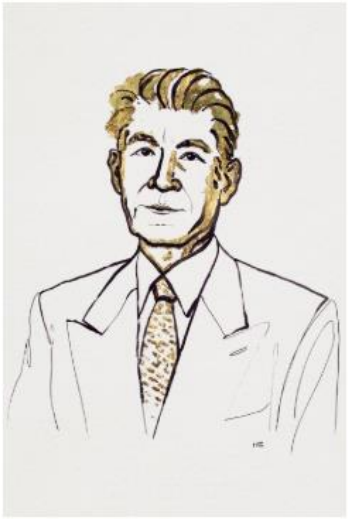
The Nobel Prize in Physiology or Medicine 2018

CTLA-4

Ill. Niklas Elmehed. © Nobel Media
James P. Allison
Prize share: 1/2



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Tasuku Honjo
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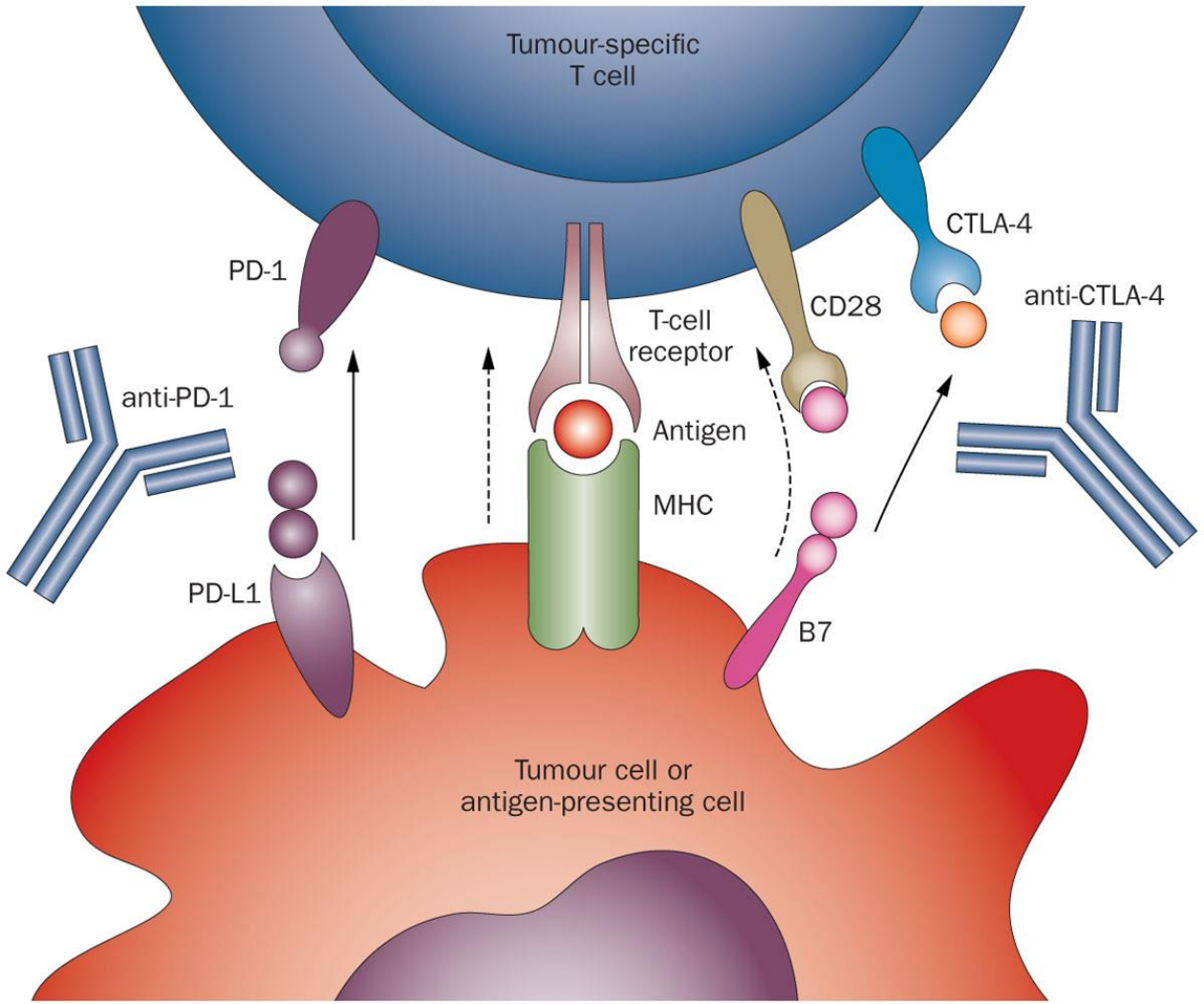
PD-1

The Nobel Prize in Physiology or Medicine 2018 was awarded jointly to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation."

Figure 2 Immune checkpoint blockade



Drake, C. G. *et al.* (2013) Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2013.208

nature REVIEWS CLINICAL ONCOLOGY

Table 1. FDA approvals of anti-PD-1/PD-L1 immunotherapeutic drugs in bladder and other cancers

Atezolizumab	Durvalumab	Avelumab	Nivolumab	Pembrolizumab
May 2016, pre-treated AMUC (bladder cancer)	May 2017, pre-treated advanced/metastatic (bladder cancer)	Mar 2017, metastatic Merkel cell carcinoma	December 2014, advanced melanoma	September 2014, advanced melanoma
October 2016, metastatic NSCLC cancer	February 2018, unresectable Stage III NSCLC cancer	May 2017, AMUC (bladder cancer)	May 2015, lung cancer	October 2015, advanced/metastatic NSCLC cancer
April 2017, first line treatment advanced/metastatic (bladder cancer)			November 2015, metastatic renal cell carcinoma	August 2016, recurrent/ metastatic head and neck squamous carcinoma
			May 2016, Hodgkin lymphoma	October 2016, first line treatment of metastatic NSCLC
			November 2016, head and neck cancer	March 2017, classical Hodgkin lymphoma
			February 2017, pre-treated AMUC (bladder cancer)	May 2017, AMUC (bladder cancer)
			August 2017, metastatic colorectal cancer with MSI or MMR deficiency	May 2017, any solid cancer with MSI or MMR deficiency
			September 2017, pre-treated hepatocellular carcinoma	September 2017, pre-treated advanced/metastatic gastric, gastroesophageal cancer
			August 2018, pre-treated SCLC	June 2018, pre-treated advanced/metastatic cervical cancer
				June 2018, pre-treated PMBCL

AMUC=Advanced/Metastatic UC

Song *et al*., J Path 2019

The NEW ENGLAND JOURNAL of MEDICINE

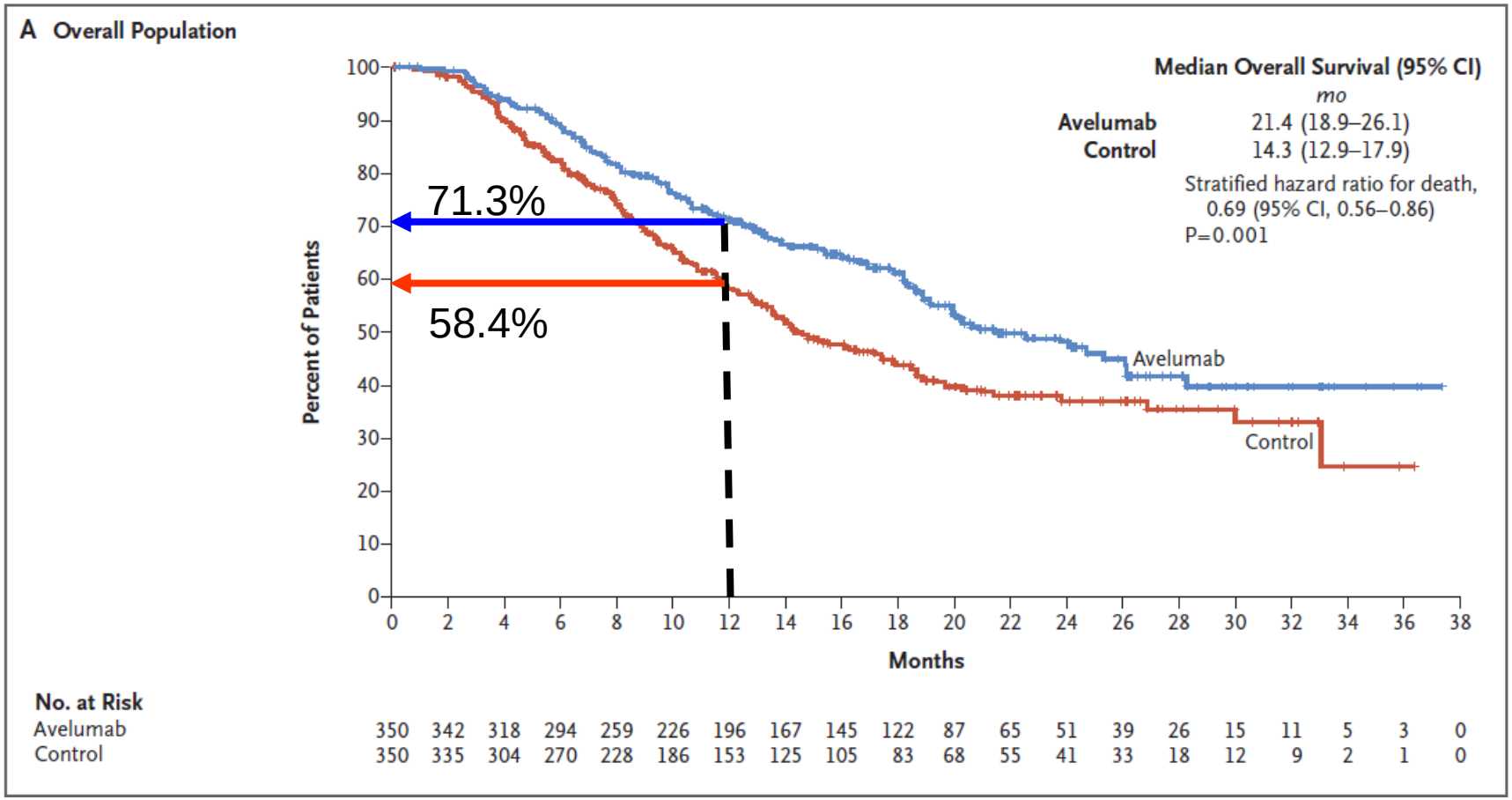
ORIGINAL ARTICLE

Avelumab Maintenance Therapy for Advanced or Metastatic Urothelial Carcinoma

T. Powles, S.H. Park, E. Voog, C. Caserta, B.P. Valderrama, H. Gurney, H. Kalofonos, S. Radulović, W. Demey, A. Ullén, Y. Loriot, S.S. Sridhar, N. Tsuchiya, E. Kopyltsov, C.N. Sternberg, J. Bellmunt, J.B. Aragon-Ching, D.P. Petrylak, R. Laliberte, J. Wang, B. Huang, C. Davis, C. Fowst, N. Costa, J.A. Blake-Haskins, A. di Pietro, and P. Grivas

N ENGL J MED 383;13 NEJM.ORG SEPTEMBER 24, 2020

JAVELIN Bladder 100 trial (NCT02603432)


A Overall Population
Median Overall Survival (95% CI)
mo
Avelumab 21.4 (18.9–26.1)
Control 14.3 (12.9–17.9)
Stratified hazard ratio for death, 0.69 (95% CI, 0.56–0.86)
P=0.001
71.3%
58.4%
Avelumab
Control
Percent of Patients
100
90
80
70
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Months
No. at Risk
Avelumab 350 342 318 294 259 226 196 167 145 122 87 65 51 39 26 15 11 5 3 0
Control 350 335 304 270 228 186 153 125 105 83 68 55 41 33 18 12 9 2 1 0

epigenomes

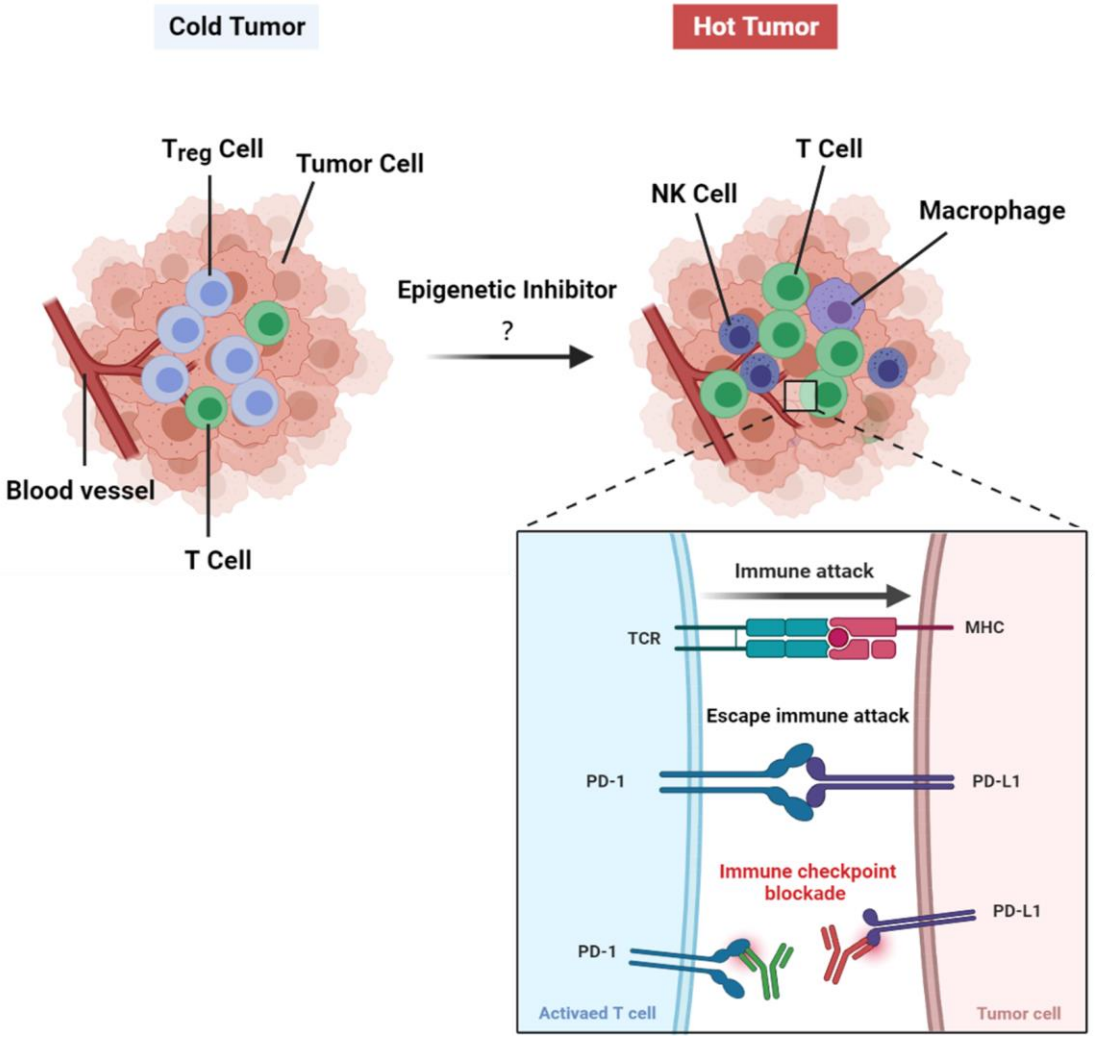
MDPI

Editorial

Firing up Cold Tumors - Targeting the Epigenetic Machinery to Enhance Cancer Immunotherapy

Guan-Ling Lin[1,2], Leah H.J Tsai[1,2], Peter J.K. Kuppen[3,*], and Michael W.Y. Chan[1,2,*]

2021 May, Vol 5 Issue 2



epigenomes

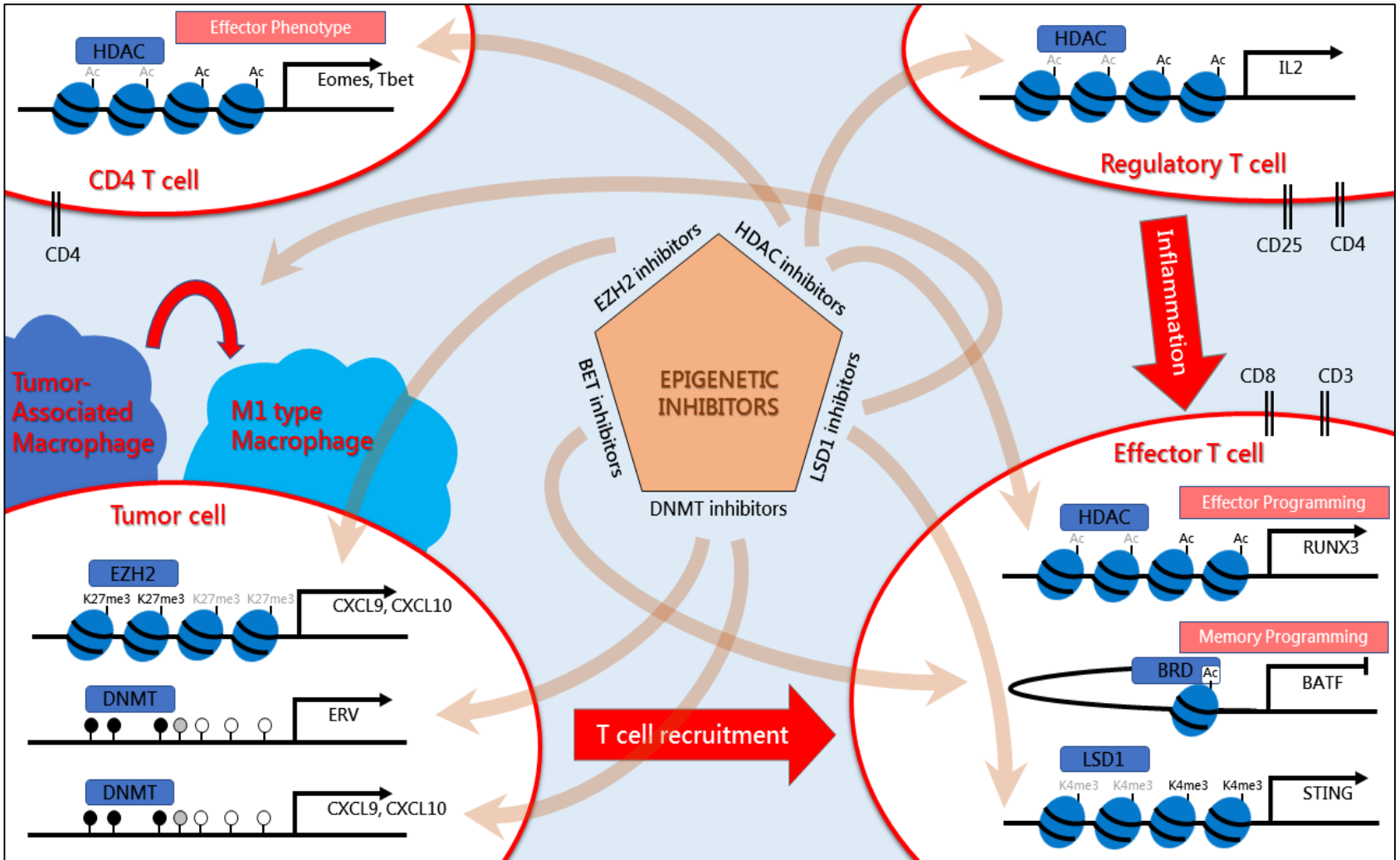
MDPI

Review

Combinatorial Epigenetic and Immunotherapy in Breast Cancer Management: A Literature Review

Yu-Ting Lee [1,2,3,4,*,†], Yu-Ming Chuang [1,2,3,†] and Michael W. Y. Chan [1,2,3,*]

2020 December, Vol 4 Issue 4



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Department of Urology

Yu-Ming Feng

Cheng-Huang Shen

Yeong-Chin Jou

Pi-Che Chen

UTHSC, San Antonio

Tim H.-M. Huang

Cedars-Sinai Medical Center, LA

Charles J Rosser

The Chinese University of HK

Ka-Fai To

Joanna Tong

Chi-Fai Ng

Hebei Medical University

Yanning Chen

Jinan University, Guangzhou

Xiaoling Wang

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