

Cancer Epigenomics

Manel Esteller

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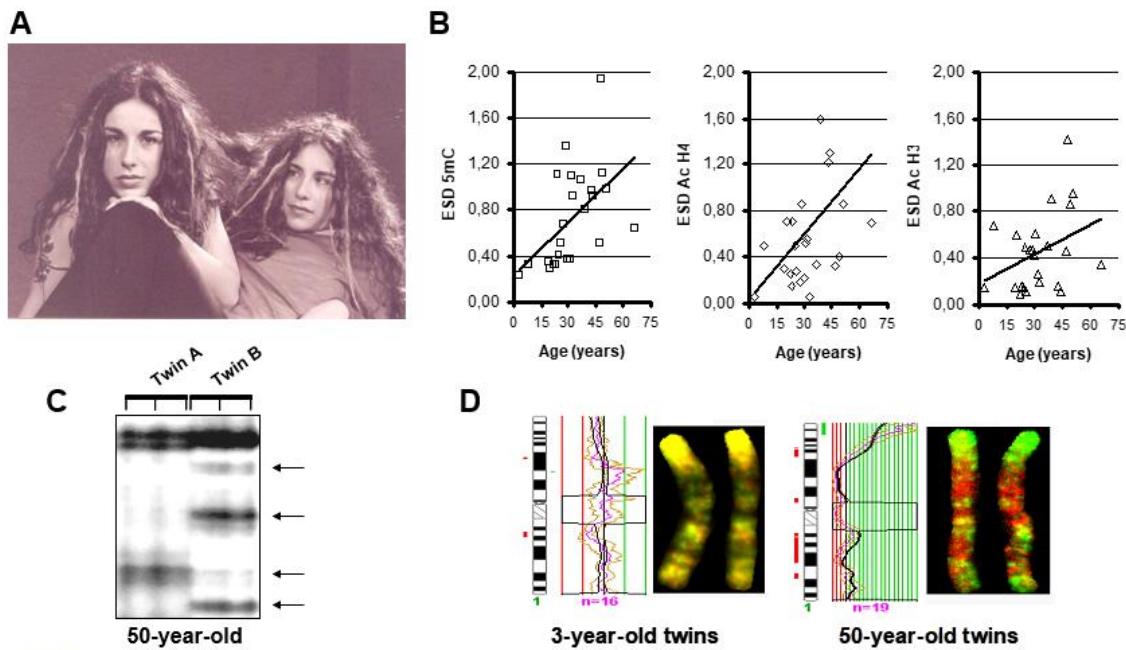


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ICREA Research Professor

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Director, Josep Carreras Leukaemia Research Institute (IJC)

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Natural Born Clones: Genotype vs Phenotype



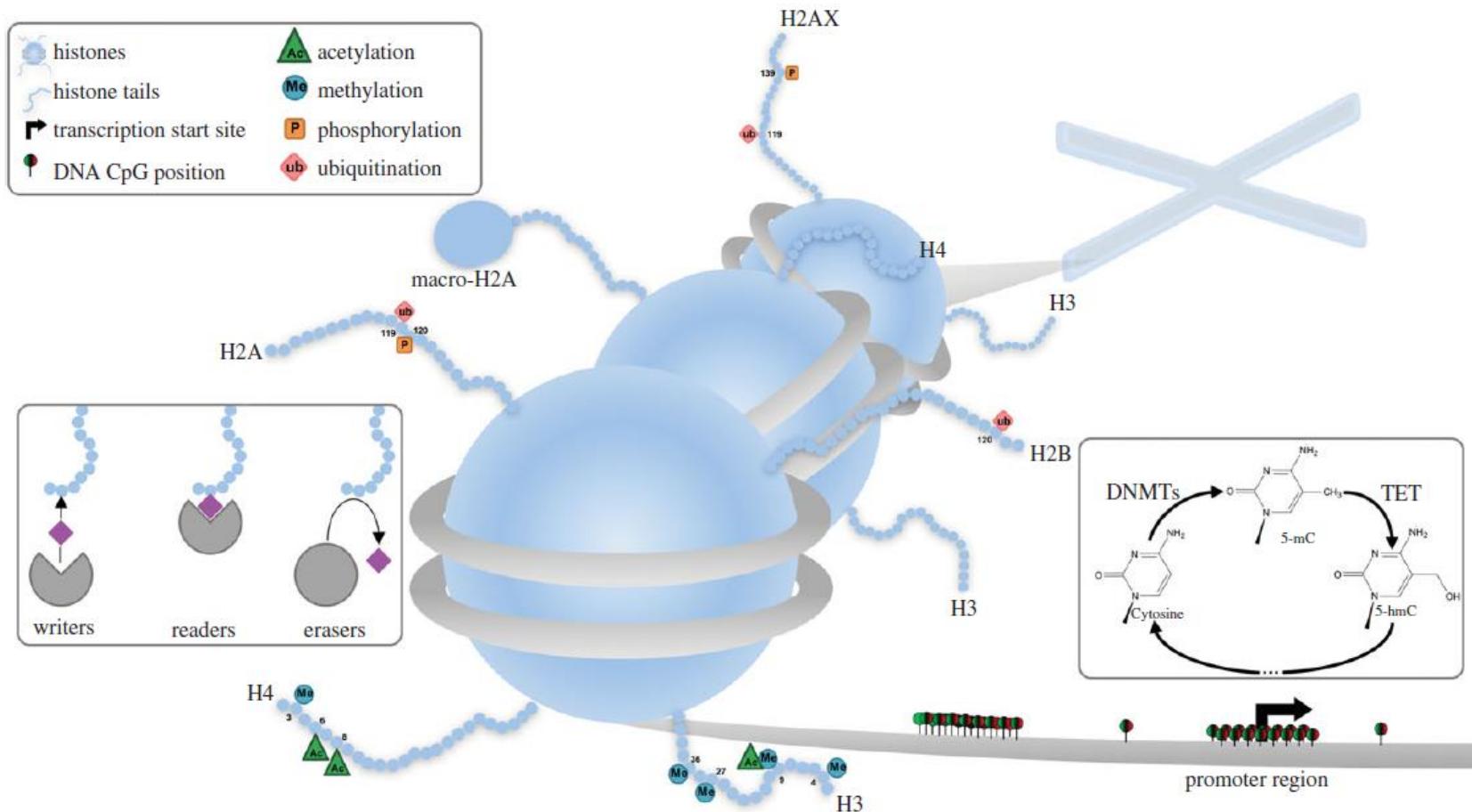
Epigenetic differences arise during the lifetime of monozygotic twins

Mario F. Fraga*, Esteban Ballestar*, María F. Paz*, Santiago Ropero*, Fernando Setien*, María L. Ballestar†, Damia Heine-Suñer‡, Juan C. Cigudosa§, Miguel Urioste‡, Javier Benítez‡, Manuel Boix-Chornet‡, Abel Sanchez-Aguilera‡, Charlotte Ling‡, Emma Carlsson‡, Per末ille Poulsen**+, Allan Vaag**, Zarko Stephan‡, Tim D. Spector‡, Yue-Zhong Wu‡, Christoph Plass‡, and Manel Esteller*§¶

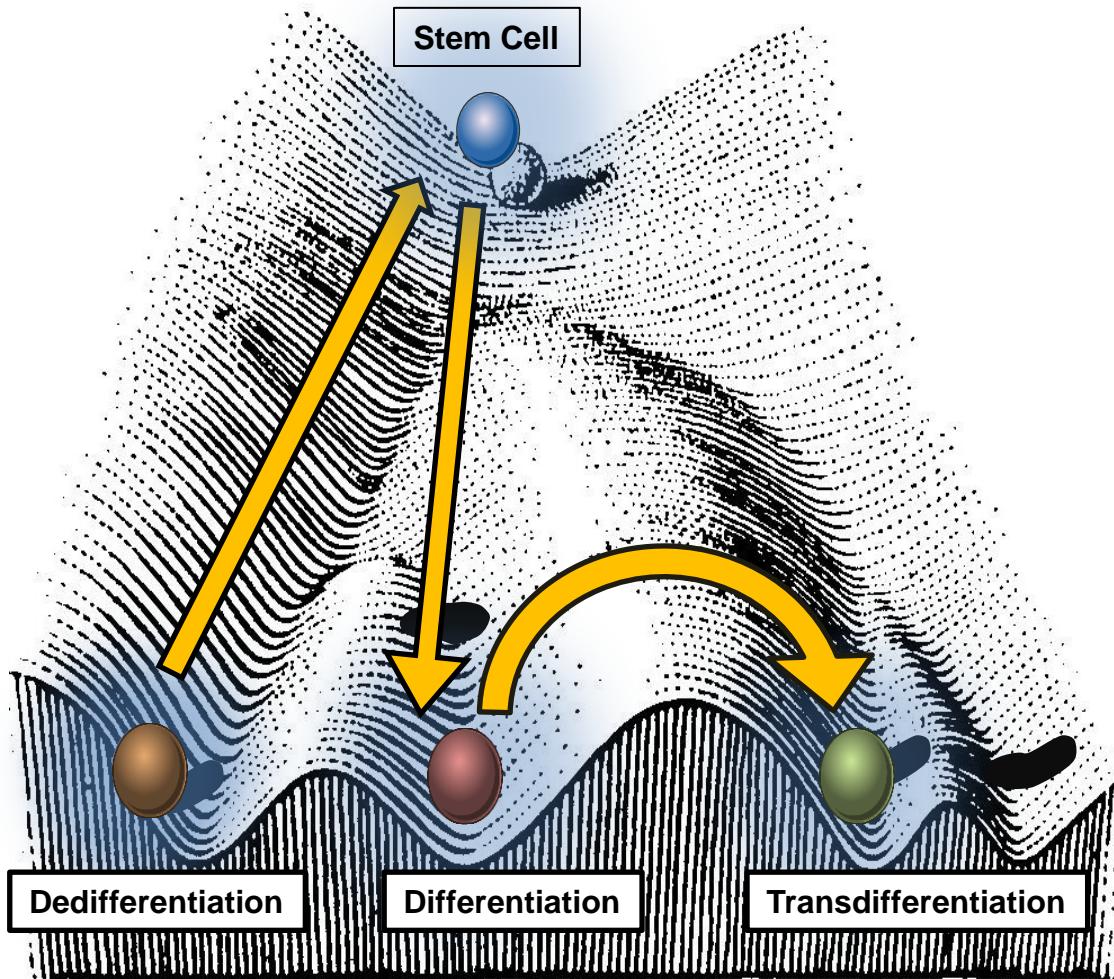
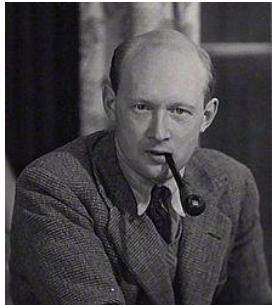
*Epigenetics, †Cytogenetics, and §Genetic Laboratories, Spanish National Cancer Centre (CNIO), Melchor Fernández Almagro 3, 28029 Madrid, Spain; ‡Department of Behavioral Science, University of Valencia, 46010 Valencia, Spain; §Molecular Genetics Laboratory, Genetics Department, Son Dureta Hospital, 07014 Palma de Mallorca, Spain; ¶Department of Clinical Sciences, University Hospital Malmö, Lund University, S-205 02 Malmö, Sweden; **Steno Diabetes Center, 2820 Gentofte, Denmark; ††Twin Research and Genetic Epidemiology Unit, St. Thomas' Hospital, London SE1 7EH, United Kingdom; and ‡‡Human Cancer Genetics Program, Department of Molecular Virology, Immunology, and Medical Genetics, Ohio State University, Columbus, OH 43210

Edited by Stanley M. Gartler, University of Washington, Seattle, WA, and approved May 23, 2005 (received for review January 17, 2005)

Epigenetic Setting: DNA Methylation and Histone Modifications



Epigenetic Roads to Cell and Tissue Identity



Differentiated Cells

Dedifferentiation

Differentiation

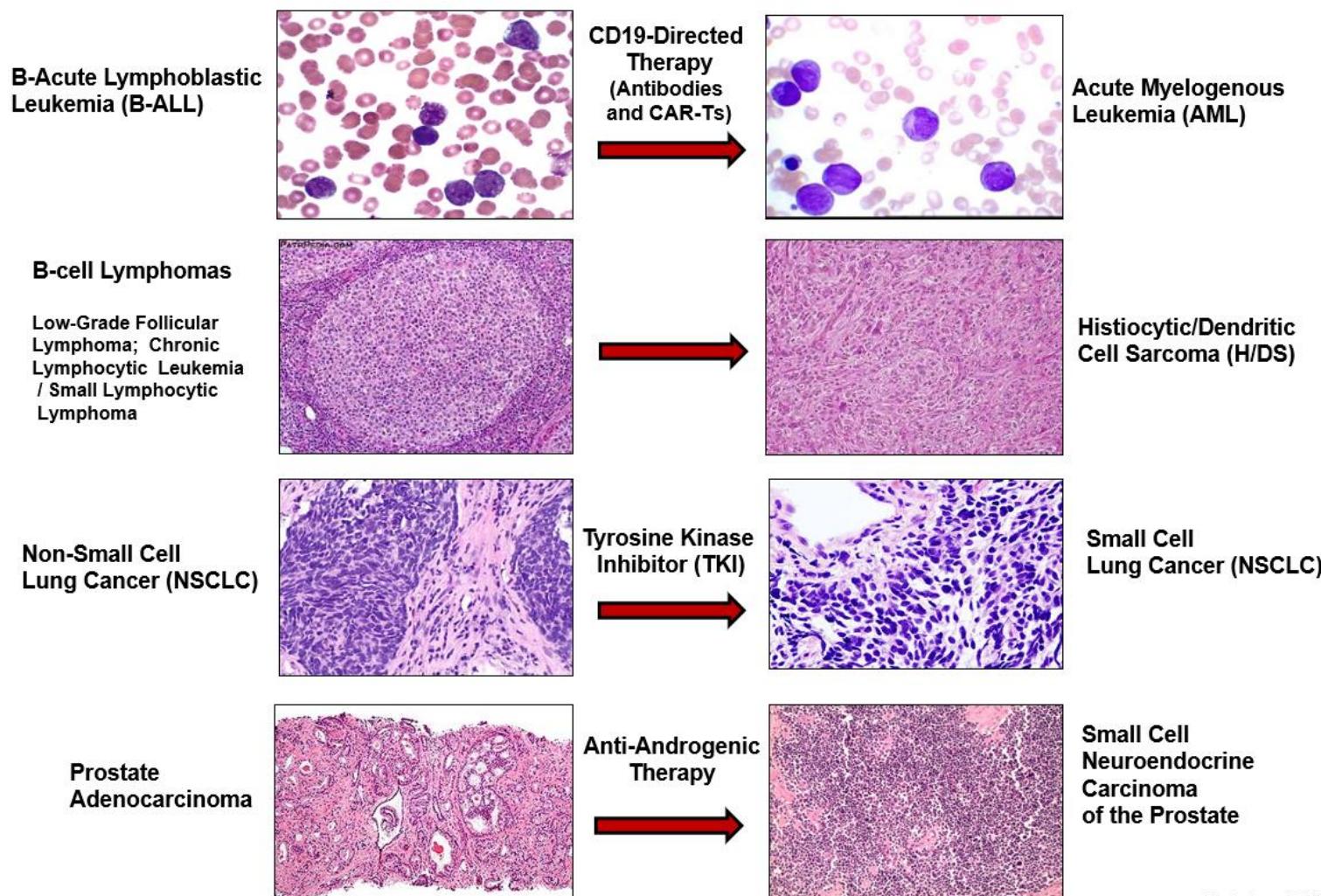
Transdifferentiation

Barrero et al.,
Stem Cells 2012

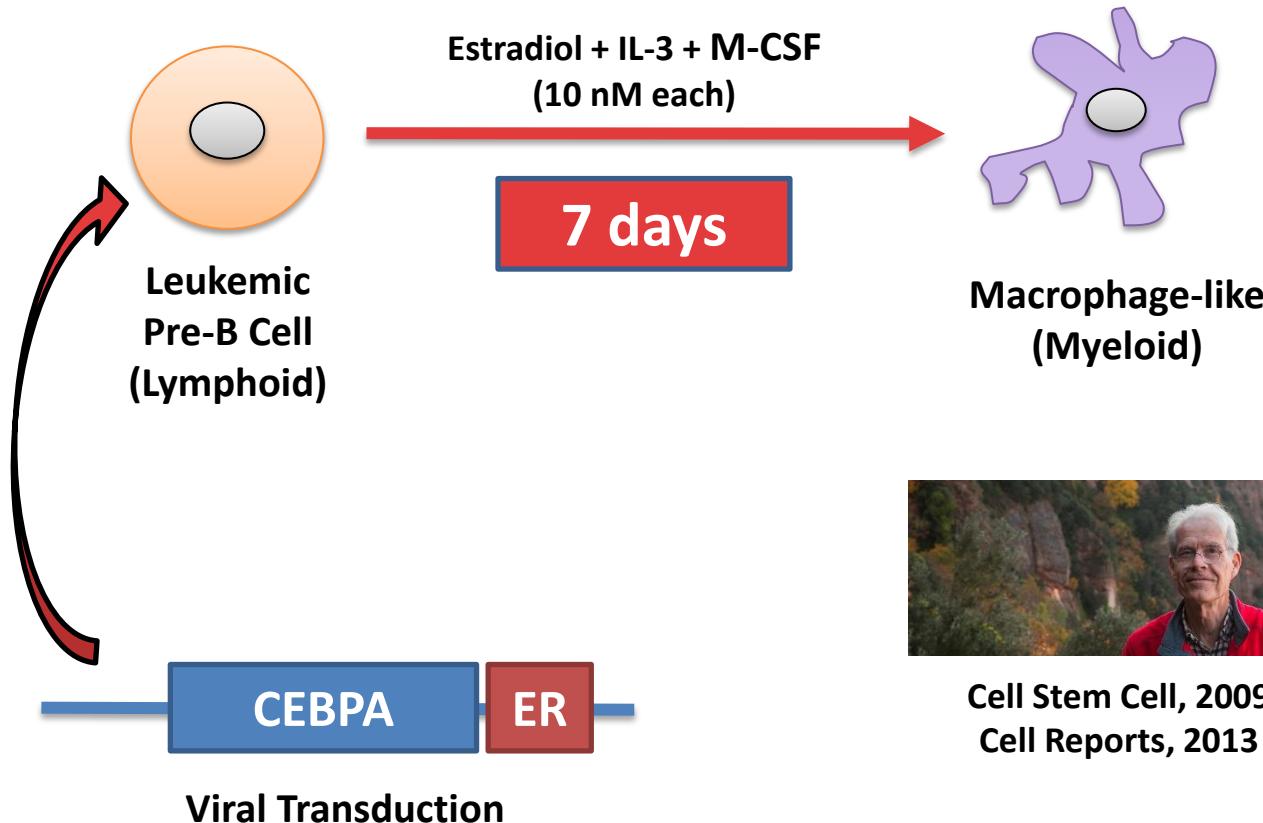
Berdasco et al.,
Am J Pathol 2012

Bueno-Costa et al.,
Leukemia 2019

Examples of Transdifferentiation in Human Cancer

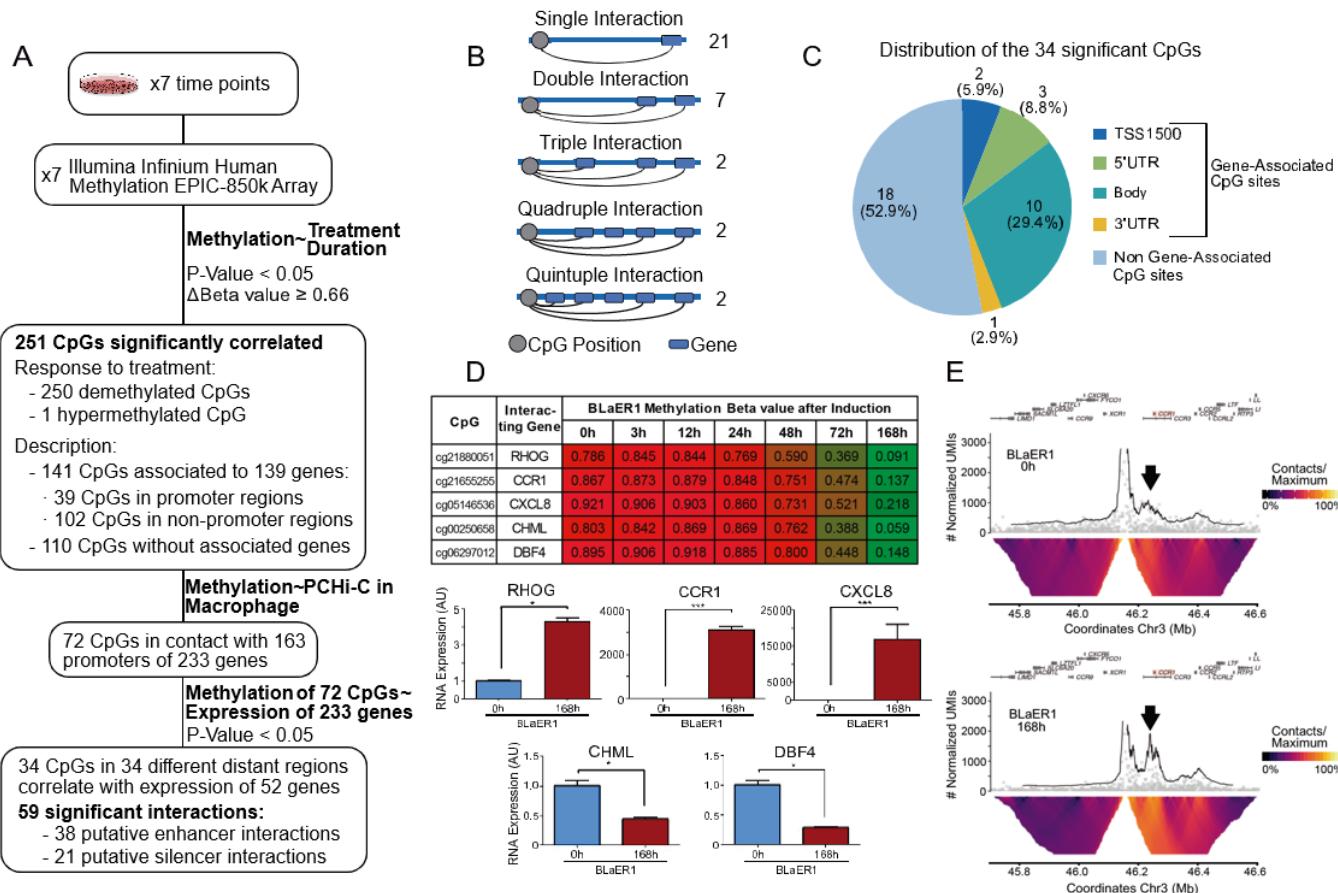


Human Leukemia Transdifferentiation Model (BLaER1)



Cell Stem Cell, 2009
Cell Reports, 2013

B-cell Leukemia Transdifferentiation to Macrophage Involves Reconfiguration of DNA Methylation for Long-Range Regulation



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I see mutated epigenetic genes...

nature
genetics

LETTERS

Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

ARID1A Mutations in Endometriosis-Associated Ovarian Carcinomas

Frequent Mutations of Chromatin Remodeling Gene *ARID1A* in Ovarian Clear Cell Carcinoma

nature
genetics

LETTERS

Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder

nature

Vol 463 | 21 January 2010 doi:10.1038/nature08672

LETTERS

Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Mutation in *TET2* in Myeloid Cancers

ARTICLE

doi:10.1038/nature10951

Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma

nature
genetics

ARTICLES

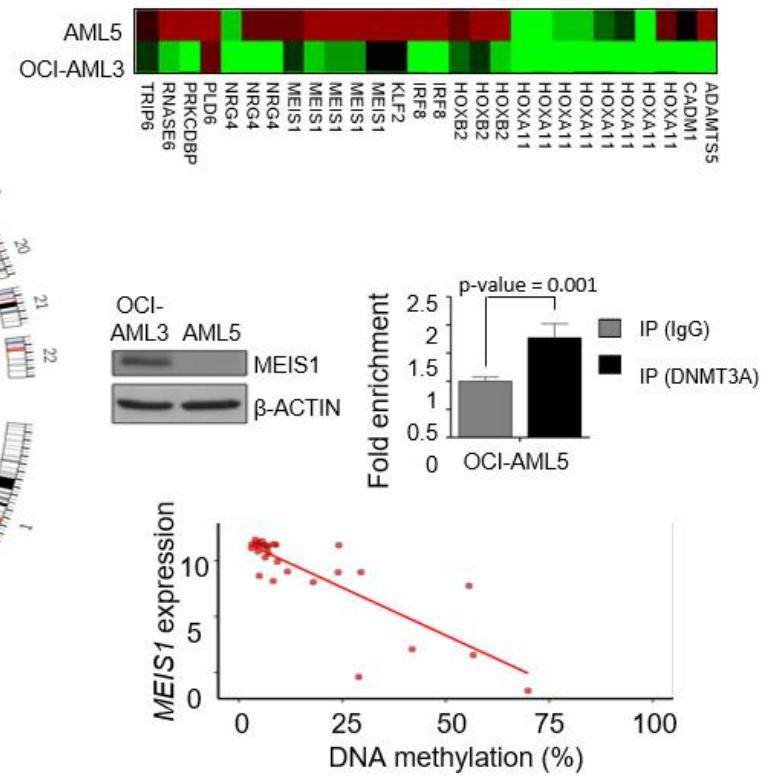
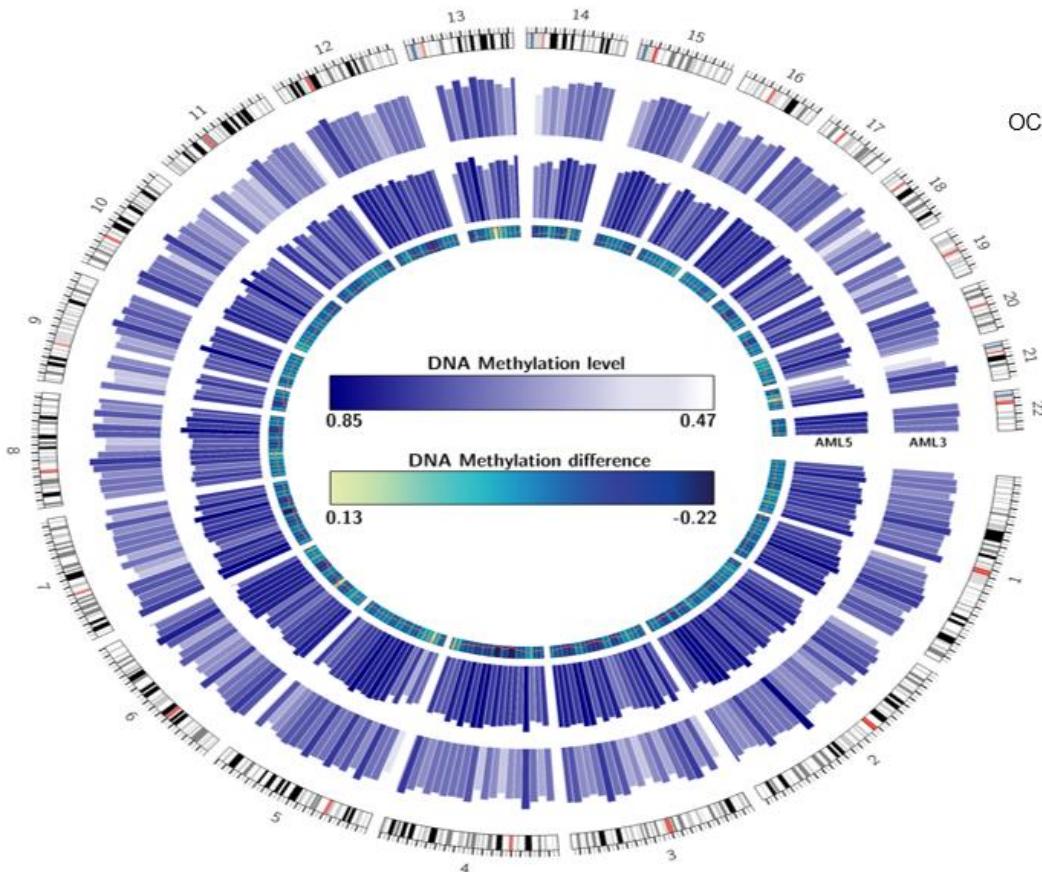
Exome sequencing identifies somatic mutations of DNA methyltransferase gene *DNMT3A* in acute monocytic leukemia

LETTER

doi:10.1038/nature09639

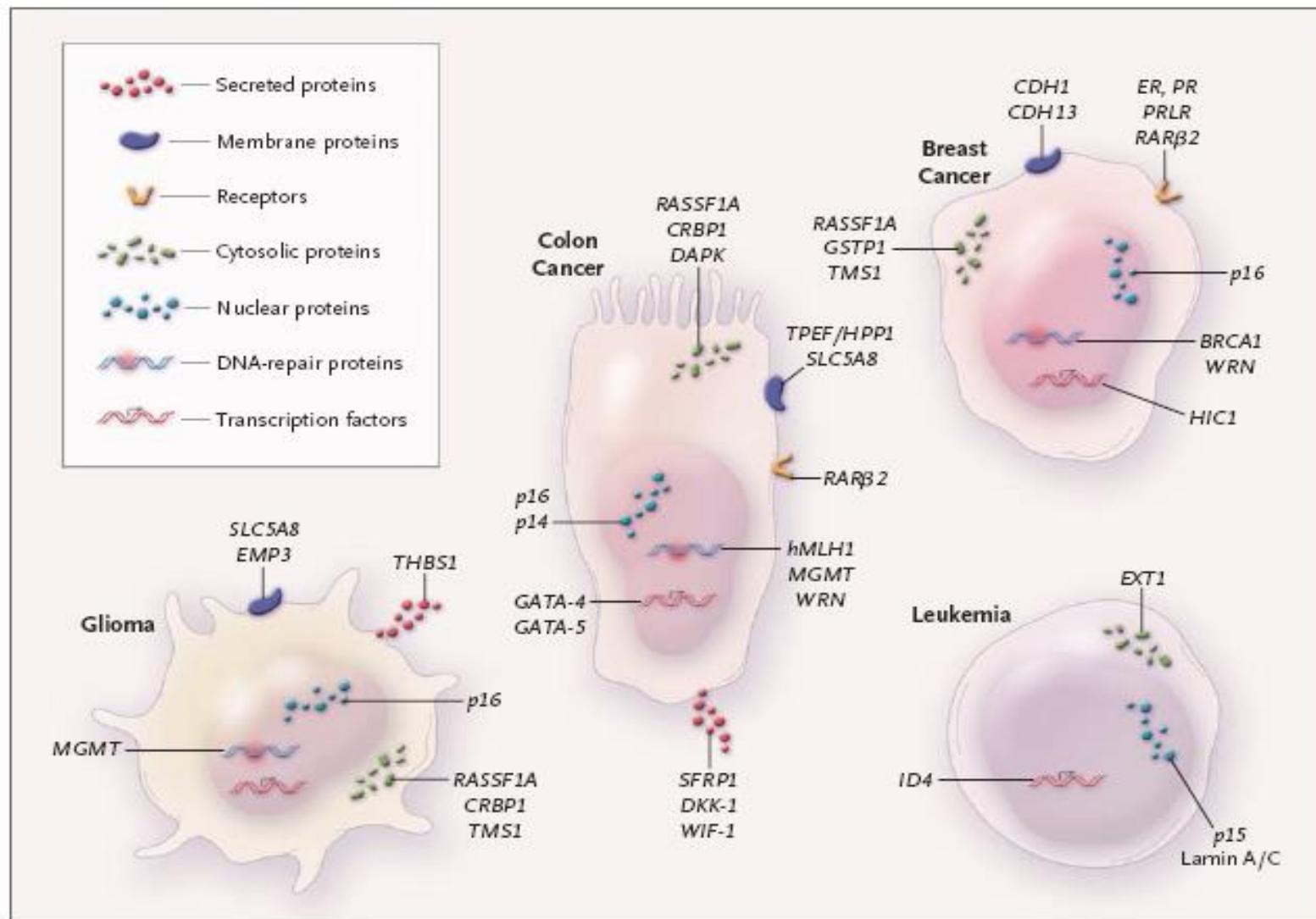
Exome sequencing identifies frequent mutation of the SWI/SNF complex gene *PBRM1* in renal carcinoma

DNMT3A mutations mediate the epigenetic reactivation of the leukemogenic factor MEIS1 in acute myeloid leukemia

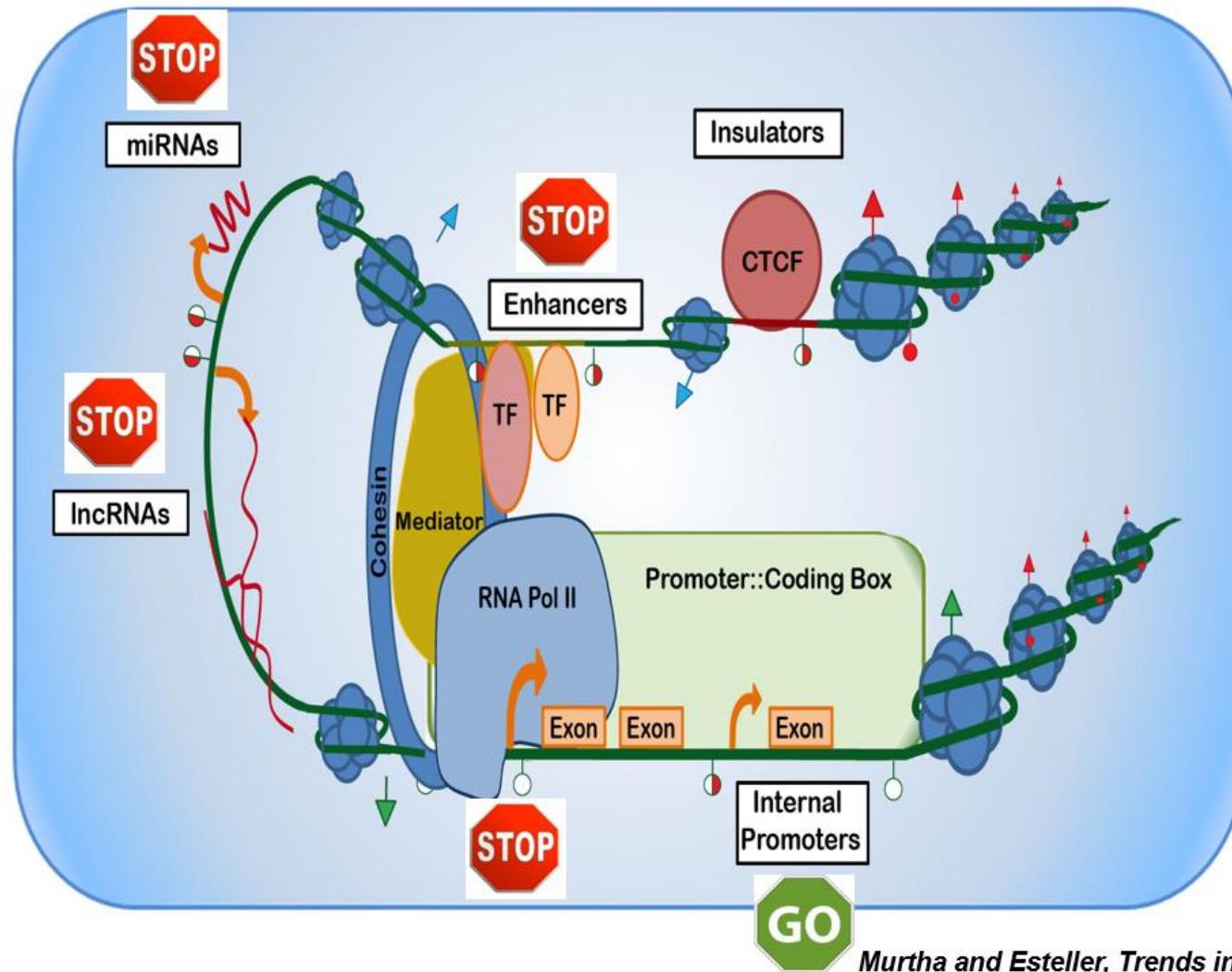


Ferreira et al., *Oncogene* 2015

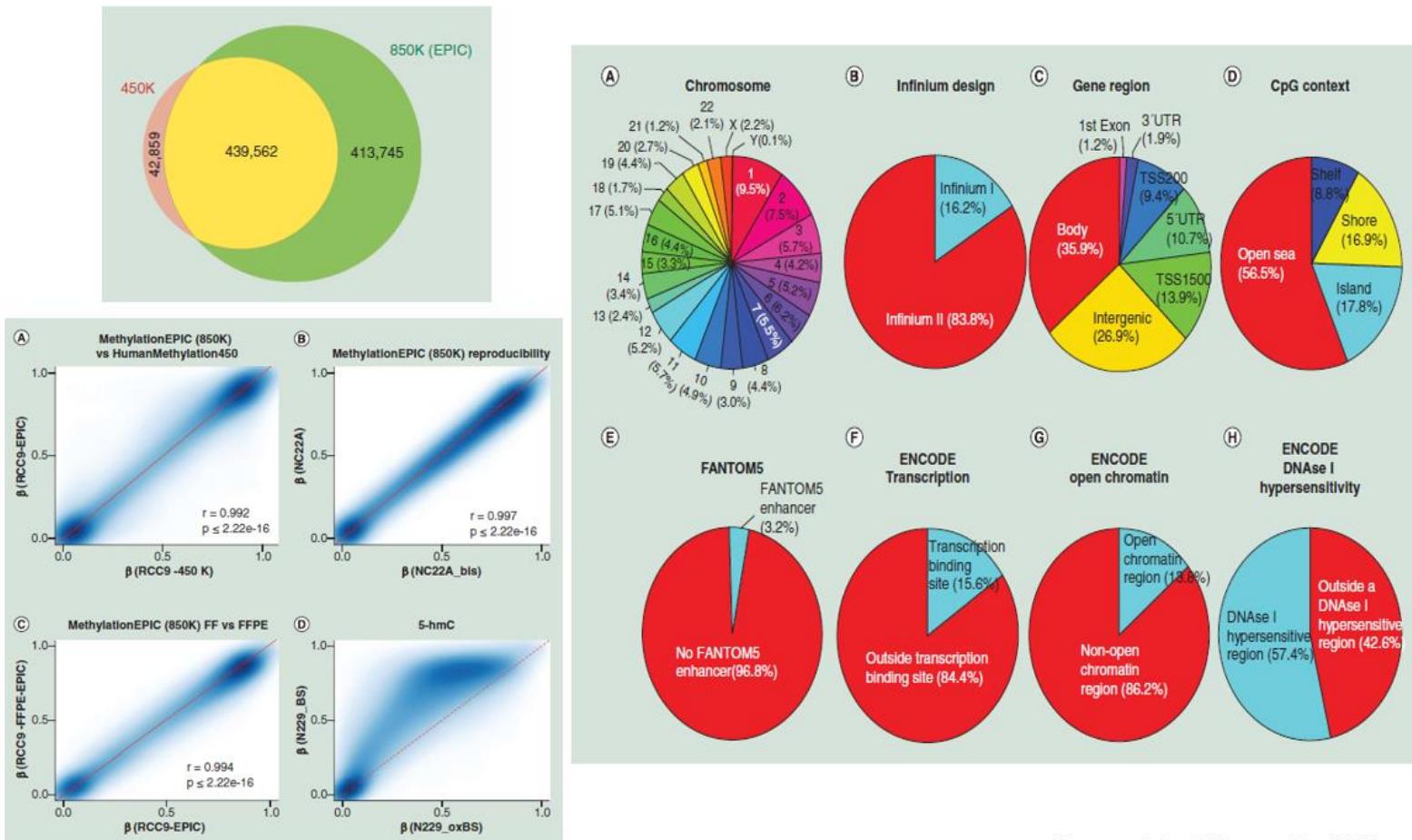
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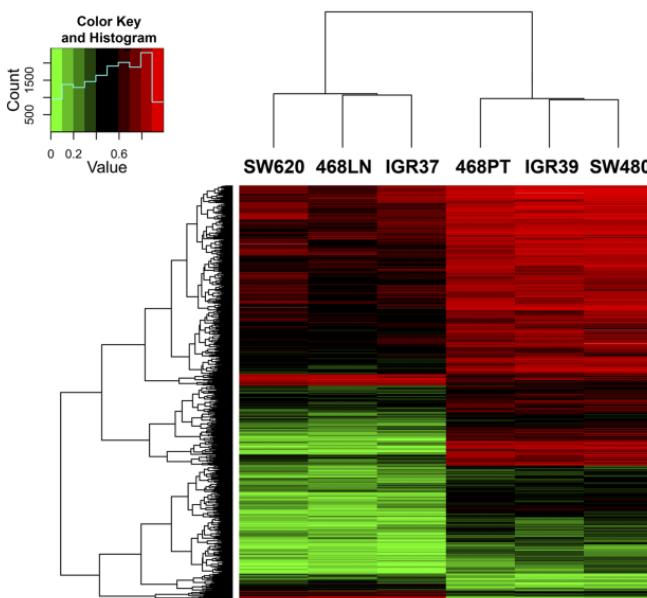
Extraordinary Cancer Epigenomics: Outside the Classical Coding and Promoter Box



Validation of a DNA methylation microarray for 850,000 CpG sites of the human genome enriched in enhancer sequences



Epigenetic Reactivation of an Isoform of the Rab GTPase Activating Protein TBC1D16 in Human Metastases



RELATION TO UCSC CPG ISLAND	UCSC REFGENE GROUP	IGR37	IGR39
Island	-1426	0.1	0.1
Island	-819	0.0	0.0
Island	-686	0.0	0.0
Island	-684	0.0	0.0
Island	-673	0.0	0.0
Island	-620	0.1	0.1
Island	-611	0.1	0.1
Island	-607	0.1	0.1
Island	-353	0.0	0.0
Island	-113	0.0	0.1
Island	310	0.1	0.1
Island	367	0.0	0.0
Island	484	0.0	0.0
Island	502	0.0	0.1

RELATION TO UCSC CPG ISLAND	UCSC REFGENE GROUP	468LN	468PT
Island	-1426	0.3	0.1
Island	-819	0.0	0.0
Island	-686	0.0	0.0
Island	-684	0.0	0.0
Island	-673	0.0	0.0
Island	-620	0.1	0.1
Island	-611	0.1	0.1
Island	-607	0.0	0.1
Island	-353	0.0	0.0
Island	-113	0.1	0.1
Island	310	0.1	0.1
Island	367	0.0	0.0
Island	484	0.0	0.0
Island	502	0.1	0.1

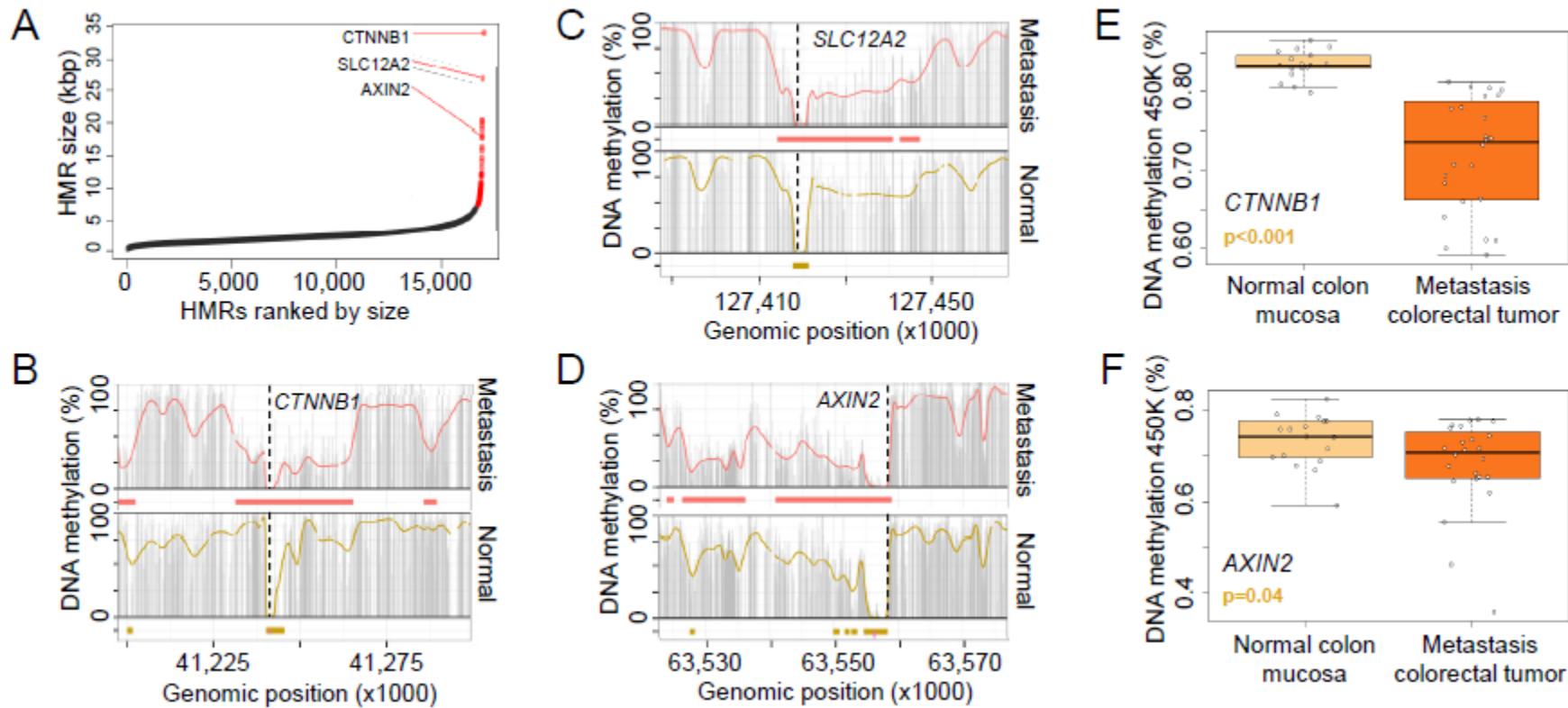
RELATION TO UCSC CPG ISLAND	UCSC REFGENE GROUP	SW620	SW480
Island	-1426	0.1	0.1
Island	-819	0.0	0.1
Island	-686	0.0	0.0
Island	-684	0.0	0.0
Island	-673	0.0	0.0
Island	-620	0.1	0.1
Island	-611	0.1	0.1
Island	-607	0.0	0.1
Island	-353	0.0	0.0
Island	-113	0.0	0.1
Island	310	0.1	0.1
Island	367	0.0	0.0
Island	484	0.0	0.1
Island	502	0.1	0.1

S Shore	-500	0.0	1.0
S Shore	-97	0.0	0.7
S Shore	-29	0.1	0.9
S Shore	54	0.1	0.9
Island	265	0.0	1.0
Island	361	0.1	0.8
Island	367	0.0	0.9
Island	456	0.0	0.9

S Shore	-500	0.1	1.0
S Shore	-97	0.0	0.9
S Shore	-29	0.1	0.9
S Shore	54	0.1	0.8
Island	265	0.0	1.0
Island	361	0.1	0.9
Island	367	0.1	0.9
Island	456	0.1	0.9

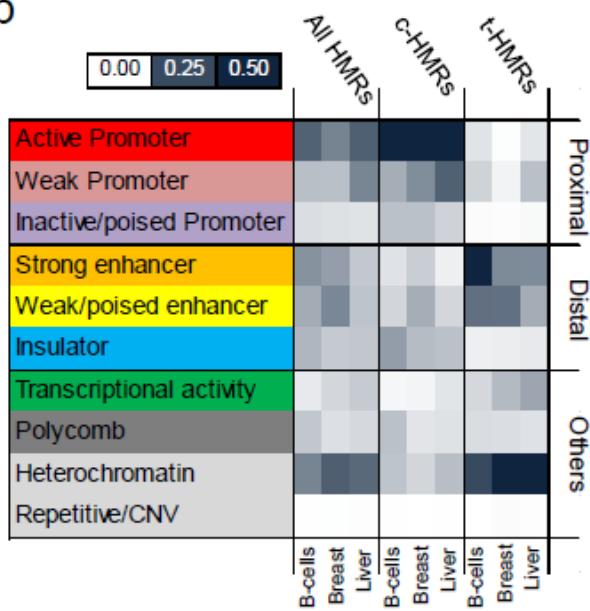
S Shore	-500	0.2	1.0
S Shore	-97	0.0	0.9
S Shore	-29	0.1	0.9
S Shore	54	0.1	0.9
Island	265	0.0	1.0
Island	361	0.4	0.8
Island	367	0.4	0.9
Island	456	0.4	0.9

Epigenomic Analysis Detects Aberrant Super-Enhancer DNA Methylation in Human Cancer

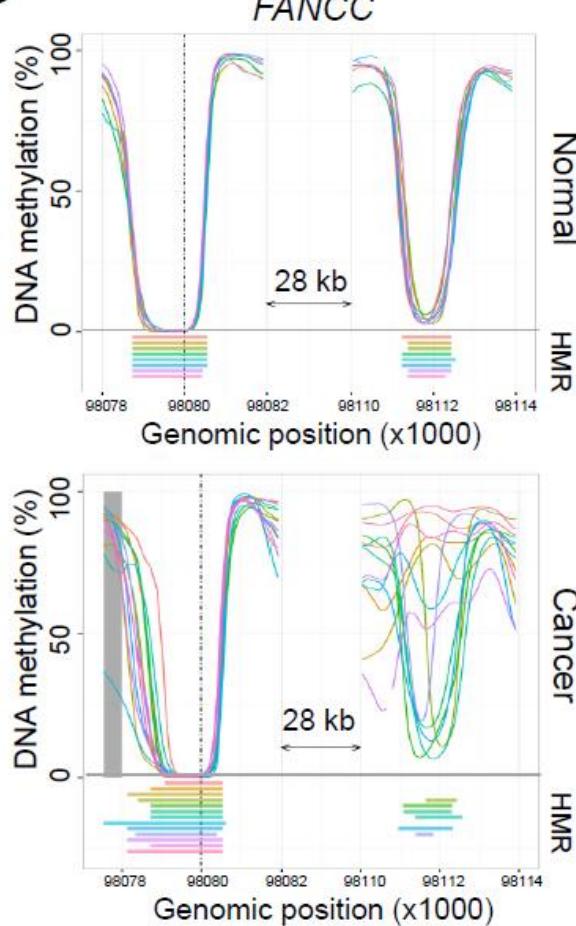


A DNA Methylation Map of Human Cancer at Single Base-Pair Resolution: A View of Distal Events

b



e

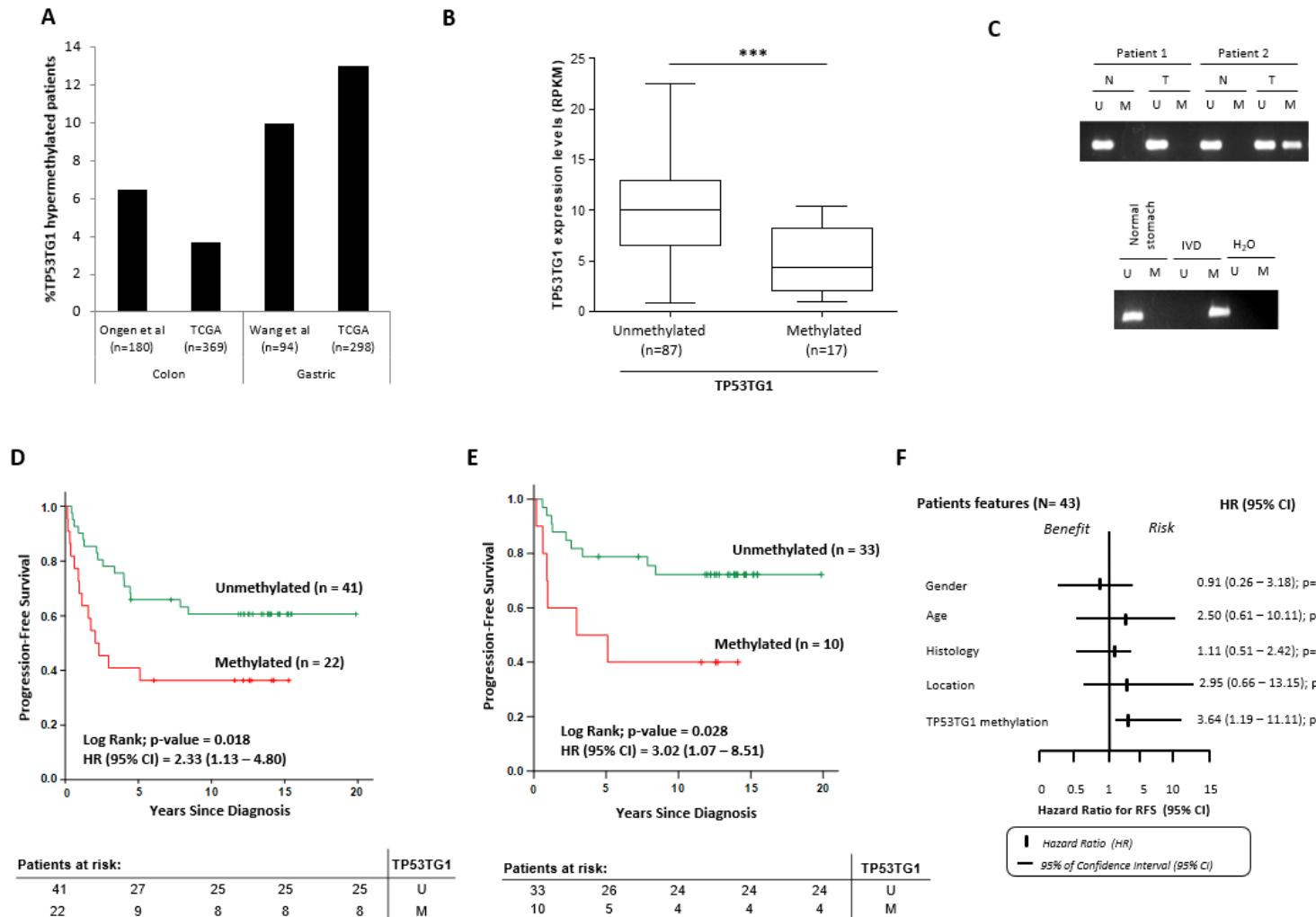


Cancer-Specific DNA Methylation Silencing of ncRNAs

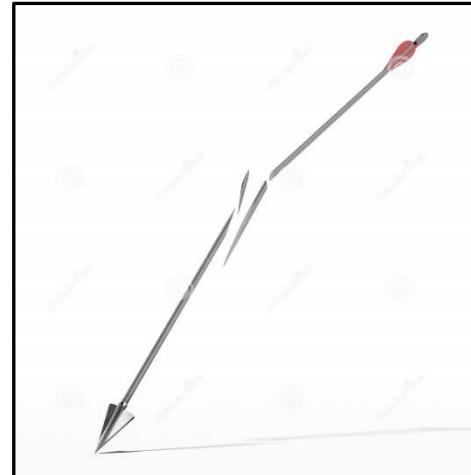
Table 1 | Types of ncRNAs*

Name	Size	Location	Number in humans	Functions	Illustrative examples	Refs
Short ncRNAs						
miRNAs	19–24 bp	Encoded at widespread locations	>1,424	Targeting of mRNAs and many others	miR-15/16, miR-124a, miR-34b/c, miR-200	 Cancer Res 2007 PNAS 2008 Oncogene 2011 Oncogene 2012
piRNAs	26–31 bp	Clusters, intragenic	23,439	Transposon repression, DNA methylation	piRNAs targeting RASGRF1 and LINE1 and IAP elements	 Epigenetics 2014
tiRNAs	17–18 bp	Downstream of TSSs	>5,000	Regulation of transcription?	Associated with the CAP1 gene	
Mid-size ncRNAs						
snoRNAs	60–300 bp	Intronic	>300	rRNA modifications	U50, SNORD	 RNA Biology 2012
PASRs	22–200 bp	5' regions of protein-coding genes	>10,000	Unknown	Half of protein-coding genes	 10
TSSa-RNAs	20–90 bp	−250 and +50 bp of TSSs	>10,000	Maintenance of transcription?	Associated with RNF12 and CCDC52 genes	35
PROMPTs	<200 bp	−205 bp and −5 kb of TSSs	Unknown	Activation of transcription?	Associated with EXT1 and RBM39 genes	36
Long ncRNAs						
lincRNAs	>200 bp	Widespread loci	>1,000	Examples include scaffold DNA–chromatin complexes	HOTAIR, HOTTIP, lincRNA-p21	 1
T-UCRs	>200 bp	Widespread loci	>350	Regulation of miRNA and mRNA levels?	uc.283+, uc.338, uc160+	 Oncogene 2010
Other lncRNAs	>200 bp	Widespread loci	>3,000	Examples include X-chromosome inactivation, telomere regulation, imprinting	XIST, TSIX, TERRAs, p15AS, H19, HYMAI	2,23–25 Mol Cell 2014

TP53TG1 methylation in gastrointestinal tumors is associated with poor outcome



PharmacoEpigenetics



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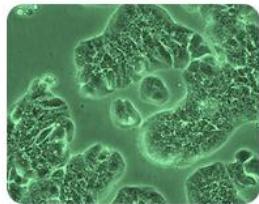
Table 3 | Hypermethylated genes predict drug sensitivity

Gene name	Gene function	Therapeutical consequences	Tumour type application	Example refs
ABCB1	Protein transport	Sensitivity to doxorubicin	Breast	120
APAF1	Apoptotic activator	Resistance to adriamycin	Melanoma	121
BRCA1	DNA damage response	Sensitivity to PARP inhibitors and alkylating agents	Breast, ovary	81
CDK10	Cell cycle control	Resistance to anti-oestrogens	Breast	122
CHFR	Ubiquitin protein ligase	Sensitivity to paclitaxel and docetaxel	Ovary, endometrium, stomach	123
ESR1	ER signalling	Resistance to anti-oestrogens	Breast	124
FANCF	DNA damage response	Sensitivity to cisplatin	Ovarian	125
GSTP1	Detoxification	Sensitivity to doxorubicin	Prostate, breast, kidney	126
IGFBP3	Signal transduction	Resistance to cisplatin	Lung	127
LINE1	Repetitive element	Resistance to fluoropyrimidines	Colon	22
MGMT	DNA repair	Sensitivity to temozolomide, BCNU, ACNU, procarbazine	Glioma, colon, lung, lymphoma	5
MLH1	DNA repair	Resistance to cisplatin	Colon, stomach, endometrium, ovary	128
MT1E	Antioxidant	Sensitivity to cisplatin	Melanoma	129
PITX2	Transcriptional regulator	Resistance to tamoxifen	Breast	130
PLK2	Cell division	Sensitivity to paclitaxel and carboplatin	Ovary	131
PRKCDPB	Signal transduction	Resistance to TNF α	Colon	132
SFN	Signal transduction	Sensitivity to cisplatin and gemcitabine	Lung	133
SLC19A1	Folate transporter	Resistance to methotrexate	Lymphomas	134
SULF2	Heparin signalling	Sensitivity to camptothecin	Lung	135
TFAP2E	Transcriptional regulator	Sensitivity to fluorouracil	Colon	136
TGM2	Apoptosis	Resistance to doxorubicin and cisplatin	Lung, breast, ovary	137
TP73	Stress response	Sensitivity to cisplatin	Renal, melanoma	138
WRN	DNA helicases	Sensitivity to irinotecan	Colon	139
ERCC5	DNA repair	Resistance to nemorubicin	Ovary	140

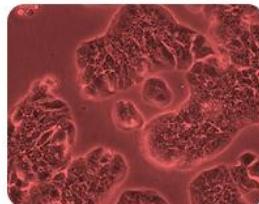
Esteller et al.,
NEJM 2000

Human colon cancer cell lines sensitive vs resistant

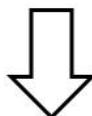
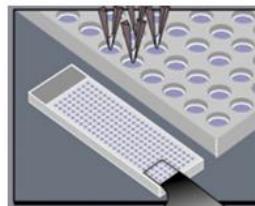
LOVO sensitive
oxaliplatin
(LOVO-S)



LOVO resistant
oxaliplatin
(LOVO-R)

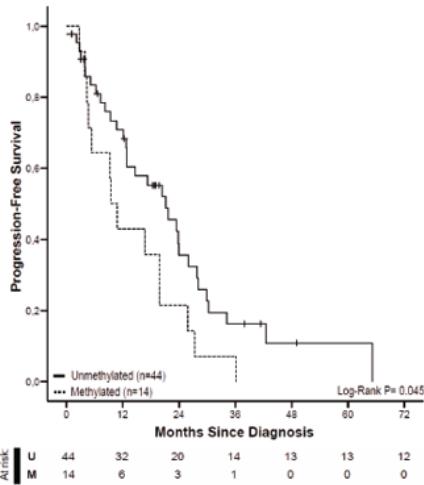
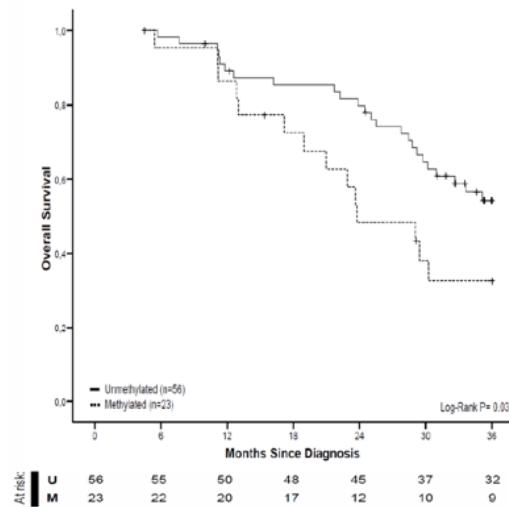
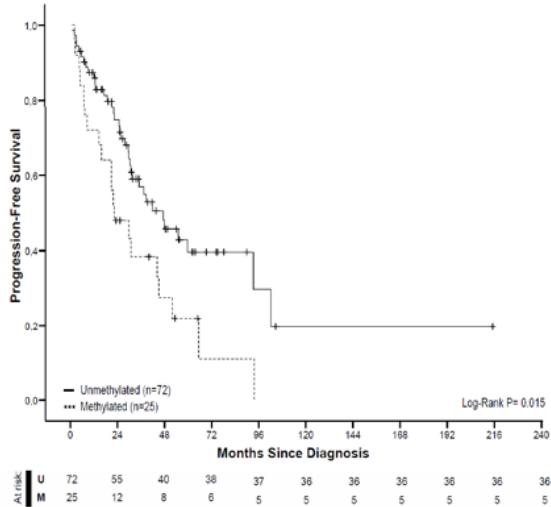


DNA Methylation Microarray



Top Scorer: The BRCA1-Interactor SRBC

With Clinical Significance



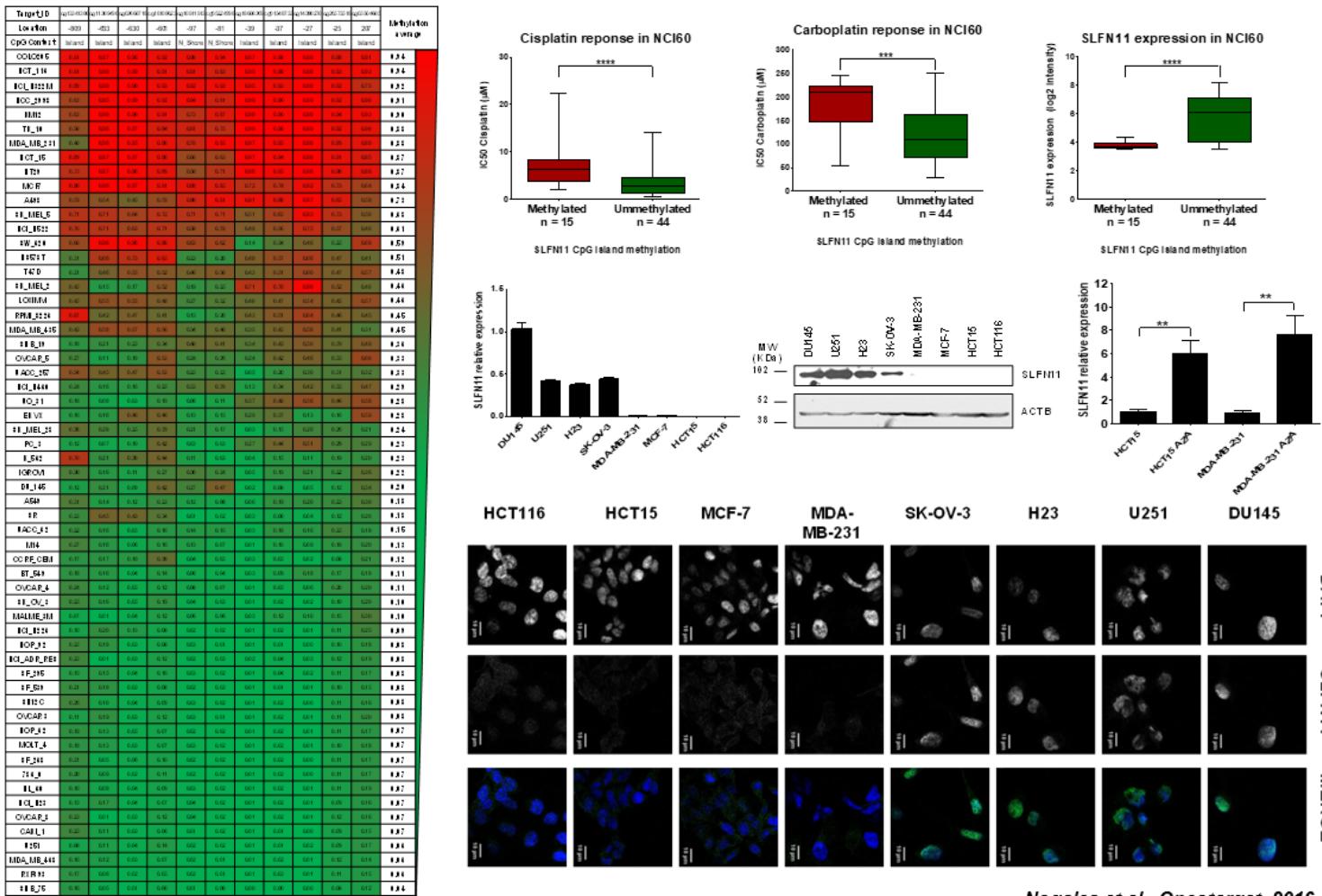
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Data Mining

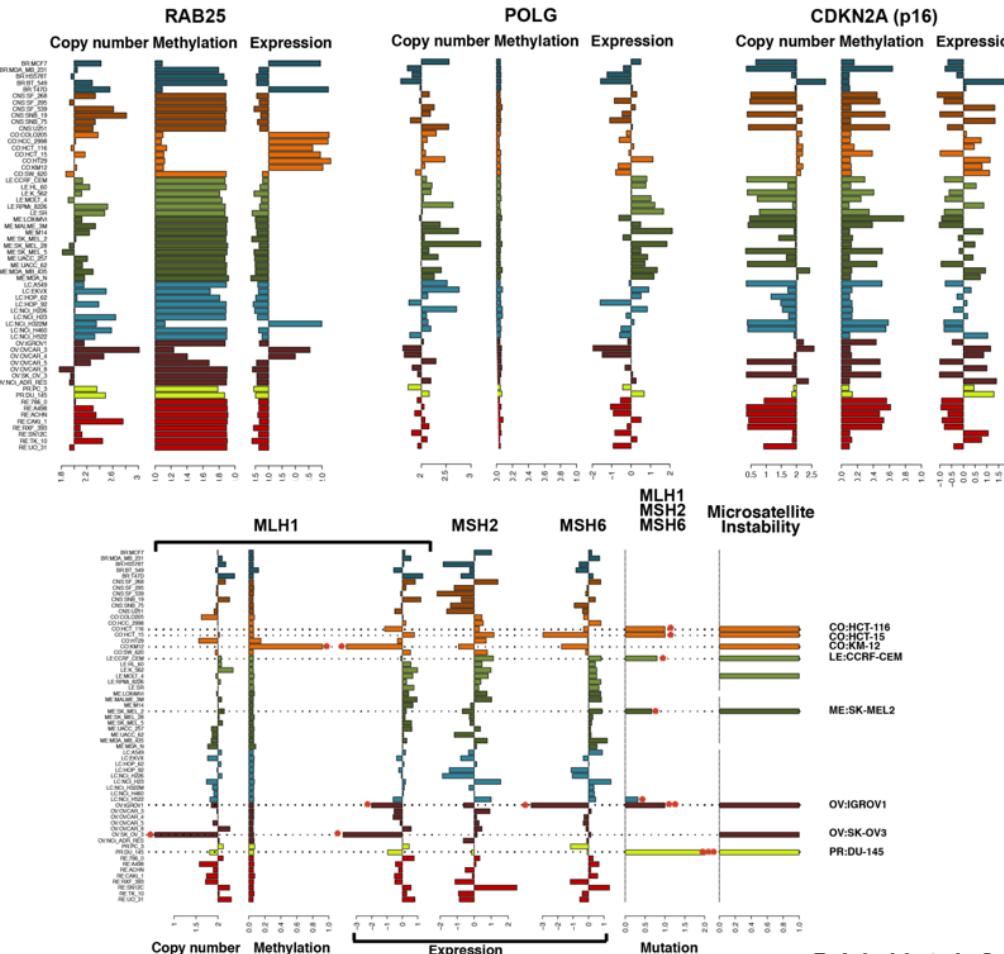


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NCI60 Hypermethylation-Associated Silencing of the Putative DNA/RNA Helicase SLFN11 and Resistance to Platinum Drugs

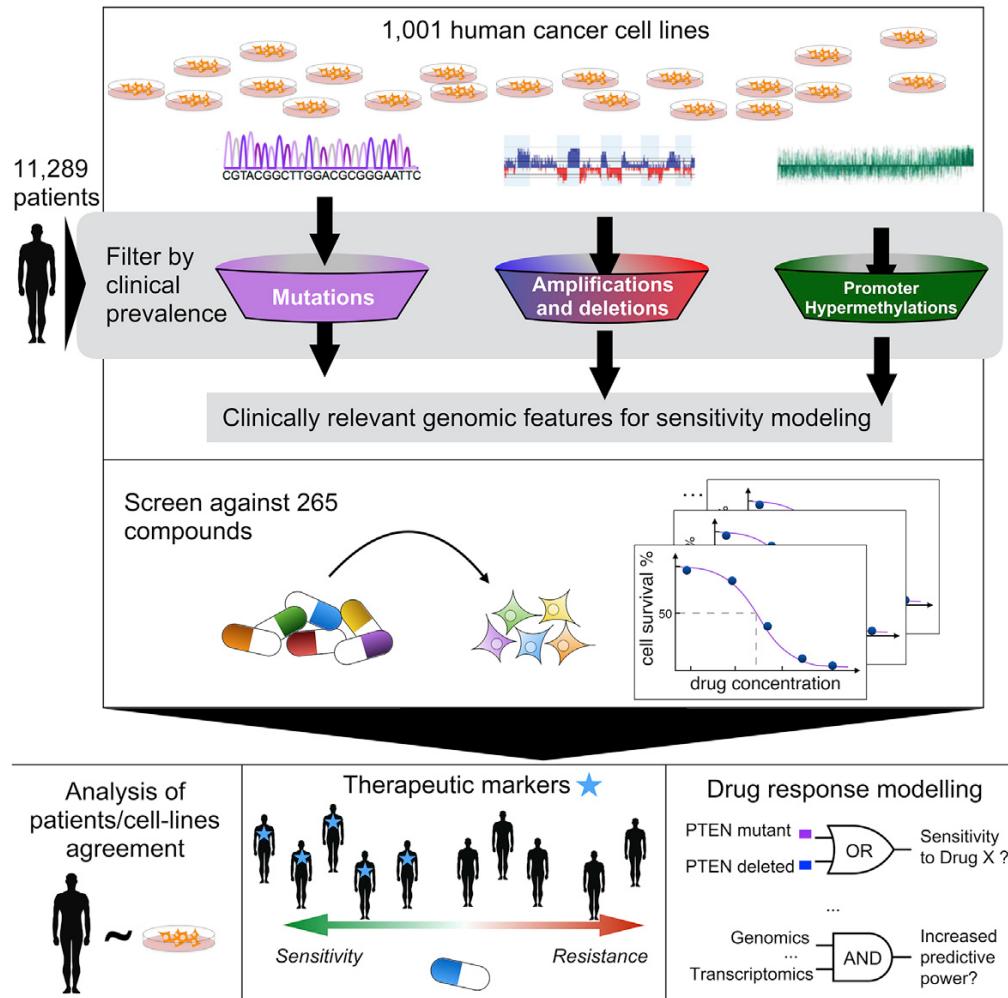


The NCI-60 DNA Methylome and its Integration into CellMiner



Reinhold et al., Cancer Research 2016

A Landscape of Pharmacogenomic Interactions in Cancer



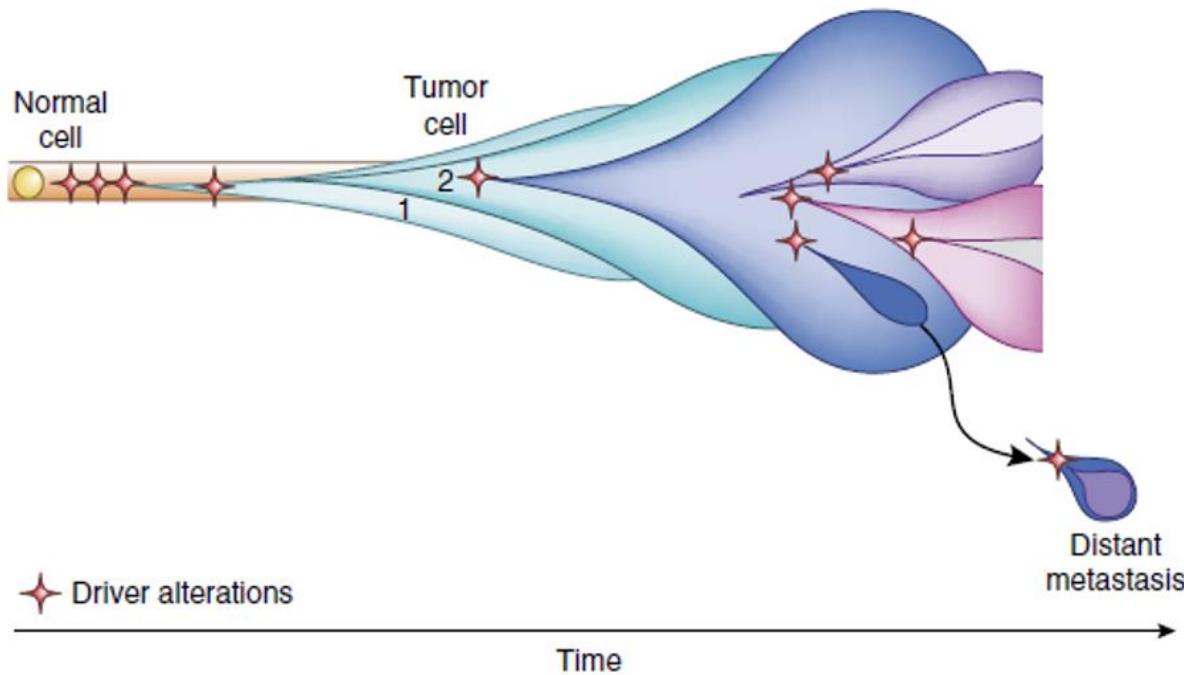
Real Life



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PERSPECTIVE

nature
medicine



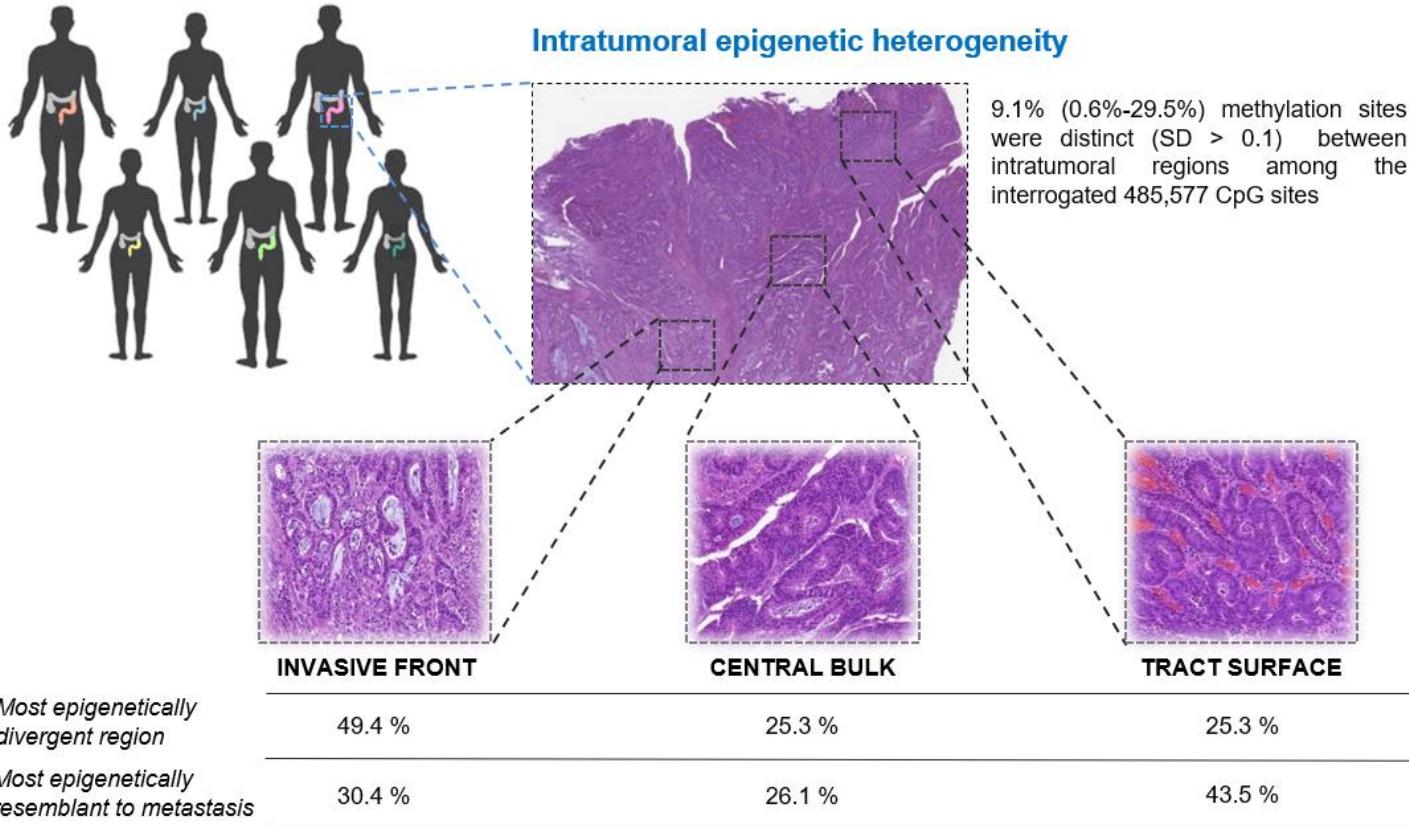
Toward understanding and exploiting tumor heterogeneity

Ash A Alizadeh^{1–3}, Victoria Aranda⁴, Alberto Bardelli^{5,6}, Cedric Blanpain⁷, Christoph Bock^{8,9}, Christine Borowski⁴, Carlos Caldas¹⁰, Andrea Califano^{11–13}, Michael Doherty¹⁴, Markus Elsner¹⁵, Manel Esteller¹⁶, Rebecca Fitzgerald¹⁷, Jan O Korbel¹⁸, Peter Lichter¹⁹, Christopher E Mason²⁰, Nicholas Navin^{21,22}, Dana Pe'er^{11,23}, Kornelia Polyak²⁴, Charles W M Roberts²⁵, Lillian Siu²⁶, Alexandra Snyder²⁷, Hannah Stower⁴, Charles Swanton^{28–30}, Roel G W Verhaak^{22,31}, Jean C Zenklusen³², Johannes Zuber³³ & Jessica Zucman-Rossi³⁴

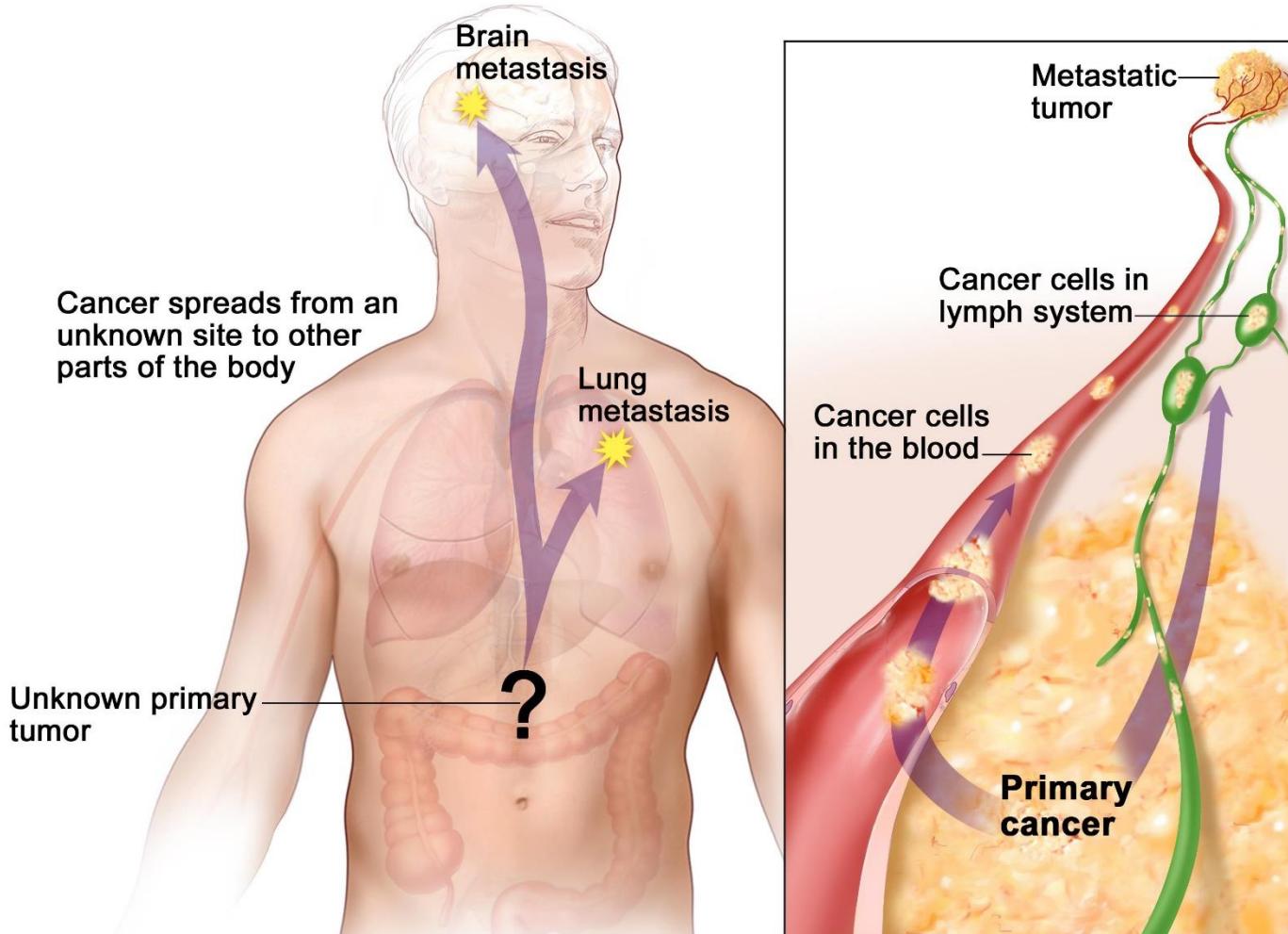
Epigenetic Heterogeneity in Human Colorectal Cancer

Intertumoral epigenetic heterogeneity

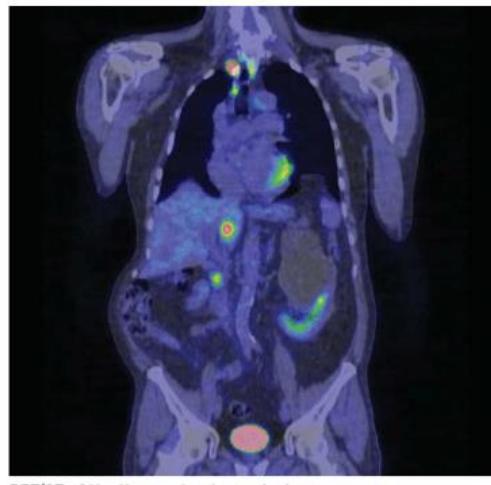
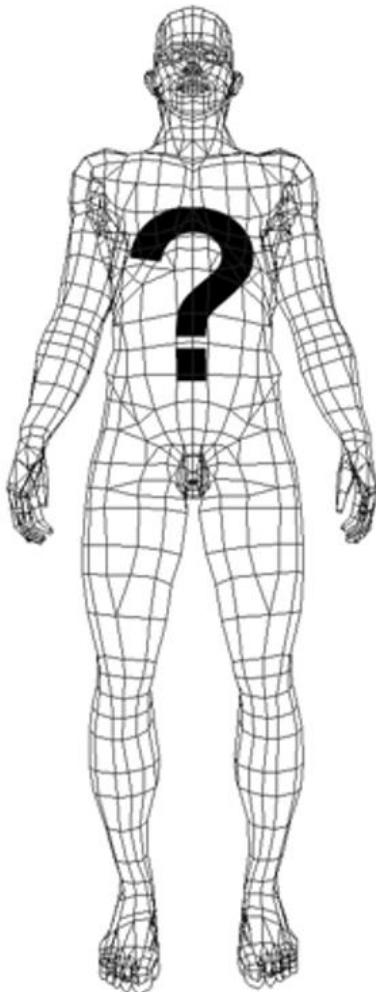
Significantly higher than intratumoral DNA methylation diversity (Chi-Square goodness of fit test: P = 0.005)



Carcinoma of Unknown Primary

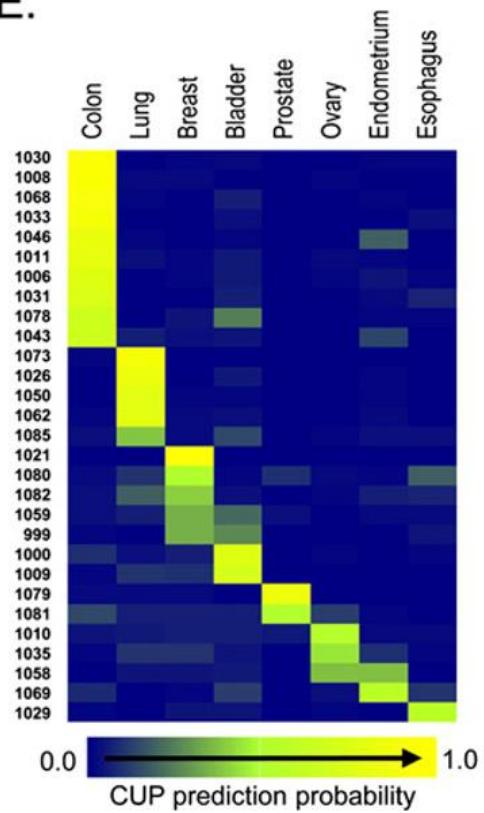


Cancer of Unknown Primary



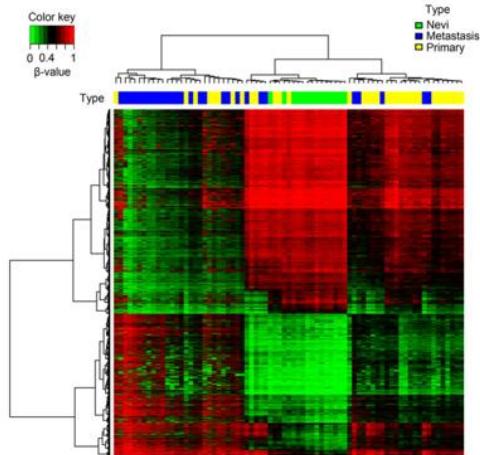
PET/CT of the thorax showing metastases.

E.

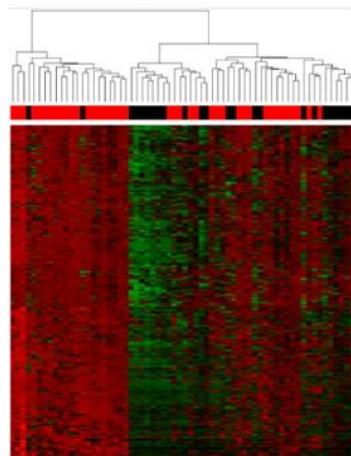


Fernandez et al., Genome Research, 2012

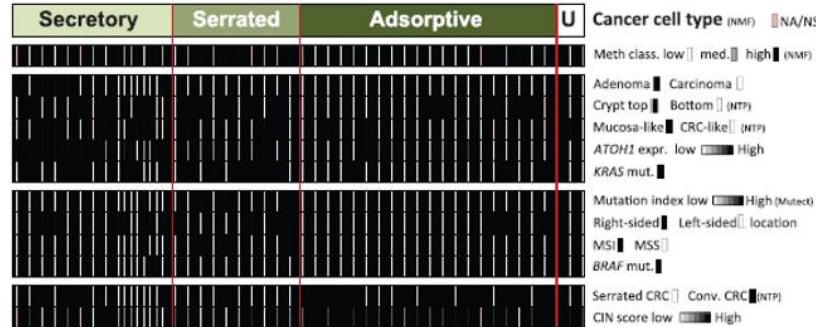
Profiling DNA Methylation in Human Cancer



Wouters et al., BMC Medicine 2017

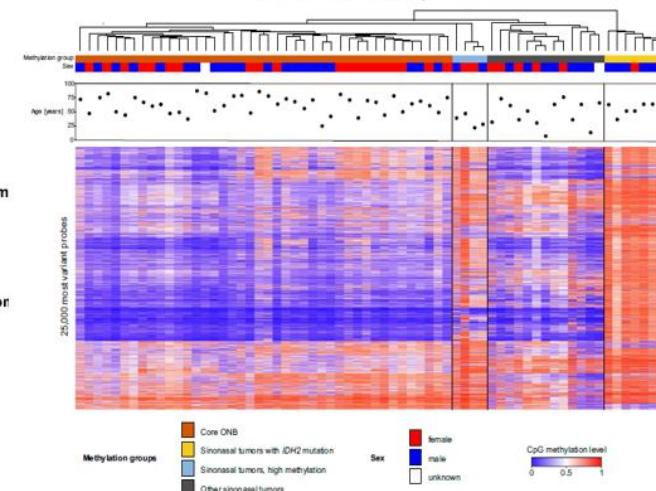


Crujeiras et al., Endocr Relat Cancer 2017



Bramsen et al., Cell Reports 2017

n = 66 tumors with institutional diagnosis of ONB

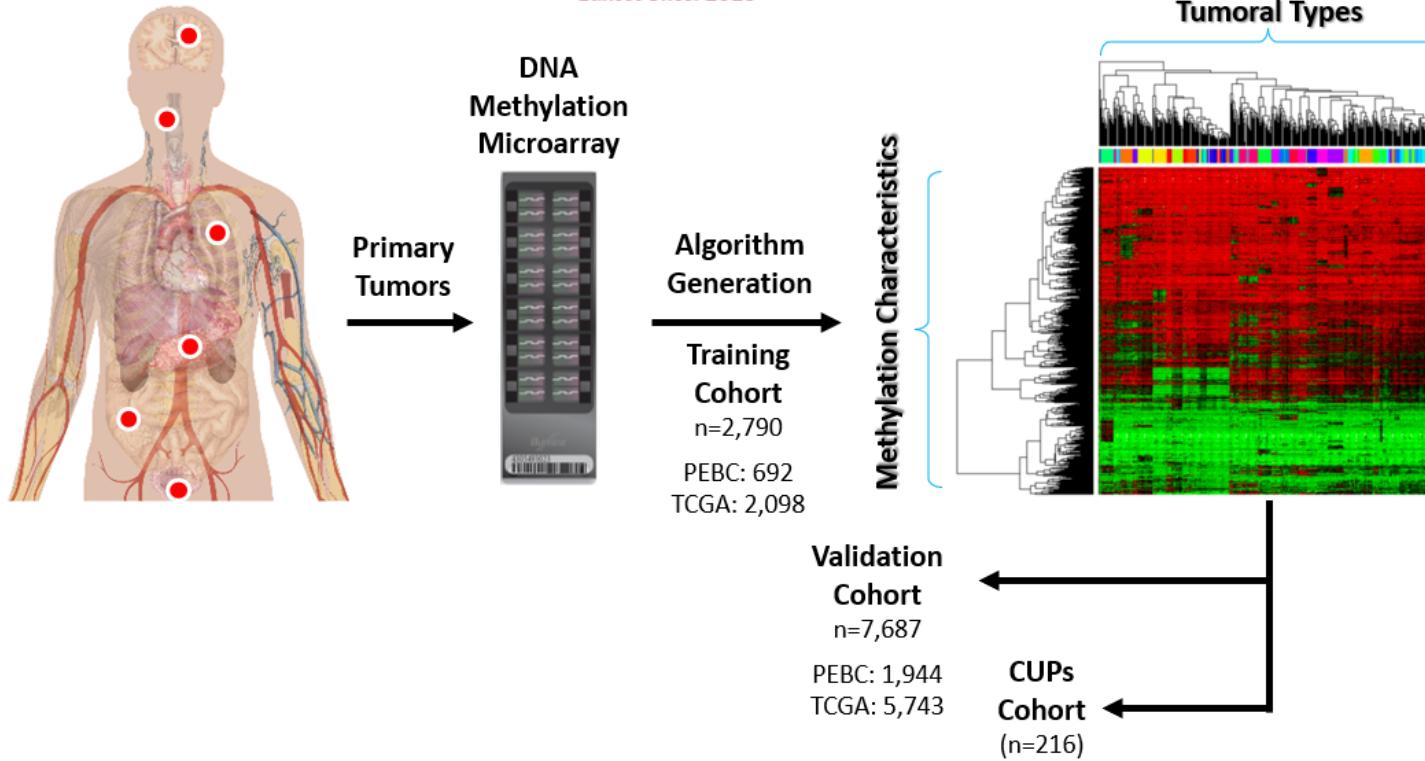


Capper et al., Acta Neuropathologica 2018

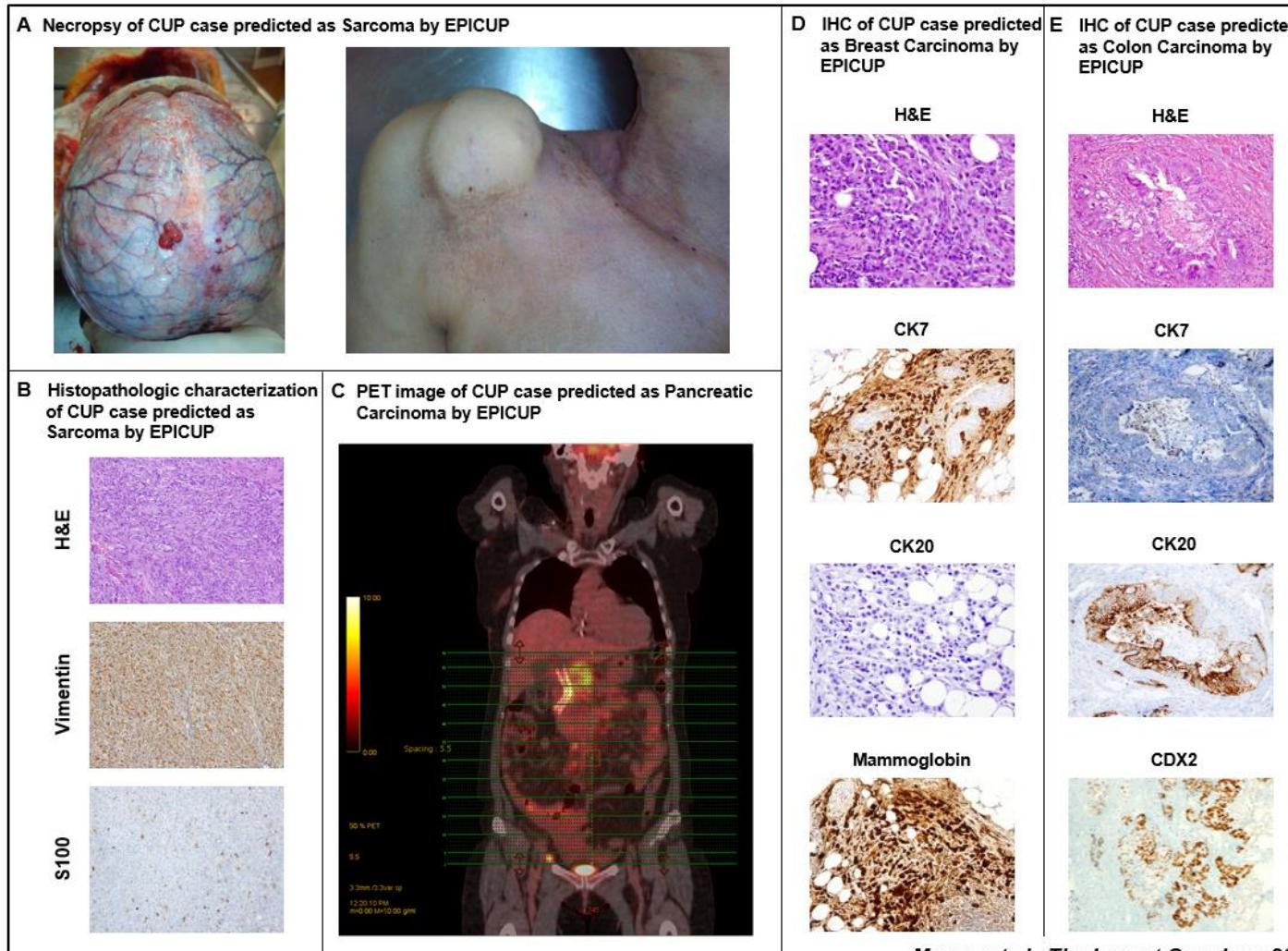
Epigenetic profiling to classify cancer of unknown primary: a multicentre, retrospective analysis

Sebastian Moran, Anna Martínez-Cardús, Sergi Sayols, Eva Musulén, Carme Balañá, Anna Estival-Gonzalez, Cátia Moutinho, Holger Heyn, Angel Diaz-Lagares, Manuel Castro de Moura, Giulia M Stella, Paolo M Comoglio, Maria Ruiz-Miró, Xavier Matias-Guiu, Roberto Pazo-Cid, Antonio Antón, Rafael Lopez-Lopez, Gemma Soler, Federico Longo, Isabel Guerra, Sara Fernandez, Yassen Assenov, Christoph Plass, Rafael Morales, Joan Carles, David Bowtell, Linda Mileskein, Daniela Sia, Richard Tothill, Josep Tabernero, Josep M Llovet, Manel Esteller

Lancet Oncol 2016

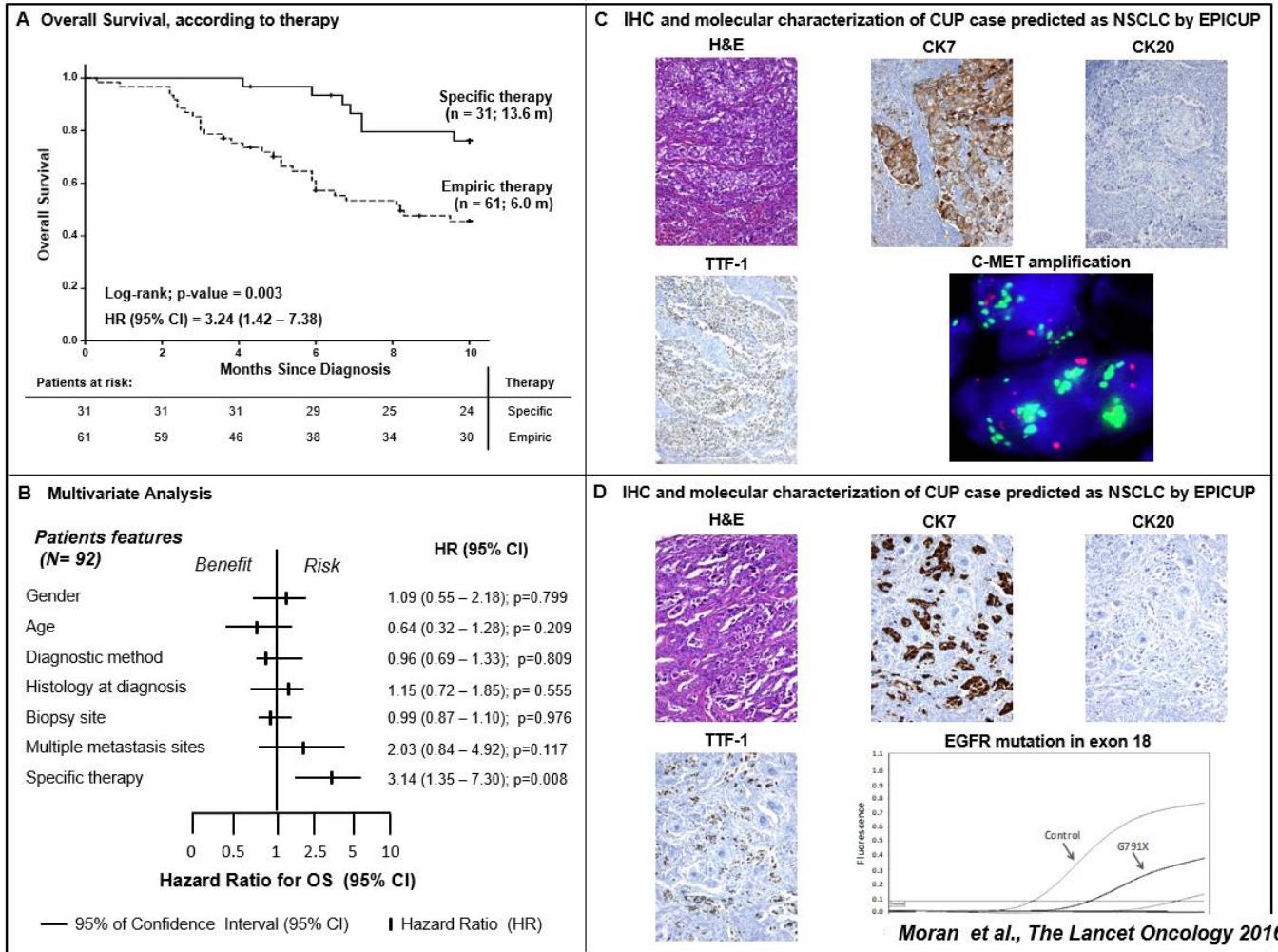


Efficacy of Epigenetic Profiling to Classify Cancer of Unknown Primary

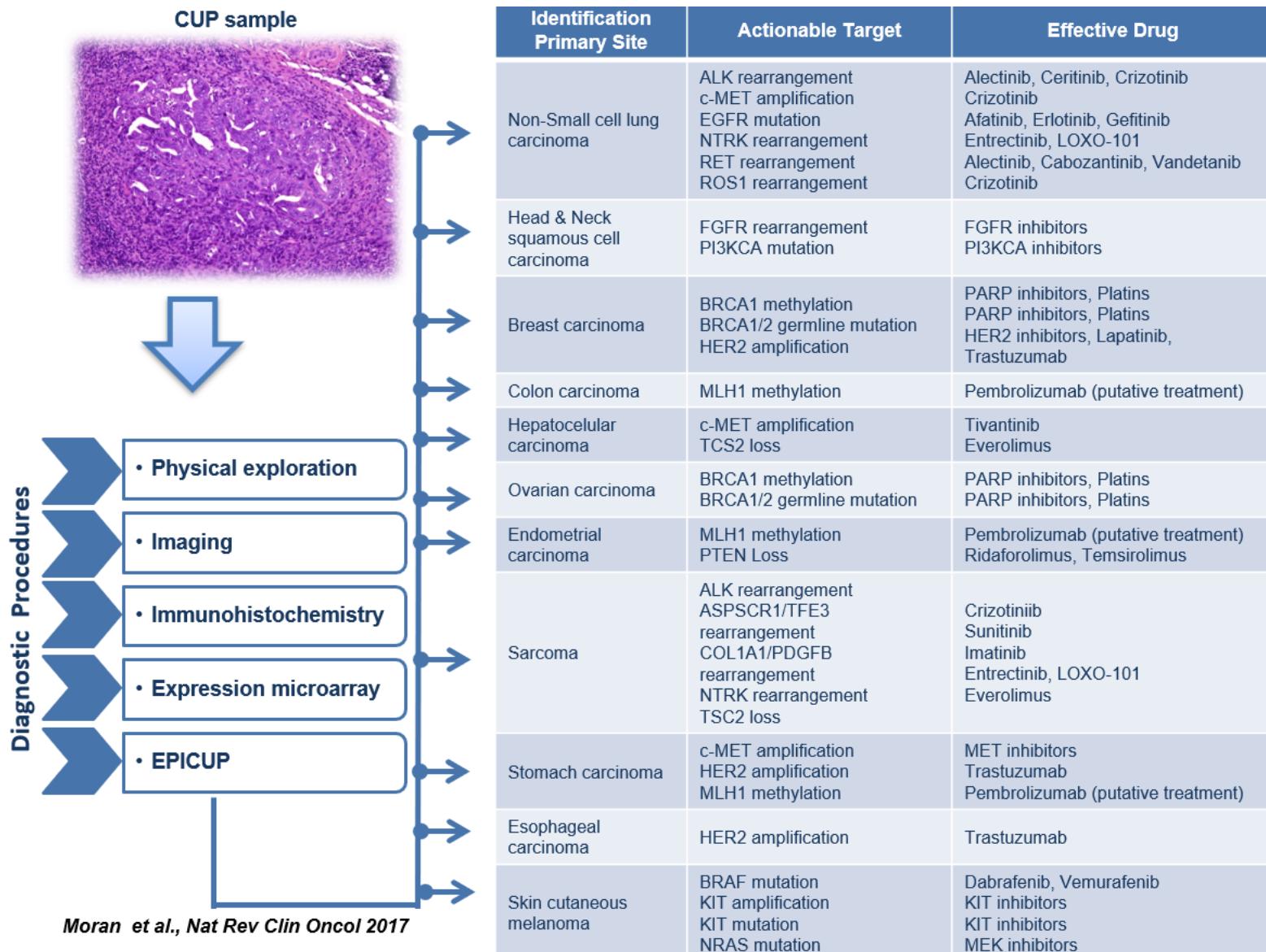


Moran et al., *The Lancet Oncology* 2016

Efficacy of Epigenetic Profiling to Classify Cancer of Unknown Primary



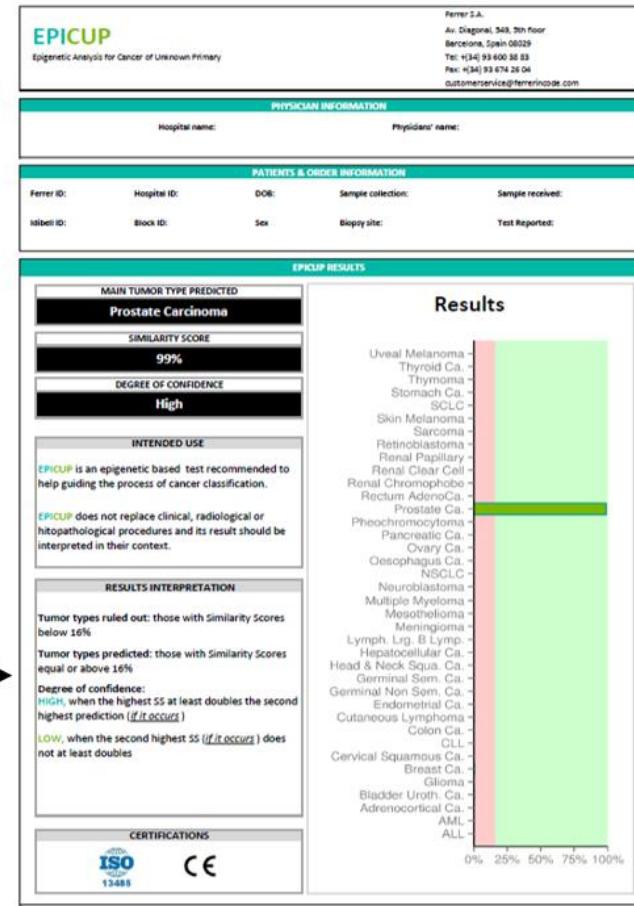
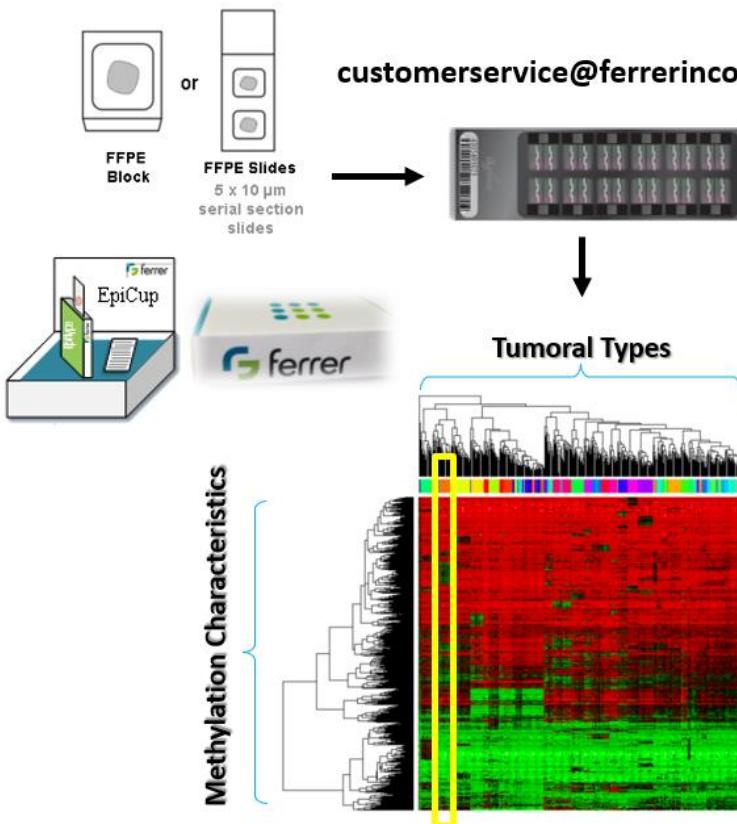
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V SIMPOSIO GETHI



Aim: Predict tumor of origin for Cancer of Unknown Primary patients



The CUP TCGA Project

The Cancer of Unknown Primary (CUP) Cancer Genome Atlas (TCGA)



Dr. Manel Esteller



Dr. Jean C. Zenklusen



THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute

Whole Genome Sequencing, Total RNA Sequencing, DNA Methylome

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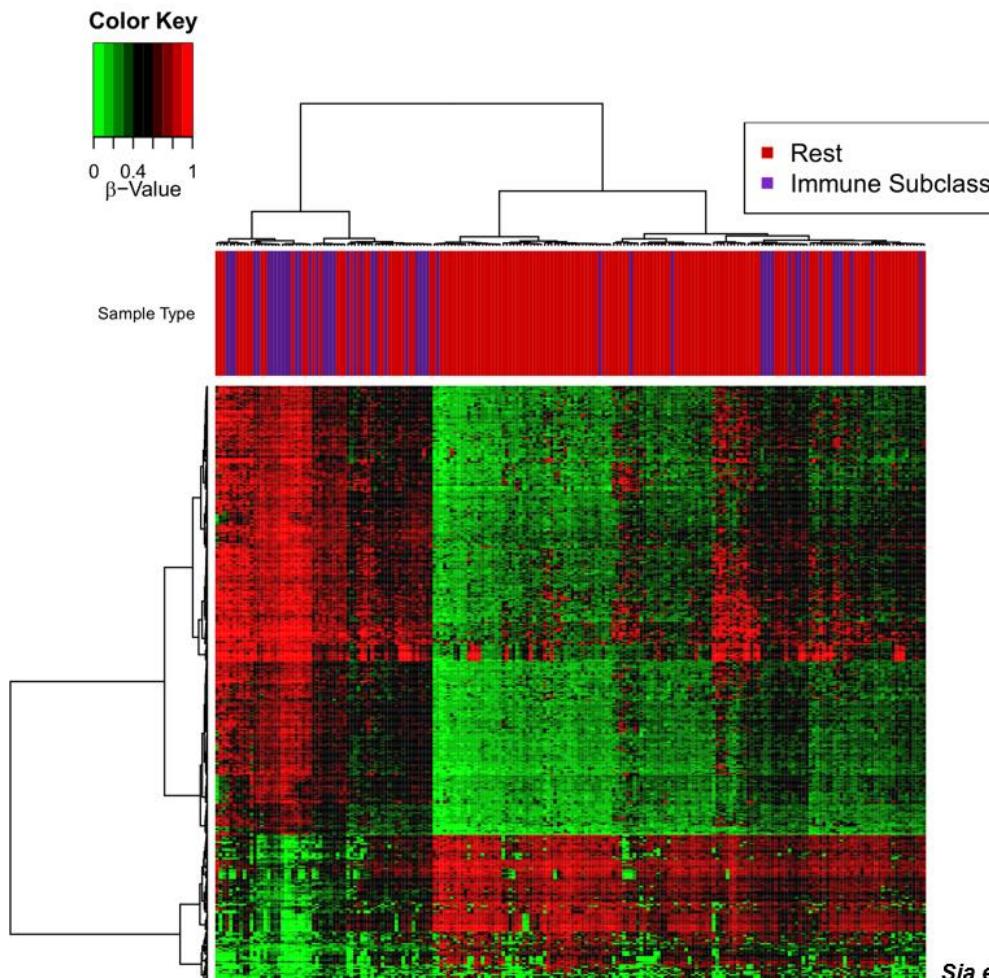


mesteller@carrerasresearch.org

THE IMMUNOTHERAPY EXPLOSION !

In a movie theatre near you

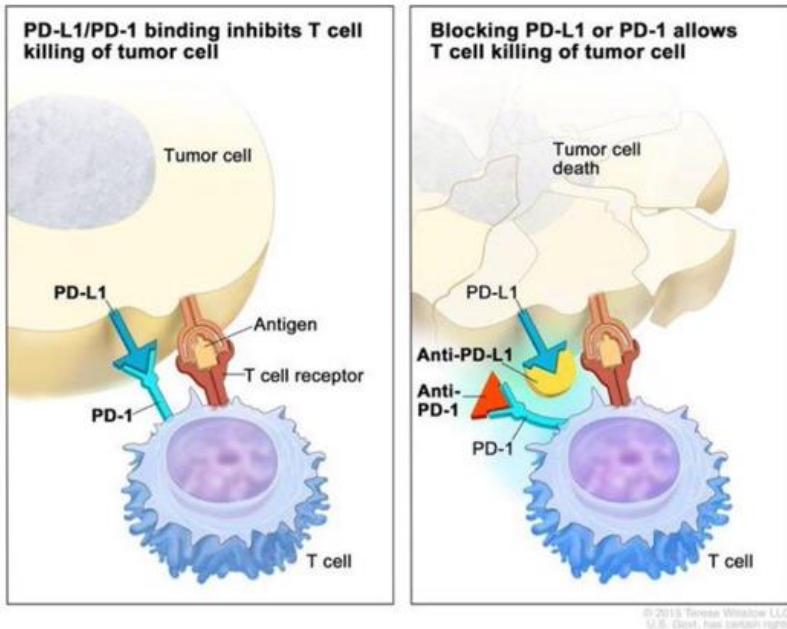
DNA Methylation Contributes to Define an Immune Subclass of Liver Cancer



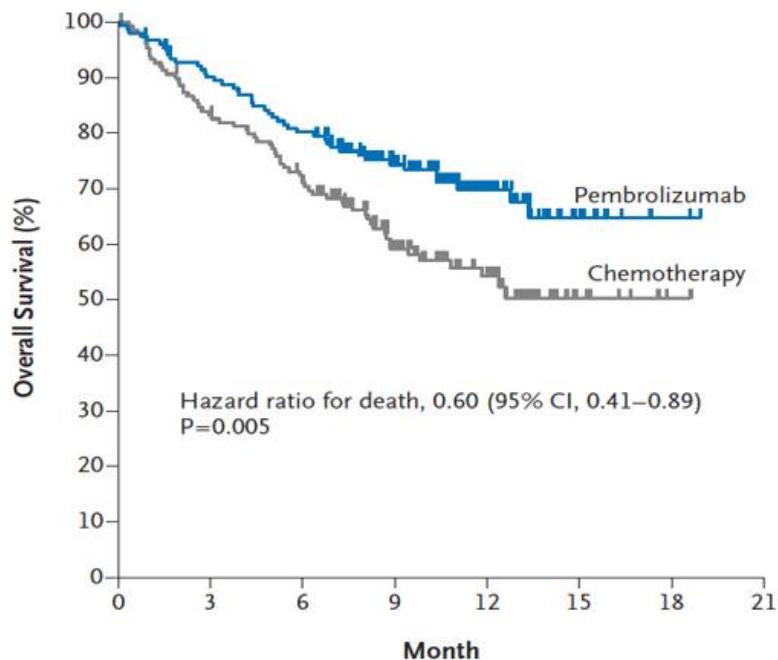
Sia et al., *Gastroenterology* 2017

Rational of anti-PD1/PD-L1 immunotherapy

Immune checkpoint PD1/PD-L1 is pivotal in adaptative immune resistance



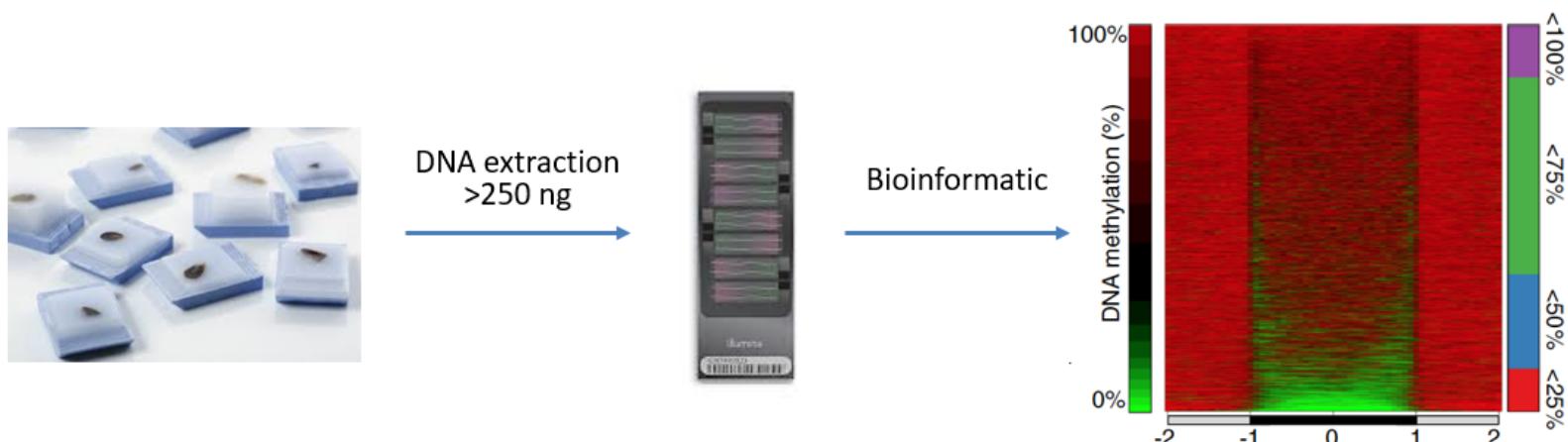
Anti-PD1 antibodies pembrolizumab and nivolumab approved in advanced non-small cell lung cancer (NSCLC)



EPIMUNE Project Purpose

Uncover epigenetic determinant of anti-PD1 efficacy in NSCLC:

Genome-wide DNA methylation profile (Illumina 850 K array beadchip) of pre-treatment FFPE samples from NSCLC patients treated with anti-PD1

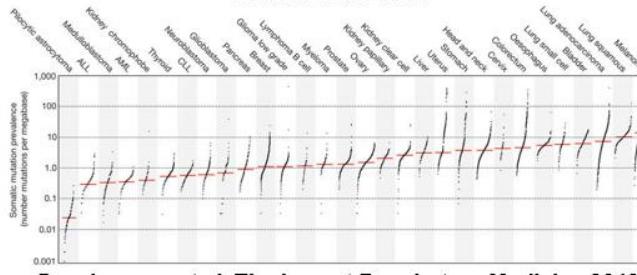
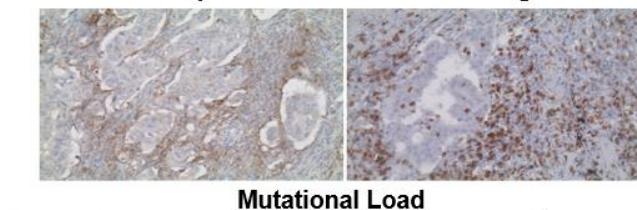
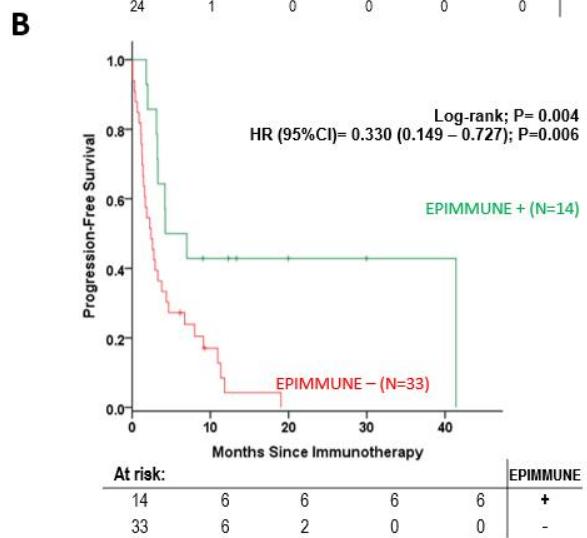
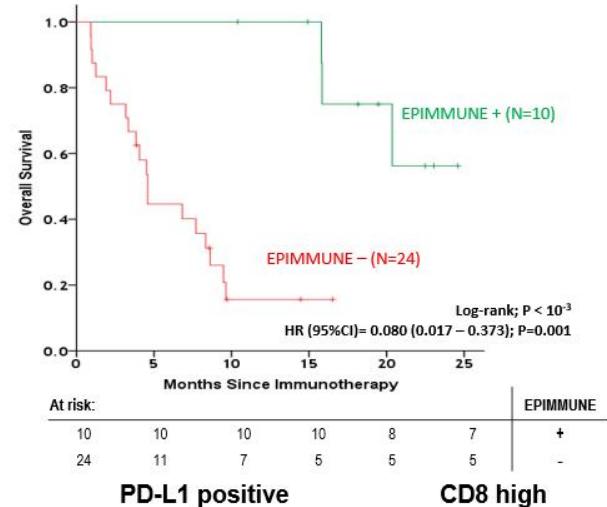
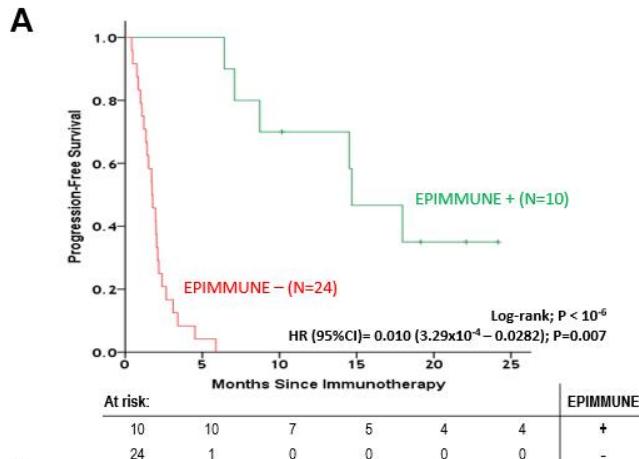


Pre-anti-PD1 FFPE
samples from NSCLC
Fully clinically annotated

Genome-wide DNA
methylation profile:
Illumina 850k array
beadchip

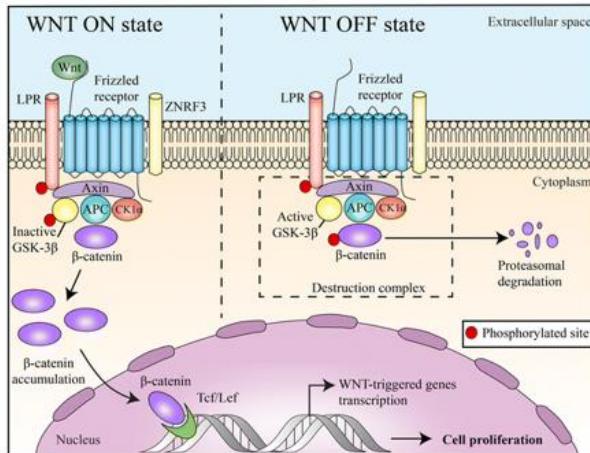
Methylation level in each
CpG explored

EPIMMUNE Positive NSCLC : Prediction of Response to Anti-PD1 Therapy

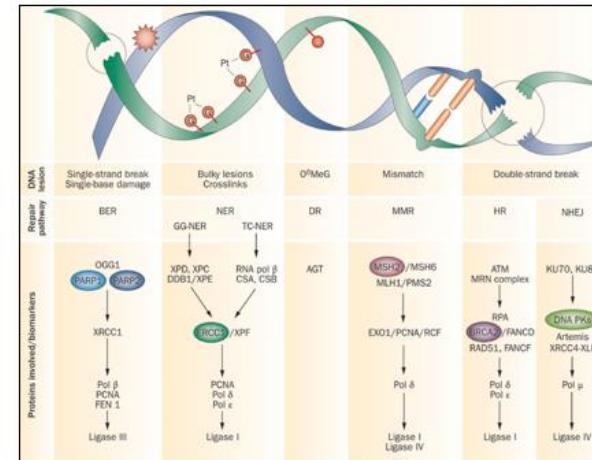


EPIMMUNE Positive NSCLC : Pathways Enrichment

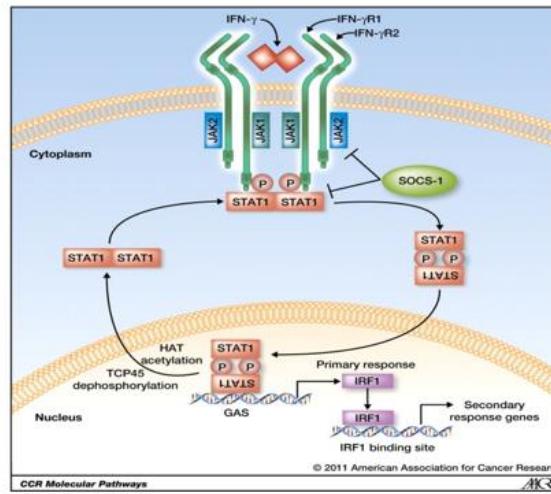
Reduced β -catenin Signaling



Deficient DNA Repair



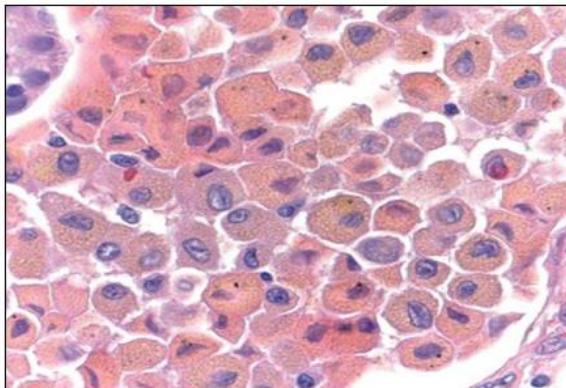
Activation of the Interferon-gamma (IFN γ) Response



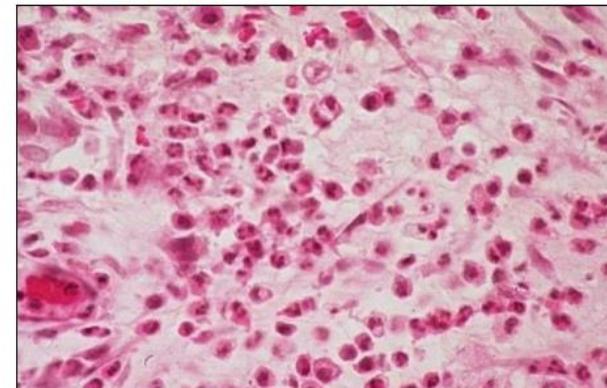
Duruisseaux, et al.
The Lancet Respiratory Medicine 2018

EPIMMUNE Negative NSCLC : Cell Type Deconvolution

Tumor-Associated Macrophages (TAMs)



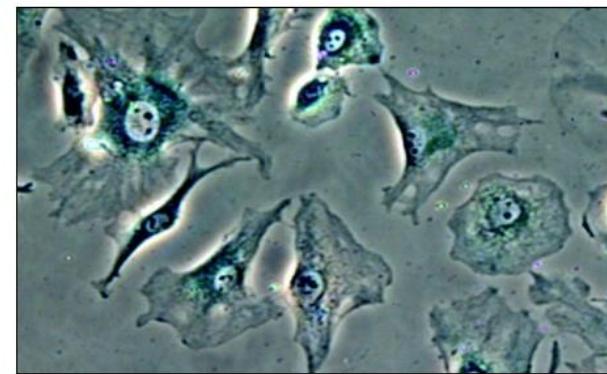
Tumor-Associated Neutrophils (TANs)



Cancer-Associated Fibroblasts (CAFs)

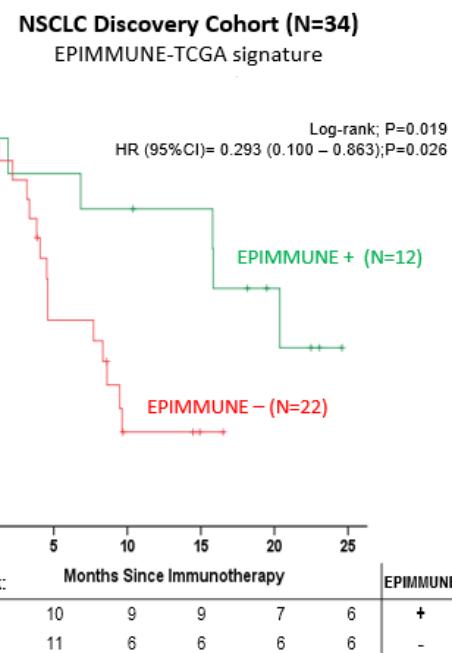
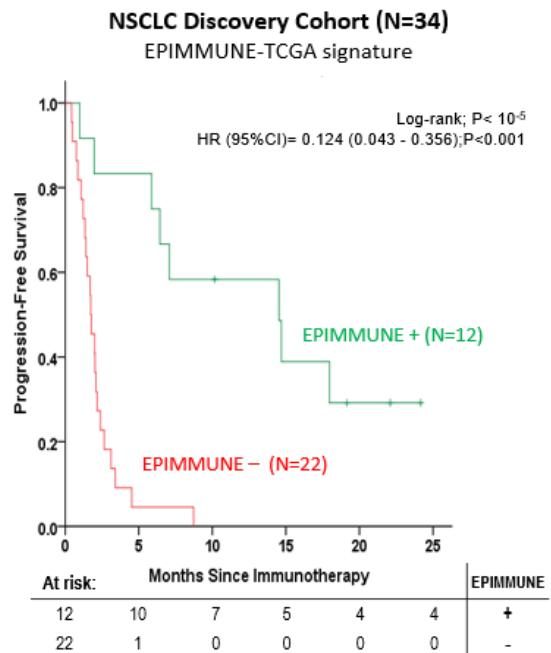


Senescent Endothelial Cells

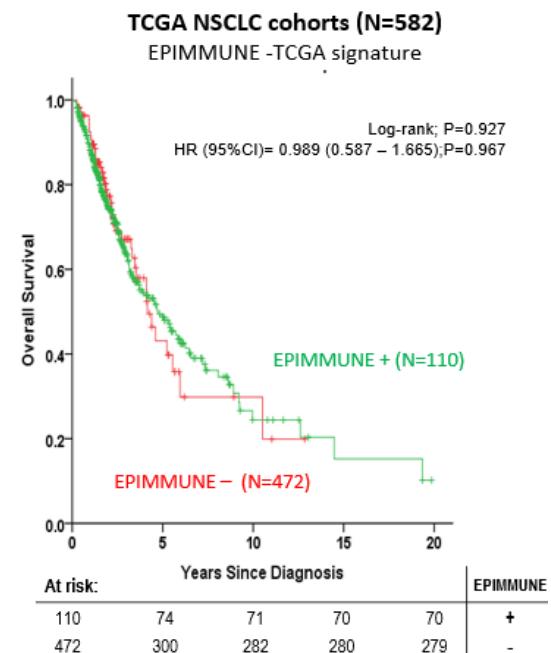


EPIMMUNE-TCGA Positive NSCLC : Prediction of Response to Anti-PD1 Therapy

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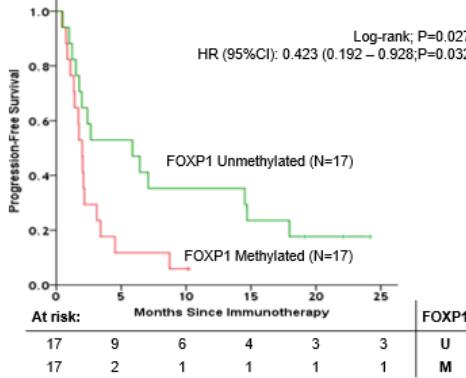


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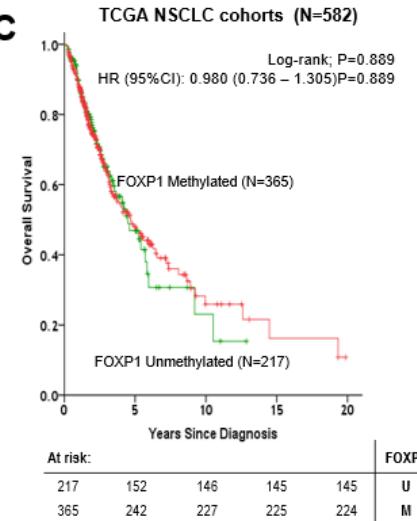


EPIMMUNE Positive Derived Biomarker: FOXP1

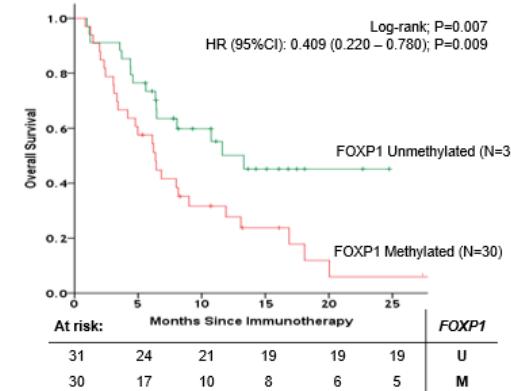
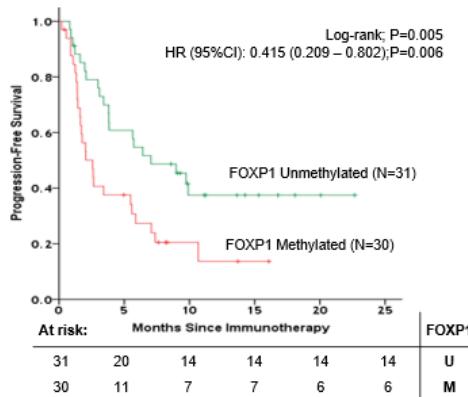
A



C

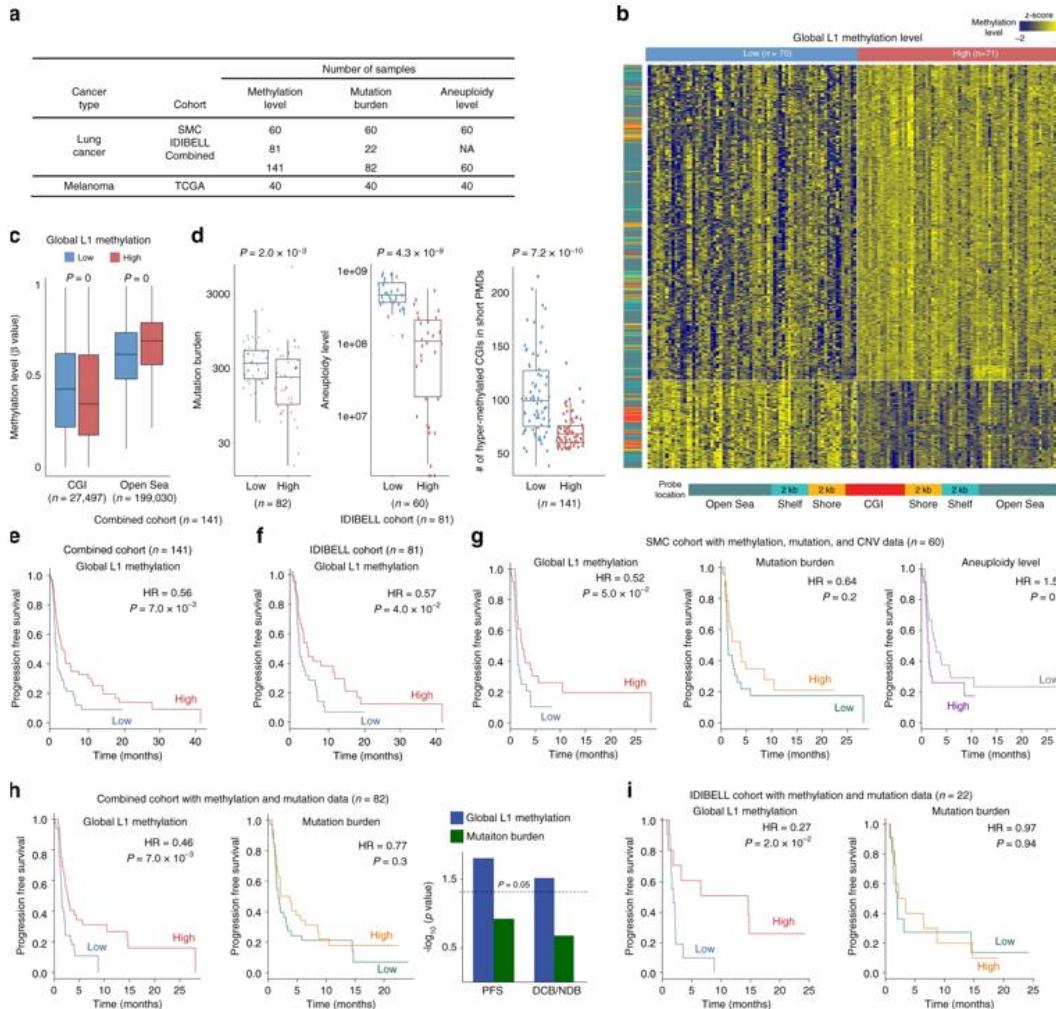


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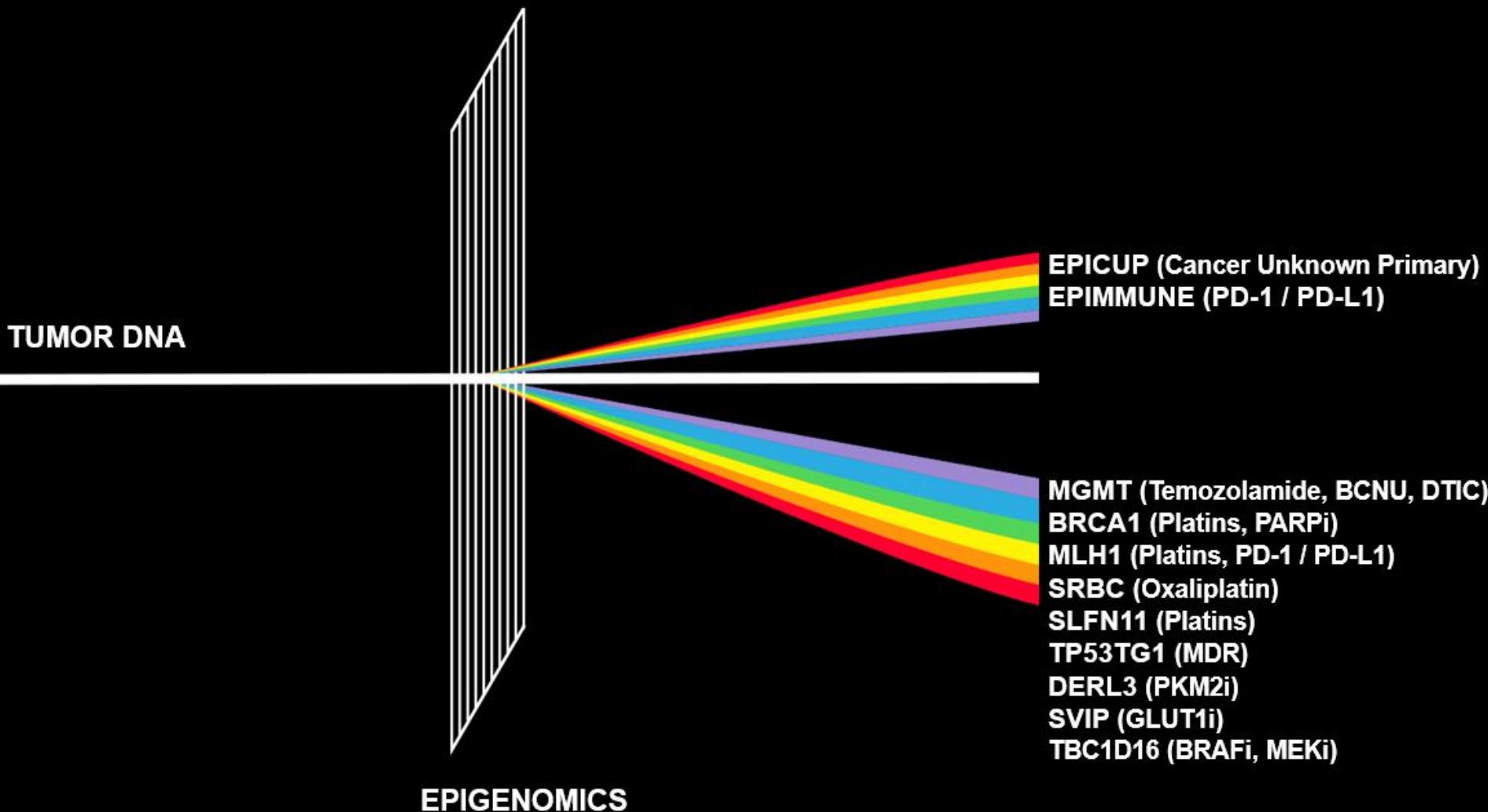


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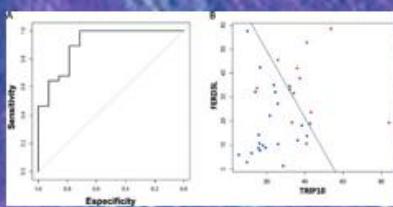
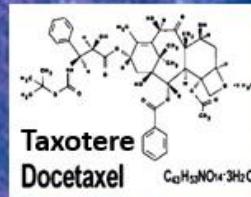
DNA methylation loss promotes immune evasion of tumours with high mutation and copy number load



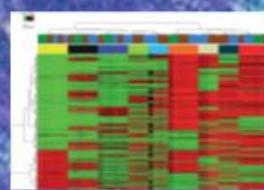
EPIGENETIC PRISM FOR CANCER PRECISION MEDICINE



Epigenetic Contribution to Chemoresistance in Breast Cancer



Pineda et al.
Clinical Epigenetics
2019



Gómez-Miragaya et al.
Molecular Cancer Research
2019



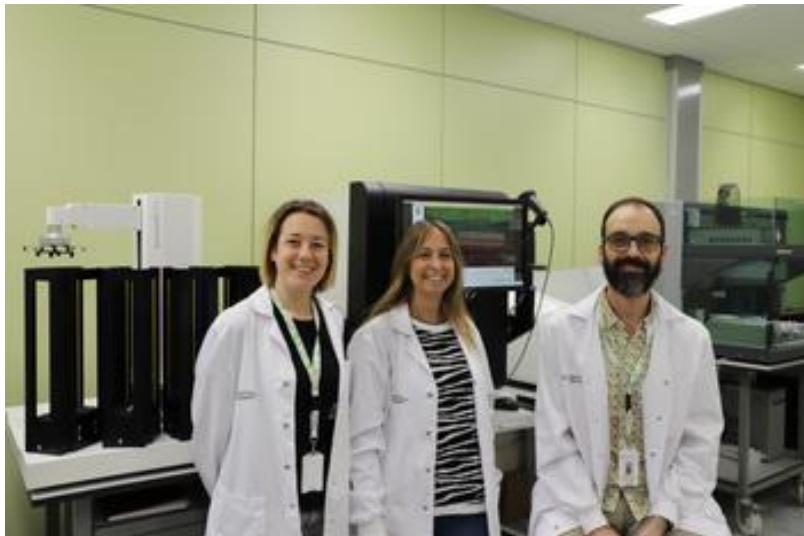
Palomeras et al.
Breast Cancer Research
2019

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Research Institute

Genomics Unit



Bioinformatics Unit



Ready to serve and to collaborate !

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