GASTRO JournalClub

L'importanza della ricerca in Oncologia

10-11 OTTOBRE 2019 - ROMA

VOI Donna Camilla Savelli Hotel - Via Garibaldi, 27

Dual targeting of histone methyltransferase G9a and DNMT1 in Gastric Cancer

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OUTLINE

BACKGROUND

- Epigenetics
- Epigenetics in cancer
- Focus on G9a and DNMT1
- Dual targeting of G9a and DNMT1 in HCC e CCA
- Epigenetics and Gastric Cancer

RESEARCH PROJECT

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Epigenetics

from transgenerational inheritance to disease

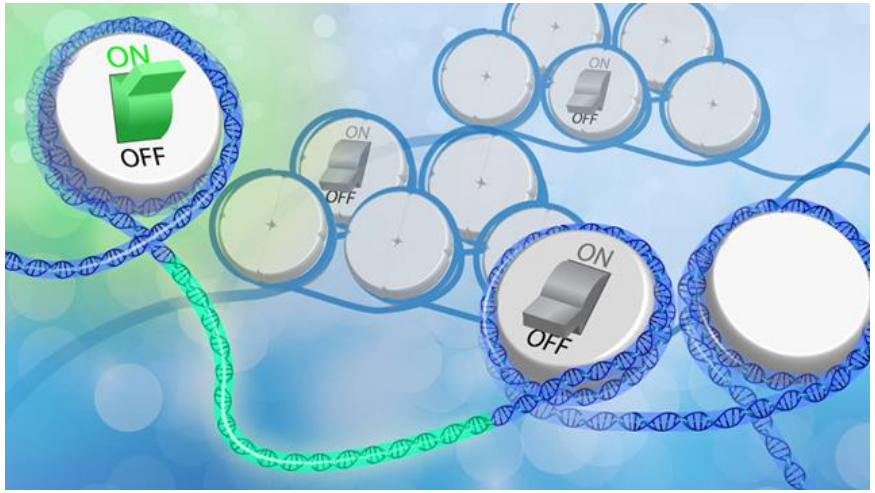
Choices you make can change your genes, and those of your kids

Time cover, 18th January 2010

Initial definition: *"The casual interactions between genes and their products, which bring the phenotype into being"* (C.H. Waddington, 1939)

Current definition: *"Heritable changes in gene expression that occur independent of changes in the primary DNA sequence"*

Epigenetics in cancer. Estellar NEJM 2008.



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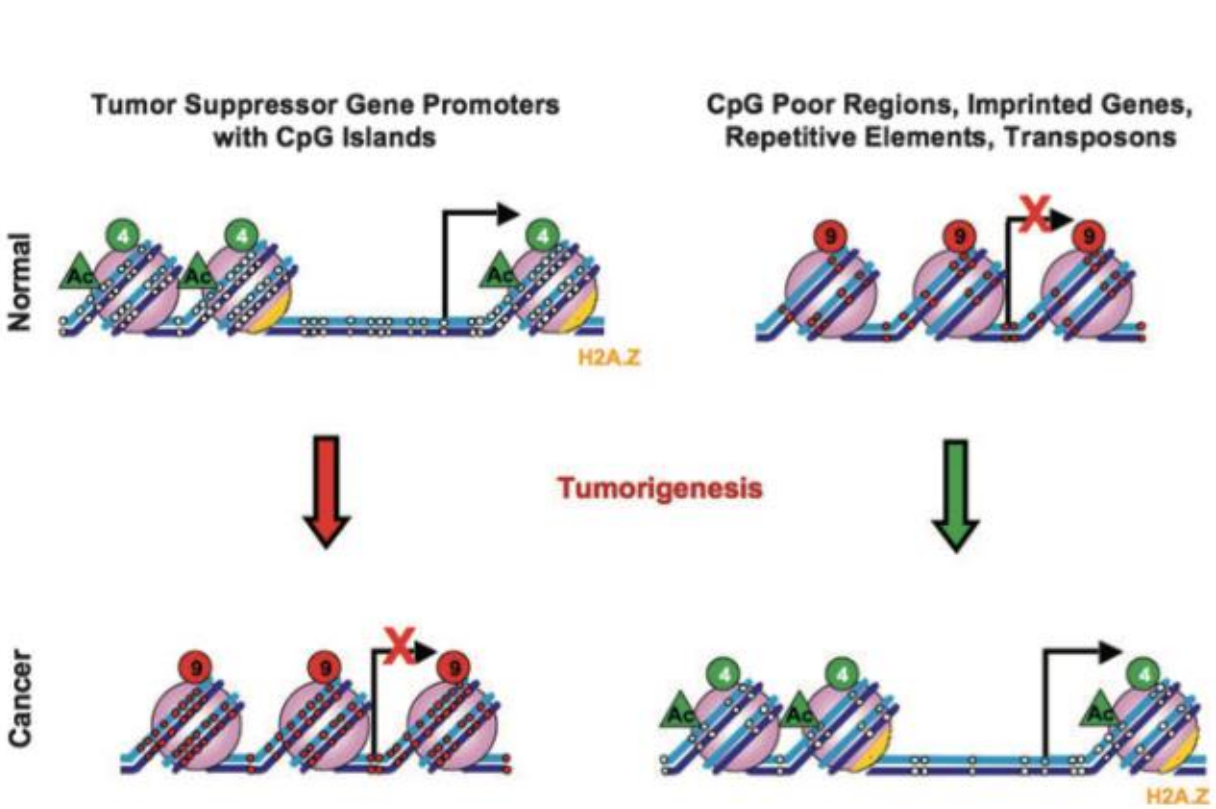
Epigenetics and cancer – 1/3

EPIGENETIC MECHANISMS:

- DNA methylation (DNMT1, DNMT3A, DNMT3B)
- Histone (H2A, H2B, H3, H4) modifications: methylation (HMT) and acetylation
- Non coding RNAs (miRNAs)



EPIGENOME CHANGES IN CANCER:

1. Genome-wide hypomethylation
2. Site-specific (TSG) CpG island promoter hypermethylation

Epigenetics in cancer. Sharma S et al. Carcinogenesis 2010.

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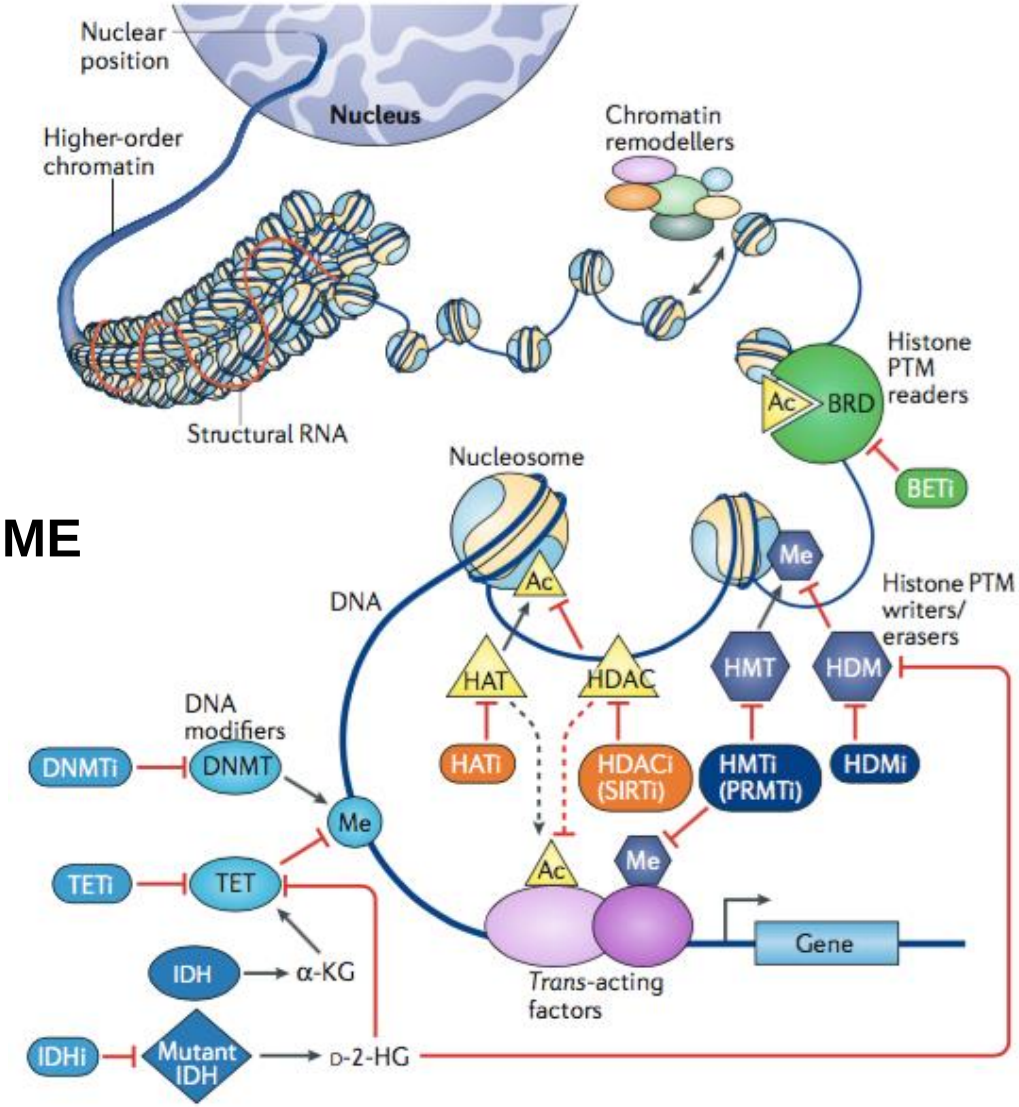
Epigenetics and cancer – 2/3

TARGETING THE EPIGENOME



Combining epigenetic drugs with other therapies for solid tumours – past lessons and future promise. Morel D et al. Nature Reviews Sept 2019.

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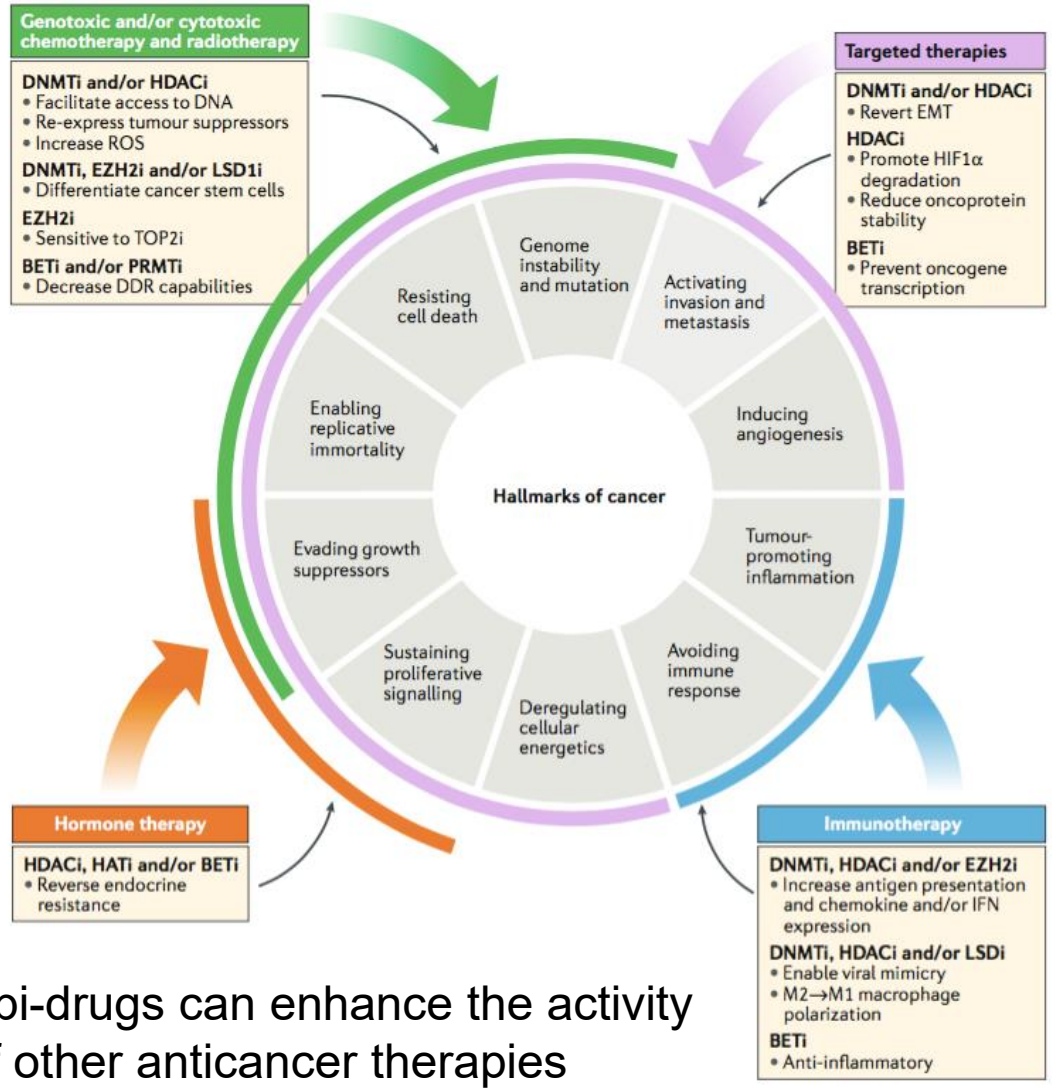
Epigenetics and cancer – 3/3

Morel D et al. Nature Reviews Sept 2019.



Epi-drugs can enhance the activity of other anticancer therapies to counteract the hallmarks of cancer

EPI-DRUGS

Drugs	classification	Approved Year	Indicated disease	ORR
Azacytidine[20]	DNMT inhibitor	2004	MDS	17.9%
Vorinostat[32]	HDAC inhibitor	2006	CTCL	30%
Decitabine[35]	DNMT inhibitor	2006	MDS	42%~54%
Romidepesin[34]	HDAC inhibitor	2009	TCL	34%
Ruxolitinib[36]	JAK1/2 inhibitor	2011	Myelofibrosis	30%
Belinostat[33]	HDAC inhibitor	2015	PTCL	25.8%
Panobinostat[25]	HDAC inhibitor	2015	MM	NA

Epigenetic targeting drugs potentiate chemotherapeutic effects in solid tumor therapy. Li J et al. Sci Rep 2017

Azacitidin

Decitabin

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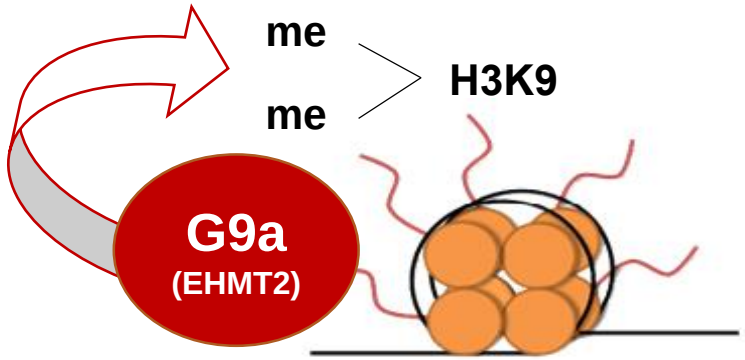
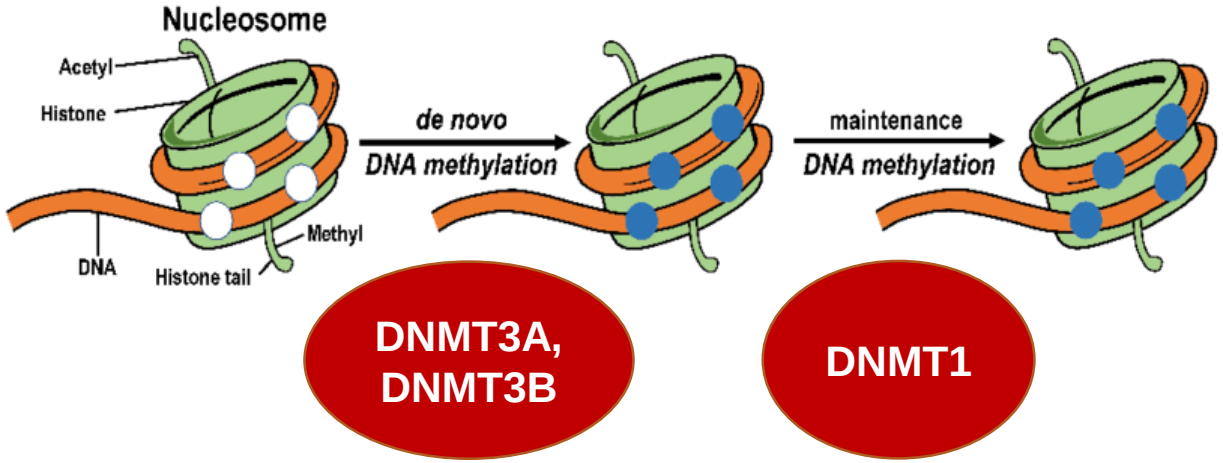
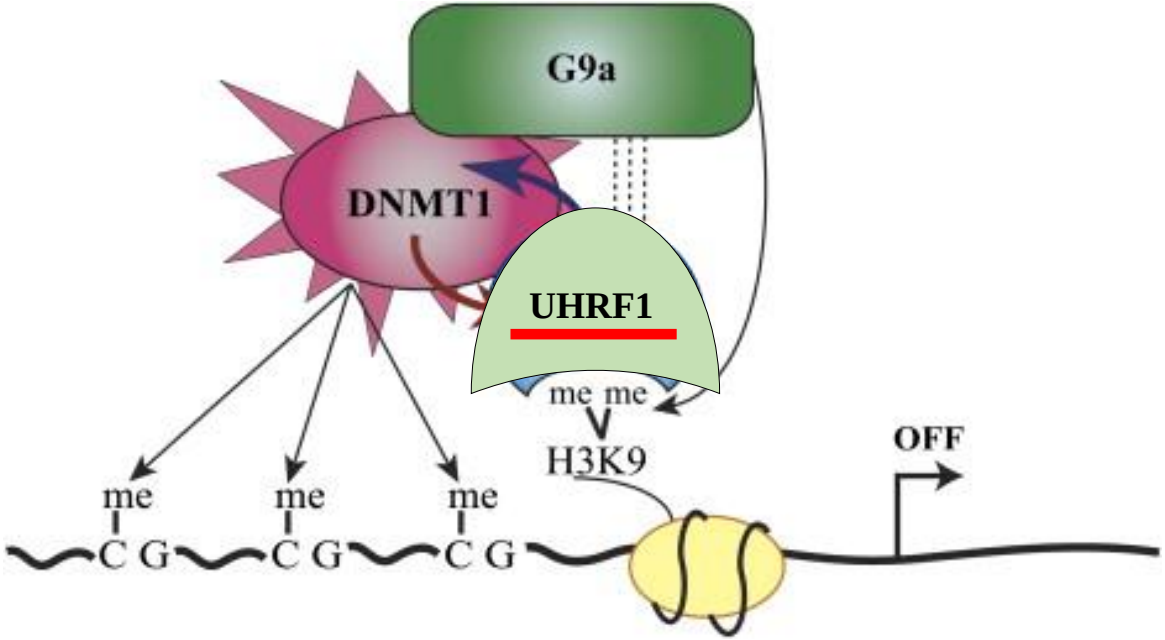
Focus on G9a and DNMT1







Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication

Estève *et al*. Genes Dev. 2006.

Dual targeting of G9a and DNMT1 in HCC

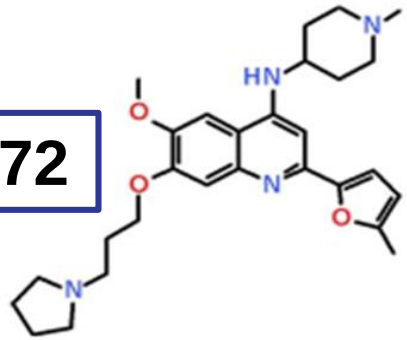
HEPATOLOGY
AASLD
HEPATOLOGY, VOL. 69, NO. 2, 2019

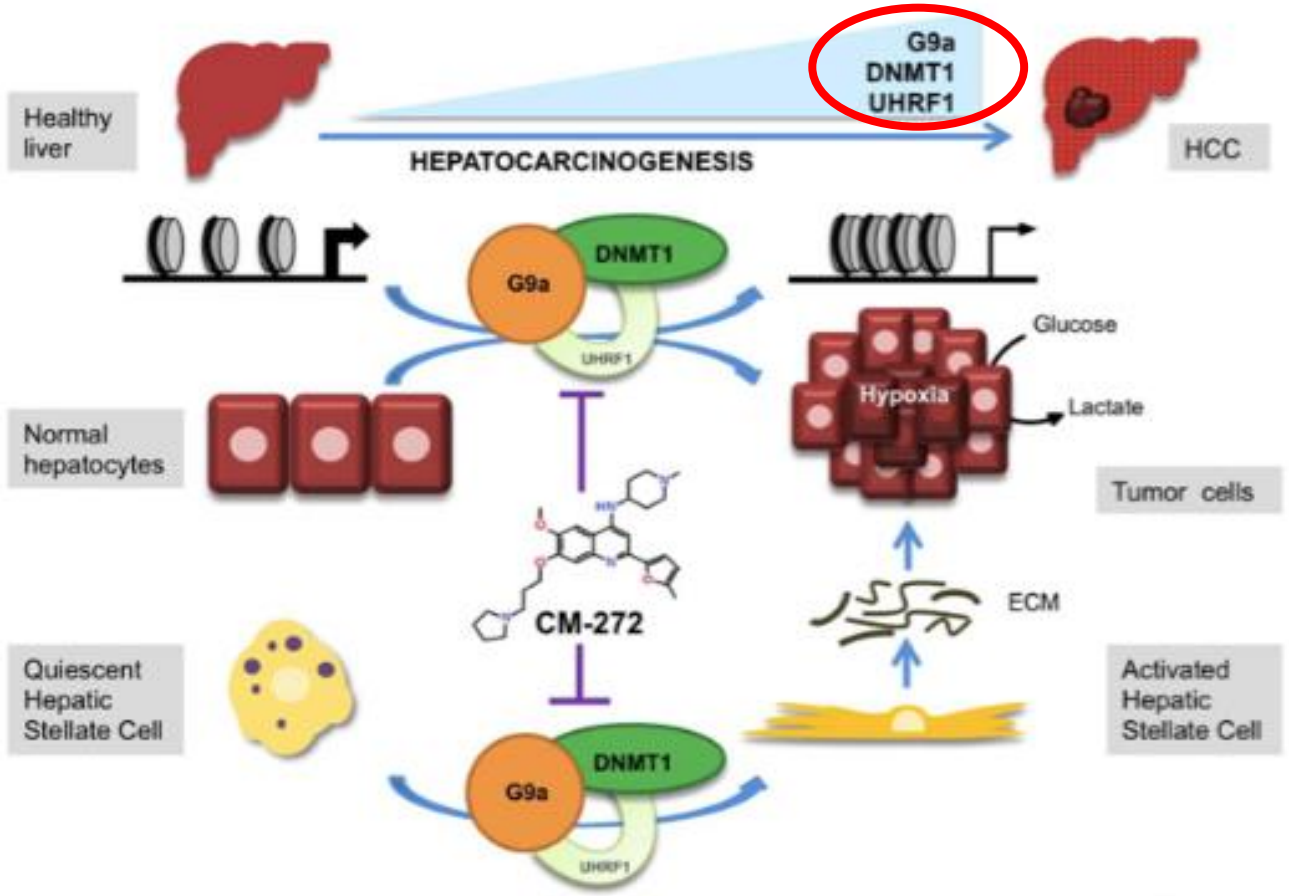
Dual Targeting of Histone Methyltransferase G9a and DNA-Methyltransferase 1 for the Treatment of Experimental Hepatocellular Carcinoma

Marina Bárcena-Varela,[1] Stefano Caruso,[2] Susana Llerena,[3,4] Gloria Álvarez-Sola,[1,4] Iker Uriarte,[1,4] M. Ujue Latasa,[1] Raquel Urtasun,[1] Sandra Rebouissou,[2] Laura Alvarez,[1] Maddalen Jimenez,[1] Eva Santamaría,[4,7] Carlos Rodriguez-Ortigosa,[1,4,7] Giuseppe Mazza,[5] Krista Rombouts,[5] Edurne San José-Eneriz,[6,7] Obdulia Rabal,[8] Xabier Agirre,[6,7] Maria Iraburu,[9] Alvaro Santos-Laso,[9,10] Jesus M. Banales,[4,9,10] Jessica Zucman-Rossi,[2] Felipe Prósper,[6,7] Julen Oyarzabal,[8] Carmen Berasain,[1,4,7*] Matías A. Ávila,[1,4,7*] and Maite G. Fernández-Barrena[1,4,7*]

- First-in-class **dual** and ***reversible*** inhibitor targeting **G9a** & **DNMT1**.
- Substrate competitive (H3K9 and DNA).

Lead compound **CM-272**





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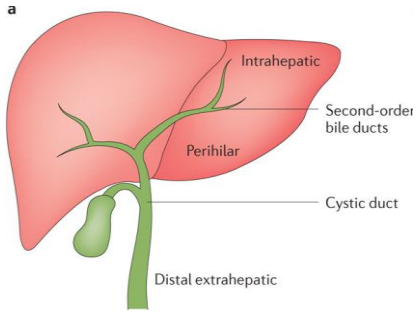
JOURNAL OF HEPATOLOGY
The Home of Liver Research

Dual targeting of G9a and DNMT1 in CCA

PS-043-Dual targeting of G9a and DNM-methyltransferase-1 for the treatment of experimental cholangiocarcinoma

Leticia Colyn, Gloria Álvarez-Sola, Maria U Latasa, Iker Uriarte, Marina Bárcena-Varela, Maria Arechederra, Maddalen Jimenez, Sergio Morini, Simone Carotti, Julen Oyarzabal, Felipe Prosper, Matteo Canale, Andrea Casadei Gardini, Maria Iraburu Elizalde, Jesus Urman, Chaobo Chen, Francisco Javier Cubero, Leonard J Nelson, Bruno Sangro, María Luz Martínez-Chantar, Jesús María Banales, Jose Marin, Carmen Berasain, Maite G Fernandez-Barrena, Matías A Avila

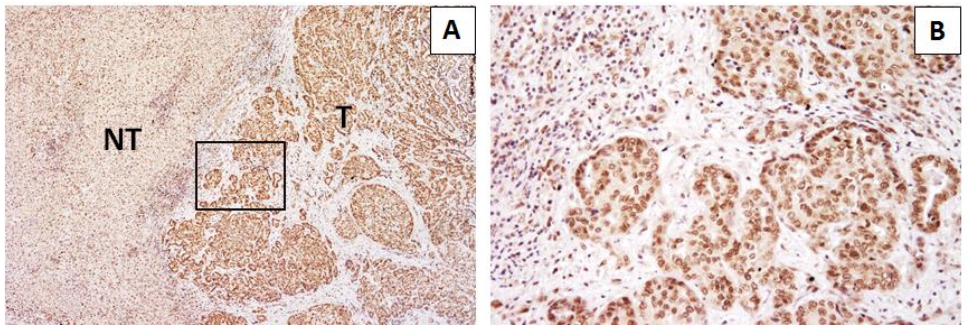


Expression levels of epigenetic modifiers G9a, DNMT1 and UHRF1 significantly increased in tumor tissue

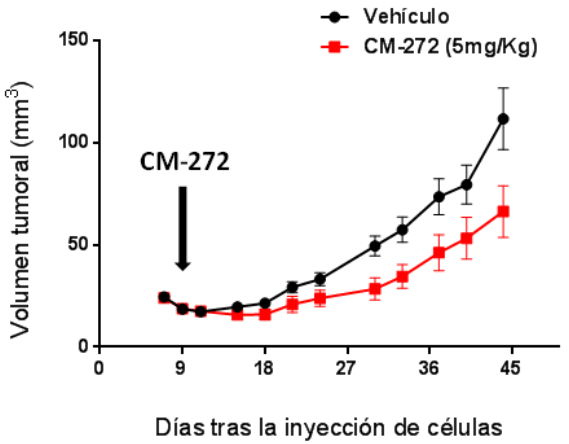
Synergistic antiproliferative effect over CCA cells by simultaneous inhibition of G9a and DNMT1.

CM-272

- Strong antiproliferative activity on different CCA cell lines
- Synergistic effect with different chemotherapeutics in the inhibition of CCA cells proliferation: Cisplatin, Mcl-1 Inhibitor and ErbB Inhibitors.
- In vivo potent inhibition of CCA growth



G9a expression in iCCA (T= tumoral tissue, NT= non tumoral tissue)



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Epigenetics and Gastric Cancer 1/5

Oncogene (2009) 28, 184–194

www.nature.com/onc

ORIGINAL ARTICLE

Hypoxic silencing of tumor suppressor *RUNX3* by histone modification in gastric cancer cells

SH Lee[1,3], J Kim[2,3], W-H Kim[2] and YM Lee[1]

Hypoxia → ↑ G9a (HMT)→ ↑ H3K9me → ↓ RUNX3 (TSG)

Cancer Res. 2005 Jun 1;65(11):4809-16.

Loss of RUNX3 expression significantly affects the clinical outcome of gastric cancer patients and its restoration causes drastic suppression of tumor growth and metastasis.

Wei D[1], Gong W, Oh SC, Li Q, Kim WD, Wang L, Le X, Yao J, Wu TT, Huang S, Xie K.

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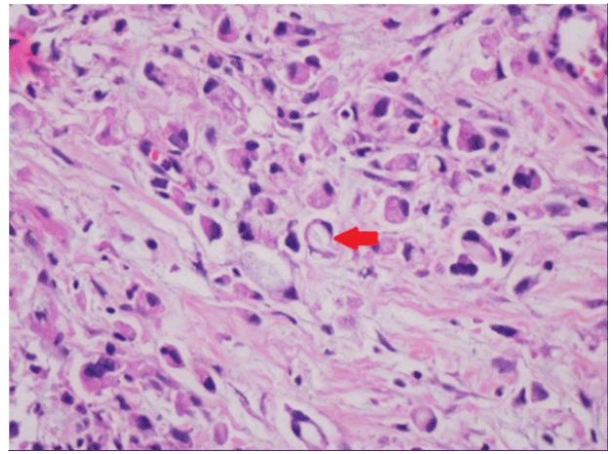
Epigenetics and Gastric Cancer 2/5

Mol Med Rep. 2013 Sep;8(3):942-8. doi: 10.3892/mmr.2013.1566. Epub 2013 Jul 2.

Expression of DNMTs and genomic DNA methylation in gastric signet ring cell carcinoma.

He M[1], Fan J, Jiang R, Tang WX, Wang ZW.



↑ ↑ ↑ DNMT1 in SRC tissue compared with matched mucosal tissue.

Positive expression of DNMT1 ←→ N+ and late TNM stages of SRC

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Epigenetics and Gastric Cancer 3/5

The FASEB Journal • Research Communication

Regulation of UHRF1 by miR-146a/b modulates gastric cancer invasion and metastasis

Lin Zhou,[1,2] Xiaodi Zhao,[1] Yanan Han,[1] Yuanyuan Lu,[1] Yulong Shang, Changhao Liu, Ting Li, Zhian Jin, Daiming Fan,[3] and Kaichun Wu[3]

UHRF1 expression in GC and adjacent nontumor tissues

Tissue	*n*	Expression level of UHRF1 −	+	++	+++	*P*
Nontumor	72	37	24	7	4	<0.01
GC	106	17	31	41	17	

The χ^2 test was used to evaluate the significance of differences between the two groups.

. Association of UHRF1 expression in the tumor tissues with demographic and clinicopathologic characteristics in 106 patients with GC

Category	*n*	Expression of UHRF1 (*n*) −	+	++	+++	*P*	Correlation coefficient
Sex						0.427	0.007^{ns}
Male	67	9	23	24	11		
Female	39	8	8	17	6		
Age (yr)						0.351	0.135^{ns}
<60	58	10	19	23	6		
≥60	48	7	12	18	11		
Differentiation						<0.05	0.286**
Well	8	5	1	2	0		
Moderate	46	7	16	17	6		
Poor	52	5	14	22	11		
Stage						<0.01	0.334***
I–II	34	11	12	8	3		
III–IV	72	6	19	33	14		
Lymph node metastases						<0.05	0.322***
0	25	8	10	6	1		
≥1	81	9	21	35	16		
Metastases to other organs						<0.05	0.249*
Present	7	0	1	2	4		
Absent	99	17	30	39	13		

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Epigenetics and Gastric Cancer 4/5

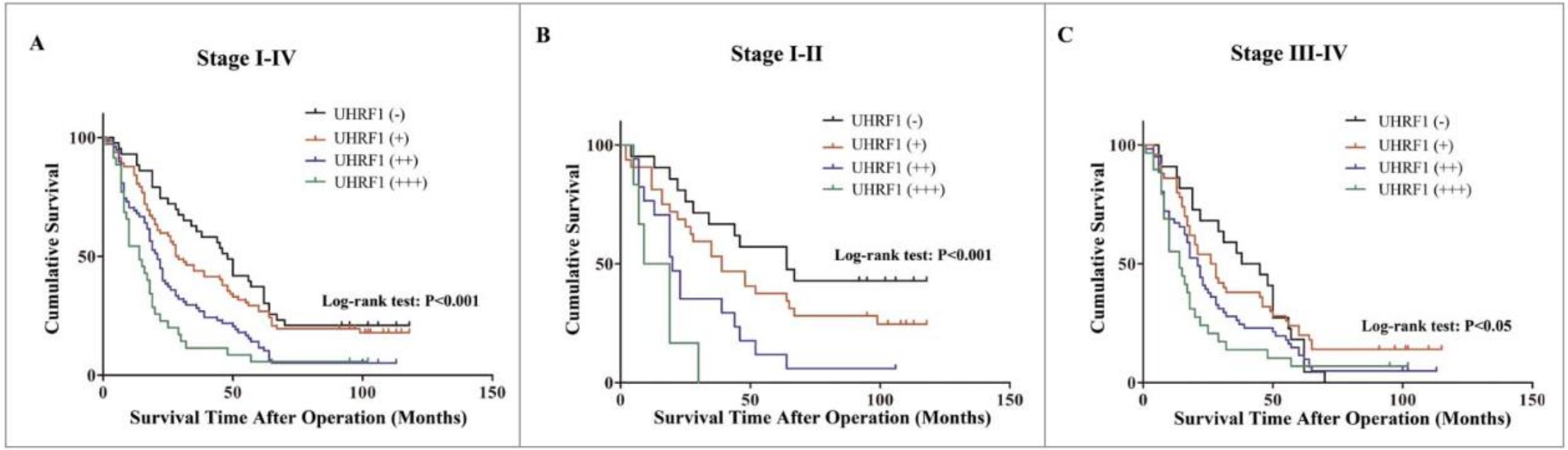
RESEARCH PAPER

Cancer Biology & Therapy 16:8, 1241–1251; August 2015; © 2015 Taylor & Francis Group, LLC

UHRF1 promotes proliferation of gastric cancer via mediating tumor suppressor gene hypermethylation

Lin Zhou[1,#,*], Yulong Shang[2,#], Zhi'an Jin[3,#], Wei Zhang[4,#], Chunlei Lv[1], Xiaodi Zhao[2], Yongqiang Liu[1], Naiyi Li[1], and Jie Liang[2,*]

Kaplan–Meier survival curves of GC patients with different level of UHRF1 expression stratified by the TNM stage of the tumor (log-rank test).



Hypermethylation of 7 TSG (CDKN2A, RUNX3, CDX2, DOXO4, PPARG, BRCA1 e PML)

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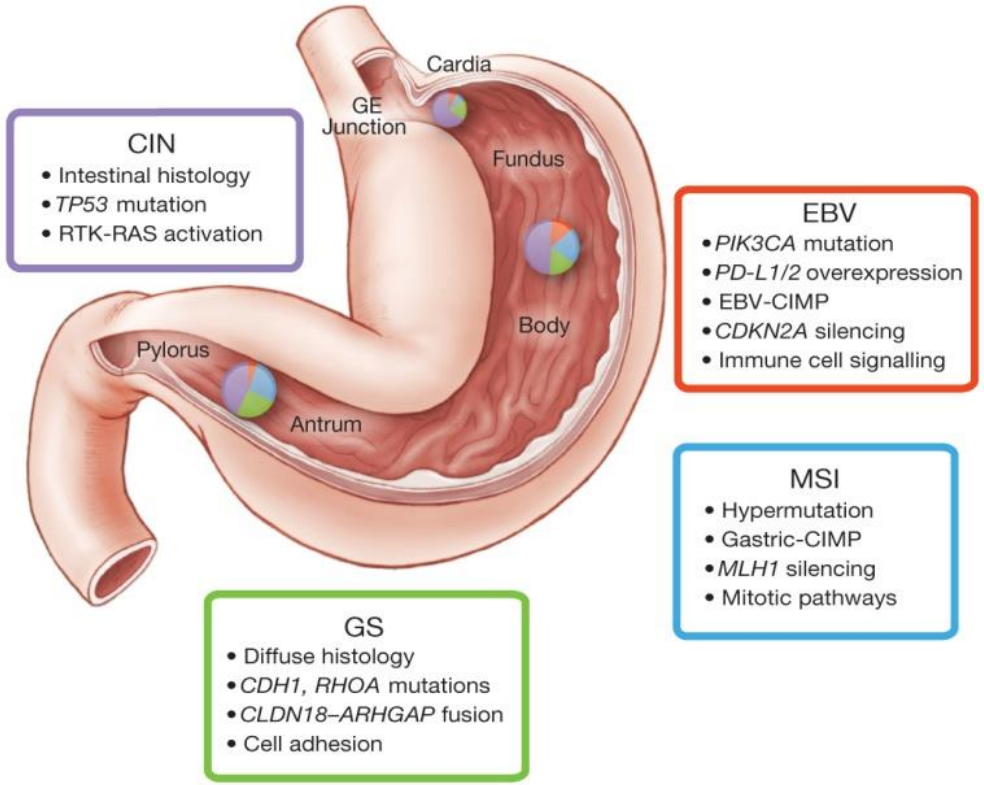
Epigenetics and Gastric Cancer 5/5

nature

Comprehensive molecular characterization of gastric adenocarcinoma

The Cancer Genome Atlas Research Network*



Key features of gastric cancer subtypes

EBV- positive tumours had a **higher prevalence of DNA hypermethylation** than any cancers reported by TCGA

All EBV-positive tumours clustered together and **exhibited extreme CIMP** (CpG island methylator phenotype)

All EBV-positive tumours assayed displayed ***CDKN2A* (*p16INK4A*) promoter hypermethylation**, but lacked the *MLH1* hypermethylation

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RESEARCH PROJECT

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Modena

Collection of human Gastric Cancer (GC) samples and clinico-pathological data (Jan 2000 – Feb 2019) → 100 samples

Preparation of tissue samples for histological examination

Inclusion Criteria

Patients with histological diagnosis of **resectable or advanced gastric cancer**

Availability of **surgical (or bioptic) specimens** for the analysis

No chemo- and/or radiotherapy prior to surgery

Written informed consent

Exclusion Criteria

Patients with uncertain histological diagnosis of gastric cancer

Chemo- and/or radiotherapy prior to surgery

Refusal of consent

INITIAL PHASES

cima
CENTRO DE INVESTIGACIÓN MÉDICA APLICADA
UNIVERSIDAD DE NAVARRA

IHC Analysis of G9a, DNMT1 and UHRF1

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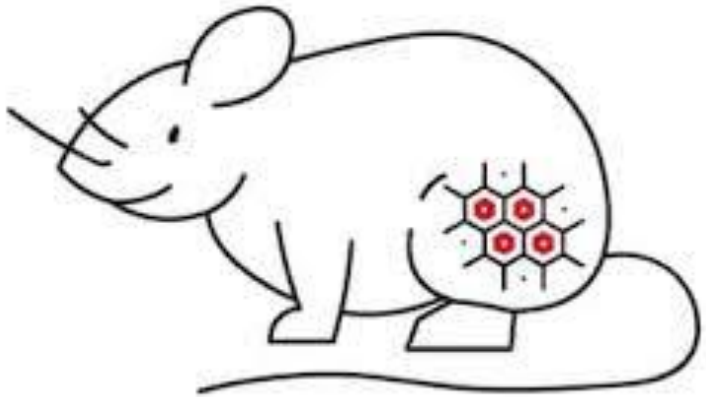
SUBSEQUENT PHASES

IN VITRO (GC Cell lines)

Evaluation of **G9a, DNMT1 and UHRF1 expression**

Evaluation of **CM-272 effect**

Evaluation of potential synergistic effect with other anticancer agents



IN VIVO (mouse xenograft models)

Evaluation of **CM-272 effect**

Aims

Primary objective:

Expression evaluation of G9a, DNMT1 and UHRF1 in GC patients

- Provide a better characterization of methylation in GC
- Identify potential novel therapeutic targets providing a background for further investigations in order to develop more specific therapeutic strategies *(Epi-drugs)*

Secondary objective:

Correlation of profile expression of G9a, DNMT1 and UHRF1 in GC **with clinico-pathological features** and **survival parameters**

- Identify possible prognostic and predictive factors

Take-home messages

- **Epigenetic mechanisms** are emerging as **attractive therapeutic target** in solid tumors
- **Epi-drugs** are already being tested
- Given the crosstalk between chromatin marks, **simultaneous targeting** of different epigenetic modifiers **may improve therapeutic efficacy**
- **CM-272** as emerged as a **promising** dual (G9a and DNMT1) targeting agent (HCC, CCA)
- **Epigenetic dysregulation** plays a crucial role in **GC development**
- **G9a and DNMT1** seems **potential epi-target** also **in** the context of **GC**, but few data are available
- Further investigation are needed

Acknowledgments

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