XVIII Encuentro de Cooperación Farma-Biotech

Non-invasive method for diagnosis and monitoring of glioblastoma

IdiPAZ
Instituto de Investigación
Hospital Universitario La Paz

Madrid, 29 de octubre de 2019

GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española

farmaindustria

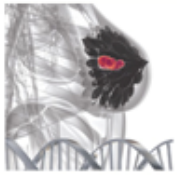
1. The Institution

Brief description of IdiPAZ:

Institute for Health Research (HULP, HUF, UAM and FIBHULP)
Constitution on December 15, 2009
Accredited institution by the Health Institute Carlos III (ISCIII)
Re-accredited on May 2015 for a 5 years period
55 research groups
6 six strategic areas

- **Neurosciences**
- **Cardiovascular**
- **Infectious diseases and immunity area**
- **Large system pathologies**
- **Cancer and human molecular genetics**
- **Surgery, transplant and health technologies area**

Certified according to ISO 9001:2015 and UNE 166002:2014



Cancer and human molecular genetics

Area Coordinator: Amparo Cano García

Research Groups

INGEMM - INSTITUTO DE GENETICA MEDICA Y MOLECULAR (INSTITUTE OF MEDICAL AND MOLECULAR GENETICS)
Pablo Lapunzina Badía

MOLECULAR GENETICS OF DYSTROGLYCANOPATHIES
Jesús Cruces Pinto

TRASLATIONAL ONCOLOGY
Jaime Feliu Battle

EXPERIMENTAL THERAPIES AND BIOMARKERS IN CANCER
Inmaculada Ibáñez de Cáceres
Javier de Castro Carpeño

OTO-NEUROSURGERY RESEARCH
Luis Lassaletta Atienza

CANCER MOLECULAR PATHOLOGY AND THERAPEUTIC TARGETS
David Hardisson Hernáez

MECHANISMS OF TUMOUR PROGRESSION
Amparo Cano García

ANIMAL AND CELL MODELS FOR DETECTION AND CHARACTERISATION OF LEUKEMIC STEM CELLS
Carmela Calés Bourguet

RESEARCH AND DIAGNOSIS OF INHERITED METABOLIC DISEASES
María Belén Pérez González

TERAPIAS EXPERIMENTALES Y BIOMARCADORES EN CÁNCER

Composición

Nombre	Cargo	Institución
María Inmaculada Ibáñez de Cáceres	**Investigadora Senior (Contrato Miguel Servet- Tipo 2) Jefe de Laboratorio**	**Hospital Universitario La Paz**
Javier de Castro Carpeño	**Jefe de Sección de Oncología Profesor Asociado**	**Hospital Universitario La Paz Universidad Autónoma de Madrid**
Cristóbal Belda Iniesta	Facultativo Especialista de Área en Oncología	Hospitales de Madrid
Jaime Carrillo García	Investigador Postdoctoral	IIB "Alberto Sols"
María Isabel Esteban Rodríguez	Facultativo Especialista de Área en Anatomía Patológica	Hospital Universitario La Paz
María Galardi Castilla	Investigadora Predoctoral	IIB "Alberto Sols"
Julia Jiménez Hernández	Investigadora Predoctoral	Universidad Autónoma de Madrid
Cristina Manguán García	Técnico de Laboratorio	IIB "Alberto Sols"
Olga Pernía Arias	Técnico de Laboratorio (Contrato PTA)	Hospital Universitario La Paz
Rosario Perona Abellón	Profesora de Investigación	IIB "Alberto Sols"
Verónica Pulido Sanz	Investigadora Predoctoral	Hospital Universitario La Paz
Garcilaso Riesco Eizaguirre	Facultativo Especialista de Área en Endocrinología y Nutrición	Hospital Universitario La Paz
Carlos Rodríguez Antolín	Bioinformático Contratado	Hospital Universitario La Paz
Rocío Rosas Alonso	Farmacéutica Interna Residente	Hospital Universitario La Paz
Isabel Sánchez Pérez	Profesora Contratada Doctor	Universidad Autónoma de Madrid
Leandro Sastre Garzón	Investigador Científico	IIB "Alberto Sols"
Javier Andrés Soto	Investigador Predoctoral	Universidad Autónoma de Madrid
Olga Vera Puente	Investigadora Predoctoral	Hospital Universitario La Paz

IdiPAZ's Innovation Indicators (2014-2018)

Nº Patents	OEPM	EPO	USPO	Other Offices
Priority applications	13	3	1	0
PCT	12	3	0	0
Regional phase	0	4	4	2
Granted patents	20	2	1	0

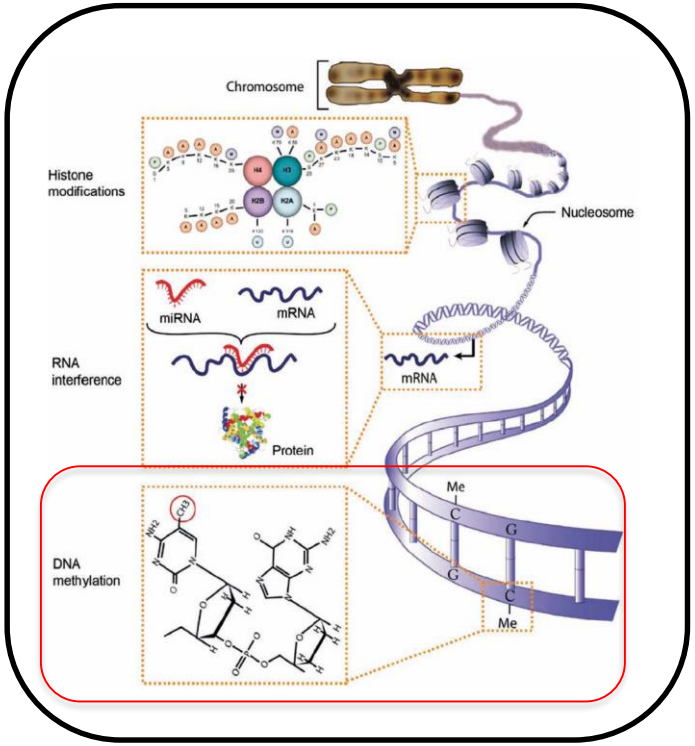
Exploita
Natio
Internat

The innovative activity carried out in IdiPAZ associated with the exploitation of its technology portfolio has generated a return that exceeds the 300,000 euros barrier in the last 5 years

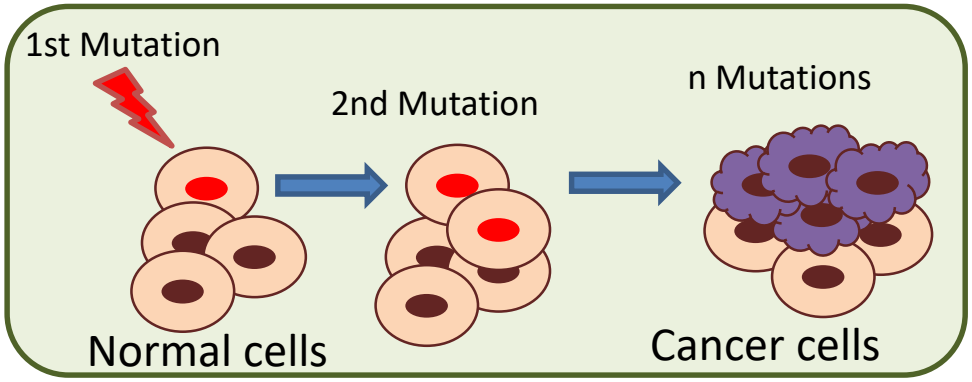
	TOTAL		TOTAL		TOTAL
Nº Trademarks (Granted vs applications)	15/17	Exploited trademarks	16	TM Licensing revenue	202.471,88 €
	TOTAL		TOTAL		TOTAL
SW/apps	18	Nº SW exploited	11	SW Licensing revenue	3.159,91 €
	TOTAL		TOTAL		TOTAL
IP licences	35	IP Licensing revenue	16.730 €	Nº Centres of implantation	35

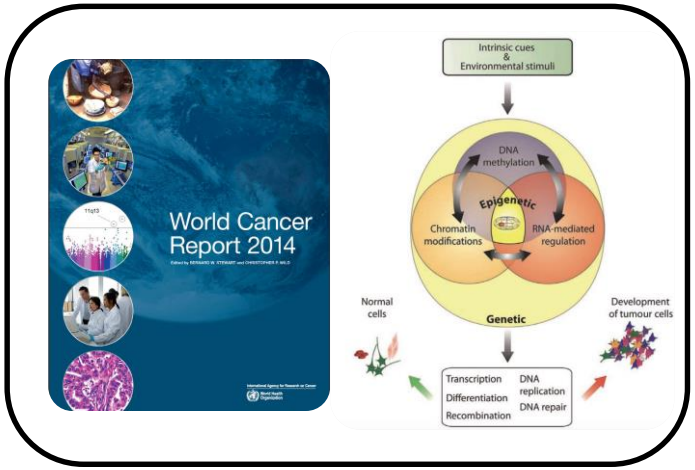
The group: Cancer epigenetics

EPIGENETIC

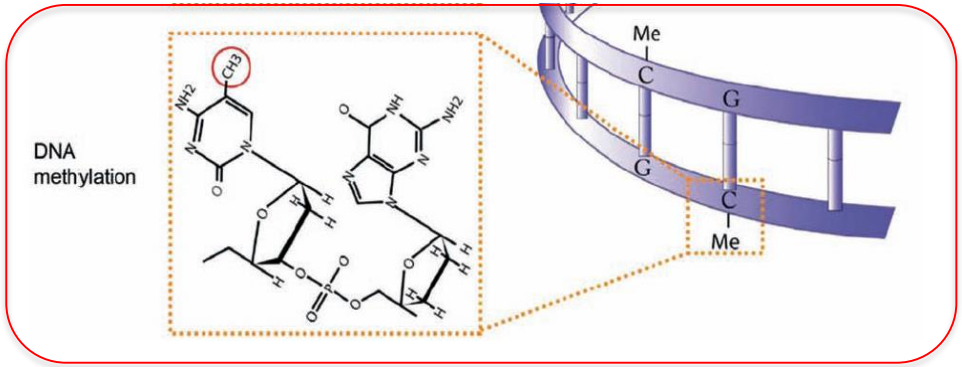


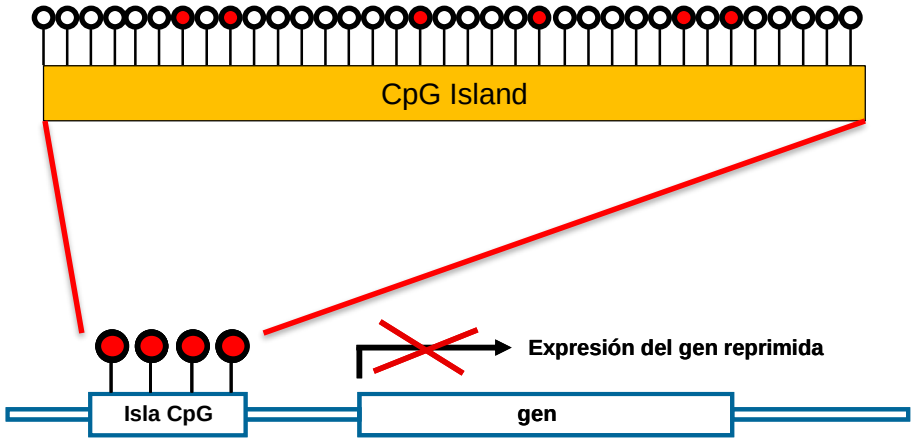
GENETIC





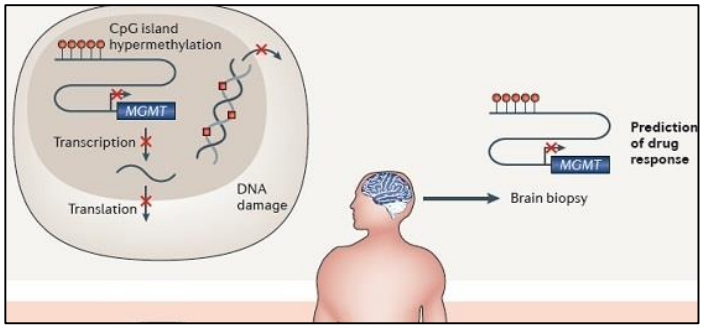
Product-Research area: DNA methylation





General overview

Glioblastoma: Most frequent and Worst prognosis



Easy commercialization without additional experimental development.
For immediate clinical use (already implanted in HULP)

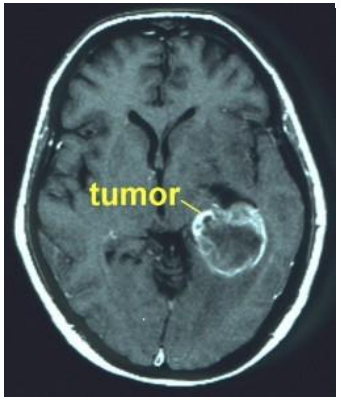
Identification **by liquid biopsy** of an approved marker of mandatory use according to clinical guidelines for glioblastoma diagnosis in tissue.

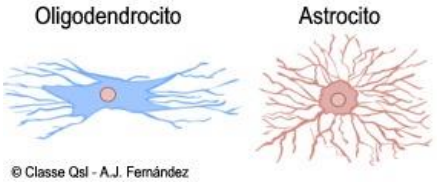
Allows for **the first time** the diagnosis and **follow-up** of patients using **non-invasive** methodology

There is an **approved** drug for the treatment of this pathology in addition to new **on-going new studies phase III** that refer to the use this marker.

Target Indications

Type \ Grade	WHO grade I	WHO grade II	WHO grade III	WHO grade IV
	Circumscript	Diffuse		
		Low-grade	High-grade	
Astrocytoma	Pilocytic astrocytoma	Low-grade astrocytoma	Anaplastic astrocytoma	Glioblastoma
Oligodendroglioma		Low-grade oligodendro-glioma	Anaplastic oligodendro-glioma	
Oligo-astrocytoma		Low-grade oligo-astrocytoma	Anaplastic oligo-astrocytoma	





- 60% Brain Neoplasias.
- All ages 17- 99 years old (more frequently 50-60 years old).
- Difficult accessibility and with little clinical progress over the last few years.
- Blood Brain barrier prevents the tumour from spreading but difficults diagnosis by ctDNA.
- Diagnosis in tumour tissue (paraffin surgical sample or biopsy).
- Standard treatment established in 2005 without modifications: maximal surgical excision followed by RT with concomitant QT.

ESMO Guidelines 2014 and 2018 for Molecular markers in gliomas

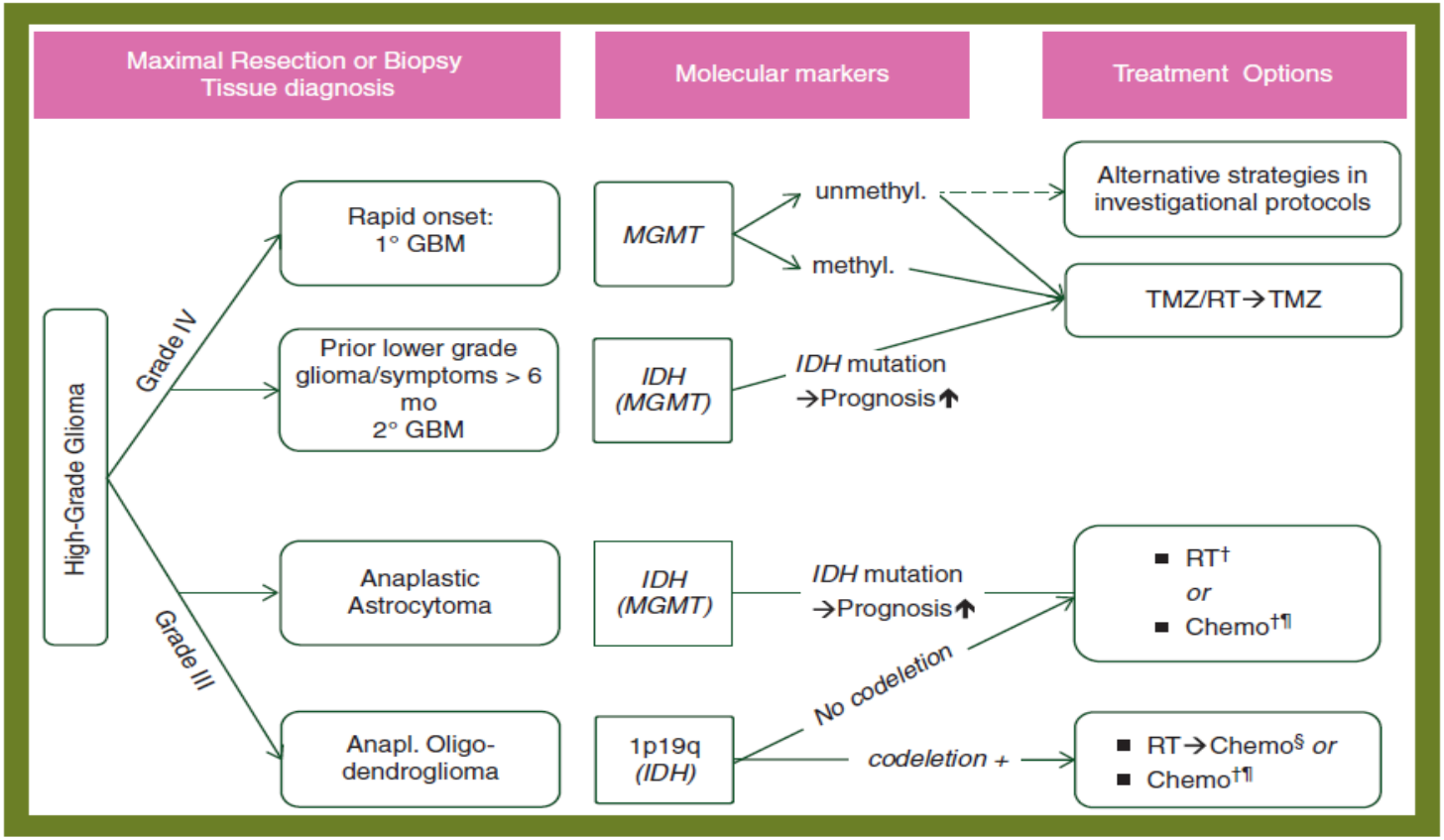
clinical practice guidelines

Annals of Oncology 25 (Supplement 3): iii93–iii101, 2014
doi:10.1093/annonc/mdu050
Published online 29 April 2014

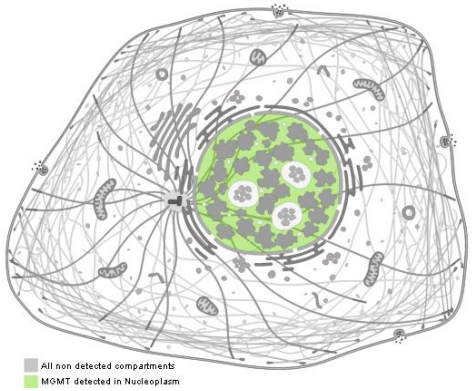
High-grade glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

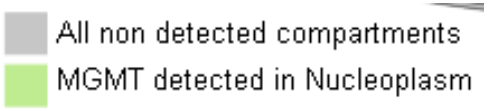
R. Stupp[1], M. Brada[2], M. J. van den Bent[3], J.-C. Tonn[4] & G. Pentheroudakis[5] on behalf of the ESMO Guidelines Working Group*

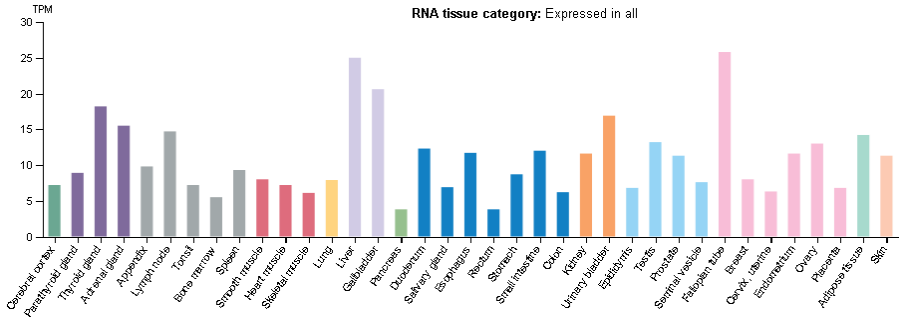
[1]Department of Oncology and Cancer Centre, University Hospital Zurich, Zurich, Switzerland; [2]Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Clatterbridge Cancer Centre, Wirral, UK; [3]Dep
Ludwig-Maximilians-University, Munich, Germany; [5]Dep



Chromosome 10: 131,265,448-131,566,271







- MGMT is expressed in all tissues.
- 30% of gliomas present MGMT silenced by DNA promoter methylation, which is associated with a best treatment response.

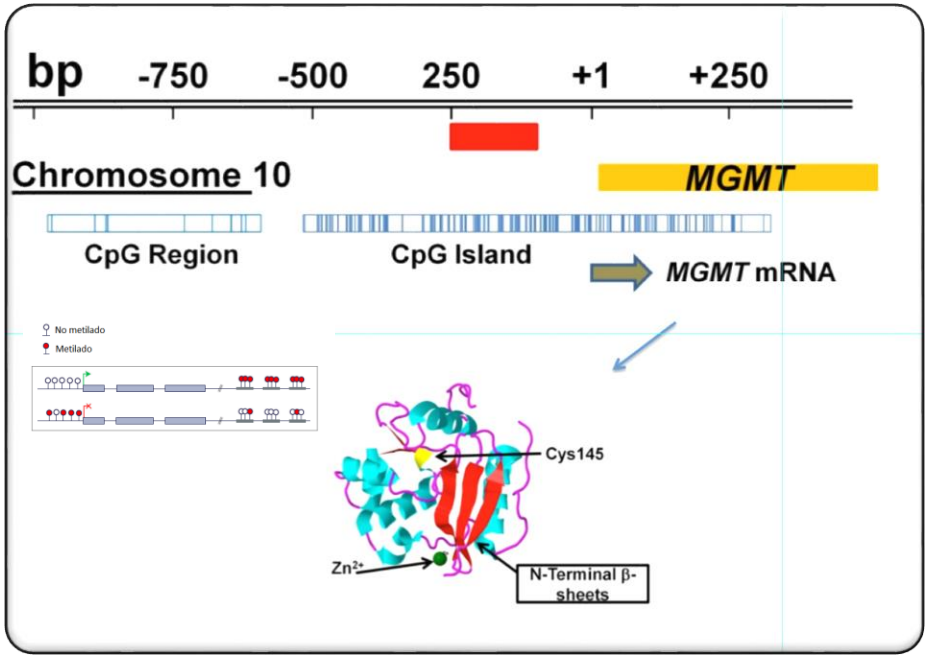
Table 1 *Hypermethylation of MGMT promoter in primary tumors*

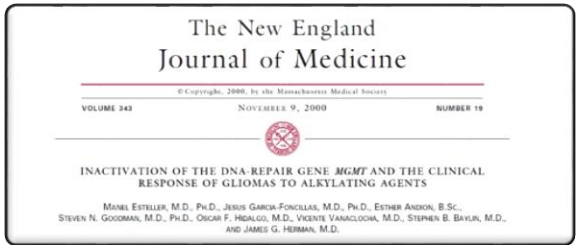
	Primary tumors
Brain tumors	55/166 (33%)
Gliomas	54/140 (38%)
Nongliomas	1/26 (3%)
Colon cancer	14/36 (38%)
Lung cancer	10/41 (24%)
NSCLC	10/34 (29%)
SCLC	0/7
Head and neck carcinoma	6/21 (28%)
Lymphomas	15/61 (25%)
Breast cancer	0/36
Ovarian cancer	0/23
Endometrial cancer	0/17
Leukemias	2/31 (6%)
Pancreatic carcinoma	2/18 (11%)
Melanoma	2/18 (11%)
Renal carcinoma	1/12 (8%)
Bladder carcinoma	2/44 (4%)

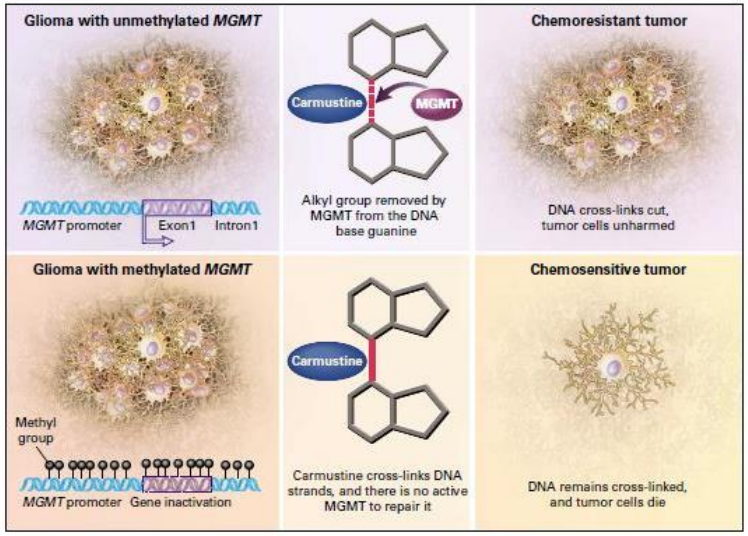
[CANCER RESEARCH 59, 793–797, February 15, 1999]

Target indications: Clinical significance of MGMT

	MGMT Methylation
	O6-methylguanine DNA methyltransferase (*MGMT*)
Function	Enzyme that repairs the DNA by removing the alkyl group (CH_3) from the O6 Guanine
Detection	MSP and QMSP
Predictive value	Glioblastoma, chemotherapy response







Target indications: Clinical significance of MGMT

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma

Roger Stupp, M.D., Warren P. Mason, M.D., Martin J. van den Bent, M.D., Michael Weller, M.D., Barbara Fisher, M.D., Martin J.B. Taphoorn, M.D., Karl Belanger, M.D., Alba A. Brandes, M.D., Christine Marosi, M.D., Ulrich Bogdahn, M.D., Jürgen Curschmann, M.D., Robert C. Janzer, M.D., Samuel K. Ludwin, M.D.,Thierry Gorlia, M.Sc., Anouk Allgeier, Ph.D., Denis Lacombe, M.D., J. Gregory Cairncross, M.D., Elizabeth Eisenhauer, M.D., and René O. Mirimanoff, M.D., for the European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups and the National Cancer Institute of Canada Clinical Trials Group*

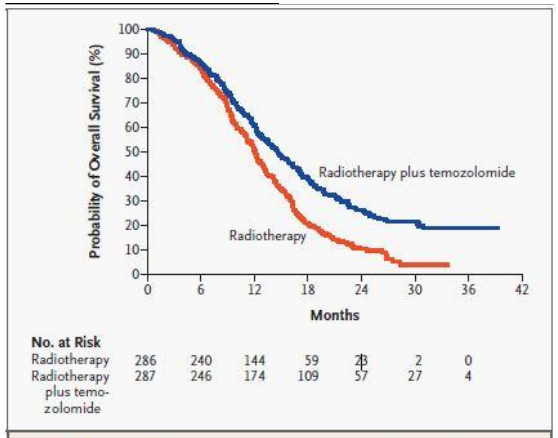


Figure 1. Kaplan–Meier Estimates of Overall Survival According to Treatment Group.
The hazard ratio for death among patients treated with radiotherapy plus temozolomide, as compared with those who received radiotherapy alone, was 0.63 (95 percent confidence interval, 0.52 to 0.75; P<0.001).

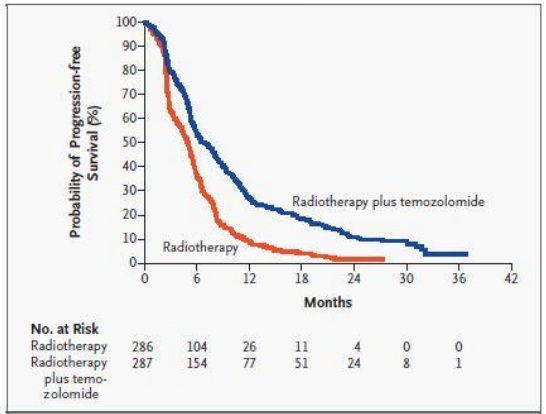


Figure 2. Kaplan–Meier Estimates of Progression-free Survival According to Treatment Group.
The hazard ratio for death or disease progression among patients treated with radiotherapy plus temozolomide, as compared with those treated with radiotherapy alone, was 0.54 (95 percent confidence interval, 0.45 to 0.64; P<0.001).

CONCLUSIONS

The addition of temozolomide to radiotherapy for newly diagnosed glioblastoma resulted in a clinically meaningful and statistically significant survival benefit with minimal additional toxicity.

N Engl J Med 2005;352:987-96.

Target indications: Clinical significance of MGMT

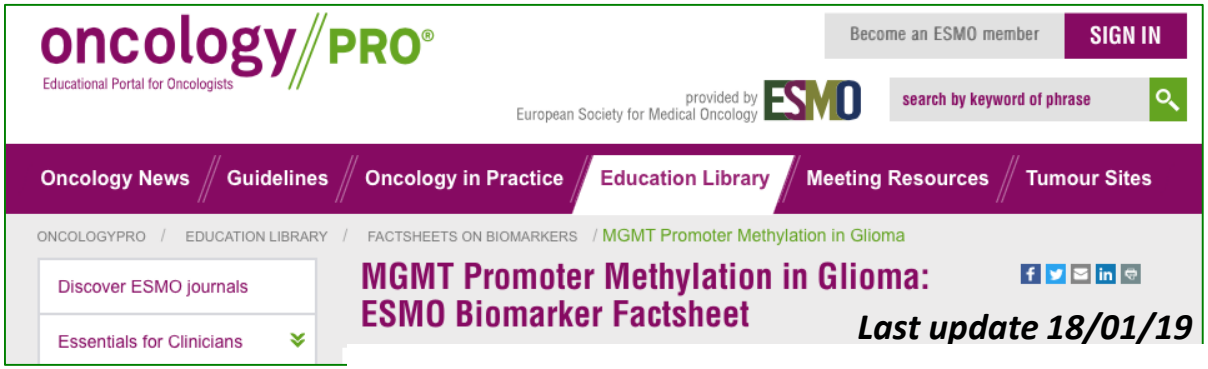


Table 1. Long-term survival results according to type of treatment and *MGMT* status in the 'Stupp trial' [5]

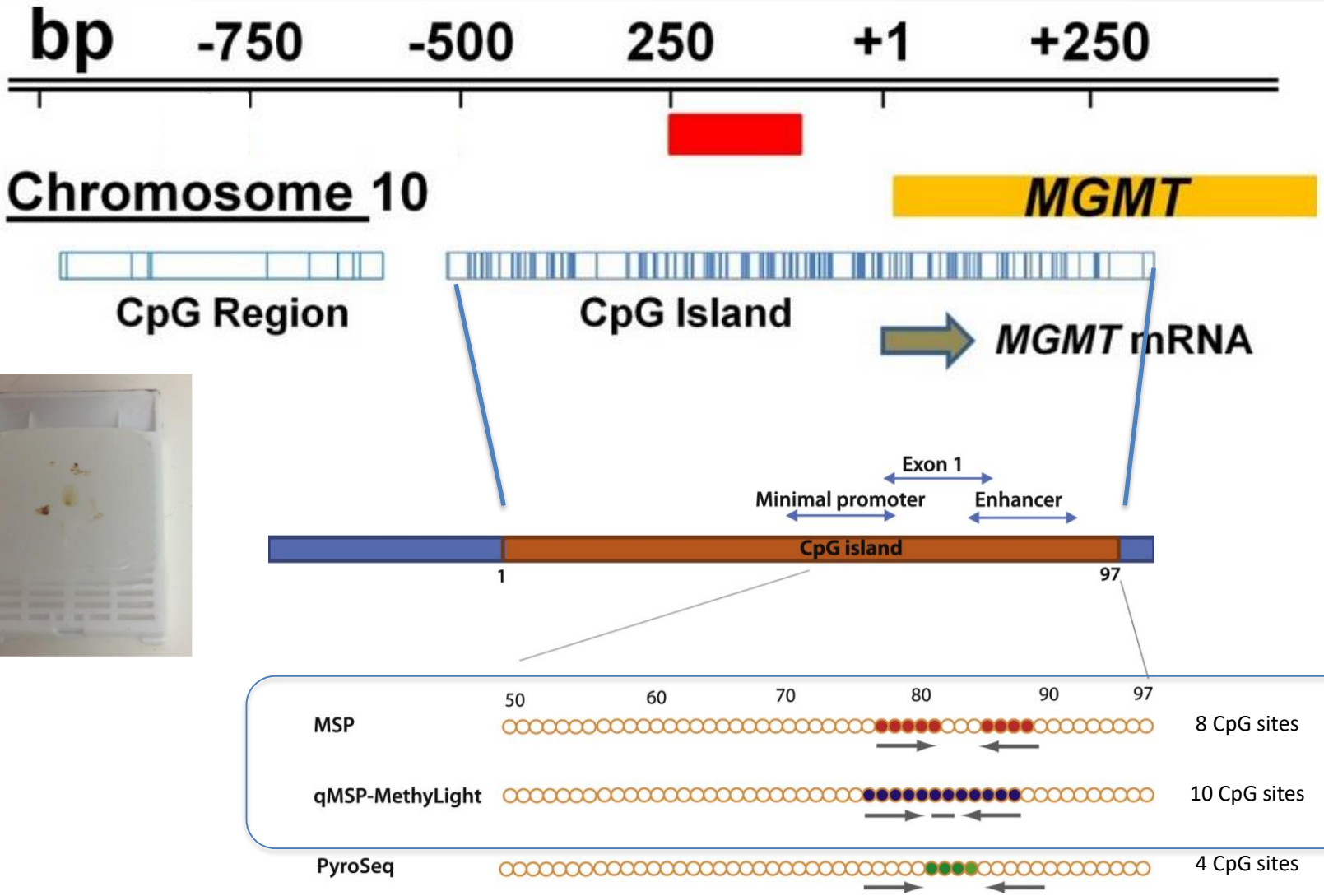
MGMT status	Treatment	Median OS (months)	2-year OS (%)	3-year OS (%)	4-year OS (%)	5-year OS (%)
Unmethylated	RT	11.8	1.8	0	0	0
	RT + TMZ	12.6	14.8	11.1	11.1	8.3
Methylated	RT	15.3	23.9	7.8	7.8	5.2
	RT + TMZ	23.4	48.9	27.6	22.1	13.8

MGMT, O^6-methylguanine-DNA methyl-transferase; OS, overall survival; RT, radiotherapy; TMZ, temozolomide

MGMT Promoter Methylation in Glioma: ESMO Biomarker Factsheet
Giulio Metro, Tiziana Pierini, Roberta La Starza. 18 January 2019

Lancet Oncol. 2009 May;10(5):459-66.

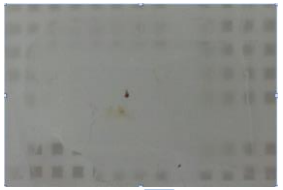
Differential features facing the market



Cankovic. 2013: The Role of MGMT Testing in Clinical Practice
A Report of the Association for Molecular Pathology

Differential features facing the market

Pathology Dpt

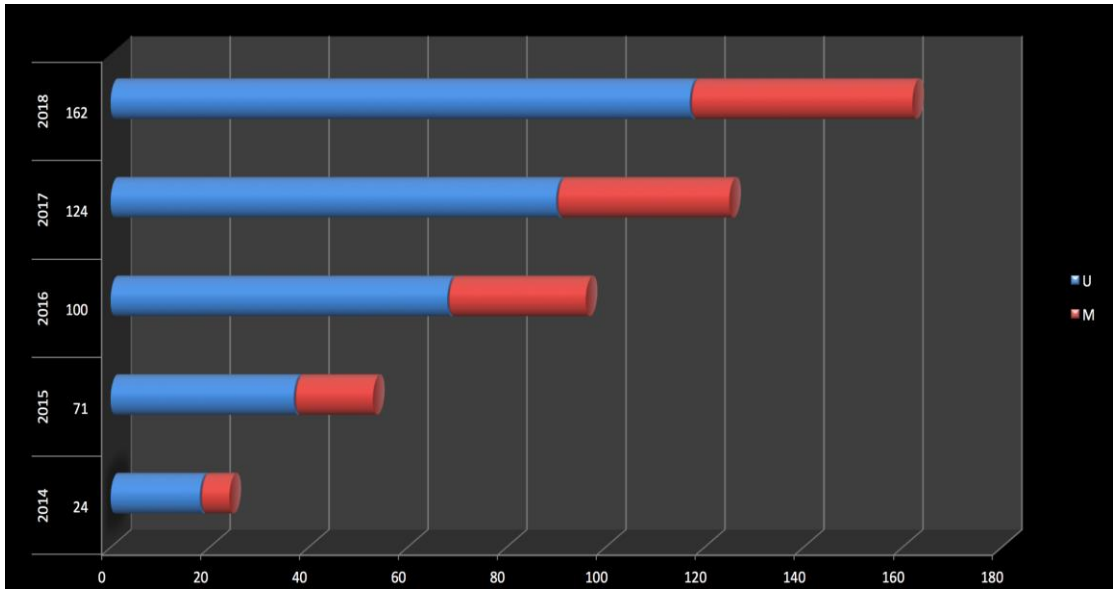


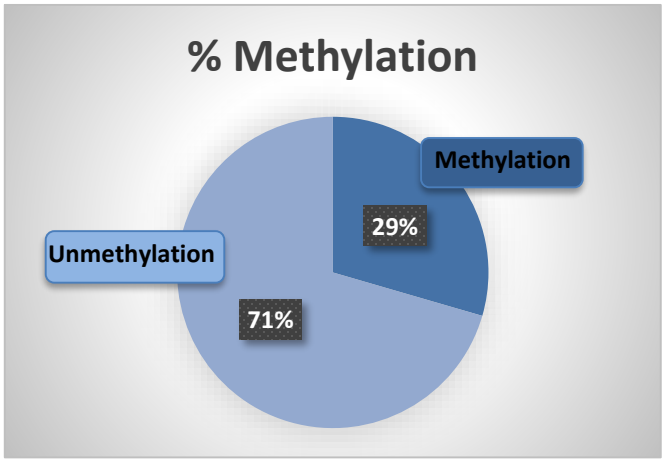
Medical Oncology Dpt

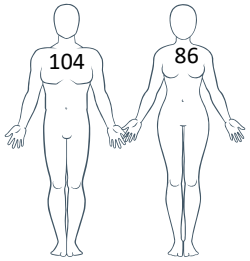
INGEMM Epigenetics

Target indications: Clinical significance of MGMT

U.H.La PAZ: Glioblastoma, Anaplastic astrocytomas and chilhood brain tumors







Response time: 7-10 days

Since 2014 there have been 15 cases in which the treatment has been modified because it did not meet the clinical criteria based on the methylation status of the MGMT gene.

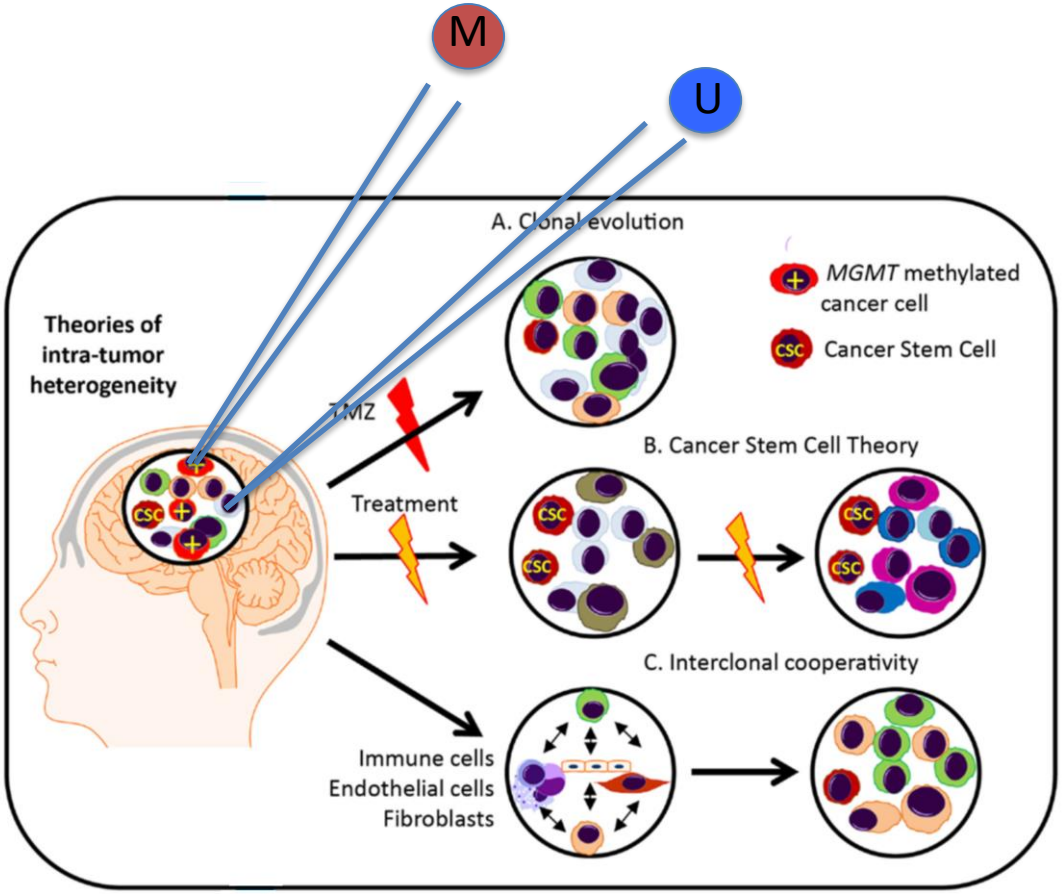
200.000 euros

Pitfalls associated to the current diagnosis

Inaccessibility of the sample
Tumour heterogeneity
Tissue necrosis
Only useful for treatment prediction

Discordance between molecular marker and patient outcome/treatment response

Therapy resistance



Differential features facing the market

MGMT methylation detection in blood.
None of them have enough sensitivity to be used in the clinical practice

JCR 2018	Year of publication	Article (PMID)	% S	N	Tumor type	Technique
NO JCR	2015	26171163	37,3 (SERUM)	89	glioma	PCR + HPLC
D1	2010	20150384	59 (SERUM)	41	glioma	Qmsp con B-actina
D1	2009	19773381	27-60 % (PLASMA)	64	colon	MSP

Pyrosequencing versus methylation-specific PCR for assessment of MGMT methylation in tumor and blood samples of glioblastoma patients

August 2019

Anna Estival, Carolina Sanz, Jose-Luis Ramirez, Jose Maria Velarde, Marta Domenech, Cristina Carrato, Ramón de las Peñas, Miguel Gil-Gil, Juan Sepúlveda, Roser Armengol, Isaac Cardiel, Alfonso Berrocal, Raquel Luque, Ana Herrero & Carmen Balana ✉

Scientific Reports **9**, Article number: 11125 (2019) | Download Citation ⬇
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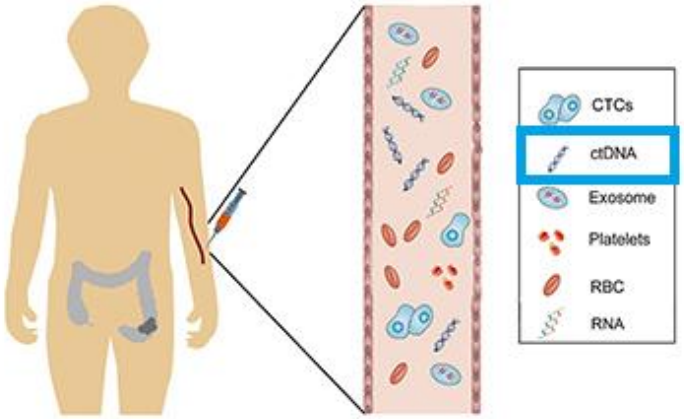
Abstract

Circulating biomarkers in blood may provide an interesting alternative to risky tissue biopsies in the diagnosis and follow-up of glioblastoma patients. We have assessed *MGMT* methylation status in blood and tissue samples from unresected glioblastoma patients who had been included in the randomized GENOM-009 trial. Paired blood and tissue samples were assessed by methylation-specific PCR (MSP) and pyrosequencing (PYR). After establishing the minimum PYR cut-off that could yield a significant difference in overall survival, we assessed the sensitivity, specificity, positive predictive value and negative predictive value (NPV) of the analyses. Methylation could be detected in cfDNA by both MSP and PYR but with low concordance with results in tissue. Sensitivity was low for both methods (31% and 38%, respectively), while specificity was higher for MSP in blood than for PYR in plasma (96% vs 76%) and NPV was similar (56 vs 57%). Concordance of results in tissue by MSP and PYR was 84.3% ($P < 0.001$) and correlated with outcome. We conclude that detection of cfDNA in the blood of glioblastoma patients can be an alternative when tumor tissue is not available but methods for the detection of cfDNA in blood must improve before it can replace analysis in tumor tissue.

MGMT methylation in free circulating DNA



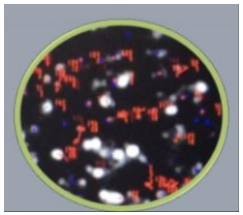
PACIENTE	MSP tumor	qMSP % M tumor	qMSP % ctDNA
GB1	M	99.9	0
GB2	M	92.6	0
GB3	M	91.5	0
GB4	M	77.0	1.5
GB5	M	73.2	13.3
GB6	M	100.0	0
GB7	U	0.4	0
GB8	U	0.0	0
GB9	U	0.0	0
GB10	U	0.0	0
GB11	U	0.0	0
GB12	U	0.0	0
GB13	U	0.0	0
GB14	U	0.0	0
GB15	U	0.0	0
GB16	U	0.0	0
GB17	U	0.0	0
GB18	U	0.0	0
GB19	U	0.0	0
GB20	U	0.0	0

Low sensitivity to detect the presence of MGMT DNA methylation in ctDNA.
There is also a low ratio of reproducibility due to the low levels of methylation found in ctDNA

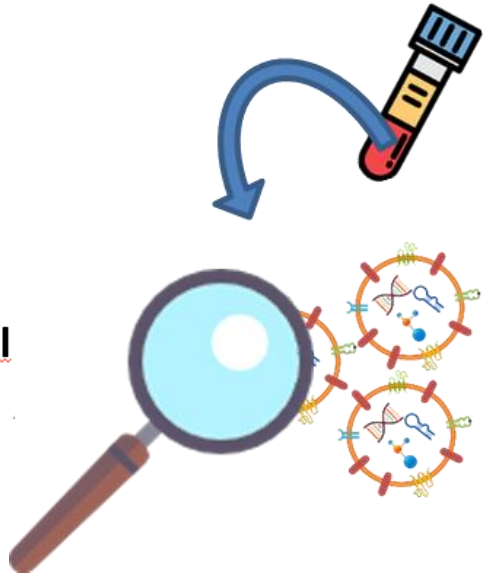
ctDNA Sensitivity **M** = 33%

ctDNA Specificity **U** = 100%

Innovative approach



Basic — Approach — **Translational**



frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 12 December 2014
doi: 10.3389/fncel.2014.00418

Gliomas and the vascular fragility of the blood brain barrier

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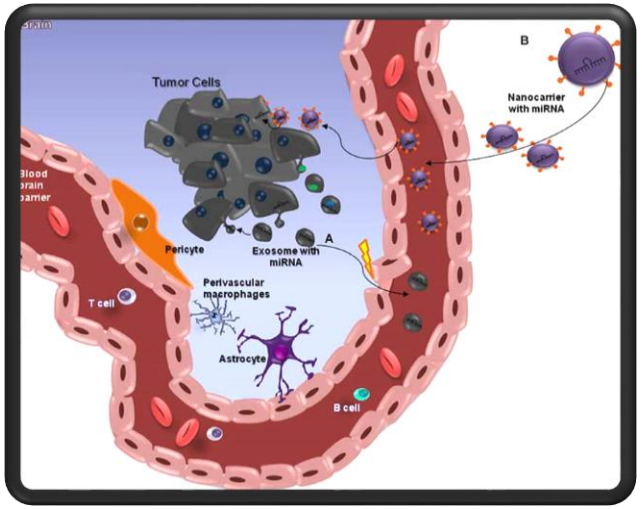
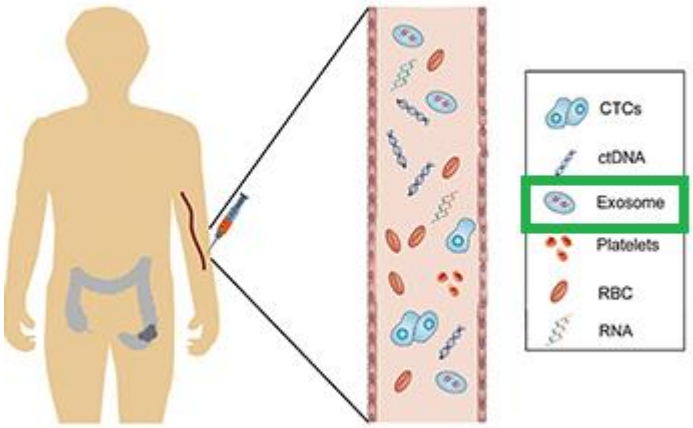
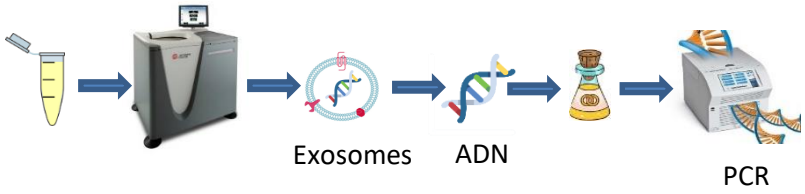


Current state of development

MGMT-methylation in DNA from Circulating Exosomes





PACIENTE	MSP t	qMSP % M tumor	qMSP% ADN exosomal
GB1	M	99.9	87.97
GB2	M	77	100
GB3	M	92.6	90,6
GB4	M	100.0	89.2
GB6	M	99.9	87.97
GB7	U	0	0
GB8	U	0	0
GB9	U	0	0
GB11	U	0	0
G			
G			
G			
G			
GB19	U	0.0	0
GB20	U	0.0	88,4
GB21	U	0.0	0
GB22	U	0.0	0
GB23	U	0.0	0
GB24	U	0.0	0
GB25	U	0.0	0
GB26	U	0.0	0
GB31	U	0.0	0
GB32	U	0.0	0
GB33	U	0.0	0
GB34	U	0.0	0

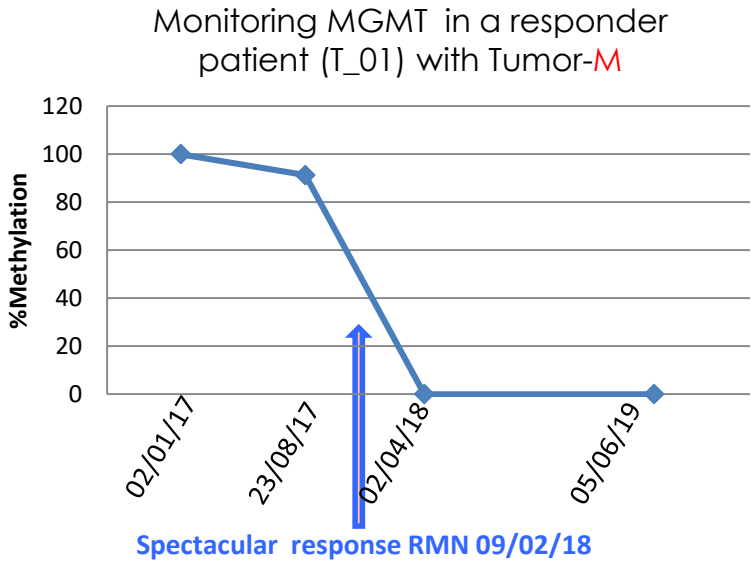
Biopsy (no surgery)
Tumor heterogeneity?

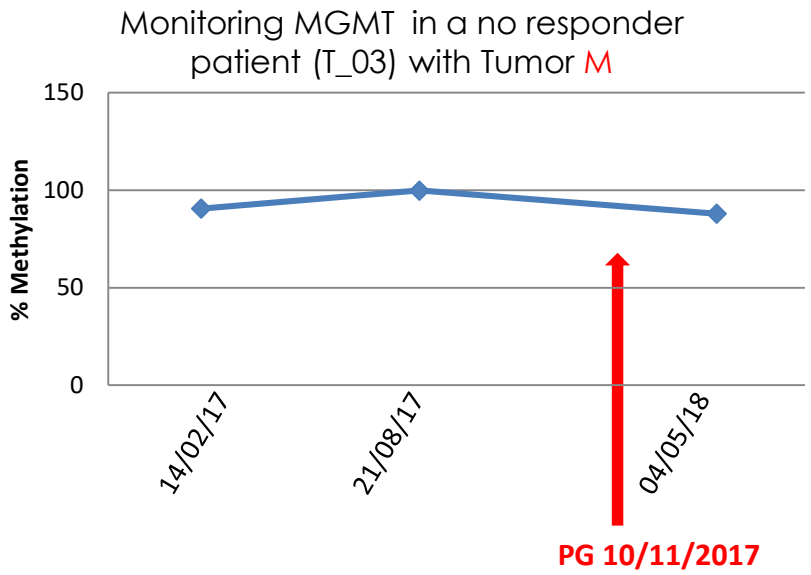
We are able to detect the presence of MGMT promoter methylation in the DNA extracted from the plasma exosomes with high sensitivity and specificity

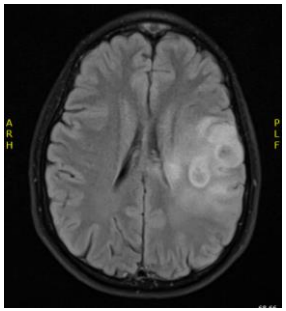
DNA exosomes Sens. **M**: 100%

DNA exosomes Speci. **U**: 95%

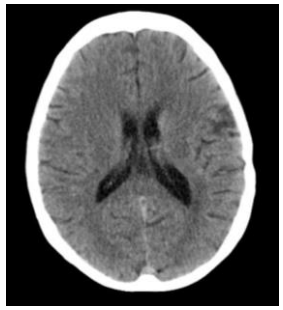
MGMT methylation as a tool for clinical monitoring of GBM patients







30-nov-2016

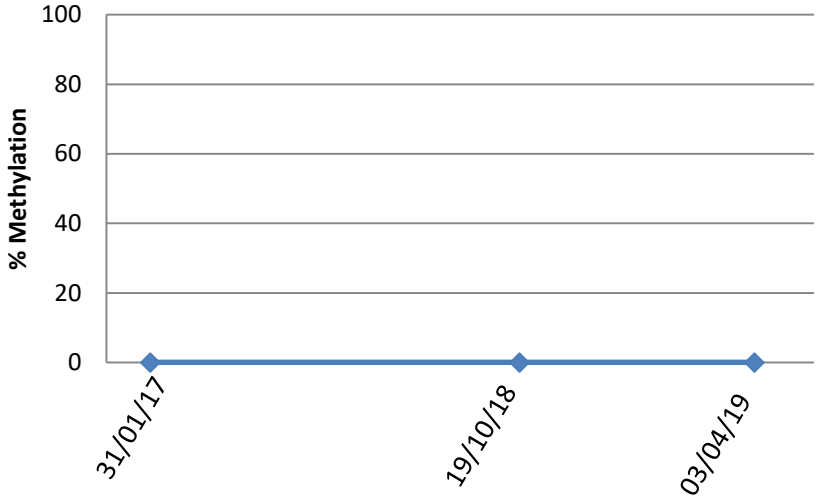


07-ago-2018

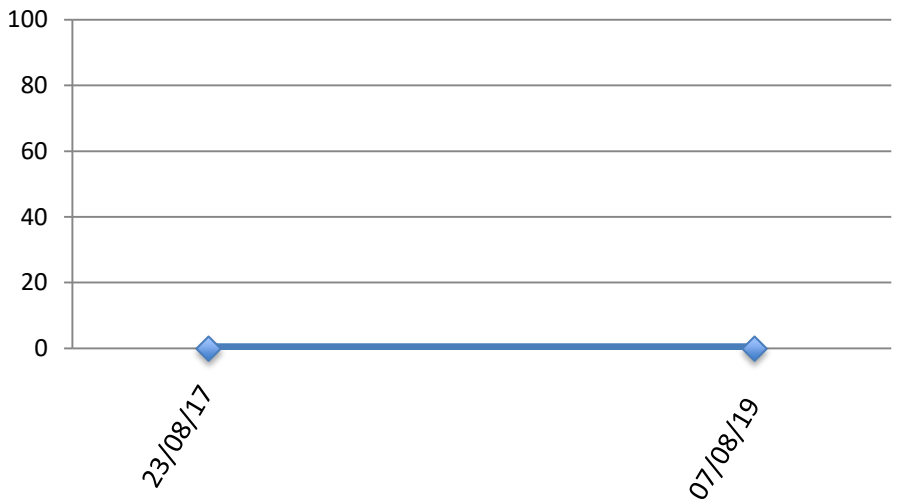
- The decrease of MGMT promoter methylation in circulating exosomes associates with a good patient response to treatment.
- The mantained levels of MGMT promoter methylation in circulating exosomes associate with bad prognosis and recurrence.

MGMT methylation as a tool for clinical monitoring of GBM patients

Monitoring MGMT in a patient with complete resection (no relapse) T_18-M



Monitoring MGMT in a patient harbouring an unmethylated tumor T-120-U



	Status	Phase	CancerType	Drug	Additional Treatment	Location
NCT02667587	MGMT METHYLATED	III	GBM	Nivolumab and Temozolomide	Radiation Therapy (RT)	USA
NCT02617589	MGMT UNMETHYLATED	III	GBM	Nivolumab and Temozolomide	Radiation Therapy (RT)	USA
NCT02685605	MGMT METHYLATED AND UNMETHYLATED	III	GBM	Temozolomide	Radiation: Intraoperative radiotherapy Radiation: Radiochemotherapy	USA
NCT03548571	MGMT METHYLATED	II and III	GBM	Adjuvant temozolomide	Biological: Dendritic cell immunization	Norway
NCT03025893	MGMT METHYLATED AND UNMETHYLATED	II and III	GBM	Sunitinib and Lomustine		Netherlands
NCT03970447	MGMT METHYLATED AND UNMETHYLATED	II and III	GBM	Lomustine, Regorafenib and Temozolomide	Radiation therapy (RT)	USA
NCT03367715	MGMT UNMETHYLATED	II	GBM	Nivolumab and Ipilimumab	Radiation Therapy (RT)	USA
NCT03643549	MGMT UNMETHYLATED	II	GBM	Bortezomib and Temozolomide		Noruega
NCT02717962	MGMT UNMETHYLATED	II	GBM	VAL-083, Dianhydrogalactitol		USA
NCT03050736	MGMT UNMETHYLATED	II	GBM	VAL-083 (Dianhydrogalactitol)		China
NCT03743662	MGMT METHYLATED	II	GBM	Bevacizumab and Nivolumab	Radiation Therapy (RT)	USA
NCT03522298	MGMT UNMETHYLATED	II	GBM	GDC-0084		USA
NCT03741244	MGMT METHYLATED AND UNMETHYLATED	II	GBM	Apatinib and Temozolomide		China
NCT03363659	MGMT UNMETHYLATED	II	GBM	Disulfiram and Temozolomide	Dietary Supplement: Copper gluconate	USA
NCT03018288	MGMT METHYLATED AND UNMETHYLATED	II	GBM	Pembrolizumab and Temozolomide	Biological: HSPPC-96	USA
NCT02179086	MGMT METHYLATED AND UNMETHYLATED	II	GBM	Temozolomide	Radiation: 3-dimensional	USA
NCT03047473	MGMT METHYLATED AND UNMETHYLATED	II	GBM	Biological: avelumab		Canada

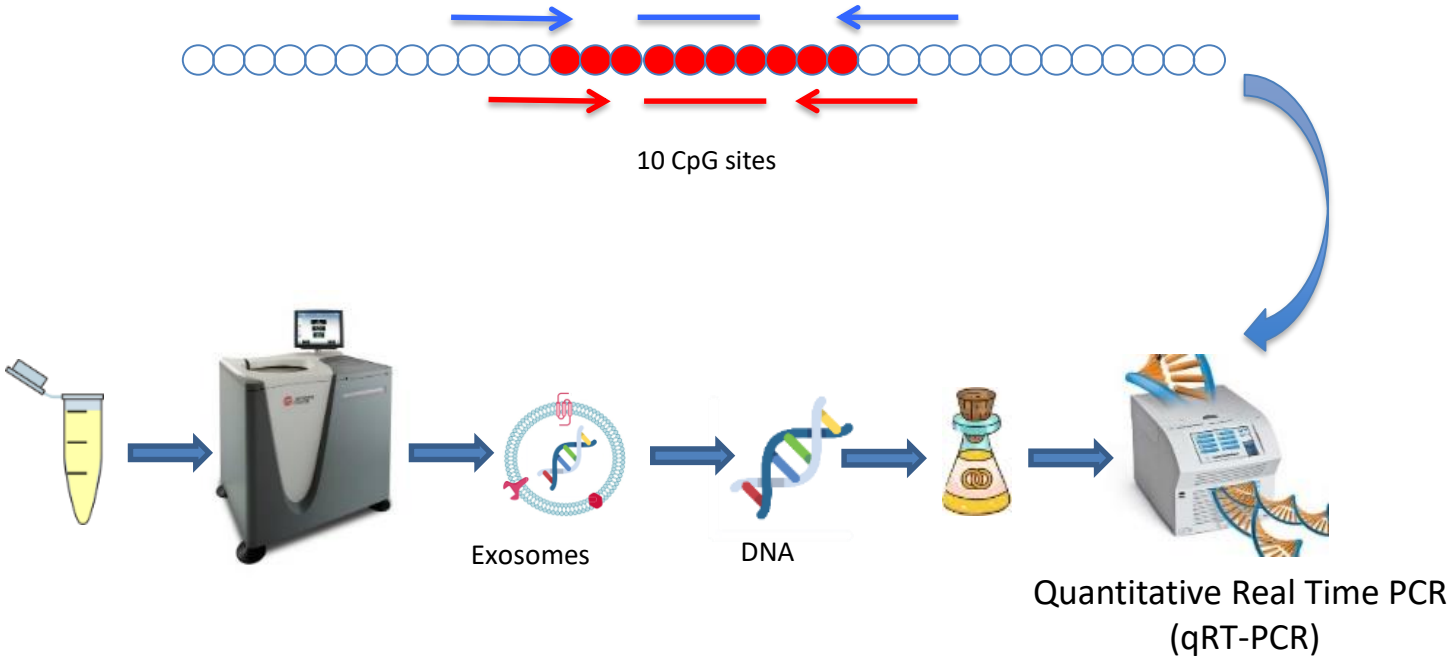
!82 MGMT clinical trials (75% GBM)

44 recruiting patients

6 at Phase III

	HYLATED	I and II	GBM	Olaptesed pegol	Radiation Therapy (RT)	Germany
	HYLATED	I and II	GBM	Temferon		Italy
	HYLATED	I and II	GBM	APG101, Alectinib, Idasanutlin, Atezolizumab, Vismodegib, Temsirolimus and Palbociclib		Germany
	ATED AND ATED	I and II	GBM	IMA950/Poly-ICLC and IMA950/Poly-ICLC and pembrolizumab		Switzerland
	HYLATED	I	GBM	Temozolomide and folinic acid	Radiation Therapy (RT)	France
	HYLATED	I	GBM	Tinostamustine	Radiation Therapy (RT)	USA
	HYLATED	I	GBM	Microtubule-Targeted Agent BAL101553	Radiation Therapy (RT)	USA
	HYLATED	I	GBM	CART-EGFRvIII T cells and Pembrolizumab		USA
NCT03535350	MGMT METHYLATED	I	GBM	Ibrutinib and Temozolomide	Radiation Therapy (RT)	USA
NCT03107780	MGMT UNMETHYLATED	I	GBM	MDM2 Inhibitor AMG-232	Radiation Therapy (RT)	USA
NCT03576612	MGMT METHYLATED AND UNMETHYLATED	I	GBM	Valacyclovir and Temozolomide	Radiation Therapy (RT)	USA
NCT03224104	MGMT METHYLATED AND UNMETHYLATED	I	GBM	TG02 and Temozolomide	Radiation Therapy (RT)	Switzerland and France
NCT03463733	MGMT METHYLATED AND UNMETHYLATED	I	GBM	Hydroxyurea and Temozolomide		Netherlands
NCT03514069	MGMT METHYLATED AND UNMETHYLATED	I	GBM	Ruxolitinib and Temozolomide	Radiation Therapy (RT)	USA
NCT04047706	MGMT METHYLATED AND UNMETHYLATED	I	GBM	Nivolumab and Temozolomide	Radiation Therapy (RT) and Biological: IDO1 Inhibitor BMS-986205	USA
NCT01849146	MGMT METHYLATED AND UNMETHYLATED	I	GBM	Adavosertib and Temozolomide	Radiation Therapy (RT)	USA

CURRENT SOLUTION	**OUR PRODUCT**
Inaccessibility of the sample	Non invasive test
Tumour heterogeneity	Global tumor profile
Tissue necrosis	Global tumor profile
Only useful for treatment prediction	-Diagnosis for treatment response -Predict recurrence -Can easily be extended to other tumor types with high % MGMT methylation



Europäisches Patentamt
European Patent Office
Office européen des brevets

Request for grant of a European patent

MINISTERIO DE INDUSTRIA, ENERGIA Y TURISMO

Oficina Española de Patentes y Marcas

Acknowledgement of receipt

We hereby acknowledge receipt of your request for grant of a European patent as follows:

Submission number	300315510
Application number	EP19382299.6
File No. to be used for priority declarations	EP19382299
Date of receipt	16 April 2019
Your reference	904 072
Applicant	Fundación para la Investigación Biomédica del Hospital Universitario La Paz (FIBHULP)
Country	ES
Title	METHOD FOR DETERMINING THE PERCENTAGE OF METHYLATION OF THE PROMOTER OF THE GENE O6-METHYLGUANINE-DNA METHYLTRANSFERASE (MGMT) IN CIRCULATING EXOSOMES
Documents submitted	package-data.xml application-body.xml OLF-ARCHIVE.zip\904 072 Final application.zip f1002-1.pdf (2 p.) ep-request.xml ep-request.pdf (5 p.) SPECNONEPO.pdf\904 072 Final application Spanish.pdf (24 p.)
Submitted by	CN=Gustavo Fuster 26814
Method of submission	Online
Date and time receipt generated	16 April 2019, 16:45:02 (CEST)
Official Digest of Submission	30:06:2F:4A:2E:26:ED:50:25:44:E0:B4:46:80:18:30:92:6B:FD:B3

Form 1002 - 1: Public inventor(s)

Designation of inventor

User reference: 904 072
Application No:

Public

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The applicant has acquired the right to the European patent:	As employer
Inventor	
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The applicant has acquired the right to the European patent:	As employer

IPR protection

Patent: European patent registration number EP19382299.6
Date of filing: 16 April 2019
Title: "Method for determining the percentage of methylation of the promoter of the gene O6-methylguanine-DNA methyltransferase (MGMT) in circulating exosomes"
Ownership: FIBHULP (100%)

Other patents of the group:

Ibáñez de Cáceres I, Belda Iniesta C, Pernía Arias O, Perona Abellón R, Cortés Sempere M; inventors. FIBHULP, CSIC, UAM, Fundación Hospital de Madrid, assignees. Method for predicting the response to a treatment consisting of radiotherapy combined with cisplatin-based chemotherapy. P201330783, PCT/ES2014/070433, EP3006572, US20160122828; 2013 May 29. **Licensed to IGEN BIOTECH.**

Ibáñez de Cáceres I, Pernía Arias O, de Castro Carpeño J, Vera Puente O, Jiménez Hernández J, Perona Abellón R, Rojo Todo F, inventors; FIBHULP, CSIC, UAM, Instituto de Investigación Sanitaria FJD, assignees. Determination of methylation and miRNA levels in response to a platinum-based antitumor compound. P201530997, PCT/ES2016/070516; 2015 July 09.

Ibáñez de Cáceres I, de Castro Carpeño J, Vera Puente O, Pernía Arias O, Rodríguez Antolín C, González Muñoz VM, Martín Palma ME, Salgado Figueroa AM, inventors; FIBHULP, FIBIOHRC, assignees. MAFG as a potential therapeutic target to restore chemosensitivity in platinum-resistant cancer cells. EP17382610.8 (Publication Number pending), PCT/EP2018/068156; 2017 September 15.

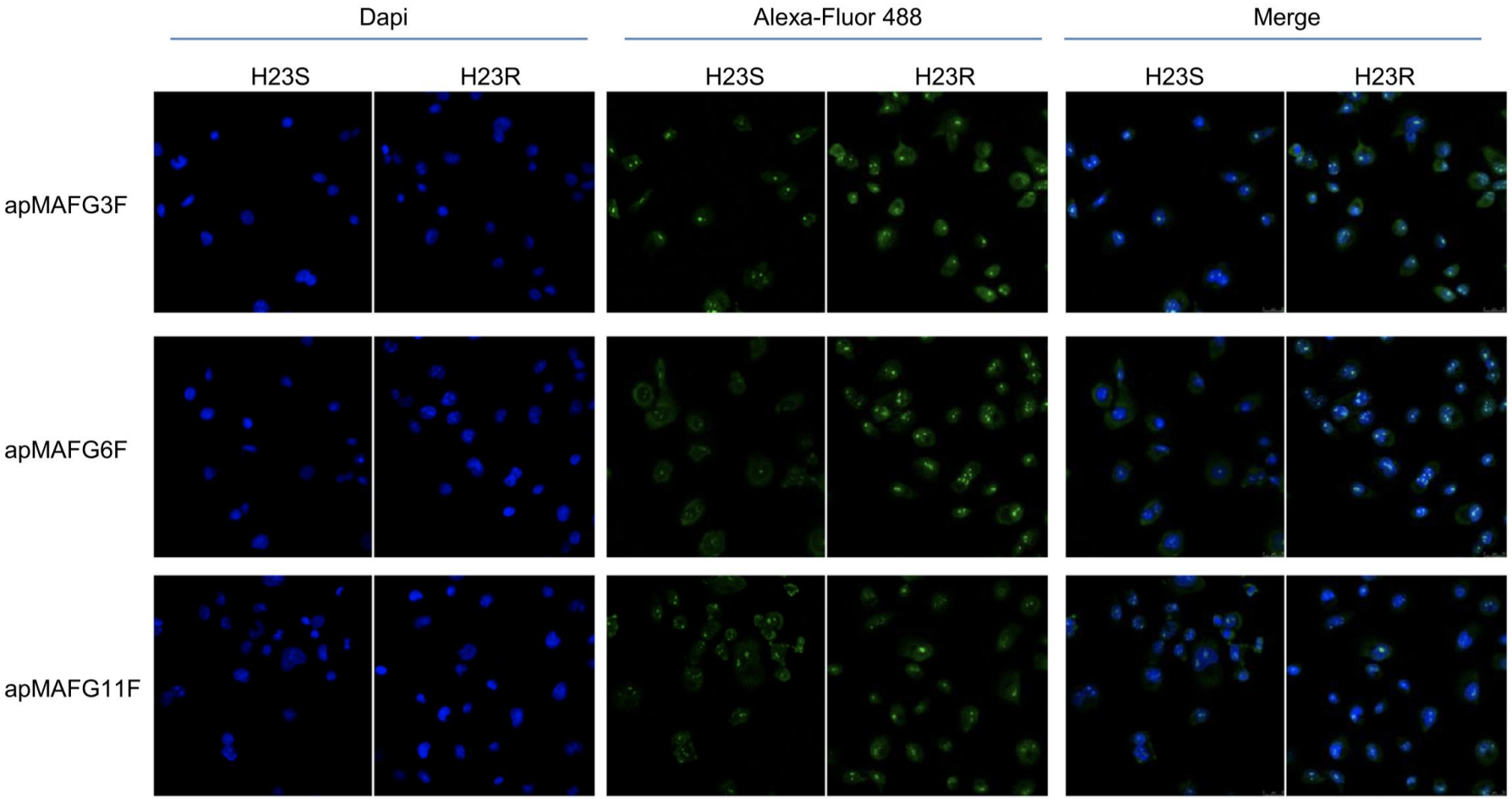
Ibáñez de Cáceres I, de Castro Carpeño J, Jiménez Hernández J, Rodríguez Antolín C, Rodríguez Jiménez C, Rosas Alonso R, Cruz Castellanos P, Burdiel Herencia M, Pernía Arias O, Diestro Tejada MD, Esteban Rodríguez MI, inventors; FIBHULP, assignee. miR-151A-3p as an universal endogenous control for exosome cargo normalization. EP19382252.5 (Publication Number pending); 2019 April 05.

Ibáñez de Cáceres I, de Castro Carpeño J, Jiménez Hernández J, Rodríguez Antolín C, inventors; FIBHULP, assignee. Method for determining the response to treatment of a patient affected by non-small cell lung carcinoma (NSCLC). EP19382614.6 (Publication Number pending); 2019 July 19.

Published data:

1.-MAFG overexpression is associated with a **poor prognosis** in patients with non-small-cell lung cancer.

2.- MAFG overexpression **induces CDDP resistance**, targeting ROS.

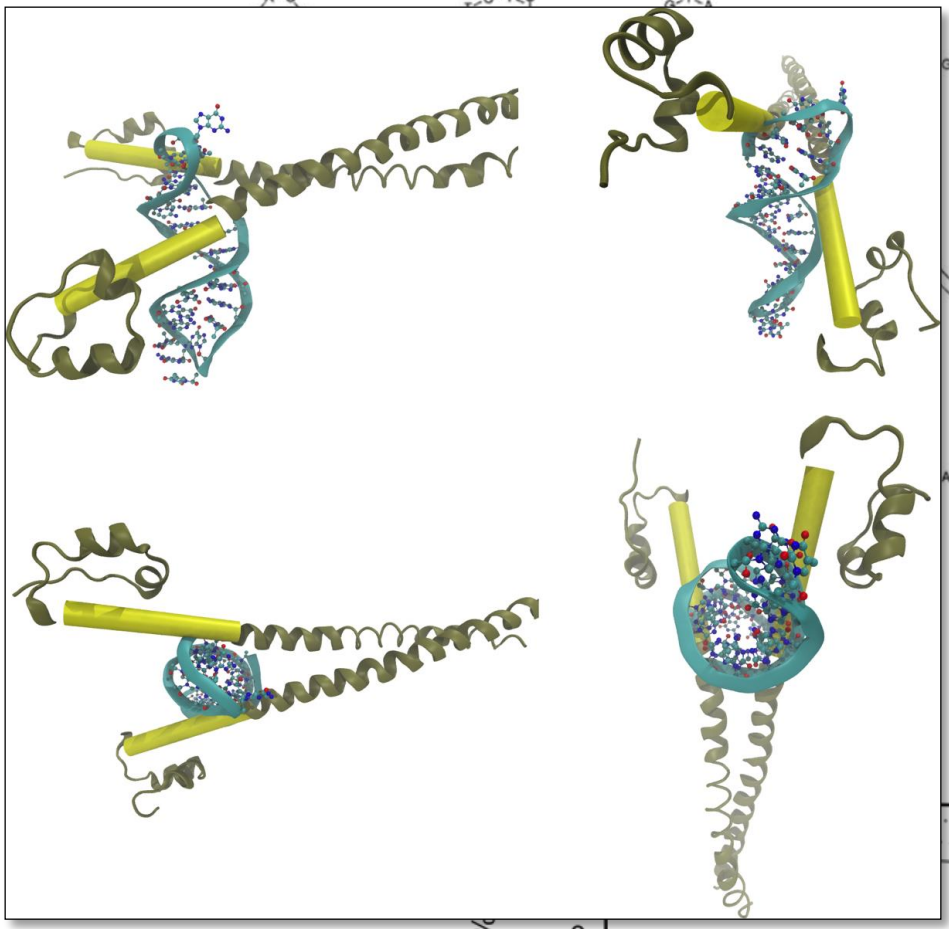


E

apMAFG3F

apMAFG6F

apMAFG11F



Published data:

3.-Targeting MAFG by specific aptamers increases ROS production and restores cell sensitivity to CDDP.

4.-MAFG aptamers can be used to detect MAFG levels in NSCLC samples.

4.54

H23S-

H23S-*MAFG*

H23S-*MAFG*-apMAFG6F

H23R-

0

0 1 2 3 4

CDDP Concentration (g/ml)

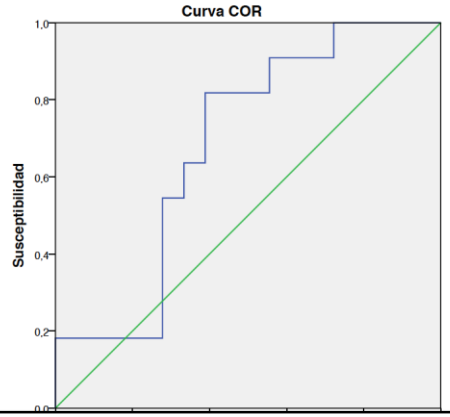
Aptamer 3. Prognostic value: 29 NSCLC patients (stages I/II)

SLE

Coordenadas de la curva

Variables resultado de contraste: apMAFG3F_T

Positivo si es mayor o igual que[a]	Sensibilidad	1 - Especificidad
-1,0000	1,000	1,000
7,5000	1,000	,889
29,8443	1,000	,833
45,6982	1,000	,778
49,8625	1,000	,722
54,0086	,909	,722
61,2423	,909	,667
68,7423	,909	,611
70,4067	,909	,556
72,5627	,818	,556
74,4596	,818	,500
74,9984	,818	,444
75,5781	,818	,389
76,4602	,727	,389
77,2131	,636	,389
78,1364	,636	,333
79,6478	,545	,333
81,6478	,545	,278
83,5705	,455	,278
84,4533	,364	,278
85,3798	,273	,278
85,9970	,182	,278
87,4444	,182	,222
89,5664	,182	,167
90,4294	,182	,111
90,6856	,182	,056
93,2391	,182	,000
97,1972	,091	,000
99,6726	,000	,000

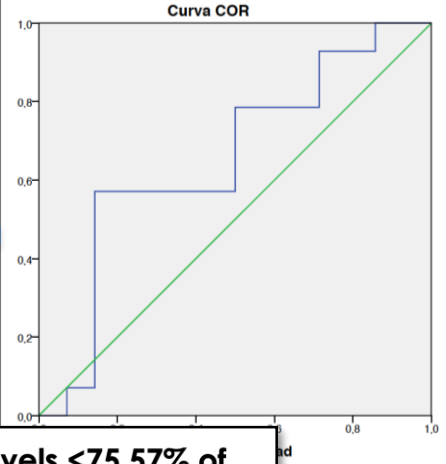


Exitus

Coordenadas de la curva

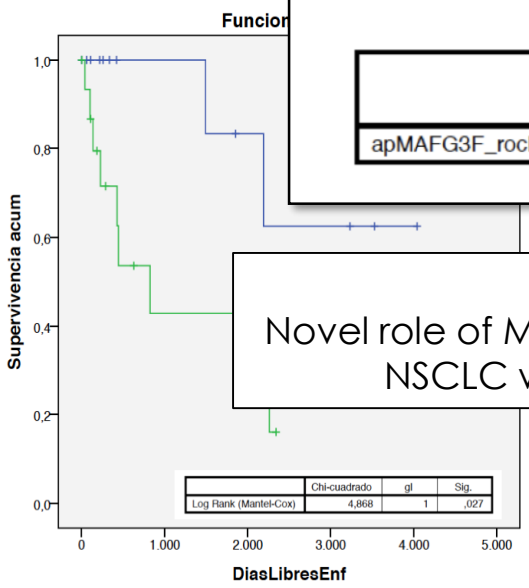
Variables resultado de contraste: apMAFG3F_T

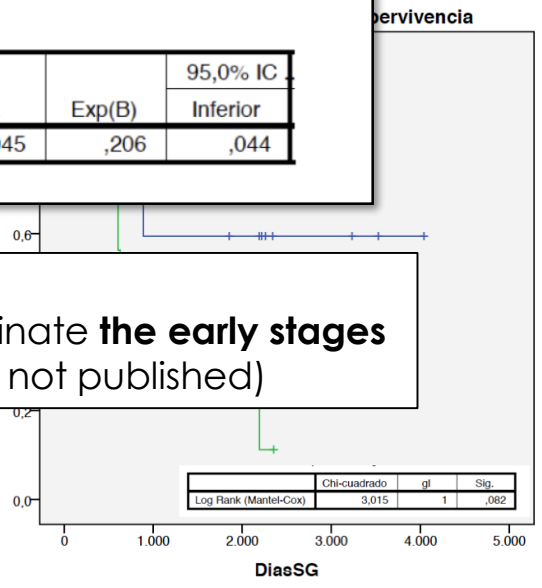
Positivo si es mayor o igual que[a]	Sensibilidad	1 - Especificidad
-1,0000	1,000	1,000
22,3443	1,000	,857
45,6982	,929	,857
49,8625	,929	,786
54,0086	,929	,714
61,2423	,857	,714
68,7423	,786	,714
70,4067	,786	,643
72,5627	,786	,571
74,4596	,786	,500
74,9984	,714	,500
75,5781	,643	,500
76,4602	,571	,500
77,2131	,571	,429
78,1364	,571	,357
79,6478	,571	,286
81,6478	,571	,214
83,5705	,571	,143
84,4533	,500	,143
85,3798	,429	,143
85,9970	,357	,143
87,4444	,286	,143
89,5664	,214	,143
90,4294	,143	,143
90,6856	,071	,143



The risk of relapse of patients harboring tumors with **MafG levels <75,57% of positive cells** (Hazard Ratio) **decreases 79.4%** (1-0.206) compared to patients with higher MafG tumor values.

	B	ET	Wald	gl	Sig.	Exp(B)	95,0% IC Inferior
apMAFG3F_rocR	-1,580	,788	4,019	1	,045	,206	,044





Data not published:

Novel role of MAFG as a marker able to discriminate **the early stages** NSCLC with high risk of recurrence (Data not published)

Theranostics 2017, Vol. 7, Issue 17

IVYSPRING INTERNATIONAL PUBLISHER

2017; 7(17): 4118-4134.

Research Paper

DNA Methylation of miR-7 is a Mechanism I Platinum Response through *MAFG* Overexpr Cancer Cells

Olga Vera[1, 2*], Julia Jimenez[1, 2*], Olga Pernia[1, 2], Carlos Rodriguez-Antolin[1, 2], Carmen Rodr Sanchez Cabo[3], Javier Soto[1, 2], Rocio Rosas[1, 2], Sara Lopez-Magallon[4], Isabel Esteban Rodri Federico Rojo[7], Cristobal Belda[8], Rafael Alvarez[8], Jaime Valentin[9], Javier Benitez[10, 11], Rosar De Castro[2✉], Inmaculada Ibanez de Caceres[1, 2✉]

MINISTERIO DE INDUSTRIA, ENERGIA Y TURISMO

Oficina Española de Patentes y Marcas

Acknowledgement of receipt

We hereby acknowledge receipt of your request for grant of a European patent as follows:

Submission number	300247372
Application number	EP17382610.8
File No. to be used for priority declarations	EP17382610
Date of receipt	15 September 2017
Your reference	902 444
Applicant	Fundación para la Investigación Biomédica del Hospital Universitario La Paz (FIBHULP)
Country	ES
Title	MAFG as a potential therapeutic target to restore chemosensitivity in platinum-resistant cancer cells

FULL TEXT ARTICLE

MAFG is a potential therapeutic target to restore chemosensitivity in cisplatin-resistant cancer cells increasing reactive oxygen species

Article in Press: Corrected Proof

Olga Vera-Puente, Carlos Rodriguez-Antolin, Ana Salgado-Figueroa, Patrycja Michalska, Olga Pernia Reid, Rocío Rosas, Alvaro Garcia-Guede, Silvia SacristÁn, Julia Jimenez, Isabel Esteban-Rodriguez Martin, Thomas A. Sellers, Rafael León, VÍctor M. Gonzalez, Javier De Castro and Inmaculada Ibane

Translational Research, Copyright © 2018 The Author(s)

Acknowledgement of receipt

We hereby acknowledge receipt of your request for the processing of an international application according to the Patent Cooperation Treaty as follows:

Submission number	6487885
PCT application number	PCT/EP2018/068156
Date of receipt	04 July 2018
Receiving Office	European Patent Office, The Hague
Your reference	903 350
Applicant	Fundación para la Investigación Biomédica del. Hospital Universitario La Paz (FIBHULP)
Number of applicants	2
Country	ES
Title	MAFG as a potential therapeutic target to restore chemosensitivity in platinum-resistant cancer cells

Partnering opportunities

The next steps to bring technology to market require more resources and infrastructure. Therefore the collaboration of industry is essential

Type of collaborations sought:

•Licensing-out type agreements

•Investor who finances the project validating with international collaborators (at present V.Quillien)

•Partner interested in getting involved in any of the different phases up to market launch

•co-development agreements, ...

Transfer actions carried out so far:
Contact with an international company, which has shown interest in the technology (Advisory board)

Non-invasive method for diagnosis and monitoring of glioblastoma

MGMT methylation

Inmaculada Ibanez de Caceres
Cancer Epigenetics Laboratory

INGEMM

IdiPAZ Instituto de Investigación Hospital Universitario La Paz