3° ANNUAL COURSE OF PHARMACOGENETICS AND PERSONALIZED MEDICINE

Thursday 9th - Friday 10th February 2012
Aula Magna Sapienza University of Roma

The emerging role of epigenetics in personalized medicine: the example of glioma

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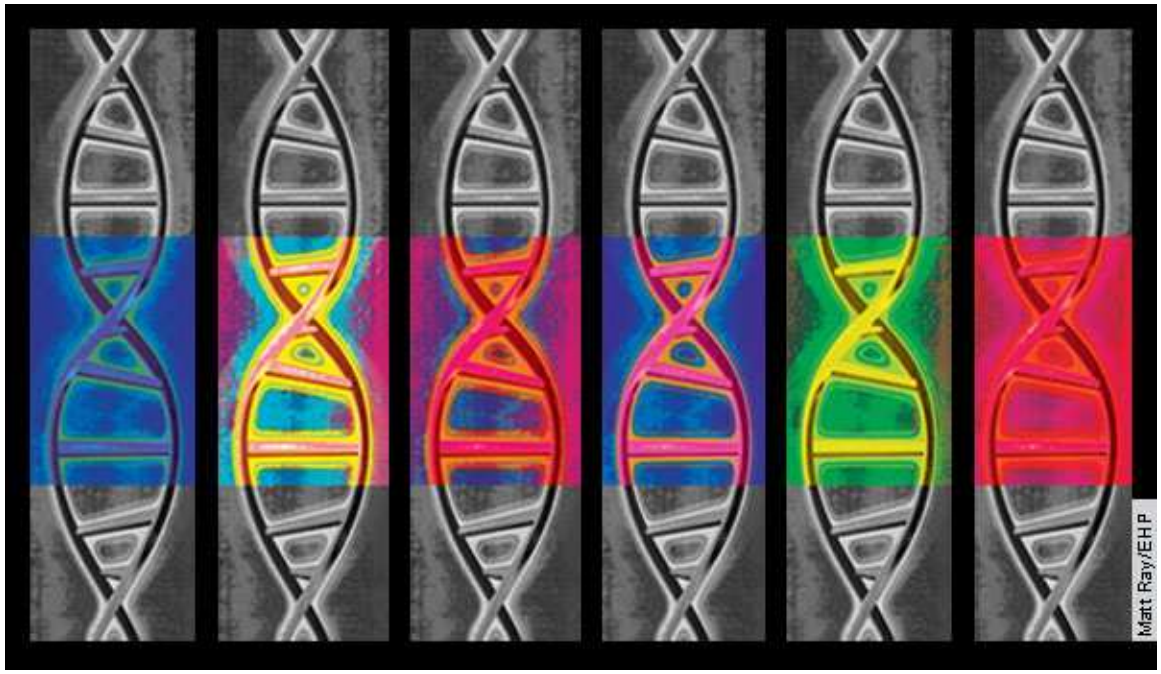
EPIGENETICS

‘over’ the genetic information



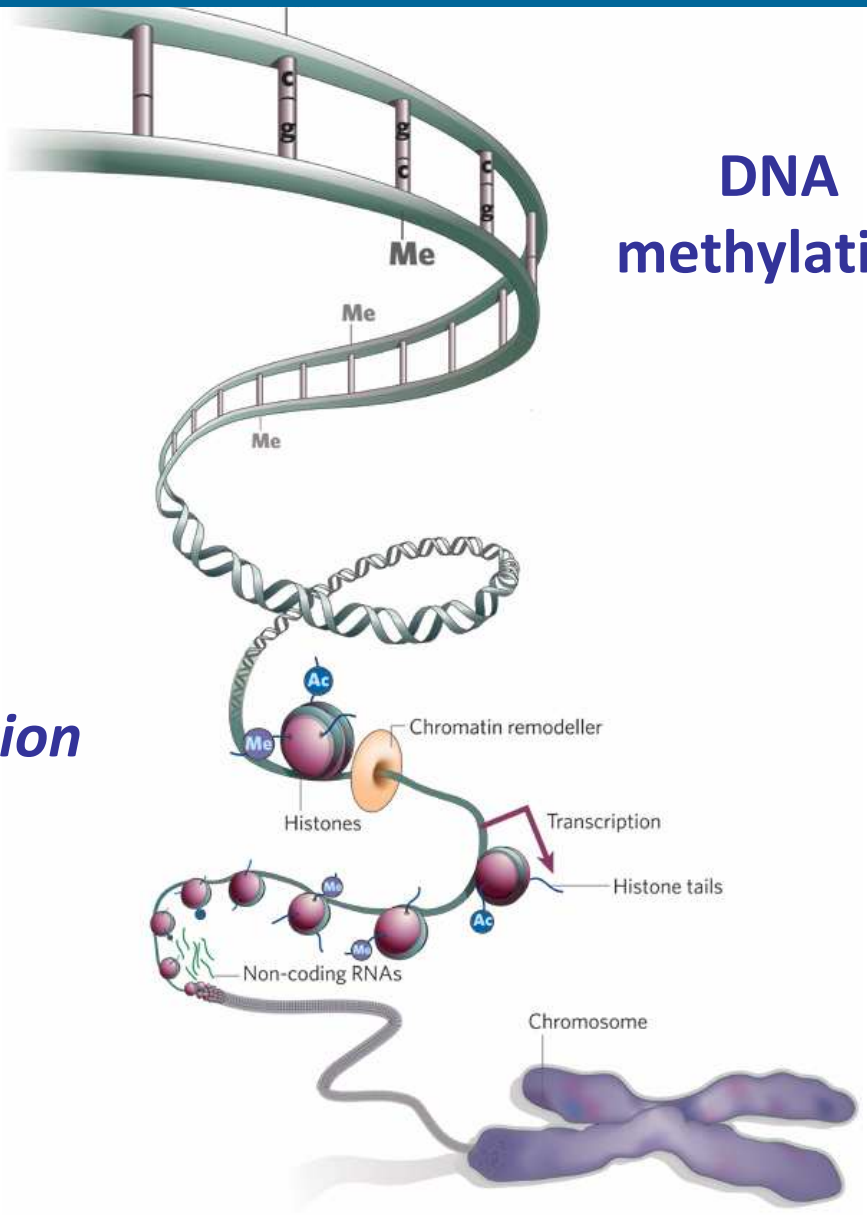
Refers to functionally relevant modifications not involving the nucleotide sequence. It is involved in controlling gene expression.

EPIGENETICS

'Dynamic'

DNA methylation

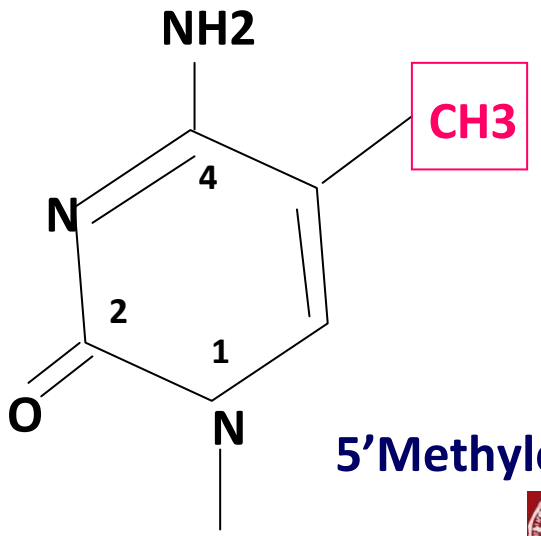
Histone modification



EPIGENETICS
‘CpG mthylation’

- CpG islands are short stretches of DNA with high frequency of the CG sequence
- DNA Methylation only occurs at CpG sites located in the promoter regions of genes. Almost all housekeeping genes are associated with at least one CpG island. About 40 % tissue specific genes are associated with islands.

5’ CpG 3’
3’ GpC 5’
CpG dinucleotides are palindromic



5’Methylcytosine

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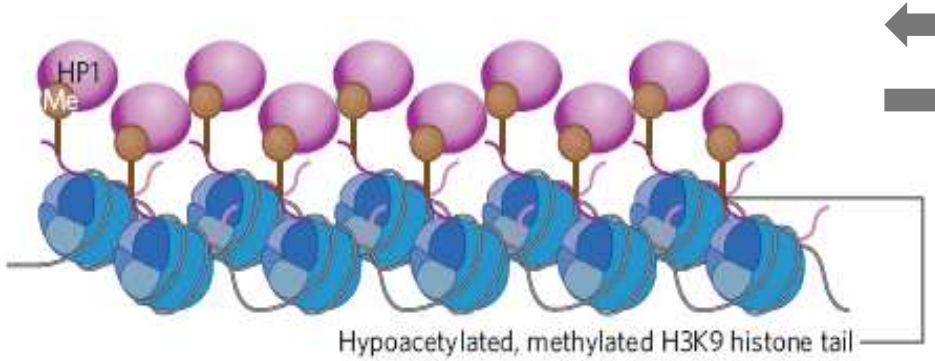
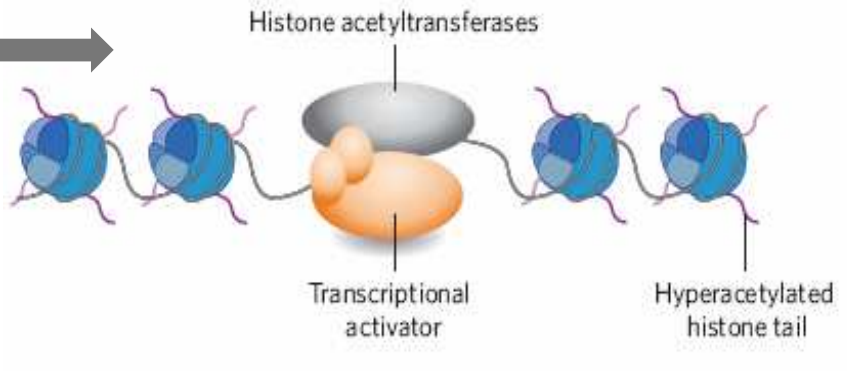
EPIGENETICS

‘Chromatin remodelling’

Heterochromatin



Euchromatin



- ✓ A tightly packed form of chromatin;
- ✓ At centromeres and telomeres;
- ✓ Contains repetitious sequences;
- ✓ Gene-poor; high DNA methylation;
- ✓ Associated with repressed transcription.

- ✓ A lightly packed form of chromatin;
- ✓ Gene-rich; low DNA methylation
- ✓ At chromosome arms;
- ✓ Associated with active transcription.

EPIGENETICS

‘Pathogenetic Mechanisms’

Improper epigenetic marks can result in gene expression changes and lead to disease (inherited and acquired)

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EPIGENETICS
'Inherited Disorders'

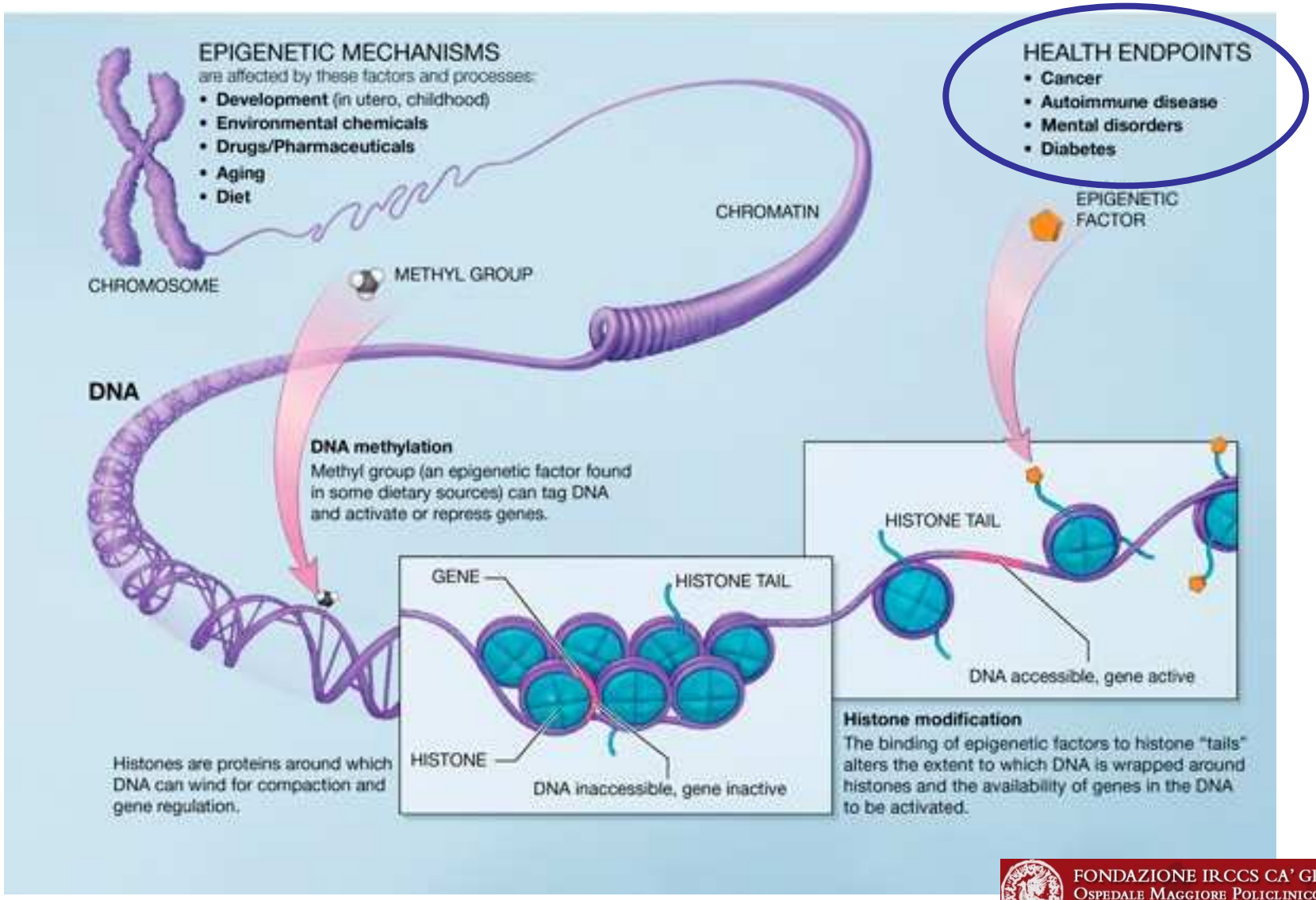
Table 1. Examples of diseases of chromatin remodelling

Disease	Chromatin defect	Clinical features
Rubinstein-Taybi syndrome (62,70,109)	Heterozygous mutations in *CBP*	Autosomal dominant inheritance; mental retardation, abnormal facial features, blunted growth
Fragile X syndrome (62,105)	Hypermethylation of DNA at the *FMR1* and *FMR2* promoters, caused by trinucleotide repeat expansion	X-linked inheritance; most common inherited form of mental retardation, signs of autistic behaviour, macrocephaly, long and narrow face with large ears, macroorchidism, hypotonia
Coffin-Lowry syndrome (70,106,107)	Mutation in *RSK2*, which can interact with CREB and CBP and can phosphorylate H3 *in vitro* (106)	X-linked inheritance; psychomotor retardation, craniofacial and skeletal abnormalities
Rett syndrome (62,70–72)	Mutations in *MeCP2*	X-linked, predominantly affecting girls; pervasive developmental disorder associated with arrested brain development, cognitive decline and autistic-like behaviour
Alpha-thalassemia/mental retardation syndrome, X-linked (ATR-X) (70)	Mutations in the *ATRX* gene encoding XH2—a member of SWI/SNF family of proteins; defective chromatin remodelling thought to downregulate the α-globin locus	X-linked inheritance; mental retardation, haemolytic anaemia, splenomegaly, facial, skeletal and genital anomalies
Immunodeficiency-centromeric instability-facial anomalies syndrome (ICF) (70)	Mutations in *Dnmt3B*; hypomethylation at centromeric regions of chromosomes 1, 9 and 16	Autosomal recessive; mild mental retardation, marked immunodeficiency, facial anomalies
Myotonic dystrophy (107)	Abnormal CTG repeat expansion at the 3′ UTR of the *DMPK* gene favours chromatin condensation, affecting the expression of many neighbouring genes	Autosomal dominant; mild mental retardation, myotonia, abnormal cardiac conduction, insulin-dependent diabetes, testicular atrophy, premature balding
Prader-Willi syndrome (108)	Rare forms caused by abnormal imprinting (DNA methylation) of paternal chromosomal region 15q11–13	Mild mental retardation, endocrine abnormalities
Angelman syndrome (108)	Rare forms caused by abnormal imprinting (DNA methylation) of maternal chromosomal region 15q11–13	Cortical atrophy, cerebellar dysmyelination, cognitive abnormalities

CBP, CREB binding protein; CREB, cyclic AMP-response element binding protein; *DMPK*, DM1 protein kinase; *Dnmt3B*, DNA methyltransferase 3B; FMR1 mental retardation protein 1; *MeCP2*, methyl-CpG-binding protein 2; RSK2, ribosomal S6 kinase 2; SWI/SNF, mating switching and sucrose non-ferment plex; UTR, untranslated region; XH2, X-linked helicase 2.

EPIGENETICS

‘Pathogenetic Mechanisms’



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EPIGENETICS

‘Cancer: genomic and epigenetic disorder’

Hypomethylation

- ✓Over-expression of oncogenes
- ✓Reactivation of transposons
- ✓Chromosome instability

Hypermethylation

- ✓Silencing of tumor suppressors
- ✓Silencing DNA repair genes (es. MLH2, MGMT)
- ✓CIMP (CpG Island Methylator Phenotype) (glioma, ca colon)

Epigenetic markers open new avenues for an early detection, diagnosis, prognosis as well as therapeutic targets in cancer.

EPIGENETICS

‘Signatures of glioma’

- ✓ MGMT promoter methylation
- ✓ G-CIMP

Genetic markers

- 1p/19q codeletion in oligodendroglioma
- IDH1/2 mutation in high grade glioma

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The New England
Journal of Medicine

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INACTIVATION OF THE DNA-REPAIR GENE *MGMT* AND THE CLINICAL RESPONSE OF GLIOMAS TO ALKYLATING AGENTS

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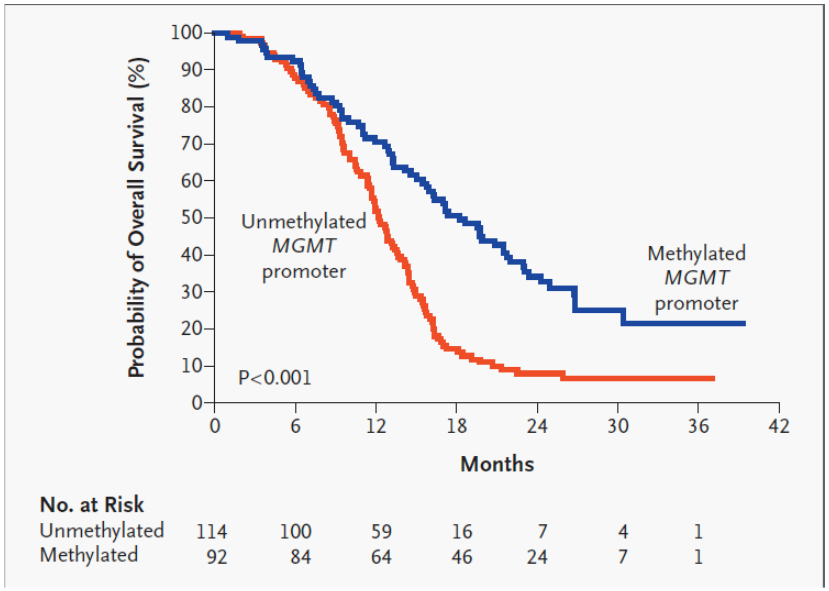

Conclusions Methylation of the *MGMT* promoter in gliomas is a useful predictor of the responsiveness of the tumors to alkylating agents. (N Engl J Med 2000; 343:1350-4.)

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Table 3 | *MGMT* promoter methylation in various human gliomas

Reference	*MGMT* methylation frequency*	Clinical significance of *MGMT* promoter methylation
Anaplastic astrocytoma		
Wick *et al.* (2009)[16]	50% (48/96)	Prolonged PFS and overall survival in response to radiotherapy or chemotherapy with temozolomide (5/28) or PCV; phase III trial
Anaplastic oligoastrocytoma		
Brandes *et al.* (2006)[83]	69% (37/54)	No prognostic significance in recurrent oligoastrocytoma or oligodendroglioma treated with temozolomide (5/28)
Wick *et al.* (2009)[16]	71% (53/75)	Prolonged PFS and overall survival in response to radiotherapy or chemotherapy with temozolomide (5/28) or PCV; phase III trial
Anaplastic oligodendroglioma		
Wick *et al.* (2009)[16]	71% (22/31)	Prolonged PFS and overall survival in response to radiotherapy or chemotherapy with temozolomide (5/28) or PCV; phase III trial
Anaplastic oligodendroglioma and anaplastic oligoastrocytoma without necrosis		
van den Bent *et al.* (2009)[17]	84% (81/97)	Prolonged PFS and overall survival in response to radiotherapy alone or radiotherapy plus chemotherapy with PCV; phase III trial
Anaplastic oligoastrocytoma with necrosis (glioblastoma)		
van den Bent *et al.* (2009)[17]	73% (29/40)	No prolonged PFS and overall survival in response to radiotherapy alone or radiotherapy plus chemotherapy with PCV; phase III trial
Recurrent anaplastic astrocytoma or oligoastrocytoma and glioblastoma		
Sadones *et al.* (2009)[51]	26% (10/38)	Prolonged overall survival in response to temozolomide (5/28 or 1 week–1 week off) in anaplastic astrocytoma and oligoastrocytoma
Grade II astrocytoma		
Komine *et al.* (2003)[63]	43% (21/49)	Decreased PFS with no treatment or radiotherapy or interferon
Grade II oligodendroglioma and oligoastrocytoma		
Everhard *et al.* (2006)[65]	93% (63/68)	Prolonged PFS in patients with oligodendroglioma ($n=42$), oligoastrocytoma ($n=18$) or astrocytoma ($n=8$) treated with temozolomide
Kesari *et al.* (2009)[64]	60% (12/20)	Prolonged PFS and OS in patients treated with temozolomide (11 weeks on–4 weeks off); phase II trial

**MGMT* promoter methylation analysis performed by gel-based methylation-specific PCR, except studies by Sadones *et al.* (quantitative methylation-specific PCR)[51] and van den Bent *et al.* (methylation-specific multiplex ligation-dependent probe amplification.)[17] Abbreviations: 5/28, 5 out of 28 days; PFS, progression-free survival; PCV, combination of procarbazine, CCNU (lomustine) and vincristine.

nature REVIEWS NEUROLOGY

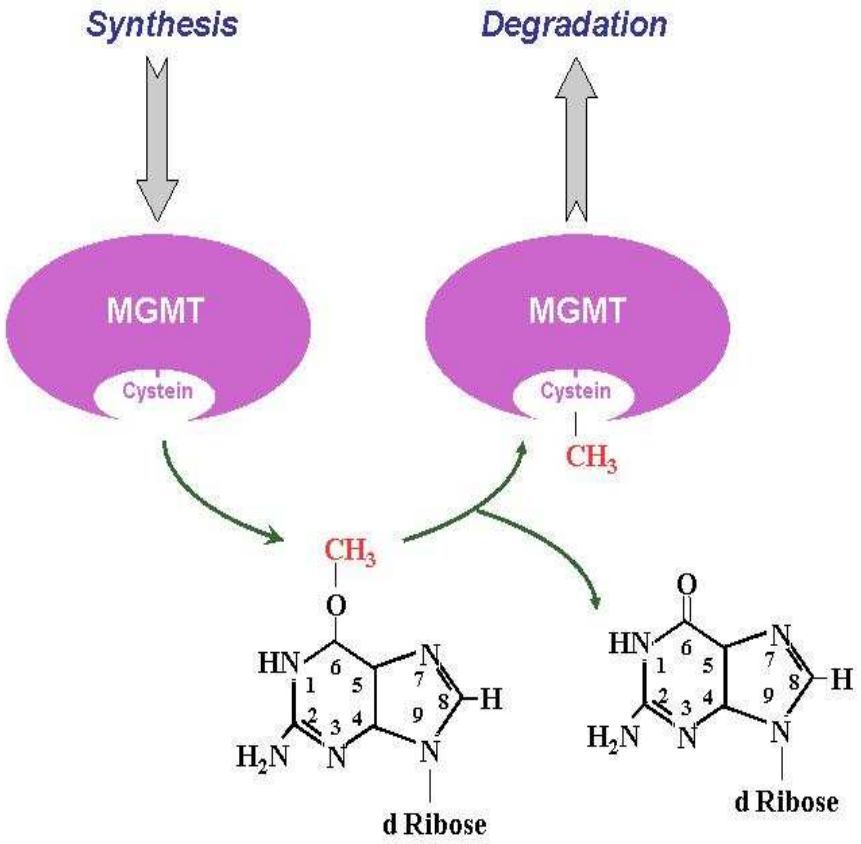
Weller, M. *et al.* (2009) *MGMT* promoter methylation in malignant gliomas: ready for personalized medicine? *Nat. Rev. Neurol.* doi:10.1038/nrneurol.2009.197

O^6-methylguanine-DNA methyltransferase (*MGMT*) 'function'

- Maps at chromosome 10q26
- 5 exons
- CpG island at the 5' of the gene (containing 98 CpG sites)

The protein removes alkyl groups from the O^6-position of G by an irreversible transfer of the alkyl group to a Cystein at its active site.

G in the DNA is thereby restored and MGMT sentenced to proteasome-mediated degradation.



MGMT
'Function'

Because of the stoichiometry of the reaction and the unavoidable fate of the MGMT protein, **the repair capacity of a cell depends on the amount of MGMT molecules in the nucleus and the cell capability of re-synthesis.**

Failure to repair the O6-alkylguanine (O^6-AG) DNA adducts increases mutagenic potential during replication:

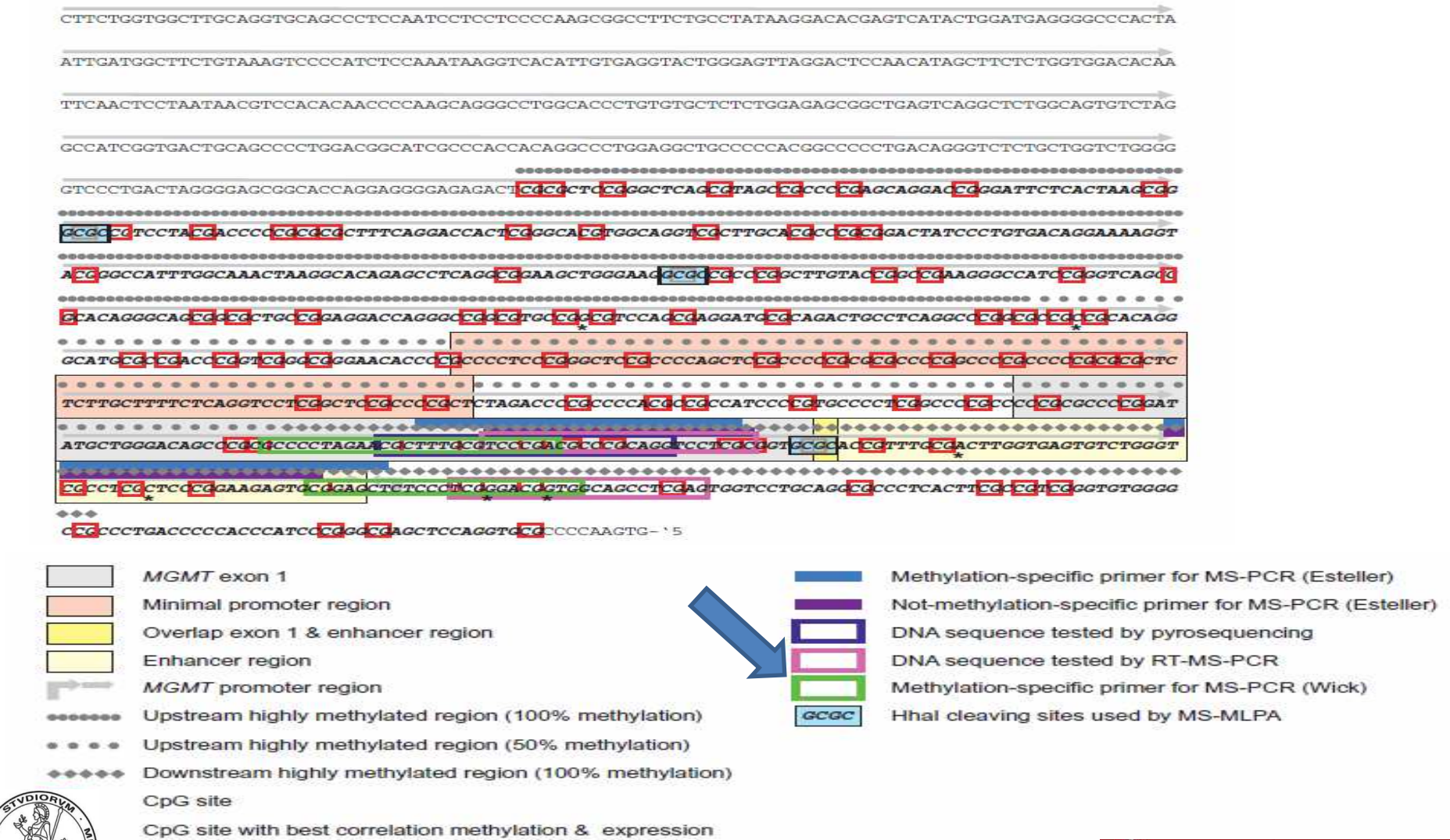
1) **O^6-AG can be mistaken for adenine** and mismatched with thymine, **giving rise to a G:C to A:T transition mutation**.

2)The adducts show a cytotoxic potential by causing **DNA double-strand breaks**.

↓

Apoptosis

MGMT
'CpGs structure'



MGMT
'the methylation test'

Sequence analyzed is located within the contig GRCh37.p2, on chromosome 10 (gi|224514688:2268342-4574265).
NCBI Reference Sequence: NT_008818.16

10 CpGs analyzed

GCCCCGGATATGCTGGGACAGCCCGCGCCCCTAGAA**C**GCTTTG**C**GTCC**C**GA**C**GCC**C**GCAGGTCC**T**C**G**C**G**GTG**C**GCAC**C**
GTTTG**C**GACTTGGTGAGTGTCTGGGTCGCCTCGCTCCCGGAAGAGTGCGGAGCTCTCCCTCGGGACGGTGGCAGCCT
CGAGTGGTCCTGCAGGCGCCCTCACTTCGCCGTCGGGTGTGGGGCC

Pyrosequencing vs MS-PCR

Most of cases showing <20% of methyl. (pyrosequencing) resulted unmethyl (MS-PCR)

Pyrosequencing is more reliable respect to MS-PCR

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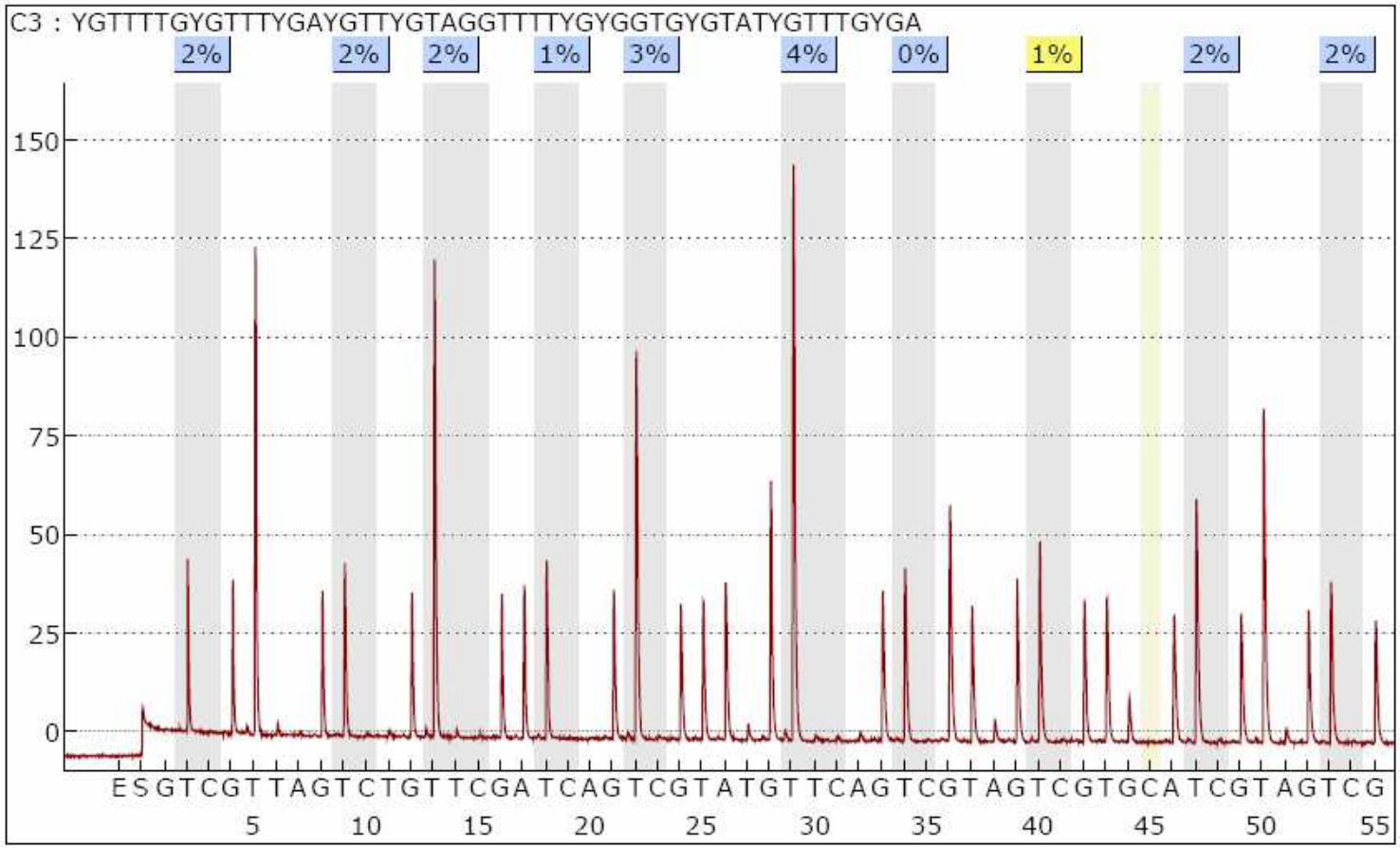
MGMT

'Pyrograms'

Normal brain (20 cases): 3±1.2 (%)



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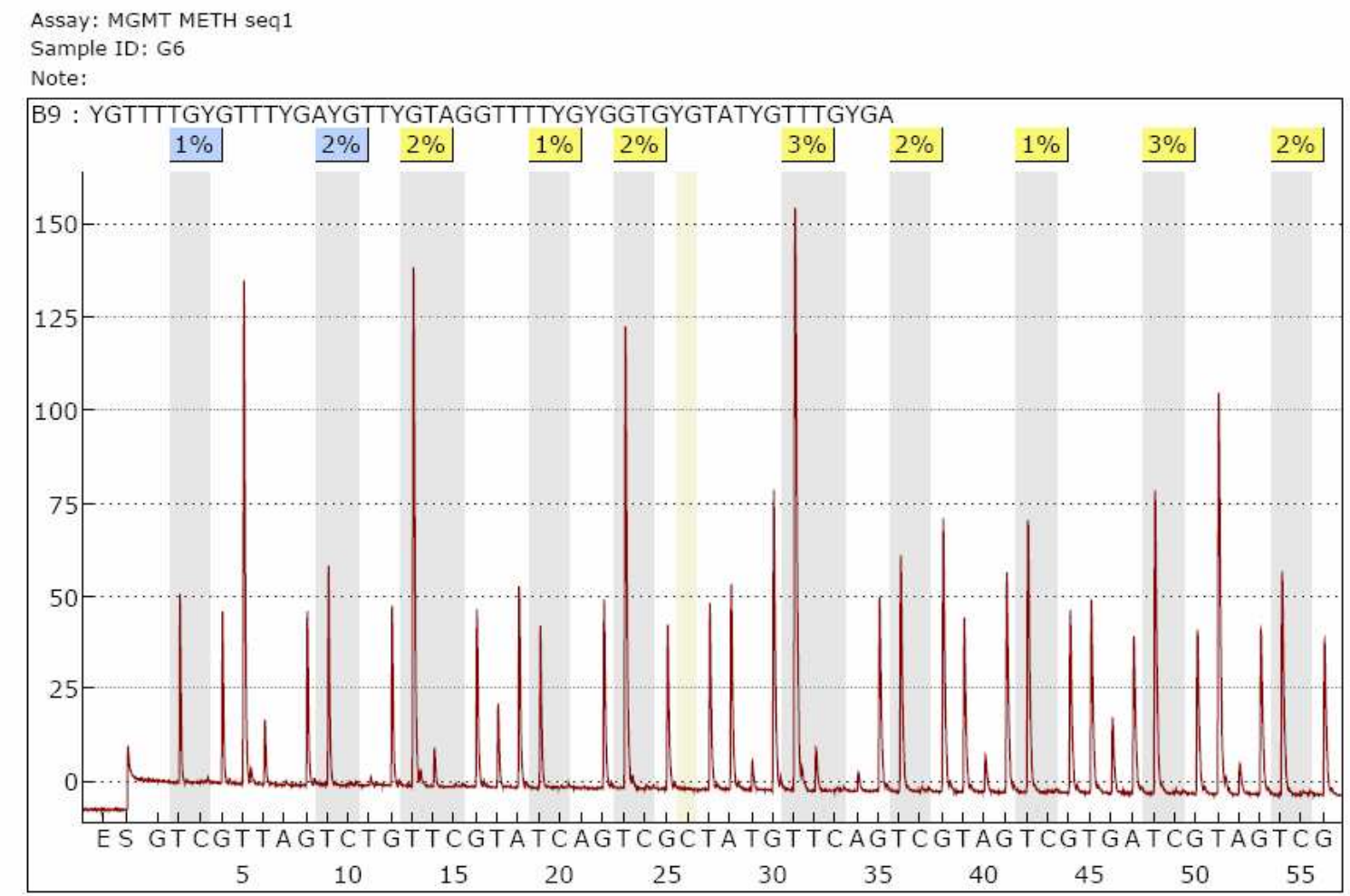
MGMT

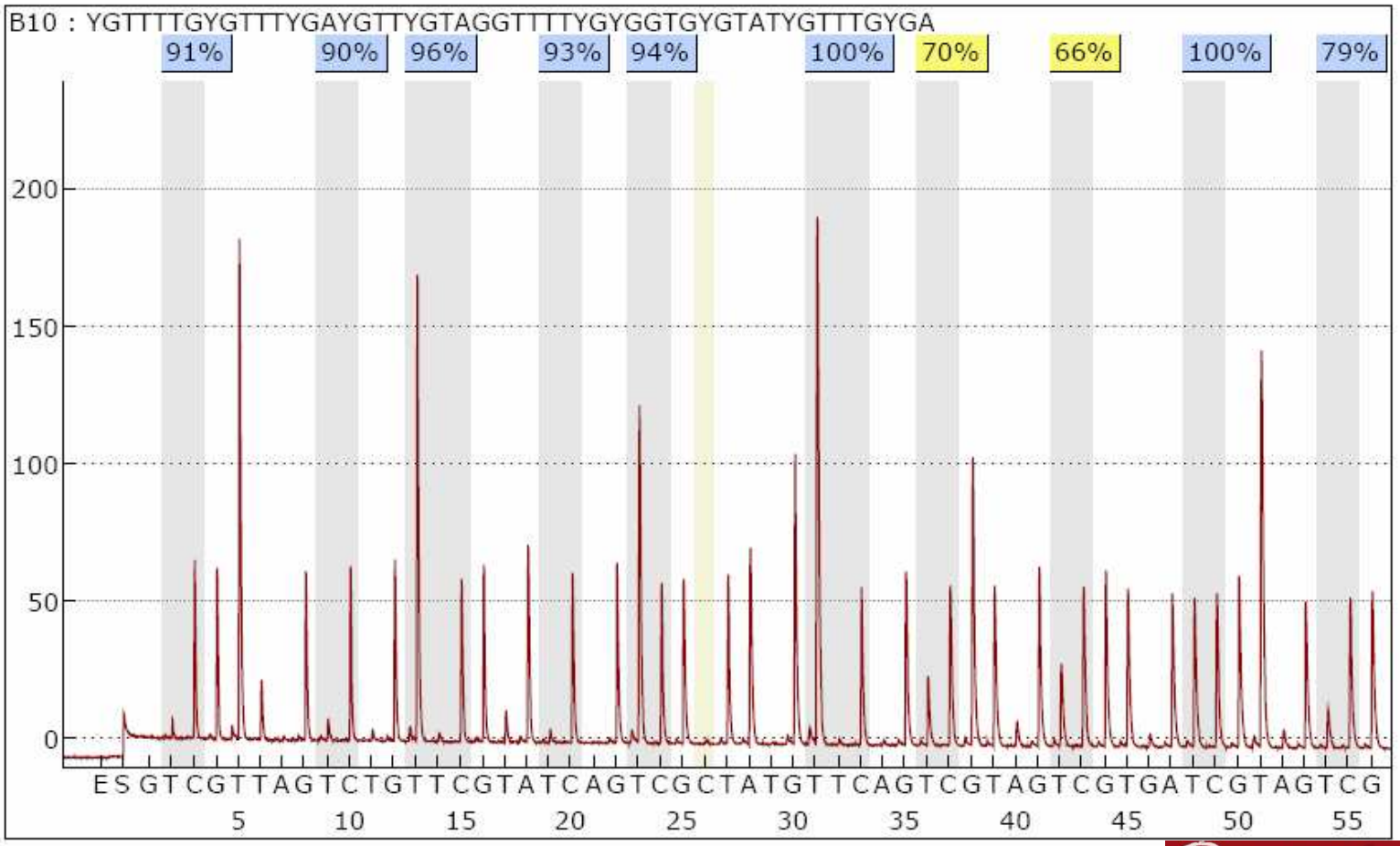
‘Pyrograms’

Glioma cell line (unmethylated)



MGMT
‘Pyrograms’

Glioma cell line (methylated)



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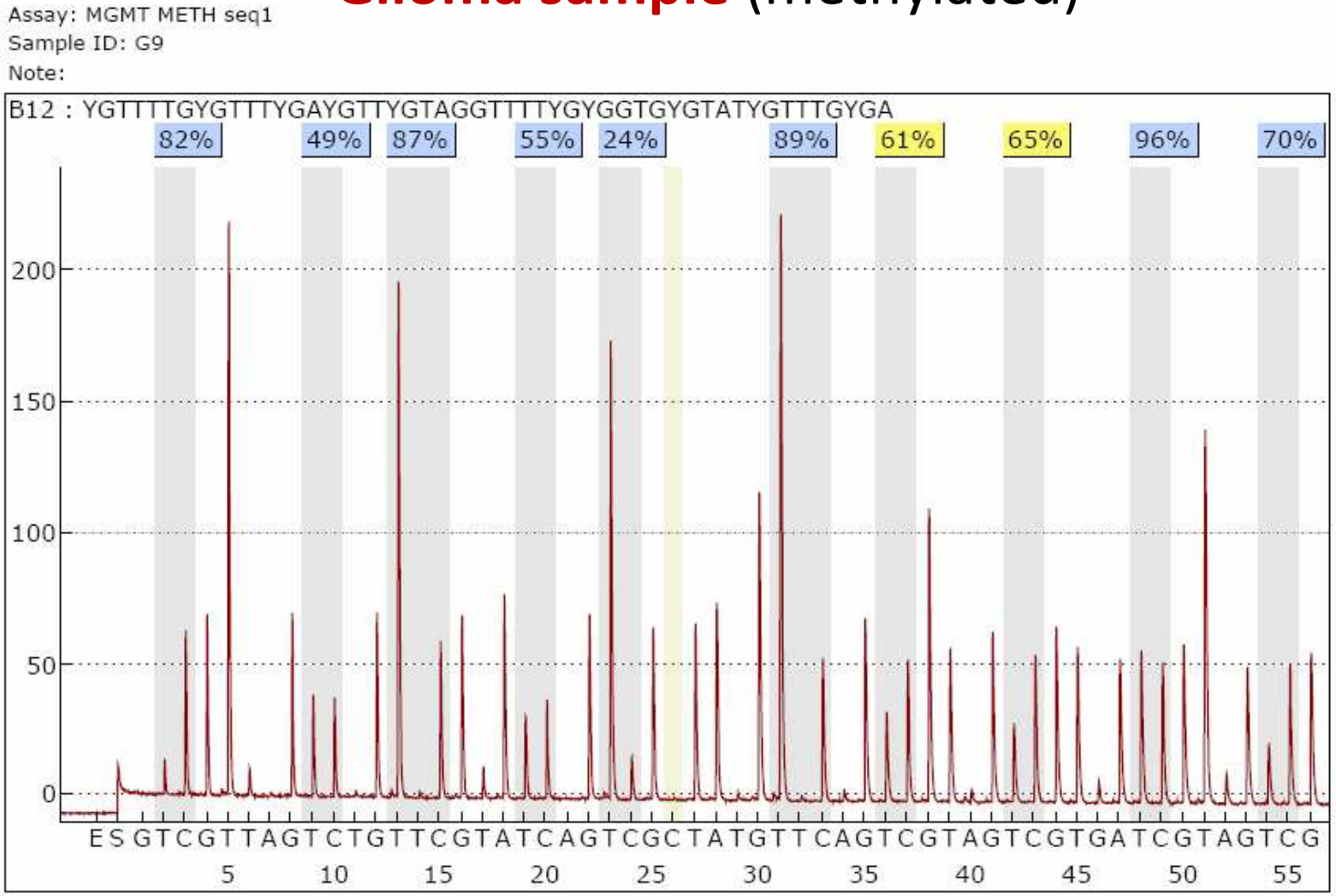
MGMT

‘Pyrograms’

Glioma sample (methylated)



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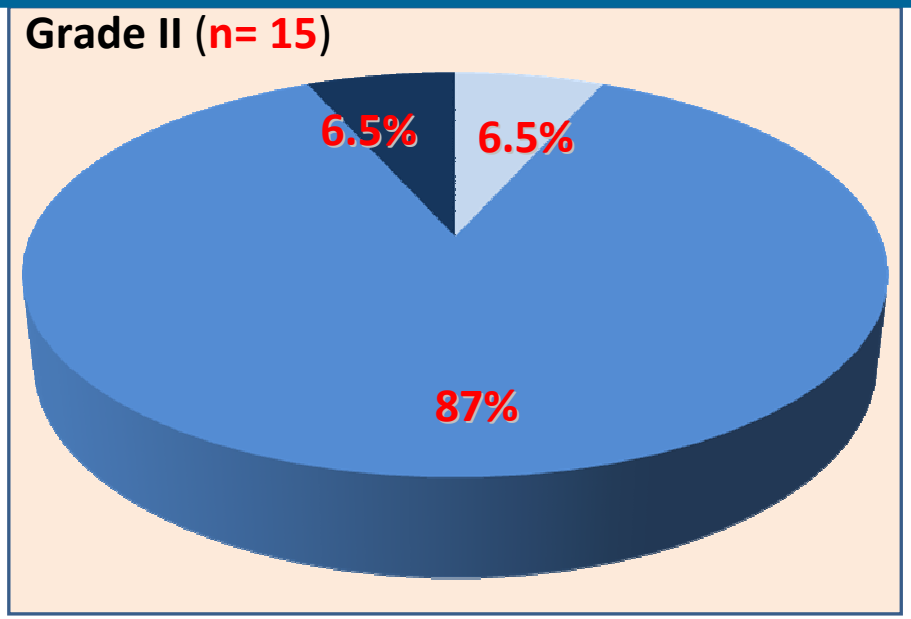
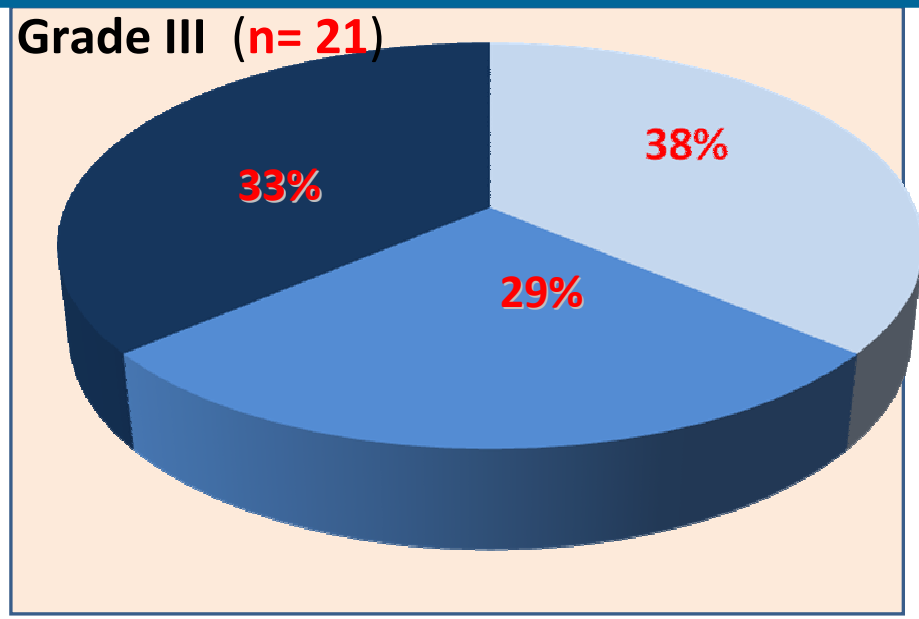
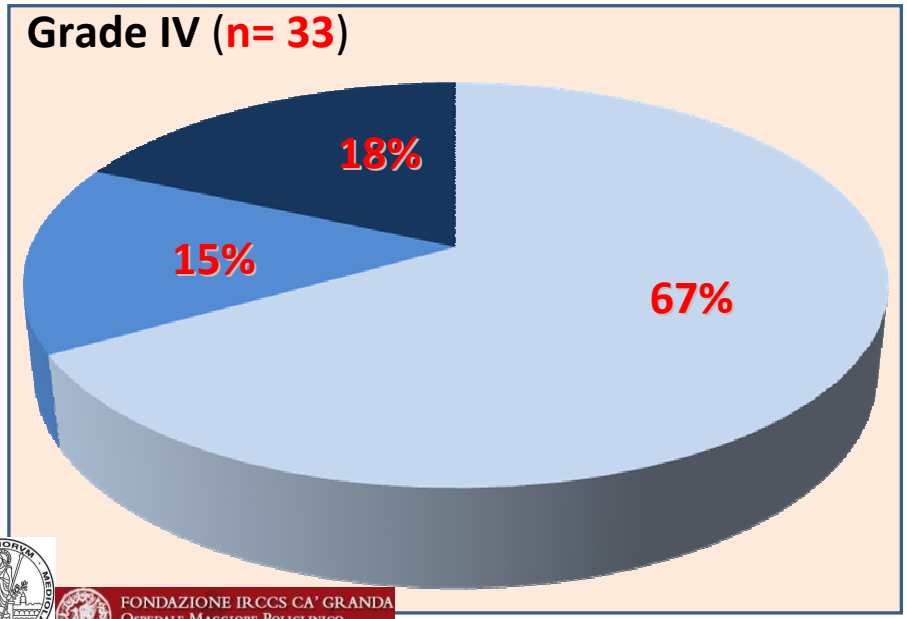
MGMT
'Results'







MGMT methyl is a frequent finding;
Unmethylated tumors are high grade;
Intermediate methyl is frequent.

- Unmethylated
- Methylated (10-50%)
- Methylated (>50%)

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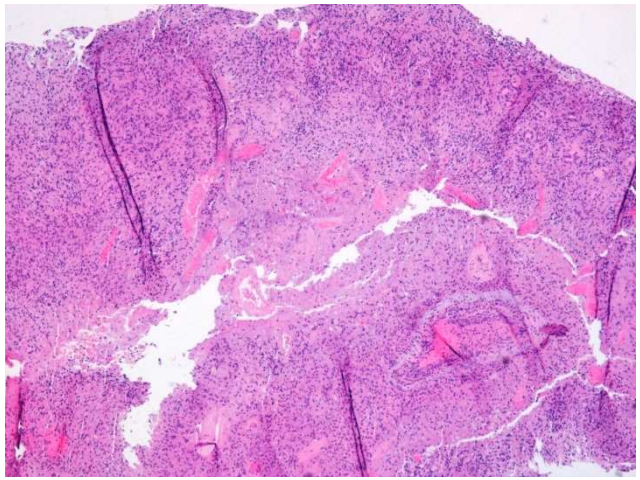
MGMT
‘Methyaltion levels significance’

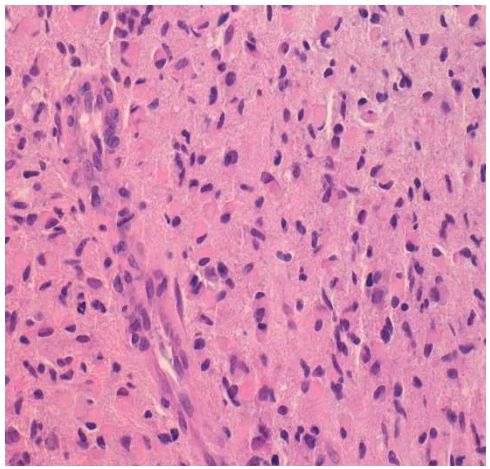
Intermediate MGMT methylation levels in glioma. WHY?

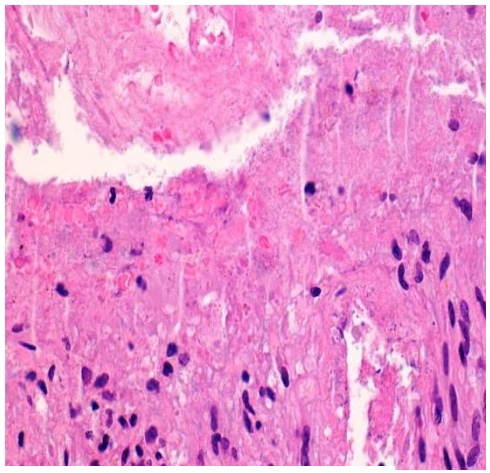
Normal cell contamination?

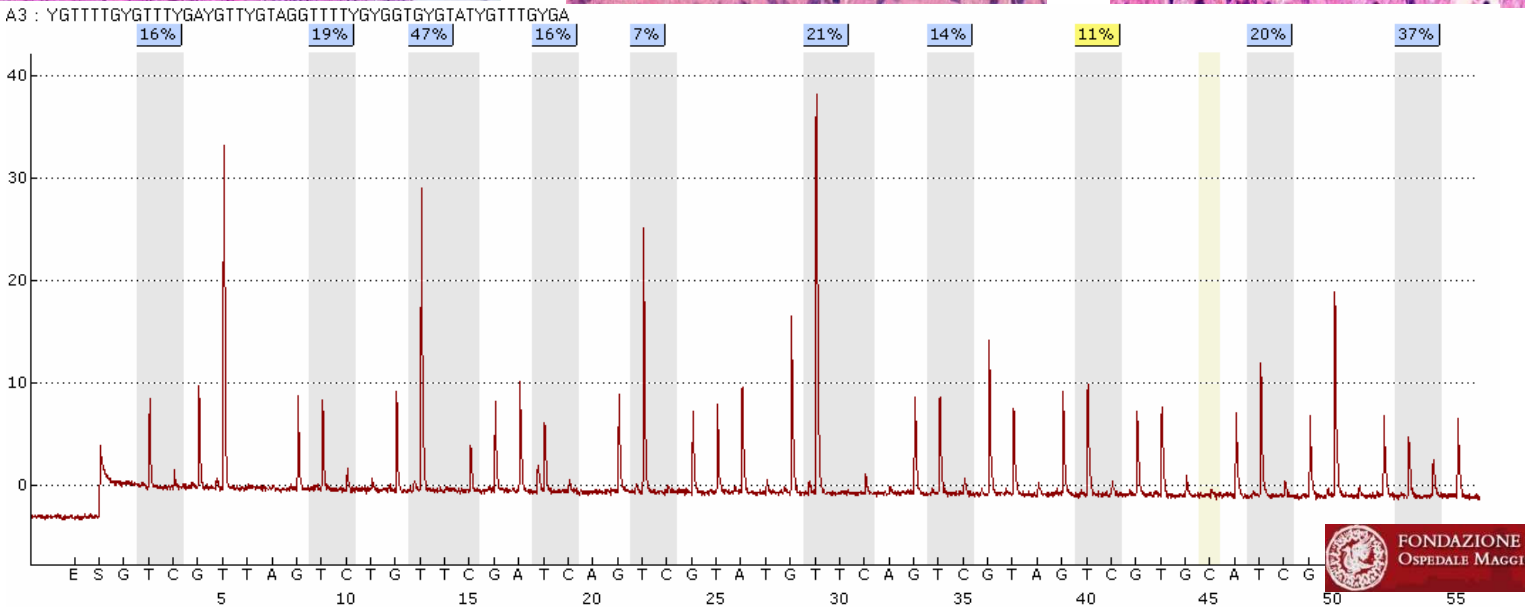
MGMT
‘Methyaltion levels significance’

Glioblastoma multiforme
MGMT methylation: 20%









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MGMT
‘Methyaltion levels significance’

Intermediate MGMT methylation levels in glioma. WHY?

Normal cell contamination?

No, or not always associated with normal cell contamination

Additional genetic/genomic events?

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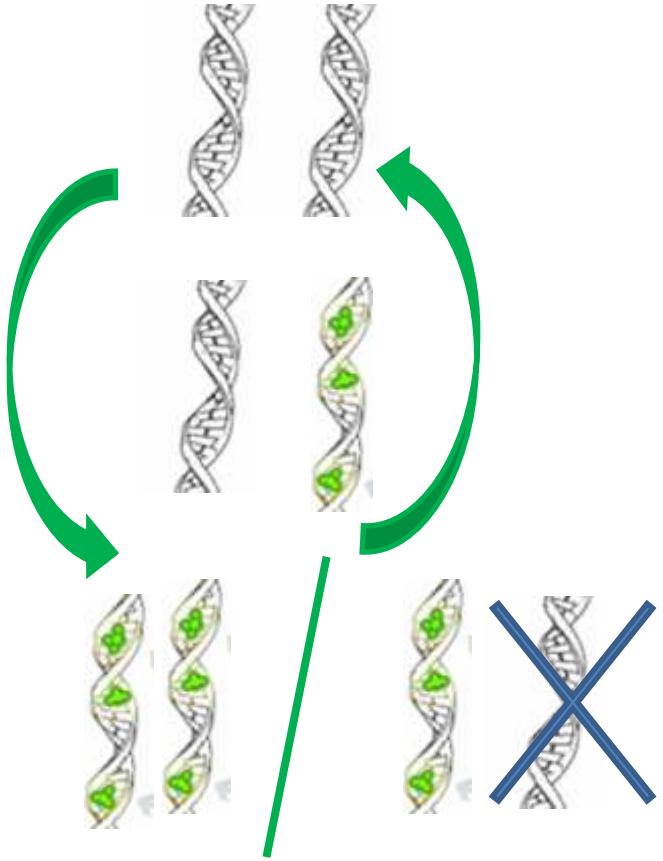
MGMT
‘Methyaltion levels significance’

The methylation levels in a single cell….

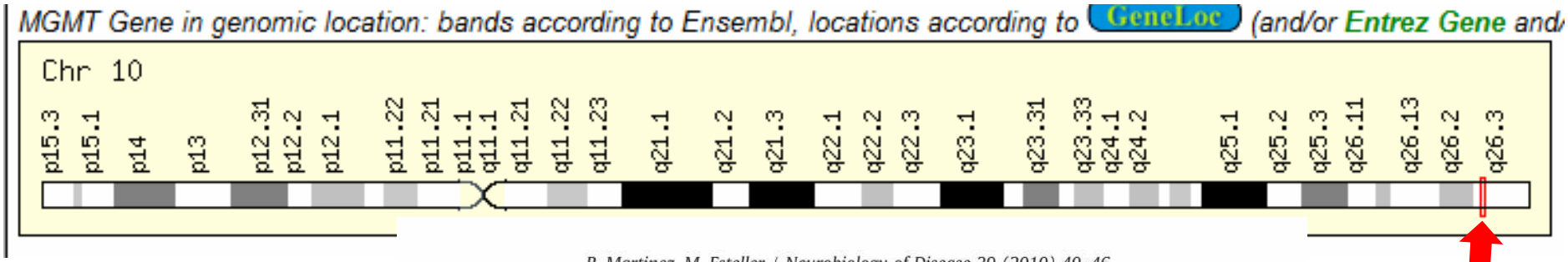
Methylation: 0%

Methylation: 50%

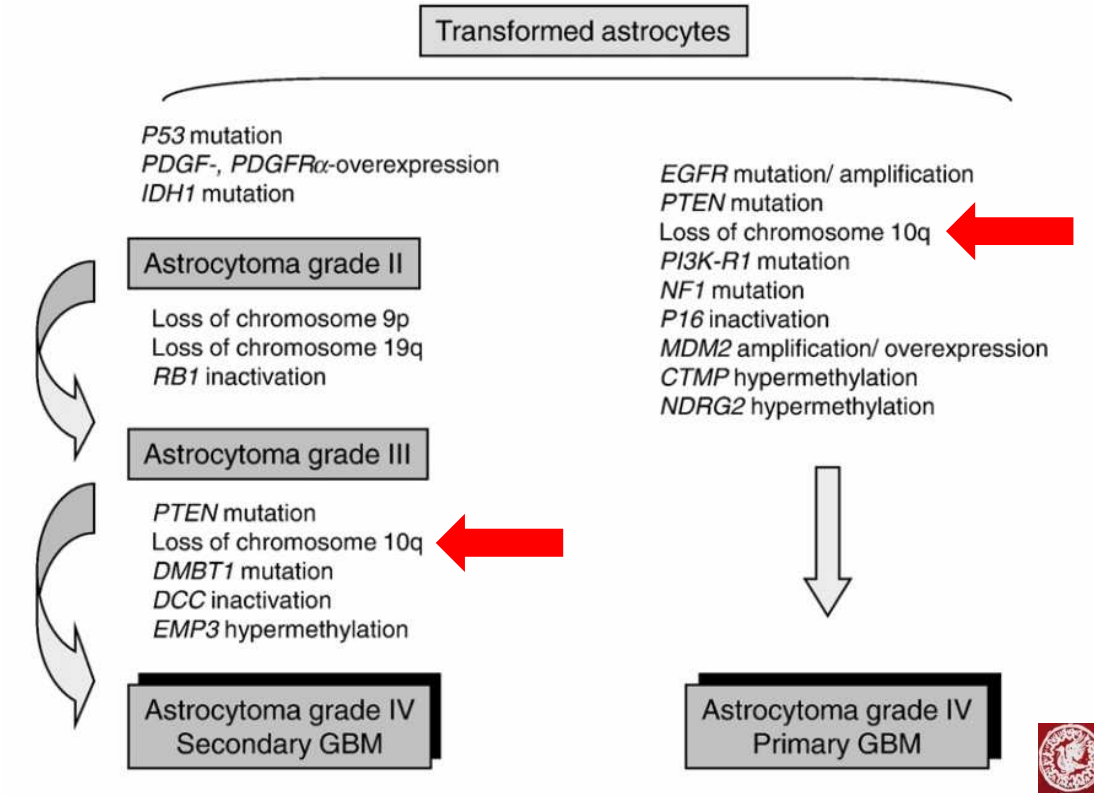
Methylation: 100%



MGMT
‘Methyaltion levels significance’



R. Martinez, M. Esteller / Neurobiology of Disease 39 (2010) 40–46



MGMT
'Methyaltion levels significance'

Intermediate MGMT methylation levels in glioma. WHY?

Concurrent genomic events (chr10q deletion, inactivation MGMT point mutations) should be considered for a better understanding of methylation levels.

The relationship between methyl level and gene expression is not obvious!

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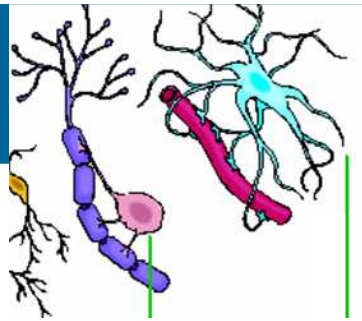
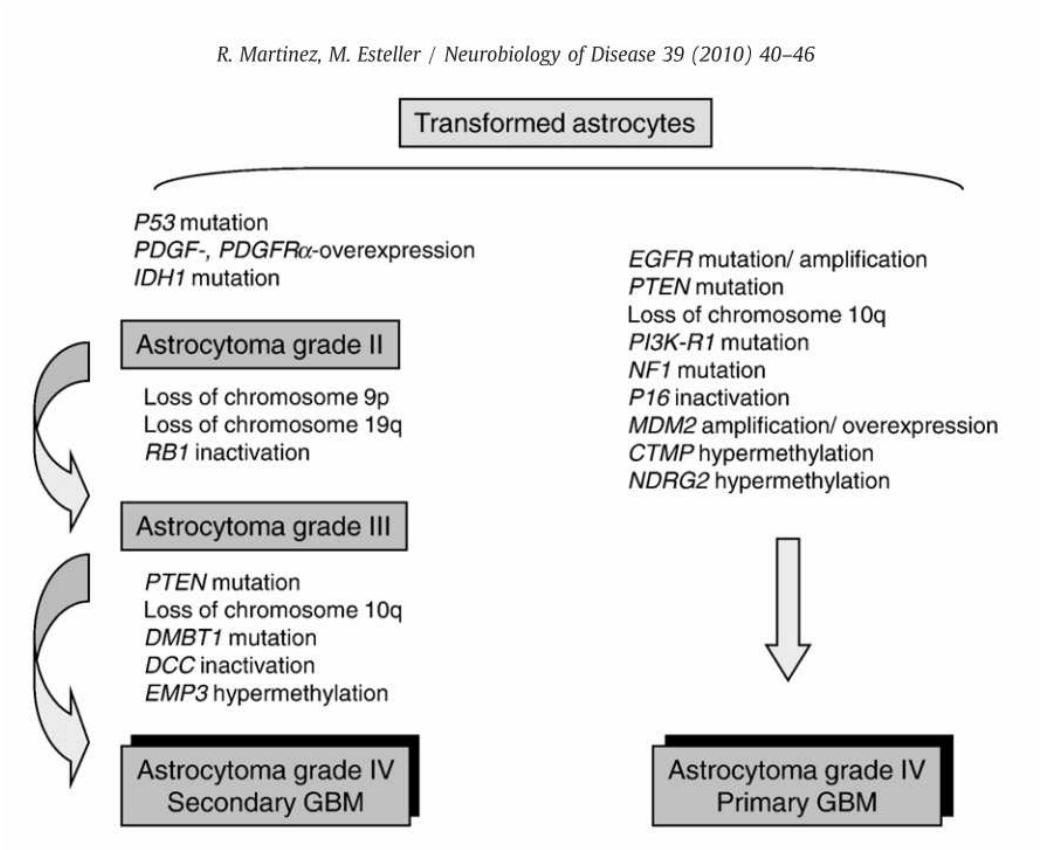
The next future...
'The Glio*Map*'





MGMT methylation, G-CIMP

\+ Pharmacogenetics markers (constitutive) of drugs response (CYTOCHROME P450 ENZYMES)

A comprehensive screen of somatic genetic and epigenetic lesions and constitutive polymorphisms: a useful clinical research tool for glioma patients.

MGMT
'Conclusions'

- ✓MGMT methyl is a common and **early event** in glioma;
- ✓**Pyrosequencing** allows a higher detection rate respect to MS-PCR
- ✓ **Unmethylated** tumors tend to **progress**;
- ✓Methylation levels tend to increase;
- ✓The effect (drug response) of intermediate methylation should be investigated considering other concurrent pathogenetic events (10q deletions, point mutations).

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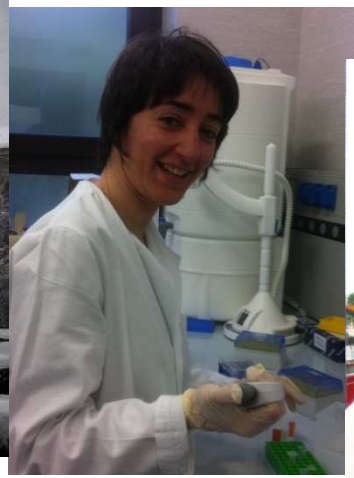
Thank You!!!

Eleonora Bonaparte, PhD

Claudia Augello, PhD



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