An introduction to epigenetics

Basic Course in Molecular Pathology

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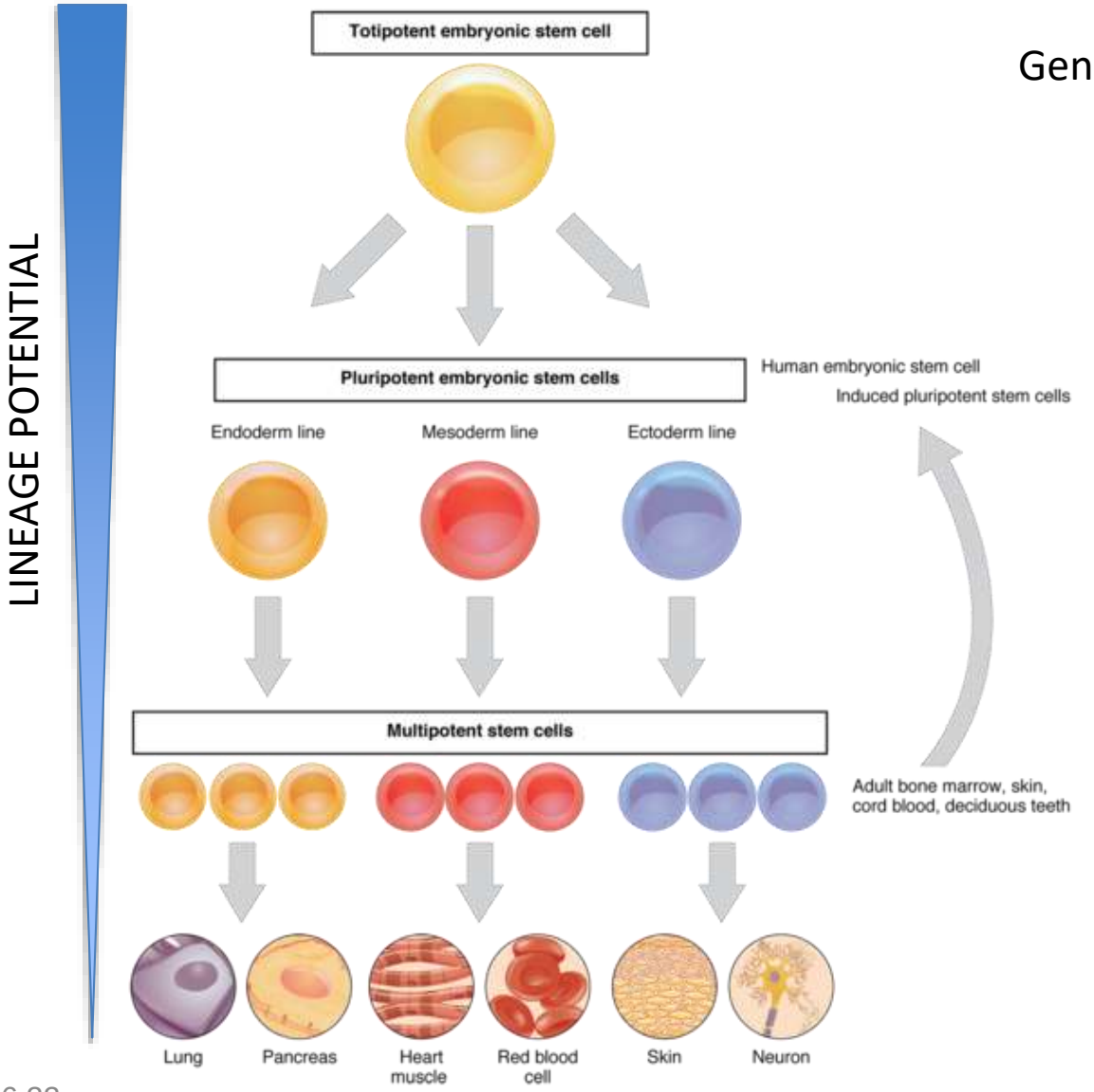
CUB Hôpital Erasme

LINEAGE RESTRICTION DURING DEVELOPMENT



Genetic material common to all ES cells

DIFFREENTIATION

Various cell types

EPIGENETICS: A LANSCAPE TAKES SHAPE

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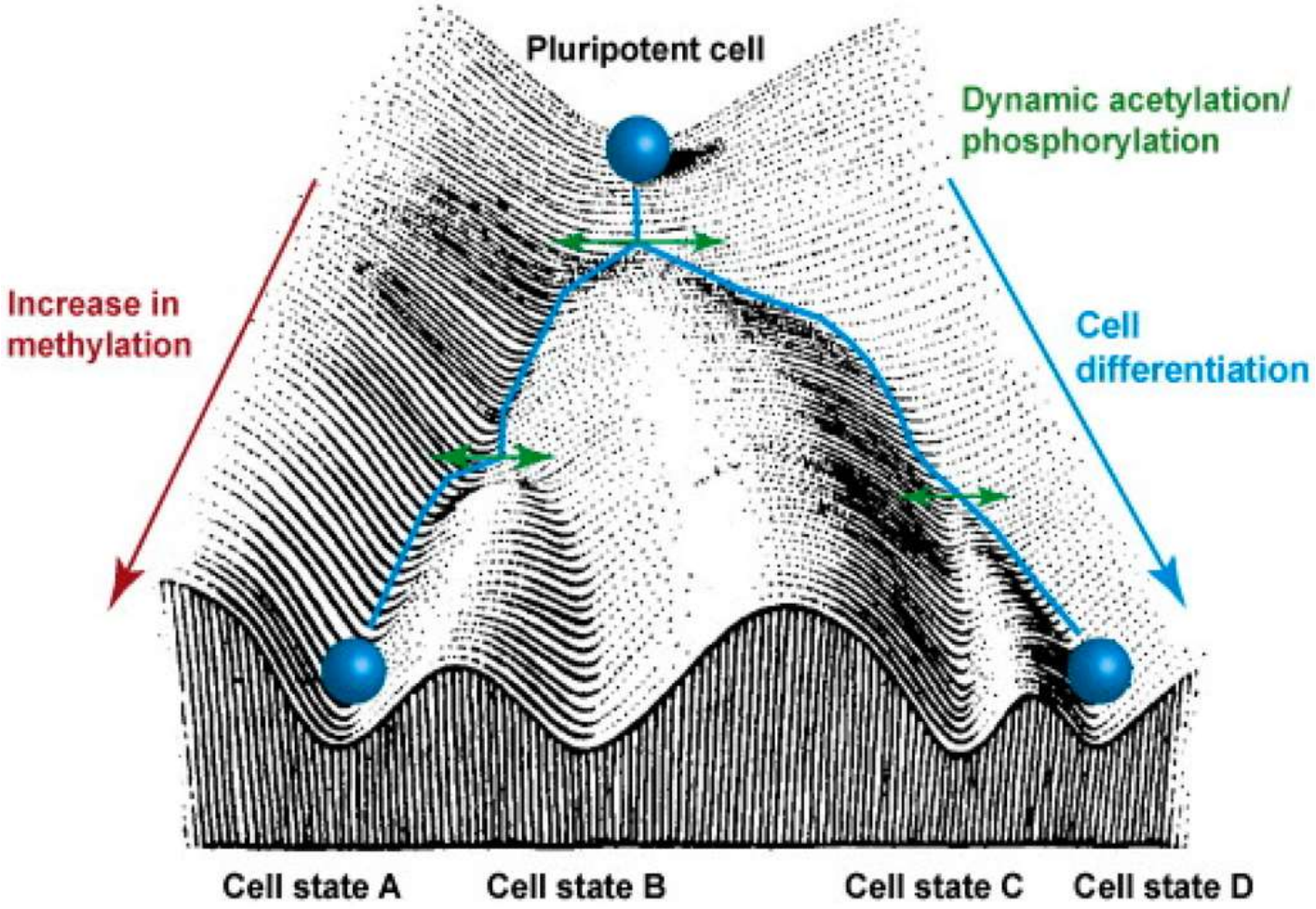
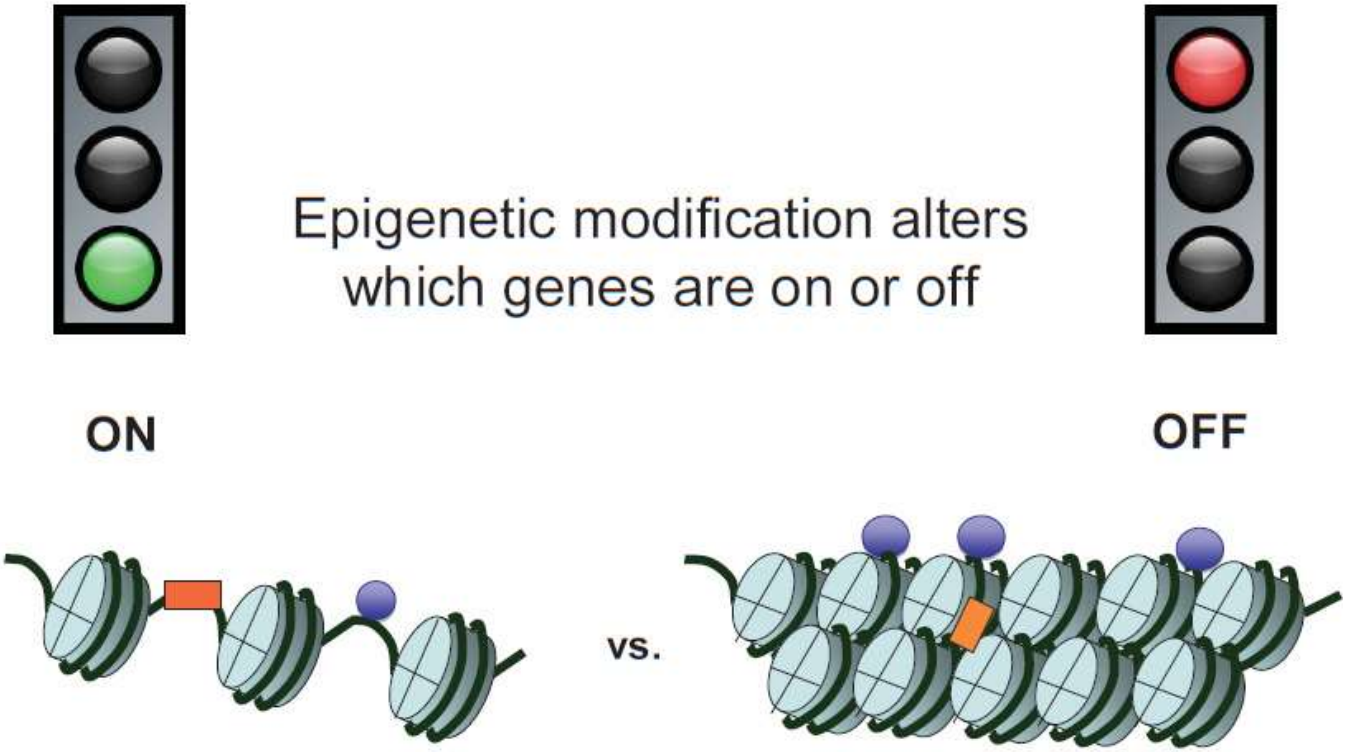
Epigenetics = events that could not be explained by genetic principles



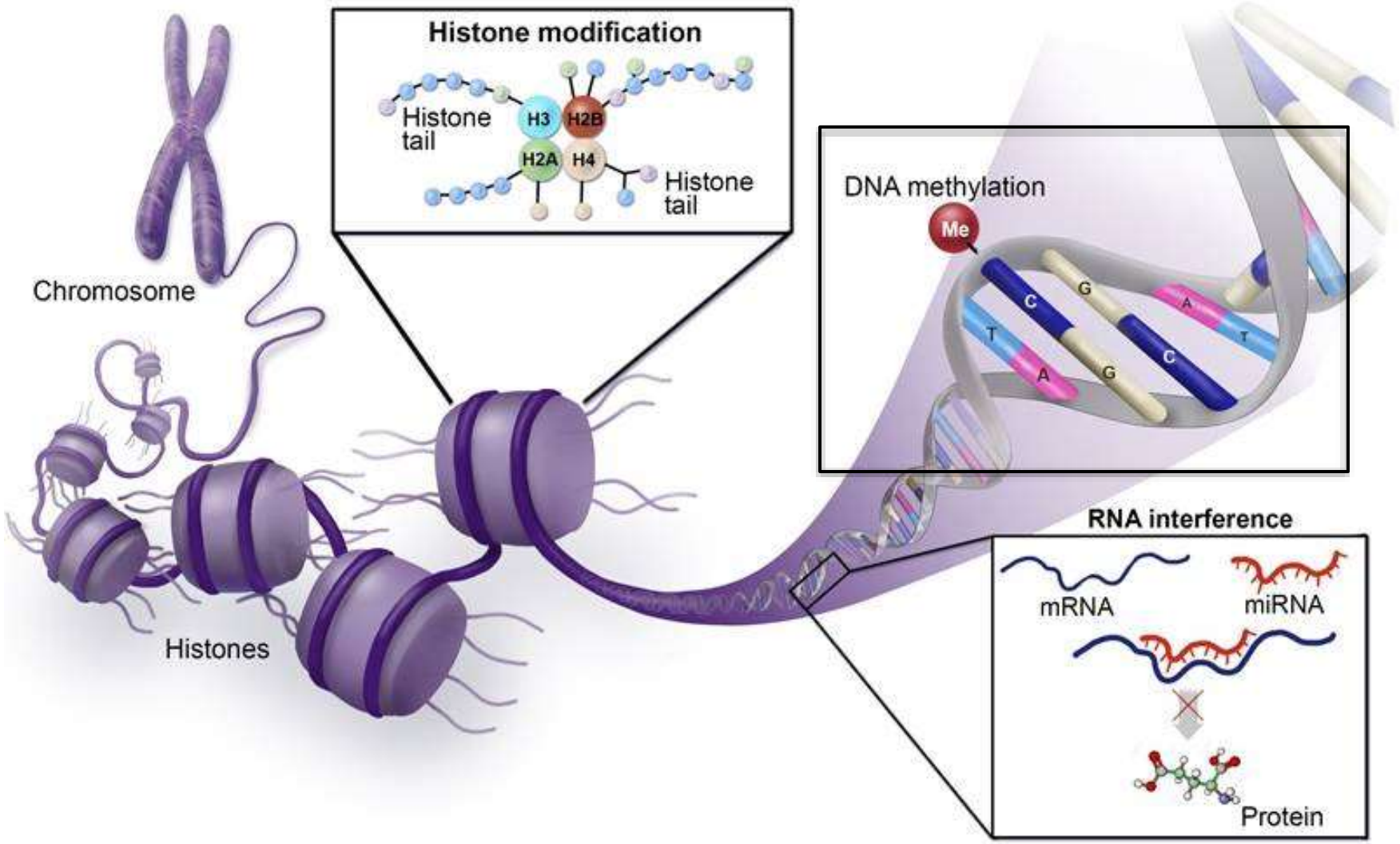
Waddington's epigenetic landscape for cell fate specification (1957)

EPI-GENETICS = ON TOP OF THE GENOME

Chemical modifications of chromosomal DNA and/or structures that changes the pattern of gene expression without altering the DNA sequence



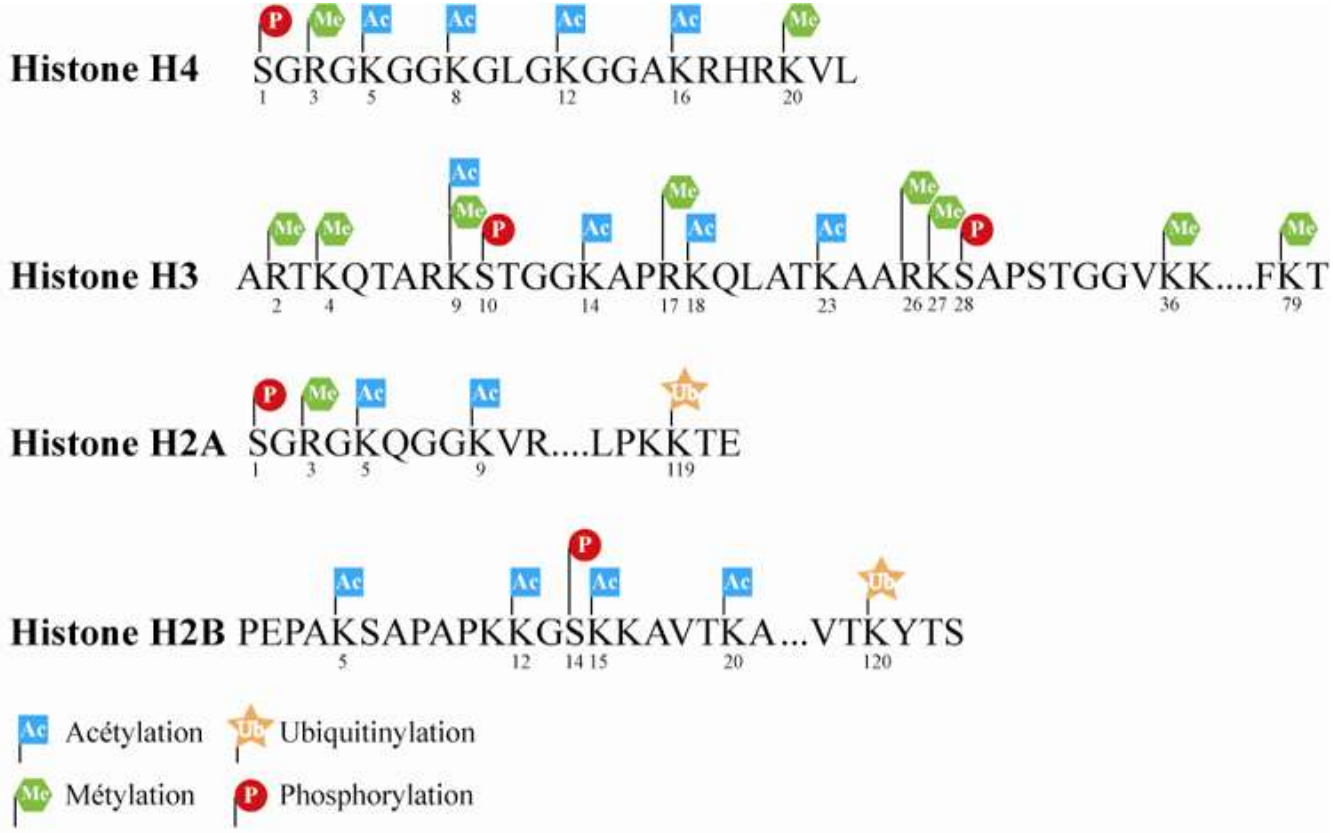
EPIGENETICS MECHANISMS



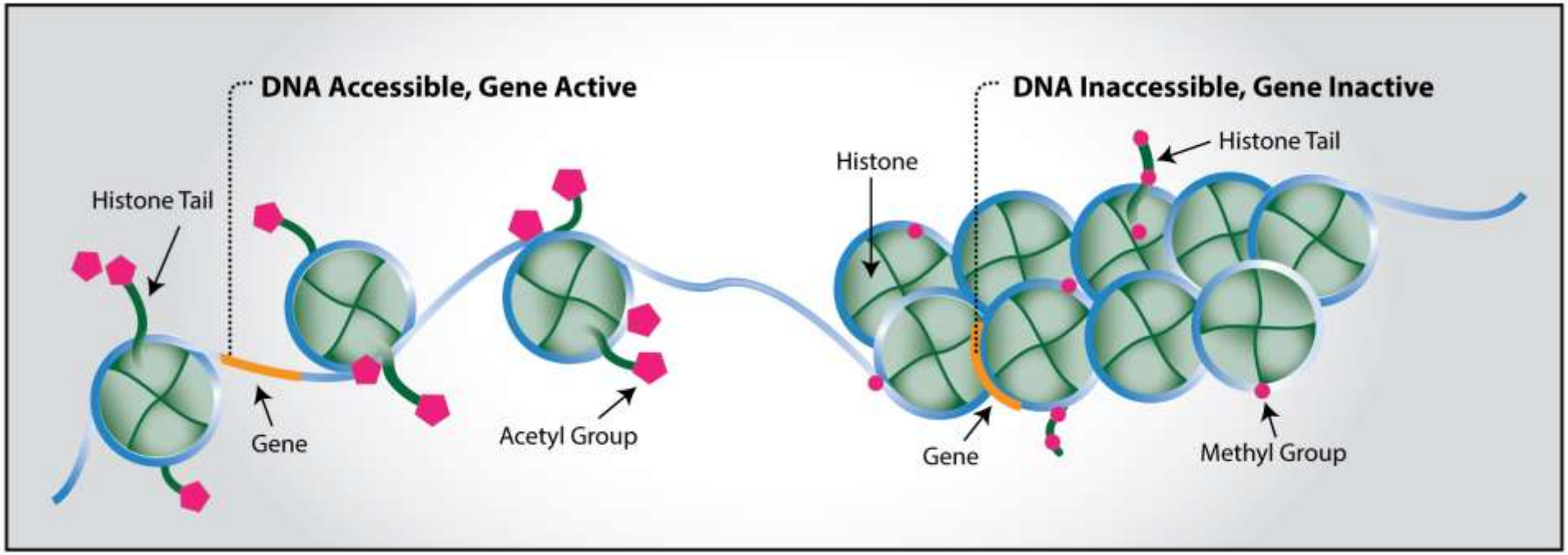
HISTONE MODIFICATIONS

Histones acétylases (HAT)
Histones méthyltranférases (HMT)
Histone kinases

Histones désacétylases (HDAC)
Histones déméthylases
Histones phosphatases



ACETYLATION AND METHYLATION OF HISTONES



Acetylation

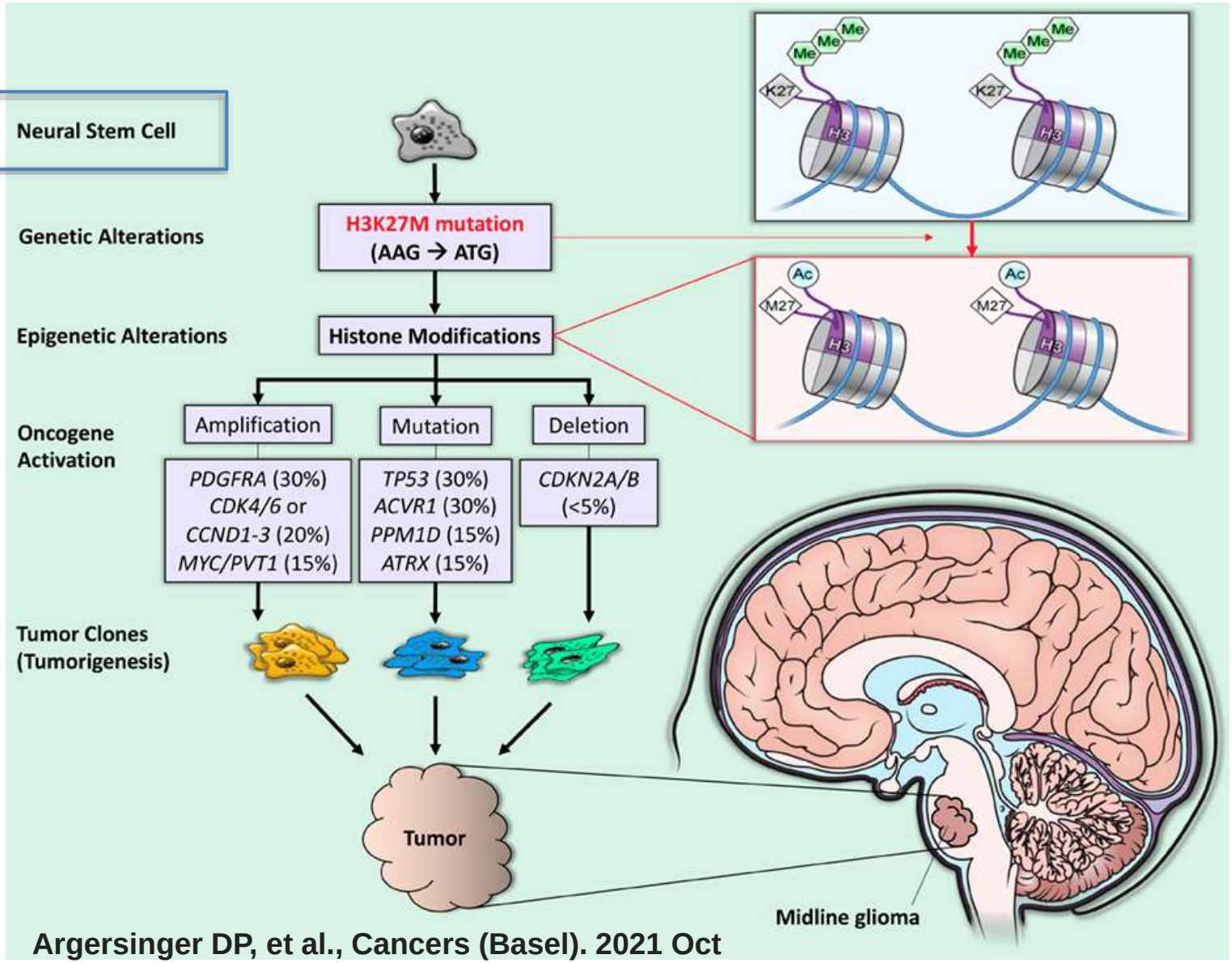
removes positive charges
=> Recuced affinity
between histones and DNA

Methylation

H3K4me2, H3K4me3, H3K79me3 **ON**

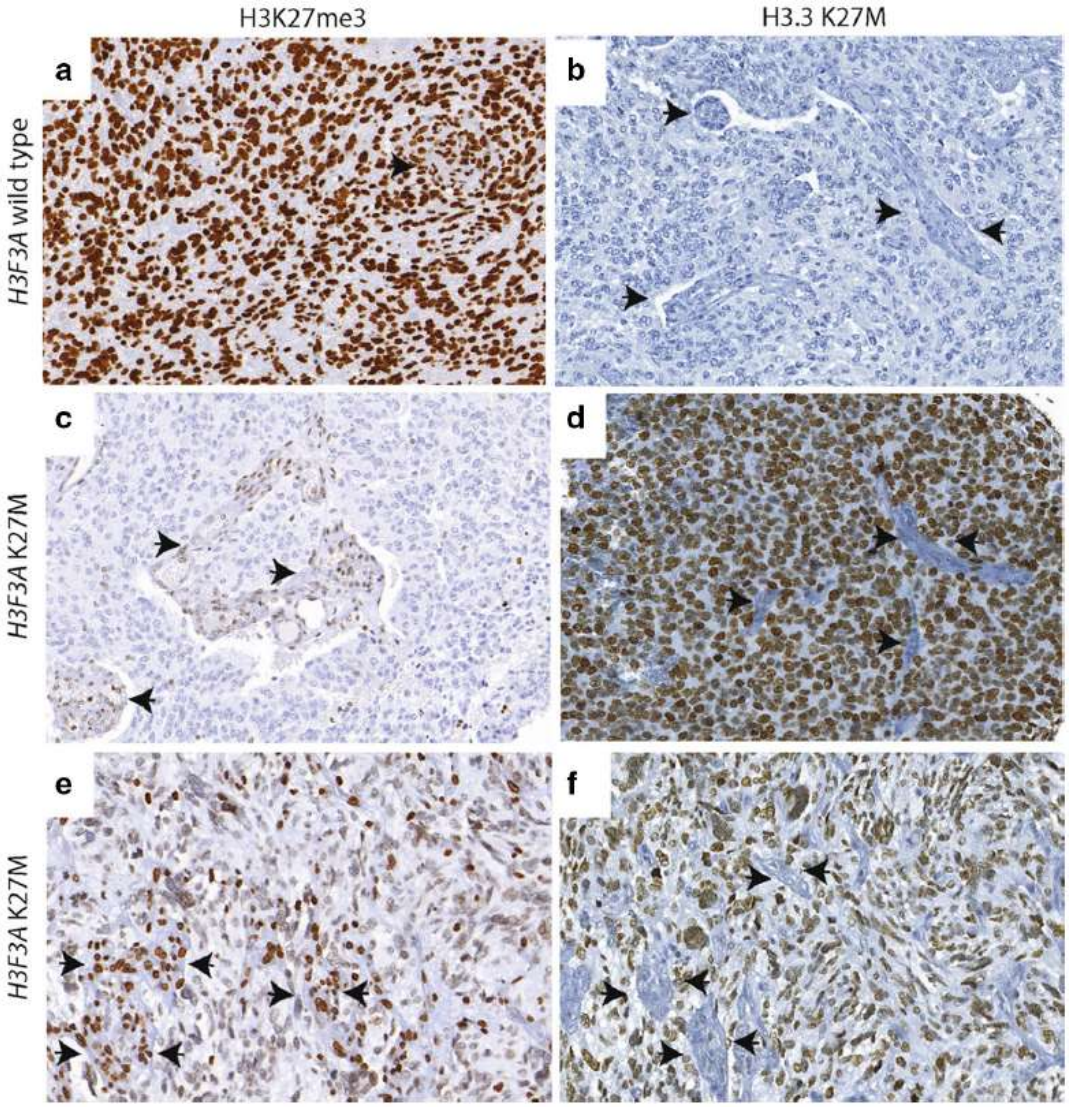
H3K9me2, H3K9me3, H3K27me2, H3K27me3, H4K20me3 **OFF**

DIFFUSE MIDLINE GLIOMAS H3K27M MUTANTS

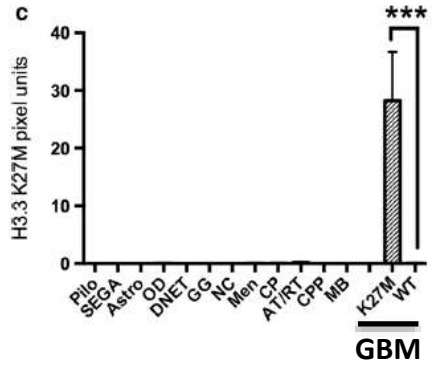


Argersinger DP, et al., Cancers (Basel). 2021 Oct 21;13(21):5280.

HISTONE H3-K27ME3 AND K27M AS DIAGNOSTIC BIOMARKERS



Detection of H3.3 K27M by IHC showed 100% sensitivity and specificty and is superior to global reduction in H3K27me3 as biomarker in diagnosing H3F3A K27M mutations.



Factor	H3.3 K27M positive	H3K27me3 low
Sensitivity	100	100
Specificity	100	98
PPV	100	70
NPV	100	100

Endothelial cells of blood vessels = internal control

Pediatric high grade gliomas

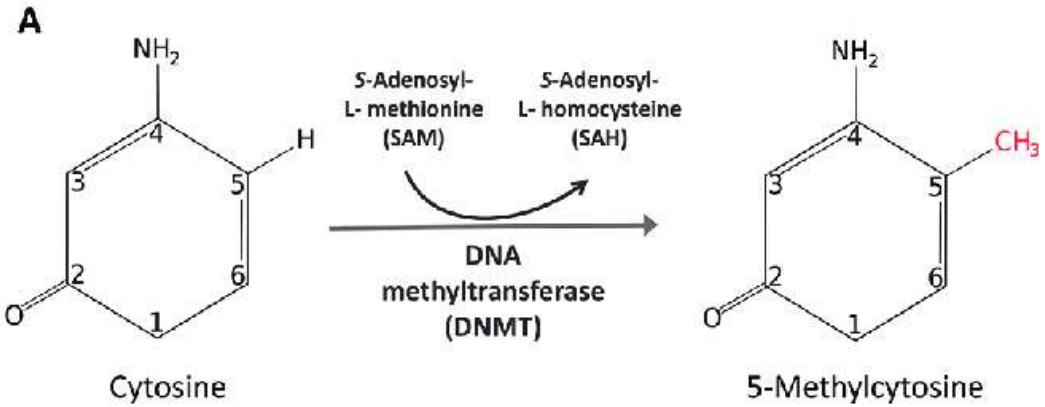
Venneti S. et al., Acta Neuropathol, 2014.

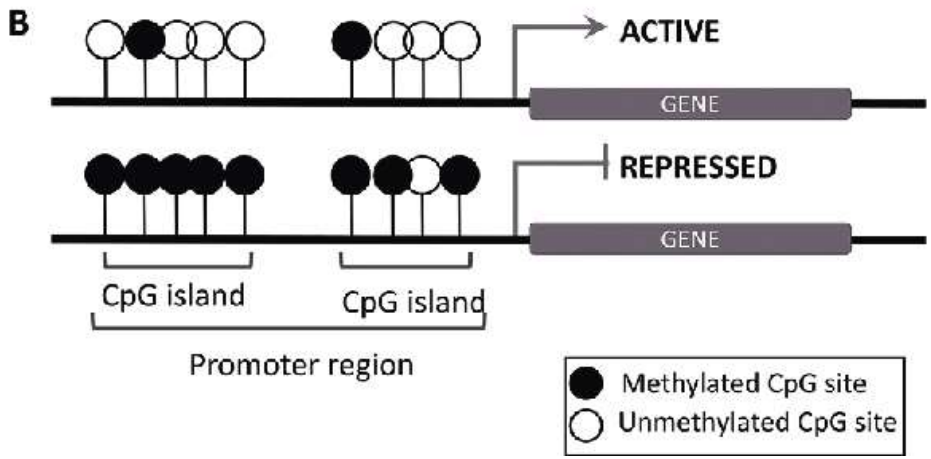
DNA METHYLATION

- CpG = regions of DNA where a cytosine is followed by a guanine along its 5' → 3' direction
- Methylation of cytosine only
- CpG sites occur with high frequency in genomic regions called CpG islands (or CG islands)
- In mammals, 70% to 80% of CpG cytosines are methylated
- About 70% of promoters located near the transcription start site of a gene contain a CpG island

DNA METHYLATION

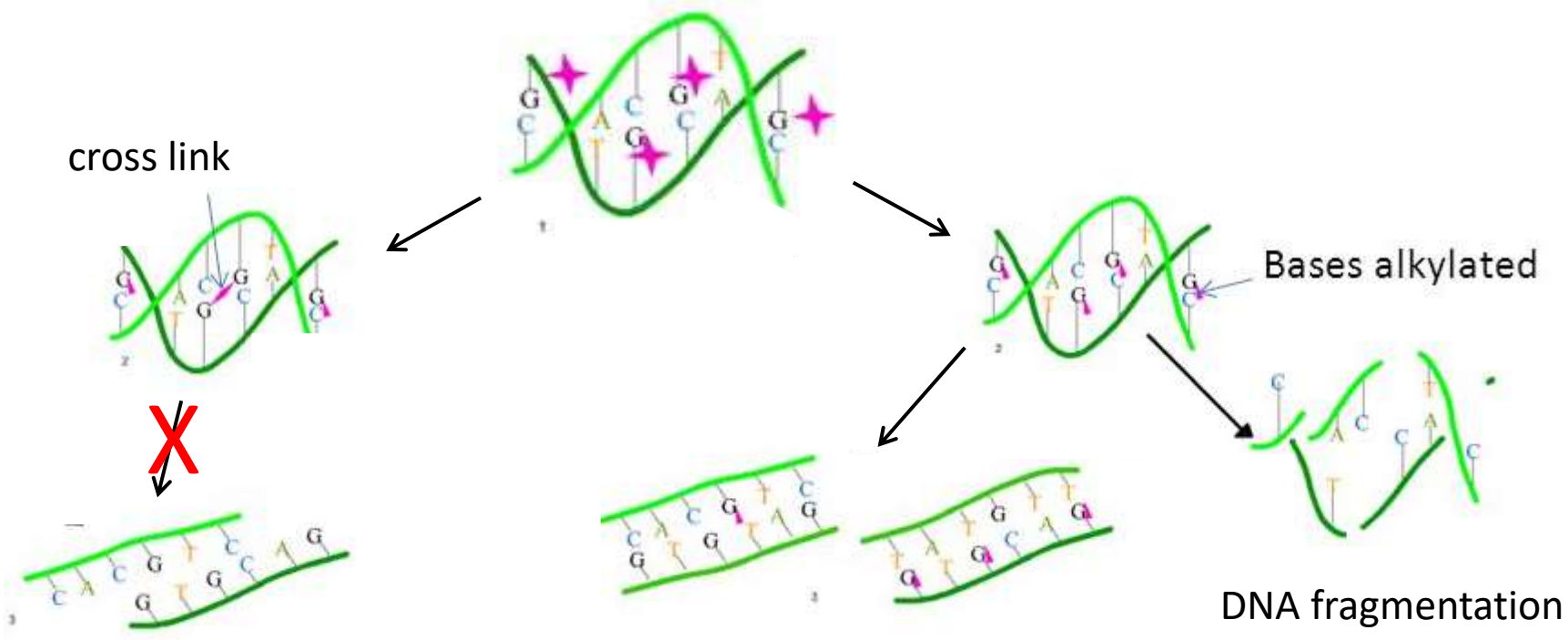
- **Covalent modification** (addition of a methyl group to the 5th carbon) of a CpG dinucleotide
- **Robust biomarker** that can be detected after fixation and tissue processing
- **Binary nature :** methylated/unmethylated makes it easier for computational analysis





MGMT O6-METHYLGUANINE-DNA METHYLTRANSFERASE

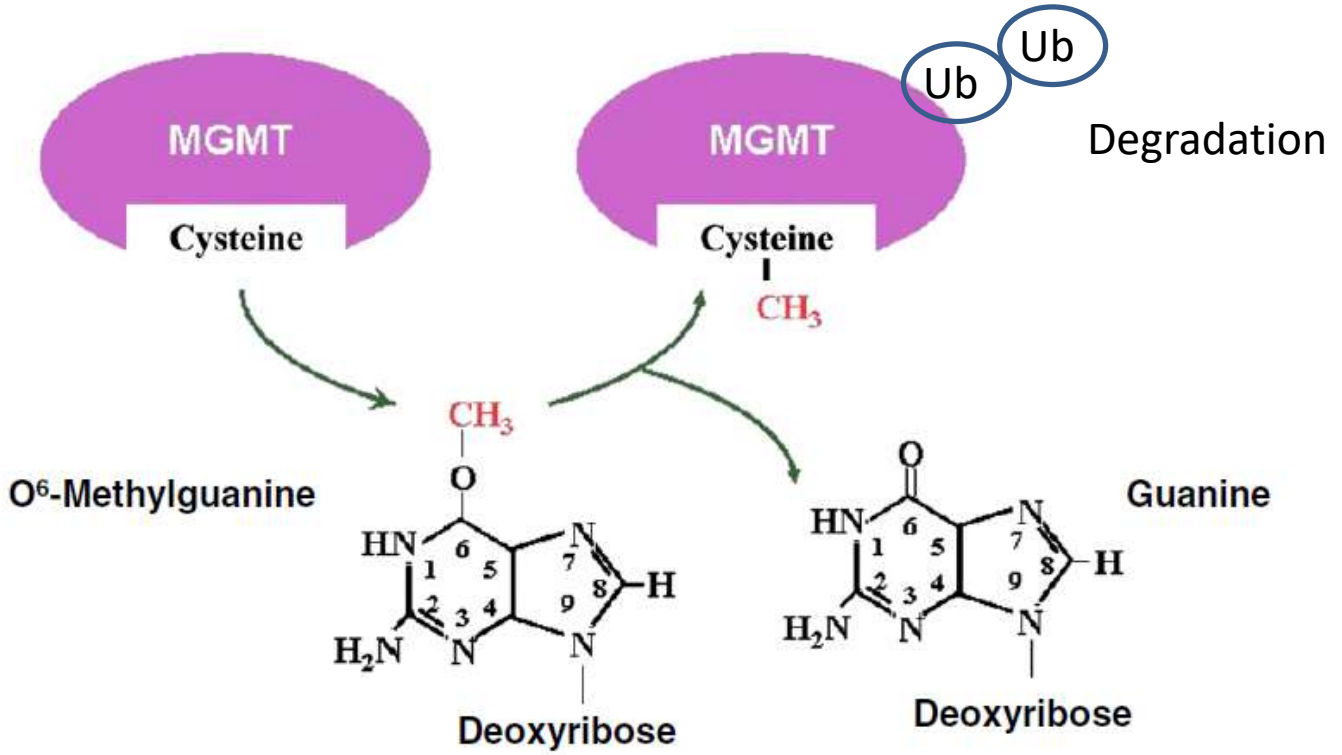
- O^6-methylguanine DNA methyltransferase (MGMT) is a key enzyme in the **DNA repair network**
- MGMT prevents the genotoxic effects of O^6-methylguanine adducts produced by exogenous and endogenous alkylating mutagens in human cells.



Cabrini et al., Int. J. of Oncology, 2015.

MGMT O6-METHYLGUANINE-DNA METHYLTRANSFERASE

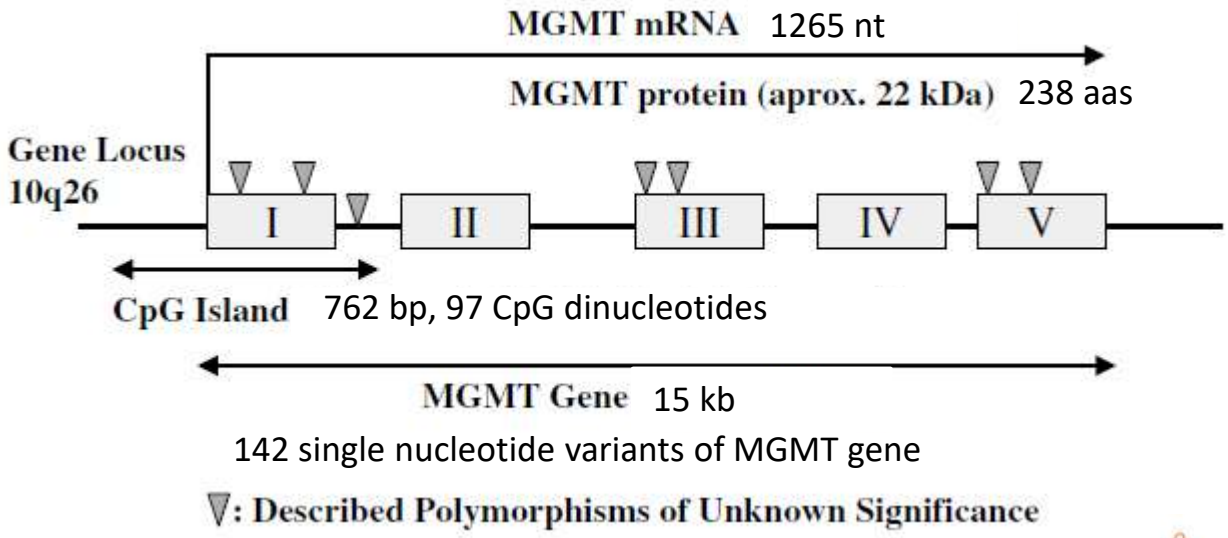
- MGMT is a ubiquitously expressed nuclear enzyme which removes alkyl groups from O^6-position of O^6-methylguanine.



- « suicide inhibition »: inactivation of one molecule of MGMT for each alkyl group removed from methylguanine.
- The number of O^6-methylguanine adducts that can be removed from DNA *in vivo* is limited by the number of MGMT molecules in cells and the rate of de novo synthesis of the protein.

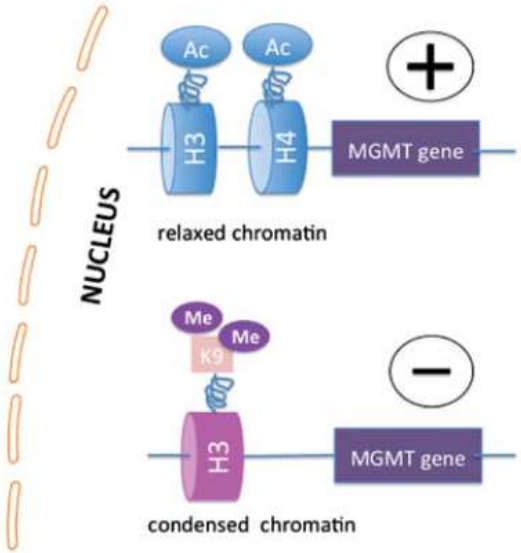
Cabrini et al., Int. J. of Oncology, 2015.
Silber et al., Biochimica et Biophysica Acta, 2012.

MGMT GENE



MGMT gene expression regulated by:

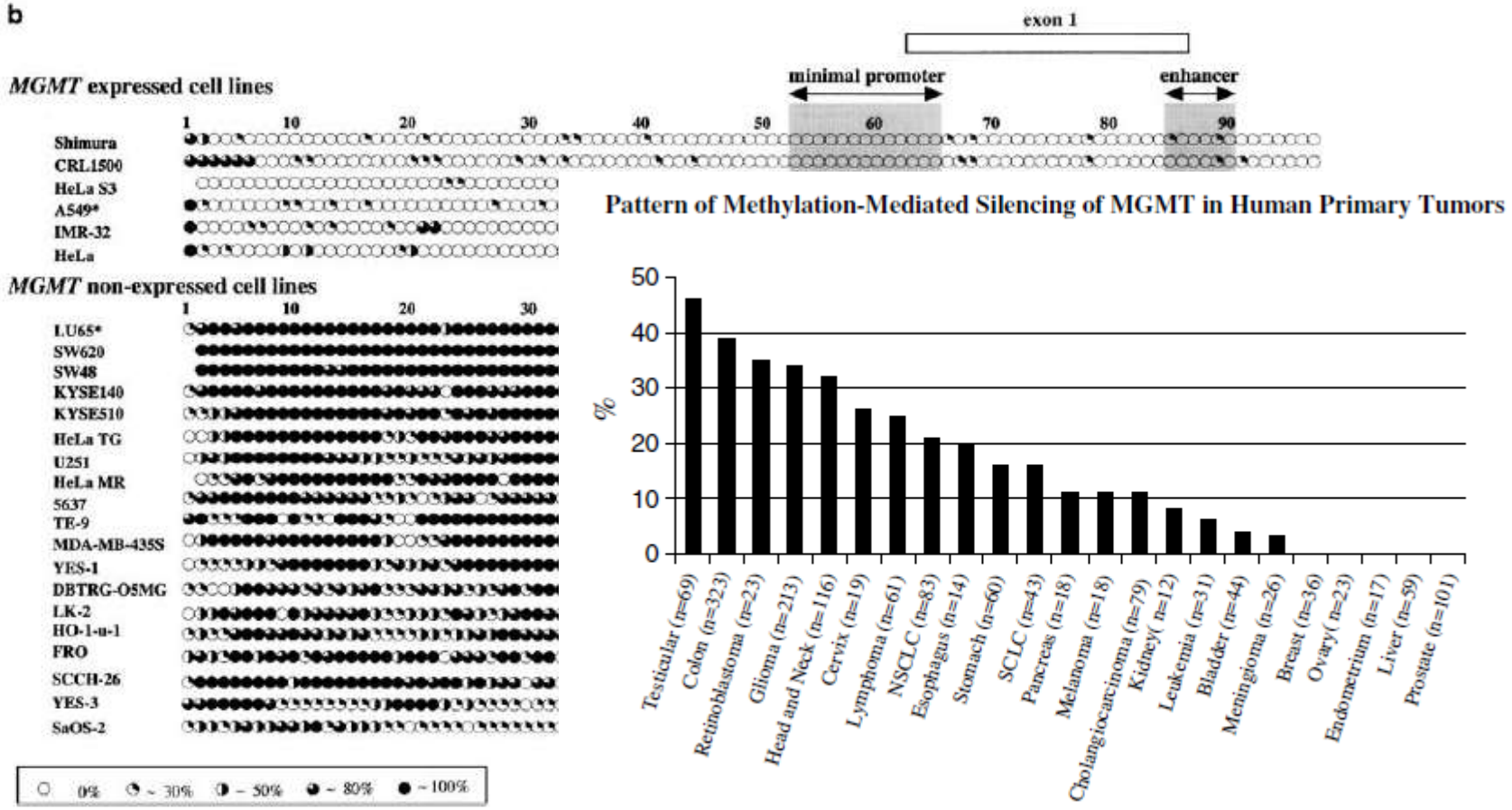
- Transcription factors (Sp1, AP-1, CEBP, NF-kB, HIF-1a
- Histone modifications
- MicroRNAs (miR-181d, miR-767-3p, miR-648…)
- **MGMT promoter methylation**



Cabrini et al., Int. J. of Oncology, 2015.
Esteller and Herman, Oncogene, 2004.

MGMT PROMOTER METHYLATION

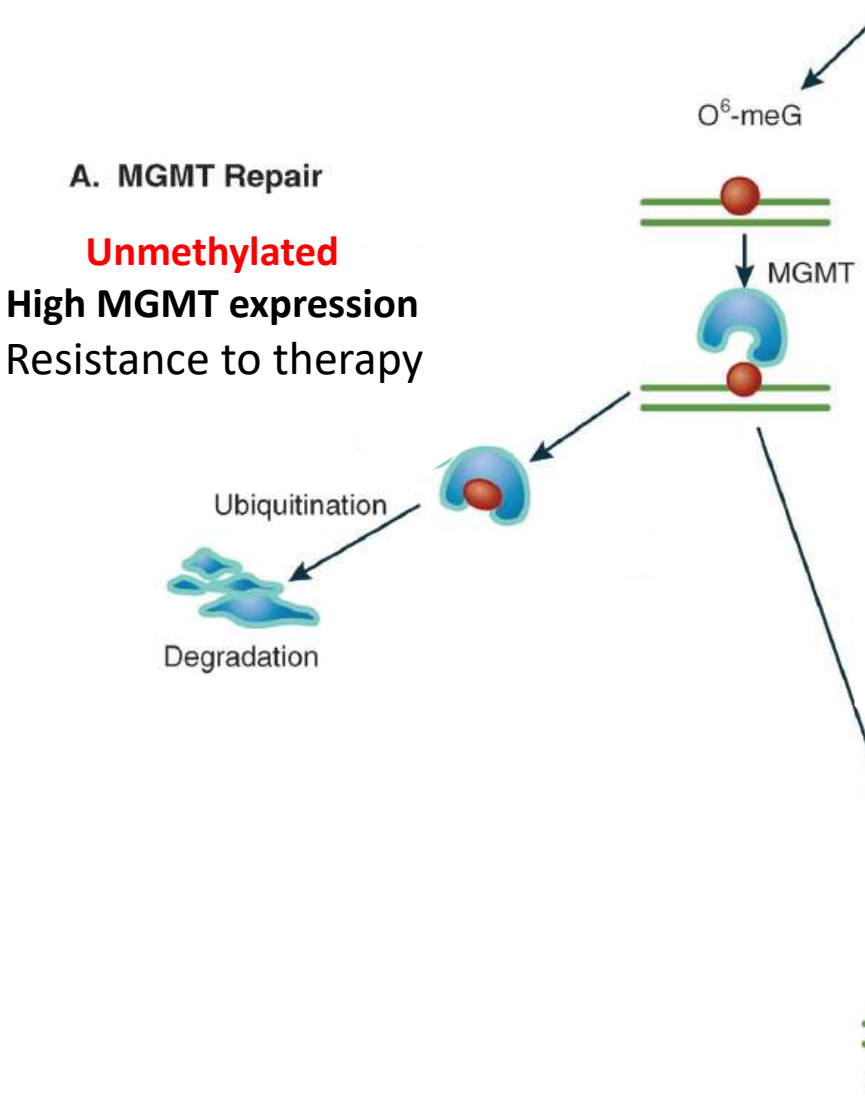
- Post-transcriptional mechanism reducing protein expression
- The extent of the methylation of CpG sites in the promoter affect the levels of expression of the protein
- Methylation of MGMT promoter is found in 40% of cancer types such as glioma and colorectal cancer and in 25% of NSCLC, lymphoma and head and neck carcinoma.



Esteller and Herman, Oncogene, 2004.
Nakagawachi et al., Oncogene, 2003.

MGMT EXPRESSION AS PREDICTIVE BIOMARKER

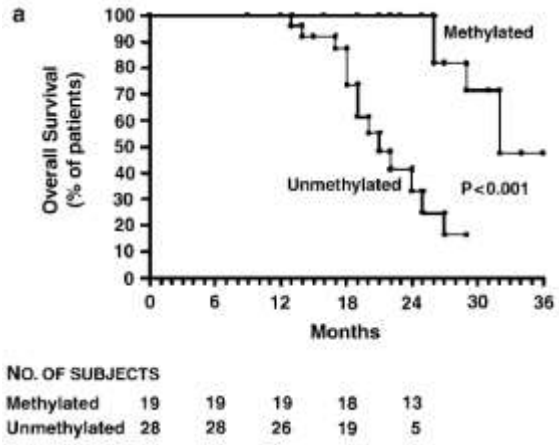
MGMT promoter methylation is a biomarker of the response to the alkylating chemotherapy



Temozolomide
Nitrosourea derivatives

Massive DNA alkylation: base mispairing

Unmethylated
High MGMT expression
Resistance to therapy

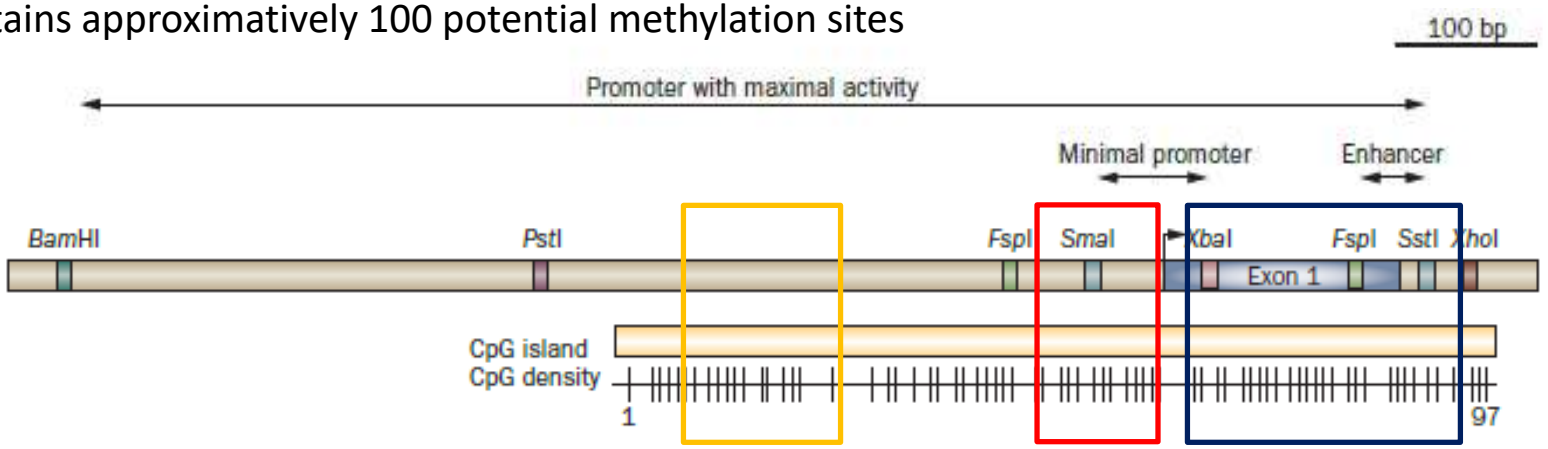


Importance of evaluating MGMT expression level

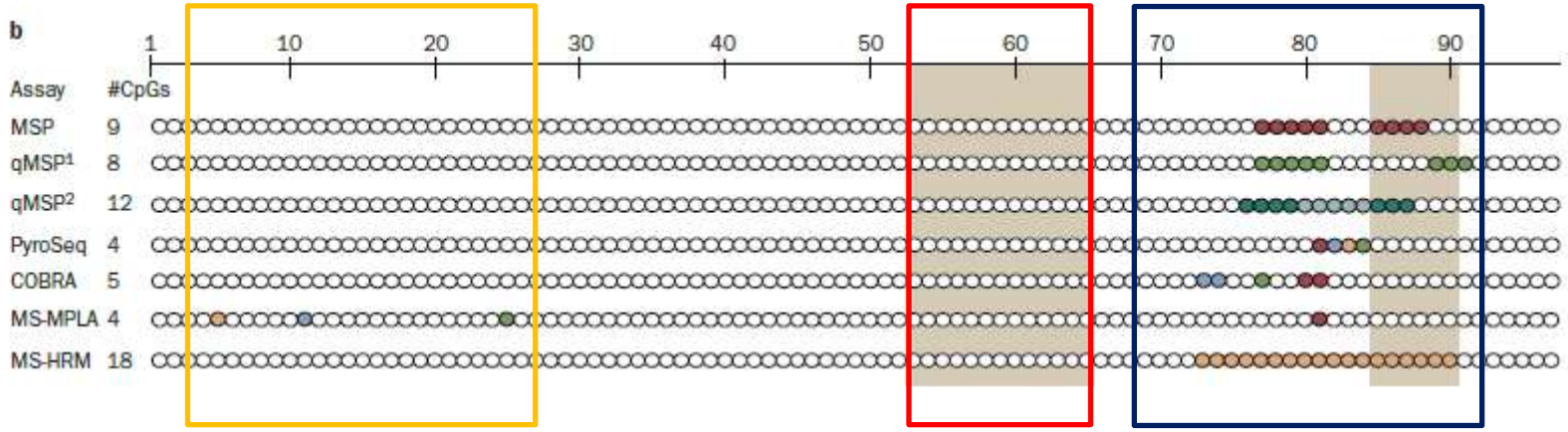
Esteller and Herman, Oncogene, 2004
Liu and Gerson, Clin. Cancer Res., 2006

Map of the CpG island region of the MGMT promoter

- MGMT promoter spans > 1000 bp
- Contains approximatively 100 potential methylation sites



Visualization of CpGs interrogated by diverse methylation-specific assays:



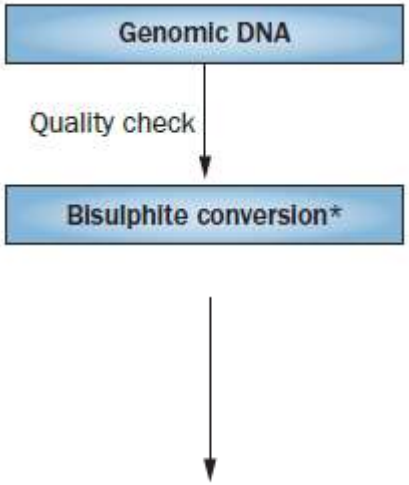
Everhard et al., 2009
Shah et al., 2011
Kanemoto et al., 2014

Qian and Brent, 1997
Watts et al., 1997

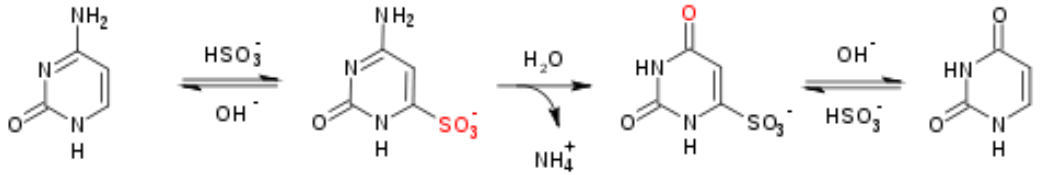
Esteller et al. 1999
Esteller et al., 2012
Evehard et al. 2009

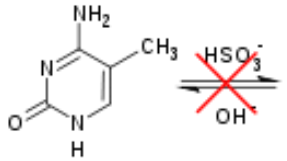
Methods to determine the methylation status of *MGMT* promoter

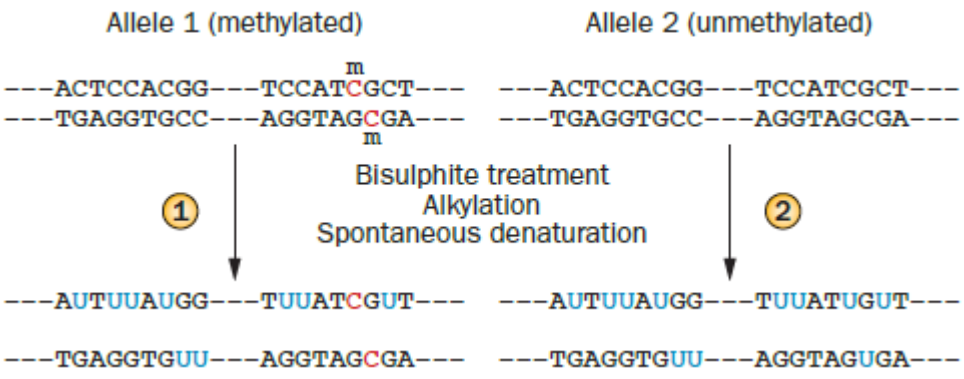
- **Quantitative methylation specific PCR**
- **Pyrosequencing**
- Methylation-sensitive high resolution melting
- NGS
- Etc.

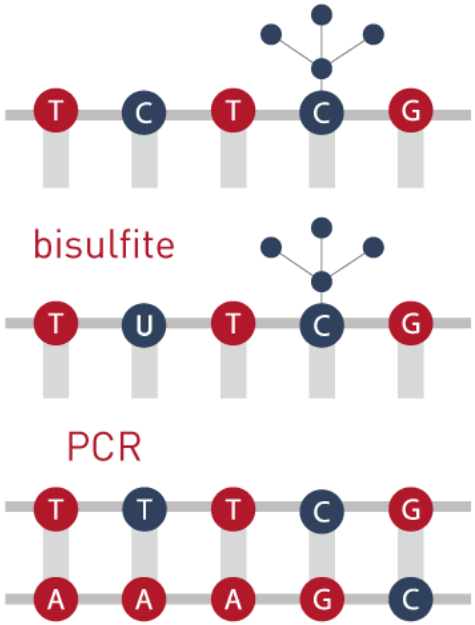


Deamination of unmethylated cytosine residues:

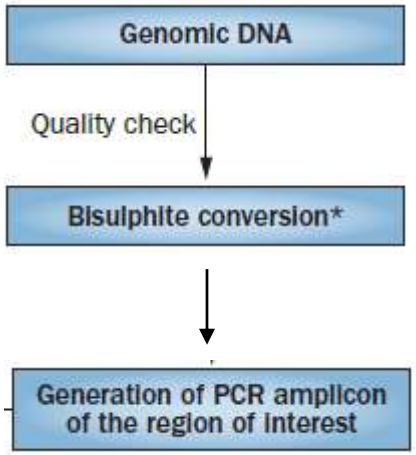


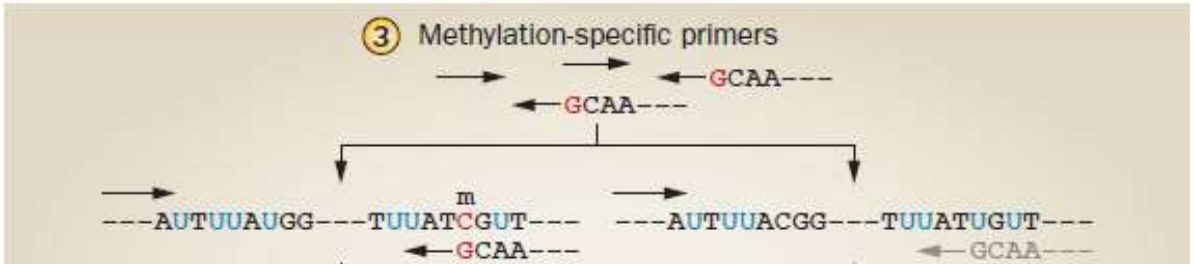


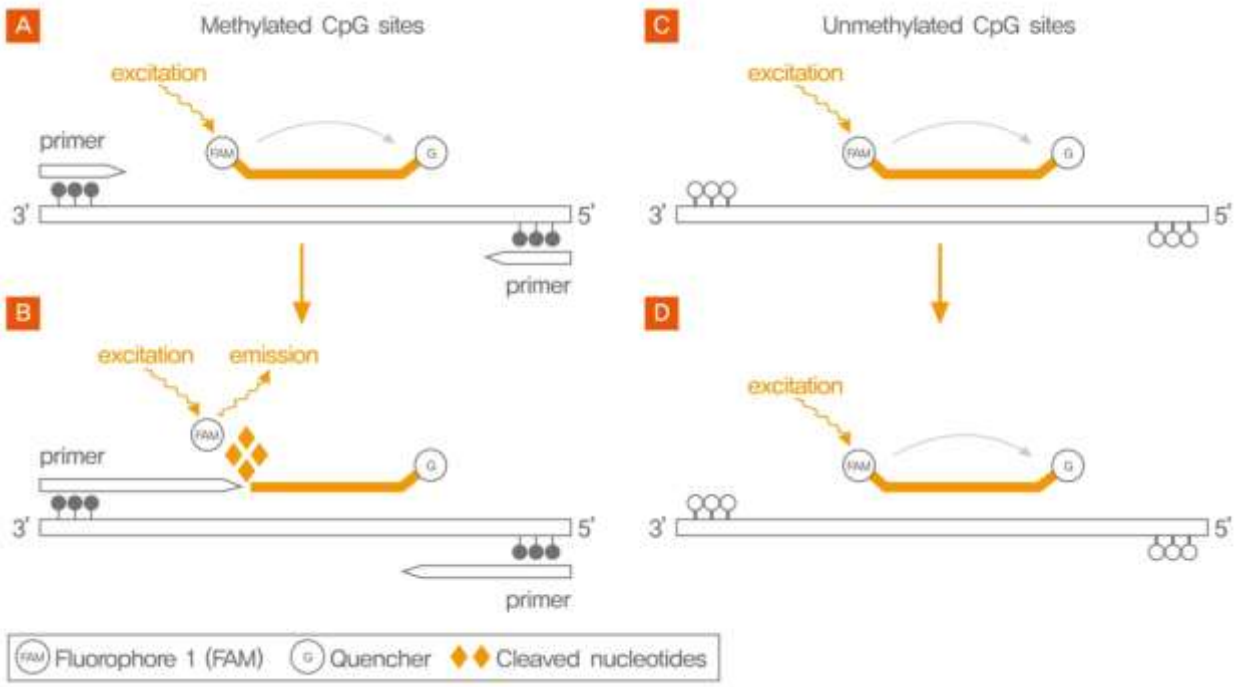




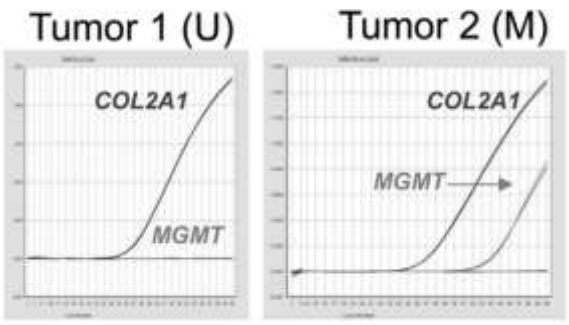
Quantitative Methylation Specific PCR (qMSP)





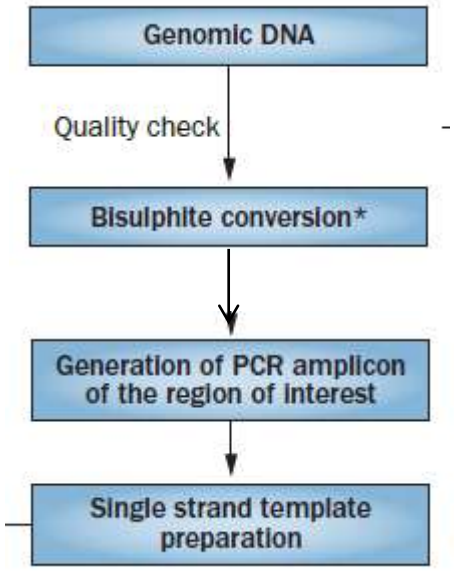


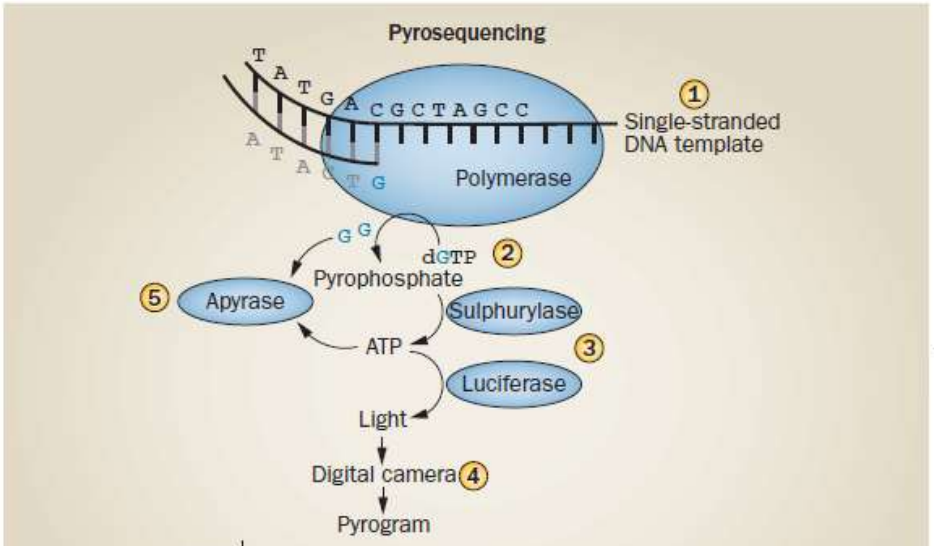
MethyLight PCR

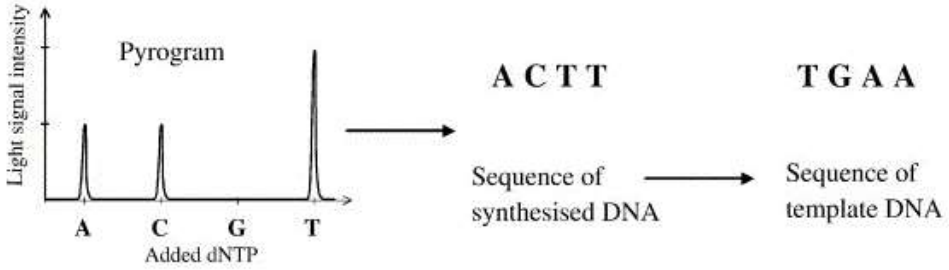


Cankovic et al., 2013

Pyrosequencing (PSQ)







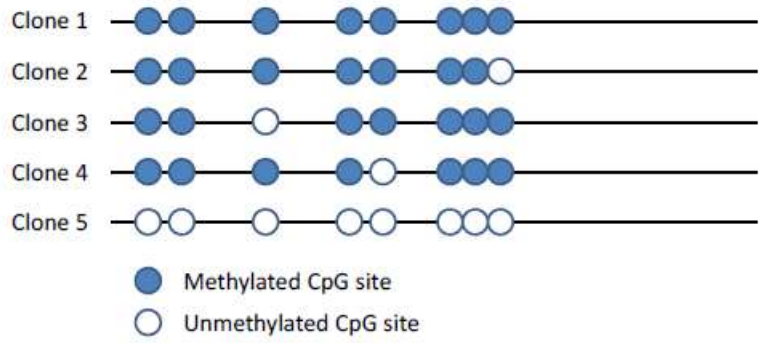
Comparison PSQ and qMSP:

	PSQ	qMSP
LOD	5%	2%
Specificity	98%	93%
Hands-on time	2 days	1 day
Costs	high	medium

350 gliomas (WHO grade I to IV) Analyzed by PSQ and qMSP:

Met.	Unmet.	Discordant
182 (52%)	152 (43%)	16 (5%)

Wang et al. Pathology 2017



Inconsistently methylated GBM cases are associated with poor OS

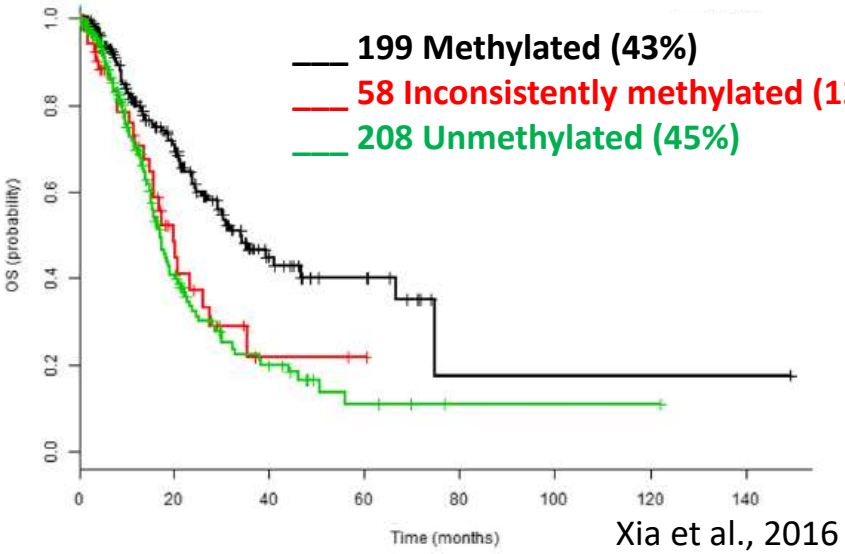
MGMT testing results :

	Méthylé	Méthylé (WHO)	Non méth.	NC/Mat ins.	Total
Astrocytome	**46% (12)**	**40-50%**	54% (14)	1	27
GBM	**41% (29)**	**35%**	59% (41)	4	74
Oligo	**45% (5)**	**60-80%**	55% (66)	2	12
Autre	**33% (4)**		67% (8)	0	37
Total	**38% (54)**		89 (62%)	7	150

	retested	total
2017	**12% (18)**	**100% (150)**
2018	**17% (18)**	**100% (106)**

- **5 to 37% of GBMs difficult to categorize**

465 GBM cases; 4 qMSP reactions per case:

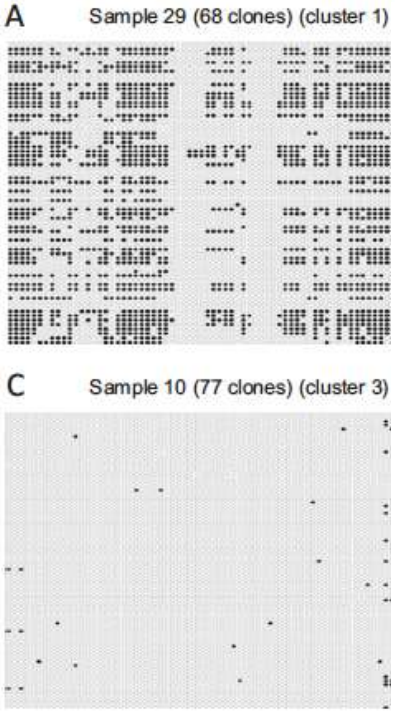


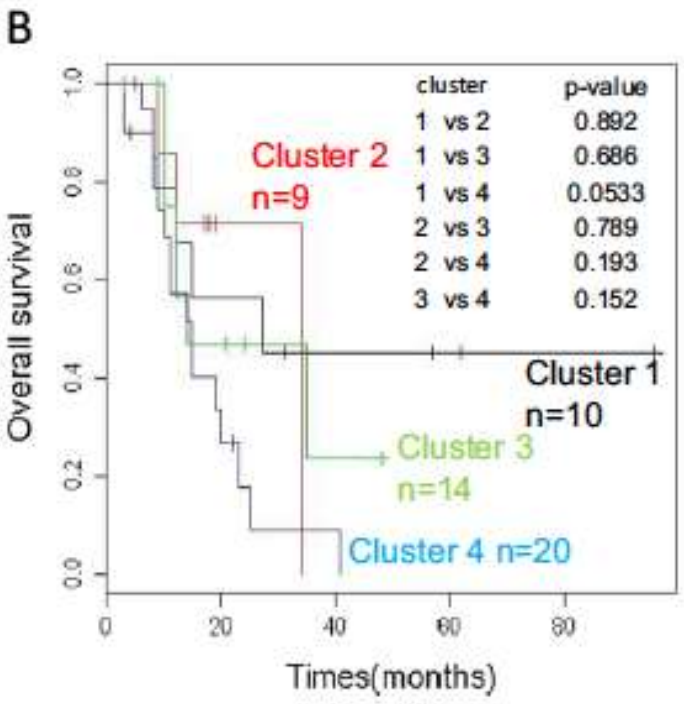
Xia et al., 2016

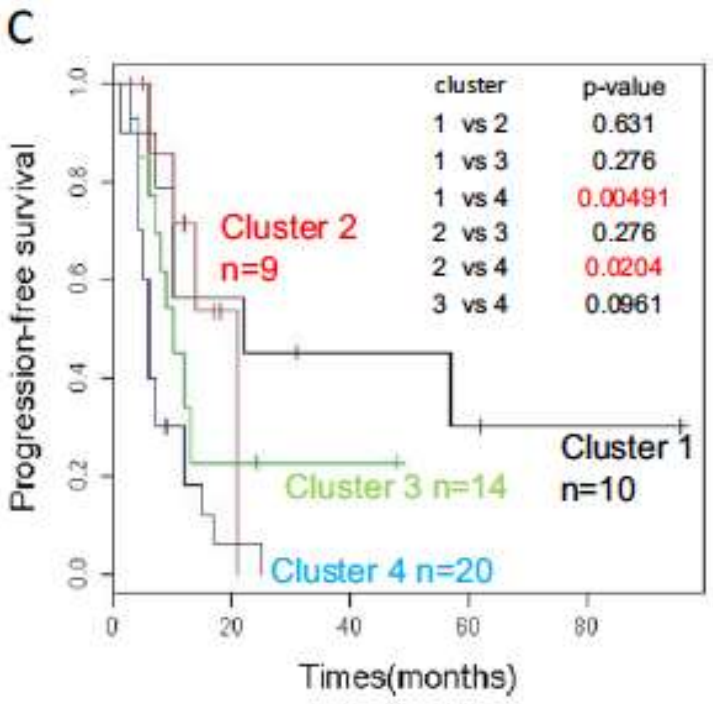
- No statistical difference between Un. and Incons.
- The Incons. forms an heterogenous group

Prognostic prediction of GBM using the entire MGMT promoter

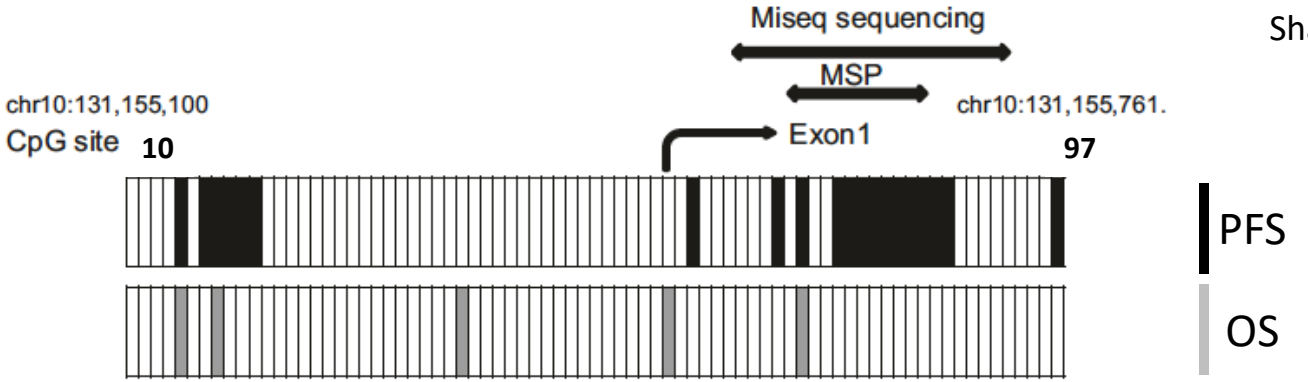
- Bisulfite conversion followed by NGS (MiSeq, Illumina)



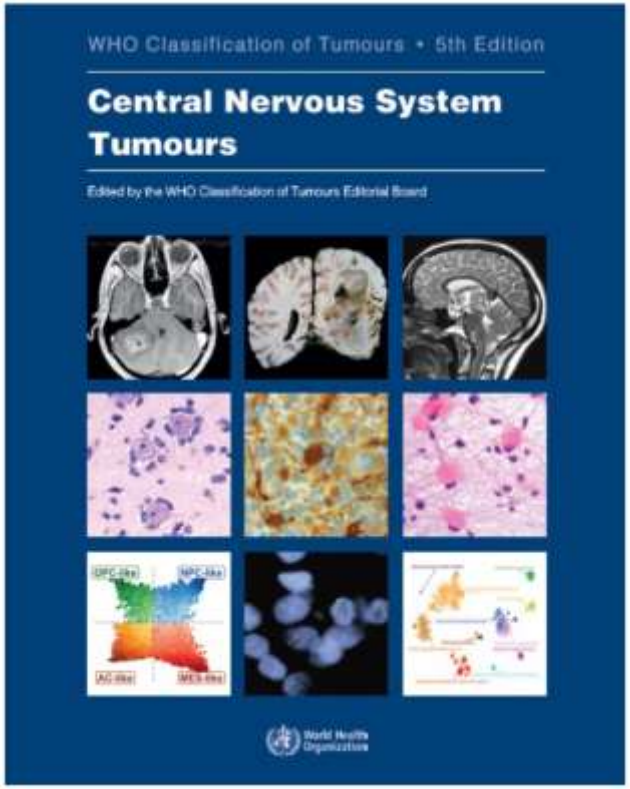


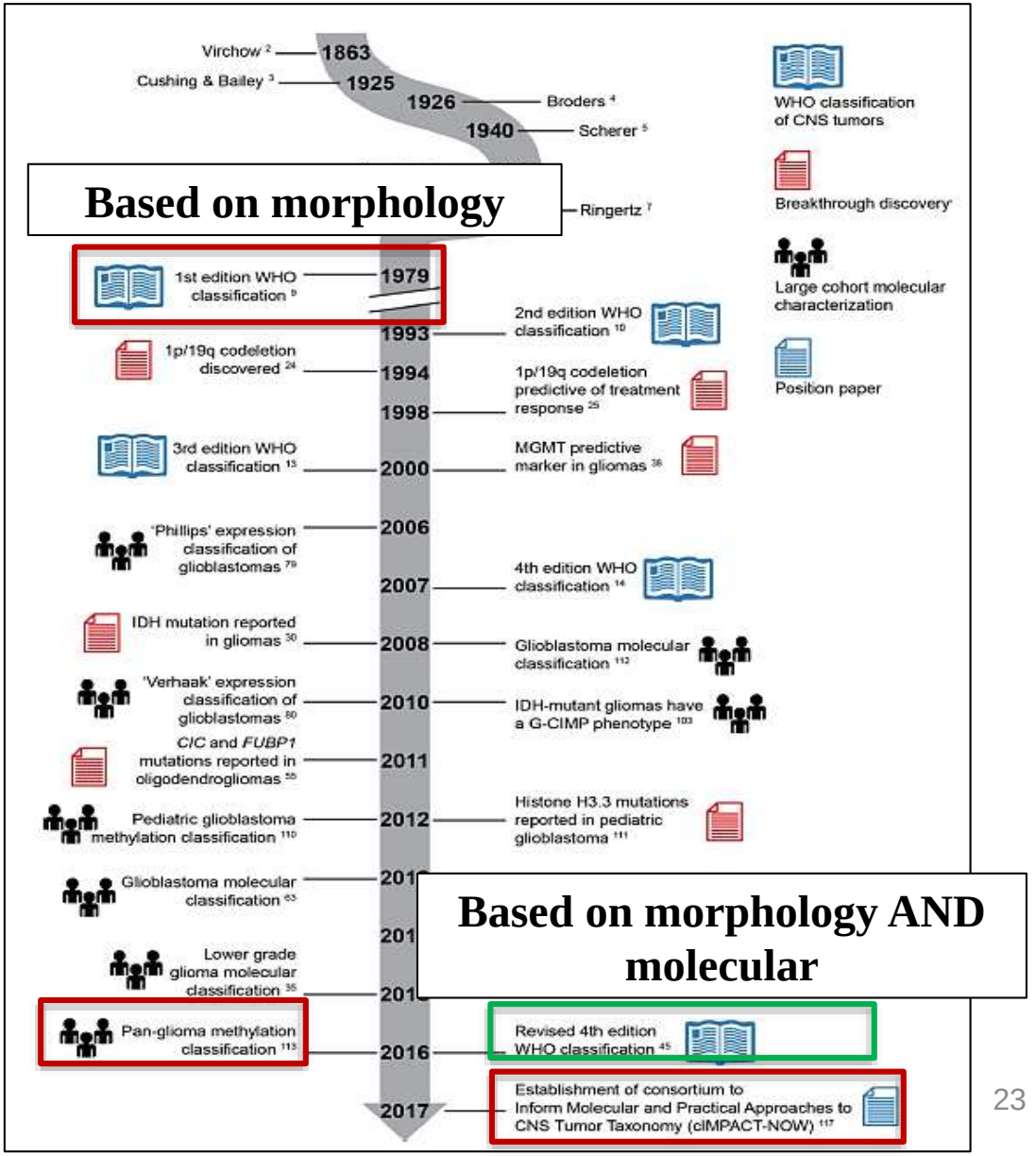


Kanemoto et al., 2014
Shah et al., 2011

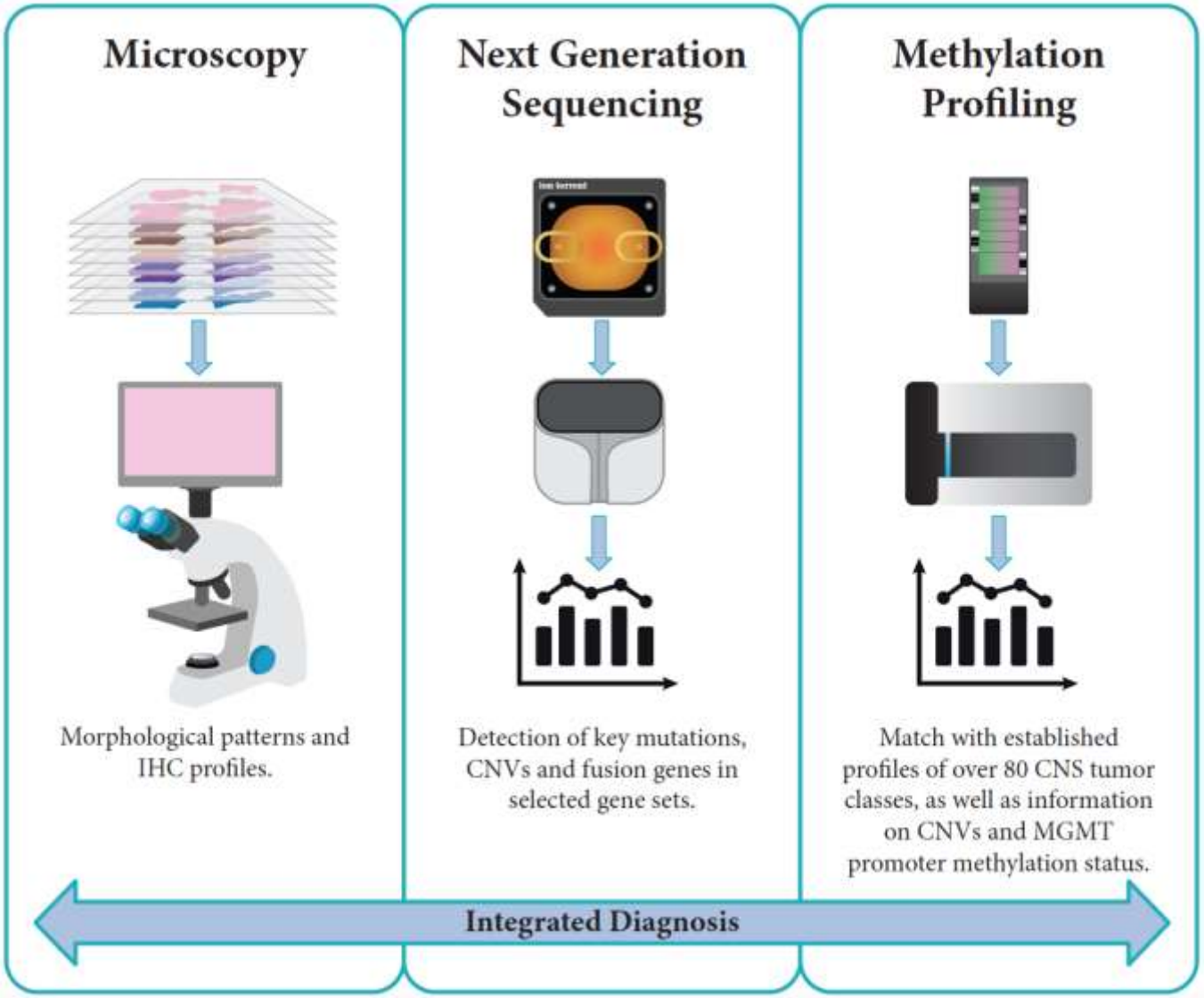


CNS TUMORS CLASSIFICATION



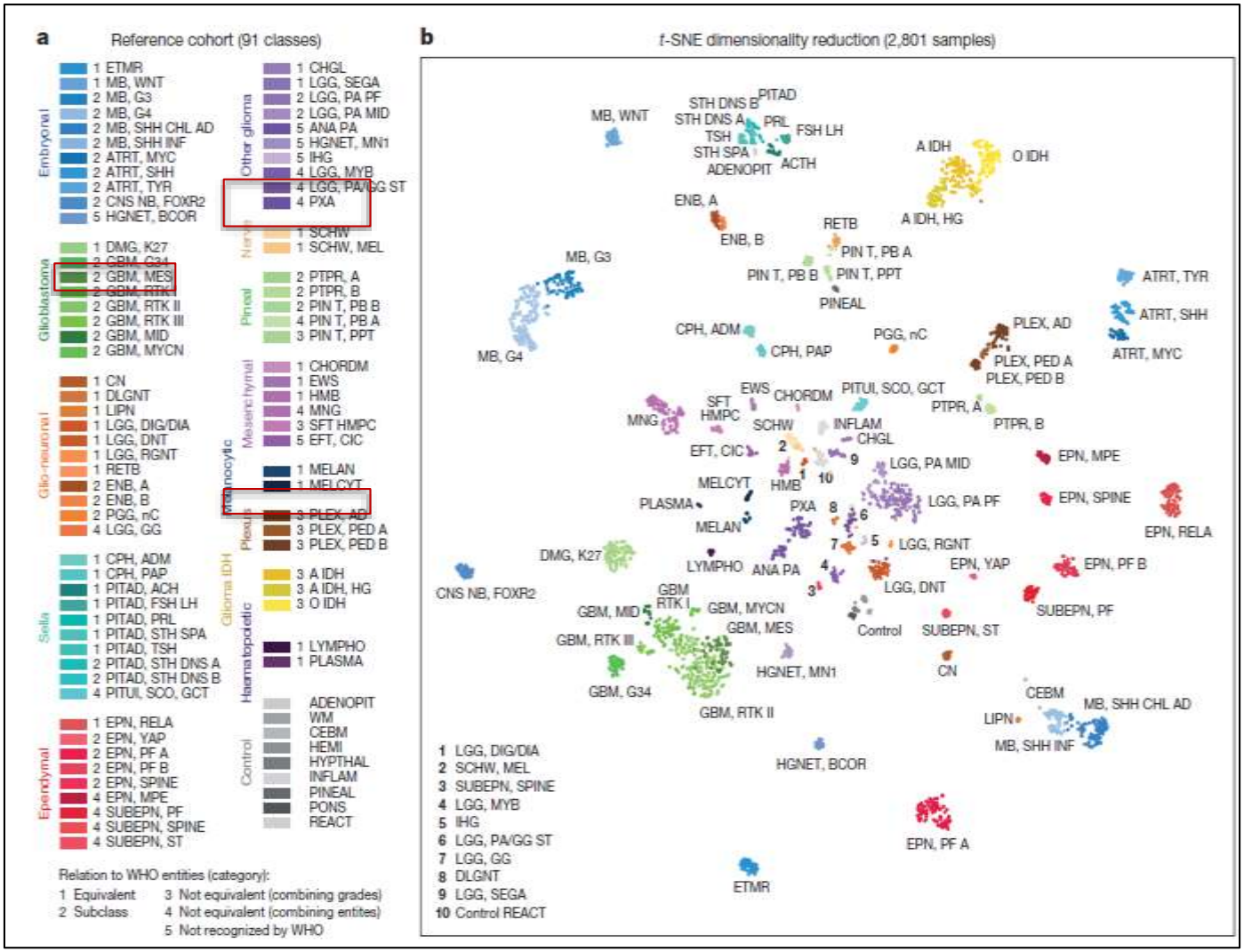


INTEGRATED DIAGNOSIS OF CNS TUMORS



Kristensen BW, et al., Ann Oncol. 2019 Aug 1;30(8):1265-1278.

DNA METHYLATION BASED CLASSIFICATION OF CNS TUMORS



82 CNS tumor classes, 9 Control classes (n=2801 samples)

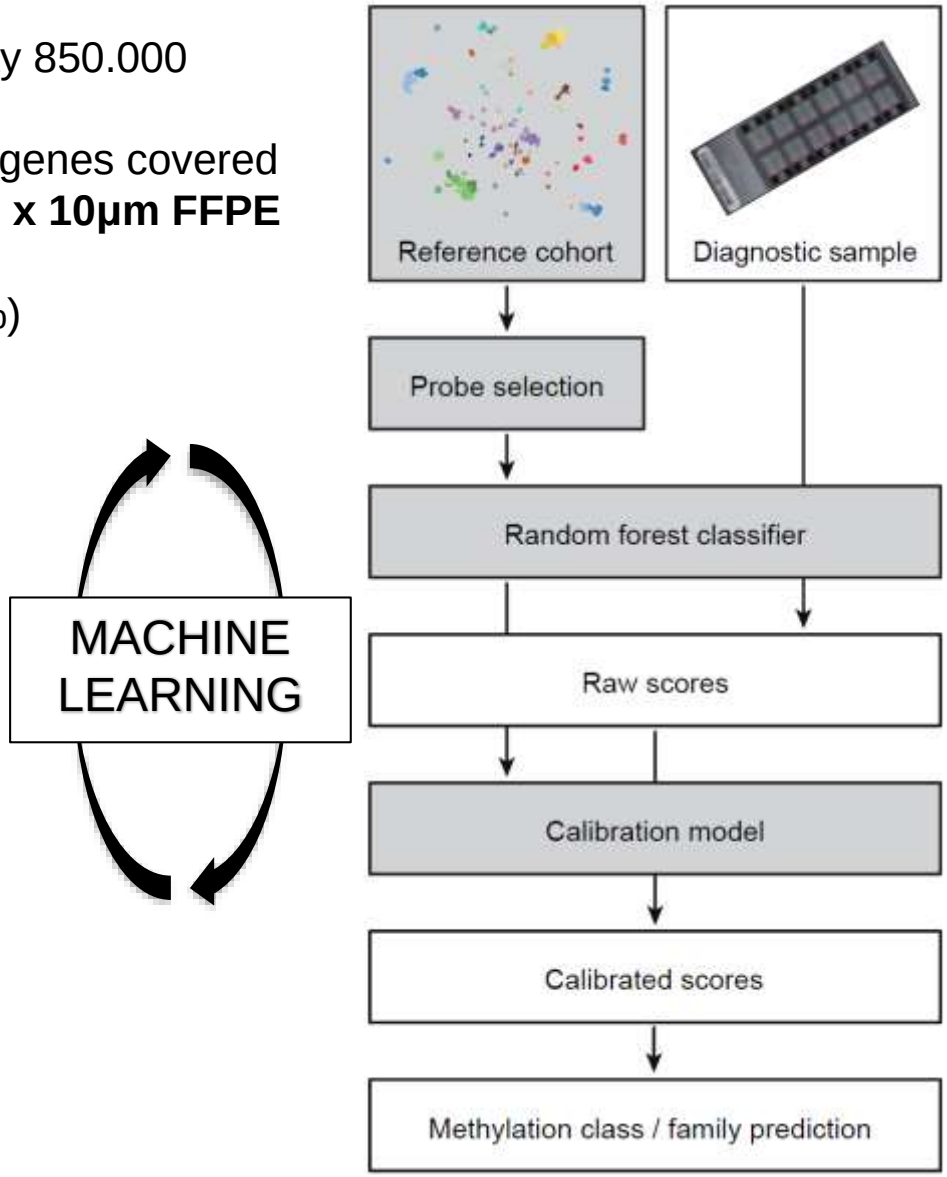
(1) 29 equivalent to the WHO 2016
(2) 29 subclasses of WHO entities
(3-4) 19 combined grades and entities
(5) 5 were not yet recognized by the WHO

Capper *et al.* Nature 2018
Capper *et al*. Acta Neuropathologica 2018

DNA METHYLATION BASED CLASSIFICATION OF CNS TUMORS

- Illumina 850k (EPIC) methylation array 850.000 individual CpG sites measured
- more than 99% of RefSeq-annotated genes covered
- **Little material** (**500 ngDNA form ~ 5 x 10µm FFPE tissue)**
- High tumor cell content required (70%)

- Training study
- Validation study
- Analytical sensitivity study



DNA METHYLATION BASED CLASSIFICATION OF CNS TUMORS

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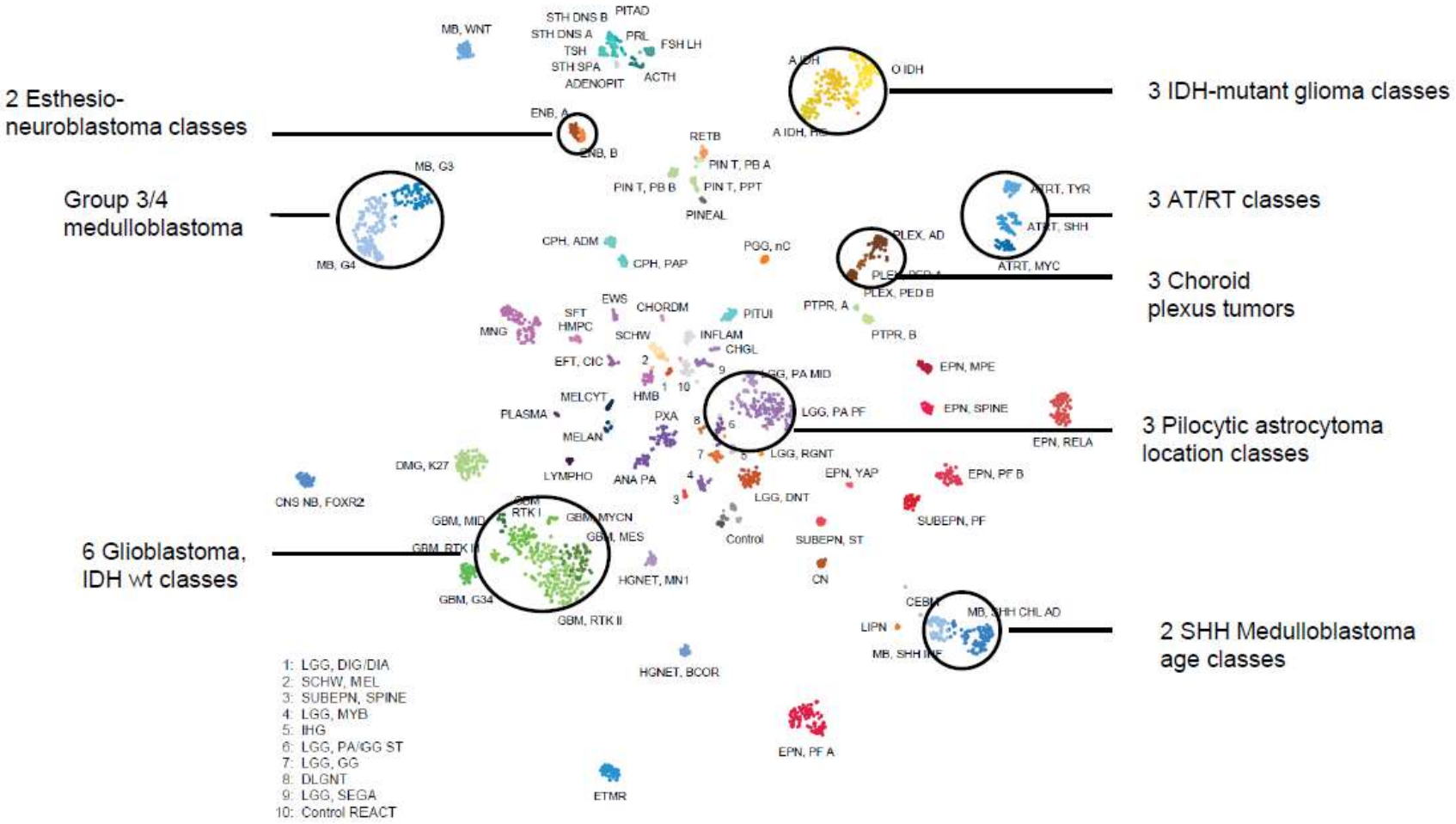
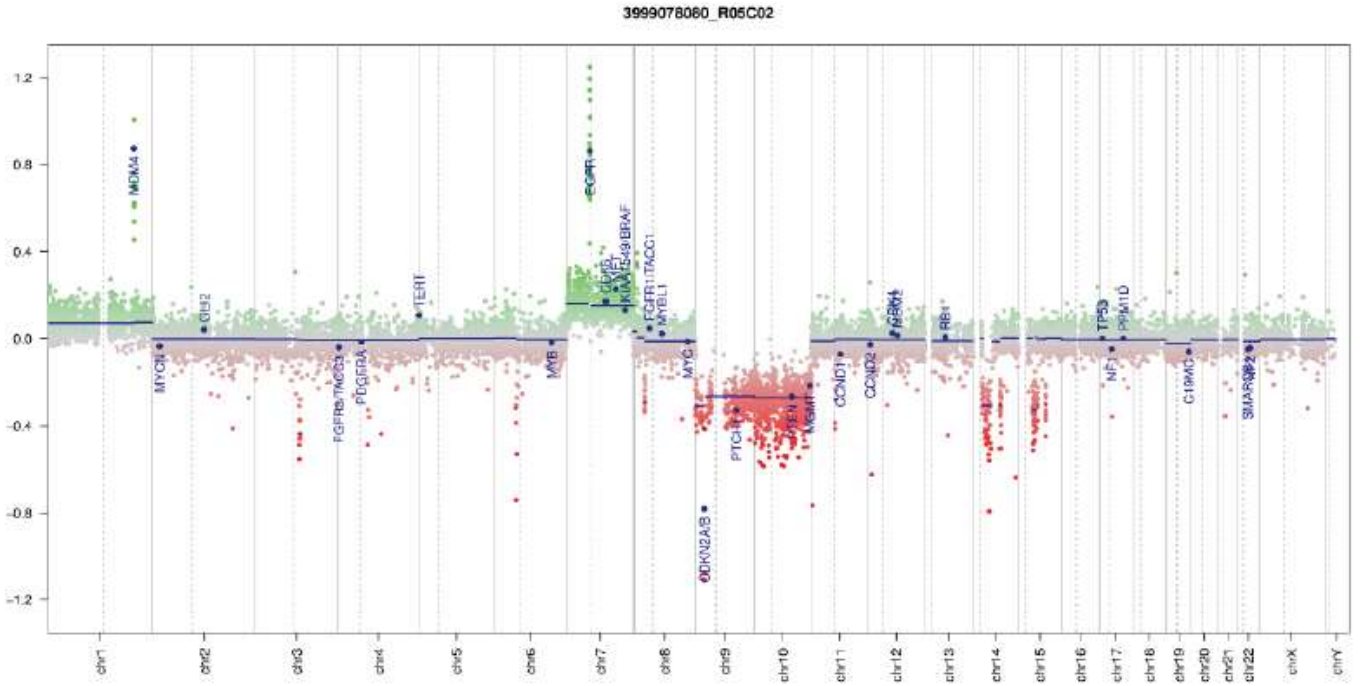
Within circles: closely related methylation classes



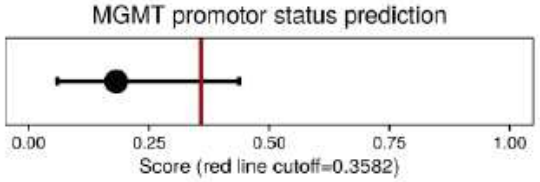
CNV AND MGMT PROMOTER METHYLATION

Copy number variation profile



Depiction of chromosome 1 to 22 (and X/Y if automatic prediction was successful). Gains/amplifications represent positive, losses negative deviations from the baseline. 29 brain tumor relevant gene regions are highlighted for easier assessment.
(see Hovestadt & Zapatka, http://www.bioconductor.org/packages/devel/bioc/html/conumee.html)

MGMT promotor methylation (MGMT-STP27)

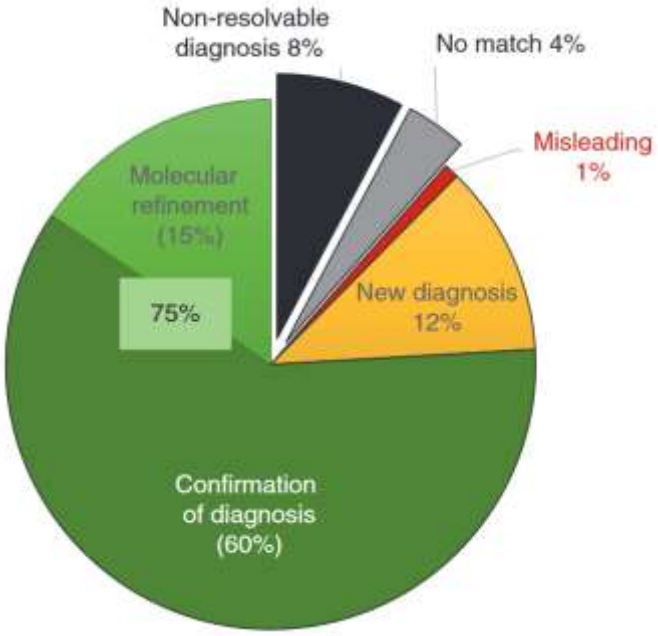


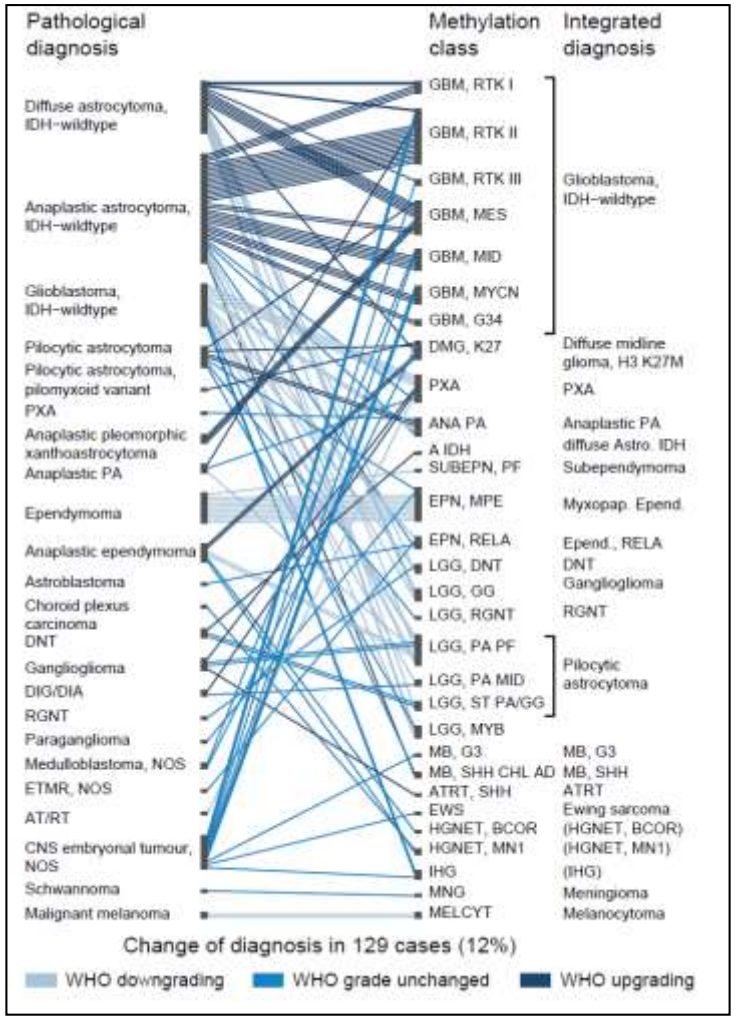
Status	Estimated	CI lower	CI upper
not determinable	0.18188	0.05985	0.43705

(see Bady et al, J Mol Diagn 2016; 18(3):350-61)

DNA METHYLATION BASED CLASSIFICATION OF CNS TUMORS

- **Capper *et al.* 2018** *:* Lead to the change of diagnosis for **12%** (129 out of 1155 cases)





Hegi *et al*. Neuro-oncology 2018
Capper *et al.* Nature 2018
Karimi et al. Clinical Epigenetics 2019

- **Karimi *et al.* 2019** : 15% of all those cases : change in the clinical decision-making for the patient

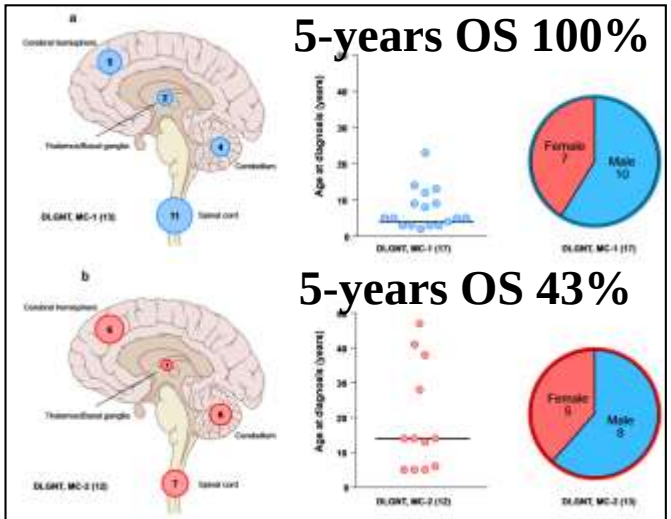
DNA METHYLATION BASED CLASSIFICATION OF CNS TUMORS

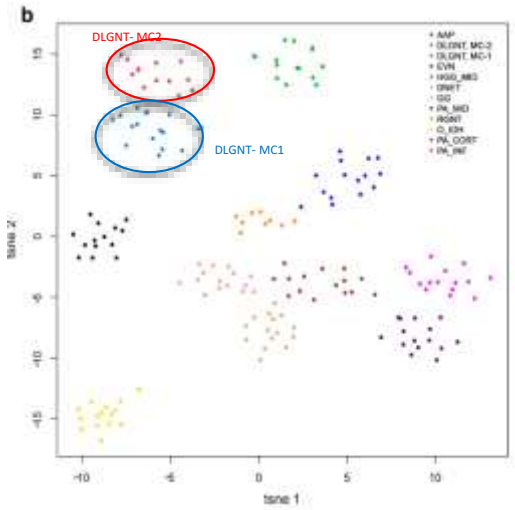
- In the 2021 WHO classification ➔ Essential criteria **for the diagnosis of 3 entities** !

Diagnostic criteria for **high-grade astrocytoma with piloid features**

Essential diagnostic criteria
Astrocytic glioma
AND
DNA methylation profile of HG astrocytoma with piloid features
Desirable diagnostic criteria
MAPK gene alteration
CDKN2A/B homozygous gene deletion or mutation or CDK4 amplification
ATRX mutation/loss of nuclear ATRX expression
Anaplastic histologic features

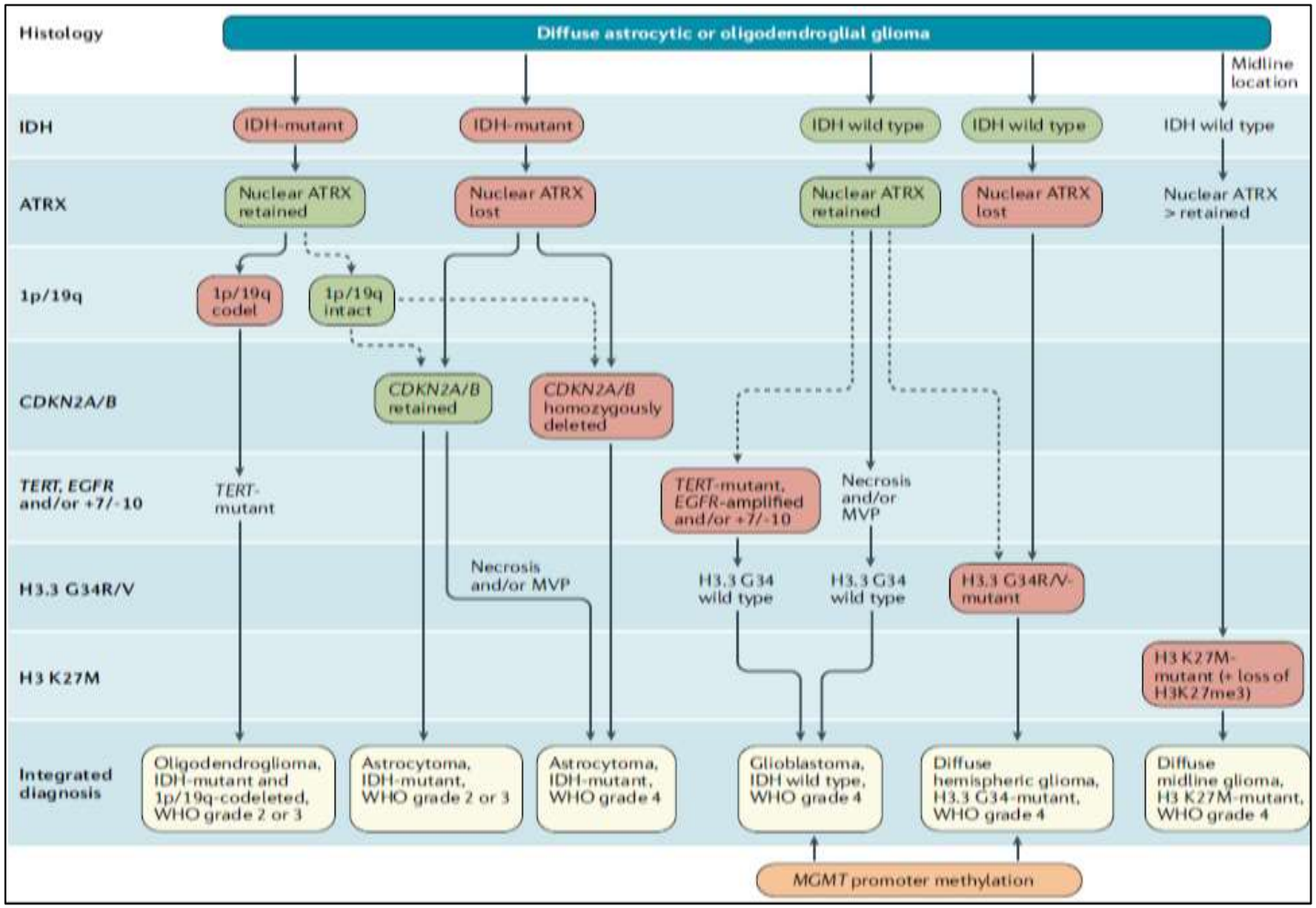
- **AND** Essential to refine prognosis in childhood tumors





THE HISTOMOLECULAR WHO 2021 CLASSIFICATION OF CNS TUMORS

- **Integrated histo-molecular classification of diffuse gliomas- 2020 EANO** (European Association of Neuro-Oncology) **guidelines- Adult-type tumors.**



Weller *et al.* Nature Reviews 2020

TAKE HOME MESSAGES

- **Epigenetics changes the pattern of gene expression**
- **Epigenetics does not alter the DNA sequence**
- **Three major mechanisms:**
  - **Histone modifications**
  - **DNA methylation**
  - **microRNAs**
- **The analysis of epigenetic marks can help with diagnosis:**
  - **Diffuse midline gliomas H3K27M mutants**
  - **Methylome analysis for CNS tumors**
  - ***MLH1* promoter methylation: sporadic colorectal cancer versus Lynch syndrome**
- **The analysis of epigenetic marks can help to guide therapy:**
  - ***MGMT* promoter methylation is a biomarker of the response to the alkylating chemotherapy**