



Memorial Sloan-Kettering  
Cancer Center



# Epigenetics in glioma

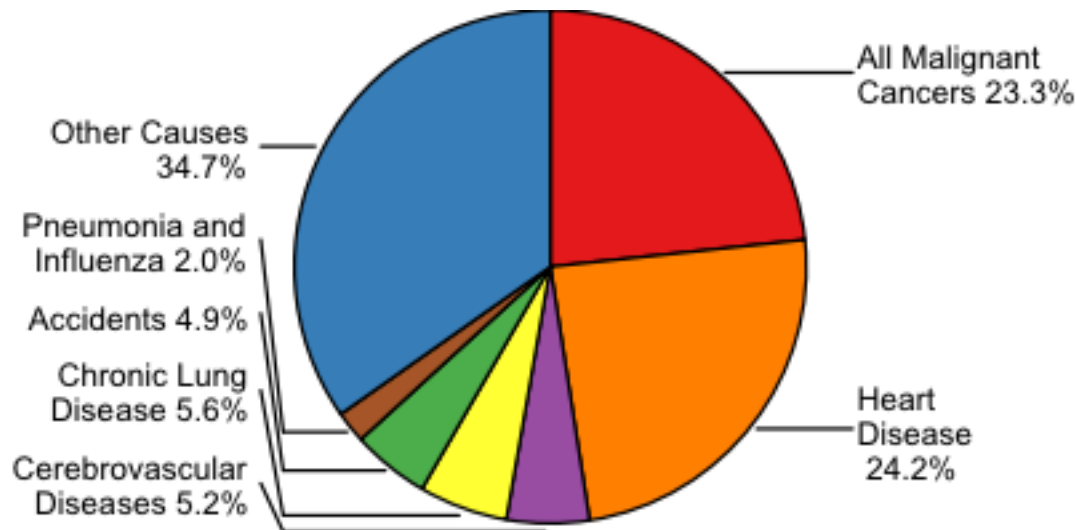
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## MGMT, ABCB1 and ABCG2 methylation in glioma

Moritz C. Oberstadt, PhD

## Tumor diseases

- One of the leading death causes



US Mortality Files, National Center for Health Statistics, Centers for Disease control and Prevention

- High malignant tumors with low 5 years overall survival (OS):

- Brain tumors (13%/15%)

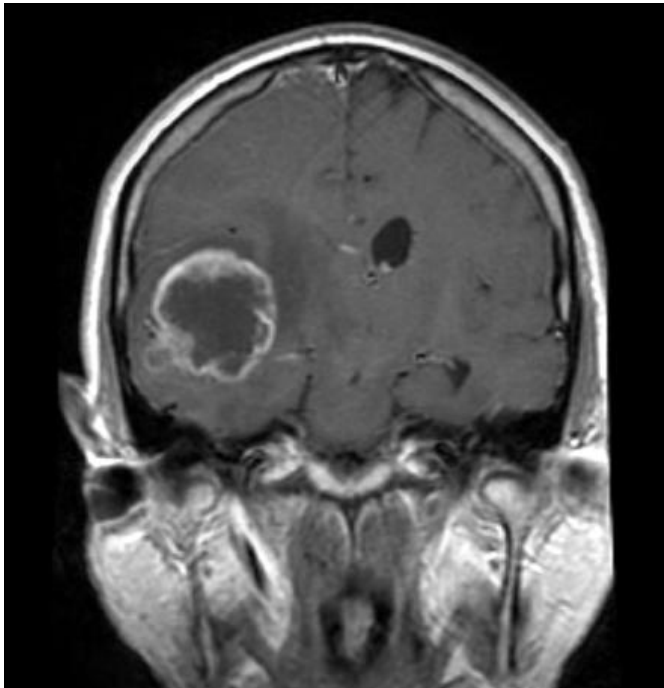
- Stomach- (12%/13%) and Oesophaguscarcinoma (7%/8%)

- Lung carcinoma (6%/6%)

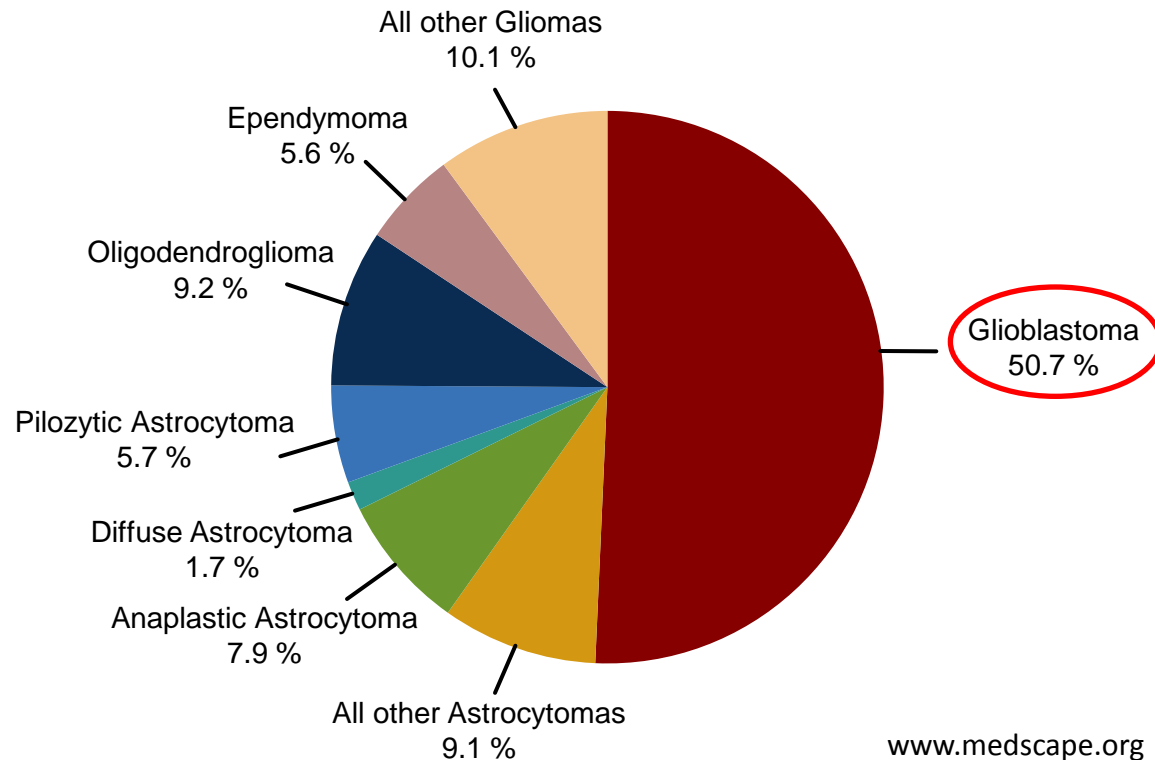
- Pancreas carcinoma (3%/2%)

# Glioblastoma multiforme

- Glioblastoma multiforme is the most frequent and aggressive primary brain tumor in adults
- Referring to WHO classification of brain tumors: Grade IV



[www.radiopaedia.org](http://www.radiopaedia.org)

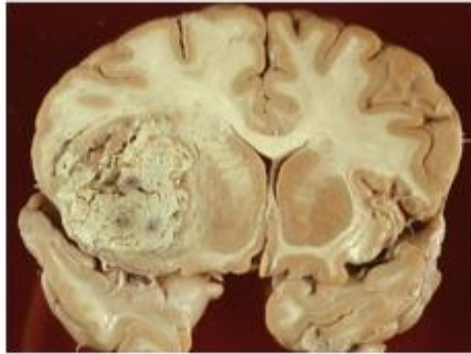


[www.medscape.org](http://www.medscape.org)

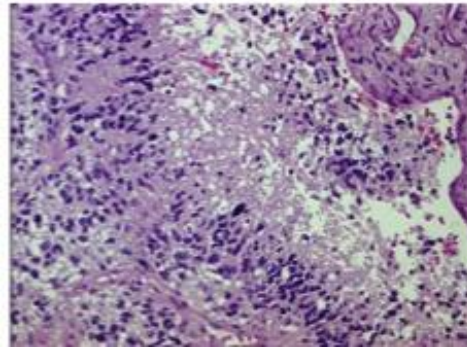


# Glioblastoma multiforme

- Markers: High cellular proliferation rate, diffuse infiltration, necrosis, angiogenesis, apoptosis resistance and genomic instability.



D.P. Agamanolis



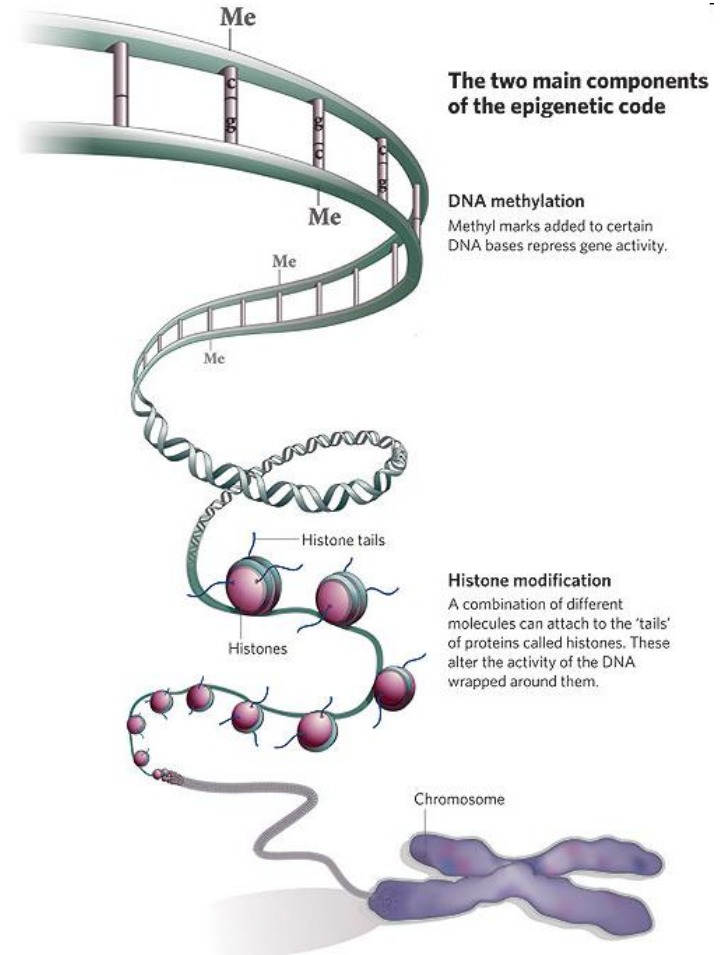
PD Dr. Vogelgesang, Greifswald

- Necrotic centers typically surrounded by hypercellular zones: pseudopallisades

# Epigenetic mechanisms

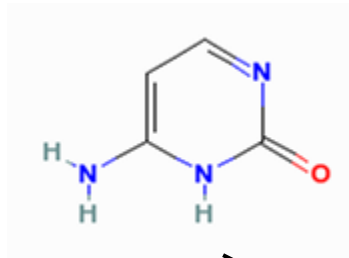
- DNA-Methylation

- Histone modification

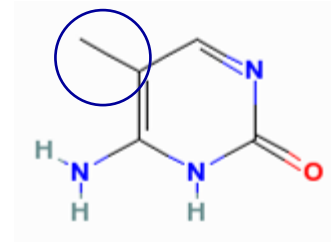


# DNA methylation

**Cytosine**



**Methyl-Cytosine**



***DNA-Methyl-  
transferase***

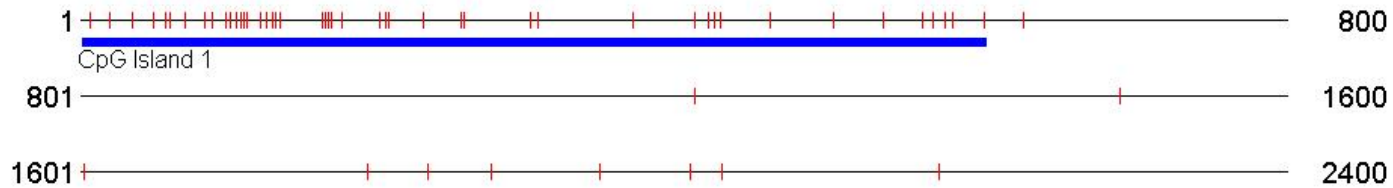
**S-Adenosylmethionine  
(SAM)**

**S-Adenosylhomocysteine  
(SAH)**

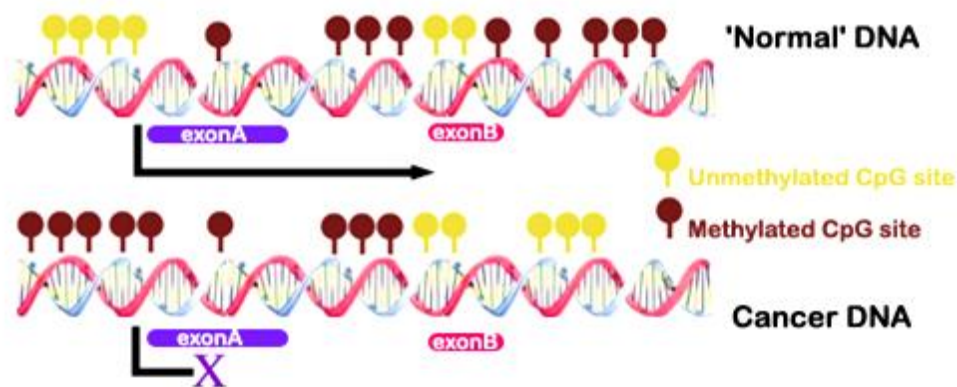
**Vitamin B12, Folate, Vitamin B6**

# DNA methylation

- Clusters of CpG sites: CpG islands

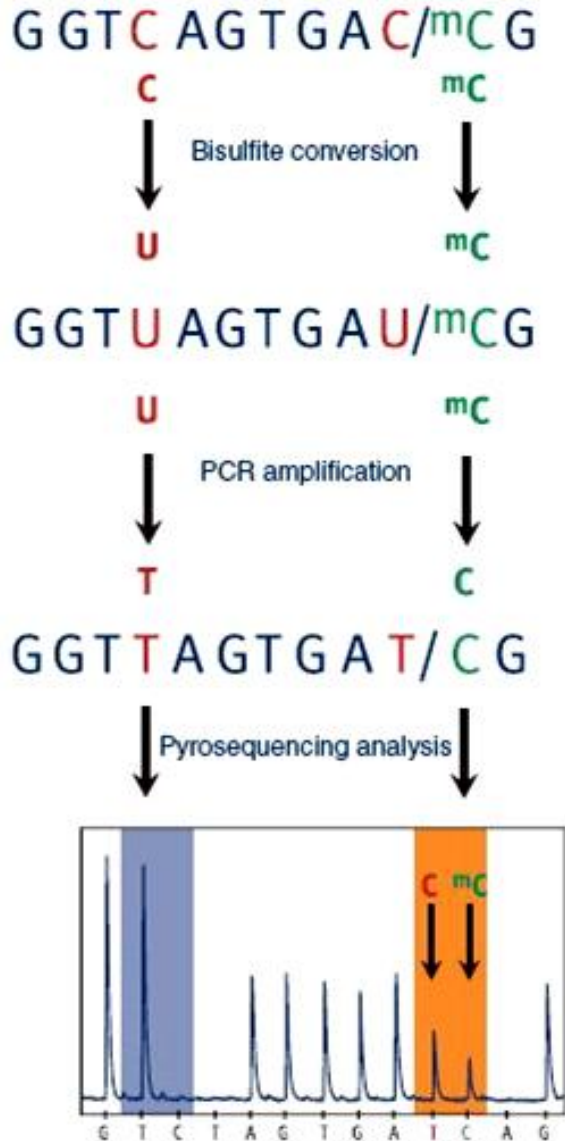


- CpG islands in promoters of about 60% of all human genes
- Loss of methylation throughout the genome in cancer cells

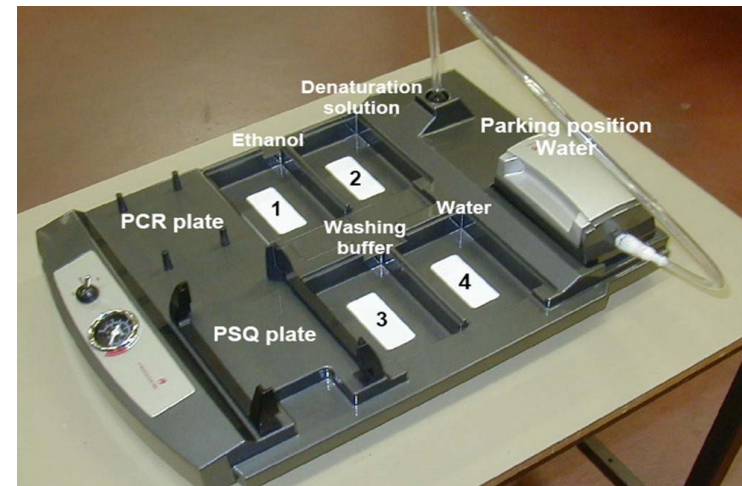




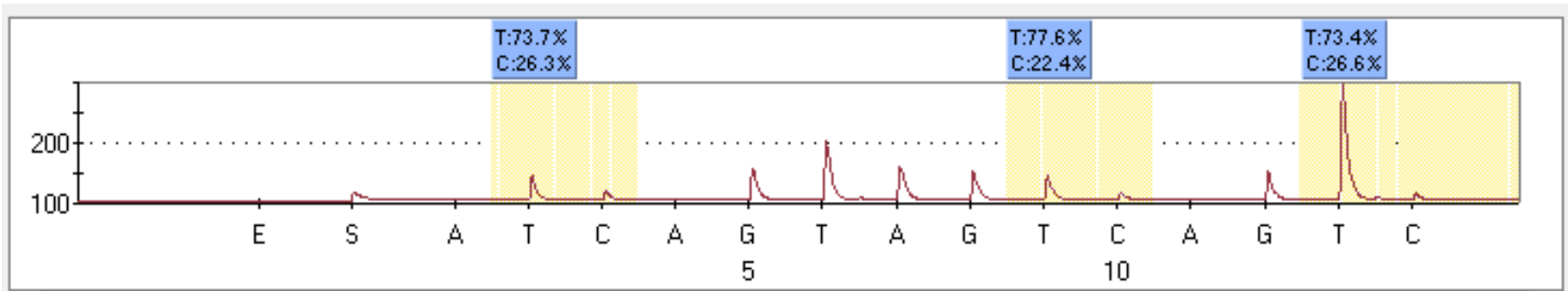
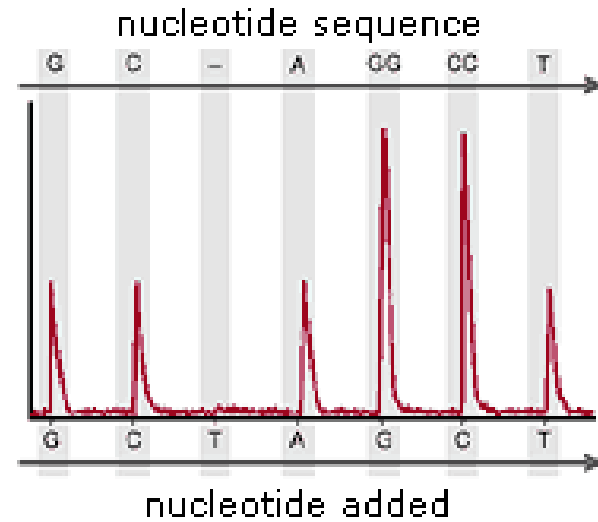
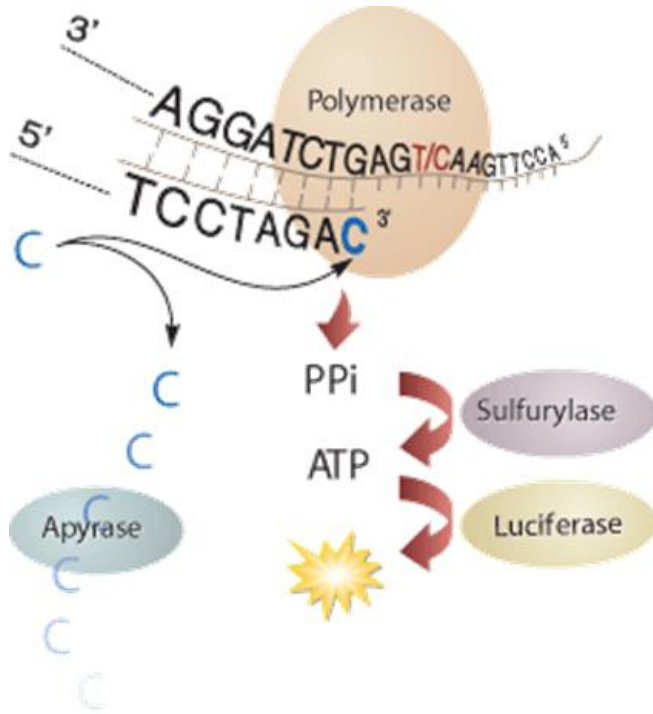
# Analysis of methylation Pyrosequencing



Pyrosequencing after  
Bisulfite treatment

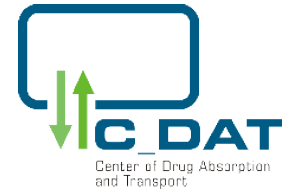


# Analysis of methylation Pyrosequencing





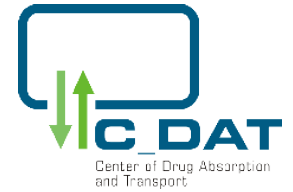
# Genes to analyze



- MGMT (O6 methylguanine methyltransferase)
- ABCB1 (P-gp)
- ABCG2 (BCRP)



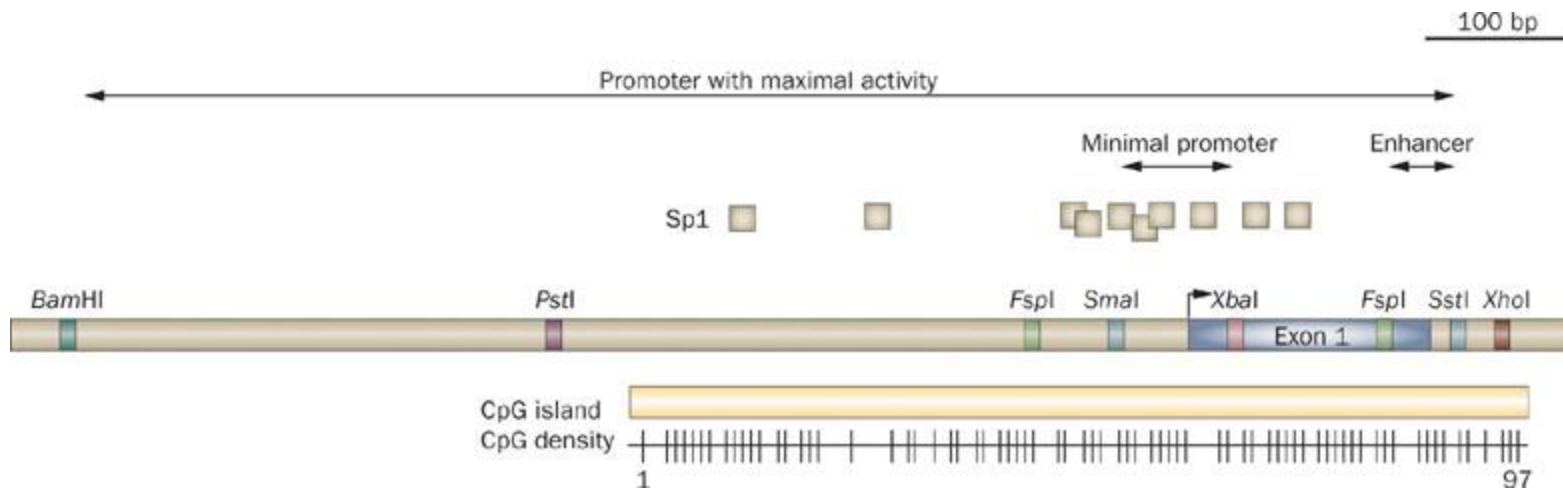
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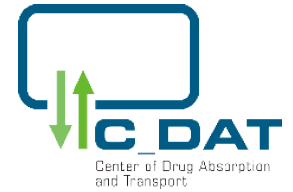
# MGMT

- DNA repair enzyme, removing mutagenic adducts from the O6 position of guanine
- MGMT causes resistance to alkylating drugs
- Survival of patients with gliomas is significantly better in previous publications, if the promoter of MGMT is methylated



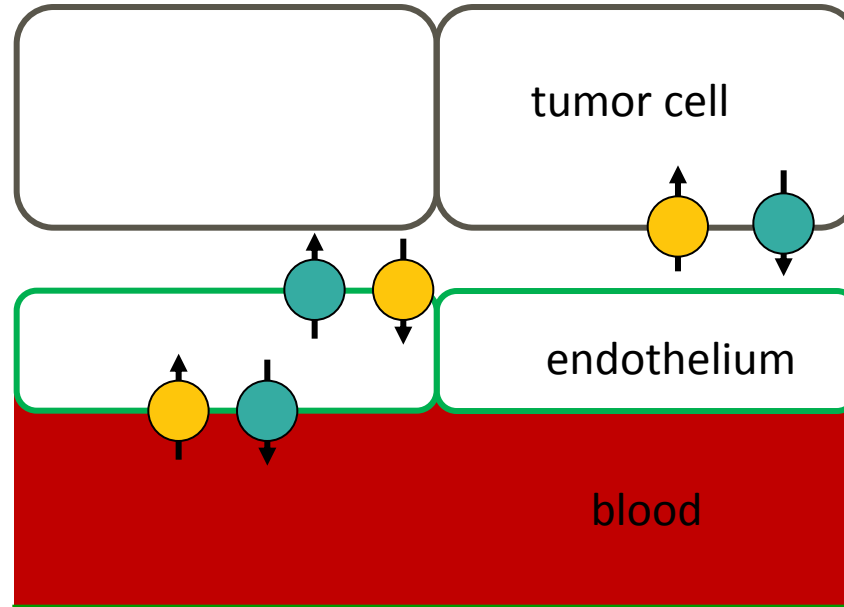


# Genes to analyze



- MGMT (O6 methylguanine methyltransferase)
- ABCB1 (P-gp)
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# Transport proteins



Effluxtransporter, e.g. ABCB1,  
ABCG2



Influxtransporter, e.g. OATP2B1



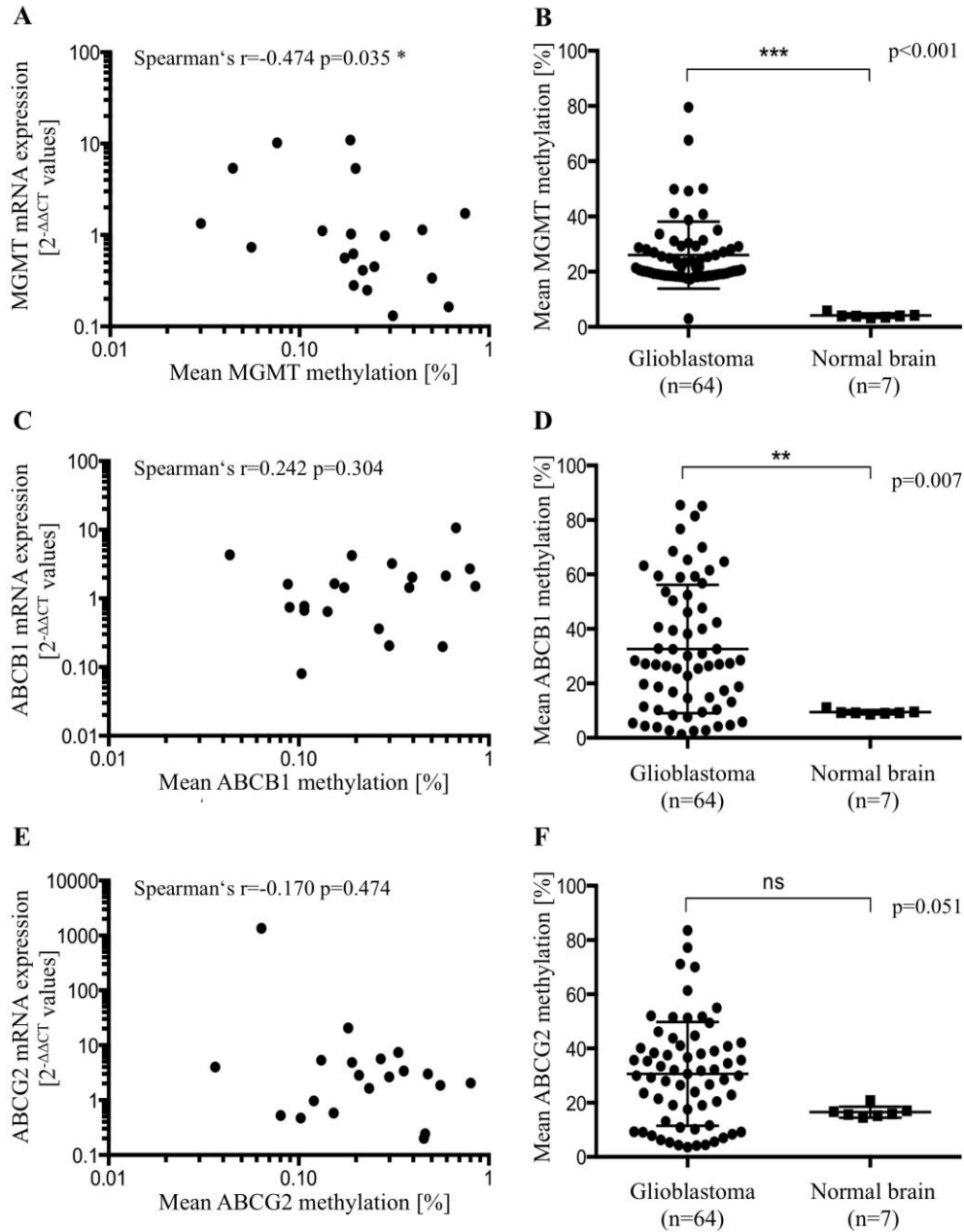
# Clinical characteristics



Characteristic	Age [Years]	
Median age at diagnosis	61.6	
Range [Min.-Max.]	40.2 - 79.9	
Patients with temozolomide therapy		
Median age at diagnosis	59.2	
Patients without temozolomide therapy		
Median age at diagnosis	64.0	
Characteristic	Number of patients	% of patients
Age classes		
<50 years	11	17.2
50 - 60 years	18	28.1
60 - 70 years	20	31.3
>70 years	15	23.4
Sex		
Male	39	60.9
Female	25	39.1
Pathohistology		
Glioblastoma multiforme	64	
Relapses of primary glioblastoma multiforme	17	
Therapy		
Only Radiotherapy	11	17.2
Radiotherapy and temozolomide	45	70.3
No adjuvant therapy	6	9.4
No therapy data applicable	2	3.1
Overall survival (OS)		
Median [Days]	459	
Range [Min.-Max.]	34 - 1954	
1-year survival	38	59.4
2-year survival	9	14.1
OS of patients with temozolomide therapy		
Median [Days]	515	
Range [Min.-Max.]	95 - 1954	
OS of patients without temozolomide therapy		
Median [Days]	87	
Range [Min.-Max.]	34 - 701	
Vital status at study end (30.06.2009)		
Dead	47	73.4
Alive	17	26.6



# Methylation and expression





# MGMT methylation and OS



Variable	Haz. Ratio	p-value	[95% Conf. Interval]	
Sex				
male (ref. female)	1.488 1.259 1.724	0.238 0.602 0.393	0.769 0.530 0.494	2.876 2.992 6.024
Age				
50-<60 years (ref. <50 years)	1.734 1.648 1.183	0.299 0.394 0.916	0.613 0.523 0.053	4.903 5.192 26.577
Age				
60-<70 years (ref. <50 years)	2.567 3.242 1.417	0.057 0.039 0.757	0.972 1.061 0.156	6.780 9.901 12.826
Age				
≥70 years (ref. <50 years)	6.427 10.700 2.442	0.001 0.000 0.445	2.194 2.998 0.247	18.824 38.191 24.152
Mean methylation level (continuous)	0.988 0.975 1.023	0.315 0.121 0.403	0.964 0.945 0.970	1.012 1.007 1.078



# ABCB1 methylation and OS



Variable	Haz. Ratio	p-value	[95% Conf. Interval]	
Sex				
male	1.457	0.276	0.740	2.866
(ref. female)	1.130	0.793	0.454	2.813
	4.222	0.043	1.045	17.060
Age				
50-<60 years	1.793	0.282	0.619	5.191
(ref. <50 years)	1.500	0.490	0.474	4.742
	5.358	0.234	0.338	84.863
Age				
60-<70 years	2.474	0.066	0.942	6.499
(ref. <50 years)	2.235	0.140	0.768	6.507
	3.596	0.290	0.336	38.441
Age				
≥70 years	6.069	0.001	2.107	17.479
(ref. <50 years)	9.872	0.000	2.786	34.988
	5.112	0.167	0.505	51.721
Mean methylation level (continuous)	0.995	0.461	0.981	1.009
	1.002	0.864	0.984	1.020
	0.973	0.032	0.950	0.998

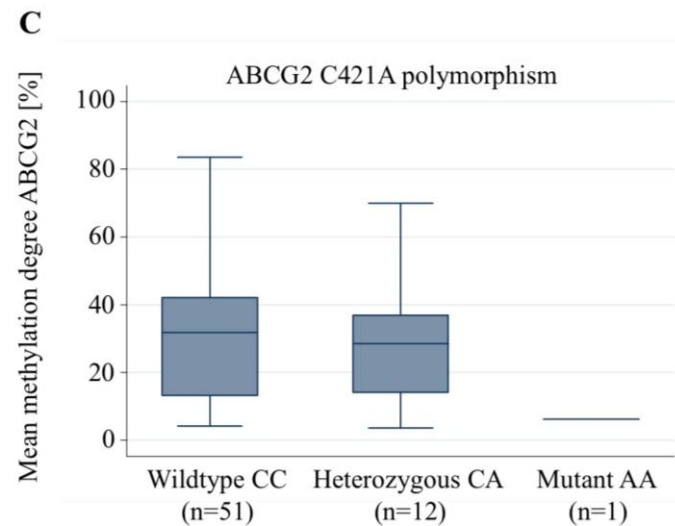
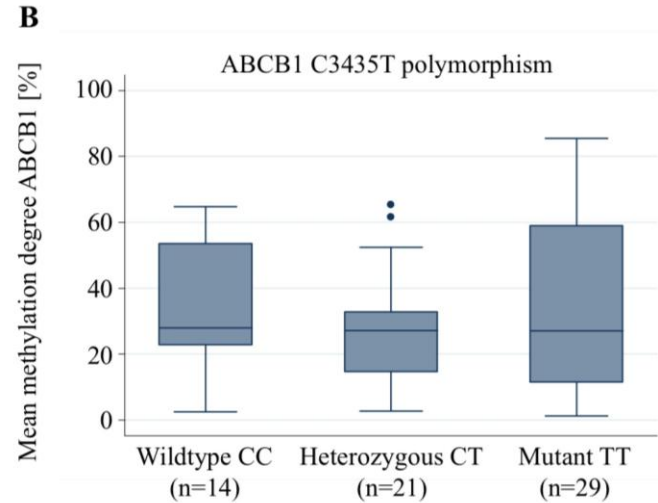
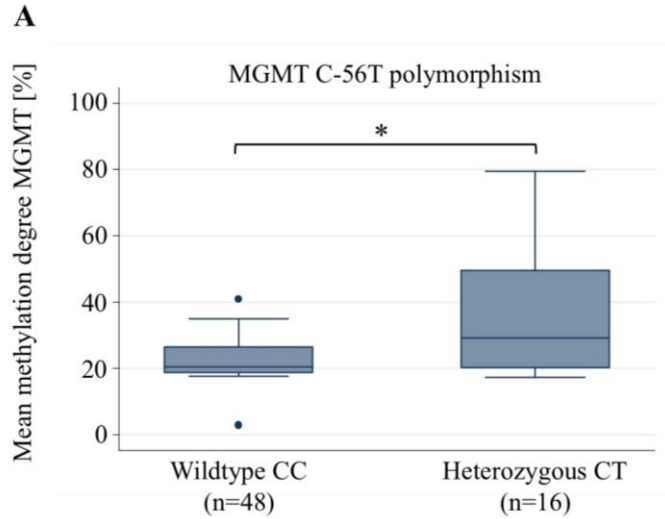


# ABCG2 methylation and OS



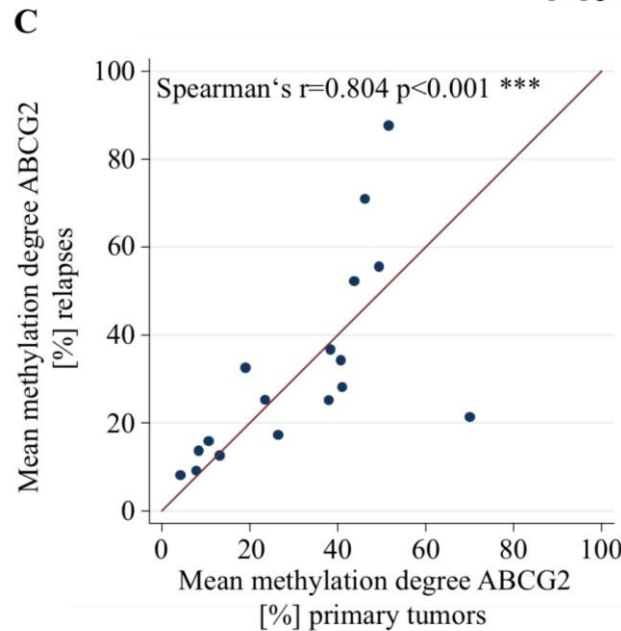
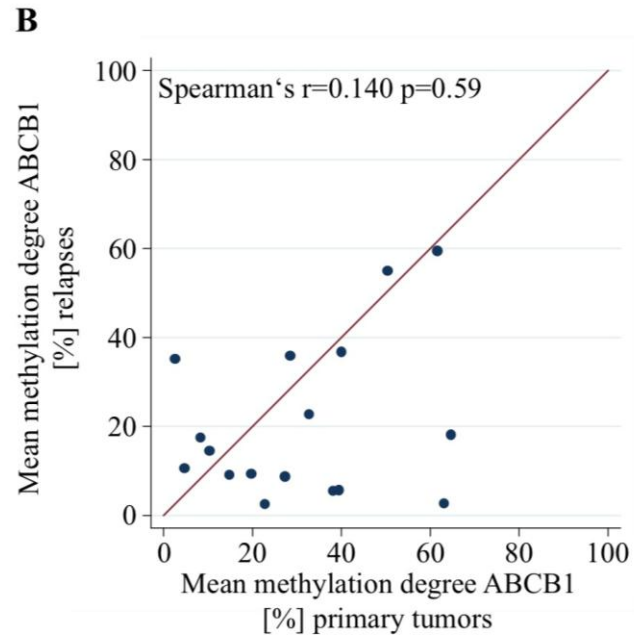
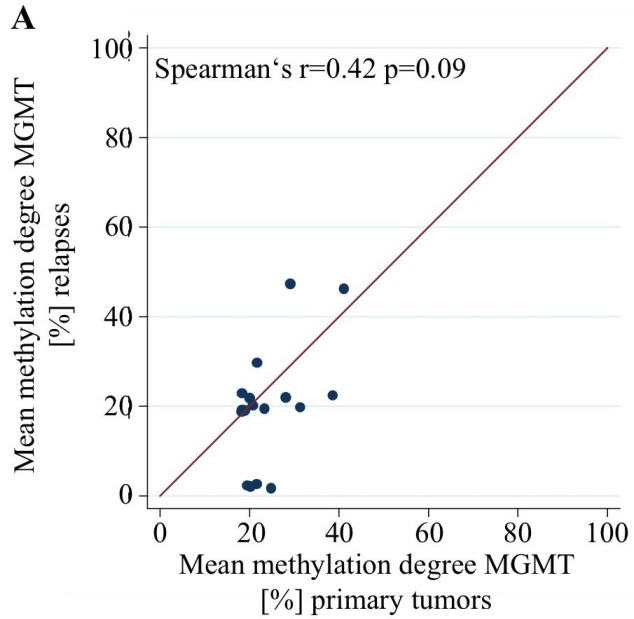
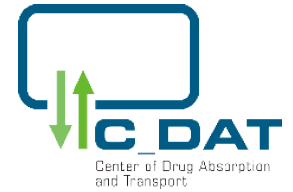
Variable	Haz. Ratio	p-value	[95% Conf. Interval]	
Sex male (ref. female)	1.463 1.092 2.489	0.271 0.842 0.211	0.743 0.459 0.596	2.879 2.600 10.389
Age 50-<60 years (ref. <50 years)	1.745 1.454 1.552	0.317 0.545 0.760	0.586 0.433 0.092	5.195 4.882 26.033
Age 60-<70 years (ref. <50 years)	2.270 2.271 1.068	0.085 0.118 0.957	0.892 0.811 0.102	5.778 6.358 11.210
Age ≥70 years (ref. <50 years)	6.112 9.923 2.774	0.001 0.000 0.376	2.087 2.808 0.290	17.903 35.062 26.572
Mean methylation level (Continuous)	1.003 0.998 1.018	0.736 0.836 0.430	0.986 0.977 0.974	1.021 1.019 1.065

# Methylation and Polymorphisms



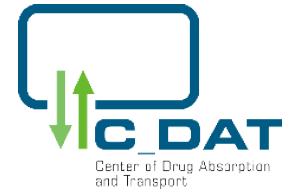


# Methylation in relapses



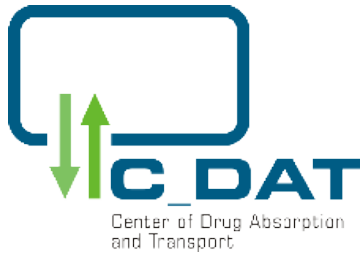


## Conclusions



- Methylation of MGMT, ABCB1 and ABCG2 have no prognostic impact for OS in glioblastoma multiforme
- Significant negative correlation between MGMT methylation and expression
- Markedly elevated MGMT and ABCB1 methylation in glioblastoma specimens
- Significant correlation between MGMT methylation and MGMT C-56T polymorphism
- Significant correlation of ABCG2 methylation in primary tumors and relapses

# Acknowledgements



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Cancer Center

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