

# Biomarkers of Brain Tumors to Temozolomide Treatment

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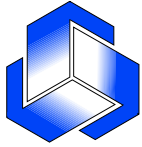
&

« NCCR Molecular Oncology », ISREC

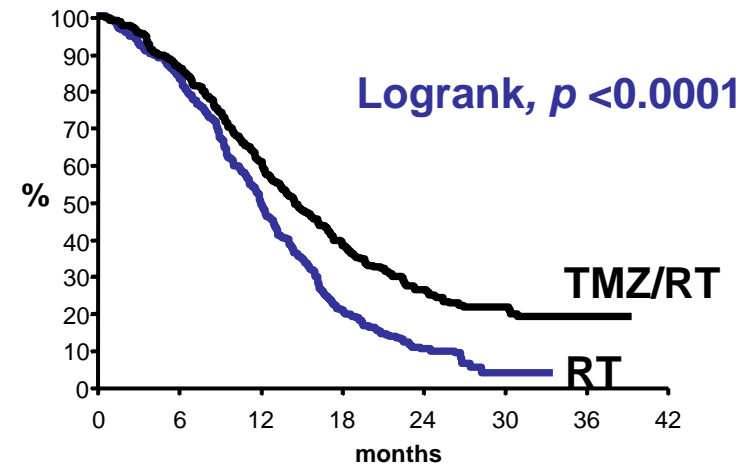
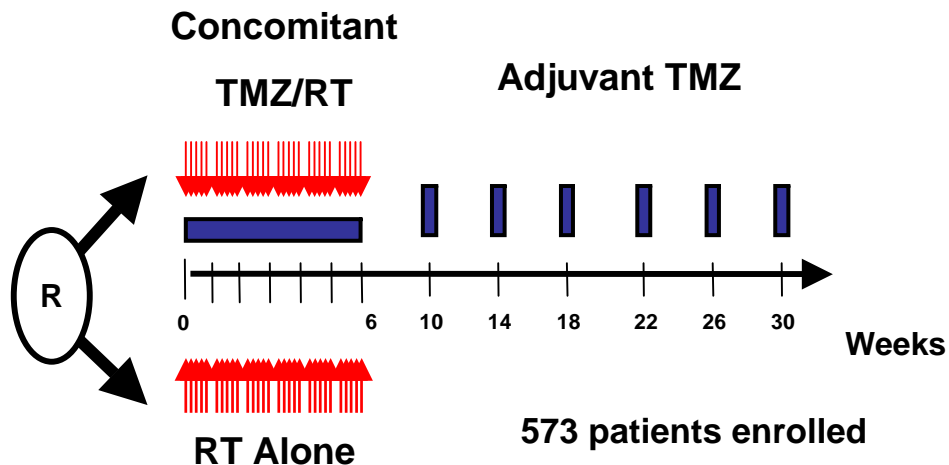


ENASCO Meeting, November 15-17, 2007





# Predictive Factor(s) for Temozolomide (TMZ) Derived Benefit for Glioblastoma Patients



**Who are the patients who benefit from TMZ ?**

	RT	TMZ/RT
Median OS, mo:	12.1	14.6
2-yr survival:	10%	26%
HR [95% CI]:	0.63 [0.52-0.75]	

# O<sup>6</sup>-Methylguanine-DNA Methyltransferase (MGMT)

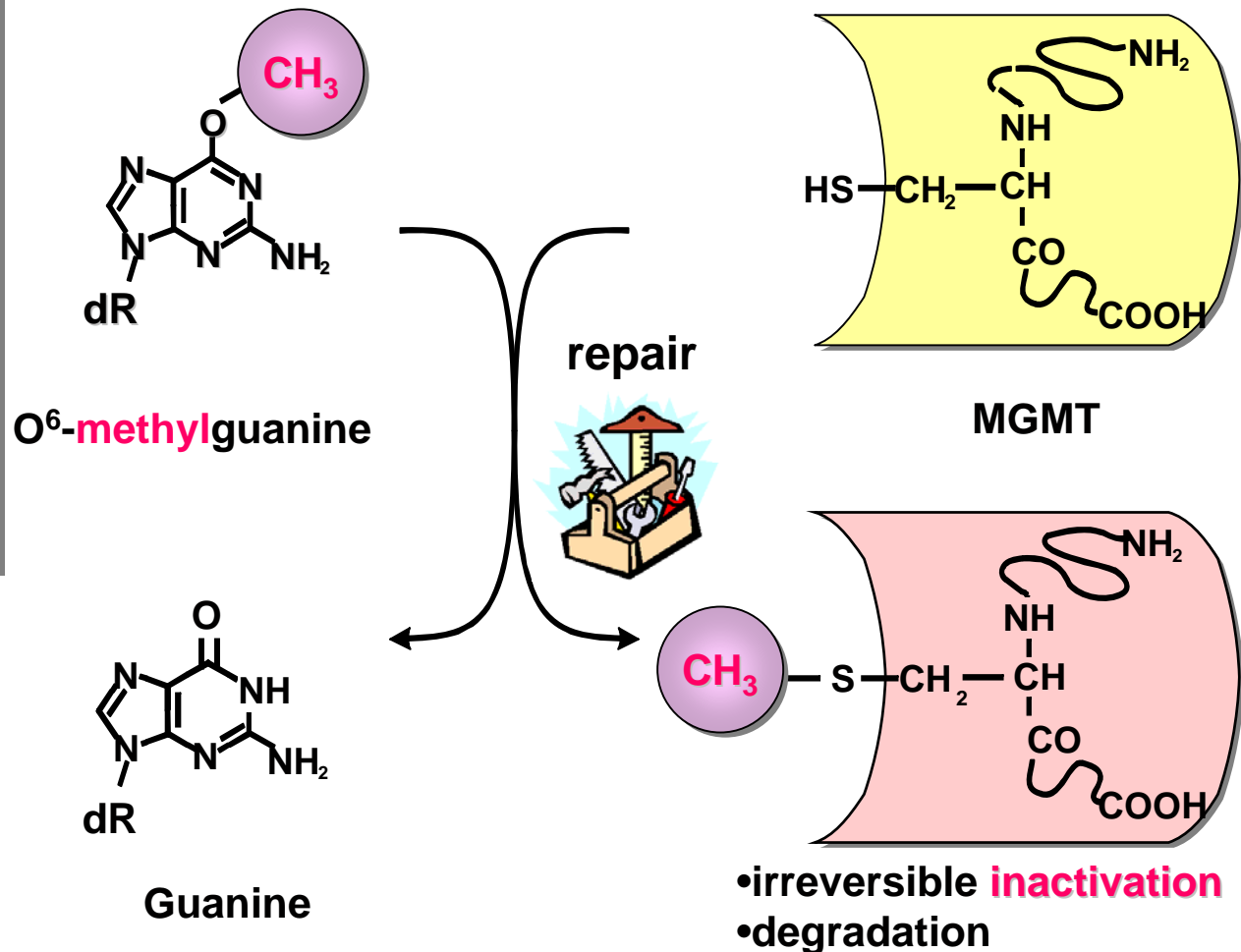
## •TMZ, alkylating agent

### **MGMT**

- CHR 10q26
- linked with resistance to alkylating agent therapy
- inducible :
  - RT
  - genotoxic agents
  - glucocorticosteroids

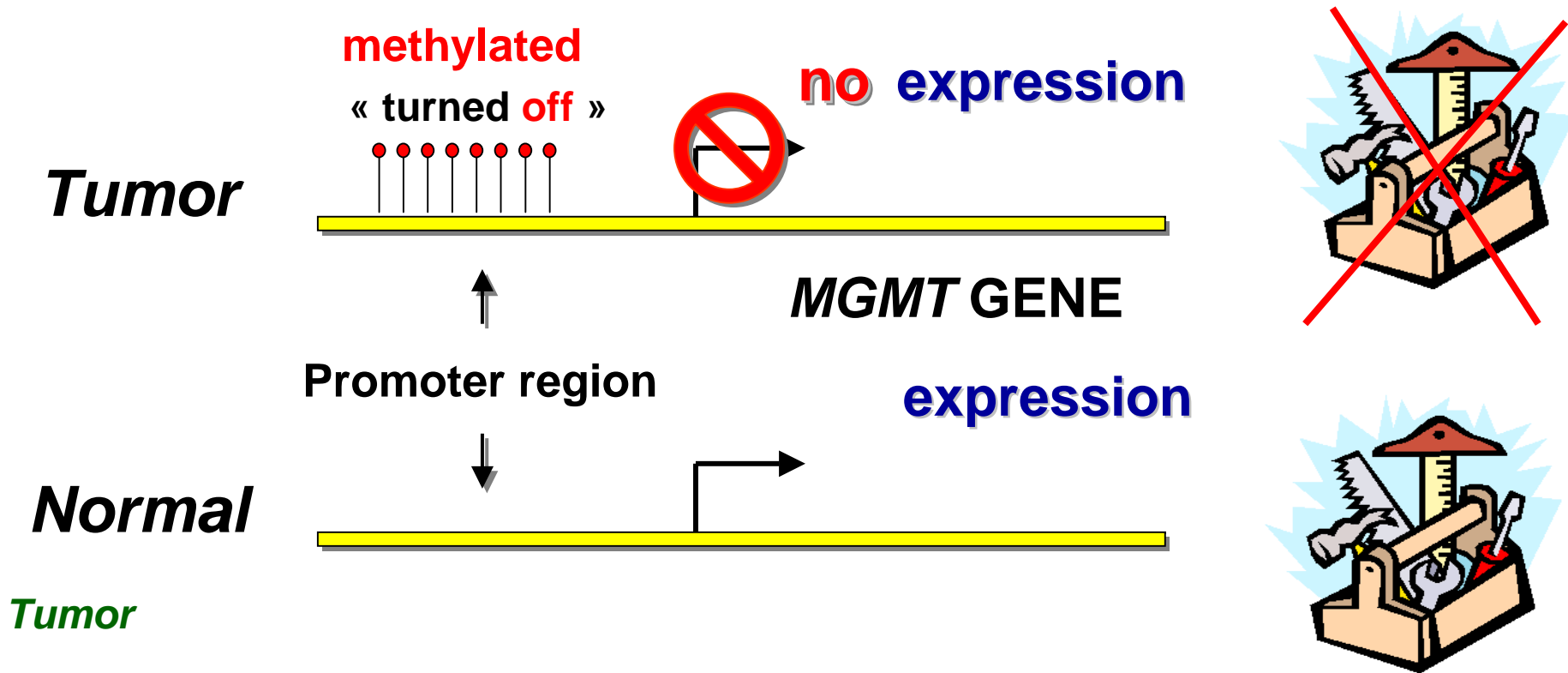
### **MOUSE MODELS & Alkylating Agents**

- *MGMT*<sup>-/-</sup> hypersensitive
- *MGMT*<sup>Tg</sup> resistant to tumor formation



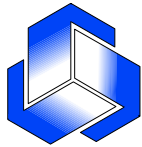
# Silencing of the *MGMT* Repair Gene by Methylation of the Gene Promoter

0<sup>6</sup>-Methylguanine-DNA Methyltransferase (MGMT)

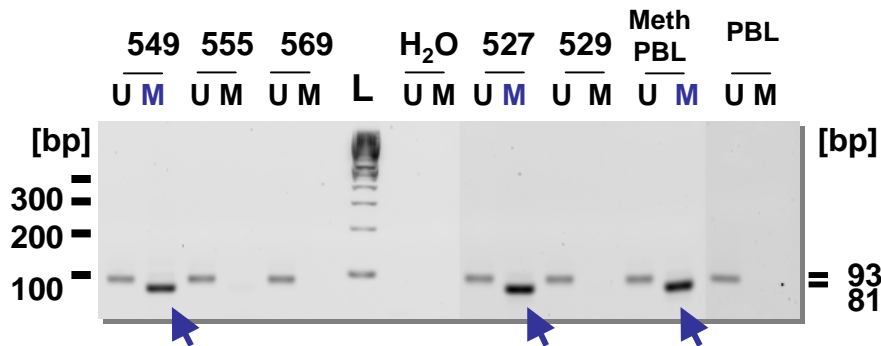


- **No** repair protein
- **No repair** of TMZ treatment induced DNA damage

- **Response to tumor treatment**
- **Improved survival of glioblastoma patient**

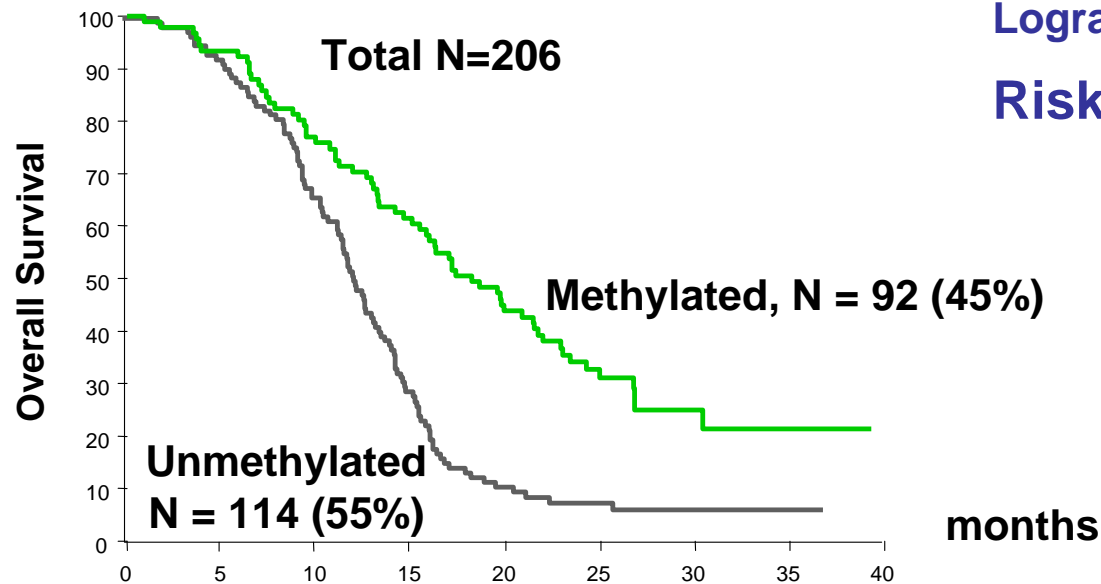


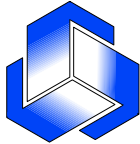
# ***MGMT* Promoter Methylation Predicts Better Outcome in Glioblastoma Patients of this Trial**



<b><i>MGMT</i></b>	<b>Unmeth</b>	<b>Meth</b>
Median OS, mo:	12.2	18.2
2-yr survival:	7.8%	34.1%
HR [95% CI]:	0.45 [0.32-0.61]	
Logrank test:	$p < 0.0001$	

**Risk of death reduced by 55%**

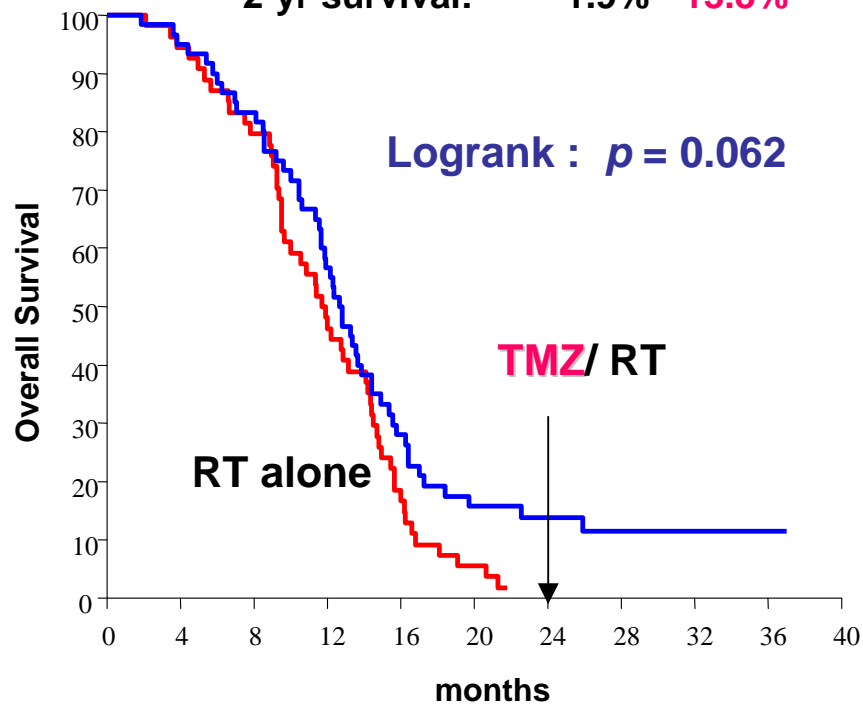




# *MGMT* Promoter Methylation Predicts Benefit from TMZ Treatment

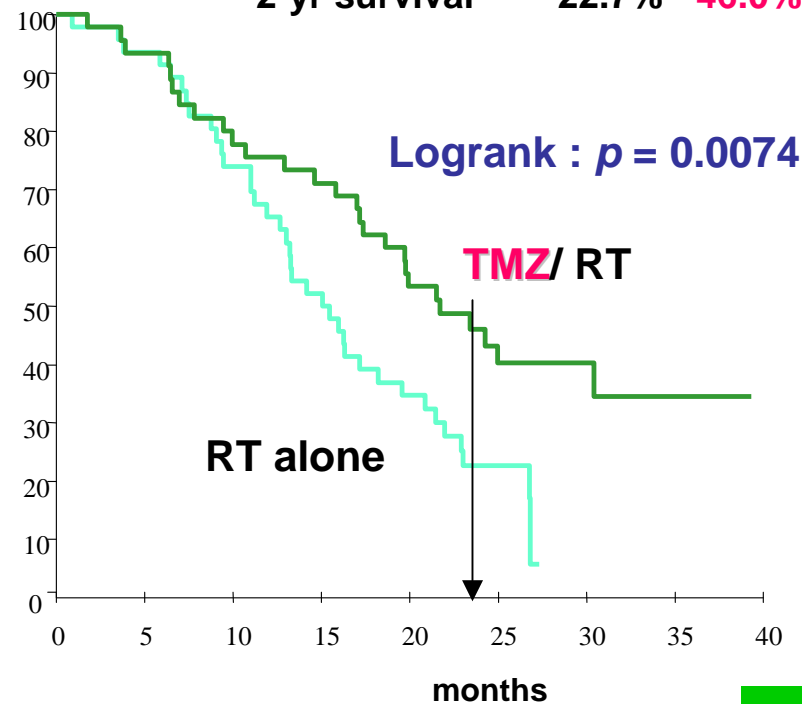
## Unmethylated *MGMT*

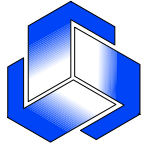
Randomization: RT      **TMZ/RT**  
Median OS mo: 11.8      **12.7**  
2-yr survival: 1.9%      **13.8%**



## Methylated *MGMT*

Randomization: RT      **TMZ/RT**  
Median OS mo: 15.3      **21.7**  
2-yr survival 22.7%      **46.0%**

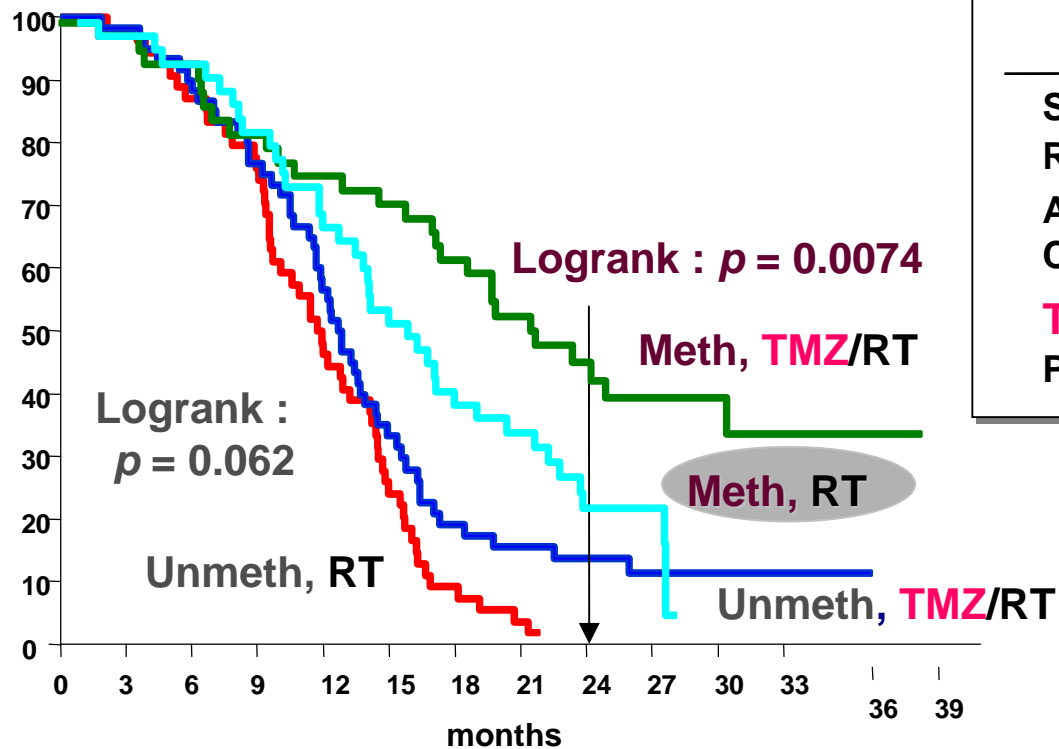




# Predictive Value of *MGMT* Methylation for Overall Survival



Overall Wald test :  $p < 0.0001$  (df=3)



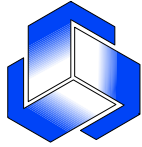
Treatment at progression  
at discretion of treating physician

Randomization	RT [%] n=286	TMZ/RT [%] n=287
Surgery	23	23
Repeat RT	4	5
Any Additional Chemotherapy	72	58
Temozolomide	60	25
Palliative Care only	17	22

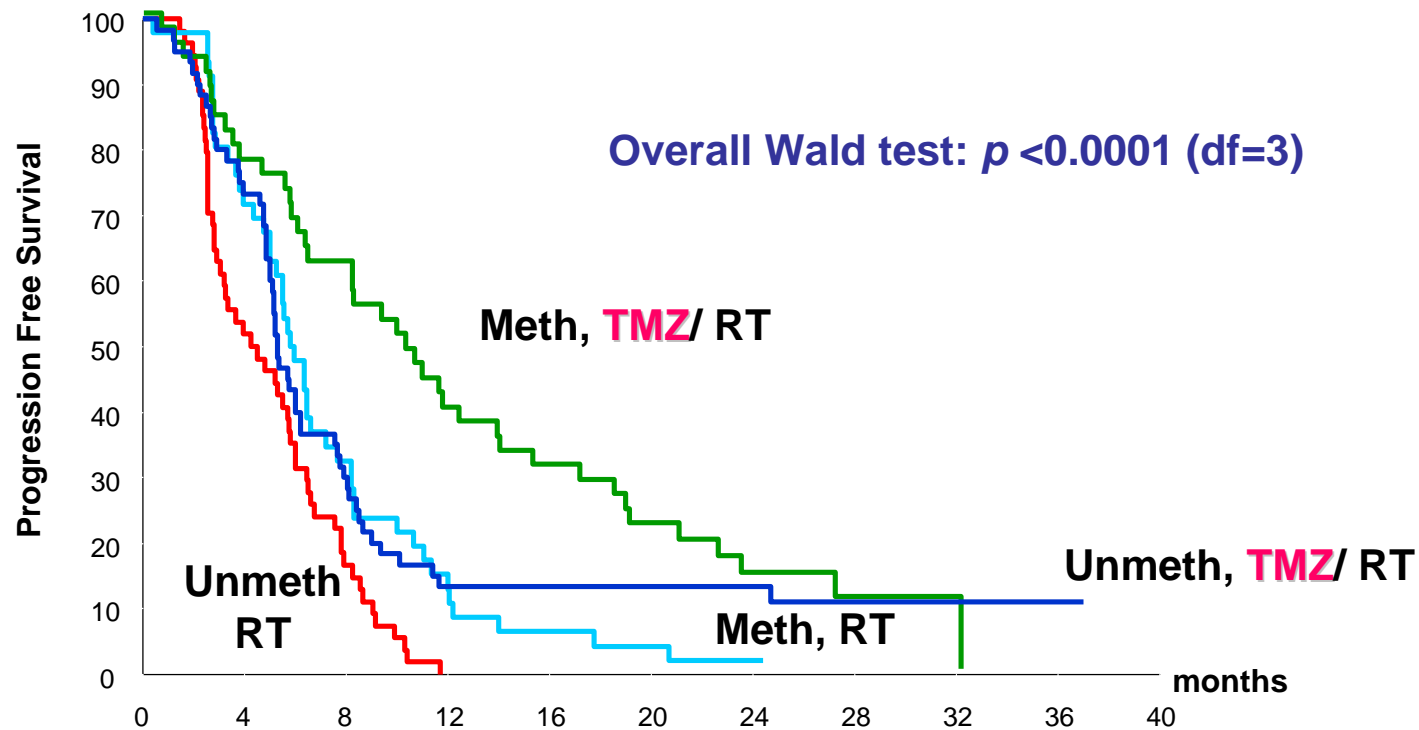
Stupp *et al*/ NEJM 2005

Hegi *et al.* N Engl J Med, 352: 997-1003, 2005





# Progression Free Survival Supports *MGMT* Methylation Status as Predictive Factor for Benefit from TMZ

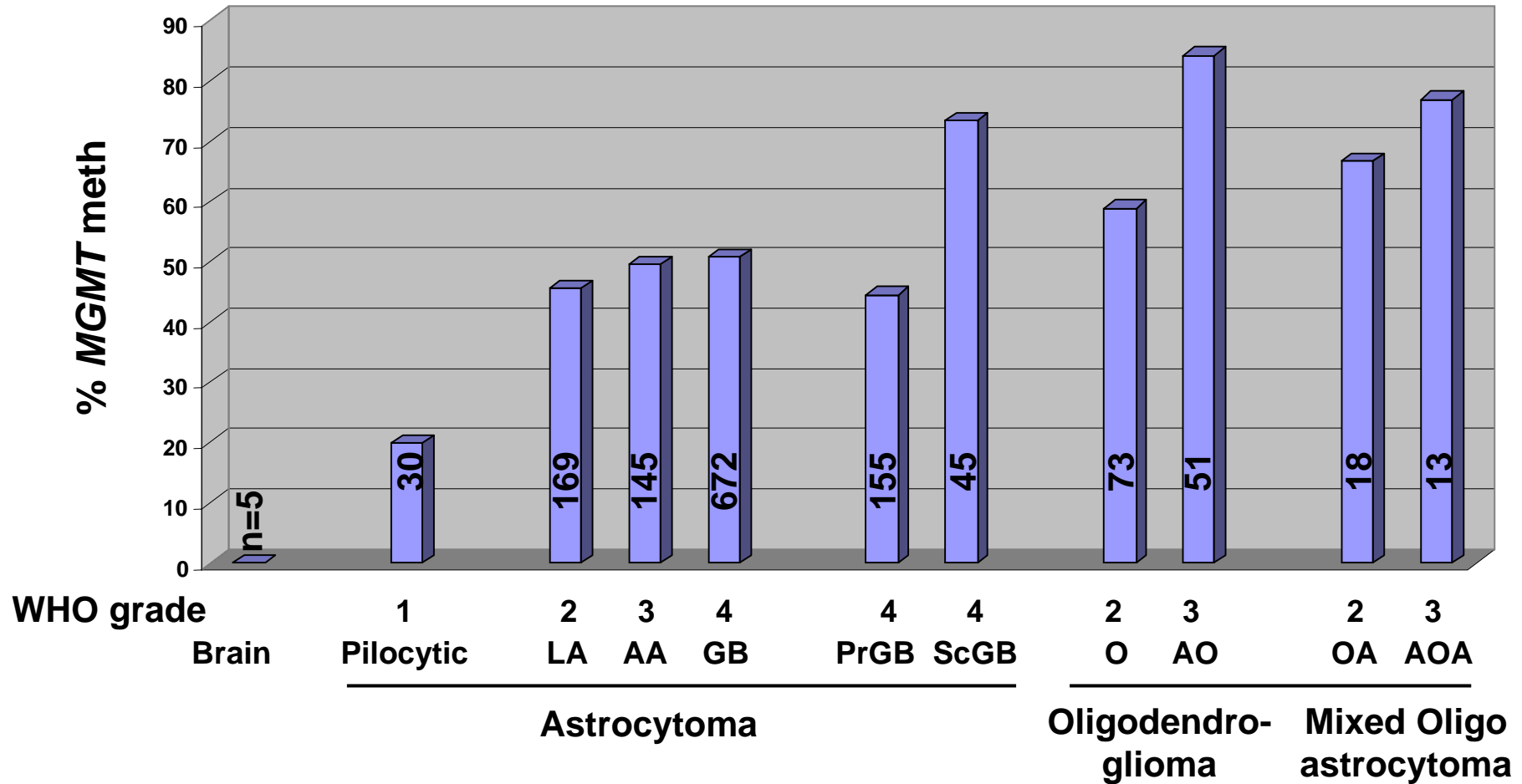


O	N	Number of patients at risk :									
54	54	28	9	0	0	0	0	0	0	0	0
53	60	44	18	8	8	8	7	5	3	1	
45	46	33	15	7	3	2	1	0	0	0	
40	46	35	28	18	14	10	6	3	1	0	

- Unmeth, RT alone
- Unmeth, TMZ/ RT
- Meth, RT alone
- Meth, TMZ/ RT

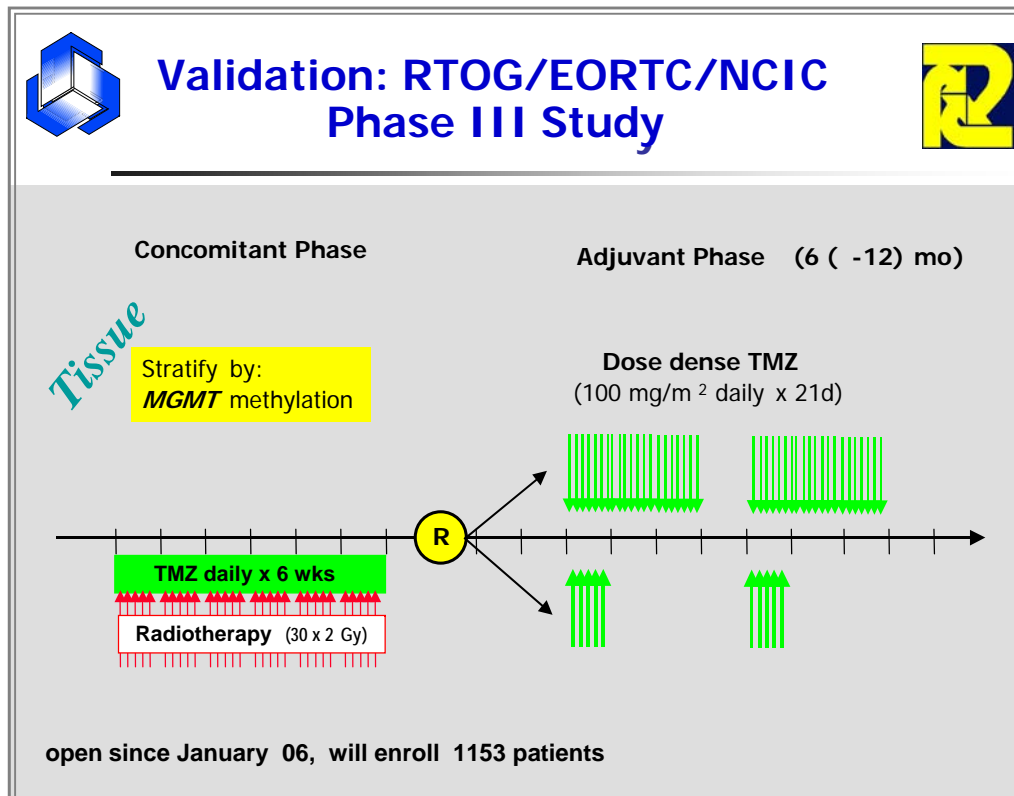


# ***MGMT* Promoter Methylation Ranges from 20 to >80% Depending on Glioma Subtype**



Review of the literature, August 2007  
 data from 1376 patients from 15 publications

# Validation of *MGMT* as Predictive Factor Depletion of *MGMT* in Tumor Cells by a Dose Dense Schedule



## *Integrated Translational Research Program:*

**Identification of other resistance factors and new targets**

### Study Chairs:

Mark R. **Gilbert**, M.D. (Medical Oncology)  
Minesh **Mehta**, M.D. (Radiation Oncology)  
Ken **Aldape**, M.D. (Neuropathology and Correlative Biology)  
Arnab **Chakravarti**, M.D. (Neuropathology and Correlative Biology)

### EORTC

Roger **Stupp**, M.D. (Medical Oncology)  
Monika **Hegi**, Ph.D. (Neuropathology and Correlative Biology)

# Trials in GBM based on the RT/TMZ→TMZ scheme

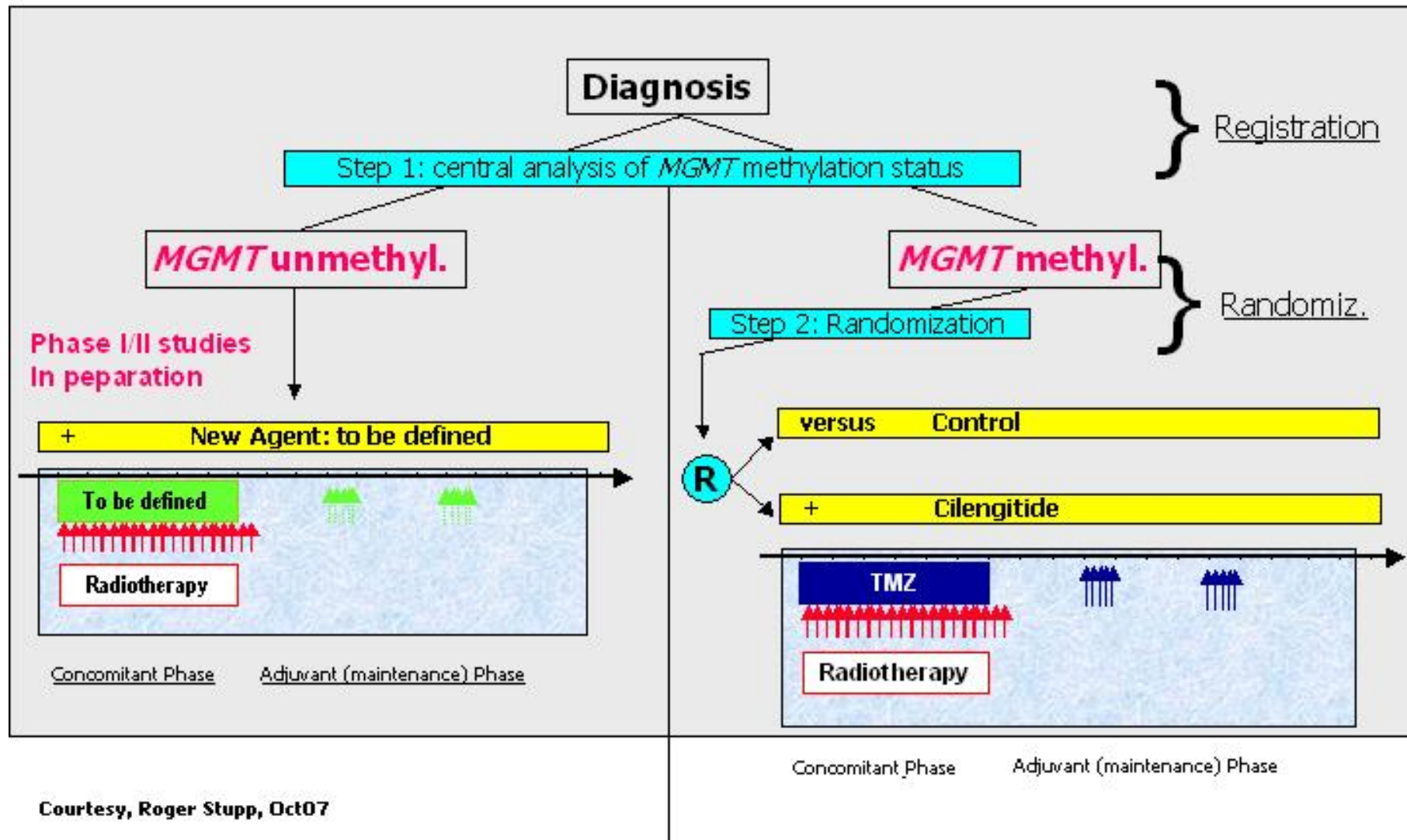
**Table 1. Ongoing Trials of Novel Agents With Concomitant to Radiotherapy**

Investigational Agent	Standard Treatment	Phase	No. of Patients	End Point(s)	Sponsor	Remarks
Cilengitide	TMZ/XRT	II	60	PFS	Merck Serono	Assess completed, multicenter (Europe), <sup>100</sup> phase III trial in preparation
Cilengitide	TMZ/XRT	I/II	112	OS	NABTT	Ongoing
Tamoxifen	TMZ/XRT	I	46	Safety and toxicity	NCCTG	Started May 2008
Cetuximab	TMZ/XRT	I/II	46	Feasibility, PFS, OS	University of Heidelberg, Germany	No maintenance TMZ
CDK-119	TMZ/XRT	II/III	90-376	PFS	Cellen	Tumor-specific vaccine for EGFRvIII expressing tumors
Valproic acid	TMZ/XRT	III (randomized)	180	Survival	EORTC	Phase I completed; further drug development still continued
Imatinib/hydroxyurea	TMZ/XRT	I/II	2 x 30T	Safety and toxicity, PFS	Novartis Germany	Phase II separate strata with/without EGFR
Bevacizumab	TMZ/XRT	II	70	Survival	UCLA	Requires fresh-frozen tumor tissue
Bevacizumab	TMZ/XRT	III	800	Survival	Berenson	Start planned summer 2007
Ercosulfurin	TMZ/XRT	I/II	72	Survival (ph1)	Lilly	Started September 2006
Ercosulfurin	XRT	II	64	PFSS	Lilly Germany	Only for patients with an unmethylated MGMT
Tipifarnib	TMZ/XRT	I	30	Safety and toxicity	NABTT	Completed
Tipifarnib	XRT	II	27	Survival	Instituto Claude Regaud, Toulouse	France: Toulouse, Clermont-Ferrand
Larotidine	XRT	I/II	80	Survival	DFCI	Phase I in phase II in combination with TMZ/radiation therapy planned
Vandetanib	TMZ/XRT	III (randomized)	160	Survival	DFCI	Start planned summer 2007. Harvard affiliate, MSKCC, University of Virginia, University of Pittsburgh
Valproic acid	TMZ/XRT	II	41	PFS, survival	National Cancer Institute	Valproic acid as a histone deacetylase inhibitor
Boreavertin	TMZ/XRT	II/III	80	Safety, PFSS	Novocis	Biosynthetic drug targeting hypoxic cells
Carmustine water	TMZ/XRT	II	72	Survival	Johns Hopkins	

Abbreviations: TMZ, temozolomide; XRT, irradiation; NABTT, New Approaches to Brain Tumor Therapy CNS Consortium; NCCTG, North Central Cancer Treatment Group; EORTC, European Organization for Research and Treatment of Cancer; EGFR, epidermal growth factor receptor; UCLA, University of California, Los Angeles; NABTT, North American Brain Tumor Consortium; DFCI, Dana-Farber Cancer Institute; MSKCC, Memorial Sloan-Kettering Cancer Center, New York; PFS, progression-free survival; OS, overall survival; PFSS, 6-month PFS rate.



# Cilengitide Phase III for GBM



Courtesy, Roger Stupp, Oct07

## Frequency of *MGMT* Methylation in Glioblastoma

Range published for GBM 34 to 68%  
(gel based, mostly on frozen tissue)

Tumor type	# samples	MGMT-meth	%	Reference
GBM	29	10	34	{Watanabe, 2005 #3670}
GBM	21	8	38	{Balana, 2003 #3672}
GBM	29	12	41	{Esteller, 2000 #1455}
GBM	12	5	42	{Yu, 2004 #2165}
GBM	74	33	45	{Kamiryo, 2004 #3677}
GBM	206	92	45	{Hegi, 2005 #2000}
GBM	44	30	68	{Blan, 2004 #3674}
GBM	38	26	68	{Hegi, 2004 #1721}
GBM	219	126	58	{Criniere, 2007 #6708}
<b>TOTAL</b>	<b>672</b>	<b>342</b>	<b>51</b>	

# Comparison of qMSP and Classic Gel Based Nested MSP for determination of the *MGMT* status

## Quantitative MSP

### OncoMethylome Sciences

Ilse Vlassenbroeck  
Stéphane Califice  
Josef Straub  
Ivano Di Stefano  
Fabrice Moreau  
Isabelle Renard,  
Bruno Flamion  
James DiGuseppi  
Katja Bierau

## Gel Based MSP

### Lab of Tumor Biology and Genetics, Neurosurgery, CHUV

Annie-Claire Diserens  
Marie-France Hamou  
Monika E. Hegi

## Statistics

### NCCR Molecular Oncology & Swiss Institute of Bioinformatics

Eugenia Migliavacca  
Mauro Delorenzi

**Tissues from Trials : Lausanne, R. Stupp; Rotterdam, M. van den Bent  
Regensburg, P. Hau**

# Experimental Workflow of the Assays

- **Evaluation of tissue** (H&E slide, tumor content, amount)
- **4 sections / sample for each center**

## GEL BASED ASSAY **CHUV**

- **Bisulfite treated DNA**
- **Nested MSP** : 1<sup>st</sup> PCR (298bp)
- 2<sup>nd</sup> PCR (discriminating)
  - PCR for **meth** *MGMT* (81bp)
  - PCR s for **unmeth** *MGMT* (93bp)
- **Results** visualized on gel

## qMSP **OncoMethylome Sciences**

- **Bisulfite treated DNA**
- **quantitative MSP** :
  - **meth** *MGMT* (136bp)
  - beta Actin (125bp)
- **Ratio of mMGMT/Actin \*1000**

**Comparison of results**

**Non-disclosed Unpublished Data**

**Vlassenbroeck *et al* submitted**



# **MGMT immunohistochemistry in GBM: Interobserver agreement in EORTC/NCIC trial26981/22981**

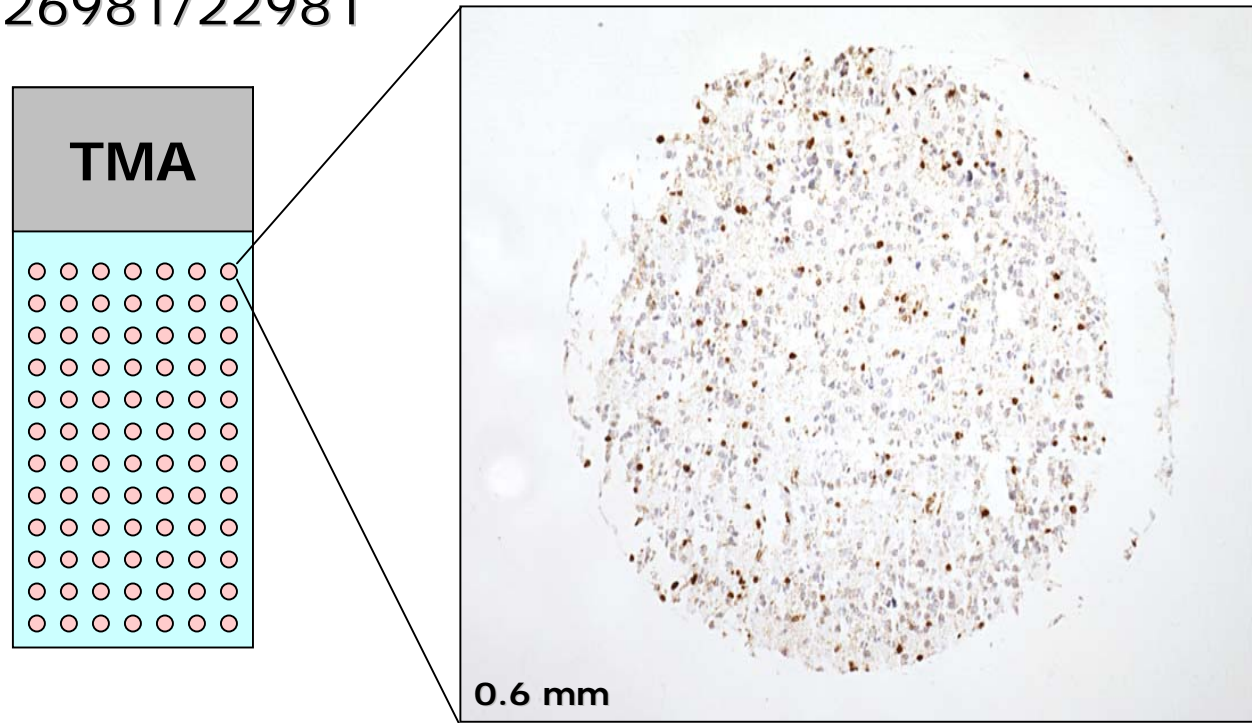
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**Preusser M, Janzer RC, Felsberg J, Reifenberger G, Hamou M-F,  
Diserens A-C, Marosi C, Heinzl H, Stupp R, Hainfellner JA, Hegi ME**



**Methods** : 2 anti-MGMT antibodies, **Dako MT3.1, Zymed MT23.2**

- Tissue micro array (TMA): 163 tissue samples from GBM trial 26981/22981

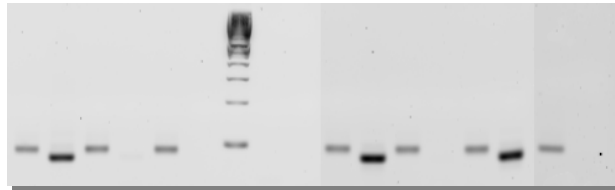


- 4 neuropathologists - 3 laboratories (RJ, GR, JAH, MP)
- Statistical analysis

**Non-disclosed Unpublished Data**

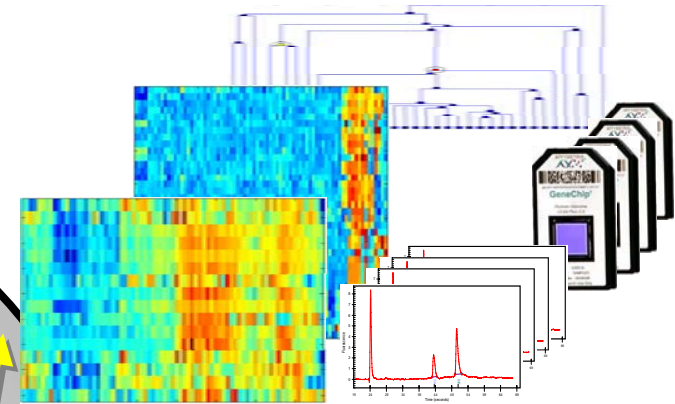
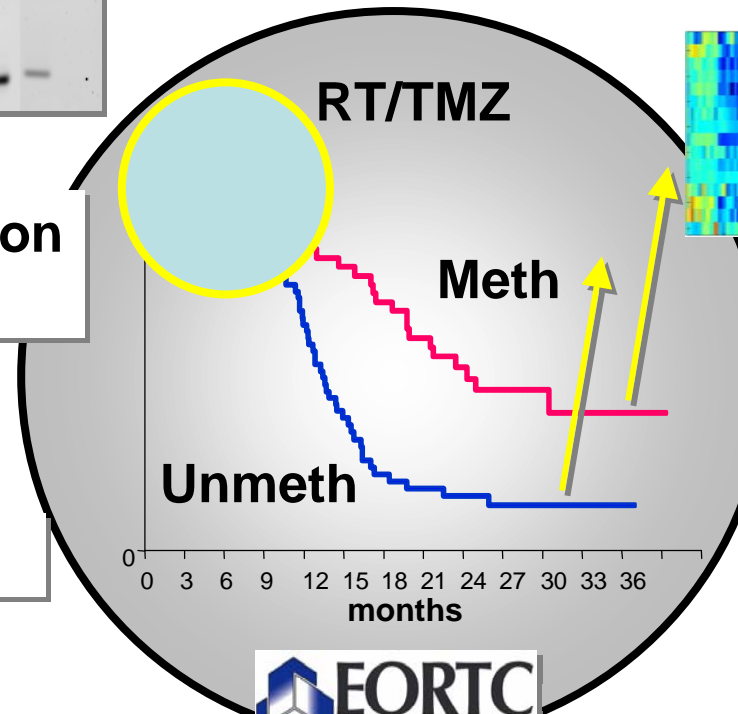
**Preusser *et al* submitted**

# New Molecular Targets



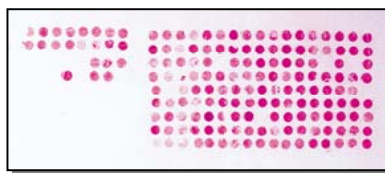
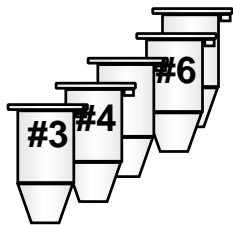
**MGMT** methylation Status

Frozen Tissue

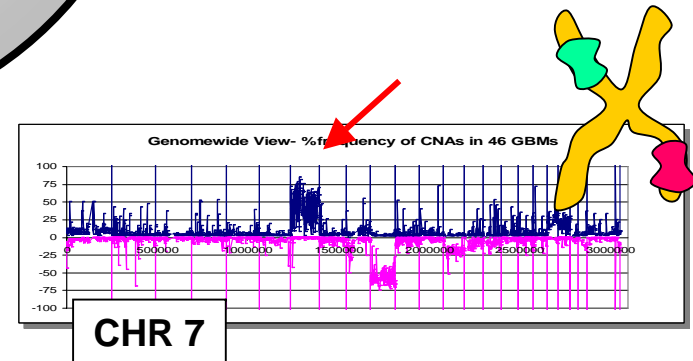


Gene Expression Profiles

Array-CGH



Tissue Array



**Non-disclosed Unpublished Data**

**Murat *et al* submitted**

# Conclusions

- The *MGMT* methylation status predicts benefit from the alkylating agent TMZ
- Standardized *MGMT*-testing required
  - Quantitative MSP is reproducible, prospective testing ongoing
  - IHC is not useful for diagnostic *MGMT*-testing
- New trials will select patients based on *MGMT* status



# The Team in the Lab



Anastasia Murat  
Wanyu Louis Lambiv  
Isabelle Desbaillets  
Annie-Claire Diserens  
Marie-France Hamou  
Yan Lachat  
Sophie Shnaper

Monika Hegi  
Nicolas de Tribolet  
Marc Levivier

**Weizmann Institute  
of Science**

**Tal Shay  
Eytan Domany**

**NCCR  
Molecular Oncology  
ISREC**

**Eugenia Migliavacca  
Mauro Delorenzi**

**NCCR  
Frontiers in Genetics  
Genève**

**Patrick Descombes  
Didier Chollet**

**UCSF  
Anjan Misra  
Burt Feuerstein**



**Oncology, CePO, CHUV**

**Roger Stupp**

**EORTC**

**Thierry Gorlia**

**Patients  
and their Families**

**85 CENTERS**



**Brain Tumor Group  
Radiotherapy Group**



**FONDATION NELIA ET AMADEO BARLETTA**

*Personalizing cancer treatment*

**85 CENTERS**

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Tübingen, D**

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**Johannes Hainfellner  
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**Warren Mason,  
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