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MGMT methylation in tissue and serum from unresectable glioblastoma (GBM) patients (p) included in the GENOM 009 Study, a multicenter randomized study by the GEINO Group comparing temozolomide (TMZ) versus TMZ-plus-bevacizumab (BEV)

(ClinicalTrials.gov NCT01102595)

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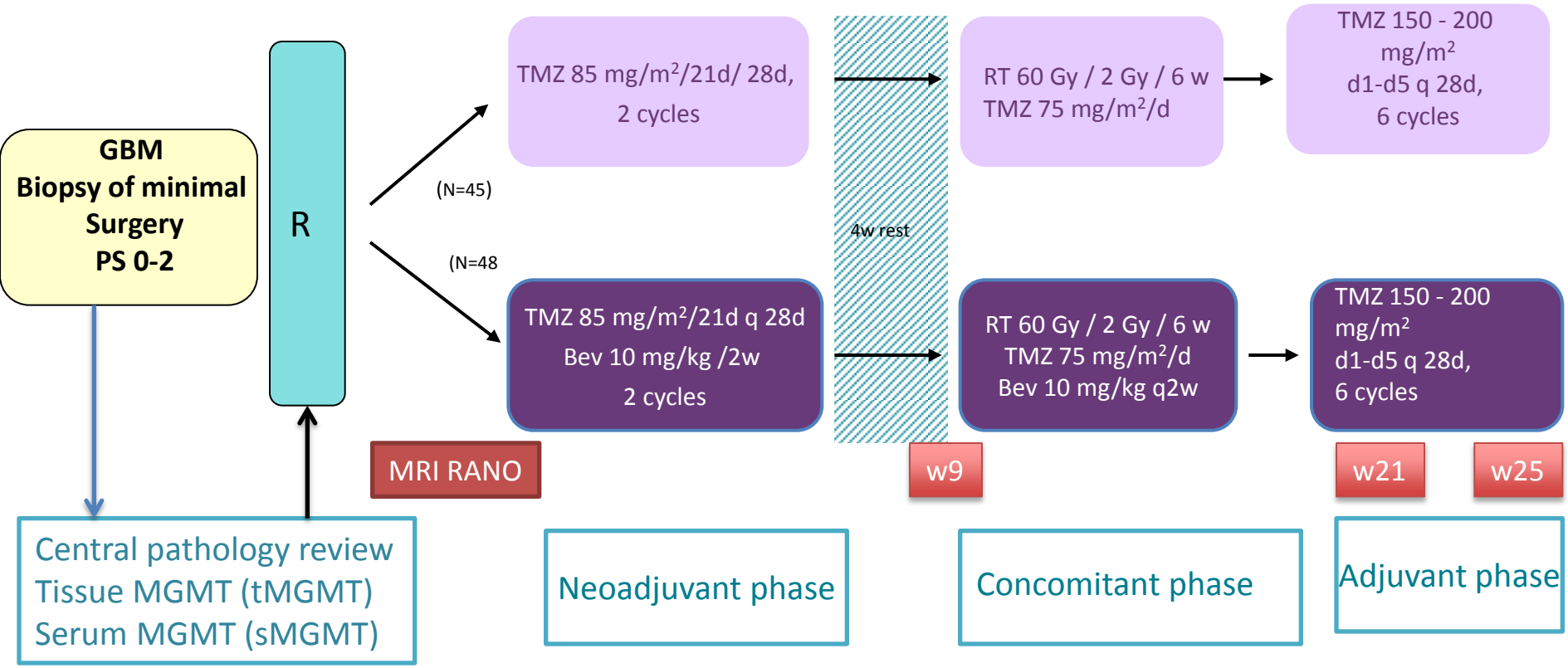
Background

- DNA fragments, proteins and miRNAs are released by CNS tumors into blood.
- There is a growing interest in identifying diagnostic, prognostic and predictive blood biomarkers in patients with CNS tumors.
- This is especially important given the often scarce amount of tissue available for molecular studies in this disease.

GENOM 009


GBM
Biopsy of minimal
Surgery
PS 0-2
R
(N=45)
TMZ 85 mg/m²/21d/ 28d,
2 cycles
(N=48
TMZ 85 mg/m²/21d q 28d
Bev 10 mg/kg /2w
2 cycles
4w rest
RT 60 Gy / 2 Gy / 6 w
TMZ 75 mg/m²/d
RT 60 Gy / 2 Gy / 6 w
TMZ 75 mg/m²/d
Bev 10 mg/kg q2w
TMZ 150 - 200
mg/m²
d1-d5 q 28d,
6 cycles
TMZ 150 - 200
mg/m²
d1-d5 q 28d,
6 cycles
MRI RANO
w9
w21
w25
Central pathology review
Tissue MGMT (tMGMT)
Serum MGMT (sMGMT)
Neoadjuvant phase
Concomitant phase
Adjuvant phase

Methods

- Endpoints:
  - Primary: ORR (RANO) after 2 pre-RT cycles
    - powered to detect a 30% difference between arms (α and β errors of 0.05 and 0.20).
  - Secondary:
    1. Toxicity
    2. % neurological deterioration before RT
    3. PFS
    4. OS
    5. 1y OS
    6. **MGMT Serum vs Tissue as predictive biomarkers**

HUGTIP Pathology Service
Tissue revision & t MGMT

Molecular Lab
sMGMT

Methods

- MGMT promoter methylation was determined by methylation-specific PCR, using specific primers for either methylated or unmethylated DNA after chemical modification.

- Using manual macrodissection, tissue was first selected from paraffin embedded tumor blocks to achieve at least 80% of tumoral DNA in the sample.

- Methods for circulating DNA analyses (and sMGMT assessment) have been described elsewhere *(Balana et al 2003; Ramirez et al 2003; Balana et al 2011)*

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Samples

TISSUE SAMPLES	All pts registered (n=103)	Pts randomized (n=93)
Received	86 (83.5%)	79 (85.0%)
Results	86 (100%)	79 (100%)
Methylated	34 (39.5%)	27 (34.2%)
Unmethylated	29 (33.7%)	31 (39.2%)
Not evaluable	5 (5.8%)	5 (6.3%)
Insufficient	18 (21.0%)	16 (20.3%)
SERUM SAMPLES		
Received	83 (80.6%)	80 (86%)
Results	83 (100%)	80 (100%)
Methylated	11 (13.2%)	11 (13.8%)
Unmethylated	63 (75.9%)	60 (75.0%)
Bad sample/No A	9 (10.9%)	9 (11.2)

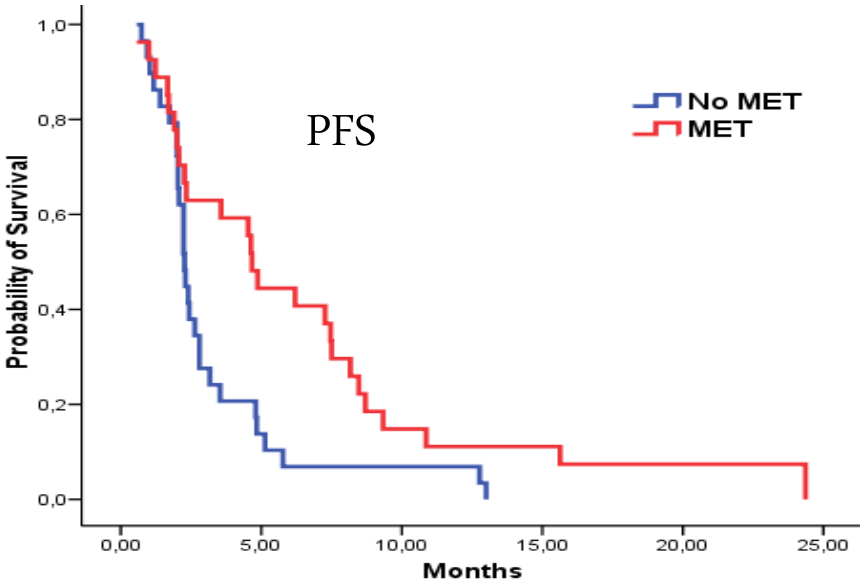
Patient characteristics

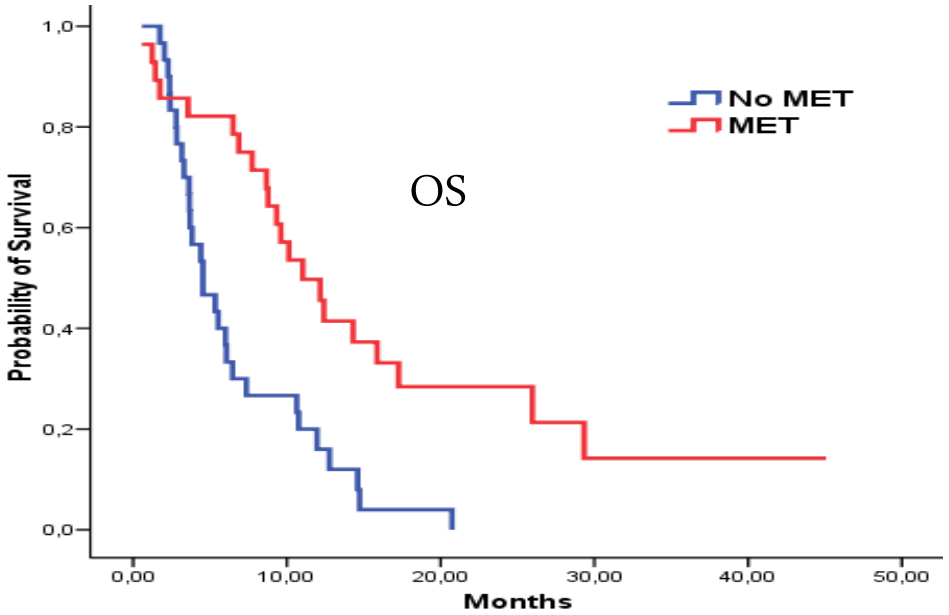
Characteristic	TMZ Arm (n=45)	BEV Arm (n=48)	*p*
Age – median±range	62±9.5	62.9±7.4	0.73
<50 / ≥50	4 / 41	1 / 47	0.19
Gender (M / F)	25 / 20	31 / 17	0.37
ECOG PS			0.819
0	9	12	
1	20	23	
≥2	16	13	
MMS			0.07
<27	23	16	
≥27	22	32	
Neurological deficit	27	27	0.71
Surgery			0.21
Biopsy (ST)	35	42	
Biopsy by Craneotomy	10	6	
DXM at inclusion (yes)	34	39	0.26

GENOM 009 Results

Endpoint	TMZ Arm (n=45)	BEV Arm (n=48)	p
ORR (ITT) PR PR&SD	 3 (6.7%) 11 (24.5%)	 11 (22.9%) 28 (68.3%)	0.003
Worse before RDT	22 (48.9%)	10 (20.8%)	0.004
Completed 6 c TMZ	22 (48.9%)	32 (66.7%)	0.08
PFS (m, 95% CI)	2.2 (2.1-2.5)	4.8 (3.6-6.1)	HR, 0.79 (0.52-1.2) p=0.28
OS (m, 95% CI)	7.7 (5.7-14.5)	10.8 (7-14.5)	HR, 0.71 (0.46-1.10) p=0.12
1-year OS	29.6 %	48.9%	0.06

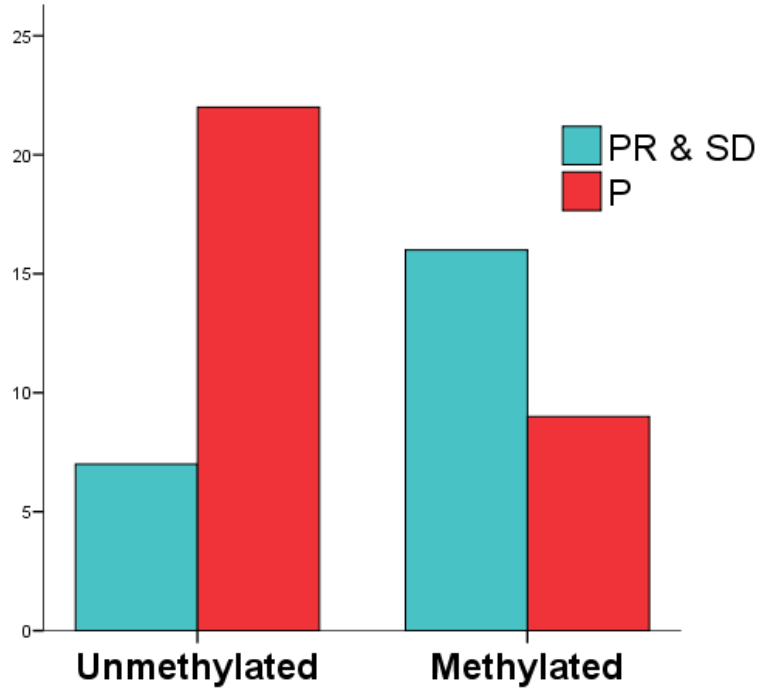
PFS and OS by tMGMT status





tMGMT STATUS	PFS (mo)	HR (95% CI)	P	OS (mo)	HR (95% CI)	P
UNMETHYLATED	2.3 (2.0-2.5)	0.49 (0.28-0.87)	0.01	4.5 (2.3-6.7)	0.36 (0.19-0.67)	0.001
METHYLATED	4.7 (4.1-5.2)			12.2 (8.7-15.6)		

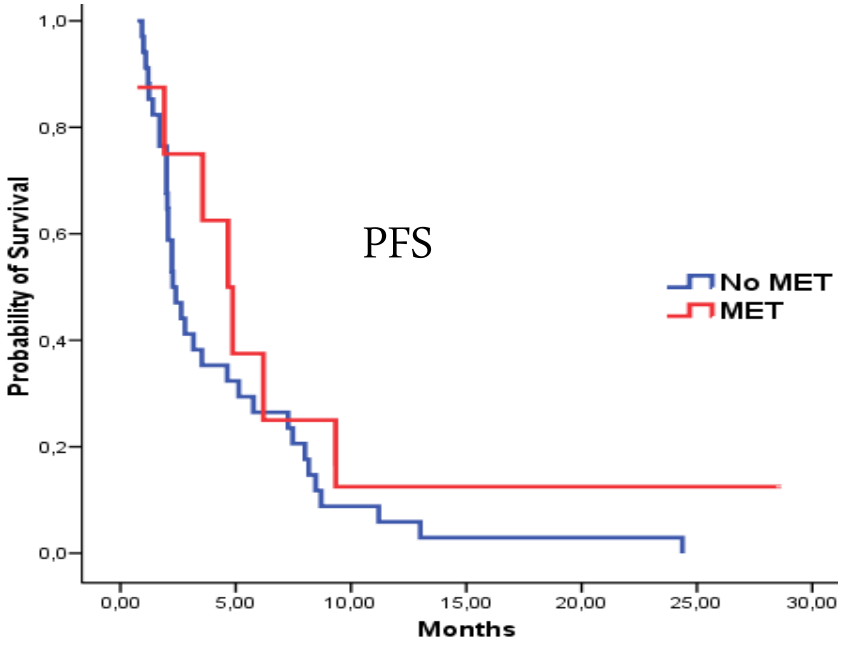
Response by tMGMT status

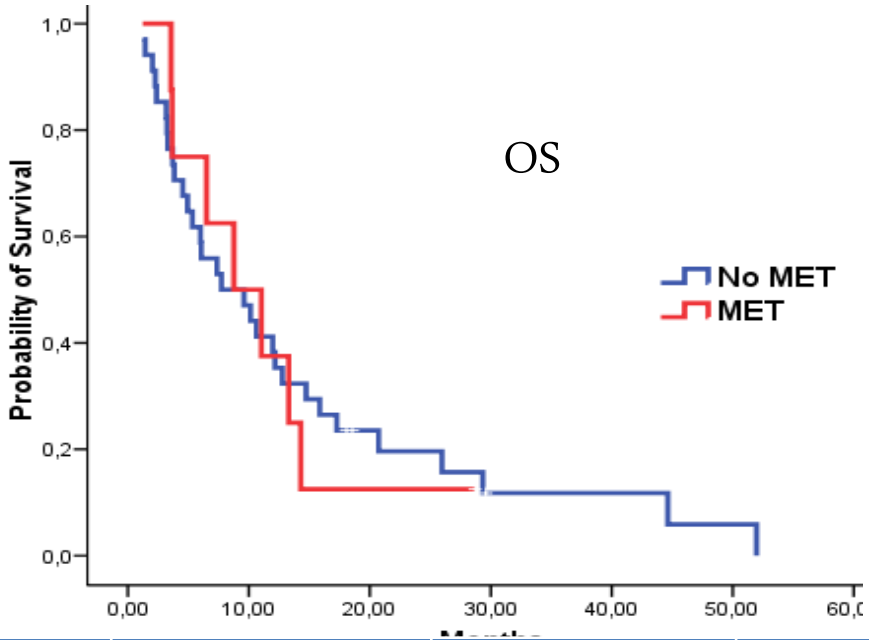


	PR & SD (%)	PD (%)	P
Unmethylated	24.1	75.9	0.005
Methylated	64.0	36.0	

N=54

PFS and OS by sMGMT status





sMGMT STATUS	PFS (mo)	HR (95% CI)	P	OS (mo)	HR (95% CI)	P
UNMETHYLATED	2.2 (1.6-2.9)	0.62 (0.27-1.4)	0.25	7.7 (1.9-13.5)	1.03 (0.45-2.3)	0.93
METHYLATED	4.6 (2.8-6.4)			8.7 (2.4-15.0)		

Correlation of MGMT status in tumor and serum

Methylation status		Serum MGMT		TOTAL
Tissue MGMT		UnMET	MET	
	UnMET	21 (95.5%)	1 (4.5%)	22
	MET	14 (66.7%)	7 (33.3%)	21

Kappa index= 0.272 (0.027)

Conclusions

- Addition of BEV significantly improved ORR (primary endpoint) and showed a trend toward improved PFS, OS and 1-y OS.
- Tissue MGMT methylation is associated with improved PFS, OS and response.
- The study of MGMT in serum failed to predict results in a blinded, randomized, multicenter study.
- **The assessment of serum MGMT with methylation-specific PCR is not sufficiently sensitive to be used as a surrogate for MGMT status in tumor tissue.**
- ONGOING RESEARCH: 12 residual tumors have been requested from the participating centers and will be included in the study. Repeated analyses of serum with real-time PCR and pyrosequencing are ongoing in order to improve sensitivity.

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