

SUPPLEMENTARY INFORMATION TO:

***MGMT* Promoter Methylation Cutoff with Safety Margin for Selecting Glioblastoma Patients into Trials Omitting Temozolomide. A pooled analysis of four clinical trials.**

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Table S1. Clinical characteristics in patients with *MGMT* promoter unmethylated versus methylated tumors (cut-off 1.27) in rand-P training cohort.

	UNMETHYLATED (%) (n=460, 53.3%)	METHYLATED (%) (n=403, 46.7%)
ECOG Performance status		
PS = 0	277 (60.2)	239 (59.3)
PS ≥ 1	183 (39.8)	164 (40.7)
Extent of resection		
Incomplete resection	218 (47.4)	190 (47.1)
Complete resection	242 (52.6)	212 (52.6)
Missing	0	1 (0.3)
Age in years		
Median (Q1,Q3)	57 (49, 63)	58 (50, 64)

Table S2. Outcome in patients with *MGMT* promoter methylated and unmethylated tumors of the rand-P training & test cohorts.

Methylation status	N (%)	Observed Events	Median survival [months]	HR (95% CI)	P-value	Adj. HR (95% CI) ^a	P-value
Training cohort							
Unmethylated	460 (53.3)	352	14.5 (14.0 – 15.3)	1.00		1.00	
Methylated	403 (46.7)	203	26.5 (25.1 – 30.2)	0.39 (0.30 – 0.50)	<0.0001	0.36 (0.28 – 0.46)	<0.0001
Test cohort							
Unmethylated	445 (51.6)	354	14.0 (13.3 – 14.7)	1.00		1.00	
Methylated	417 (48.4)	219	25.6 (23.2 – 28.4)	0.41 (0.32 – 0.52)	< 0.0001	0.39 (0.30 – 0.49)	< 0.0001

^aadjusted for age, ECOG performance status, and extent of resection

Table S3. Retest classification, unsupervised cut-off (1.27)

		Retest cohort		
		Unmethylated (%)	Methylated (%)	Total
Original cohort	Unmethylated (%)	107 (49.1)	7 (3.2)	114
	Methylated (%)	1 (0.5)	103 (47.2)	104
	Total	108	110	218

Table S4. Retest classification including grey zone

		Retest cohort			
		Truly unmethylated (%)	Grey zone (%)	Methylated (%)	Total
Original cohort	Truly unmethylated (%)	79 (36.2)	9 (4.1)	0	88
	Grey zone (%)	8 (3.7)	11 (5.1)	7 (3.2)	26
	Methylated (%)	1 (0.5)	0	103 (47.2)	104
	Total	88	20	110	218

Table S5. Retest classification with grey zone and limit of detection rule ^a

		Retest cohort (*MGMT<10 rule applied)			
		Truly unmethylated (%)	Grey zone (%)	Methylated (%)	Total
Original cohort (*MGMT<10 rule applied)	Truly unmethylated (%)	99 (45.4)	4 (1.8)	2 (0.9)	105
	Grey zone	1 (0.5)	3 (1.4)	5 (2.3)	9
	Methylated	1 (0.5)	0	103 (47.2)	104
	Total	101	7	110	218

^a MGMT copy number < 10, set to unmethylated (10 copies = limit of detection)

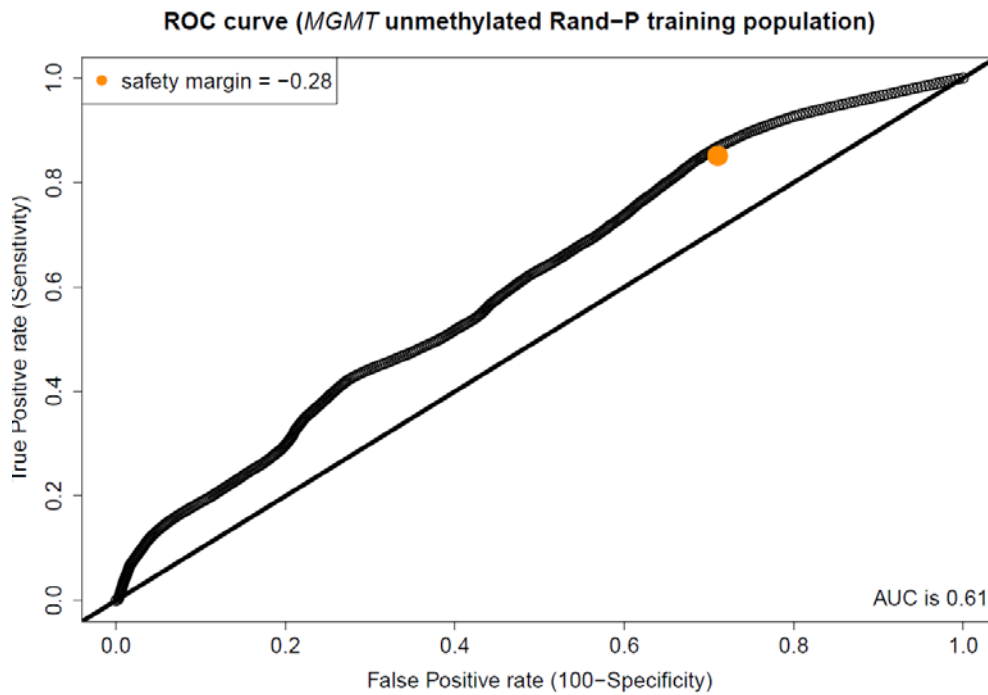
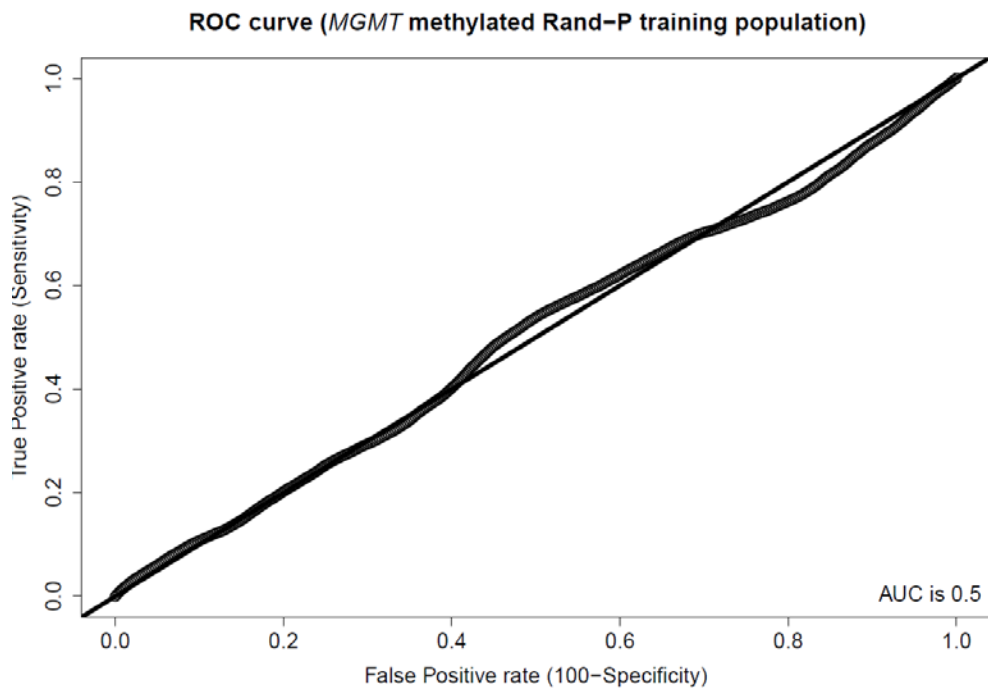
Table S6. Comparison of classification based on original and new cut-offs

ORIGINAL			NEW Corrected log ₂ MGMT ratio, cut-off = 1.27, lower safety margin = -0.28		
			Unmethylated	Grey zone	Methylated
Uncorrected log ₂ MGMT ratio, cut-off = 1 lower safety margin = -0.75 MGMT = 0 = UNMET	all-P training cohort	Unmethylated	1076 (53.4%)	39 (1.9%)	0
		Grey zone	25 (1.2%)	174 (8.6%)	1
		Methylated	0	18 (0.9%)	688 (34.0%)
	all-P test cohort	Unmethylated	1080 (53.5%)	22 (1.2%)	0
		Grey zone	17 (0.8%)	158 (7.8%)	2
		Methylated	0	17 (0.8%)	724 (35.8%)

Table S7 Comparison of classification based on original and new procedure with limit of detection rule ^a

ORIGINAL			NEW Corrected log ₂ MGMT ratio, cut-off = 1.27, lower safety margin = -0.28, *MGMT<10 rule applied		
			Unmethylated	Grey zone	Methylated
Uncorrected log ₂ MGMT ratio cut-off = 1 lower safety margin = -0.75 *MGMT<10 rule applied	all-P training cohort	Unmethylated	1280 (63.3%)	0	0
		Grey zone	8 (0.4%)	55 (2.7%)	0
		Methylated	0	16 (0.8%)	662 (32.8%)
	all-P test cohort	Unmethylated	1237 (61.2%)	0	0
		Grey zone	8 (0.4%)	51 (2.5%)	0
		Methylated	0	15 (0.7%)	709 (35.1%)

^a MGMT copy number < 10, set to unmethylated (10 copies = limit of detection)

A**B****Figure S1. Time-dependent ROC analysis.**

A Time-dependent ROC analysis of the *MGMT* promoter unmethylated subgroup of the training randomized patient (rand-P) cohort resulted in a ROC curve with an AUC = 0.61. The optimal cutoff point corresponded to a corrected *MGMT* \log_2 ratio value of -0.28 (marked in orange). **B** For the *MGMT* methylated subgroup of the training rand-P cohort an AUC = 0.50 was obtained, no cutoff point was not calculated.

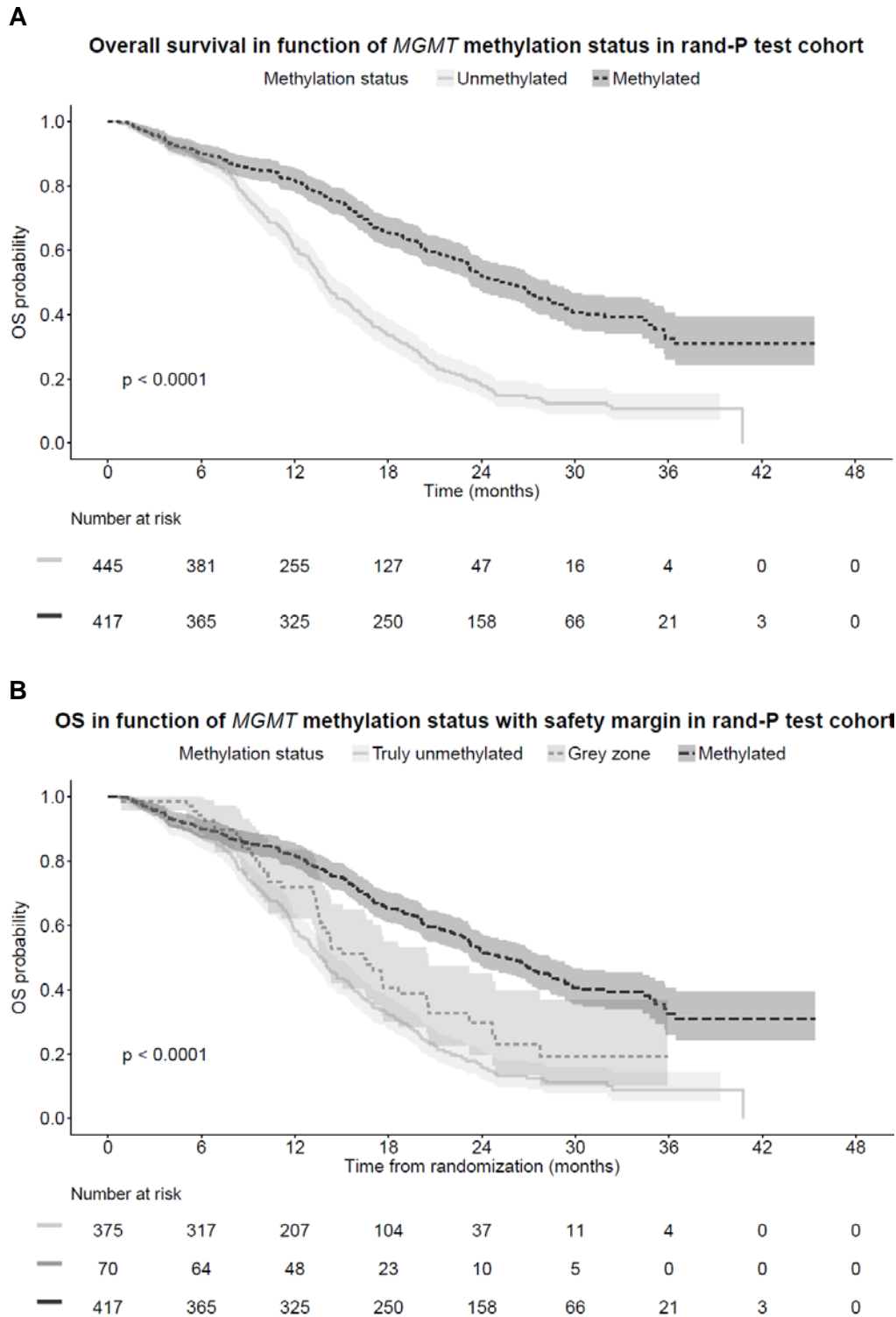


Figure S2. Validation of the new *MGMT* classification in the rand-P Test cohort.

A Kaplan Meyer plot visualizes OS in the randomized patient (rand-P) test cohort separated into *MGMT* promoter methylated and unmethylated patients. OS was significantly different between the groups ($p < 0.0001$, logrank test). **B** The outcome of patients in the rand-P test cohort separated into *MGMT* methylated (< 1.27), grey zone (-0.28 , and ≤ 1.27), and truly unmethylated patients (< -0.28) ($p < 0.0001$, logrank test) are shown in a Kaplan Meyer plot. The shaded area represents the 95% confidence interval.