Title: Withholding temozolomide in glioblastoma patients with unmethylated MGMT promoter–still a dilemma?

Authors: Hegi ME, Stupp R

Journal: Neuro-oncology

Year: 2015 Nov

Issue: 17

Volume: 11

Pages: 1425-7

DOI: 10.1093/neuonc/nov198

Withholding TMZ in GBM patients with unmethylated *MGMT – still a dilemma ?*

Monika E. Hegi and Roger Stupp

Neuroscience Research Center, Service of Neurosurgery, Lausanne University Hospital, Lausanne (CHUV) (MEH); Department of Oncology, University Hospital Zurich, Zurich, Switzerland

Corresponding author: Monika E. Hegi, Department of Clinical Neurosciences, CHUV CLE C306, chemin des Boveresses 155, 1066 Epalinges, Switzerland (Monika.Hegi@chuv.ch)

Ten years ago we established *methyl-guanin methyl transferase* (*MGMT)* gene promoter methylation as the first predictive marker in neuro-oncology, and the strongest prognostic factor for treatment outcome in newly diagnosed glioblastoma (GBM). But rather than embracing a marker that allows identification and selection of patients likely to derive some benefit from the addition of alkylating agent chemotherapy, we have been challenging the validity of the findings, are striving for the one and perfect molecular test and treat the majority of patients with temozolomide (TMZ) chemotherapy irrespective of the tumors MGMT status. Isn't the data convincing enough, or is it the lack of effective alternative treatments to be offered to patients with an unmethylated *MGMT* ?

Following a large body of mechanistic evidence for the role of MGMT in repairing lesions of alkylating agents, MGMT expression has been advanced as a resistance factor in glioma in the nineties. Subsequently in a seminal work by Esteller and colleagues a correlation with promoter methylation of the *MGMT* gene was demonstrated in an analysis of samples from patients treated in Spain with chemotherapy comprising the alkylating agent carmustine (BCNU) [1]. We confirmed this observation in an unplanned analysis of patients treated within our phase II trial with upfront TMZ [2]. In 2005 finally, our retrospective analysis of prospectively treated patients within a randomized phase III trial demonstrated a clear predictive value of *MGMT* promoter methylation status [3]. Since, numerous additional trials have consistently demonstrated the prognostic effect of the MGMT status, but as all patients are now receiving upfront TMZ chemotherapy, the predictive value cannot be evaluated again. The one exception is elderly glioblastoma patients where the

relative benefit of adding chemotherapy was of lesser magnitude. Two randomized trials compared single agent TMZ chemotherapy versus radiotherapy (RT) [4,5]. In this more fragile patient population it was shown that treating *MGMT* unmethylated tumors with TMZ was detrimental, while patients with methylated tumors fared best if treated with TMZ (even in the absence of radiotherapy). These two trials confirm the predictive value of the MGMT status. Together, the data allows the conclusion that alkylating agent chemotherapy is of marginal benefit, if any for patients with an *MGMT* unmethylated GBM.

By continuing to treat the majority of *MGMT* unmethylated patients with TMZ chemotherapy we are missing an opportunity to do better. Innovative treatment approaches, novel agents in combination with radiotherapy may provide a better chance for improved outcome than sticking to an agent with marginal activity. From the patient's point of view it may be perceived as "wasting the last opportunity" for trying a potentially efficacious new agent. Evidently this patient population would benefit most from drugs with other mechanisms of action. To date, only a few trials have selected patients and assigned treatments according to the *MGMT* promoter status [6-9].

Adding a new drug or agent on top of the previously established combined modality regimen may cause undue toxicity or drug interaction, thus requiring dose reduction and treatment with potentially subtherapeutic doses. As an example, adding polygulatmated paclitaxel to the combination of TMZ/RT lead to early discontinuation due to prohibitive toxicity [10], but incited a follow-up trial in *MGMT* unmethylated patients only, omitting TMZ during RT (www.clinicaltrials.gov: NCT01402063). Still, patients with an unmethylated *MGMT* promoter are in greatest need for improved treatments, and may benefit from the opportunity to replace TMZ by novel agents. In a randomized EORTC trial for patients with an unmethylated *MGMT* promoter only; temsirolimus was combined with RT followed by temsirolimus maintenance and compared to standard TMZ/RT →TMZ. Similarly, Herrlinger and colleagues randomized patients with an unmethylated *MGMT* promoter to either standard TMZ/RT→TMZ, or RT combined with irinotecan and bevacizumab followed by maintenance irinotecan/bevacizumab. Although both trials failed to show improved outcome as compared to the standard, it is important to note, that dropping TMZ was not detrimental (Table 1).

Treatment selection according to a molecular marker is intimately dependent on the validity and reproducibility of the molecular test. Standardizing the MGMT assay and determining the optimal cutoff based on outcome prediction have been a challenge. It is obvious that choice of methodology, and quantity and quality of the sample may yield different limits of detection and levels of precision for prediction. Of note, unlike a mutation that is present or absent, promoter methylation creates a pattern recognized by so called methyl-binding proteins, which are relevant for inhibition of expression. These patterns are identified by different means depending on the methodology. This can result in some discrepancies of classification, affecting mostly samples with incomplete methylation. However, like for any test in medicine, appropriate validation is required, including but not limited to reproducibility and association with outcome in an independent prospective cohort. Prospective testing in the trials reported earlier has been performed centrally using a quantitative methylation specific PCR assay that is commercially available [11]. In this assay the technical cutoff between methylated and unmethylated is set at the nadir of the bimodal distribution of the methylated *MGMT* measured (ratio with a normalizing gene) in a large population of samples. Evidently, there is a grey zone around the cutoff that can be approximated by a confidence interval. In two of the trials dropping TMZ [8,9] the lower bound of the 95% confidence interval was used to select unmethylated patients (cutoff with a “safety margin”), in order to avoid withholding TMZ from a patient who could potentially profit. The in and outs of *MGMT* testing have been reviewed extensively elsewhere [12].

Additional biomarkers are required for appropriate testing of new targeted drugs allowing for enrichment of the potentially sensitive patient population. However, the frequency of a potentially druggable target may be so low (e.g. 3% for FGFR3-TACC3 fusions [13]) that conducting prospective and controlled clinical trials is practically almost impossible. Quality assurance and the paucity of material available in the brain require platforms that will provide an array of biomarkers, rather than individual tests.

Patients with unmethylated GBM are in need for better treatments, and this population not only offers the opportunity to test novel treatments, but actually require more than others that they are offered innovative therapies right from the diagnosis of a GBM. The extended experience of the predictive value of the MGMT status in GBM, and the reassuring first results from trials selecting patients with an unmethylated *MGMT* for experimental therapy omitting TMZ, provide sufficient confidence for such an adapted trial designs. Recruiting patients according to their

MGMT status opens opportunities for innovative new therapies not limited by the treatment scheme of TMZ and respective toxicity. This will a llow focusing on new drugs - which need to be developed together with their proper biomarkers.

References:

1. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med.* 2000; 343(19):1350-1354.

2. Hegi ME, Diserens AC, Godard S, et al. Clinical trial substantiates the predictive value of O-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. *Clin Cancer Res.* 2004; 10(6):1871-1874.

3. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005; 352(10):997-1003.

4. Malmstrom A, Gronberg BH, Marosi C, et al. Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. *Lancet Oncol.* 2012; 13(9):916-926.

5. Wick W, Platten M, Meisner C, et al. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. *Lancet Oncol.* 2012; 13(7):707-715.

6. Stupp R, Hegi ME, Gorlia T, et al. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol.* 2014; 15(10):1100-1108.

7. Nabors LB, Fink KL, Mikkelsen T, et al. Two cilengitide regimens in combination with standard treatment for patients with newly diagnosed glioblastoma and unmethylated MGMT gene promoter: results of the open-label, controlled, randomized phase II CORE study. *Neuro Oncol.* 2015; 11.

8. Wick W, Gorlia T, van den Bent MJ, et al. Radiation therapy and concurrent plus adjuvant temsirolimus (CCI-779) versus chemoirradiation with temozolomide in newly diagnosed glioblastoma without methylation of the MGMT gene promoter. *J Clin Oncol.* 2014; 32: (5s):suppl; abstr 2003.

9. Herrlinger U, Schäfer N, Steinbach JP, et al. The randomized, multicenter glarius trial investigating bevacizumab/irinotecan vs standard temozolomide in newly diagnosed, mgmt-non-methylated glioblastoma patients: final survival results and quality of life. *Neuro-Oncology.* 2014; 16(suppl 2):ii23-ii24.

10. Jeyapalan S, Boxerman J, Donahue J, et al. Paclitaxel poliglumex, temozolomide, and radiation for newly diagnosed high-grade glioma: a Brown University Oncology Group Study. *Am J Clin Oncol.* 2014; 37(5):444-449.

11. Vlassenbroeck I, Califice S, Diserens AC, et al. Validation of real-time methylation-specific PCR to determine O6-methylguanine-DNA methyltransferase gene promoter methylation in glioma. *J Mol Diagn.* 2008; 10(4):332-337.

12. Wick W, Weller M, van den Bent M, et al. MGMT testing-the challenges for biomarker-based glioma treatment. *Nat Rev Neurol.* 2014; 10(10):100.

13. Singh D, Chan JM, Zoppoli P, et al. Transforming Fusions of FGFR and TACC Genes in Human Glioblastoma. *Science.* 2012.

14. Gilbert MR, Wang M, Aldape KD, et al. Dose-Dense Temozolomide for Newly Diagnosed Glioblastoma: A Randomized Phase III Clinical Trial. *J Clin Oncol.* 2013.

15. Gilbert MR, Dignam JJ, Armstrong TS, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med.* 2014; 370(8):699-708.

16. Chinot OL, Wick W, Mason W, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N Engl J Med.* 2014; 370(8):709-722.

Table 1: Outcome of *MGMT* unmethylated glioblastoma

Trial name experimental regimen	Publicatation year/ref.	Median Overall Survival [months]		
		Standard arm:	Experimental arm:	
		TMZ/RT→TMZ	TMZ/RT + novel agent	RT + novel agent
EORTC 26981 RT alone	2005 [3]	12.6		11.8 *
Glarius Beva/RT	2014 [9]	17.3		16.6
EORTC 26082: Temsirolimus	2014 [8]	16		14.8
CORE Cil (2x/wk)/TMZ/RT→Cil/TMZ Cil (5x/wk)/TMZ/RT→Cil/TMZ	2015[7]	13.4	16.3 14.5	
RTOG 0525 † TMZ/RT → TMZ (21/28d)	2012 [14]	16.6	15.4	
RTOG 0825† Beva/TMZ/RT → beva/TMZ	2014 [15]	14.6	14.0	
AvaGlio† Beva/TMZ/RT → beva/TMZ	2014 [16]	16.7	16.8	

† subgroup of *MGMT* unmethylated tumors; * RT alone
RT, radiotherapy; TMZ, temozolomide; beva, bevacizumab; cil, cilengitide
EORTC, European Organisation for Research and Treatment of Cancer; RTOG, Radiation Therapy Oncology Group