Neuro-Oncology

Neuro-Oncology 17(11), 1425–1427, 2015
doi:10.1093/neuonc/nov198
Advance Access date 15 September 2015

Withholding temozolomide in glioblastoma patients with unmethylated *MGMT* promoter—still a dilemma?

Monika E. Hegi and Roger Stupp

Neuroscience Research Center, and Service of Neurosurgery, Lausanne University Hospital, Lausanne (CHUV) (M.E.H); Department of Oncology, University Hospital Zurich, Zurich, Switzerland (R.S)

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

Ten years ago we established O-6-methylguanine-DNA methyltransferase (*MGMT*) gene promoter methylation as the first predictive marker in neuro-oncology, and the strongest prognostic factor for treatment outcome in patients with 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 still striving for the one perfect molecular test, and are treating the majority of patients with temozolomide (TMZ) chemotherapy irrespective of the tumor's MGMT promoter status. Aren't the data convincing enough, or is it because of the lack of effective alternative treatments to be offered to patients with an unmethylated *MGMT* promoter?

Following a large body of mechanistic evidence for the role of MGMT in repairing lesions of alkylating agents, MGMT expression was advanced as a resistance factor in glioma in the 1990s. Subsequently, seminal work by Esteller and colleagues[1] demonstrated a correlation with promoter methylation of the *MGMT* gene in an analysis of samples from patients in Spain treated with chemotherapy comprising the alkylating agent carmustine (BCNU). We confirmed this observation in an unplanned analysis of patients treated within our phase II trial with upfront TMZ.[2] Finally, in 2005, 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 then, 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 could not be evaluated again. The one exception is elderly glioblastoma patients in whom the relative benefit of adding chemotherapy is 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 RT). These 2 trials confirm the predictive value of the MGMT status. Together, the data allow the conclusion that alkylating agent chemotherapy is of marginal benefit, if any, for patients with *MGMT* unmethylated GBM.

By continuing to treat the majority of *MGMT* unmethylated patients with TMZ, we are missing an opportunity to do better. Innovative treatment approaches with novel agents in combination with RT may provide a better chance for improved outcome than adhering to the use of an agent with marginal activity. From the patient's point of view, it may be perceived as "wasting the last opportunity" to try a potentially efficacious new agent. Clearly, 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 *MGMT* promoter methylation 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, the addition of polyglutamated paclitaxel to the combination of TMZ/RT led to early discontinuation due to prohibitive toxicity,[10] but this resulted in 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 of improved treatments and may benefit from the opportunity to replace TMZ by novel agents. In a randomized European Organisation for Research and Treatment of Cancer (EORTC) trial for patients with an unmethylated *MGMT* promoter only, temsirolimus was combined with RT followed by temsirolimus maintenance and compared with standard TMZ/RT followed by TMZ.[8] Similarly, Herrlinger and colleagues[9] randomized patients with an unmethylated *MGMT* promoter to either standard TMZ/RT followed by TMZ or RT combined with irinotecan and bevacizumab followed by maintenance irinotecan/bevacizumab. Although both trials failed to show improved outcome compared with 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

Table 1. Outcome of *MGMT* unmethylated glioblastoma

Trial Name Experimental Regimen	Publication Year/Ref.	Median Overall Survival, mo		
		Standard Arm	Experimental Arm	
		TMZ/RT→TMZ	TMZ/RT + Novel Agent	RT + Novel Agent
EORTC 26981	2005[3]			
RT alone		12.6		11.8*
Glarius	2014[9]			
Beva/RT		17.3		16.6
EORTC 26082	2014[8]			
Temsirolimus		16		14.8
CORE	2015[7]			
Cil (2×/wk)/TMZ/RT→Cil/TMZ		13.4	16.3	
Cil (5×/wk)/TMZ/RT→Cil/TMZ			14.5	
RTOG 0525†	2012[14]			
TMZ/RT→TMZ (21/28d)		16.6	15.4	
RTOG 0825†	2014[15]			
Beva/TMZ/RT→beva/TMZ		14.6	14.0	
AVAglio†	2014[16]			
Beva/TMZ/RT→beva/TMZ		16.7	16.8	

Abbreviations: Beva, bevacizumab; cil, cilengitide; RTOG, Radiation Therapy Oncology Group; AVAglio, Avastin in Glioblastoma.
†Subgroup of *MGMT* unmethylated tumors.
*RT alone.

optimal cutoff for 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 that mostly affect samples with incomplete methylation. As with any test in medicine, however, 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 was set at the nadir of the bimodal distribution of the methylated *MGMT* measured (ratio with a normalizing gene) in a large population of samples.[6] Evidently, there is a gray zone around the cutoff that can be approximated by a confidence interval. In 2 of the trials dropping TMZ,[8,9] the lower boundary of the 95% CI 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 challenges of *MGMT* testing have been reviewed extensively elsewhere.[12]

Additional biomarkers are required for appropriate testing of new targeted drugs allowing for selective enrichment of the potentially sensitive patient population. The frequency of a potentially druggable target, however, may be so low (eg, 3% for fibroblast growth factor receptor 3–transforming acidic coiled-coil protein 3 fusions[13]) that conducting prospective and controlled clinical trials is practically 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 of better treatments. This population not only offers the opportunity to test novel treatments but actually requires—more than other patients—that they be offered innovative therapies right from the diagnosis of GBM. The extended experience of the predictive value of the MGMT status in GBM and the reassuring first results from trials selecting patients with unmethylated *MGMT* for experimental therapy omitting TMZ provide sufficient confidence for such an adapted trial design. Recruiting patients according to their MGMT status opens opportunities for innovative new therapies not limited by the treatment scheme of TMZ and its toxicity. This will allow us to focus on new drugs that need to be developed together with their corresponding 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;17(5):708–717.
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;abstract # 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(7):372–385.
13. Singh D, Chan JM, Zoppoli P, et al. Transforming fusions of FGFR and TACC genes in human glioblastoma. *Science*. 2012; 337(6099):1231–1235.
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;31(32):4085–4091.
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.