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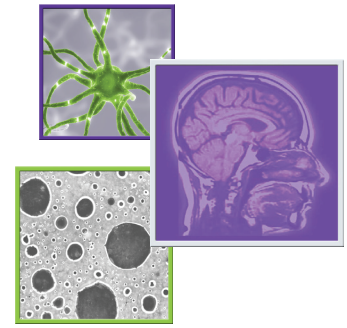

Assessing *MGMT* methylation status and its current impact on treatment in glioblastoma

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SUMMARY *MGMT* promoter methylation status is a strong and independent prognostic factor in patients with newly diagnosed glioblastoma and a clinically relevant predictive marker in the subpopulation of elderly glioblastoma patients. However, there is still lack of consensus on the optimal assay for reliable *MGMT* promoter methylation testing and a variety of test are being used in different laboratories. Pyrosequencing is the only method for which an adequately high analytical performance (high intra- and inter-laboratory repeatability and reproducibility) has been demonstrated in a fully published ring trial. For clinical decision-making *MGMT* promoter methylation testing should be performed only in experienced laboratories using meticulous validation of assay accuracy. Ideally, such laboratories should undergo regular accreditation through a quality control consortium.

KEYWORDS
biomarker • glioma • *MGMT* promoter methylation • O6-methylguanine-methyltransferase • temozolomide

Glioblastoma is the most common primary brain tumor of adults and has a high morbidity and mortality. The median overall survival times across contemporary clinical trials enrolling patients with newly diagnosed glioblastoma are in the range of 1–1.5 years with a wide range of outcomes with some patients surviving only few weeks or months and others achieving relatively long survival of several years [1]. The established treatment options comprise maximal safe resection, radiotherapy and alkylating chemotherapy with temozolomide [1–3]. Despite extensive and sophisticated research into the biology of glioma and the availability of vast datasets on molecular alterations in this tumor type, only few biomarkers with potential clinical relevance have emerged. The most important molecular biomarker with proven clinical impact in glioblastoma is the promoter methylation status of the *MGMT* gene [4–6].

MGMT: function & pathobiology

MGMT is a DNA-repair protein that removes methyl groups from the O6-position of guanine and in 2000 Esteller *et al.* described a sensitizing effect to alkylating chemotherapy in glioblastoma [7,8]. The *MGMT* gene is localized on the telomeric end of the long arm of the chromosome 10 and contains 98 CpG sites in the first of five exons and the promoter region. Hypermethylation of the *MGMT* gene promoter is associated with gene silencing and reduced expression of the MGMT protein, although it has yet to be elucidated which of the CpG sites determine *MGMT* expression [9,10].

Prognostic value of *MGMT* promoter methylation status in glioblastoma

A large number of studies have confirmed the positive prognostic value of *MGMT* promoter hypermethylation in adult glioblastomas since its identification in the trial population of the European

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Organisation for Research and Treatment of Cancer/National Cancer Institute Canada (EORTC/NCIC) study published in 2005 (Table 1) [11–15]. Thus, *MGMT* testing may aid clinical management of adult glioblastoma patients by indirectly influencing therapy and follow-up strategies. However, in adult patients below the age of 65–70 years, the *MGMT* promoter methylation status does not carry predictive information as it is not unequivocally associated with response to a particular therapy (Figure 1).

The initial data on the prognostic role of the *MGMT* status were generated with semiquantitative methods yielding a binary test result (*MGMT* promoter methylated versus unmethylated) such as MS-PCR [11]. Variants of this method or other techniques methods, e.g., methylation-specific quantitative real-time PCR or pyrosequencing of bisulfite-modified DNA, result in quantitative assay readouts that provide information on the relative extent of promoter methylation (i.e., the number of methylated CpG sites per gene allele) [18–21]. Interestingly, some studies seem to imply that the percentage of methylated CpG sites is positively correlated with patient survival times [22,23]. This association is of potential clinical value, but needs verification and further study in large and prospective patient populations.

Predictive value of *MGMT* promoter methylation status in glioblastoma

The results of two prospective randomized trials and one prospectively collected large case series show that the *MGMT* promoter methylation status is a clinically relevant predictive marker in the population of elderly patients with newly diagnosed glioblastoma [16–17,23]. Importantly, combination of radiation and chemotherapy is not a practicable treatment option in the majority of elderly patients, as the overall constitution and comorbidities limit the treatment tolerance. Further, a decreasing benefit from chemotherapy and a high risk of cognitive decline from cranial radiation were described previously for elderly patients treated with combination chemoradiotherapy [24].

The NOA-08 study enrolled 412 patients with anaplastic astrocytoma or glioblastoma aged 65 years or older and with a Karnofsky performance score of 60% or higher into a chemotherapy alone arm (temozolomide 100 mg/m^2 given on days 1–7, 1 week on, 1 week off) or radiotherapy alone (60 Gy over 6–7 weeks in 30 fractions of 1.8–2.0 Gy) [17]. Concerning eventfree survival, *MGMT* promoter methylated tumors responded better to temozolomide than to radiotherapy, while the opposite was true for unmethylated tumors. A similar, albeit statistically nonsignificant, result was observed for overall survival.

The Nordic glioma trial randomized 291 patients with newly diagnosed glioblastoma and aged 60 years and older to either chemotherapy alone (temozolomide 200 mg/m^2 d1–5 of each 28-day cycle) or radiotherapy alone (either 34.0 Gy in 3.4 Gy fractions over 2 weeks or 60.0 Gy in 2.0 Gy fractions over 6 weeks) [16]. Among patients treated with temozolomide, those with *MGMT* methylated tumors had significantly longer survival than those with *MGMT* unmethylated tumors, while no survival differences were apparent between patients with methylated or unmethylated tumors treated with radiotherapy.

The German Glioma network prospectively collected a series of 233 glioblastoma patients aged 70 years or more and compared survival outcomes according to therapy and *MGMT* promoter methylation status [23]. Patients with *MGMT* methylated tumors had longer progression-free survival times when their treatment contained temozolomide as compared with patients treated with sole radiotherapy. In contrast, patients with *MGMT* unmethylated tumors did not show a survival benefit from chemotherapy.

An interesting unresolved issue concerns the optimal treatment choice for patients in whom

Table 1. Landmark trials on the predictive and prognostic value of *MGMT* promoter methylation status in adult glioblastoma patients.

Study	Patient age (years)	Prognostic value	OS unmethylated *MGMT* promoter (months)	OS methylated *MGMT* promoter (months)	Predictive value for response to temozolomide	Ref.
Hegi *et al.*	18–70	Yes	12.2	18.2	No	[11]
Gilbert *et al.*	>18	Yes	21.2	14.0	No	[13]
Malmström *et al.*	>60	No	6.9	9.0	Yes	[16]
Wick *et al.*	>65	Yes	8.2	11.9	Yes	[17]

OS: Overall survival.

MGMT testing is not possible or shows inconclusive results. The available evidence suggests that such patients should be treated with radiotherapy, because in the NOA-08 trial patients with *MGMT* unmethylated tumors treated with temozolomide had the worse prognosis than patients with *MGMT* unmethylated or methylated tumors treated with radiotherapy [17].

An important an unresolved issue relates to the identification of elderly patients that are fit enough to be offered combined radiochemotherapy, as the individuality of genetic constitution and the accumulated lifelong aging process results in significant diversity among persons of the same chronological age. A comprehensive geriatric assessment or at least a shortened evaluation of the individual´s comorbidities, resources and deficits for the tolerance of oncologic therapies has been found helpful before choosing treatment for cancer [25–30]. Further studies are needed to identify helpful strategies for standardized geriatric assessment and its impact on treatment recommendations in glioblastoma patients.

MGMT promoter methylation testing: technical issues

A variety of test methods are in use for *MGMT* promoter methylation testing. There is widespread agreement that RNA-based and protein-based (e.g., activity assays, western blot, immunohistochemistry) MGMT assays lack the necessary requirements to be of clinical use [20,31–33]. Most of these methods are limited because they require unfixed tumor tissue specimens, need special equipment and are time-consuming and for MGMT immunohistochemistry a low interobserver reliability and lack of a robust correlation with the promoter methylation status has been shown [34]. Therefore, methods directly assessing the *MGMT* promoter methylation status at the DNA-level need to be preferred for clinical purposes. However, there is lack of consensus on the optimal laboratory method that fulfills all criteria needed for clinical decision making, that is, high analytical performance (reproducibility and repeatability) and clinical performance (robust correlation with patient outcome).

Most available studies including several large prospective clinical trials have used Methylation Specific Polymerase Chain Reaction (MS-PCR) for *MGMT* testing [11,17,35]. However, this methodology is commonly criticized for poor analytical performance with lack of reproducibility and repeatability of test results. One study documented consistent results of MS-PCR in only 1of 6 specimens in four repetitive runs [18]. Furthermore, several publications show relatively high percentages of MS-PCR runs, e.g., 54.2% [18], 44% [17] and 32.9% [11], yielding inconclusive results, particularly in cases with small tumor tissue samples; for example, from neurosurgical biopsies. The reasons for the suboptimal performance of MS-PCR in clinical tumor samples are unclear, but may be related to chemical DNA alterations associated with formalin-fixation, as frozen samples or samples preserved by alternative fixation methods such as RCL2 seem to yield better results [36]. Of note, singular centers seem to be satisfied with MS-PCR performance (personal communications) and it seems conceivable that strict protocol adherence, rigorous quality control and experienced staff are indeed able to produce reliable results even with a challenging analytical technique. However, the lack of published detailed MS-PCR protocols with proven high analytical performance limits evidence-based critical evaluation and widespread application of this method. In particular, there is a need for well-designed investigations assessing the intra- and interlaboratory reliability of MS-PCR in routine formalin-fixed and paraffin-embedded glioblastoma samples. Ideally, such studies should include samples that were subject to preanalytical processing in several different neuropathology laboratories, as there is a high interlaboratory variability in several important parameters such as exact formalin formula or fixation times.

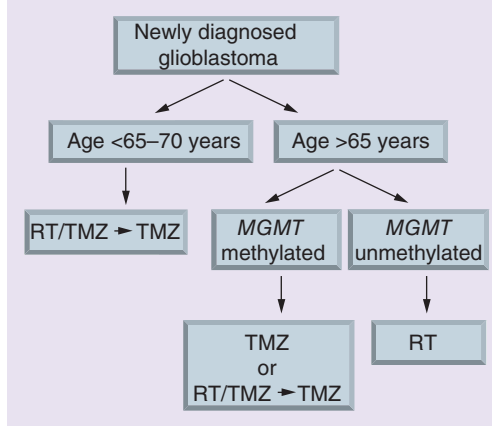

Figure 1. Decision tree diagram according to age and *MGMT* promoter methylation status in patients with newly glioblastoma.
MGMT: O6-methyl-guanine-methyl-transferase.

So far, the results of only one interlaboratory ring trial assessing the analytical performance of a DNA-based method for *MGMT* promoter methylation testing have been reported in a full publication [32]. In this project, 18 specimens of 9 patients with known *MGMT* promoter methylation as determined by control analyses from frozen tissue parts in two independent laboratories using methylation-sensitive restriction enzyme (MSRE)-based quantitative PCR (qPCR) and a MALDI-Epityper assay were included. Of each case, one formalin-fixed/paraffin-embedded and one RCL2- fixed/paraffin-embedded sample were repeatedly (at least twice and up to five times) and independently analyzed using pyrosequencing in two laboratories. Concordance rates between the individual analytical runs were assessed in a separate and independent laboratory. There was a perfect reproducibility and repeatability of MGMT pyrosequencing with 100% concordance rate among all individual test results irrespective of tissue fixation conditions, testing laboratory, testing technician and time point of testing. These data suggest that pyrosequencing is a useful method for MGMT testing in the routine setting. However, some issues remain to be resolved in order to refine its clinical application, for example the identification of the CpG islands with the highest clinical (i.e., prognostic and predictive) value and the definition of unequivocal cutoffs for defining *MGMT* promoter methylated vs. unmethylated cases based on the quantitative test results (the percentage of methylated alleles for each of the investigated CpG sites). A cutoff of around 8% has been used in several studies, but needs validation in larger investigations [22,37]. Although the conclusions are limited by the patient number and need further validation in larger patient cohorts and more laboratories, this interlaboratory trial ring trial is the first to clearly address the important issue of analytical performance, i.e., the reproducibility within one laboratory and between different laboratories.

Another ring trial has been performed but has only been reported in abstract form so far [38]. In this study, *MGMT* promoter methylation status was tested in DNA extracted from frozen and formalin-fixed and paraffin embedded tumor tissue samples of 16 glioblastoma cases in 23 academic institutions in Germany, Austria and the Netherlands. The methods facilitated in the various centers included MS-PCR, pyrosequencing of bisulfite-modified DNA, multiplex ligation-dependent probe amplification (MLPA), methylation-quantification of endonuclease-resistant DNA (MethyQESD), and PCR-based fragment analysis. Apparently, suboptimal concordance of test results, particularly in cases with weak or partial *MGMT* promoter methylation was documented [38]. The results of this study seem to underscore once more that MGMT testing is technically far from trivial and represents an unresolved problem in neurooncology. However, full publication of this study is required for critical interpretation of its implications for clinical practice and further research projects.

Conclusion & future perspective

In the recent years, the prognostic and predictive role of the *MGMT* promoter methylation status has been established. At least for the population of elderly glioblastoma patients direct clinical consequences can be derived from MGMT testing and therefore it needs to be considered as a part of the current standard of care [1,39]. Importantly, *MGMT* promoter methylation is not an 'absolute' predictive marker for response to temozolomide, as other molecular factors also influence the response. The main current challenge is the lack of commonly accepted standard operating procedures that clearly define how to reliably asses the *MGMT* promoter methylation status in the clinical setting. Due to the technical difficulties associated with *MGMT* testing, it should be performed only in experienced laboratories and using meticulous validation of assay accuracy for clinical decision making. Ideally, such laboratories should undergo regular accreditation through a quality control consortium.

An interesting approach that also warrants further research is the determination of the *MGMT* promoter methylation in circulating DNA in the blood [40]. *MGMT* analysis from blood samples could potentially help to assess prognosis and make treatment choices in patients not amenable to neurosurgical intervention, which could be of particular interest in recurrent or progressive disease.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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EXECUTIVE SUMMARY

- MGMT promoter methylation status is a strong prognostic factor in patients with newly diagnosed glioblastoma and a clinically relevant predictive marker in the subpopulation of elderly patients (>65–70 years) with newly diagnosed glioblastoma.
- MGMT promoter methylation status should be determined in elderly patients with newly diagnosed glioblastoma who do not qualify for combined radiochemotherapy to recommend them to either radiotherapy alone (MGMT promoter unmethylated or inconclusive) or temozolomide alone (MGMT promoter methylated).
- MGMT promoter methylation testing is technically challenging and for clinical decision evaluation should be performed in experienced laboratories using elaborate quality control measures.
- For clinical MGMT promoter methylation testing, DNA-based assays should be preferred over RNA-based or protein-based (e.g., immunohistochemistry) assays.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Weller M, Van Den Bent M, Hopkins K *et al.* EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. *Lancet Oncol.* 15(9), e395–e403 (2014).

2 Preusser M, De Ribaupierre S, Wohrer A *et al.* Current concepts and management of glioblastoma. *Ann. Neurol.* 70(1), 9–21 (2011).

3 Stupp R, Mason WP, Van Den Bent MJ *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* 352(10), 987–996 (2005).

4 Weller M, Pfister SM, Wick W, Hegi ME, Reifenberger G, Stupp R. Molecular neuro-oncology in clinical practice: a new horizon. *Lancet Oncol.* 14(9), e370–e379 (2013).

5 Weller M, Stupp R, Hegi ME *et al.* Personalized care in neuro-oncology coming of age: why we need MGMT and 1p/19q testing for malignant glioma patients in clinical practice. *Neuro Oncol.* 14(Suppl. 4), iv100–iv108 (2012).

6 Tabatabai G, Stupp R, Van Den Bent MJ *et al.* Molecular diagnostics of gliomas: the clinical perspective. *Acta Neuropathol.* 120(5), 585–592 (2010).

7 Liu L, Gerson SL. Targeted modulation of MGMT: clinical implications. *Clin. Cancer Res.* 12(2), 328–331 (2006).

8 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.* 343(19), 1350–1354 (2000).

9 Bhakat KK, Mitra S. Regulation of the human O(6)-methylguanine-DNA methyltransferase gene by transcriptional coactivators cAMP response element-binding protein-binding protein and p300. *J. Biol. Chem.* 275(44), 34197–34204 (2000).

10 Watts GS, Pieper RO, Costello JF, Peng YM, Dalton WS, Futscher BW. Methylation of discrete regions of the O6-methylguanine DNA methyltransferase (MGMT) CpG island is associated with heterochromatinization of the MGMT transcription start site and silencing of the gene. *Mol. Cell. Biol.* 17(9), 5612–5619 (1997).

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

•• **The prognostic impact of O6-methyl-guanine-methyl-transferase (MGMT) promoter methylation in patients with newly diagnosed glioblastoma.**

12 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.* 15(10), 1100–1108 (2014).

13 Gilbert MR, Wang M, Aldape KD *et al.* Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. *J. Clin. Oncol.* 31(32), 4085–4091 (2013).

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

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

16 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.* 13(9), 916–926 (2012).

• **Phase III trial showing predictive value of MGMT status for temozolomid treatment in elderly patients.**

17 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.* 13(7), 707–715 (2012).

• **Phase III trial showing predictive value of MGMT status for temozolomid treatment in elderly patients.**

18 Preusser M, Elezi L, Hainfellner JA. Reliability and reproducibility of PCR-based testing of O6-methylguanine-DNA methyltransferase gene (MGMT) promoter methylation status in formalin-fixed and paraffin-embedded neurosurgical biopsy specimens. *Clin. Neuropathol.* 27(6), 388–390 (2008).

•• **A low reproduceability of MGMT status using PCR-based testing, indicating a low analytical performance of PCR-based testing.**

19 Pulverer W, Hofner M, Preusser M, Dirnberger E, Hainfellner JA, Weinhaeusel A. A simple quantitative diagnostic alternative for MGMT DNA-methylation testing on RCL2 fixed paraffin embedded tumors using restriction coupled qPCR. *Clin. Neuropathol.* 33(1), 50–6 (2013).

20 Quillien V, Lavenu A, Karayan-Tapon L *et al.* Comparative assessment of 5 methods (methylation-specific polymerase chain reaction, MethyLight, pyrosequencing,

methylation-sensitive high-resolution melting, and immunohistochemistry) to analyze O6-methylguanine-DNA-methyltranferase in a series of 100 glioblastoma patients. *Cancer* 118(17), 4201–4211 (2012).

21 Mikeska T, Bock C, El-Maarri O *et al.* Optimization of quantitative MGMT promoter methylation analysis using pyrosequencing and combined bisulfite restriction analysis. *J. Mol. Diagn.* 9(3), 368–381 (2007).

22 Dunn J, Baborie A, Alam F *et al.* Extent of MGMT promoter methylation correlates with outcome in glioblastomas given temozolomide and radiotherapy. *Br. J. Cancer* 101(1), 124–131 (2009).

23 Reifenberger G, Hentschel B, Felsberg J *et al.* Predictive impact of MGMT promoter methylation in glioblastoma of the elderly. *Int. J. Cancer* 131(6), 1342–1350 (2012).

24 Weller M, Platten M, Roth P, Wick W. Geriatric neuro-oncology: from mythology to biology. *Curr. Opin. Neurol.* 24(6), 599–604 (2011).

25 Puts MT, Hardt J, Monette J, Girre V, Springall E, Alibhai SM. Use of geriatric assessment for older adults in the oncology setting: a systematic review. *J. Natl Cancer Inst.* 104(15), 1133–1163 (2012).

26 Puts MT, Santos B, Hardt J *et al.* An update on a systematic review of the use of geriatric assessment for older adults in oncology. *Ann. Oncol.* 25(2), 307–315 (2014).

27 Kenis C, Bron D, Libert Y *et al.* Relevance of a systematic geriatric screening and assessment in older patients with cancer: results of a prospective multicentric study. *Ann. Oncol.* 24(5), 1306–1312 (2013).

28 Brunello A, Loaldi E, Balducci L. Dose adjustment and supportive care before and during treatment. *Cancer Treat. Rev.* 35(6), 493–498 (2009).

29 Girre V, Falcou MC, Gisselbrecht M *et al.* Does a geriatric oncology consultation modify the cancer treatment plan for elderly patients? *J. Gerontol. A Biol. Sci. Med. Sci.* 63(7), 724–730 (2008).

30 Extermann M, Boler I, Reich RR *et al.* Predicting the risk of chemotherapy toxicity in older patients: the Chemotherapy Risk Assessment Scale for High-Age Patients (CRASH) score. *Cancer* 118(13), 3377–3386 (2012).

31 Berghoff AS, Preusser M. Clinical neuropathology practice guide 06–2012: MGMT testing in elderly glioblastoma patients – yes, but how? *Clin. Neuropathol.* 31(6), 405–408 (2012).

32 Preusser M, Berghoff AS, Manzl C *et al.* Clinical Neuropathology practice news 1–2014: pyrosequencing meets clinical and analytical performance criteria for routine testing of MGMT promoter methylation status in glioblastoma. *Clin. Neuropathol.* 33(1), 6–14 (2014).

•• **Ring trial on MGMT pyrosequencing showing 100% concordance between experiments, suggesting a very high analytical performance of MGMT pyrosequencing.**

33 Karayan-Tapon L, Quillien V, Guilhot J *et al.* Prognostic value of O6-methylguanine-DNA methyltransferase status in glioblastoma patients, assessed by five different methods. *J. Neurooncol.* 97(3), 311–322 (2010).

34 Preusser M, Charles Janzer R, Felsberg J *et al.* Anti-O6-methylguanine-methyltransferase (MGMT) immunohistochemistry in glioblastoma multiforme: observer variability and lack of association with patient survival impede its use as clinical biomarker. *Brain Pathol.* 18(4), 520–532 (2008).

35 Wick W, Weller M, Van Den Bent M *et al.* MGMT testing – the challenges for biomarker-based glioma treatment. *Nat. Rev. Neurol.* 10(7), 372–385 (2014).

36 Preusser M, Plumer S, Dirnberger E, Hainfellner JA, Mannhalter C. Fixation of brain tumor biopsy specimens with RCL2 results in well-preserved histomorphology, immunohistochemistry and nucleic acids. *Brain Pathol.* 20(6), 1010–1020 (2010).

37 Havik AB, Brandal P, Honne H *et al.* MGMT promoter methylation in gliomas-assessment by pyrosequencing and quantitative methylation-specific PCR. *J. Transl. Med.* 10, 36 (2012).

38 Reifenberger G, Malzkorn B, Acker T *et al.* Results of the international interlaboratory comparison of MGMT promoter methylation analysis involving twenty-three academic centers in Germany, Austria and the Netherlands. *Neuro-oncology* 16, iii42–iii52 (2014).

39 Weller M, Stupp R, Reifenberger G *et al.* MGMT promoter methylation in malignant gliomas: ready for personalized medicine? *Nat. Rev. Neurol.* 6(1), 39–51 (2010).

40 Fiano V, Trevisan M, Trevisan E *et al.* MGMT promoter methylation in plasma of glioma patients receiving temozolomide. *J. Neur Oncol.* 117(2), 347–357 (2014).

•• **Study on MGMT status analysis from plasma specimens in glioblastoma patients.**