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CLINICAL STUDY

# Assessment of molecular markers demonstrates concordance between samples acquired via stereotactic biopsy and open craniotomy in both anaplastic astrocytomas and glioblastomas

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**Abstract** The classification, treatment and prognosis of high-grade gliomas has been shown to correlate with the expression of molecular markers (e.g. *MGMT* promotor methylation and IDH1 mutations). Acquisition of tumor samples may be obtained via stereotactic biopsy or open craniotomy. Between the years 2009 and 2013, 22 patients initially diagnosed with HGGs via stereotactic biopsy, that ultimately underwent open craniotomy for resection of their tumor were prospectively included in an institutional glioma database. *MGMT* promotor analysis was performed using methylation-specific (MS)-PCR and IDH1R132H mutation analysis was performed using immunohistochemistry. Three patients (13.7%) exhibited IDH1R132H mutations in samples obtained via stereotactic biopsy. Tissue derived from stereotaxic biopsy was demonstrated to have *MGMT* promotor methylation in ten patients (45.5%), while a non-methylated *MGMT* promotor was demonstrated in ten patients (45.5%); inconclusive results were obtained for the remaining two patients (9%) within our cohort. The initial histologic grading, IDH1R132H mutation and *MGMT* promotor methylation results were confirmed using samples obtained during open craniotomy in all but one patient; here inconclusive *MGMT* promotor analysis was obtained in contrast to that which was obtained via stereotactic biopsy. Tumor samples acquired via stereotactic biopsy provide accurate information with regard to clinically relevant molecular markers that have been shown to impact patient care decisions. The profile of markers analyzed in our cohort was nearly concordant between those samples obtained via stereotactic biopsy or open craniotomy thereby suggesting that clinical decisions may be based on the molecular profile of the tumor samples obtained via stereotactic biopsy.

**Keywords** Glioblastoma · Anaplastic astrocytoma · Molecular markers · *MGMT*-promotor methylation · IDH1 mutations · Stereotactic biopsy

**Abbreviations**

| | |
|---|---|
| HGG | High-grade glioma |
| MGMT | O6-methyl- guanine-DNA methyltransferase |
| IDH | Isocitrate dehydrogenase |
| WHO | World Health Organization |
| CT | Computed tomography |
| MRI | Magnetic resonance imaging |
| IHC | Immunohistochemistry |

## Introduction

The prognosis of patients suffering from high-grade gliomas (HGGs) remains poor with a median survival of approximately 12 months in population based studies [1]. Malignant gliomas account for about 70% of all primary brain tumors in adult patients and recent work has centered

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on understanding molecular markers in an effort to personalize prognostics/treatment regimens for patients in need [2]. Such work has led to the identification of molecular markers capable of stratifying HGG patients into subgroups thereby influencing treatment decisions [3].

One of the major molecular markers developed in glioma patients in recent years is the promoter methylation status of the gene encoding the enzyme O6-methyl-guanine-DNA methyltransferase (MGMT); the methylation status of the promoter is predictive of a better response to temozolomide (TMZ) as an adjuvant post-operative chemotherapeutic agent [4]. In line with such findings, the NOA-08 study demonstrated the value of treatment decisions based on *MGMT* promoter methylation status in elderly patients with GBM [5]. The clinical significance of such findings highlights the importance of *MGMT* promotor methylation assessment and currently, *MGMT* promotor methylation assessment is the most commonly performed molecular analysis in neuro-oncologic patients suffering from GBM [6].

A second major molecular marker employed in the diagnosis/management of both primary and secondary GBM patients is that of isocitrate dehydrogenase 1 (IDH1) mutations [7, 8]. The most common IDH1 mutation is located on chromosome 2q33 at amino acid residue 132 and is predominately found in grade II/III gliomas as well as in secondary GBM (sGBM) (~85%) [9]. Accordingly, such findings imply, that the presence of an IDH1 mutation may be used as a diagnostic and/or prognostic marker indicative of sGBM [10]. Recently, novel therapies targeting IDH1 mutations have shown promising results for tumor therapy [11, 12]; it is therefore tempting to speculate that IDH1R132 mutational status may result in clinically relevant treatment decisions.

It is prudent to note that HGGs are frequently unresectable due in part to proximity with eloquent and/or critical areas within the brain. In these patients, stereotactic biopsies, for both histological confirmation of the clinico-radiological diagnosis and/or evaluation of a tumors molecular marker profile are necessary. Accordingly, we performed histology, and assessed *MGMT* promotor methylation and IDH1R132 mutational status in specimens derived from patients who underwent stereotactic biopsies due to inconclusive preoperative imaging. Those patients that ultimately underwent open surgery for their now diagnosed glioma (i.e. via the stereotactic sample) were included within our analysis. Please note, biopsy was only performed because of inconclusive preoperative imaging (e.g. differential diagnosis of cerebral lymphoma having not been ruled out). Specimens derived from open craniotomy were ultimately compared to those that had been derived from stereotaxic biopsy in an effort to understand if results derived from both methods would in fact correlate.

## Patients and methods

All patients diagnosed with intracranial gliomas are entered into a prospective data registry at the University of Frankfurt. This study was approved by the University Hospital Institutional Review Board (reference # 04/09 SNO 01/08).

Attending neuropathologists participated in every stereotactic biopsy in an effort to confirm the pathology of the lesion. Stereotactic trajectories were planned by the attending neurosurgeon who would perform the procedure. During planning the surgeon accounted for tumor location, contrast enhancement, peritumoral edema, central necrosis and patient history. The number of biopsies, trajectories and all results from histopathological and molecular analyses were prospectively entered into our institutional glioma database.

Stereotactic planning was based on magnetic resonance imaging (MRI) acquired using a 3T scanner (Siemens Medical Solutions, Erlangen, Germany) which employed 3D isovolumetric T2 weighted and T1 weighted sequences with a spatial resolution of 1 $mm^3$. T1 weighted magnetization-prepared rapid-acquisition gradient echo (MPRAGE) sequencing (TR = 1900 ms; TE = 2.7 ms; inversion time = 900 ms; flip angle = 9°; field of view = 256 × 256 $mm^2$) was performed after intravenous injection of gadobutrol (1 mmol/ml) at a dose of 0.1 ml/kg body weight [13].

For all stereotactic biopsies, a stereotactic frame was utilized (Leksell Coordinate Frame G; Elekta Instruments, Stockholm, Sweden). On the day of surgery, cranial computed tomography (CT) with 1.5 mm slice thickness was performed after fixation of the head to the frame. Automated image co-registration with the preoperative MRI and trajectory planning was performed using iPlan software (BrainLAB, Feldkirchen, Germany). After skin incision and burr hole trepanation, the biopsy needle was inserted into the border of the lesion. Serial biopsies were obtained using micro-forceps with a diameter of 1.4 mm. The procedure was performed or supervised by one of three neurosurgeons with clinical expertise in stereotactic neurosurgical procedures.

Intraoperatively, single specimens (n = 1–3) were selected for smear preparation and stained with methylene blue in an effort to provide a preliminary intraoperative neuropathological diagnosis and to confirm that an adequate sample was retrieved from the pathologic areas for subsequent diagnostic procedures [14]. The remaining samples were fixed in formalin for 24–48 h before further processing. All tumor specimens were evaluated using classic hematoxylin & eosin (HE) staining and were classified according to the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) [15]. Immunohistochemical (IHC) analyses were employed

for the analysis of IDH1 (R132H). Microscopic evaluation of samples was performed using an Olympus BX50 light microscope (Olympus, Hamburg, Germany).

Two to 3 (median: 3) tumor specimens displaying the largest amount of vital tumor tissue (goal: >70% vital tumor tissue) were selected for methylation specific (MS)-PCR. Briefly, four slides of 10 µm thickness cut from each paraffin block were deparaffinized using xylene and two times 96% ethanol. DNA isolation, PCR and gel electrophoresis were performed and interpreted as has been previously described [13].

During open craniotomy, random samples were allocated for both *MGMT* promotor methylation assessment and IHC for IDH1 mutation analysis. In so doing we provide an unbiased approach for the assessment of molecular markers post-tumor resection.

### Statistical analyses

Nominal factors (e.g. type of sample acquisition) related to histology/MS-PCR /IHC results were analyzed using a contingency table followed by $\chi 2$ analysis. Cohen's kappa was analyzed in order to assess correlation between specimens obtained via stereotactic biopsy or via open craniotomy. A significance level of $\alpha = 0.05$ was selected for all tests and deemed to be significant. Statistical analyses were performed using SPSS software (SAS, Cary, USA).

## Results

We analyzed 22 consecutive patients from our institutional glioma database who initially underwent stereotactic biopsies and then went on to have an open resection procedure for their definitively diagnosed intracranial glioma (Fig. 1).

The median age of patients included within our study was 58 years (IQR 48–66 years). Thirteen patients were male (59.1%) and nine patients were female (40.9%). The Karnofsky Performance Score (KPS) of the patients included within our analyses is presented in Table 1 and ranged from 100 to 50. Three patients (13.6%) had a histopathologic diagnosis of WHO°III lesions (anaplastic astrocytomas) and 19 patients (86.4%) had a histopathologic diagnosis of WHO°IV (GBM). Only one patient (4.5%) had a prior history of glioma, while 21 patients (95.5%) had been newly diagnosed with glioma. The median time interval between stereotactic biopsy and open craniotomy was 13 days (IQR 9–17 days). It is important to note that patients included within this study cohort underwent both procedures without receiving additional therapy (i.e. between the stereotactic biopsy and open craniotomy). The adjuvant therapeutic regimen after open craniotomy (45.6%) consisted of concomitant radio/chemotherapy and temozolomide for 12 patients; four patients (18.2%) were participants in experimental trials, whilst the remaining eight patients (36.4%) had received other therapies (Table 1).

Within our cohort, MS-PCR of stereotactic biopsies yielded conclusive results in 20 (90.9%) of the included cases with both methylated and non-methylated *MGMT* promotors having been described (i.e. $n = 10$ patients for each of the aforementioned). Of note, inconclusive *MGMT* promoter methylation status was described in two (9.1%) of the patients analyzed (Fig. 2c).

We went on to examine if conclusive *MGMT* promoter methylation was associated with the pattern in which the stereotaxic samples were acquired. Hence, we dichotomized trajectories into those which were directed at the lesion center ("centered") and those which were tangentially directed at the lesion border ("tangential"). No significant differences were observed with regard to a higher level of conclusive results in MS-PCR obtained from patients biopsied via a tangential stereotactic trajectory (92.9% conclusive MS-PCR results) in comparison to those patients biopsied via centered trajectories (87.5% conclusive MS-PCR results; Table 2).

No significant differences were observed in the total number of tumor specimens taken by the neurosurgeon ($p = 0.88$) between the patients showing conclusive (median 17; IQR 14–19) and those showing inconclusive results (median 17; IQR 14–18) in MS-PCR for *MGMT* promoter methylation (data not shown). In line with such findings, the absolute number of paraffin-embedded samples ($p = 0.9$) taken for intraoperative neuropathological diagnosis was not associated with conclusiveness of results in MS-PCR.

Critically within our cohort, no complications with the exception of minor bleeding were observed. In 6 patients (27.3%) the attending surgeon documented an intraoperative observation of blood effusion in the trajectory path. All six patients had minor postoperative signs of hemorrhage evident on postoperative CT scan; none of the other patients had postoperative imaging suggestive of bleeding. In total, two patients (9.1%) developed temporary neurological worsening, yet recovered over the observed clinical course (i.e. no patient suffered a permanent deficit).

In the samples acquired via stereotactic biopsy, conventional histology revealed WHO°III tumors in 3 (12.4%) patients and WHO°IV GBM in 19 patients (87.6%). The same results were obtained, when using samples acquired via open craniotomy. Cohen's kappa, when comparing both modes of sample acquisition, was 1 ($p < 0.001$; Fig. 2a).

Assessment of molecular markers consisted of IHC staining of IDH1R132H and MS-PCR for *MGMT* promotor analysis. Analysis of markers revealed IDH1R132H mutation in three patients in those specimens obtained during

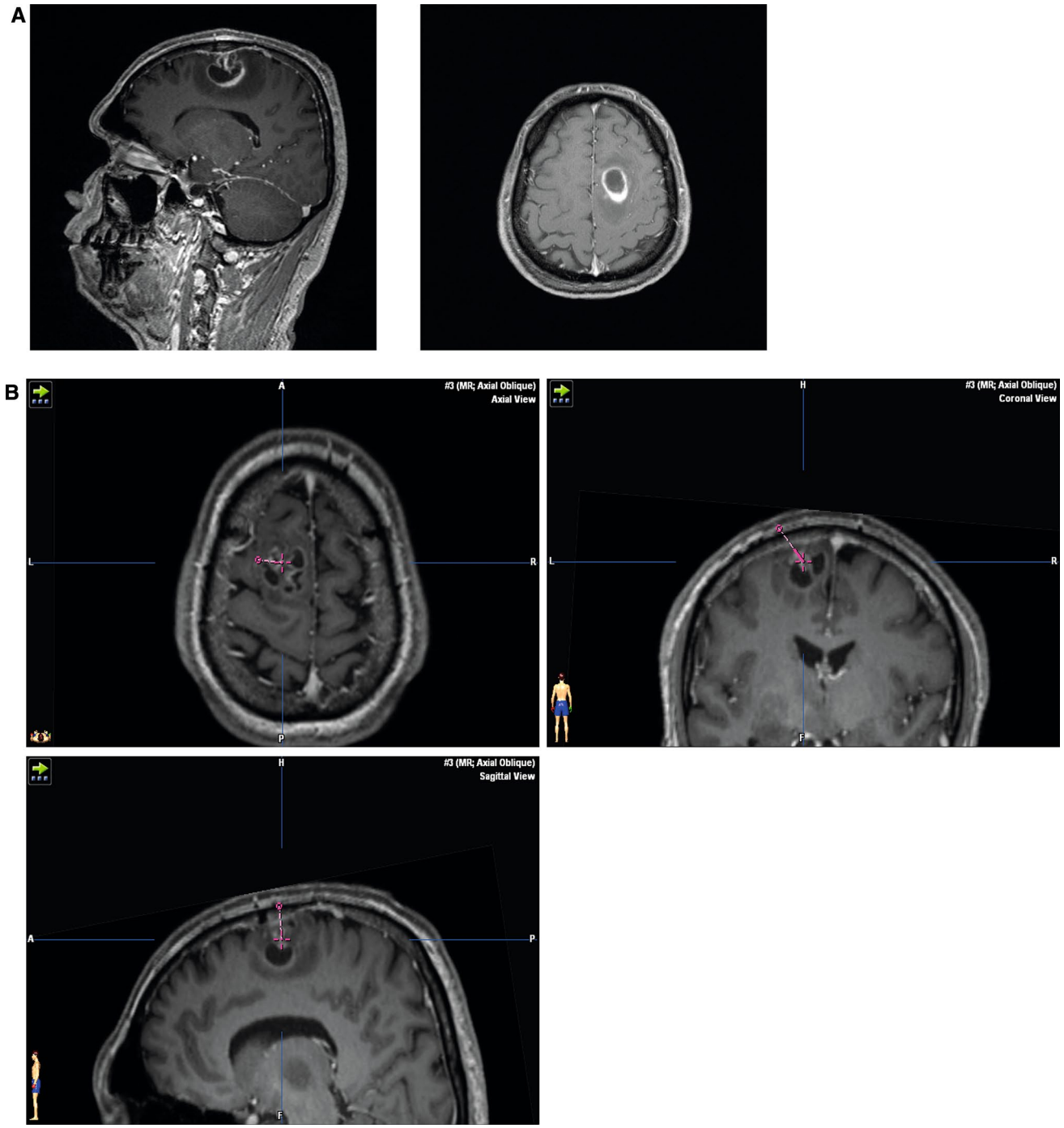


**Fig. 1** Illustrative case. **a** Sagittal and axial MR imaging of an exemplary patient. Based on imaging, no definitive diagnosis of glioma was made. **b** Stereotactic biopsy and sample acquisition using a trajectory tangential to the contrast enhancement was performed and a WHO °IV glioblastoma diagnosed. Open craniotomy and resection of the tumor was performed, followed by postoperative MRI demonstrating complete resection of the tumor. *WHO* World health organization

both stereotactic biopsy and open craniotomy. The remaining patients were immunonegative using IHC and were considered wildtype with regard to IDH1 mutational status. Between both modes of sample acquisition, Cohen's kappa was 1 ($p < 0.001$; Fig. 2b).

Assessment of *MGMT* promotor methylation using samples obtained via stereotactic biopsy resulted in conclusive results in 20 patients (90.9%). Of these, ten patients displayed a methylated *MGMT* promotor and ten patients an unmethylated *MGMT* promotor. In samples acquired via

**Table 1** Demographic and clinical patient data

| | All patients (n = 22) |
|---|---|
| Age (years) | 58 (48–66) |
| Sex (n [%]) | |
| Male | 13 (59.1) |
| Female | 9 (40.9) |
| KPS (n [%]) | |
| 100 | 4 (18.2) |
| 90 | 2 (9.1) |
| 80 | 5 (22.7) |
| 70 | 6 (27.3) |
| ≤60 | 5 (22.7) |
| Histological diagnosis (n [%]) | |
| WHO III Anaplastic Astrocytoma | 3 (13.6) |
| WHO IV Glioblastoma | 19 (86.4) |
| Diagnosis (n [%]) | |
| *De novo* | 21 (95.5) |
| Recurrent glioma | 1 (4.5) |
| Interval between biopsy and craniotomy (d [IQR]) | 13 (9–17) |
| Adjuvant therapy (n [%]) | |
| Stupp | 12 (45.6) |
| Trial | 4 (18.2) |
| Other | 8 (36.4) |

Data are given as median. Values given in parentheses indicate range unless otherwise defined

*KPS* Karnofsky performance score, *WHO* World Health Organization, *IQR* Interquartile range

open craniotomy, 19 patients displayed conclusive results with one patient previously diagnosed with a methylated *MGMT* promotor (using a stereotaxic sample) now having been diagnosed with an inconclusive *MGMT* promotor status. Cohen's kappa, when comparing both modes of sample acquisition, was 0.92 ($p < 0.001$; Fig. 2c).

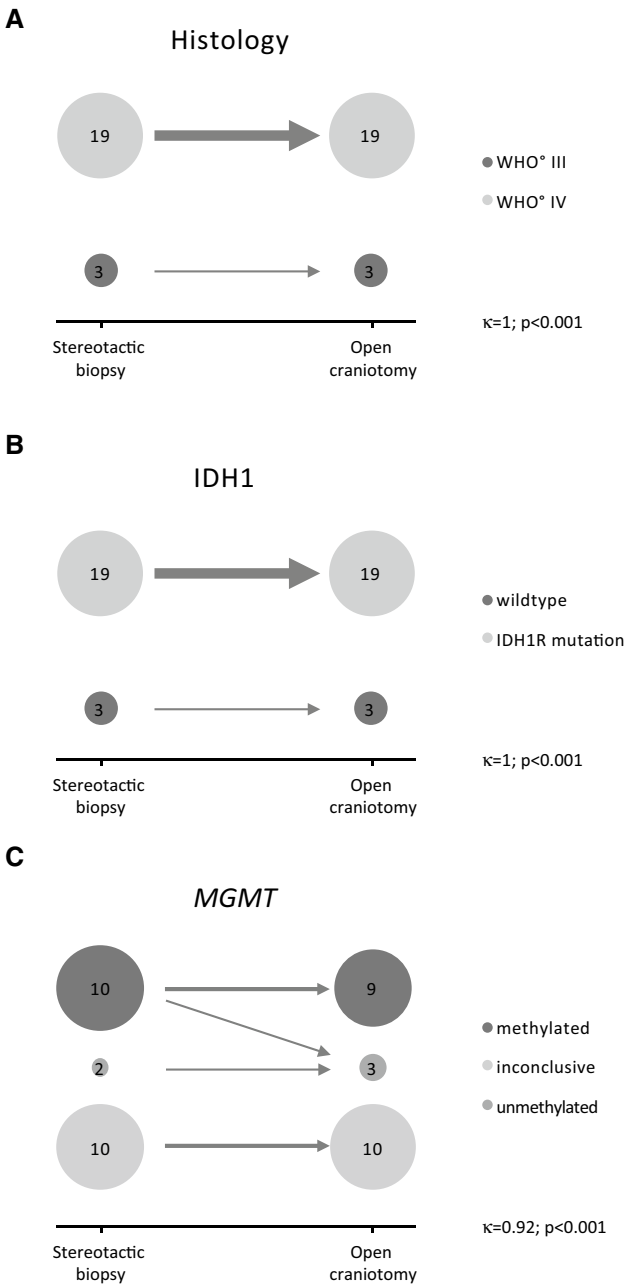


**Fig. 2** Correlation between stereotactic and open surgical sample acquisition. **a** Correlation of conventional histopathologic grading, **b** *MGMT* promotor methylation analysis and **c** IDH1 mutation were assessed in samples acquired by both stereotactic biopsy and open craniotomy. A highly significant correlation was observed in all three parameters; *MGMT* O6-methylguanine-DNA methyl-transferase, *IDH* Isocitrate dehydrogenase, *WHO* World health organization; data comparison was facilitated by employing Cohen's κ. *p* values are indicated for the respective parameters

## Discussion

Within the neurosurgical community consensus has emerged to suggest that HGG patients may in fact benefit from the complete resection of their tumor followed by adjuvant radio and chemotherapy [16–20]. Recent literature has also come to suggest that an incomplete tumor resection may not superior to combined radio- and chemotherapy alone [21].

Accordingly, in those patients, where diffuse infiltration of glioma cells into eloquent/critical areas of the CNS may hinder complete resection, tumor samples are nonetheless required for an accurate neuropathological diagnosis. Further, the assessment of such molecular markers will also be required for the development of novel therapeutic regimens and/or evaluation of clinical prognosis.

In the literature, controversial data regarding the tumor homogeneity of both low-grade and high-grade glioma

**Table 2** Surgery-related patient data

| | All patients (n = 22) |
|---|---|
| Trajectory orientation (n [%]) | |
| Centered | 14 (63.6%) |
| Tangential | 8 (36.4%) |
| Total samples/patient (n) | 17 (12–21) |
| Number of FFPE samples/patient (n) | 15 (10–20) |
| Number of biopsies for MGMT analysis/patient (n) | 3 (1–3) |
| Complications (n [%]) | |
| Postoperative radiological signs of bleeding > 1 cm | 3 (13.6%) |
| Neurological worsening | 2 (9.1%) |

Values given in parentheses indicate range unless otherwise defined

*FFPE* Formalin fixed, paraffin embedded, *MGMT* O6-methylguanine-DNA methyl-transferase

exists; while the intratumoral homogeneity of low grade gliomas with respect to clinically relevant markers has been demonstrated [22], other groups have observed a high level of intratumoral heterogeneity, increasing the risk of undergrading a low-grade glioma (e.g. when missing anaplastic foci in stereotactic sample acquisition) [23, 24]. Further, recent reports have highlighted intratumoral heterogeneity in HGGs with regard to certain molecular markers and this must also be factored into the reliability of stereotactically centered diagnoses [25–29]. With regard to HGG, reports have also suggested that there may be intratumoral heterogeneity with regard to *MGMT* promotor methylation status [27, 30, 31]. Contrary to such reports, high intratumoral homogeneity for *MGMT* promotor methylation status has been described for high-grade anaplastic astrocytomas as well as for GBM [23, 24]. One possible explanation for such discordant findings might be the "contamination" with necrotic tissue sample as discussed by Grasbon-Frodl et al. [24].

However, the literature critically lacks analyses confirming the homogeneity of clinically relevant molecular markers in HGGs as well as concordance between samples obtained via stereotactic biopsy and open craniotomy.

Approaches centered around a stereotactic biopsy are much less invasive when compared to open craniotomy procedures [32] which might result in craniotomy-associated complications [33]. Further, the utilization of improved stereotactic techniques for sample acquisition has resulted in a diagnostic neuropathological accuracy of up to 100% [34–37]; such findings are in line with the work presented herein.

Beyond an accurate histopathological diagnosis, molecular markers are required for GBM patient treatment stratification [38]. While in the past, *MGMT* promoter methylation status was considered to be the most important marker, novel molecular markers such as IDH1 have emerged [6, 10]. The prognostic value of IDH1 mutations in GBM has been demonstrated with the reduced survival of GBM patients who display a wild-type IDH1 protein [39]. Such diagnostic and therapeutic implications further strengthen the importance of proper tumor sampling by the neurosurgeon, who must provide adequate specimens for both microscopic and molecular pathologic diagnostics [11, 22].

Regarding histologic grading, all patients had the same diagnosis independent of the type of sample acquisition as evidenced by the Cohen's kappa value of 1. Such a high level of concordance is superior to that which has been published, with correlations between samples acquired via stereotactic surgery and open craniotomy being as low as 63% [40–43]. Such disparate findings may be due in part to the advancement of novel imaging tools/paradigms (e.g. CT, MRI and/or positron emission tomography [PET]), which are now routinely incorporated into the planning of stereotactic procedures [44–47].

When evaluating IDH1 mutational status, we were able to demonstrate a high degree of concordance in all patients included within our study when comparing both modes of sample acquisition (i.e. stereotactic biopsy versus open craniotomy) (Cohen's $\kappa = 1$). As per the above-mentioned, IDH1 mutations occur early in the development of gliomas and are observed to be highly homogenous in low-grade gliomas [23, 48]. Such findings clearly imply that stereotactic sample acquisition in low-grade glioma patients would be of value. Beyond low grade gliomas our data also suggest that stereotaxic sample acquisition may be reliably performed if one seeks to determine the IDH1 status of HGGs. This is of particular interest, as recent literature has come to suggest the importance of IDH1 status in the accurate histopathological grading of HGGs in which limited tissue is available [49].

As described, our department relies on MS-PCR analysis for the assessment of *MGMT* promotor methylation status [13]. The results reported herein fall within the expected range of conclusive results previously published (i.e. 56–100% of all cases) [4, 5]. When we compared samples obtained via stereotactic biopsy or open craniotomy, only one patient displayed a difference in MS-PCR findings. This patient displayed an inconclusive result when the open craniotomy sample was analyzed, while previously having had a conclusive (i.e. methylated) result in the sample acquired via stereotactic biopsy. Our findings result in an exceptionally high correlation as observed by a Cohen's $\kappa$ of 0.92. Again, these results may be explained when considering that advanced/novel imaging techniques have been employed and that biopsies were performed using non-centered trajectories in the majority of cases. It is prudent to note that the cohort analyzed within this manuscript is comprised of newly diagnosed glioblastoma patients;

however as has been previously demonstrated *MGMT* promotor methylation may change over time [50]. Hence, extrapolation of the results presented with regard to *MGMT* promoter methylation should take the aforementioned into consideration.

## Conclusions

The value of stereotactic sample acquisition in neurosurgery has become widely acknowledged. In this study, we have demonstrated that the analysis of stereotactically obtained samples provides a high rate of conclusive *MGMT* promotor methylation and can be used to detect the presence of IDH1 mutations. Further, we have shown for the first time, that there is nearly complete concordance with results obtained from samples derived from open craniotomies with regard to histologic grade, *MGMT* promotor methylation and IDH1 mutational status in HGGs.

Accordingly, our data indicate that treatment decisions incorporating molecular markers in HGGs may in fact be based on stereotactic biopsies alone. Being that the morbidity of a biopsy is less than that of a craniotomy, a stereotactic biopsy may in fact be employed if molecular profiling is the main reason for surgery (e.g. in non-resectable, eloquent tumors).

#### Compliance with ethical standards

**Conflict of interest** The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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