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RESEARCH LETTER

Tumoral MGMT content predicts survival in patients with aggressive pituitary tumors and pituitary carcinomas given treatment with temozolomide

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Temozolomide (TMZ) has recently been recommended as the first line chemotherapy in patients with aggressive pituitary tumors (APT) and pituitary carcinomas (PC) not controlled by standard treatment, i.e., surgery, radiotherapy and/or medical therapy (dopamine agonists, somatostatin analogs) [1]. About 40% of patients respond initially to TMZ, complete tumor regression is observed in <10% of patients [2, 3]. The predictive value of the DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT) as a biomarker for response to TMZ has been debated. Here we present survival data after TMZ treatment in relation to tumoral MGMT content.

TMZ acts by donating a methyl group to DNA bases, especially guanine. As modified guanine are recognized by DNA mismatch repair proteins (MMRs), this defense system tries unsuccessfully to match the methylated guanine with thymidine on its sister chromatid. Futile cycles follow, and eventually the DNA strand breaks and promotes apoptosis. Other DNA repair systems, including base excision repair (BER) and nucleotide excision repair (NER) are involved in the action of TMZ, but considered to have less prominent roles (recently reviewed by Syro et al. [4]). MGMT is an enzyme that counteracts the effect of TMZ by removing methyl groups added to DNA (direct repair). In glioblastomas and advanced neuroendocrine tumors, low tumor MGMT content is associated with good response to TMZ treatment [5–7], while data on its predictive value in APT/PC have been inconsistent. Most results in APT/PC are derived from case reports or small multi-center series in which MGMT amount was analyzed with different techniques at various laboratories. Unlike the situation in glioblastomas, where there is an agreement between methylation status of the MGMT gene, assessed by PCR, and tumor protein expression determined by immunohistochemistry (IHC), such a correlation has not been demonstrated in APT/PC [8]. Other factors than methylation are possibly of importance for gene transcription. IHC is presently recommended for analysis in APT/PC [1]. Besides inter-observer variations of the MGMT ICH staining, processing of the tissue, lack of a positive control

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Table 1 Patients treated with a second course of temozolomide

Sex/age[a]	Tumor type; hormone secreted	MGMT IHC (%)	Nr of cycles at 1st TMZ course	Response to the 1st course	Months between stop of 1st TMZ course to start of 2nd	Nr of cycles at 2nd TMZ course	Response to the 2nd course
F/40	PC; GH (initially silent)	9	6	Complete regression; normalized IGF1	69	12	30% regression of tumor volume; IGF-1 normalized
F/49	PC; PRL/GH	9	23	Complete regression; normalized hormones	117	12	27% regression of tumor volume; PRL normalized
M/70	PC; ACTH	9	2	50% regression of tumor volume; UFC decreased by 98%	2	4	Progressive disease. Metastases 3 mo after stop of 2nd TMZ course
M/57	APT; NF	95	3	Progressive disease	19	9	Progressive disease
M/22	APT; PRL	90	12	25% regression of tumor volume; PRL unchanged	5	3	Progressive disease

PC pituitary carcinoma, *APT* aggressive pituitary tumor, *GH* growth hormone, *PRL* prolactin, *ACTH* adrenocorticotropic hormone, *NF* non-functioning, *UFC* urinary free cortisol

[a]Age at diagnosis of the pituitary tumor

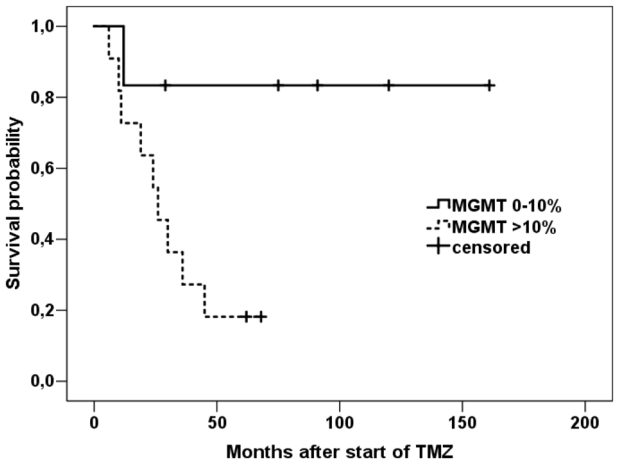

Fig. 1 Kaplan–Meier estimates showing survival in patients with low (0–10%) vs. high (>10%) MGMT tumor content. The low MGMT group survived longer, $p = 0.032$, Log rank (Mantel-Cox). In the low MGMT group one patient died from unrelated cause and was censored at 29 months. All other deceased patients died from progressive disease

(e.g., vascular endothelial cells in the tumor tissue), no consensus on cut off levels, and heterogeneity in occasional tumors make comparison between studies difficult.

By using a robust method and an experienced laboratory, we previously found that low tumor MGMT determined by IHC predicted a good primary response to TMZ [9]. Here we report the long-term follow-up of the 17 patients (11 APT and 6 PC) treated with TMZ, in whom tumoral MGMT staining was homogeneous (three cases with heterogeneous staining were excluded from this analysis). Tumor tissues from the last surgery preceding treatment with TMZ were examined. A single pathologist, blinded to the outcome of treatment, performed the MGMT IHC. MGMT was low (0–10%) in 6 patients and high (>10%) in 11. Outcome data were collected and survival was analyzed by the Kaplan–Meier method (log rank test, Mantel-Cox).

The median (range) time from diagnosis to start of TMZ was 100 months (3–285), and the median follow-up time after start of TMZ was 30 months (6–161). Age, sex, tumor type (APT vs PC), number of TMZ cycles and duration from diagnosis to start of TMZ did not differ between the MGMT groups. Two of six patients (33%) with low MGMT were deceased, one from an unrelated cause and one due to tumor progression, whereas 9 out of 11 patients (82%) with high MGMT had died from tumor progression. The group of patients with low MGMT survived longer after start of TMZ, $p = 0.032$. The estimated median survival time from start of TMZ was 36 months (95% CI 13–59) in the whole cohort, and 26 (14–38) in the group with high MGMT (Fig. 1). In the group with low MGMT, with a median (range) follow-up time of 83 months (12–161), only one patient died from pituitary tumor progression, (Fig. 1).

Five patients with progressive/recurrent disease received a second course of TMZ 19 months (range 2–117) after completing the first course. Tumor regression by ≥30% was achieved in one of three patients with low MGMT. The decrease in tumor size was borderline, 27% in another, and there was no effect in the third. The tumors progressed in both patients who had high MGMT (Table 1). It is thus important to note that patients with low MGMT may respond to a second course of TMZ.

The impression from the literature has been that an additional treatment course with TMZ had limited effects [1], possibly due to changes in tumor properties over time. The role of MGMT as a predictor of the outcome of a second course of TMZ in patients with APT/PC has previously been

addressed only in five patients who received TMZ for at least 3 months [2, 10–12]. In this follow-up study we add another five cases to the overall experience. MGMT tumor content in the earlier five cases were analyzed at four different labs, and reported as high in one, and low in two 2 [2, 10, 11]. None of these three patients responded to a second treatment course. The two remaining patients were difficult to evaluate regarding MGMT [10, 12]. In one of these patients MGMT was high at the first surgery and low at the second, possibly reflecting tumor heterogeneity [10]. In the other patient there was loss of function of the MMR protein MSH6 (MutS Homolog 6 Protein) in parallel with a transformation from an aggressive prolactinoma to a PC [12]. No effect of TMZ would be anticipated since intact function of DNA mismatch repair proteins is necessary for a cytotoxic effect of the drug, irrespective of MGMT status. Other mechanisms of primary or acquired resistance to TMZ have been discussed, e.g., adaptive response to alkylating damage, expression of DNA methylpurine-N-glycosylase (MPG) in the BER pathway, and DNA damage response. This might explain why a second course of TMZ is ineffective, why some MGMT-negative patients do not respond to TMZ, and why some MGMT-positive cases do [4, 13].

The present data illustrate the value of MGMT measurements in predicting the primary response to TMZ, and the overall survival. The clinical usefulness of MGMT as a biomarker for a second treatment course remains to be proven. We now call upon expert pituitary pathologists to standardize the MGMT techniques, and to establish reference laboratories. This is of crucial importance for treatment to be guided by tumor characteristics, especially in the future when alternative therapies emerge.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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