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ORIGINAL PAPER

Efficacy of monoterpene perillyl alcohol upon survival rate of patients with recurrent glioblastoma

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Abstract
Purpose The monoterpene perillyl alcohol (POH) a Ras inhibitor with potential capacity to arrest gliomagenesis is being used in a phase I/II clinical trial in adults with recurrent malignant glioma. The present study aimed to investigate the efficacy of intranasal administration of monoterpene POH upon survival rate of patients with recurrent glioblastoma (GBM) in comparison with historical control group of GBM patients.
Patients and methods It was included 89 adults with recurrent GBM receiving daily intranasal administration of 440 mg POH and 52 matched GBM patients as historical control untreated group only with supportive treatment.
Results Patients with recurrent primary GBM treated with POH survived significantly longer (log rank test, $P < 0.0001$) than untreated group. Patients with recurrent primary GBM in deep location survived significantly longer than with lobar location (log rank test, $P < 0.0001$). Median survival rate of secondary GBM was 11.2 months, longer (log rank test, $P = 0.0366$) than primary GBM (5.9 months). Radiographic improvement and reduction of corticosteroid dosage (36%) further associated with a delay towards progression.
Conclusion Intranasal administration of POH increased the overall survival of patients with recurrent GBM in comparison with historical untreated controls, but especially patients with secondary GBM and primary GBM with tumor localized in deep regions of the brain. The side effects of POH treatment were almost nonexistent, even in patients treated for over 4 years.

Keywords Glioblastoma · Perillyl alcohol · Terpene · Intranasal administration · Tumor topography

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Introduction

Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor in adults (Diehn et al. 2008). Standard therapy for newly diagnosed GBM includes surgical resection, radiotherapy, and chemotherapy with temozolomide administered both during and after radiotherapy. However, most patients develop tumor recurrence or progression after this multimodality treatment (Sathornsumetee and Rich 2008) thus emphasizing the importance to develop therapeutic strategies to overcome limitations of conventional therapy. Glioblastoma is currently defined by histological features of cellular atypia, mitotic figures, necrotic foci with peripheral cellular pseudopalisading and microvascular hyperplasia (Rong et al. 2006) but based on clinical characteristics and distinct genetic mechanisms of tumorigenesis it is classified in

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primary and secondary (Gravendeel et al. 2009). Secondary GBM develops slowly through progression from low-grade (WHO grade II) or anaplastic (WHO grade III) glial tumors frequently presenting mutations in the *p53* gene (60%), overexpression of platelet-derived growth factor receptors, and loss of heterozygosity (LOH) at 17p, 19q, and 10q (8, 9). In contrast, primary GBM develops rapidly with high grade lesion characterized by amplification/overexpression of epidermal growth factor receptor (EGFR) and mouse double minute 2 (MDM2); *PTEN* mutations and lack or partial loss of chromosome 10 (Kleihues and Ohgaki 1999). The epidermal growth factor receptor (EGFR) and its ligand-independent mutant EGFRvIII (Heimberger et al. 2005) activate downstream pathways including PI3 K-AKT and RAS—MAPK that induces cell proliferation and inhibits apoptosis {Chakravarti, 2002 #180}. These findings suggest that strategies to inactivate RAS—MAPK signaling may be critical to improve not only the efficacy of single-agent therapy but also of combined modality therapy in gliomas.

Perillyl alcohol (POH) a dietary monoterpene found in a variety of plants, known to suppress several signaling pathways such as Ras/Raf/ERK, the extracellular signal-regulated kinase for nuclear factor NFkB; and also the post-translational isoprenylation of the Ras small GTPase superfamily of proteins that stimulate tumor-associated angiogenesis {Hohl, 1995 #252;Holstein, 2003 #255; Chaudhary, 2009 #204;Loutrari, 2004 #251} may be an effective agent for treating patients with glioma. Therefore we set up a protocol for using the intranasal route to deliver POH to patients with glioma (da Fonseca et al. 2006a, 2008a, 2009). Such strategy was based upon the evidence that drugs reach the brain parenchyma, spinal cord, and cerebrospinal fluid (CSF) within minutes by an extracellular route through perineural and/or perivascular channels along the olfactory and trigeminal nerves without binding to any receptor or using axonal transport (Hashizume et al. 2008). Previous data from our group showed that patients with recurrent glioma presenting similar histological features but distinct tumor location (basal ganglia versus lobar) had different therapeutic response to intranasal POH (da Fonseca et al. 2009). The present study aimed to analyze the clinical efficacy of intranasal POH administration upon overall survival rate of patients with recurrent malignant glioma in comparison with control untreated GBM patients (historical controls) with similar retrieved clinical database but not included in the POH protocol.

Patients and methods

The Hospital Medical Research Ethics Committee and the Brazilian Ministry of Health (CONEP 9681 no. 124 25000.009267/2004-25) approved this study that complies with the principles laid down in the Declaration of Helsinki. Before inclusion in the protocol, patients and their surrogates gave a written informed consent. This prospective study was carried out in the Antonio Pedro Hospital Medical School of the Fluminense Federal University between 2005 and 2009 for a Phase I/II clinical trial to assess therapeutic efficacy of intranasal administration of perillyl alcohol (POH) 440 mg daily.

The cohort included patients older than 18 years-old with recurrent glioblastoma, with measurable contrast—enhancing tumor image on magnetic resonance, Karnofsky index = 70% or higher; adequate hematologic function and clinic laboratory-based measures; stable heart rhythm and no clinical evidence of congestive heart failure or unstable angina.

Patient selection

As criteria for inclusion in the POH protocol, eligible GBM patients ($n = 89$) were just receiving symptomatic treatment, had at least three relapses and already did current available treatment (surgery and/or radiation) including multimodal chemotherapy specific for glioblastoma. Clinical data and radiographic images from 52 matched GBM patients that received multimodal therapy (surgery, radiotherapy and chemotherapy) between January 2005 and January 2009 were reviewed and included as historical control group. The control group also included patients older than 18 years-old with recurrent malignant glioma, measurable contrast enhancing tumor on CT scan and/or MRI, Karnofsky index = 70% or higher and clinical database similar to POH-treated patients.

Patients were stratified into groups according to therapeutic strategy, clinical presentation (primary or secondary GBM) and tumor location (deep grey matter or lobar regions). The treated group ($n = 89$) received intranasal administration of 440 mg POH, four times daily at intervals of 6 h. Patients with recurrent primary GBM ($n = 83$) were selected based upon previous clinical history of the 3 to 6 months disease and strict histological criteria confirming GBM diagnosis at the first biopsy. Diagnosis of secondary GBM ($n = 6$) required at least two biopsies, clinical history of more than 2 years and histological evidence of progression from low grade or anaplastic astrocytoma to type IV GBM. The following parameters were assessed: initial symptoms, clinical diagnosis, tumor size control and overall survival.

Drug administration and dose escalation

Perillyl alcohol (POH) was administered by inhalation 4 times daily. Initially patients received 0.3% v/v POH

(55 mg) totaling 220 mg/day but owing to the efficacy of the intranasal delivery and lack of toxicity patients received an increase in POH dose, with escalation up to 440 mg daily as limiting dose to avoid nasal discomfort.

Statistical analysis

Statistical analysis was carried out using Kaplan–Meier curves, log rank tests with no censored data, method D. Collet. Software Bioestat 5.0 and multivariate Cox regression model. Additional clinical and radiological variables were evaluated for prognostic significance.

Results

Clinical data

One hundred and forty-one subjects with recurrent GBM were enrolled (male = 80; female = 61) in this study. Before entering the clinical trial, all patients received prior conventional therapy (surgery, radiotherapy and chemotherapy), presented at least 3 events of recurrence and were considered out of therapeutic possibilities. Tumor recurrence was defined as the appearance of residual tumor growth or new lesion on MRI and manifestation of new clinical symptoms after conventional treatment (Figarella-Branger et al. 2008). Demographic characteristics of patients are specified in Table 1.

A clinical course of disease varied among patients with primary GBM with 69% ($n = 57$) presenting a course less than 3 months, 17% ($n = 14$) between 3 to 6 months and 14% ($n = 11$) had clinical history longer than 6 months, but this was not associated with progression from a low grade glioma as confirmed by histological analysis (data not shown). Only 7% (6/89) had diagnosis of secondary GBM with histological evidence of a precursor low grade or anaplastic astrocytoma. Such patients presented approximately 2.2 years mean time progression from anaplastic astrocytoma to recurrent GBM and 4.9 years from grade II. Commonly observed combination of symptoms and signs indicating neurological deficits associated with recurrence were headache (38%), seizures (21.5%) and/or symptoms related to increase intracranial pressure (52%).

The median age of primary GBM patients was 62 years (IQR range IQR: 39–85) and 45 years (IQR range: 42–47) for secondary GBM. Considering gender differences, primary GBM developed more frequently in men (1.37; M/F ratio) and secondary GBM in women (0:66; M/F ratio). As control (historical) group, it was included clinical data from 52 matched GBM patients that received multimodal therapy (surgery, radiotherapy and chemotherapy) but were not included in the POH protocol. This group included subjects with similar male/female ratio (1.36); age range (42 to 78 years; mean 63) and clinical course as patients with primary GBM under POH treatment.

Tumor location in the deep grey matter (basal ganglia, thalamus) was present in 18 (20.7%) patients with primary

Table 1 Demographic characteristics

Patients	Primary	Secondary	Control group
Total of patients	83 (93%)	6 (7%)	52
Age	62	45	63
IQR	(39–85)	(42–47)	(42–78)
Gender			
Male	48	2	30
Female	35	4	22
Ratio M/F	1.37	0.5	1.36
Mean length progression			
<3 months	57 (69%)	*	36 (68%)
3 to 6 months	14 (17%)	*	9 (18%)
>6 months	11 (14%)	*	7 (14%)
From grade II	*	4.9 years	NA
From grade III	*	2.2 years	NA
Mean survival time after recurrence (month)	5.9	11.2	2.3
Palliative care	Yes	Yes	Yes
POH treatment	Yes	Yes	No
Site of tumor			
Lobar	65 (78.3%)	5 (83.3%)	40 (76.6%)
Deep	18 (20.7%)	1 (16.6%)	12 (23.3)%

**ND* not done, *NA* not assessed, *IQR* interquartile range

GBM and one (16.6%) with secondary GBM, whereas lobar location was present in 65 (79.3%) patients with primary GBM and all patients ($n = 6$) with secondary GBM. Historical control group showed similar tumor topography of primary GBM patients.

Efficacy of POH treatment

In order to establish whether POH could modify the course of the disease by improving the overall survival, it was carried out a comparison between groups of patients under POH treatment and control receiving only supportive care. Patients with primary recurrent GBM treated with POH showed 5.9 months survival advantage compared with 2.3 months mean survival of historical control group (Fig. 1a), a difference with statistical significance ($P < 0.0001$).

Comparison between primary and secondary GBM patients under treatment with POH (Fig. 1b) showed a significant difference ($P = 0.0366$) in overall survival among patients with secondary GBM.

Clinical observations indicated that survival rate differed significantly according to the location of tumor lesion. Patients with tumor at deep site (thalamus, basal ganglia) survived significantly longer ($P < 0.0001$) than those with tumor at lobar region (Fig. 1c). MRI (Fig. 2) obtained from patients with recurrent GBM and tumor lesion at deep location, showed marked difference between images taken at the time of inclusion in the protocol and 16 months after exclusive treatment with POH. In addition, patients with secondary GBM even with tumor at lobar site had clinical evolution as good responders to POH intranasal administration (Fig. 3). Magnetic resonance images showed efficient response to intranasal POH administration before and 4 years after treatment.

Discussion

Despite advances in multimodality treatment, survival of patients with GBM remains poor, with median survival of 12–15 months from onset and only few months after the first recurrence. The present study aimed to define the clinical efficacy of intranasal POH administration among patients with recurrent malignant glioma in comparison with historical untreated GBM controls shows that: (1) patients with recurrent disease that evolve from a lower grade precursor lesion (secondary or progressive GBM) survived significantly longer (11.2 months) than patients with primary or de novo GBM with a high-grade lesion from onset (5.9 months); (2) increased survival was consistently observed in patients with tumor located in the basal ganglia; (3) patients with primary GBM treated with

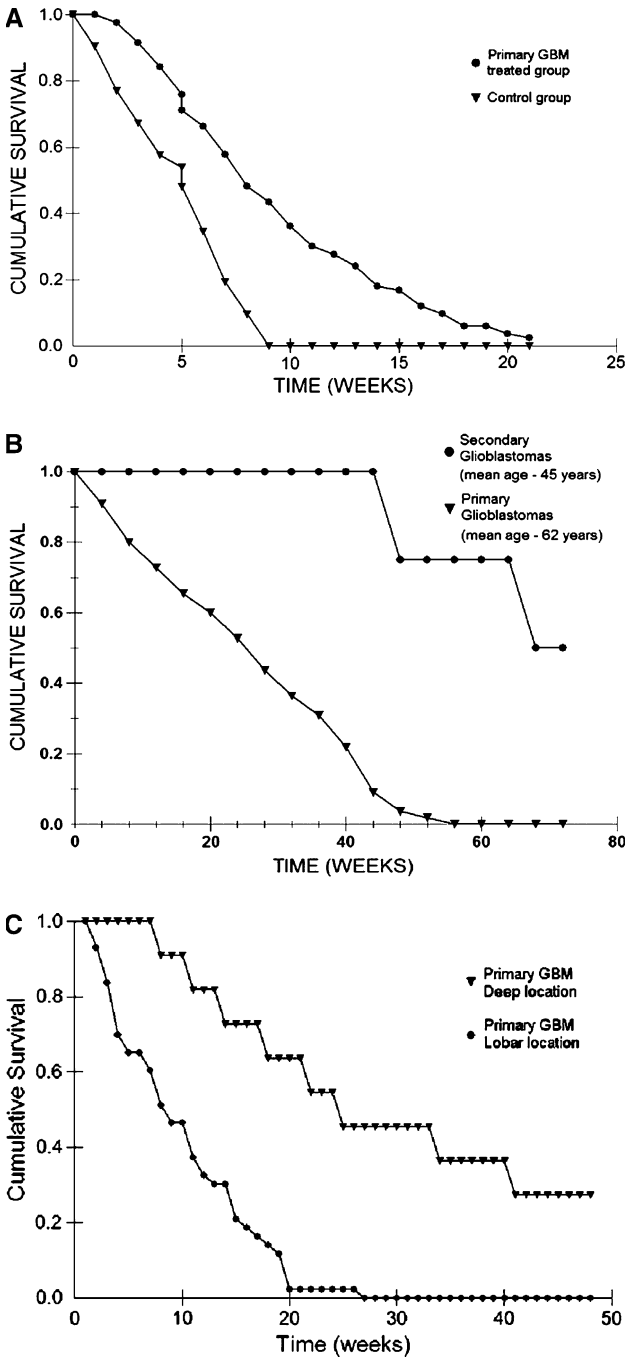

Fig. 1 Kaplan–Meier graphs showing significant difference ($P < 0.0001$) in the overall survival of patients **a** with recurrent primary GBM treated with POH and untreated group; **b** difference ($P = 0.0366$) in the survival rate of patients with recurrent primary and secondary GBM under POH intranasal treatment; **c** patients with recurrent primary GBM under treatment with intranasal POH administration according to the tumor site ($P = 0.0001$)

POH showed survival (5.9 months) advantage compared with control group untreated group (2.3 months).

There is considerable variability of median survival time among GBM patients, but despite multimodal therapy, the 5-year survival rate for patients with GBM after initial

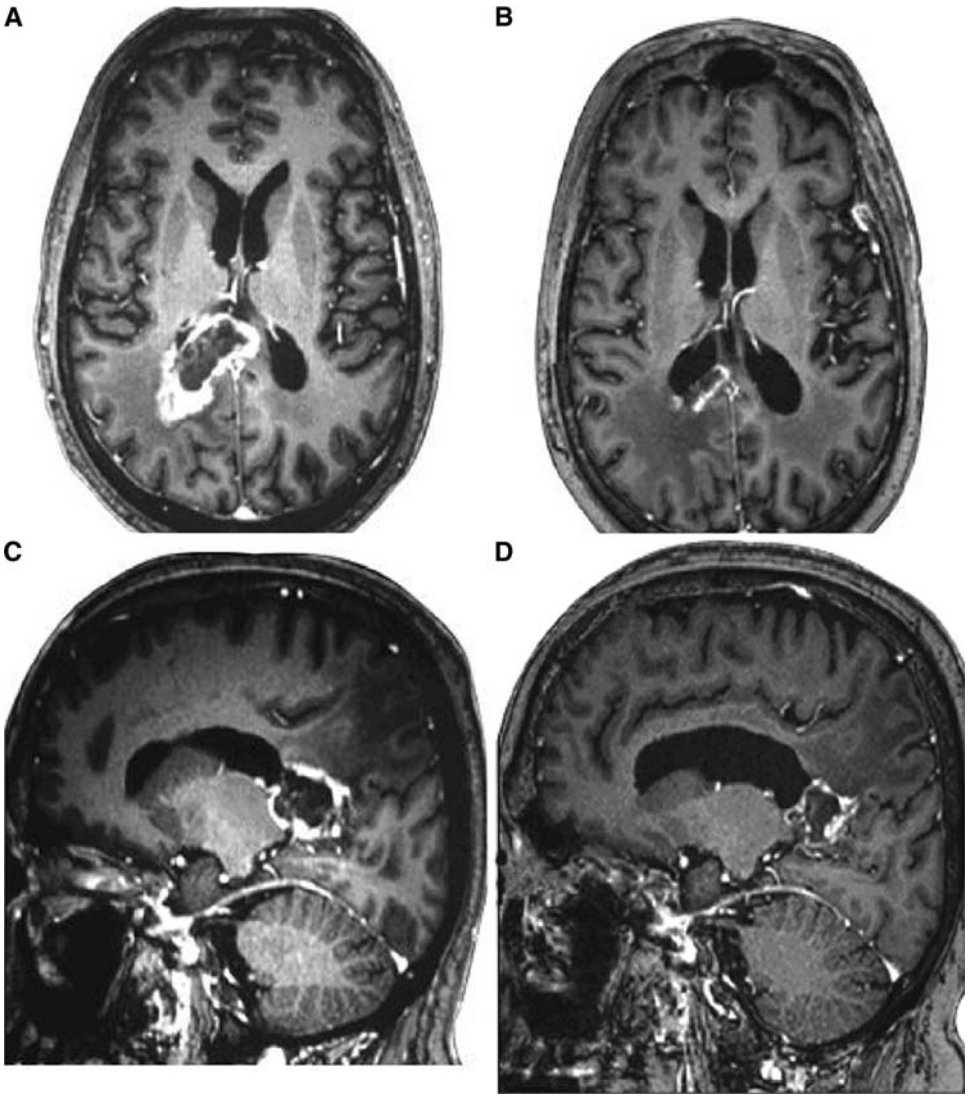

Fig. 2 Magnetic resonance images of a patient with recurrent primary GBM and tumor at deep location showing efficient response to intranasal POH administration. Note the partial response in tumor size between the initial MRI (5.5 cm × 4.0 cm × 3.5 cm) (**a**, **c**) and performed after 16 months of treatment (2.3 cm × 2.1 cm × 1.9 cm) (**b**, **d**)

diagnosis is less than 5% (Bokstein et al. 2008; Sathornsumetee and Rich 2008). Anaplastic astrocytoma frequently present within a year recurrence and progression to glioblastoma, even with the use of aggressive therapeutic strategies including cytotoxic alkylating drugs that cause DNA damage in both normal and transformed cells. Radiotherapy with concomitant adjuvant triazene derivative temozolomide (TMZ) is now extensively used as postoperative first-line treatment for GBM (van Genugten et al. 2010). However such treatments still remain insufficient as resistance towards radio and chemotherapy frequently occurs. Drug resistance may arise through several distinct DNA repair mechanisms that can restore the integrity of TMZ-induced alkylated DNA bases (Johannessen et al. 2008; van Genugten et al. 2010). Drugs against key components of cellular pathways critical for cancer initiation and tumor progression offer the potential advantage to increase therapeutic efficacy and decrease systemic toxicity compared with traditional cytotoxic agents. In this context, intranasal administration of perillyl alcohol (POH), a Ras inhibitor, has been used as potential adjuvant therapeutic strategy for inhibiting gliomagenesis of patients with recurrent malignant glioma (da Fonseca et al. 2006b, 2008b).

As favorable prognostic factors for malignant glioma, the literature report young age at onset, absence of neurological deficits, the histological grade, and lesion in non-eloquent areas (Stark et al. 2005). Current findings support indications that glioblastoma cell invasion is not random, transformed glial cells interact with specific structures in their immediate microenvironment and preferentially invade along myelinated axons, vascular basement membranes, and the subependyma (Auvinen et al. 2000; Lefranc et al. 2007). This ultimately would influence patient overall survival, whether tumor is located in the deep gray matter or lobar region (Rainov et al. 2006). We noticed that patients

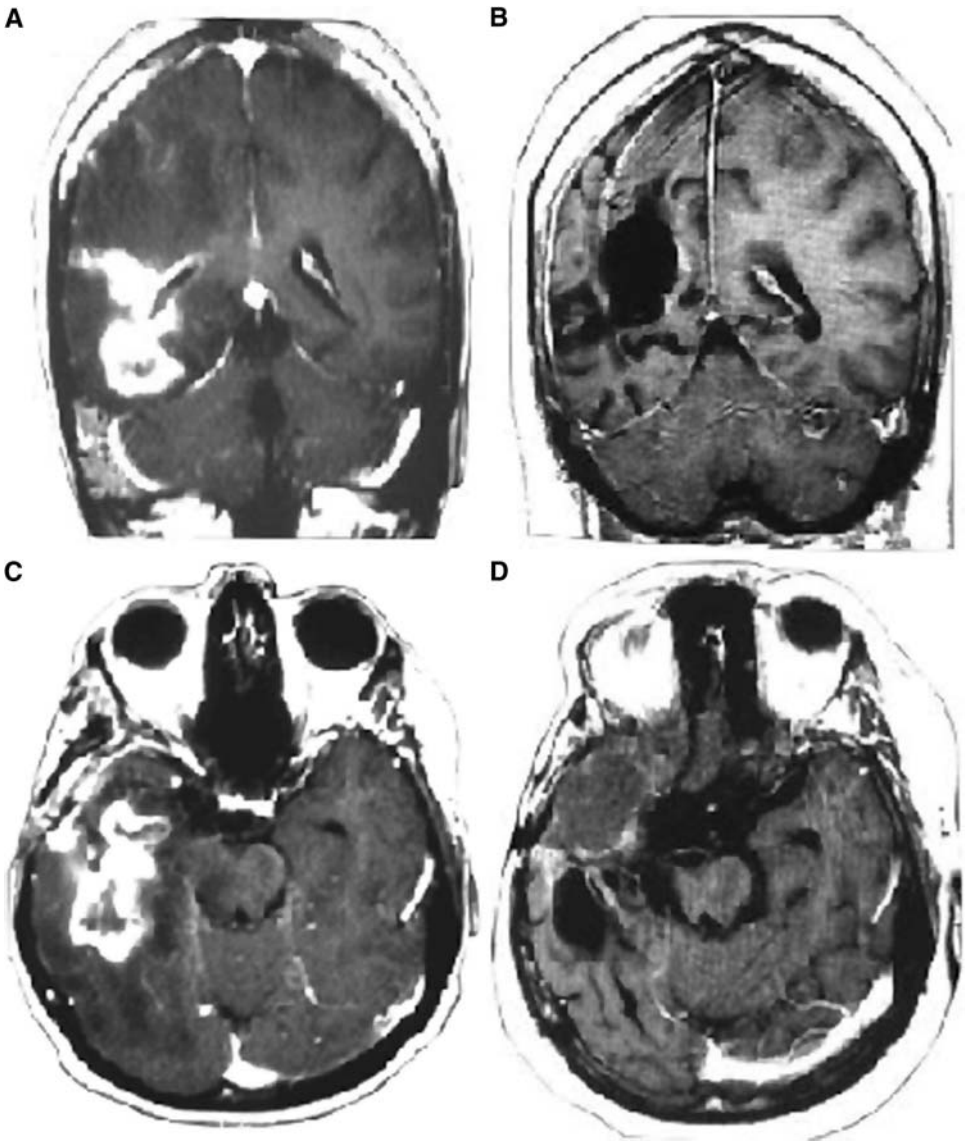

Fig. 3 Magnetic resonance images of a patient with recurrent secondary GBM with efficient response to intranasal POH administration. Note a decrease in the tumor size between the initial MRI (**a**, **c**), and 4 years (**b**, **d**) after treatment

with high-grade lesion from onset (primary or de novo GBM) treated with POH after at least 3 events of recurrence had survival advantage (5.9 months) compared with historical control untreated group (2.3 months). Recent studies showed that patients with recurrent GBM treated with fotemustine had median survival of 9.1 months (Scoccianti et al. 2008) and patients with recurrent high-grade glial tumors receiving bevacizumab and irinotecan had a 6-month progression free survival of 25% and 4.7 months median time to progression (Bokstein et al. 2008) In the present study we observed that patients with recurrent GBM that progressed from anaplastic astrocytoma had mean survival longer than those with primary (de novo) GBM. Given the likely biological differences concerning molecular alterations, it would not be surprising to learn that each subtype requires a specific and unique set of treatment. In addition, differential invasion of glioma cells within distinct brain microenvironments highlight the importance to establish novel prognostic parameters.

Altogether the present study shows the efficacy of intranasal administration of the monoterpene perillyl alcohol improving the overall survival of patients with recurrent glioblastoma. In addition, it also emphasizes the importance to include as predictive factors determining the outcome of tumor progression and overall survival, the location of tumor lesion (deep or lobar) because patients with tumor location in the basal ganglia had longer survival and efficient response to treatment.

Conclusion

Intranasal administration of POH increased the overall survival of patients with recurrent GBM in comparison

with historical controls, but especially patients with secondary GBM and those with tumor localized in deep regions of the brain. In addition, the side effects of treatment were almost nonexistent, even in patients treated for over 4 years.

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Conflict of interest statement None.

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