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# Mutant IDH1 and seizures in patients with glioma



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## ABSTRACT

**Objective:** Because the D-2-hydroxyglutarate (D2HG) product of mutant isocitrate dehydrogenase 1 (IDH1<sup>mut</sup>) is released by tumor cells into the microenvironment and is structurally similar to the excitatory neurotransmitter glutamate, we sought to determine whether IDH1<sup>mut</sup> increases the risk of seizures in patients with glioma, and whether D2HG increases the electrical activity of neurons.

**Methods:** Three WHO grade II-IV glioma cohorts from separate institutions (total N = 712) were retrospectively assessed for the presence of preoperative seizures and tumor location, WHO grade, 1p/19q codeletion, and IDH1<sup>mut</sup> status. Rat cortical neurons were grown on microelectrode arrays, and their electrical activity was measured before and after treatment with exogenous D2HG, in the presence or absence of the selective NMDA antagonist, AP5.

**Results:** Preoperative seizures were observed in 18%–34% of IDH1 wild-type (IDH1<sup>wt</sup>) patients and in 59%–74% of IDH1<sup>mut</sup> patients ( $p < 0.001$ ). Multivariable analysis, including WHO grade, 1p/19q codeletion, and temporal lobe location, showed that IDH1<sup>mut</sup> was an independent correlate with seizures (odds ratio 2.5, 95% confidence interval 1.6–3.9,  $p < 0.001$ ). Exogenous D2HG increased the firing rate of cultured rat cortical neurons 4- to 6-fold, but was completely blocked by AP5.

**Conclusions:** The D2HG product of IDH1<sup>mut</sup> may increase neuronal activity by mimicking the activity of glutamate on the NMDA receptor, and IDH1<sup>mut</sup> gliomas are more likely to cause seizures in patients. This has rapid translational implications for the personalized management of tumor-associated epilepsy, as targeted IDH1<sup>mut</sup> inhibitors may improve antiepileptic therapy in patients with IDH1<sup>mut</sup> gliomas. **Neurology® 2017;88:1805-1813**

## GLOSSARY

**AUC** = area under the receiver operating characteristic curve; **CI** = confidence interval; **D2HG** = D-2-hydroxyglutarate; **GBM** = glioblastoma; **HR** = hazard ratio; **IDH1<sup>mut</sup>** = mutations in isocitrate dehydrogenase 1; **IDH1<sup>wt</sup>** = wild-type isocitrate dehydrogenase 1; **MEA** = multielectrode array; **NMH** = Northwestern Memorial Hospital; **NYU** = New York University; **OR** = odds ratio; **RR** = relative risk; **TAE** = tumor-associated epilepsy.

WHO grade II–III gliomas are more likely to induce epilepsy than grade IV glioblastomas (GBMs), though the reason for this is unclear.<sup>1</sup> One possible explanation is that the former group of tumors usually have mutations in isocitrate dehydrogenase 1 (IDH1<sup>mut</sup>), whereas the latter usually do not. IDH1 is a metabolic enzyme located in the cytosol and peroxisomes, catalyzing the oxidative decarboxylation of isocitrate to  $\alpha$ -ketoglutarate. In contrast, mutant enzyme reduces  $\alpha$ -ketoglutarate to D-2-hydroxyglutarate (D2HG). This is so efficient that the concentration of D2HG can exceed 30 mM in IDH1<sup>mut</sup> gliomas.<sup>2</sup> IDH1<sup>mut</sup> cells release D2HG into their surroundings in culture, in vivo, and into the CSF of patients with glioma.<sup>2,3</sup> Furthermore, infiltrative glioma cells readily migrate along axons and dendrites in the gray and white matter, forming close physical associations with neurons (figure 1, A and B). Thus, IDH1<sup>mut</sup> glioma cells can expose neurons to D2HG.

The D2HG product of IDH1<sup>mut</sup> bears a strong structural resemblance to glutamate, the most widespread excitatory neurotransmitter in the CNS (figure 1C). Because glutamate is the

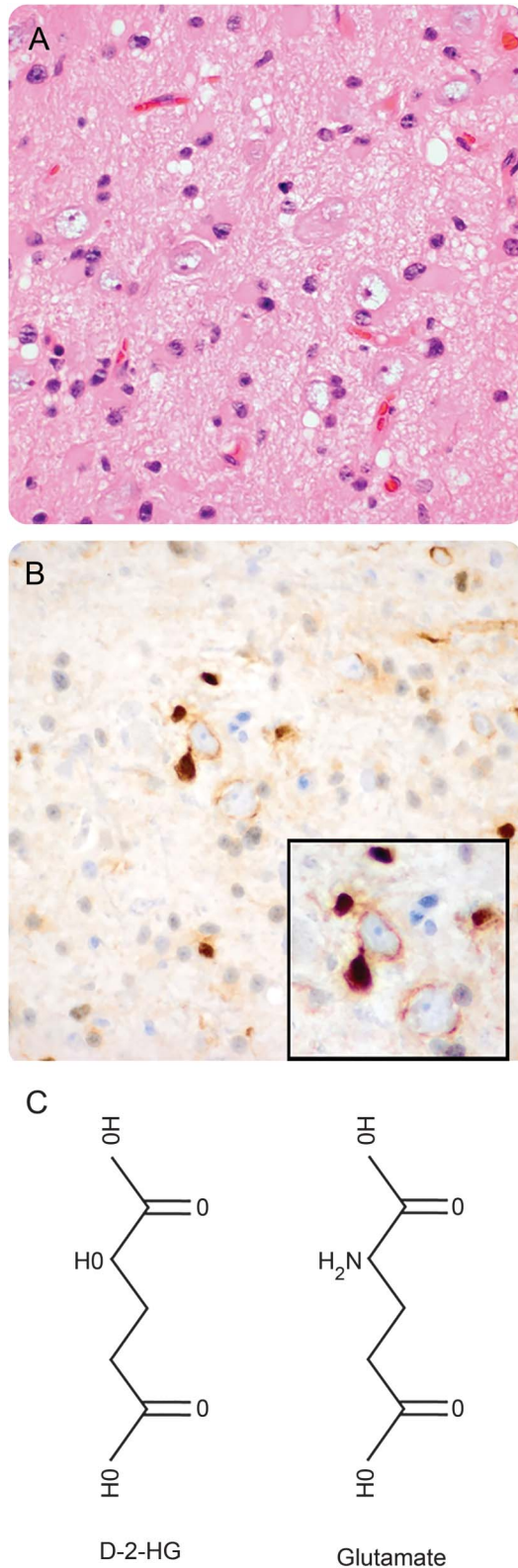
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Supplemental data  
at Neurology.org

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Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

**Figure 1** Chemical structure of D-2-hydroxyglutarate (D2HG) and the close relationship of mutations in isocitrate dehydrogenase 1 (IDH1<sup>mut</sup>) glioma cells to neurons



(A) Hematoxylin & eosin section of the left frontal cortex from a 34-year-old man who presented with seizures shows scattered infiltrative oligodendroglioma cells that were

primary excitatory neurotransmitter in the brain, its baseline concentration is tightly regulated in the extracellular space around neurons.<sup>4</sup> Thus, infiltrative glioma cells releasing a glutamate mimetic could disrupt the balance between inhibition and excitation, and potentially lead to seizures. In this study, we analyzed clinical data from 712 patients representing 3 separate institutions, and examined the effects of D2HG on cortical neuron activity, in order to determine whether there is a link between IDH1<sup>mut</sup> and seizures in patients with glioma.

**METHODS Patient cohorts.** This was a retrospective cohort study of WHO grade II–IV infiltrative astrocytomas, oligodendrogliomas, and GBM, from patients aged 18 years and older, with known *IDH1/2* mutation status (collectively referred to as IDH1<sup>mut</sup>). Cohort 1 consisted of 159 gliomas that had been tested for IDH1<sup>mut</sup> as part of routine clinical care at the University of Kentucky (table 1). This cohort included 13 grade II astrocytomas, 14 grade II oligodendrogliomas, 16 grade III astrocytomas, 4 grade III oligodendrogliomas, and 112 GBMs from 2009 to 2014. (Some cases had originally been resected prior to 2009, but for clinical reasons required retrospective testing.) Cohort 2 consisted of 136 gliomas that had been tested for IDH1<sup>mut</sup> at New York University (NYU) from 2010 to 2014, including 4 grade II astrocytomas, 6 grade II oligodendrogliomas, 10 grade III astrocytomas, 4 grade III oligodendrogliomas, and 112 GBMs. Both of these cohorts have recently been described in a separate study.<sup>5</sup> Cohort 3 consisted of 417 gliomas from 2010 to 2016, including 26 grade II astrocytomas, 14 grade II oligodendrogliomas, 70 grade III astrocytomas, 24 grade III oligodendrogliomas, and 283 GBMs with known IDH1<sup>mut</sup> status from Northwestern Memorial Hospital (NMH). In all 3 cohorts, patient charts were retrospectively reviewed for the presence of any kind of seizures as part of the documented preoperative clinical presentation. A remote history of childhood seizures, or seizures attributed to other causes (e.g., alcohol withdrawal, head trauma), were excluded. Fewer than 1% of all patients with glioma had such a seizure history. To avoid potential therapy-related confounding factors, only seizures in new patients, with no prior surgical or adjuvant therapy, were included. To avoid bias, chart reviewers were blinded to the IDH1<sup>mut</sup> status of each tumor. Average and maximal follow-up for each patient, from the time of initial surgery, in cohort 1 was 18 and 243 months, respectively. In cohort 3, average and maximal follow-up from the time of initial surgery was 13 and 141 months, respectively. Survival data were obtained in cohort 1 via the Kentucky Cancer Registry, and in cohort 3 by the Northwestern University Tumor Registry, but were not available for cohort 2.

Mutation screening was done via R132H IDH1 immunohistochemistry (Dianova, Hamburg, Germany). Immunonegative tumors were tested for less common *IDH1/2* mutations via

highlighted by R132H IDH1 immunohistochemistry (B). Many tumor cells were closely apposed to neurons, even wrapping their processes around the neurons (B, inset). (C) The chemical structure of D2HG, compared with the structure of the excitatory neurotransmitter, glutamate.

**Table 1** Patient cohort characteristics

Characteristics	No. of patients	Cohort 1	Cohort 2	Cohort 3	$\chi^2$	p Value
Total	712	159	136	417	—	—
Mean $\pm$ SEM age at diagnosis, y	—	54.0 $\pm$ 1.2	60.7 $\pm$ 1.3	53.4 $\pm$ 0.79	—	<0.001 <sup>a</sup>
<b>Sex</b>						
Male	400 (56.2)	82 (51.6)	81 (59.6)	237 (56.8)	0.5	0.48
Female	312 (43.8)	77 (48.4)	55 (40.4)	180 (43.2)		
<b>WHO grade</b>						
II	77 (10.8)	27 (17.0)	10 (7.4)	40 (9.6)	0.11	0.74
III	128 (18.0)	20 (12.6)	14 (10.3)	94 (22.5)		
IV	507 (71.2)	112 (70.4)	112 (82.4)	283 (67.9)		
<b>IDH1 status</b>						
Wild-type	535 (75.1)	112 (70.4)	105 (77.2)	318 (76.3)	0.43	0.51
Mutant	177 (24.9)	47 (29.6)	31 (22.8)	99 (23.7)		
<b>1p/19q status</b>						
Intact	68 (9.6)	18 (11.3)	12 (8.8)	38 (9.1)	0.03	0.86
Codeleted	644 (90.4)	141 (88.7)	124 (91.1)	379 (90.9)		
<b>Location</b>						
Temporal	191 (27.1)	44 (27.7)	30 (23.4)	117 (28.1)	0.09	0.76
Nontemporal	513 (72.9)	115 (72.3)	98 (76.6)	300 (71.9)		
<b>Preoperative seizures</b>						
Yes	276 (38.8)	55 (34.6)	54 (39.7)	167 (40.0)	0.15	0.70
No	436 (61.2)	104 (65.4)	82 (60.3)	250 (60.0)		

A total of 712 patients from 3 separate institutions produced the clinical data in this study. Differences in age among the cohorts were calculated by one-way analysis of variance followed by post hoc Tukey test. All other statistical comparisons were made by  $\chi^2$ , with Yates correction for continuity. Tumor location was unknown for 8 cases in cohort 2.

<sup>a</sup>Mean patient age was higher in cohort 2 than in cohorts 1 and 3.

pyrosequencing (University of Kentucky, NMH) or by 450K methylation array profiling (NYU) as described previously.<sup>6</sup>

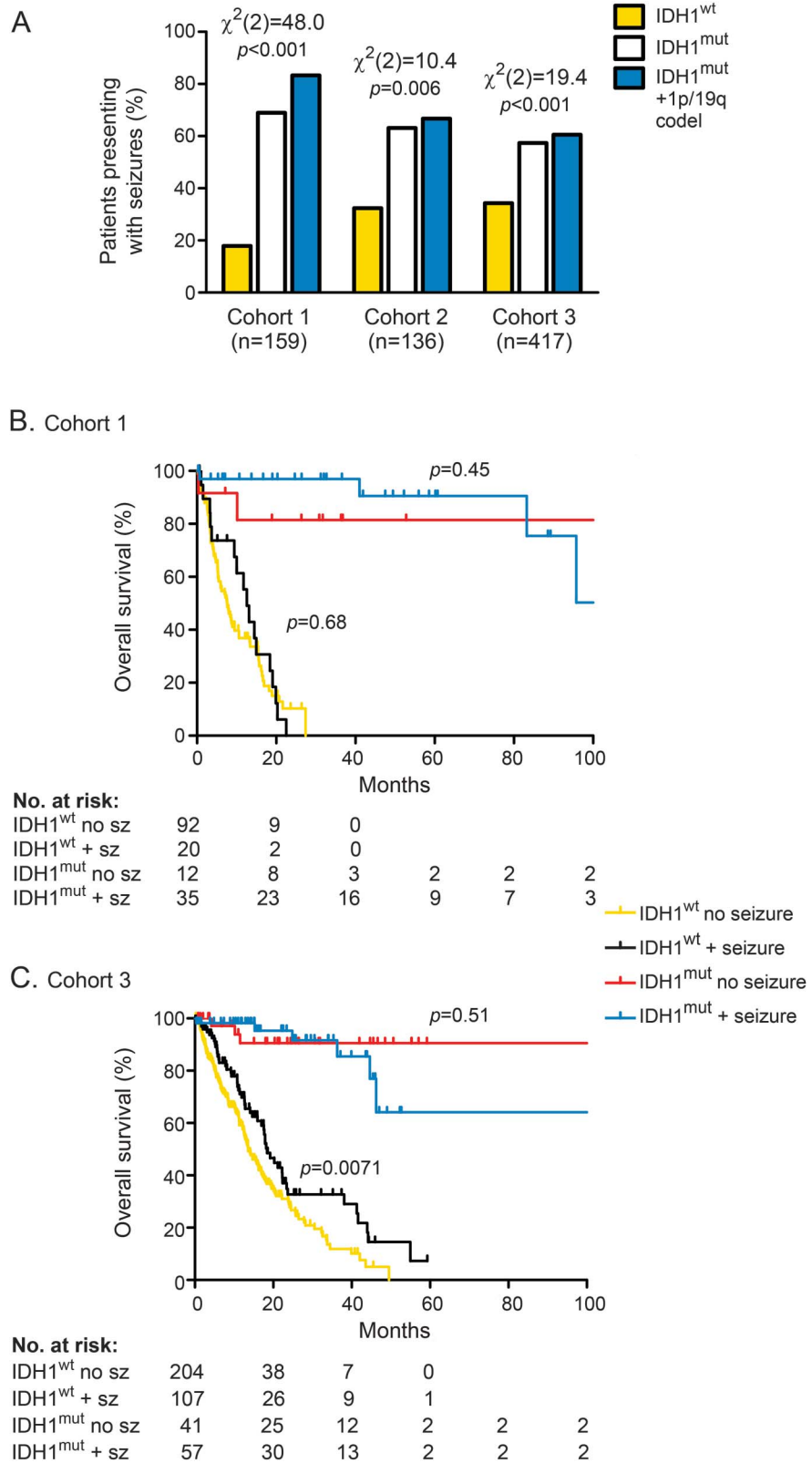
**Multielectrode array recording in cell culture.** Primary dissociated cortical cultures were prepared from postnatal day 0–1 Sprague-Dawley rat pups (Charles River, Malvern, PA) for multi-electrode array (MEA) plates as previously described.<sup>7</sup>  $1.5 \times 10^5$  neurons were plated on 12-well MEA plates (Axion Biosystems, Atlanta, GA) precoated with poly-L-lysine (0.5 mg/mL) (Sigma-Aldrich, St. Louis, MO) and laminin (20  $\mu$ g/mL) (Gibco, Langley, OK). Cultures were maintained in NeuralQ basal medium supplemented with 2% GS21 (MTI-GlobalStem, Gaithersburg, MD) and GlutaMAX (Thermo Fisher Scientific, Waltham, MA) with 1% Penn/Strep (Gibco) at 37°C in 5% CO<sub>2</sub>. Cytidine (Sigma-Aldrich) (2.5  $\mu$ M) was added 4 days after plating.

MEA recordings were performed on DIV 14. Extracellular potentials were recorded at 37°C using Maestro channel amplifier with AxIS software (Axion Biosystems, Atlanta, GA) with 1,200 $\times$  gain and a 12.5 kHz/channel sampling rate. Plates were placed on the amplifier and equilibrated for 30 minutes before recording. Baseline and treatment measurements were recorded for 10 minutes each. D- $\alpha$ -Hydroxyglutaric acid (Sigma-Aldrich) was prepared in culture media at specified concentrations on the day of recording. DL-2-Amino-5-phosphopentanoic acid (Tocris, Bristol, UK) (100  $\mu$ M) was utilized to block NMDA receptors and was added between the baseline and treatment measurements for 10 minutes.

MEA data were analyzed, while blinded to experimental groups, using AxIS software and MATLAB-based Neural Metric Tool (Axion Biosystems). Recordings were passed through a Butterworth bandpass filter (300–5,000 Hz) and spikes were detected using an adaptive threshold crossing ( $6 \times$  SD). Bursts were classified as having a minimum of 5 spikes with a max ISI of 100 milliseconds. Raster plots were generated with Neural Metric Tool.

**Developing IDH1<sup>mut</sup> prediction model.** We used previously described patient cohorts to evaluate whether seizures can improve the accuracy for the prediction of IDH1<sup>mut</sup> in addition to age, GBM, and history of grade II/III glioma, which were 3 predictors identified in that article.<sup>8</sup> For this purpose, we calculated the difference in the area under the receiver operating characteristic curve ( $\Delta$ AUC) between the logistic regression model with seizures and the later 3 predictors and the logistic regression model only with the later 3 predictors by using the ROCR package of R software version 3.2.3. We also calculated the 95% confidence interval (CI) of  $\Delta$ AUC by the following bootstrap procedure. We generated 1,000 bootstrap samples of the original data. For each bootstrap sample, we fitted 2 logistic regression models, one with seizures and age, GBM, and history of grade II/III glioma and the other one only with the later 3 predictors, and calculated the  $\Delta$ AUC between those 2 models. We then obtained the lower and upper limits of the 95% CI by calculating the 2.5 and 97.5 percentiles of  $\Delta$ AUC in those 1,000 bootstrap samples.

**Figure 2** Seizures and mutations in isocitrate dehydrogenase 1 (IDH1<sup>mut</sup>) in patients with glioma



(A) Patients with newly diagnosed infiltrative gliomas from 3 cohorts were assessed according to whether seizures were a part of their presenting symptoms, segregated into the 3 main molecular subgroups of gliomas: wild-type isocitrate dehydrogenase 1 (IDH1<sup>wt</sup>) (yellow), IDH1<sup>mut</sup> (white), and IDH1<sup>mut</sup> with 1p/19q codeletion (blue). (B, C) Overall survival in cohorts 1 (B) and 3 (C), according to IDH1<sup>mut</sup> and seizures on clinical presentation. *p* Values were calculated based on log-rank tests between IDH1<sup>wt</sup> or IDH1<sup>mut</sup> curves. (Cohort 2 lacked sufficient follow-up data for survival analyses.)

**Statistical evaluation.** Statistical significance of differences between groups was determined via 2-sided *t* test, one-way analysis of variance with post hoc Tukey test, Fisher exact test, or log-rank test, as appropriate, using GraphPad software (La Jolla, CA). Odds ratios (ORs) were based on exact logistic regression. Multivariable hazard ratios (HRs) were based on Cox proportional hazards models. In all analyses, the significance target was  $p \leq 0.05$ .

**Standard protocol approvals, registrations, and patient consents.** Institutional research board approval for the retrospective collection and analysis of deidentified patient data was obtained at University of Kentucky, NYU, and NMH prior to study initiation. Institutional Animal Care and Use Committee approval was obtained from the University of California, Davis, prior to neuronal harvesting and culture.

**RESULTS** To determine whether there is an association between IDH1<sup>mut</sup> and seizures in patients with glioma, we obtained clinical data from 3 separate cohorts of patients, totaling 712 newly diagnosed WHO grade II–IV infiltrative gliomas (table 1). Aside from cohort 2 having a higher mean patient age at presentation, the cohorts were similar in proportions of sex, WHO grades, IDH1<sup>mut</sup> frequency, 1p/19q codeletion, temporal lobe vs extratemporal tumor location, and patients who presented with seizures. Combining all 3 cohorts, IDH1<sup>mut</sup> frequency was 80.5% for grade II gliomas, 54.7% for grade III gliomas, and 8.9% for grade IV gliomas.

Current data indicate that testing for IDH1<sup>mut</sup> and 1p/19q effectively stratifies gliomas into 3 major clinical–pathologic groups: (1) IDH1<sup>mut</sup> with 1p/19q codeletion (i.e., oligodendroglioma), (2) IDH1<sup>mut</sup> without 1p/19q codeletion (i.e., astrocytoma), (3)

neither IDH1<sup>mut</sup> nor 1p/19q codeletion (i.e., GBM).<sup>9–11</sup> Whole-arm 1p/19q codeletion does not occur in the absence of IDH1<sup>mut</sup>.<sup>12</sup> In all 3 cohorts, IDH1<sup>mut</sup> gliomas were more likely to present with seizures than gliomas with only wild-type IDH1 (IDH1<sup>wt</sup>) (figure 2A). This increase in seizure risk was 56.6% in cohort 1 (74.5% vs 17.9%,  $\chi^2$  44.4,  $p < 0.001$ ), 32.1% in cohort 2 (64.5% vs 32.4%,  $\chi^2$  9.0,  $p = 0.003$ ), and 25.3% in cohort 3 (59.4% vs 34.1%,  $\chi^2$  17.6,  $p < 0.001$ ). In all 3 cohorts, there was no difference in seizure frequency between IDH1<sup>mut</sup>, 1p/19q-codeleted gliomas, and IDH1<sup>mut</sup>, 1p/19q-intact gliomas (83.3% vs 69.0%,  $p = 0.32$  in cohort 1; 66.7% vs 63.2%,  $p = 1.0$  in cohort 2; 60.5% vs 57.4%,  $p = 0.84$  in cohort 3). On multivariable analysis of other major factors associated with seizure risk, including 1p/19q codeletion, WHO grade, and temporal lobe location, IDH1<sup>mut</sup> was an independent correlate with seizures (OR 2.5, 95% CI 1.6–3.9,  $p < 0.001$ ) (table e-1 at Neurology.org).

We had previously created a web-based algorithm to help guide the efficient molecular workup of gliomas by providing accurate pretest probability of IDH1<sup>mut</sup>.<sup>8</sup> That model included patient age, history of glioma, and GBM diagnosis, as those were the variables that contributed to the predictive accuracy of the overall model. We found that, in the combined cohorts of that study, incorporating seizures at clinical presentation into the algorithm improved the model's accuracy ( $\Delta$ AUC 0.008, 95% CI 0.0004–0.017), further underscoring the relationship between IDH1<sup>mut</sup> and seizures in patients with glioma.

In cohorts 1 and 3, where survival data were available, there was no difference in median survival among IDH1<sup>mut</sup> gliomas without and with seizures (undefined vs undefined in cohort 1, HR 2.1, 95% CI 0.29–15.5,  $p = 0.45$ ; undefined vs undefined in cohort 3, HR 0.64, 95% CI 0.17–2.4,  $p = 0.51$ ) (figure 2, B and C). Among IDH1<sup>wt</sup> gliomas, only cohort 3 showed a modest increase in median survival when patients presented with seizures (7.6 vs 12.6 months in cohort 1, HR 1.1, 95% CI 0.66–1.9,  $p = 0.25$ ; 13.6 vs 18.4 months in cohort 3, HR 1.5, 95% CI 1.1–2.1,  $p = 0.01$ ). On multivariable analysis, however, only IDH1<sup>mut</sup> was an independent correlate with overall survival in both cohorts, not seizures (IDH1<sup>mut</sup> relative risk [RR] 0.027 and 0.20, 95% CI 0.006–0.12 and 0.57–1.1,  $p < 0.001$  and  $< 0.001$ ; seizures RR 0.78 and 0.79, 95% CI 0.45–1.4 and 0.57–1.1,  $p = 0.39$  and 0.15) (table 2).

We found that exogenous D2HG markedly increased the in vitro electrical activity of rat cortical neurons (figure 3, A and B, video). D2HG specifically increased the duration of synchronized bursts of firing in the neuronal networks (figure 3C), a characteristic effect of epileptogenic agents.<sup>13,14</sup> Along with an increase in network burst duration,

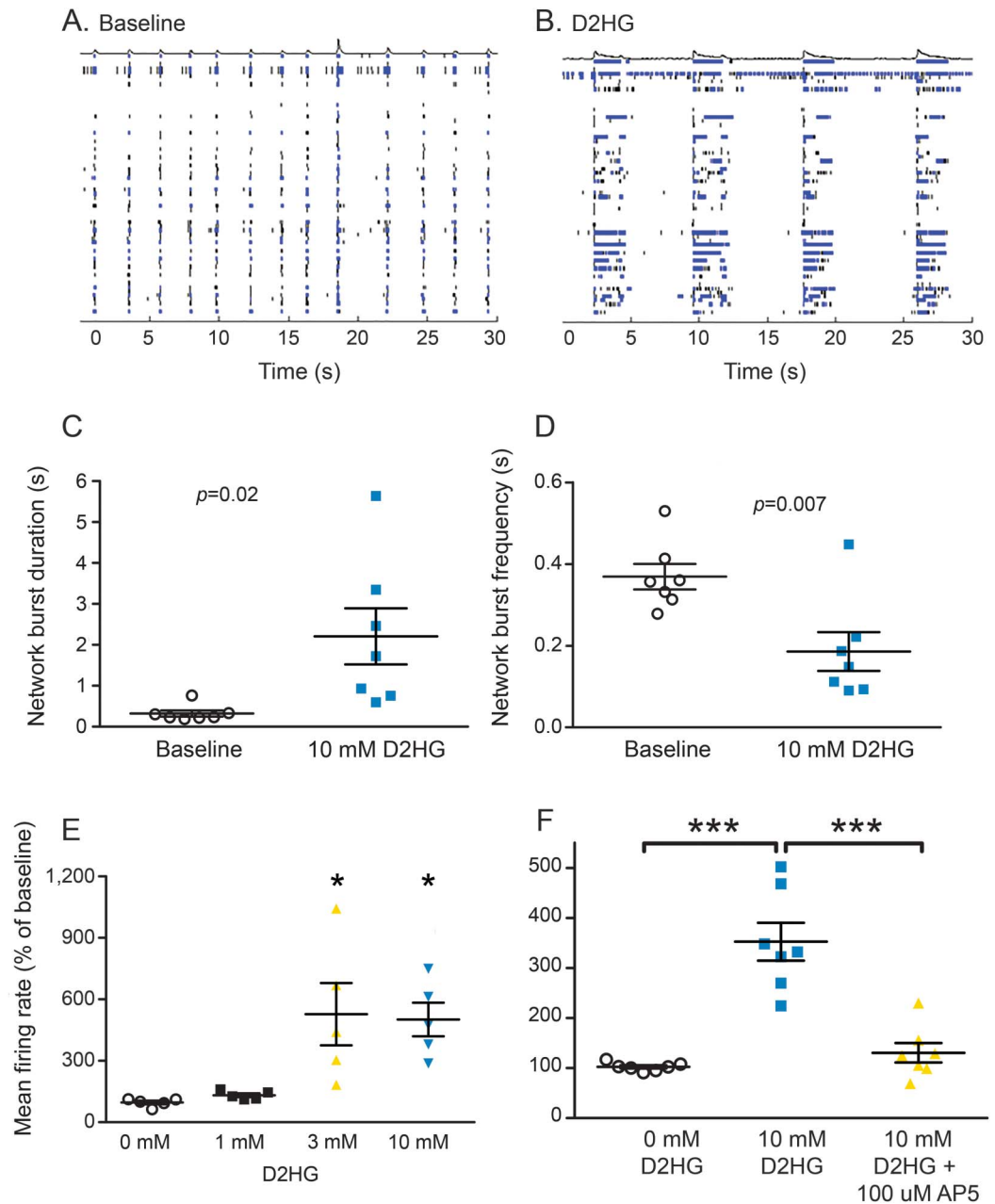
**Table 2** Multivariable analysis of survival in gliomas

	RR	95% CI	p Value
<b>Cohort 1 (n = 159)</b>			
GBM	0.62	0.27–1.44	0.26
1p/19q	0.77	0.14–4.3	0.76
IDH1 <sup>mut</sup>	0.027	0.006–0.12	<0.001
Seizures	0.78	0.45–1.4	0.39
<b>Cohort 3 (n = 409)</b>			
GBM	2.7	1.7–4.2	<0.001
1p/19q	0.0	0.0–7.0	0.96
IDH1 <sup>mut</sup>	0.20	0.10–0.39	<0.001
Seizures	0.79	0.57–1.1	0.15

Abbreviations: CI = confidence interval; GBM = glioblastoma; IDH1<sup>mut</sup> = mutations in isocitrate dehydrogenase 1; RR = relative risk.

Cox proportional hazards modeling of overall survival in cohort 1 (upper) and cohort 3 (lower) outcomes, including GBM diagnosis, 1p/19q codeletion, IDH1<sup>mut</sup>, and presentation with seizures. Survival data were not available for cohort 2, and were missing for 8 patients in cohort 3.

**Figure 3** Effects of D-2-hydroxyglutarate (D2HG) on neuronal activity

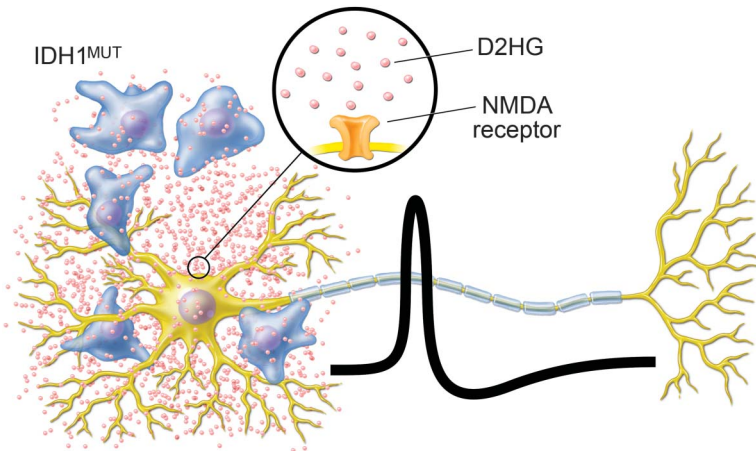


(A, B) Raster plots of cultured rat cortical neurons, before (A) and after (B) treatment with 10 mM D2HG. (C, D) Network burst duration (C) and frequency (D), before and after treatment with 10 mM D2HG. (E) Neuronal activity, expressed as mean firing rate relative to baseline, in the presence of increasing concentrations of D2HG.  $p = 0.003$  Via one-way analysis of variance,  $*p < 0.05$  vs 0 and 1 mM via post hoc Tukey test. (F) Mean firing rate of cultured neurons with control vehicle (0 mM D2HG), 10 mM D2HG, or 10 mM D2HG + 100  $\mu$ M AP5.  $***p < 0.001$ . (C, D, F) Seven wells per condition from 2 biological replicates; E shows 5 wells per condition from 2 biological replicates.

D2HG decreased network burst frequency (figure 3D). IDH1<sup>mut</sup> gliomas contain up to 30 mM D2HG, cells expressing IDH1<sup>mut</sup> release D2HG into their environment, and elevated D2HG has been detected in the circulating CSF of IDH1<sup>mut</sup> patients with glioma.<sup>2,3</sup> Infiltrative glioma cells readily migrate along axons and dendrites in the gray and white matter, forming close physical associations with neurons (figure 1). Thus, IDH1<sup>mut</sup> glioma

cells are poised to expose neurons to very high local concentrations of D2HG. We found a dose-response relationship between D2HG and neuronal activity, with increased activity detectable at 3 and 10 mM (figure 3E). The selective NMDA receptor antagonist, (2R)-amino-5-phosphonovaleric acid; (2R)-amino-5-phosphonopentanoate (AP5), completely blocked the excitatory effect of D2HG on neurons (figure 3F).

**Figure 4** Proposed proseizure effect of isocitrate dehydrogenase 1 (IDH1<sup>mut</sup>) glioma cells



IDH1<sup>mut</sup> glioma cells infiltrate gray matter and track along neurons. The D-2-hydroxyglutamate (D2HG) product of the mutant IDH1 enzyme is released by tumor cells and activates neuronal NMDA receptors, thereby creating excitatory postsynaptic potentials and increasing the likelihood of action potentials.

**DISCUSSION** Seizures are a common manifestation of infiltrative gliomas. Over 50% of patients with an infiltrative glioma will experience at least one seizure during the course of their disease, and over 30% will develop tumor-associated epilepsy (TAE), defined as multiple seizures.<sup>15,16</sup> This is a major cause of tumor-associated morbidity and greatly diminishes quality of life. At least 10% of patients with glioma will develop status epilepticus, a life-threatening condition wherein a seizure lasts longer than 5 minutes, or when multiple seizures occur without regaining consciousness.<sup>1</sup> TAE is managed by a combination of antitumor therapies (including surgical resection, chemotherapy, and radiation), as well as antiepileptic drugs (such as lamotrigine, levetiracetam, and valproate). Even multimodal strategies fail to control seizures in up to a third of patients.<sup>17</sup> A better understanding of the risk factors and mechanisms associated with TAE are therefore needed in order to improve patient quality of life.

The relationship between IDH1<sup>mut</sup> and seizures in patients with glioma has been controversial.<sup>1,10,18–26</sup> A direct mechanistic link between the D2HG product of IDH1<sup>mut</sup> and neuronal activity strengthens the hypothesis that IDH1<sup>mut</sup> gliomas are more likely to induce TAE than IDH1<sup>wt</sup> gliomas. Glutamate binds to and activates multiple receptors in neurons, including ligand-gated NMDA and AMPA ion channels, allowing influx of Na<sup>2+</sup>, K<sup>2+</sup>, and sometimes Ca<sup>2+</sup> ions. Depolarization caused by glutamate thus increases the likelihood that a neuron will fire an action potential. In an artificial model of human embryonic kidney cells expressing NMDA or AMPA receptors, D2HG bound and activated ionotropic NMDA receptors, but not AMPA receptors.<sup>27</sup> In the current

study, we directly tested the effect of D2HG on cultured neurons using MEAs, which are widely used to measure neuronal activity in vitro.<sup>28</sup> Unlike intracellular recordings that are limited to measurement of electrophysiologic activity in a single cell for a short duration, MEAs allow for noninvasive, simultaneous, and long-term recordings of local field potentials and extracellular action potentials from a population of neurons within a millisecond time scale.<sup>28</sup> MEAs can quantitatively measure properties of electrical activity across neuronal networks that are altered when changes in the inhibitory/excitatory balance lead to seizures.<sup>29–31</sup> Our data demonstrate that D2HG may activate neuronal NMDA receptors, thereby promoting the same kind of heightened network synchrony that is characteristic of seizure-inducing agents (figure 3, video).

A direct excitatory role for the D2HG product of IDH1<sup>mut</sup> helps explain a number of patterns regarding epilepsy in gliomas. First, older studies reported that oligodendrogliomas are more likely to cause seizures than those of astrocytic lineage.<sup>32–34</sup> Our data suggest that this is because the molecular profile of true oligodendrogliomas necessarily includes IDH1<sup>mut</sup>, whereas the molecular profile of astrocytic gliomas (including GBM) is more heterogeneous. Second, seizures are more common in lower-grade gliomas than in GBM, even though the latter are more invasive and destructive.<sup>1,35</sup> Our multivariable data show that IDH1<sup>mut</sup> is a significant correlate of seizures, independent of WHO grade (figure 2A, table e-1). Third, presenting with seizures is commonly thought to correlate with longer overall survival in patients with glioma, possibly because such tumors are detected earlier.<sup>16</sup> However, IDH1<sup>mut</sup> gliomas are much less aggressive than those harboring only IDH1<sup>wt</sup>,<sup>36</sup> raising the possibility that seizures at clinical presentation only predict longer survival to the extent that they are linked with IDH1<sup>mut</sup>. Our data suggest that the association between seizures at clinical presentation and better prognosis is driven mostly by the intrinsic biological differences between IDH1<sup>mut</sup> and IDH1<sup>wt</sup> gliomas, rather than by seizures prompting earlier clinical intervention (figure 2, B and C, and table 2).

Although seizures were more common in IDH1<sup>mut</sup> gliomas, a sizeable proportion of IDH1<sup>wt</sup> gliomas also caused pretherapy seizures (figure 2A). This underscores the fact that tumors can cause seizures by a variety of mechanisms, such as abnormal regulation of excitatory and inhibitory receptors, altered expression of ion transporters, increased mTOR activity, and inflammation.<sup>37,38</sup> Our data suggest that the production of D2HG, which is a central, defining feature of IDH1<sup>mut</sup> gliomas, may be another seizure-inducing mechanism.

While these findings suggest a mechanistic link between IDH1<sup>mut</sup>, its D2HG product, and



glioma-induced seizures, there are some limitations to this study. First, although the clinical data consist of a large number of patients from 3 separate institutions, it is a retrospective study and the presence of seizures is based on history-taking and subjective clinical impressions, not EEG data. Second, while MEA studies firing patterns among interconnected neurons over long periods of time, and high-density *in vitro* neuronal networks show synapse-specific modifications similar to what is seen *in vivo*,<sup>39</sup> single electrode approaches in low-density neuronal culture allow for the detailed study of D2HG-evoked currents. It will therefore be necessary to extend the current findings in future studies, using prospective patient-based analyses, single-electrode recordings, and *in vivo* models, in order to more fully understand the effects of D2HG on neuronal activity and epilepsy.

To date, the majority of glioma research on D2HG and IDH1<sup>mut</sup> has focused on their effects within the tumor cell. Our data indicate that IDH1<sup>mut</sup> may also exert a considerable effect on surrounding non-neoplastic cells, including neurons, through the release of its D2HG oncometabolite into the tumor microenvironment. In a preclinical model of IDH1<sup>mut</sup> glioma, pharmacologic inhibition of IDH1<sup>mut</sup> effectively lowered D2HG production and showed promising effects against tumor growth.<sup>40</sup> Currently, there are multiple clinical trials aimed at evaluating IDH1<sup>mut</sup> inhibitors in gliomas, including AG-120 (NCT02073994), AG-881 (NCT02481154), BAY1436032 (NCT02746081), and IDH305 (NCT02381886). To our knowledge, none of these trials include seizure monitoring as a primary or secondary endpoint. Yet, even if none of these drugs effectively controls glioma growth, they might help control TAE in patients with IDH1<sup>mut</sup> gliomas, either as a monotherapy or in conjunction with other antiepileptic drugs, by directly blocking the production of D2HG (figure 4).

#### AUTHOR CONTRIBUTIONS

Hao Chen performed the *in vitro* neuronal experiments and assisted with the manuscript. Pamela Lein designed the *in vitro* experiments. Laith Khoury collected patient data from University of Kentucky. Cheddhi Thomas, Carolina Benjamin, Donato Pacione, John Golfinos, and Matija Snuderl collected patient data from NYU. Jonathon Judkins, Farhad Ghamsari, and Priya Kumthekar collected patient data from NMH. Meijing Wu performed biostatistical analyses on all 3 patient cohorts. Li Chen provided the IDH1<sup>mut</sup> prediction modeling. Dane Chetkovich assisted with the manuscript. Craig Horbinski conceived the project, designed the study, and wrote the manuscript.

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#### DISCLOSURE

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#### REFERENCES

1. Kerkhof M, Vecht CJ. Seizure characteristics and prognostic factors of gliomas. *Epilepsia* 2013;54(suppl 9):12–17.
2. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 2009;462:739–744.
3. Kalinina J, Ahn J, Devi NS, et al. Selective detection of the D-enantiomer of 2-hydroxyglutarate in the CSF of glioma patients with mutated isocitrate dehydrogenase. *Clin Cancer Res* (in press 2016).
4. Moussawi K, Riegel A, Nair S, Kalivas PW. Extracellular glutamate: functional compartments operate in different concentration ranges. *Front Syst Neurosci* 2011;5:94.
5. Unruh D, Schwarze SR, Khoury L, et al. Mutant IDH1 and thrombosis in gliomas. *Acta Neuropathol* 2016;132:917–930.
6. Sturm D, Witt H, Hovestadt V, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell* 2012;22:425–437.
7. Cotterill E, Hall D, Wallace K, Mundy WR, Eglen SJ, Shafer TJ. Characterization of early cortical neural network development in multiwell microelectrode array plates. *J Biomol Screen* 2016;21:510–519.
8. Chen L, Voronovich Z, Clark K, et al. Predicting the likelihood of an isocitrate dehydrogenase 1 or 2 mutation in diagnoses of infiltrative glioma. *Neuro Oncol* 2014;16:1478–1483.
9. Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. *N Engl J Med* 2015;372:2499–2508.
10. Brat DJ, Verhaak RG, Aldape KD, et al. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N Engl J Med* 2015;372:2481–2498.
11. Reuss DE, Sahn F, Schrimpf D, et al. ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an “integrated” diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. *Acta Neuropathol* 2015;129:133–146.
12. Labussiere M, Idbaih A, Wang XW, et al. All the 1p19q codeleted gliomas are mutated on IDH1 or IDH2. *Neurology* 2010;74:1886–1890.
13. Jiruska P, Csicsvari J, Powell AD, et al. High-frequency network activity, global increase in neuronal activity, and synchrony expansion precede epileptic seizures *in vitro*. *J Neurosci* 2010;30:5690–5701.
14. Truccolo W, Ahmed OJ, Harrison MT, et al. Neuronal ensemble synchrony during human focal seizures. *J Neurosci* 2014;34:9927–9944.
15. van Breemen MS, Wilms EB, Vecht CJ. Epilepsy in patients with brain tumours: epidemiology, mechanisms, and management. *Lancet Neurol* 2007;6:421–430.
16. Lote K, Stenwig AE, Skullerud K, Hirschberg H. Prevalence and prognostic significance of epilepsy in patients with gliomas. *Eur J Cancer* 1998;34:98–102.

17. Vecht CJ, Kerkhof M, Duran-Pena A. Seizure prognosis in brain tumors: new insights and evidence-based management. *Oncologist* 2014;19:751–759.
18. Zhong Z, Wang Z, Wang Y, You G, Jiang T. IDH1/2 mutation is associated with seizure as an initial symptom in low-grade glioma: a report of 311 Chinese adult glioma patients. *Epilepsy Res* 2015;109:100–105.
19. Zou Y, Bai HX, Wang Z, Jiang Y, Yang L. Association of IDH1/2 mutation with preoperative seizure in low-grade gliomas: how strong is the evidence? *Epilepsy Res* 2015; 112:154–155.
20. Wang Z, Zhang Z, Wang Y, You G, Jiang T. Response to “Association of IDH1/2 mutation with preoperative seizure in low-grade gliomas: how strong is the evidence?” *Epilepsy Res* 2015;115:145–146.
21. Liubinas SV, D’Abaco GM, Moffat BM, et al. IDH1 mutation is associated with seizures and protoplasmic subtype in patients with low-grade gliomas. *Epilepsia* 2014; 55:1438–1443.
22. Morokoff A, Liubinas S. In response: IDH1 mutation expression is associated with seizures and protoplasmic subtype in patients with low-grade glioma. *Epilepsia* 2014;55:1679–1680.
23. Gonen T, Grossman R, Sitt R, et al. Tumor location and IDH1 mutation may predict intraoperative seizures during awake craniotomy. *J Neurosurg* 2014;121:1133–1138.
24. Mulligan L, Ryan E, O’Brien M, et al. Genetic features of oligodendrogliomas and presence of seizures: the relationship of seizures and genetics in LGOs. *Clin Neuropathol* 2014;33:292–298.
25. Stockhammer F, Misch M, Helms HJ, et al. IDH1/2 mutations in WHO grade II astrocytomas associated with localization and seizure as the initial symptom. *Seizure* 2012;21:194–197.
26. Yang P, You G, Zhang W, et al. Correlation of preoperative seizures with clinicopathological factors and prognosis in anaplastic gliomas: a report of 198 patients from China. *Seizure* 2014;23:844–851.
27. Kolker S, Pawlak V, Ahlemeyer B, et al. NMDA receptor activation and respiratory chain complex V inhibition contribute to neurodegeneration in d-2-hydroxyglutaric aciduria. *Eur J Neurosci* 2002;16:21–28.
28. Obien ME, Deligkaris K, Bullmann T, Bakkum DJ, Frey U. Revealing neuronal function through microelectrode array recordings. *Front Neurosci* 2014;8:423.
29. Cao Z, Hammock BD, McCoy M, Rogawski MA, Lein PJ, Pessah IN. Tetramethylenedisulfotetramine alters Ca<sup>2+</sup>(+) dynamics in cultured hippocampal neurons: mitigation by NMDA receptor blockade and GABA(A) receptor-positive modulation. *Toxicol Sci* 2012;130:362–372.
30. Cao Z, Zou X, Cui Y, et al. Rapid throughput analysis demonstrates that chemicals with distinct seizurogenic mechanisms differentially alter Ca<sup>2+</sup> dynamics in networks formed by hippocampal neurons in culture. *Mol Pharmacol* 2015;87:595–605.
31. Robinette BL, Harrill JA, Mundy WR, Shafer TJ. In vitro assessment of developmental neurotoxicity: use of microelectrode arrays to measure functional changes in neuronal network ontogeny. *Front Neuroeng* 2011;4:1.
32. Chang EF, Potts MB, Keles GE, et al. Seizure characteristics and control following resection in 332 patients with low-grade gliomas. *J Neurosurg* 2008;108:227–235.
33. Whittle IR, Beaumont A. Seizures in patients with supratentorial oligodendroglial tumours: clinicopathological features and management considerations. *Acta Neurochir* 1995;135:19–24.
34. Lebrun C, Fontaine D, Ramaoli A, et al. Long-term outcome of oligodendrogliomas. *Neurology* 2004;62:1783–1787.
35. Ruda R, Soffietti R. What is new in the management of epilepsy in gliomas? *Curr Treat Options Neurol* 2015;17:351.
36. Horbinski C. What do we know about IDH1/2 mutations so far, and how do we use it? *Acta Neuropathol* 2013;125: 621–636.
37. Buckingham SC, Campbell SL, Haas BR, et al. Glutamate release by primary brain tumors induces epileptic activity. *Nat Med* 2011;17:1269–1274.
38. Huberfeld G, Vecht CJ. Seizures and gliomas: towards a single therapeutic approach. *Nat Rev Neurol* 2016;12:204–216.
39. Jimbo Y, Tateno T, Robinson HP. Simultaneous induction of pathway-specific potentiation and depression in networks of cortical neurons. *Biophysical J* 1999;76:670–678.
40. Rohle D, Popovici-Muller J, Palaskas N, et al. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* 2013;340:626–630.

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