Detection of perillyl alcohol and its metabolite perillic acid in postsurgical glioblastoma tissue after intranasal administration of NEO100: illustrative case

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BACKGROUND Intranasal delivery of NEO100, a pharmaceutical-grade version of the natural monoterpene perillyl alcohol (POH), is undergoing clinical phase IIa testing as a treatment for glioblastoma (GBM). However, so far there is no evidence that intranasal delivery of NEO100 indeed results in POH reaching intracranial malignancies in a patient.

OBSERVATIONS After surgical removal of her recurrent GBM tumor, a patient received daily intranasal NEO100 therapy for more than 3 years before a second recurrence emerged. At that time, a ﬁnal dose of NEO100 was given shortly before the tumor tissue was surgically removed, and the tissue was processed for high-performance liquid chromatography analysis of POH and its primary metabolite, perillic acid (PA). Both molecules could readily be detected in the tumor tissue.

LESSONS This is the ﬁrst demonstration of POH and PA in brain tumor tissue from any patient. It reveals that intranasal administration of NEO100 is a valid approach to achieve delivery of this agent to a brain tumor. In view of the noninvasive and safe nature of this method, along with tentative indications of activity, our ﬁndings add conﬁdence to the notion that intranasal administration of NEO100 holds potential as a new treatment option for brain-localized malignancies.

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KEYWORDS blood-brain barrier; case report; monoterpene; recurrent glioblastoma

Malignant gliomas comprise the most common primary brain tumors in adults, and 5-year survival remains less than 10% for grade IV IDH wildtype gliomas (glioblastoma [GBM]).1,2 Secondary GBM (grade IV IDH mutant) shares a distinct molecular signature compared to primary GBM and accounts for approximately 10% of GBMs.3,4 Prognostic mutations in secondary GBM include O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, isocitrate dehydrogenase (IDH) 1 and 2 mutations, and 1p19q deletions.5 Despite a large number of investigations, targeted therapies have not proved effective to date in GBM, and standard of care beyond surgery and radiotherapy has not progressed from temozolomide.

Prognosis for GBM patients further worsens in the recurrent setting, in which median overall survival is less than 12 months.6 Perillyl alcohol (POH; also called p-metha 1,7-diene-6-ol) is a naturally occurring monoterpene derived from limonene, which is present in the essential oils of citrus fruits and other plants.7 Preclinical data have demonstrated chemopreventive and cancer therapeuetic activity of POH in a number of preclinical tumor models, including GBM.8 However, a series of phase I and II trials with three- to four-times-daily oral POH formulated in gelatin capsules failed to demonstrate convincing anticancer activity, and unreliant gastrointestinal toxicity proved severely taxing for these patients.8

ABBREVIATIONS BBB = blood-brain barrier; CSF = cerebrospinal ﬂuid; GBM = glioblastoma (multiforme); IDH1 = isocitrate dehydrogenase 1; MGMT = O6-methylguanine-DNA methyltransferase; OS = overall survival; PA = perillic acid; POH = perillyl alcohol; WHO = World Health Organization.

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Although the presence of POH metabolites, such as perilllic acid (PA), could be demonstrated in patient serum, POH itself was undetectable, presumably because of its well-known high susceptibility to metabolic liver enzymes.\(^9\)

To circumvent the above limitations of oral delivery, POH has been assessed for clinical use by means of intranasal administration to patients with glioma, and ongoing studies in Brazil have yielded encouraging responses.\(^{10,11}\) In the United States, a highly pure, pharmaceutical-grade version of POH, termed “NEO100,” has been developed and investigated in a recently published phase I trial involving patients with recurrent GBM, in whom intranasal delivery showed negligible toxicity and preliminary signs of activity.\(^{12}\)

The general concept of intranasal drug delivery is based on the premise that this noninvasive route of administration may employ, at least in part, direct nose-to-brain transport, which avoids rapid drug metabolism by the liver, circumvents drug rejection by the blood-brain barrier (BBB), and minimizes the need for flooding the entire systemic circulation with drug to deliver sufficiently high drug levels to the brain lesion.\(^{13,14}\) It has remained unclear, however, whether the intranasal route of POH/NEO100 administration is indeed able to achieve its key objective, namely to enable the drug to reach its intended intracerebral tumor target. Such confirmation is critical because it will lend much-needed support to the model that intranasal NEO100 represents a viable, safer, and potentially superior means of treating patients with brain cancer.

In the following report, we present the case of a patient with recurrent grade IV IDH\(^1\) mutant glioma who received intranasal NEO100 for more than 3 years with good success and in whom repeat surgery allowed us to obtain tumor tissue after NEO100 dosing, which enabled the intratumoral detection of POH and its metabolite PA.

**Illustrative Case**

A 39-year-old woman was diagnosed with a left frontal low-grade glioma upon workup for new-onset seizure. She was initially treated with two craniotomies over a 4-year period, with histopathological examination revealing World Health Organization (WHO) grade 2 oligoastrocytoma. Upon second recurrence, she underwent gross-total resection of recurrent disease, with histological and molecular features demonstrating WHO grade 3 anaplastic gemistocytic astrocytoma, \(\text{IDH1}\) (R132H) mutant, with no evidence of 1p/19q codeletion (Fig. 1A and B). The patient received standard-of-care intensity-modulated radiotherapy (57 Gy in 30 fractions) with concomitant temozolomide followed by six cycles of adjuvant temozolomide. Radiographic recurrence was demonstrated 10 months from the time of the last resection. The patient then underwent subtotal resection with a small volume of residual enhancing disease (Fig. 2A).

Pathological examination at that time revealed grade IV glioma, MGMT unmethylated, \(\text{IDH1}\) mutant (Fig. 1C). The patient was enrolled in an open-label, phase II/IIa trial (NCT02704858),\(^{12}\) in which she self-administered intranasal NEO100 using a nebulizer and nasal mask. Dosing was four times a day, every day, at 144 mg/dose (576 mg/day). Plasma levels of POH and PA at different time intervals after dosing are shown in Figure 3A, confirming uptake of NEO100 into the systemic circulation. PA plasma levels were approximately 100-fold higher than POH plasma levels. Despite being much lower, POH levels were readily detectable at the 5-minute
time point, but in two of three serial samplings the levels dropped below the detection threshold within 30 minutes, consistent with the known very short half-life of POH in plasma and the resulting difficulty of detecting it in biological systems.8

An excellent clinical outcome was achieved, with tumor regression and lack of progression for more than 3 years (Fig. 2). No significant toxicities were appreciated, based on physical examination, vital signs, clinical laboratory test results, and National Cancer Institute Common Terminology Criteria for Adverse Events.15 Mild dermatitis related to the inhalation mask was successfully treated with cortisone during cycle 21. Tumor recurrence was demonstrated after 40 months (Fig. 2E), and the patient was taken for resection given good performance status.

On the day of surgery, the patient self-administered one dose of 144 mg intranasal NEO100. After surgery, the resected GBM tissue was processed for pharmaconalytical examination and for molecular analysis, which revealed MGMT methylated, IDH1 mutant disease. High-performance liquid chromatography was applied to two separate regions of the tumor tissue, both of which readily revealed the presence of POH and its metabolite PA (Fig. 3B). This result is especially notable on two accounts: (1) despite the unavoidable delay between NEO100 administration and retrieval of the tissues sample (approximately 4 hours), POH was still present in the tissue and (2) the ratio of PA to POH was very low (approximately 1.4), altogether indicating a very slow metabolism of POH in this tissue.

Discussion
Observations
The key observation of our study is the detection of both POH and PA in GBM tissue resected after intranasal administration of NEO100. This represents the first documentation that POH indeed reaches the intracranial malignant tissue after intranasal administration in a human patient. It has implications not only for the treatment of GBM but possibly also for other malignancies, such as brain metastasis.

The intranasal route of drug delivery is postulated to have significant advantages over other means of drug delivery. For purposes of brain cancer treatment, it is envisioned that direct nose-to-brain transport of a pharmaceutical agent may be able to achieve higher

*FIG. 2. A: Postoperative magnetic resonance imaging scan at time of diagnosis of GBM demonstrating small residual enhancing disease (white arrow). B–D: Representative scans after 1, 2, and 3 years of daily intranasal NEO100 therapy, demonstrating no enhancing disease. E: Progressive disease (white arrow) demonstrated posterior to the prior resection cavity after 40 months of therapy. Radiographic response was evaluated using both Macdonald and Response Assessment in Neuro-Oncology criteria*.
drug concentrations at the intracranial lesion site, may circumvent the obstacle posed by the BBB, and may reduce systemic side effects because overall lower dosages can be used. However, there is a paucity of evidence that these benefits can be achieved with cancer drugs. POH (in Brazil) and NEO100 (in the United States) are the first cancer therapeutic agents that have advanced to phase I/II trials investigating intranasal delivery to patients with glioma. Despite promising signs of activity\(^{10-12}\), it has frequently been criticized that investigators in these trials are lacking the most basic knowledge, namely, proof that POH indeed reaches the tumor.

Although we succeeded in demonstrating the presence of POH and PA in tumor tissue, a limitation of our study is that it is based on only one patient. In general, it remains a challenge to measure drug concentrations directly in human brain lesions because of the invasive nature of brain biopsies, sensors, or surgery. It also requires careful logistic planning to align drug treatment with timing of surgery and proper securing and processing of tumor tissue. Based on the known short plasma half-life of PA and the even shorter half-life of POH\(^5,9\), it was a valuable surprise to actually be able to detect both compounds in the brain tumor sample, considering that several hours had passed between intranasal NEO100 delivery and acquisition of the tumor sample after surgery. This finding indicates greater stability of POH once it has entered tumor tissue, which is of particular interest in view of in vitro studies showing that POH exerts substantially greater cytotoxic activity against cancer cells than its PA metabolite\(^{16,17}\). Similarly, we find that the ratio of PA to POH in tumor tissue is very low (approximately 1.4), which contrasts the very high ratio (approximately 100-fold) found in plasma. A low ratio is much preferred because it indicates slow metabolism (and inactivation) of POH, providing prolonged biological activity.

Our findings derived from a clinical sample are in agreement with preclinical observations published recently. Nehra et al.\(^{18}\) performed intranasal administration of POH to rats, followed by measurements of cerebrospinal fluid (CSF)/plasma ratios of POH and PA. They reported 10-fold higher CSF/plasma ratios for both compounds after intranasal delivery as compared to equal dose intravenous delivery, implying direct transport from the nasal mucosa to the CSF, and providing further evidence that intranasal delivery...
achieves overall higher brain exposure to POH. Cho et al.\textsuperscript{19} reported that intranasal delivery of POH to mice harboring orthotopic, temozolomide-resistant human U251 resulted in statistically significant (p < 0.009) survival of animals, providing the first in vivo indication that intranasal POH can result in antitumor activity against brain-localized disease.

The mechanism of POH’s antitumor activity appears to be pleiotropic. A large number of in vitro and in vivo studies with this monoterpene have yielded a variety of targets, including components of the cell cycle machinery, the endoplasmic reticulum stress response pathway, isoprenylation of small G proteins including p21-Ras, immediate-early genes, telomerase reverse transcriptase (hTERT), eukaryotic translation initiation factors eIF4E and eIF4G, sodium/potassium adenosine triphosphatase (Na/K-ATPase), and numerous others.\textsuperscript{20} Furthermore, POH has shown inhibitory effects not only against tumor cell proliferation and survival but also against tumor cell migration and tumor angiogenesis, either alone or in combination with standard chemotherapeutic drugs.\textsuperscript{21–23} The combination of these pleiotropic molecular and cellular activities is thought to contribute to a joint, multipronged impact against tumor growth.

The patient’s IDH1 mutant status deserves additional consideration, because IDH1 gene mutation in tumor tissue generally is a predictor of improved overall survival (OS) in patients with malignant glioma.\textsuperscript{24} However, while this link has been well established for newly diagnosed patients, it has remained less clear for the recurrent GBM setting. A study by Mandel et al.\textsuperscript{25} reported no statistically significant difference in median OS when patients with recurrent GBM with IDH1 wild-type status (n = 75; OS, 8.6 months) were compared to those with IDH1 mutant status (n = 17; OS, 9.6 months). However, when this analysis was limited to patients at first recurrence only, those with IDH1 mutant tumor status (n = 5) seemed to gain a survival advantage (OS, 19.3 months). In contrast, Tabei et al.\textsuperscript{26} were unable to detect a positive correlation between IDH1 mutation and OS after first progression; they reported OS of 10.1 months for the mutant IDH1 group (n = 13) and 10.5 months for the wild-type IDH1 group (n = 109), concluding that “IDH1 mutation may not be a prognostic factor for survival at the first progression.” Considering these rather short survival times even for patients with mutant IDH1, we deem it extremely encouraging that our patient experienced more than 36 months without progression while on intranasal NEO100 therapy. At the time of this writing (May 2022), she is still alive 54 months after recurrence.

The encouraging survival of our patient is in line with an updated OS analysis of other patients in the phase I part of our phase I/IIa study of intranasal NEO100 for recurrent GBM. Results from phase I were published in early 2021.\textsuperscript{12} This earlier study described a total of 5 patients with mutant IDH1, and at the 24-month data cutoff point, 4 patients (80%) were still alive. Since then, 1 of these 4 patients succumbed to disease at 33 months, leaving 3 patients (60%; including the subject of this case study) alive at 48 months after recurrence. Intriguingly, those 2 deceased patients were assigned to the lowest of four dose-escalation cohorts (384 mg NEO100 per day), whereas current survivors were in higher 576 mg/day (n = 1) and 768 mg/day (n = 2) cohorts. In all, while none of the patients with wild-type IDH1 (n = 7) survived beyond 18 months, median OS of the 5 patients with mutant IDH1 has surpassed the 48-month mark, representing a strikingly promising outcome.

### Lessons

Intranasal delivery of POH and NEO100 has been shown to be very well tolerated, along with indicating promising signs of activity.\textsuperscript{10–12} However, up until now, a key question has remained unanswered: namely, whether the active pharmaceutical ingredient in these treatments, POH, indeed reaches the tumor tissue. Our report now provides this critical piece of the puzzle. Quite clearly, POH could be detected and measured in GBM tissue after intranasal delivery, providing the first documentation of POH in the tumor tissue from any patient. Combined with the updated survival data from our phase I study, this finding provides substantial support to the view that intranasal NEO100/POH is a promising treatment option for patients with brain tumor and that using the nose-to-brain pathway—perhaps in combination with other chemotherapeutic agents—may offer a number of benefits for future patients.

### References


Disclosures
Thomas C. Chen reports a patent for 8507734 issued and is an officer and shareholder of NeOnc Technologies. Vincent F. Simmon reports stock options in NeOnc Technologies during the conduct of the study. No other disclosures were reported.

Author Contributions
Conception and design: Chen, Wagle. Acquisition of data: Chen, Swenson, Bonney, Wagle. Analysis and interpretation of data: Chen, Schönthal, Swenson, Wagle. Drafting the article: Bonney, Wagle. Critically revising the article: Chen, Schönthal, Wagle, Simmon. Reviewed submitted version of manuscript: Chen, Schönthal, Swenson, Bonney, Wagle, Simmon, Hurth. Approved the final version of the manuscript on behalf of all authors: Chen. Administrative/technical/material support: Schönthal, Simmon. Study supervision: Chen, Wagle. Pathology: Mathew. Assessment of tissue: Hurth.

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