Functional precision medicine assay for recurrent meningioma: a proof of principle.
Illustrative case

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BACKGROUND Meningiomas are the most prevalent primary central nervous system tumors. Although low-grade meningiomas are considered benign tumors, a subset of these can behave aggressively, showing progression and recurrence. In such cases, functional assays could influence treatment decisions and improve patient outcomes.

OBSERVATIONS A 78-year-old female presented with a long-standing history of a supratentorial meningioma that was initially resected and treated with Gamma Knife radiosurgery. Surveillance revealed progression. She began systemic therapy with everolimus and octreotide but was lost to follow-up and did not continue the treatment. She returned because of a rapid decline in her neurological status. Biopsy with advanced molecular characterization by next-generation sequencing revealed NF2 and CREBBP mutations, and a commercial functional assay was done. This assay successfully isolated cancer stem cells (CSCs) from biopsy cores and identified potential drugs based on cellular sensitivity profiles. This is the first reported case in which a commercial functional drug screen was used for a meningioma.

LESSONS In cases in which meningiomas exhibit specific genetic alterations and characteristics of aggressiveness, functional assays can be a useful tool for isolating CSCs. The authors report success in obtaining drug-screen profiling for a World Health Organization grade 1 meningioma. Multimodal approaches utilizing multi-omics analyses with functional assays can improve patient outcomes.

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KEYWORDS functional precision medicine; meningioma; NF2 mutation; CREBBP mutation; low-grade meningioma

Meningiomas are the most common primary brain tumors, accounting for 38.7% of all central nervous system (CNS) tumors, with an incidence rate of 9.51 per 100,000 persons in the United States. They are classified by the World Health Organization (WHO) into 3 major groups: grade 1 (benign), grade 2 (atypical), and grade 3 (malignant). The majority of these tumors are benign grade 1 meningiomas, representing approximately 80% of all diagnosed meningioma cases. Around 20% will present a more aggressive nature with elevated recurrence rates and an increased probability of progressing to a higher-grade tumor. While CNS tumor grading has traditionally reflected the natural behavior of the clinical course and the potential “curability” of the disease, recent guidelines have started to incorporate a tumor-type grading approach. Nonetheless, there is still a correlation between the grade of the tumor and the expected clinical/biological behavior in the most updated CNS WHO classification. For instance, higher-grade (WHO grade 2 and 3) meningiomas are expected to be more aggressive, with higher rates of recurrence and mortality, while lower-grade meningiomas are generally considered to be benign, even though they are not exempt from this rule, as they can also behave aggressively and recur.

The incorporation of molecular features in the 2021 WHO classification introduced a prognostic significance to meningiomas. The inclusion of different genetic alterations, such as gene mutations, copy number variations, and epigenetic modifications, has improved the diagnostic approaches. For example, one of the most frequent genetic mutations in this tumor is the tumor-suppressor gene NF2 located on 22q12. This mutation has been reported in approximately 40%–60% of sporadic meningioma cases, with over 50% of the tumors showing loss of heterozygosity in the 22q12 chromosomal region responsible for codification of the NF2 gene. Advances in sequencing technologies have made it possible to identify a wide variety of relevant genes responsible for the pathogenesis and phenotypes of this disease. Not

ABBREVIATIONS CEVOREM = Combination of Everolimus and Octreotide in Aggressive Recurrent Meningiomas; CNS = central nervous system; CSC = cancer stem cell; FPM = functional precision medicine; GKRS = Gamma Knife radiosurgery; LITT = laser interstitial thermal ablation; MRI = magnetic resonance imaging; mTOR = mechanistic target of rapamycin; WHO = World Health Organization.

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only can the mutational statuses of certain alterations serve as a correlation for the phenotype of the disease, such as NF2 and SMARCE1 in convexity meningiomas or TERT gene promoter mutation and CDKN2 homozygous deletion in grade 2 and grade 3 meningiomas, but they can also serve as fundamental markers for establishing a more accurate prognosis. For example, the presence of genetic alterations, such as ATM and CREBBP, is associated with accelerated meningioma recurrence and worse overall outcomes of the disease.9,10

On the other hand, recent studies have demonstrated the importance of integrating multi-omics analyses such as epigenomics, transcriptomics, and genomics for defining clinically distinct meningioma subgroups in histologically challenging cases.4,11-14 These advanced molecular pathogenetic markers have slowly been integrated into clinical guidelines, but some are still not part of routine clinical practice. Additionally, while surgery remains the mainstream treatment, systemic therapies may be beneficial for clinically aggressive, recurrent meningiomas. In such cases and due to the advent of precision oncology technologies, functional assays could be useful for therapy guidance. In functional precision medicine (FPM), tumor cells are isolated ex vivo and screened for a variety of therapies. As an illustration, in this case report, we describe the first known case of successfully sending a commercial functional assay based on cancer stem cell (CSC) isolation from tumor tissue for an aggressive WHO grade 1, NF2- and CREBBP-mutated meningioma.

Illustrative Case

A 78-year-old female had dural-based frontal meningiomas that were initially resected in 2008. Subsequent serial radiographic surveillance revealed interval growth suggestive of recurrent/residual meningioma in multiple regions in 2013 (Fig. 1). Brain magnetic resonance imaging (MRI) showed interval growth suggestive of recurrent/residual meningioma in multiple regions. She underwent Gamma Knife radiosurgery (GKRS) with 15 Gy to all 3 lesions. Continued surveillance demonstrated recurrent masses on imaging and an interval increase of the meningioma along the right frontal resection cavity. She was scheduled for a second session of GKRS in 2017.

In 2021 serial imaging showed further progression, with the right falx cerebri lesion measuring 7 cm anteroposteriorly compared to 1.5 cm in 2019. The patient, deemed unsuitable for surgery due to a high surgical risk stemming from her age, uncontrolled hypertension, and diabetes mellitus, was also at risk for radiation necrosis. Systemic therapy using the Combination of Everolimus and Octreotide in Agressive Recurrent Meningiomas (CEVOREM) protocol (everolimus + octreotide) was initiated,15 which she underwent for 10 months. Unfortunately, the patient was lost to follow-up and did not continue the established protocol.

A year later, the patient returned to the clinic with a rapid decline in mental and functional status. A trial of steroids was initiated, resulting in mild improvement. Brain MRI showed further progression of the disease. Given the amount of bilateral brain compression and the patient’s age and comorbidities, traditional resection was deemed to carry excessive morbidity. Multidisciplinary neuro-oncology tumor board discussions highlighted different strategies for approaching this case, including medical management, fractionated radiation (imposing a higher risk of worsening cerebral edema), and biopsy with laser interstitial thermal ablation (LITT). Discussions with the neuro-oncology tumor board and the patient’s family resulted in a biopsy with advanced molecular characterization of the tumor with next-generation sequencing as well as sending for an ex vivo FPM assay. Laser

FIG. 1. Timeline illustrating the disease progression on MRI of the brain. Contrast T1-weighted sequences showing a heterogeneous enhancing mass, initially situated in the parafalcine area. Subsequent scans demonstrate gradual progression toward the bilateral frontal lobes, extending into the overlying extra-axial space, adjacent subarachnoid spaces, and the anterior and mid superior sagittal sinuses. SRS = stereotactic radiosurgery; Sx = surgery.
Thermal ablation was done along multiple trajectories. Importantly, LITT was introduced as an option in this case given that different studies have shown how this method could be beneficial for patients with recurrent, aggressive, and refractory meningiomas.16-18

The procedure was done without complications, and the patient was discharged days later with improvement in her cognitive function. Surprisingly, pathological analysis of both the index and recurrent tumor did not fulfill the WHO criteria for a grade 2 meningioma. Mitotic figures were difficult to find, with few confluent areas of necrosis and without a clear demonstration of definitive neuroglial tissues or brain invasion with immunostaining. However, there was a high Ki-67 proliferation index of 8%–10% in both pathological samples (Fig. 2). Changes attributable to radiation effect were not identified.

Meningiomas with atypical findings and a high proliferative index may exhibit more frequent recurrences and/or more aggressive behavior (i.e., grade 1.5).19-21 Furthermore, genomic analysis of the tumor showed an NF2 c.448-2A>G loss-of-function mutation as well as a CREBBP mutation. No other molecular alterations were reported, with immunohistochemical staining for BAP-1 showing retained nuclear expression and no additional molecular evidence such as a CDKN2A/B homozygous deletion, TERT gene promoter mutation, H3K27 trimethylation, or copy number alterations. Methylation profiling was not done in this case.

A fresh tumor sample (i.e., 4 biopsy cores) at the time of surgery was sent for ChemoID analysis, an FPM assay that isolates CSCs with previous neuro-oncological applications in gliomas.22,23 To date, no report of the utilization of an FPM assay for meningioma exists. ChemoID analysis of the tumor (Fig. 3) displayed the different chemotherapies used in vitro against the CSCs and bulk tumor cells of this tumor. Based on these findings, the neuro-oncology team proposed bevacizumab, as some clinical trials have shown meningioma shrinkage in patients with neurofibromatosis type 2,24,25 and imatinib, guided by the ChemoID assay as one of the most effective drugs for eliminating CSCs. Although there is some controversy, it may be a possible therapeutic option for this drug in select meningioma cases.26 Nevertheless, the patient refused chemotherapy and radiation therapy. She continued to experience a progressive decline in her neurological status and her refractory cerebral edema. Eventually, it was decided to transition her to comfort care. She died 4 months after her last surgery.

**Table 1.** Comparative Values for Bulk of Tumor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Cell Kill</th>
<th>Graphic Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCNU 100 mg/m2</td>
<td>100.0% ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Imatinib 200 mg + Temodar 50 mg/m2</td>
<td>67.2% ± 0.2</td>
<td></td>
</tr>
<tr>
<td>CCNU 100 mg/m2</td>
<td>60.9% ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Vincristine 1.4 mg/m2 + CCNU 100 mg/m2 + Procarbazine 60 mg/m2</td>
<td>56.5% ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Etoposide 50 mg/m2 + Carboplatin 350 mg/m2</td>
<td>41.5% ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Irinotecan 125 mg/m2 + Carboplatin 350 mg/m2</td>
<td>33.3% ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Comparative Values for Cancer Stem-Like Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Cell Kill</th>
<th>Graphic Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib 200 mg</td>
<td>83.3% ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Etoposide 50 mg/m2</td>
<td>21.6% ± 0.1</td>
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**Reference**

<10 - 30 Non-responsive (<10 - 30)

30 - 60 Intermediate response (30 - 60)

60 - 100 Responsive (60 - 100)

Patient Informed Consent

The necessary patient informed consent was obtained in this study.

Discussion

Observations

In this case, the pathology did not meet the WHO CNS 5th edition criteria for atypical meningioma but did exhibit a high Ki-67 proliferative index. Meningiomas with a high degree of proliferative index are more likely to recur and behave aggressively.27-29 Additionally, molecular profiling provided critical insights into the prognosis of this patient by identifying NF2 and CREBBP mutations. The NF2 mutation, reported as the most common mutation in meningiomas, has been shown to be a predictor of overall prognosis in WHO meningiomas.
grade 1 meningiomas, especially when coupled with the anatomical location of the tumor. A study done by Teranishi et al. conducted a retrospective long-term follow-up on 281 WHO grade 1 meningioma patients. That study demonstrated how the prognosis of the tumors differed depending on the mutations and the anatomical region: supratentorial tumors with NF2 mutations were associated with worse progression-free survival rates compared to the rates for infratentorial tumors without NF2 mutations. Additionally, the multivariate analysis done in this study showed how supratentorial tumors harboring NF2 mutations and a high Ki-67 index (defined as ≥ 4) can serve as significant predictors for a poor prognosis and recurrence. Similarly, CREBBP mutations have also been associated with meningioma recurrence.

Due to the aggressive nature of our patient’s tumor, an approach with everolimus and octreotide was first initiated. NF2 mutations dysregulate the moesin-ezrin-radixin-like protein (also known as merlin). This “cytoskeleton scaffolding protein,” best known as a tumor suppressor, regulates cellular functions such as intracellular signaling or cell-cell adhesion, which converge in essential pathways that influence cellular survival and proliferation. A relevant pathway controlled by the merlin protein involves the downregulation of the mechanistic target of rapamycin (mTOR). Abnormally activated mTOR has been correlated with uncontrolled cell growth and proliferation in cancer cells. The use of everolimus, a Food and Drug Administration–approved mTORC1 inhibitor, in combination with octreotide, a somatostatin receptor subtype 2 (SST2) agonist, was endorsed by the CEVOREM protocol, a prospective phase II study that demonstrated antitumor activity for meningiomas in patients who were given a combination of these 2 drugs.

On the other hand, the multimodal treatment approach used in this patient required the exploration of alternative options, including FPM and LITT, given the aggressiveness of this meningioma. This decision was prompted by promising results of functional assays observed in other CNS tumors such as glioblastoma. FPM has been transforming the practices of medical oncology. By individually analyzing patients’ samples, these assays allow for a deeper understanding of the most crucial oncogenic processes by providing a sensitivity panel of the most relevant oncological drugs. Given the complexity and dynamism of cancer, where a single tumor can harbor cellular subclones with numerous related somatic mutations while simultaneously containing unrelated cells of varying nature, the use of functional assays may offer a more nuanced understanding into the optimal treatment at a specific point in time for a tumor. In the same manner, addressing the sensitivity profile of tumors by isolating CSCs, responsible for “tumor initiation, invasiveness, recurrence, and drug resistance,” may improve patients’ survival.

The assay utilized in this case report specifically screens CSCs in solid tumors to identify cytotoxic therapies. Previous studies have shown the isolation of CSCs in low-grade meningiomas. Recent advancements in three-dimensional tumor organoids have allowed for advanced modeling of brain tumors. Considering a functional assay was a valid approach and was the preferred option for this patient. Indeed, it successfully helped to identify potential drugs for this aggressive tumor. Despite the unfortunate outcome of this case, it provided insights into potentially usable drugs for approaching this case, highlighting this report as one of the first known cases in which this approach has been used.

Lessons
Consideration of a functional assay may be of clinical significance for aggressive meningiomas, particularly when several indicators of poor prognosis, such as supratentorial location, NF2 mutation, CREBBP mutation, and high Ki-67 index, are present. This should prompt consideration for a multimodal approach, and functional assays could be a valuable option in such cases.

References


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Author Contributions
Conception and design: Rodriguez. Acquisition of data: Shelton, Horta, Nix, Gokden. Analysis and interpretation of data: all authors. Drafting the article: Shelton. Critically revising the article: Rodriguez, Horta, Nix, Gokden. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Rodriguez. Administrative/technical/material support: Rodriguez, Gokden. Study supervision: Rodriguez.

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