Mismatch repair deficiency in high-grade meningioma: a rare but recurrent event associated with dramatic immune activation and clinical response to PD-1 blockade

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Disclosure of Potential Conflicts of Interest
PKS is a member of the Scientific Advisory Board of RareCyte Inc., which manufactures the CyteFinder slide scanner used in this study. PKS is also co-founder of Glencoe Software, which contributes to and supports the open-source OME/OMERO image informatics software used in this paper. SS has consulted for RareCyte Inc.. Other authors have no competing financial interests to disclose.

FOOTNOTE
We obtained all permissions required by law and by the Dana–Farber/Harvard Cancer Center to allow for the publication of images from the patient.
INTRODUCTION

Meningiomas are the most common primary tumor of the central nervous system, with ~28,000 new diagnoses annually in the United States\textsuperscript{1}. Currently, there are no approved systemic therapies for meningiomas that recur following local treatment: chemotherapy and hormonal agents have demonstrated minimal benefit in numerous clinical trials\textsuperscript{2–4}.

Meningioma comprises a heterogeneous group of neoplasms driven by mutations in a wide array of tumor suppressor genes and oncogenes\textsuperscript{5–17}. Characterization of these mutations has revealed opportunities for rational therapy\textsuperscript{18–20}. For example, a durable therapeutic response has been reported for a metastatic AKT1(E17K)-mutant meningioma treated with a pan-AKT inhibitor\textsuperscript{21}.

Studies also suggest the potential for treating meningioma with immune checkpoint modulators\textsuperscript{22–24}: programmed death receptor 1 ligand (PD-L1) is expressed in a subset of meningiomas and the tumor microenvironment is immunosuppressive\textsuperscript{22–28}. Higher-grade meningiomas also harbor mutations predicted to generate neoantigens, which may foster susceptibility to immunotherapies\textsuperscript{29}.

Based on these data, we initiated a phase II study of nivolumab, a humanized IgG4 PD-1 blocking monoclonal antibody, in patients with higher-grade meningiomas that recurred following surgery and radiotherapy (NCT02648997). We report here a patient with an atypical meningioma that was not controlled by repeated surgery and radiation but which was highly responsive to nivolumab.

CASE REPORT

A 50-year-old women with progressive headaches underwent a gross total resection (Surgery\#1) of a gadolinium-enhancing right frontal convexity atypical meningioma (WHO grade II) (Figure 1A). The tumor recurred 10 months later and was treated with radiosurgery (SRS\#1; 25 Gy/5 fractions). Recurrent atypical meningioma was debulked 17 (Surgery\#2) and 21 months (Surgery\#3) after the original diagnosis. The patient then underwent conventional radiotherapy (Conformal RT; 54 Gy/27 fractions). Three months later, she again underwent radiosurgery (SRS\#2; 14 Gy/1 fraction) due to tumor spread to the sphenoid ridge and infratemporal fossa. Additional debulking surgeries for recurrent atypical meningioma were performed at 46 (Surgery\#4) and 49 (Surgery\#5) months. She then received bevacizumab for eleven months, mifepristone for seven months and temozolomide for four months. Each therapy was discontinued after disease progression. A sixth debulking surgery (Surgery\#6) at 70 months confirmed recurrent atypical meningioma.

Five weeks later, the patient enrolled in our phase II nivolumab clinical trial. At that time, 75 months after the original diagnosis, painful subcutaneous masses overlaid the right convexity. The patient required oxycodone for scalp pain and dexamethasone (4mg/day) for worsening headache. Pretreatment MRI showed an enlarging enhancing right sphenoid-wing

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mass, plaque-like dural enhancement over the right convexity, tumor erosion through the occipital skull, and an increased FLAIR (fluid attenuated inversion recovery) signal abnormality involving the right hemisphere (Figure 1B).

The patient received 3mg/kg of nivolumab on days 1 (Dose#1) and 15 (Dose#2) of cycle 1 without incident but her headaches and scalp pain worsened. The scalp masses were notably larger, erythematous, and more tender by day 28. Brain MRI showed significantly enlarged dural based enhancing lesions, increased T2 and FLAIR signal abnormalities and enlarged scalp masses (Figure 1C). Her physical examination and MRI appeared consistent with progressive disease and we withheld further nivolumab. She underwent palliative debulking surgery 3 weeks later (Surgery#7). Immediately prior to surgery, her scalp masses were modestly smaller and less tender. Post-operative MRI showed a subtotal resection (Figure 1D).

**MATERIALS AND METHODS**

**Patient Consent**

Our patient provided informed consent for our IRB approved study and consented to this publication.

**Immunohistochemistry**

We used immunohistochemistry (IHC) to evaluate protein expression using Envision Plus detection (Dako, Carpinteria, CA; Table S1) and published protocols.

**Multiplexed Immunofluorescence**

The protocol for tissue-based multiplexed cyclic immunofluorescence (t-CyCIF) of conventionally prepared formalin-fixed, paraffin-embedded (FFPE) specimens and image analysis are described elsewhere. Antibodies are listed in Table S1.

**Oncopanel sequencing**

We used targeted next generation exome sequencing (Oncopanel-v3) to detect mutations and copy number variations in 447 cancer genes. We processed and annotated data as previously described. We calculated tumor mutational burden (TMB) by determining the number of non-synonymous mutations per megabase (Mb) of exonic sequence data across sequenced genes.

**RESULTS**

Histopathologic examination of the tissue resected five weeks before nivolumab treatment (Sample#6) revealed a highly proliferative atypical meningioma (Figure 2A). In contrast, tissue resected three weeks after nivolumab dose#2 (Sample#7) had dense fibrosis with extensive immune cell infiltration, and necrosis (Figure 2C). Tumor cells were absent in the H&E stained slides but IHC using a marker of meningioma, SSTR2a (Figure 2B, D), revealed one small cluster (~1000 μm²) of possible residual tumor cells (representing <0.0001% of resected tissue; not shown).
To characterize the effects of nivolumab, we profiled the tumor and its microenvironment using t-CyCIF, a method for highly multiplexed immunofluorescence imaging of FFPE specimens\(^{32}\). We imaged 11 markers (Table S1) on pre- and post-treatment samples to measure changes in immune cell types and the relative density of the immune infiltrate (Figure 2E-H, Table 1, Fig. S1, Table S2). We have previously shown that PD-L1 is overexpressed in tumor cells of some higher-grade meningiomas\(^{22}\), but, in this pre-treatment specimen (Sample#6), we found PD-L1 restricted to immune cells. Post-treatment (in Sample#7), we observed a marked increase in IBA1+/CD14+ macrophages, CD4+ and CD8+ T cells, CD20+ B cells, and FOXP3+ Treg cells (Figure 2E-H, Table 1, Fig. S1). The fraction of CD8+ T cells increased from 7% to 73% (Table 1) and CD8+ T-cell density increased from 0.5 to 304 cells/mm\(^2\); the CD8+ T-cell to Treg ratio increased 20-fold (from 5.8 to 115; Figure 2G-H, Table S2). These data are consistent with a highly active anti-tumor immune response post-treatment.

We used targeted exome sequencing to characterize genomic aberrations in specimens from the original resection (Sample#1), the resection preceding bevacizumab, mifepristone and temozolomide (Sample#4) and the resection preceding nivolumab (Sample#6) (Figure 1A; Tables S3–5). This analysis revealed a significantly elevated tumor mutational burden (TMB)\(^{37}\) of 20.5, 26.6, and 38.0 mutations/Mb in the samples, respectively (Table S6). These levels were greater than those of 228 other meningiomas sequenced as part of the BWH/DFCI Profile Initiative\(^{35}\). Copy number variation in the three samples was characteristic of higher-grade aggressive meningioma\(^{38–41}\) (Figure 3A, Tables S7–9). Notably, homozygous deletion of exons 2 to 5 of the DNA mismatch repair (MMR) gene MSH2 was present in all three samples (Figure 3A-B) but was absent in a peripheral blood specimen.

Immunohistochemistry showed that tumor cells were negative for MSH2 protein and its heterodimeric partner, MSH6, but that expression of MLH1 and PMS2 was retained (Figure 3C-F). Loss of MSH2/MSH6 in sample#1 demonstrated that MSH2 had been inactivated prior to any therapy and independently of temozolomide exposure, a known driver of acquired MMR deficiency in gliomas\(^{42, 43}\). Thus, the tumor was MMR-deficient at the original diagnosis and there was a progressive increase in TMB (Table S6).

The patient resumed nivolumab following sugery#7 and has continued biweekly therapy for over 2 years. MRI scans have shown gradual and continued regression of enhancing lesions and associated T2/FLAIR signal abnormalities (Figure 1E). Scalp lesions have disappeared and narcotics and dexamethasone are no longer required for pain control and cerebral edema.

Given the dramatic response of this MSH2-deficient tumor to nivolumab, we investigated the prevalence of elevated TMB and MMR deficiency in meningioma (Figure 3G; Table S10, S16–18). We used sequencing data from the BWH/DFCI Profile Initiative (228 cases)\(^{35}\) or BWH/DFCI cases screened only by MMR-protein IHC (237 cases\(^{22, 44}\)) to study specimens from 465 patients (288 grade I, 132 grade II, 45 grade III). Among the 228 sequenced specimens, the cohort mean TMB was 4.2 mutations/Mb (grade I:4.0; grade II: 4.4; grade III:6.5, Table S10–11). Seven of the 228 specimens had TMBs ≥10 mutations/Mb.
a commonly used threshold for hypermutation\textsuperscript{36}; one of these meningiomas (TMB=18 mutations/Mb) had a truncating mutation in the DNA MMR regulator \textit{SETD2} as well as a splice site mutation in \textit{MSH2} of unclear functional significance. We discovered one case with MMR-protein loss discovered by IHC (1 of 237 cases), and sequencing confirmed bilallelic inactivation of \textit{MSH6} and TMB=10 mutations/Mb (Figure 3G, Table 2; Table S12–15). Among the sequenced meningiomas, elevated TMB was significantly positively associated with anaplastic histology, MIB-1 proliferative index, and chromosomal instability (CAS)\textsuperscript{38} but not with prior radiotherapy, radiation induced meningioma, or recurrent tumor (Table S16–17).

In a second cohort of 615 sequenced meningiomas (Foundation Medicine), 14 tumors had a TMB >10 mutations/Mb. Among these 14 specimens, two grade III meningiomas had inactivating mutations in \textit{MSH2} and \textit{MSH6}; one grade II meningioma had a substitution in the DNA polymerase domain of the \textit{POLE} subunit of DNA polymerase epsilon and another grade III meningioma had a truncating mutation in \textit{SETD2} (Figure 3G, Table 2; Table S18). Thus, across two cohorts of sequenced meningioma samples (n=843), 21 (2.5\%) had high TMB\textsuperscript{36}. Three of 1,080 meningiomas screened by sequencing or IHC (0.3\%) had detectable inactivating mutations in \textit{MSH2} or \textit{MSH6}. The number of notable tumors increased to 6 (0.6\%) when considering MMR-related genes (e.g. \textit{SETD2}, \textit{POLE}).

\section*{DISCUSSION}

In our patient with an \textit{MSH2}-deficient meningioma, nivolumab treatment generated a robust anti-cancer immune response, as evidenced by dramatically increased infiltrating CD8+ T cells and a durable therapeutic response. Notably, the enlargement of lesions and increased signal abnormalities seen on the MRI post-nivolumab reflected pseudo-progression, not tumor growth. In addition to inactivation of \textit{MSH2}, sequencing of the patient’s tumors revealed missense mutations in \textit{POLD1} (R639C), \textit{RAD50} (P571L) and \textit{POLE} (G203E, S30L), but these changes are of unclear significance and are predicted to be non-pathogenic\textsuperscript{45}. An increase in TMB from 26.6 mutations/MB in Sample #4 (before experimental therapies) to 38.0 in Sample #6 raises the possibility that temozolomide can augment TMB in meningioma. However, this neoplasm was MMR-deficient at the time of original diagnosis, thus, temozolomide did not drive acquired MMR deficiency.

Our work also shows that ~2.5\% of meningiomas have a high mutation burden, a phenotype that has been linked with neoantigen expression in other tumor types\textsuperscript{46, 47}. In a subset of mutation-rich meningiomas, loss of function alterations in MMR and MMR-related genes can be detected. It is possible that other, as-yet unknown or undetected, mutations contribute to high TMB in the remaining tumors. In all, this first report of a dramatic response of a MMR-deficient meningioma to immunotherapy and our characterization of meningioma cohorts from two different institutions indicate that screening meningiomas is warranted to identify a molecularly-defined subtype likely responsive to immunotherapy.

\section*{Supplementary Material}

Refer to Web version on PubMed Central for supplementary material.
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REFERENCES


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Figure 1. Patient treatment and radiographic assessment.
(A) Timeline of patient therapy. Abbreviations: Nivo, Nivolumab; RT, radiation therapy; SRS, stereotactic radiosurgery; wks., weeks; mos., months. Sagittal T1 gadolinium-enhanced images (B) prior to initiation of study nivolumab therapy (before Surgery #6), (C) after two doses of nivolumab therapy (before Surgery #7), (D) following subtotal resection (Surgery #7) demonstrating necrosis and no viable tumor, and (E) ongoing response one year after initiating study nivolumab therapy. White arrows indicate bulky tumor burden including occipital lesion growing into soft tissue external to skull.
Figure 2. Histologic, immunohistochemical and tissue-based multiplexed cyclic immunofluorescence (t-CyCIF) characterization of meningioma resection samples.

Representative images of H&E stained section of meningioma resection (A) before treatment with nivolumab (Surgery #6/Sample #6, S6) and (C) after two doses of nivolumab (Surgery #7/Sample #7, S7). Representative images of immunohistochemistry for meningioma marker SSTR2a on resections (B) before treatment with nivolumab (Surgery #6/Sample #6, S6) and (D) after two nivolumab doses (Surgery #7/Sample #7, S7). The inset in (A) shows a mitotic figure and cells with prominent nucleoli; an elevated mitotic index of >4 mitoses per 10 high powered fields was used for grading. (E) A representative field of view of tissue-based cyclic immunofluorescence (t-CyCIF) imaging data of 11 biomarkers (IBA1, CD45RB, CD14, CD3, Ki-67, CD20, CD4, CD8A, PD-1, FOXP3, PD-L1) generated from a single section of pre-treatment meningioma (Surgery #6/Sample #6) and (F) from a single section of the post-treatment meningioma (Surgery #7/Sample #7). Comparison of the images in (E) and (F) showed a marked increase in macrophages, T lymphocytes (CD3+) including CD4+ T cells and CD8A+ T cells, and B lymphocytes (CD20+) following nivolumab treatment. The majority of T cells following treatment were CD8A+. The antibodies used for this characterization and a detailed analysis of the absolute number, relative number and density of various immune subtypes is provided in Table 1 and...
Table S2. (G) Bar graph of the percentage of immune cell subtypes relative to all cells (immune and non-immune) before (red bars) and after (blue bars) nivolumab treatment. (H) Bar graph of cell density before (red bars) and after (blue bars) nivolumab treatment. The analysis was performed on 10 representative views (tiles) from the t-CyCIF data collected from Samples #6 and #7. t-test statistical analysis was performed. ** p < 0.01, *** p< 0.001, **** p<0.0001. The bar graphs represent the most pertinent data derived from the immune profile of the pre-nivolumab (Sample #6) and post-nivolumab (Sample #7) treated meningioma samples using t-CyCIF. Detailed data is presented in Table 1 and Table S2.
Figure 3. Genomic and immunohistochemical characterization of meningioma samples. (A) Copy number analysis from targeted next-generation sequencing data from Sample #6 identified a genome-wide profile characteristic of a high-grade meningioma, including loss of 1p, 9p and monosomy of chromosome 18 and 22. Focal homozygous deletion of CDKN2A/CDKN2B and intragenic deletion of MSH2 were present. (B) Gene-level view of MSH2 showed a homozygous intragenic deletion of exons 2 through 5 of MSH2 (NM_000251) (log2 ratio from $-2.21$ to $-2.73$) in the setting of 2p arm-level single copy loss. Copy number is depicted as a log2-ratio value. Immunohistochemistry on pre-treatment meningioma resection (Surgery #4/Sample #4; S4) for (C) MSH2, (D) MSH6, (E) MLH1 and (F) PMS2. MSH2 and MSH6 staining was present only in non-tumor cells. (G) Box and whiskers plot (5–95 percentile) of tumor mutation burden (mutations per megabase) for BWH/DFCI Profile Initiative cohort (228 sequenced cases; Table S10) plus sequencing data for the MMR-deficient case BWH/DFCI-2 and for Foundation Medicine cohort (615 cases).
sequenced cases; Table S18). Cases with loss of function changes in MMR and MMR-related genes (from Table 2) are highlighted in blue (dots and squares). For the BWH/DFCI Profile Initiative cohort, the mean TMB was 4.25 mutations per megabase (Standard Deviation: 2.55; Standard Error of the Mean: 0.17; Lower 95% CI of mean: 3.91; Upper 95% CI of mean: 4.58). For the Foundation Medicine cohort, the mean TMB was 2.77 mutations per megabase (Standard Deviation: 8.08; Standard Error of the Mean: 0.33; Lower 95% CI of mean: 2.14; Upper 95% CI of mean: 3.41).
Table 1.

Immune profile of the pre-nivolumab (Sample #6) and post-nivolumab (Sample #7) treated meningioma samples using t-CyCIF

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Pre-treatment total cell number (percentage of total cells)</th>
<th>Post-treatment total cell number (percentage of total cells)</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cells</td>
<td>160967 (100%)</td>
<td>128307 (100%)</td>
<td></td>
</tr>
<tr>
<td>CD45RB+ (Lymphocytes)</td>
<td>1743 (1.08%)</td>
<td>65896 (51.36%)</td>
<td>47.56</td>
</tr>
<tr>
<td>IBA1+/CD14+ (Macrophages)</td>
<td>29614 (18.4%)</td>
<td>69378 (54.07%)</td>
<td>2.34</td>
</tr>
<tr>
<td>CD45RB+ or IBA1+ or CD14+ (Immune cells)</td>
<td>62970 (39.12%)</td>
<td>106483 (82.99%)</td>
<td>2.12</td>
</tr>
<tr>
<td>CD45RB-/IBA1-/CD14- (Tumor cells, Fibroblast)</td>
<td>97997 (60.88%)</td>
<td>21823 (17.01%)</td>
<td>0.28</td>
</tr>
<tr>
<td>CD45RB+/CD3+ (T cells)</td>
<td>513 (0.32%)</td>
<td>42273 (32.95%)</td>
<td>102.97</td>
</tr>
<tr>
<td>CD45RB+/CD20+ (B cells)</td>
<td>43 (0.027%)</td>
<td>3307 (2.58%)</td>
<td>95.56</td>
</tr>
<tr>
<td>CD45RB+/CD3+/CD4+ (CD4+ T cells)</td>
<td>27 (0.017%)</td>
<td>8457 (6.59%)</td>
<td>387.65</td>
</tr>
<tr>
<td>CD45RB+/CD3+/CD8A+ (CD8A+ T cells)</td>
<td>35 (0.022%)</td>
<td>30906 (24.09%)</td>
<td>1095.00</td>
</tr>
<tr>
<td>CD45RB+/CD3+/CD4-/CD8A-</td>
<td>452 (0.28%)</td>
<td>6665 (5.19%)</td>
<td>18.54</td>
</tr>
<tr>
<td>CD45RB+/CD3+/PD1+</td>
<td>13 (0.0081%)</td>
<td>79 (0.062%)</td>
<td>7.65</td>
</tr>
<tr>
<td>CD45RB+/PDL1+</td>
<td>2 (0.0012%)</td>
<td>100 (0.078%)</td>
<td>65.00</td>
</tr>
<tr>
<td>IBA1+/CD14+/PDL1+</td>
<td>5 (0.0031%)</td>
<td>59 (0.046%)</td>
<td>14.84</td>
</tr>
<tr>
<td>CD45RB+/PD1+/PDL1+</td>
<td>0 (0%)</td>
<td>25 (0.019%)</td>
<td></td>
</tr>
<tr>
<td>CD45RB+/CD3+/CD4+/FOXP3 (Treg cells)</td>
<td>6 (0.0037%)</td>
<td>270 (0.21%)</td>
<td>56.76</td>
</tr>
<tr>
<td>Ratio of CD8A+ T cells vs Treg cells</td>
<td>5.83</td>
<td>114.47</td>
<td>19.63</td>
</tr>
<tr>
<td>Ki67+</td>
<td>22239 (13.82%)</td>
<td>7317 (5.7%)</td>
<td>0.41</td>
</tr>
<tr>
<td>CD45RB+/Ki67+</td>
<td>443 (0.28%)</td>
<td>4824 (3.76%)</td>
<td>13.43</td>
</tr>
<tr>
<td>CD45RB+/CD3+/Ki67+</td>
<td>217 (0.13%)</td>
<td>3635 (2.83%)</td>
<td>17.77</td>
</tr>
<tr>
<td>CD45RB+/CD3+/CD8A+/Ki67+</td>
<td>8 (0.005%)</td>
<td>3116 (2.43%)</td>
<td>486</td>
</tr>
<tr>
<td>CD45RB+/CD3+/CD4+/Ki67+</td>
<td>9 (0.0056%)</td>
<td>634 (0.49%)</td>
<td>87.5</td>
</tr>
<tr>
<td>CD45RB+/CD3+/CD4+/CD8A+/Ki67+</td>
<td>200 (0.12%)</td>
<td>328 (0.26%)</td>
<td>1.71</td>
</tr>
<tr>
<td>IBA1+/CD14+/Ki67+</td>
<td>4872 (3.03%)</td>
<td>4153 (3.24%)</td>
<td>1.07</td>
</tr>
<tr>
<td>CD45RB-/IBA1-/CD14-/Ki67+</td>
<td>12968 (8.06%)</td>
<td>1035 (0.81%)</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Table 2.
Additional meningiomas with mutations in MMR genes and MMR-related genes from the BWH/DFCI Profile Initiative cohort and the Foundation Medicine cohort

<table>
<thead>
<tr>
<th>Patient</th>
<th>WHO grade</th>
<th>Tumor Mutational Burden (mutations/MB)</th>
<th>Gene</th>
<th>Amino Acid Alteration</th>
<th>MMR IHC in tumor cells</th>
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<tbody>
<tr>
<td>BWH/DFCI-1</td>
<td>III</td>
<td>18</td>
<td>MSH2</td>
<td>SETD2 1510+8delT Splice site (VUS)</td>
<td>MSH2/MSH6 retained</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E1595Sfs*15</td>
<td></td>
</tr>
<tr>
<td>BWH/DFCI-2</td>
<td>II</td>
<td>10</td>
<td>MSH6</td>
<td>F1088Sfs*2</td>
<td>MSH2/MSH6 negative</td>
</tr>
<tr>
<td>FMI-4</td>
<td>III</td>
<td>30</td>
<td>MSH2</td>
<td>Homozygous deletion</td>
<td>NA</td>
</tr>
<tr>
<td>FMI-6</td>
<td>III</td>
<td>28</td>
<td>MSH6</td>
<td>C559fs*3</td>
<td>NA</td>
</tr>
<tr>
<td>FMI-7</td>
<td>III</td>
<td>91</td>
<td>SETD2</td>
<td>N34fs*77</td>
<td>NA</td>
</tr>
<tr>
<td>FMI-10</td>
<td>II</td>
<td>25</td>
<td>POLE</td>
<td>R762W</td>
<td>NA</td>
</tr>
</tbody>
</table>

(VUS) Variant of unknown significance
(NA) Tissue sections not available for analysis
(fs) frameshift