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Checkpoint inhibitor failure in hypermutated and mismatch repair-mutated recurrent high-grade gliomas

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Abstract

Background. Recurrent high-grade gliomas in adults remain a deadly cancer with median survival of less than 1 year. In the absence of effective agents, immunotherapy with checkpoint inhibitors has been adopted as a potentially beneficial next step for recurrences with hypermutated or mismatch repair-mutated phenotypes. The rationale for their use, however, is based on case reports and studies with other types of cancer.

Methods. We reviewed 4 cases of hypermutated or mismatch repair-mutated recurrent high-grade gliomas treated with checkpoint inhibitors.

Results. All cases had recurrent high-grade glioma that harbored either a hypermutated phenotype and/or a mismatch repair mutation. Treatment with checkpoint inhibitor therapy resulted in no significant response.

Conclusions. In our experience, hypermutated or mismatch repair-mutated high-grade gliomas in adults do not respond to checkpoint inhibitors alone. This lack of efficacy is in agreement with underwhelming results of clinical trials examining checkpoint inhibitors in high-grade gliomas. The case reports of responders have been in pediatric patients with glioma and are likely a different subtype altogether.

Keywords

glioma | glioblastoma | hypermutation | immunotherapy | mismatch-repair

Recurrent high-grade gliomas remain a deadly cancer with a median survival of less than 1 year. In spite of numerous clinical trials examining promising new therapies, the prognosis remains poor.[1] The success of immunotherapy with other types of cancer has prompted interest in the use of checkpoint inhibitor (CPI) therapy for the treatment of recurrent high-grade gliomas (WHO Grade III and IV gliomas). Recognition of tumor characteristics that identify patients more likely to benefit from this approach is crucial in the design of clinical trials.

Several reports suggest that in non-CNS cancers and high-grade gliomas alike there is an association between tumors exhibiting hypermutated phenotypes and/or mismatch repair (MMR) mutations and response to CPI therapy.[2–5] The hypothesis is that the hypermutated genomes provide more neoantigens through which the CD8+ T cells may recognize and target the neoplastic cell.[6] Gliomas carry a relatively low tumor mutational load (TML) vs other types of cancer,[7] but about 26% of recurrent glioblastomas (GBMs; WHO Grade IV glioma) have a higher mutation frequency.[8]

Similarly, tumors carrying MMR mutations, such as mutations in the mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), mutS homolog 6 (*MSH6*), and PMS1 homolog 2, mismatch repair system component (*PMS2*) genes are more responsive to CPI. Several trials suggest that defective MMR is associated with clinical response to CPI in non-CNS cancers.[4,9,10] In the setting of GBM, MMR deficiency may arise at recurrence because of treatment, even if absent at onset.[8,11,12] According to The Cancer Genome Atlas, 26% of recurrent GBMs acquire *MSH6* mutations[13] after treatment with temozolomide (TMZ), indicating appearance of MMR mutations as the tumor becomes resistant to alkylating agents. An association between high TML, although infrequent, and loss of expression of *MLH1*, *MSH2*, *MSH6*, and *PMS2* in recurrent GBMs would suggest

this select group of patients may be more likely to benefit from CPI.[6] A report of 2 siblings with recurrent GBMs and biallelic MMR deficiency with homozygous *PMS2* mutation who had a dramatic response to nivolumab provides further support for this association.[4]

There have been no clinical trials showing benefit of CPI use in recurrent adult hypermutated or MMR-mutated GBMs. However, neuro-oncologists often use CPI in this setting, based on the aforementioned non-CNS data and reported pediatric glioma cases. In our experience, we have not been able to replicate the expected response. We present 4 cases of recurrent, high-grade, MMR-deficient, and/or hypermutated glioma that did not respond to CPI therapy (summarized in Table 1).

Case Reports

Case 1

A 46-year-old man with diagnosis of grade III oligodendroglioma had initial resection 11 years earlier followed by 12 cycles of adjuvant TMZ. Three years later, he had disease progression and received an additional 19 cycles of adjuvant TMZ. He had further progression 1 year later and underwent his second resection followed by radiation with TMZ. Two years later, he had progression and had a third resection followed by 6 cycles of lomustine and procarbazine. He had further progression treated with a fourth surgery. It was decided to obtain genomic profiling using the commercially available FoundationOne assay (Foundation Medicine company), which characterizes mutations and copy number alterations in key cancer-related genes. Based on the assay's report of a MET (proto-oncogene for tyrosine kinase receptor) mutation, he was briefly treated with cabozantinib, but it was stopped early because of side effects. The FoundationOne assay also revealed a moderately hypermutated phenotype (TML = 66 Muts/Mb) as well as homozygous mutation in the MMR gene *MSH6*. He then received pembrolizumab at 2 mg/kg, every 3 weeks. After 3 infusions, there was radiographic and clinical progression, at which time bevacizumab was added. He continued bevacizumab and pembrolizumab for 1.25 years, but the tumor continued slow radiographic progression. The patient was switched to bevacizumab and oliparib. He died 20 months after having started pembrolizumab.

Case 2

A 60-year-old man was diagnosed with GBM 3 years prior; he had surgery followed by concomitant TMZ and fractionated radiation therapy. Afterward, treatment consisted of 17 cycles of adjuvant TMZ and use of the Novocure Optune device. Two years later, he had progression and re-resection. Genomic testing with FoundationOne assay revealed a hypermutated phenotype (TML = 187 Muts/Mb) as well as a homozygous mutation in the MMR gene *MSH6*. He received pembrolizumab at 2.5 mg/kg, every 3 weeks. After 12 weeks, there were radiographic changes suggestive of progression. Because the patient was clinically stable, pembrolizumab was continued for the possibility of immunotherapy-related changes. After 12 weeks, progression was confirmed and treatment changed to bevacizumab and irinotecan. The patient died 11 months after having started pembrolizumab.

Case 3

A 49-year-old man was initially diagnosed with isocitrate dehydrogenase (NADP+) 1 (*IDH1*) mutant grade II oligodendroglioma, 7 years prior. A year later, he had progression and was started on TMZ. Two years later, he had progression again and underwent gross total resection. Pathology now showed grade III oligodendroglioma. Further treatment consisted of fractionated radiation therapy, followed by procarbazine and lomustine. Three months later he was switched to bevacizumab and irinotecan. Owing to cytopenias he was switched to vorinostat. At his next progression, 4 years from initial diagnosis, genomic testing with the FoundationOne assay was performed on tissue from the second resection. This revealed a mutation in *MSH6*. Tumor mutational load testing was not performed. He was treated with nivolumab 3 mg/kg every 2 weeks. After 12 weeks, he had radiographic progression. Nivolumab was continued in hopes that changes were immunotherapy related. There was further progression on repeat MRI, and everolimus was added. The patient died 11 months after the initiation of nivolumab with no sign of response during that time.

Case 4

A 27-year-old woman was diagnosed with *IDH1*-mutant grade III astrocytoma, and underwent resection 21 years prior. She had progression 9 years later and underwent a second resection. A third recurrence and resection, 1 year later, was followed with 12 cycles of adjuvant TMZ. She was observed until a fourth recurrence and resection, 3 years later, at which time pathology revealed GBM and genomic profiling using the FoundationOne assay showed hypermutation (TML = 165 Muts/Mb) and homozygous MMR mutations in the *MSH6* and *MLH1* genes. She was initially treated with fractionated radiotherapy and irinotecan, but then switched to pembrolizumab given the FoundationOne results indicating MMR deficiency. After about 4 months, she had radiographic progression and bevacizumab was added. Pembrolizumab was stopped about 10 weeks later. Afterward she was on bevacizumab with irinotecan, but changed because of progression to bevacizumab and lomustine, which she continues currently.

Discussion

These 4 cases of adults with recurrent high-grade glioma with the hypermutation phenotype and/or carrying MMR mutations did not respond to CPI therapy. They all had progression after starting immunotherapy, and CPI treatment did not significantly affect the expected outcome of recurrent high-grade gliomas. Our results suggest the mutation signature of recurrent gliomas in adults does not correlate with response to single-agent programmed cell death-1 (PD-1) blockade.

Table 1 Patient characteristics

Age at Dx	Sex	Diagnosis	Chemotherapy	RT	Surgery	Checkpoint Inhibitor	Mutation (Foundation One Testing)	PFS After CPI (Weeks)	Overall Survival (Years)
35	M	Grade III oligodendroglioma *IDH1* mutant, 1p/19q codeleted	TMZ ×2, CCNU/procarbazine, cabozantinib, pembrolizumab, olaparib	59.4 Gy after second recurrence	4 subtotal resections	Pembrolizumab	*MSH6*, hypermutation (66 Muts/Mb)	9 weeks	13
60	M	GBM *IDH1* wild-type, 1p/19q intact	TMZ, pembrolizumab, irinotecan, bevacizumab	60 Gy at diagnosis with TMZ	2 subtotal resections	Pembrolizumab	*MSH6*, hypermutation (187 Muts/Mb)	12 weeks	3
49	M	Grade II to grade III *IDH1* mutant, 1p/19q codeleted	TMZ, procarbazine, CCNU, bevacizumab, vorinostat, nivolumab	60 Gy after second recurrence	2 subtotal resections	Nivolumab	*MSH6* (TML unavailable)	12 weeks	15
27	F	Grade III astrocytoma to secondary GBM *IDH1* mutant, 1p/19q intact	TMZ, pembrolizumab, bevacizumab, irinotecan, CCNU	50 Gy after third recurrence	4 subtotal resections	Pembrolizumab	Hypermutated, *MSH6*, *MLH1* (165Muts/Mb)	36 weeks	21+

Abbreviations: CCNU, lomustine; CPI, checkpoint inhibitor; Dx, diagnosis; F, female; GBM, glioblastoma; Gy, gray, radiation unit; *IDH1*, isocitrate dehydrogenase (NADP+) 1; IMRT, intensity modulated radiation therapy; M, male; *MLH1*, mutL homolog 1; *MSH6*, mutS homolog 6; Muts/Mb, mutations/megabase; PFS, progression-free survival; RT, radiation therapy; TMZ, temozolomide.

The differences between adult and pediatric high-grade gliomas could be an explanation for the lack of expected efficacy. In the cases reported by Bouffet et al, both responders to CPI were pediatric patients with GBMs showing extremely high TML in the setting of germline MMR mutations in the DNA polymerase epsilon, catalytic subunit (*POLE*) gene.[4] While these 2 patients did have a clear response to nivolumab, it should be noted that patients with high-grade gliomas with *POLE* mutations tend to be young and to have longer progression-free survival.[14] Furthermore, recurrent high-grade gliomas can carry high TML, but these are mostly somatic mutations secondary to insults from chemotherapy and radiation.[2,15,16] Lung tumors with chemotherapy-induced subclonal neoantigens were not as responsive to pembrolizumab as tumors with high mutation load and low neoantigen subclonal fraction.[17] Therefore, it is possible that cancer treatment-induced mutation burden does not predict response to CPI, unlike the situation in tumors that have a high TML prior to therapy. The results of a forthcoming clinical trial (NCT03557359) that will examine the response rate of nivolumab in patients with recurrent *IDH*-mutated tumors previously treated with alkylating agents may help answer this question.

In the case of gliomas, other factors like CD8+ T cell infiltration and PD1/programmed death-ligand 1 (PD-L1) expression may be more relevant than the mutation phenotype as a biomarker for CPI response. Hodges and colleagues demonstrated no significant association between TML and PD-1/PDL-1 expression[6] or CD8+ T-cell influx into the tumor.[6] De Groot et al corroborated this finding, with studies of 35 immune markers by mass cytometry revealing that GBM pathology specimens exhibit no influx of effector T cells after treatment with pembrolizumab.[18] In gliomas, the poor expression of PD-1/PD-L1 in addition to the scant inflammatory infiltrate is unlikely to yield an effective antineoplastic immune response to CPI.

The findings of lack of efficacy of CPI are consistent with several reports on their use in adults with recurrent GBM (not specifically hypermutated or with MMR mutation).[19] The CheckMate 143 study (NCT02017717) was a randomized clinical trial comparing nivolumab and bevacizumab in patients with recurrent GBM.[20] Nivolumab did not extend overall survival and had a significantly lower response rate in comparison with bevacizumab. A phase II study showed minimal activity, having 6-month progression-free survival as primary outcome, of pembrolizumab alone or in combination with bevacizumab in patients with recurrent GBM.[19] The analysis of correlative biomarkers to determine if a subgroup of patients would benefit remains pending. Preliminary results of another phase II study including 35 patients with recurrent GBM revealed that pembrolizumab has no efficacy as monotherapy.[19] A retrospective study of 31 patients with recurrent high-grade glioma revealed that salvage therapy with pembrolizumab or nivolumab did not improve their survival.[21] In spite of these results, the possibility remains that a subset of patients with high-grade glioma could benefit from CPI monotherapy, including inhibition of other costimulatory molecules, or in combination with other therapies.

In the case reports showing CPI responses in GBM, one caveat worth mentioning is that both reported pediatric cases did initially have clinical and radiographic worsening prior

to improving. Presumably, this was due to the inflammatory immune response against the tumor, and it was good clinical judgment to continue the therapy. Furthermore, in the CheckMate 143 study, neuropathologic analysis of 13 tissues obtained from surgery for radiologic progression after CPI therapy revealed that in 4 cases there were treatment effects with ≤50% viable tumor.[22] It is possible that in our cases, early discontinuation of the CPI occurred because of pseudoprogression and a benefit would appear later on. The Immunotherapy Response Assessment in Neuro-Oncology (iRANO) criteria, although not validated, guides the assessment of radiologic changes reflecting delayed responses or therapy-induced inflammation when using immunotherapy in neuro-oncology patients.[23] However, we considered the iRANO criteria at progression and each case met criteria for true progression.

Our series of 4 cases does not support treatment with CPI alone for recurrent high-grade glioma, even if hypermutated and/or with MMR mutation, in routine clinical practice. Given the preclinical and non-CNS evidence supporting the use of CPI in hypermutated GBM, there is a need for large studies that can assess their efficacy in this population. It is possible that gliomas with hypermutation or MMR mutation present prior to therapy, suggesting germline mutations, would be better suited for CPI therapy. Finally, our reports do not rule out the possible efficacy of CPI in combination therapy with other agents, which should be evaluated in future clinical trials.

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