Immune Checkpoint Inhibition for Hypermutant Glioblastoma Multiforme Resulting From Germline Biallelic Mismatch Repair Deficiency


ABSTRACT

Purpose

Recurrent glioblastoma multiforme (GBM) is incurable with current therapies. Biallelic mismatch repair deficiency (bMMRD) is a highly penetrant childhood cancer syndrome often resulting in GBM characterized by a high mutational burden. Evidence suggests that high mutation and neoantigen loads are associated with response to immune checkpoint inhibition.

Patients and Methods

We performed exome sequencing and neoantigen prediction on 37 bMMRD cancers and compared them with childhood and adult brain neoplasms. Neoantigen prediction bMMRD GBM was compared with responsive adult cancers from multiple tissues. Two siblings with recurrent multifocal bMMRD GBM were treated with the immune checkpoint inhibitor nivolumab.

Results

All malignant tumors (n = 32) were hypermutant. Although bMMRD brain tumors had the highest mutational load because of secondary polymerase mutations (mean, 17,740 ± standard deviation, 7,703), all other high-grade tumors were hypermutant (mean, 1,589 ± standard deviation, 1,043), similar to other cancers that responded favorably to immune checkpoint inhibitors. bMMRD GBM had a significantly higher mutational load than sporadic pediatric and adult gliomas and all other brain tumors (P < .001). bMMRD GBM harbored mean neoantigen loads seven to 16 times higher than those in immunoresponsive melanomas, lung cancers, or microsatellite-unstable GI cancers (P < .001). On the basis of these preclinical data, we treated two bMMRD siblings with recurrent multifocal GBM with the anti–programmed death-1 inhibitor nivolumab, which resulted in clinically significant responses and a profound radiologic response.

Conclusion

This report of initial and durable responses of recurrent GBM to immune checkpoint inhibition may have implications for GBM in general and other hypermutant cancers arising from primary (genetic predisposition) or secondary MMRD.

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INTRODUCTION

Glioblastoma multiforme (GBM) is a highly malignant brain tumor and the most common cause of death among children with CNS neoplasms. Despite primary management, which consists of surgical resection followed by radiation therapy and chemotherapy, most GBMs will recur, resulting in rapid death. Patients with recurrent disease have a particularly poor prognosis, with a median survival of fewer than 6 months; no effective therapies currently exist.

In contrast to adult CNS malignancies, a significant proportion of childhood brain tumors occur in the context of cancer predisposition syndromes. Pediatric GBMs are associated with germline mutations in TP53 (Li-Fraumeni syndrome) and the mismatch repair (MMR) genes (biallelic MMR deficiency syndrome [bMMRD]).

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Patients with bMMRD are unique in both the molecular events that lead to GBM formation and opportunities for innovative management of these tumors to possibly improve survival.

bMMRD is caused by homozygous germline mutations in one of the four MMR genes (PMS2, MLH1, MSH2, and MSH6) and is arguably the most penetrant cancer predisposition syndrome, with 100% of biallelic mutation carriers developing cancers in the first two decades of life. These are most commonly malignant gliomas, hematologic malignancies, and GI cancers.3–4 Understanding the relationship between the bMMRD somatic mutational landscape and tumor biology can lead to development of novel therapies and improved patient outcomes.

bMMRD GBMs harbor the highest mutation load among human cancers.7 Combined germline mutations in the MMR genes and somatic mutations in DNA polymerase result in complete ablation of proofreading during DNA replication and underpin this phenomenon. bMMRD GBMs, in contrast to other childhood cancers and adult MMR-proficient gliomas, exhibit a molecular signature characterized by single-nucleotide changes present in exponentially higher numbers. An important characteristic of non-bMMRD cancers exhibiting high mutation loads—subsets of malignant melanomas and lung, bladder, and microsatellite-unstable GI cancers—is responsiveness to immune checkpoint inhibitors.6–9

Checkpoint inhibitors target the immunomodulatory effect of CTLA-4 (cytotoxic T lymphocyte–associated protein 4) and programmed death-1 (PD-1)/programmed death-ligand 1, restoring effector T-cell function and antitumor activity. Recent reports have shown that patients whose tumors bear a high mutation load and/or defined tumor-associated antigen (neo-antigen) signatures derive enhanced clinical benefit from checkpoint inhibitor therapy.10

Nivolumab is an anti–PD-1–directed immune checkpoint inhibitor approved for use in the treatment of non–small-cell lung cancer11 and melanoma and under clinical investigation in multiple adult and pediatric tumors.12,13 However, this response is currently unknown in bMMRD-associated cancers and the uniformly lethal GBM.

PATIENTS AND METHODS

We performed exome sequencing on a large cohort of bMMRD cancers (Appendix Table A1), including 21 GBMs. Sequencing, alignment, and variant calling were performed as previously described.1 Tumors were defined as hypermutant if they harbored more than 100 mutations per exome based on this threshold as predictor of response to immune checkpoint inhibitors.8

To assess neoantigen load, we used each nonsynonymous somatic variant (ie, amino acid sequence changing, except stop gains). We generated all possible peptide sequences of eight, nine, and 10 amino acids overlapping it (ie, eight-, nine-, and 10-mers), with (ie, mutated) and without (ie, wild type) the variant, using the ANNOVAR accessory program (coding_change.pl; http://annovar.openbioinformatics.org) to translate the DNA reference sequence into peptide sequences. In addition, we generated all possible wild-type peptides from RefSeq transcripts (database of the National Center for Biotechnology Information, Bethesda, MD) and removed mutant peptides already present in the human transcriptome. Generated peptides were submitted to the standalone version of NetMHC 3.4 (Center for Biological Sequence Analysis, Copenhagen, Denmark) for predicting the binding affinity of the peptides to the major histocompatibility complex I (MHC-I) alleles of the patient.

On the basis of the somatic variant annotation and peptide affinity predictions, we generated peptide counts for each patient’s MHC-I alleles at all binding affinities (NetMHC affinity nM < = 500). To compare the bMMRD GBM neoantigen data with those of other cancers, we modified the method for neoantigen counting to generate comparable counts for the respective cohorts of immunoreponsive tumors (ie, lung,14 melanoma,15 and colon16).

A white girl age 6 years from a nonconsanguineous union (Fig 1A) was diagnosed with a left parietal GBM. The patient underwent near-total resection and subsequent focal irradiation to a total of 59.4 Gy delivered over 6.5 weeks. She remained in clinical remission for 3 months, when surveillance magnetic resonance imaging (MRI) revealed tumor
recurrence with a 6 × 7 mm nodule in the initial tumor bed and a second 16 × 17 mm lesion in the left temporal lobe.

Six months earlier, her brother, age 3.5 years, was diagnosed with a right frontoparietal GBM, which was treated with surgery, focal irradiation, and temozolomide. Ten months after the initial diagnosis, surveillance MRI revealed an asymptomatic diffuse multinodular GBM recurrence. Both tumors harbored TP53 mutations and lack of MGMT promoter hypermethylation, which are poor prognostic markers in childhood GBM.16-18

Importantly, both children exhibited café-au-lait spots, a shared feature of neurofibromatosis type 1 and bMMRD, prompting clarifying molecular investigations. These revealed germline homozygous c.2117delA, p. Lys706SerfsX19 mutation in PMS2, loss of PMS2 staining in the tumor and normal tissue (Fig 1B), and normal NF1 germline testing. Together, these clinical and molecular tests confirmed a diagnosis of bMMRD.3

RESULTS

To examine whether immune checkpoint inhibitors would be applicable for bMMRD cancers, we surveyed the extent of hypermutation across bMMRD tumors from various tissues. Exome sequencing of 37 cancers collected from the bMMRD consortium revealed that all malignant tumors (n = 32) were hypermutant. Although bMMRD brain tumors had the highest mutational load resulting from secondary polymerase mutations (mean, 17,740 ± standard deviation [SD], 7,703), all other high-grade tumors were hypermutant, harboring more than 100 exonic mutations (mean, 1,589 ± SD, 1,043; Fig 2A). Lower-grade bMMRD tumors (n = 5) did not exhibit hypermutation (mean, 40 ± SD, 18). Importantly, bMMRD GBMs had a significantly higher mutational load than sporadic pediatric and adult gliomas and all other brain tumors (P < .001; Fig 2A). To test the extent to which hypermutation translates to a strong neoantigen signature, a current predictor of response to immune checkpoint inhibition, we performed genome-wide somatic neoepitope analysis using similar algorithms previously used for melanoma, lung, and colon cancers.9,14,15 For each study, we compared our cohort of tumors with other tumors that were reported to respond to immune checkpoint inhibitors (Fig 2B). Strikingly, bMMRD GBMs had a significantly higher number of predicted neoantigens, whereas other tumors responded with a fraction of the neoantigens found in our patients (P < .001; Fig 2B). The mean neoantigen load was seven to 16 times higher than those of immunoresponsive melanomas, lung cancers, and microsatellite-unstable GI cancers.

Because of this information, the index patient and her brother’s tumors were sequenced using the rapid SickKids Cancer Sequencing Program (Toronto, Ontario, Canada) protocol, KiCS,5 as a part of the international bMMRD consortium effort (Appendix Table A1, online only). Both tumors harbored driver mutations in POLE (Appendix Fig A1, online only) and were found to harbor 24,680 and 21,919 mutations per exome, respectively. Given the rationale that tumors with molecular signatures similar to those developing in children with bMMRD are responsive to immunomodulators and the lack of effective therapies for children with recurrent disseminated GBM, the index patient’s parents were
approached to consider a therapeutic trial of nivolumab. After institutional ethics approval and parental written informed consent, the patient received her first course of nivolumab 1 month postsurgery at a regimen of 3 mg/kg every 2 weeks. Ten days after the initial injection, the patient presented with an onset of seizures. A repeat MRI suggested tumor progression (Fig 3A), with significant edema in the three areas of previously noted tumor dissemination. The patient was treated with levetiracetam, a rapid dexamethasone tapering regimen, and a second dose of nivolumab was administered 2 weeks after the first infusion. She again presented 1 week later with recurrent seizures and hyponatremia, requiring a brief hospital admission for supportive care. Because no evidence of neurologic deterioration was noted and no additional complications occurred, treatment with nivolumab was continued as planned without modification. A follow-up MRI performed after the sixth infusion showed significant improvement, with tumor shrinkage in the parietal lesion and complete disappearance of imaging abnormalities in the frontal and temporal lobes (Fig 4A).

Given the index patient’s clinical and objective radiologic responses to nivolumab therapy, her brother was treated with an identical regimen upon recurrence. Similarly, the patient presented 11 days after treatment initiation with a seizure and apparent disease flaring detected by MRI (Fig 3B). However, a repeat MRI scan performed 2 months after treatment initiation revealed significant reduction of peritumoral edema and nodular lesions (Fig 4B). Importantly, after 9 and 5 months of therapy, respectively, the patient and her brother resumed normal schooling and daily activities.

**DISCUSSION**

We describe two pediatric patients with recurrent multifocal GBM refractory to current standard therapies who exhibited remarkable and durable responses to immune checkpoint inhibition with single-agent nivolumab. This observation is especially encouraging because these children are still clinically stable, whereas most relapsed pediatric GBMs will progress within 1 to 2 months despite salvage treatment, and survival is usually 3 to 6 months postrecurrence. Furthermore, bMMRD GBMs have outcomes similar to those of sporadic childhood GBMs, and data gathered from the consortium reveal a mean time from relapse to death of 2.6 months in bMMRD GBM. To our knowledge, this is the first report of such a response in childhood or adult GBM. It also highlights the utility of germline

Although the mutational load in all bMMRD GBMs suggests that these tumors are attractive candidates for immune checkpoint inhibitors, this specific report also highlights several important issues with this therapeutic approach. The blood–brain barrier is an ongoing challenge to the effective delivery of both chemotherapeutic compounds and other therapies, such as antibody-directed immunotherapy. The disappearance of multiple foci of disease with nivolumab, including areas where surgery was not performed, suggests that the antibody-mediated T-cell tumor interaction was not constrained by the blood–brain barrier and that effector T cells can in fact infiltrate the brain.

The specific pattern of mutations observed in bMMRD cancers, especially when somatic mutations in DNA polymerase are present, did not have a detrimental effect on the predicted formation of neoantigens or their affinity to the MHC complex (Fig 2B). Furthermore, even non–polymerase-mutated and non-GBM bMMRD cancers are hypermutant, with predicted high neoantigen load. The current concept suggests that cancers with greater numbers of mutations have an increased chance of responding to immunotherapy because they have an increased chance of having so-called winning neoantigens that activate T cells (the neoantigen roulette). bMMRD cancers have a higher chance of harboring these winning neoantigens because of their unique degree of ultrahypermutation. Moreover, in addition to having a higher mutational load and more neoantigens than other cancers, each bMMRD GBM lacks replication repair in every cell cycle, exponentially increasing the number of non-synonymous mutations available to encode novel neoantigens. Consequently, even a tumor that does not currently harbor the winning neoantigens will acquire new neoantigens continuously, which may be responsive to immunotherapy. Together, these data suggest that all bMMRD malignant cancers may benefit from immunotherapy regardless of the tissue of origin.

Preventive treatment of cancer predisposition syndromes is highly sought after, and surveillance protocols are identifying low-grade lesions. Interestingly, low-grade bMMRD tumors, including low-grade gliomas that later transform to GBMs, do not harbor excess mutations and would therefore be predicted to not respond to PD-1 blockade. Additional studies are required to assess the role of immunotherapy for prevention of transformation by surveying malignant cells that acquire new mutations.

The initial flare demonstrated both clinically and radiographically has been reported with other therapies (eg, radiotherapy pseudoprogression and other immunotherapies). This phenomenon is important to recognize and appreciate, because in these cases it marks a positive response by which activated T cells cause local inflammation. These adverse events are different than common adverse effects associated with treatment with immune checkpoint inhibitors, because the latter appear later and are not tumor specific. Misinterpretation of this tumor-specific flare can result in inappropriate management of these patients with prolonged courses of immunosuppressive drugs or can lead to premature treatment cessation. Indeed, careful management of the two patients, including rapid tapering of corticosteroids, enabled continuation of therapy, allowing the patients to achieve delayed, encouraging clinical responses to nivolumab.

These cases may have implications for other patients and cancers worldwide. bMMRD is not uncommon in countries where consanguinity is high and MMR mutations are more prevalent. In a recent study, up to 40% of pediatric GBMs in Jordan were attributable to MMR deficiency and therefore would be expected to be hypermutant. Heterozygous carriers of the MMR genes have a higher frequency of GI, urogenital, and other cancers, including gliomas. These tumors also harbor the hypermutation phenotype and may respond to immunotherapy. Finally, recurrent cancers of patients who do not harbor germline MMR defects tend to have secondary somatic MMR deficiency and hypermutation. This can be acquired stochastically or as a result of MMR deficiency–associated resistance to previous therapies, such as purine analogs or nitrosoureas. For example, gliomas treated with temozolomide acquire secondary MSH6 mutations, conferring temozolomide resistance and resulting in a hypermutation phenotype.

In summary, to our knowledge, this is the first report of initial and durable responses of recurrent GBM to immune checkpoint inhibition. This study may have implications for GBM in general and other hypermutant cancers arising from primary (genetic predisposition) or secondary MMRD. Given the increasing availability of commercial sequencing platforms, analysis of mutation burden and neoantigens can play a role in transforming treatment of these patients. Multinational prospective clinical trials of these bMMRD-driven hypermutant cancers are required to validate our encouraging results for patients with these devastating and heretofore universally lethal cancers.

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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GLOSSARY TERMS

glioblastoma multiforme (GBM): by far the most common and most malignant of the glial tumors. Composed of a heterogeneous mixture of poorly differentiated neoplastic astrocytes, glioblastomas primarily affect adults, and they are located preferentially in the cerebral hemispheres. Much less commonly, GBMs can affect the brainstem in children and the spinal cord. These tumors may develop from lower-grade astrocytomas (WHO grade II) or anaplastic astrocytomas (WHO grade II), but more frequently, they manifest de novo, without any evidence of a less malignant precursor lesion. The treatment of glioblastomas is palliative and includes surgery, radiotherapy, and chemotherapy.


mismatch repair genes (MMR): genes that recognize and correct errors in DNA replication leading to single base-pair mismatches or insertions/deletions in small repetitive tracts of DNA known as microsatellites.

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Immune Checkpoint Inhibition for Hypermutant Glioblastoma Multiforme Resulting From Germline Biallelic Mismatch Repair Deficiency

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## Table A1. bMMRD Cancers With Exome-Sequencing Data

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Abbreviations: GBM, glioblastoma multiforme; MB, medulloblastoma; PNET, primitive neuroectodermal tumor.
Fig A1. POLE mutations found in both patients with biallelic mismatch repair deficiency glioblastoma multiforme. Both mutations (boxed in red) occurred at highly conserved amino acids in the POLE exonuclease domain (indicated in blue) and compromised exonuclease proofreading activity. Sequences were aligned according to data from the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool and NCBI Conserved Domain Database. Amino acids critical for catalysis are highlighted in gold.