Received: 19 March 2024 | Revised: 18 September 2024 | Accepted: 30 September 2024

DOI: 10.1111/cas.16370

Cancer Science WILEY

CASE REPORT

Pembrolizumab efficacy in a tumor mutation burden-high glioblastoma patient: A case study and implications for precision oncology

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Abstract

A glioblastoma (GBM) patient with a high tumor mutation burden (TMB-high) and mismatch repair deficiency (dMMR) exhibited a significant response to pembrolizumab, an immune checkpoint inhibitor (ICI), despite prior treatment with temozolomide (TMZ), known to induce hypermutation and potential resistance to ICIs. The rapid disease progression, indicated by 80% Ki67 positivity, was markedly countered by the positive outcome of pembrolizumab treatment. This case challenges traditional GBM treatment paradigms, demonstrating the potential of precision oncology in patients with significant TMB and dMMR, and underscores the importance of comprehensive genomic profiling in guiding clinical decisions in GBM management.

KEYWORDS
glioblastoma, mismatch repair deficiency, pembrolizumab, precision oncology, tumor mutation burden

1 | BACKGROUND

Glioblastoma (GBM) is the most frequent and aggressive primary malignant brain tumor in adults. Therapeutic options for GBM remain limited, and patients with GBM typically have a poor prognosis. High tumor mutation burden (TMB) and mismatch repair (MMR) deficiency (dMMR) are considered tumor agnostic markers capable of predicting response to immune checkpoint inhibitors (ICIs).[1,2] Pembrolizumab, an effective ICI to treat solid tumors harboring these markers, has gained worldwide approval. Germline or somatic mutations in MMR genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) lead to DNA replication repair deficiency, resulting in hypermutant tumors owing to the accumulation of multiple somatic mutations. These tumors are characterized by ultra-hypermutations (>100 mutations/Mb).[3]

Temozolomide (TMZ), the standard chemotherapy for GBM, is known to directly induce hypermutagenesis through MMR pathway inactivation. GBMs with TMZ-associated hypermutation appear insensitive to ICIs.[4] Conversely, GBMs with TMB-high, due to inherent MMR gene defects, respond to ICIs.[5]

2 | CASE PRESENTATION

A 29-year-old female presented with a headache and mild left weakness. After a thorough examination and initial surgery, she was diagnosed with GBM, and was negative for the *IDH* mutation and *TERT* promoter mutation, but with *MGMT* promoter methylation. She underwent the Stupp regimen, followed by maintenance therapy with 200 mg/body/day TMZ, achieving a partial response. This response lasted for 2 years across 23 courses of TMZ maintenance. Following disease progression, she underwent treatment at the Department of Neurosurgery, Kanazawa University Hospital, where TMZ therapy was continued (Figure 1A). Twelve months later, a local recurrence accompanied by brain edema resulted in herniation and a second surgery. Pathological examination revealed similarities to giant cell GBM (Figure 1B–D). Immunostaining revealed 99% p53 positivity, 80% Ki67, and 10% GFAP positivity (Figure 1E–G); negative Olig 2, IDH1 R132H, and BRAF V600E; and an ATRX nucleus elimination rate of 20%. Additionally, given that the primary site of this patient's tumor was the right basal ganglia, categorized as a midline primary, we performed immunostaining for a histone H3 mutation (Figure S2C,D). The tumor was classified as a high-grade glioma with giant cells. As adjuvant therapy, the patient received TMZ plus bevacizumab. This treatment lasted for 5 months, with bevacizumab administered only twice. Gradually, the recurrent lesion began to enlarge. One month after the conclusion of this therapy, at the age of 33, she underwent comprehensive genomic profiling (CGP) using the FoundationOne CDx assay. The CGP, based on the specimen from the second surgery, confirmed the absence of *IDH* and *TERT* promoter mutations, which was in line with the previous GBM diagnosis. The tumor had a TMB of 714 mutations/Mb and an equivocal microsatellite status (Figure 1H). Subsequently, this status was confirmed as dMMR through immunostaining, indicated by *MLH1* and *PMS2* negativity (Figure S1). Following the CGP, she underwent a third operation with photodynamic therapy at another institution. One month after this surgery, MRI findings indicated enhanced lesions, suggesting disease progression (Figure 2A,B). Pembrolizumab therapy for TMB-high and dMMR GBM tumors led to a favorable clinical trajectory with minimal toxicity, predominantly presenting as a grade 1 skin rash. An initial therapeutic response was noted after two courses of pembrolizumab, which was sustained for over 23 months (Figure 2). Pathogenic variants *MLH1* T117H and *TP53* R273H were identified with high allele frequencies, suggesting potential germline pathogenicity (Table 1). There was no familial history of malignancies, and subsequent genetic screening for Lynch syndrome and Li-Fraumeni syndrome yielded negative results, suggesting that these mutations were of a somatic nature. The details of the genetic test are as follows: the genome gene sequence is analyzed using targeted next-generation sequencing analysis by hybrid capture method for the region including two bases before and after the genome position. The obtained base sequence is compared with the published human genome reference sequence (GRCh38/hg38), and the presence or absence of low-frequency base substitutions and short base sequence deletions/insertions is analyzed by computer.

3 | DISCUSSION

To the best of our knowledge, the current case represents the first documented instance in which ICIs elicited a response in a GBM patient with high TMB within the adolescent and young adult cohort. With reference to reports that pediatric patients with GBM harboring germline DNA replication repair deficits exhibit a response to

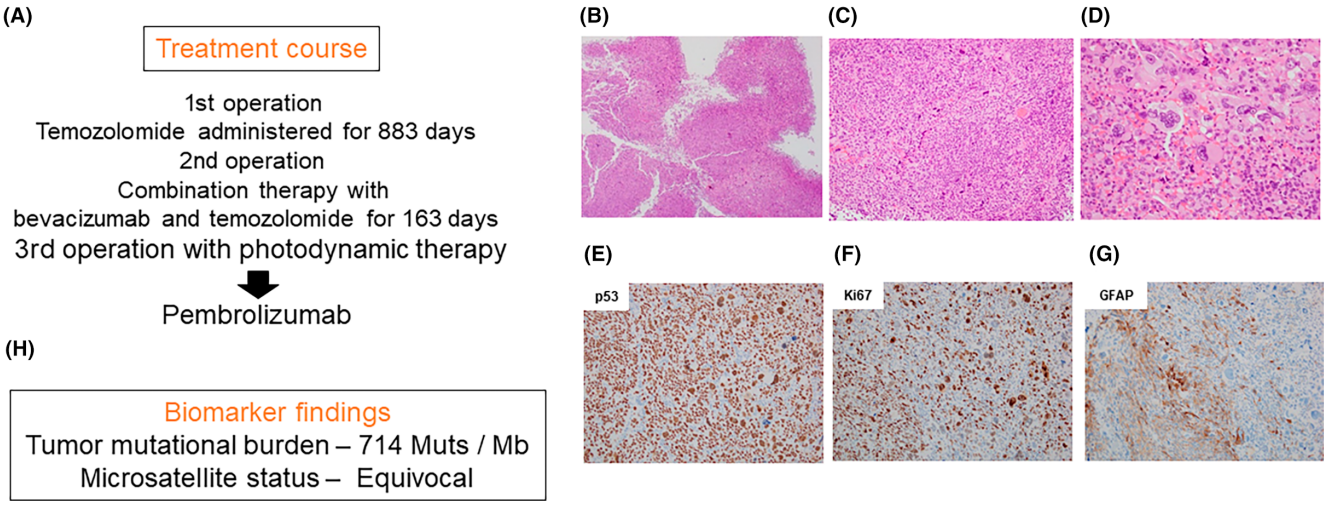

FIGURE 1 Clinical course including the comprehensive genomic profile test and pathologic findings. (A) Overview of the clinical course. (B) Low-power field image of H&E-stained surgical sample from the right insular gyrus, reveals a tumor cell content rate of 80%, as determined by a pathologist at Kanazawa University Hospital. (C) High-power field image displays scattered giant cells within the surgical specimen. (D) Detailed view of the giant cells. (E) p53 staining shows 99% positivity. (F) Ki67 staining with 80% positivity. (G) GFAP staining demonstrates 10% positivity. (H) Biomarker analysis revealed a tumor mutation burden of 714 Muts/Mb and an undetermined microsatellite status.

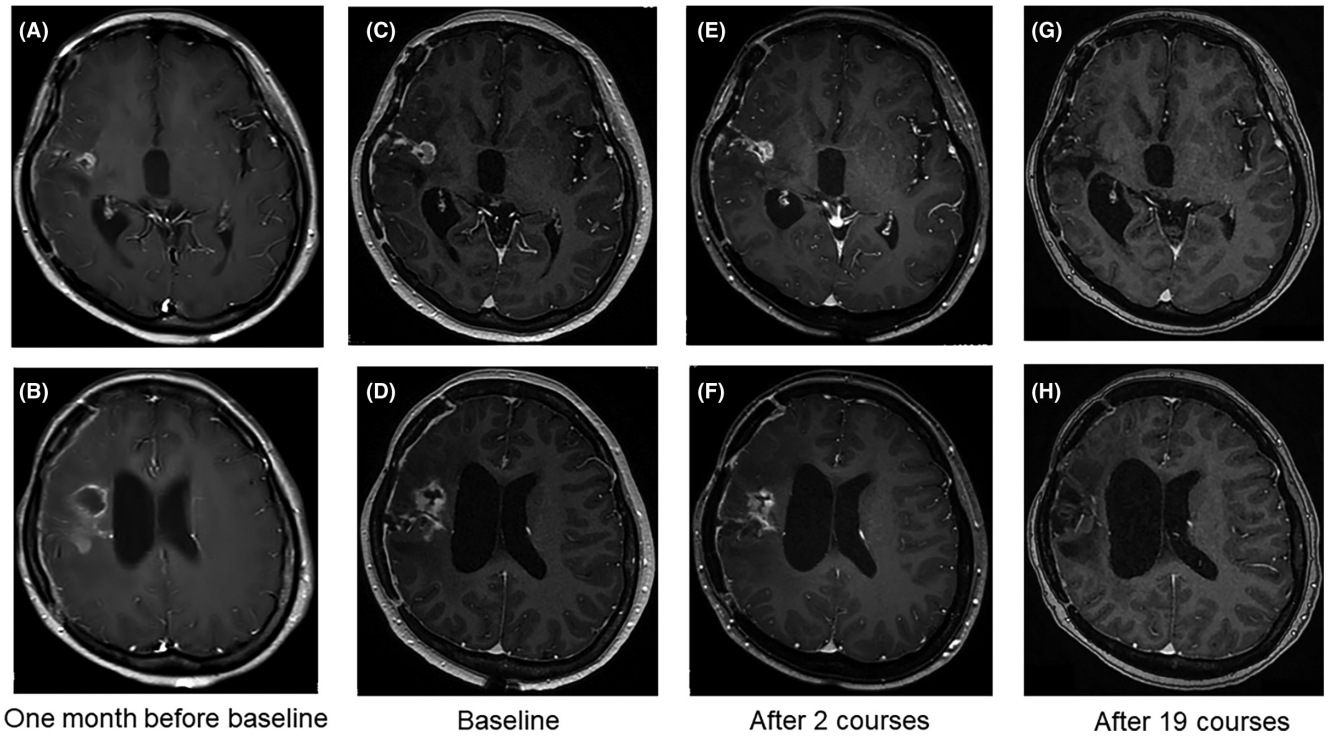

FIGURE 2 Radiographic response to pembrolizumab. (A, B) One month after the third operation, enhanced lesions represent tumor recurrence. (C, D) Prior to the administration of pembrolizumab, the recurrent lesions are highlighted with a contrast medium. (E, F) An initial response can be observed after just two courses of pembrolizumab, indicating a reduction in lesion size. (G, H) Following 19 courses of pembrolizumab, the recurrent lesions appear to have mostly disappeared, demonstrating a notable therapeutic response.

programmed death 1 (PD-1) blockade,[5] and given the positive clinical outcome observed in our patient, we initially postulated that the elevated TMB could be attributed to germline mutations in MMR genes and not as a repercussion of TMZ therapy. Nevertheless, subsequent genetic assays confirmed that mutations in *MLH1* and *TP53* were somatic. This finding, coupled with the identified high TMB characterized by numerous C>T transitions (Table 1), indicated a potential association with antecedent alkylating chemotherapy,[5] thus suggesting that hypermutation could be correlated with TMZ exposure. Interestingly, even in the presence of high TMB or deficient MMR, a previous clinical trial emphasized that 82% of patients experienced progressive disease as their best overall response to ICI therapy.[6] This stark contrast underscores the importance of re-evaluating TMB thresholds to predict ICI responsiveness in GBM. Moreover, an ongoing clinical trial (NCT02658279) probing the efficacy of pembrolizumab in patients with recurrent glioma with a TMB of over 30 mutations/Mb promises to shed further light on this criterion.

Our study suggested that clonal neoantigens with low neoantigen diversity may enhance the response to ICIs, but further validation is necessary to confirm their ability to elicit an immune response. Future studies should consider using bioinformatics tools for epitope prediction, along with experimental methods such as peptide synthesis and T-cell activation assays. Additionally, a comprehensive analysis of immune cell infiltration in the tumor microenvironment (TME), including CD8+ T cells, macrophages, NK cells, and other immune cells, could provide deeper insights into the shifts in immune profiles and their impact on ICI efficacy. Incorporating these approaches in future research would help clarify the mechanisms by which neoantigens influence ICI response, paving the way for more personalized immunotherapeutic strategies for GBM patients.

The current case report facilitates our understanding of the involvement of mutations in the exonuclease domains of the DNA polymerases *POLE* and *POLD1*, which have been implicated in compromising proofreading capabilities, thereby precipitating the proliferation of genetic mutations.[7] In the current patient, four distinct *POLE* mutations and four *POLD1* mutations were detected, with the majority deemed to be of uncertain significance or likely benign (Table S1) and situated outside the exonuclease domain. Moreover, the frequencies of these mutations turned out to be less than 15%, which reflected not genetic mutation but somatic mutation (Table S1). This finding is consistent with the accumulated literature, indicating that patients with *POLE* mutations that do not affect the exonuclease domain may achieve durable responses to ICI treatment, a phenomenon mirrored in the clinical progression of our patient.[7]

The influence of neoantigens on the efficacy of immunotherapy warrants further investigation. Although a TMB-high status is associated with an increased probability of immunogenic neoantigen production by tumor cells, potentially triggering an immunotherapy response,[8] this effect is not universally observed across all cancers. Specifically, in GBM, the neoantigen load does not seem to directly

TABLE 1 Genomic characteristics of GBM tumor identified by the FoundationOne CDx assay.

Gene	VAF (%)	Alteration	Coding sequence effect
APC	8.0	Splice site 532-8G>A	532-8G>A
ARID1A	2.0	Q1856*	5566C>T
ATM	7.2	W1710*	5130G>A
ATM	15.6	Splice site 2251-1G>A	2251-1G>A
ATR	27.3	Splice site 3819+1G>A	3819+1G>A
ATR	9.5	Splice site 6078+1G>A	6078+1G>A
AURKA	8.9	P32S	94C>T
BCL2	1.3	D31N	91G>A
BCOR	12.9	Splice site 4493+1G>A	4493+1G>A
BRAF	1.5	G464E	1391G>A
BRCA1	6.6	E1559K	140G>A
BRCA1	4.0	C47Y	4675G>A
BRCA2	0.94	D23N	67G>A
CBL	2.1	R830K	2489G>A
CDC73	7.3	M1I	3G>A
CDKN2A/B	2.3	P16INK4a P81S and p14ARF T95I	241C>T
CHEK2	15.1	D293fs*1	876-877insT
CIC	27.5	Splice site 765+1G>A	765+1G>A
CREBBP	5.1	A1071T	3211G>A
DNMT3A	26.0	Splice site 449-1G>A	449-1G>A
EGFR	17.4	P794L	2381C>T
EGFR	2.4	A597T	1789G>A
EGFR	19.1	P772L	2315C>T
EP300	14.9	P1337S	4009C>T
ERBB2	3.5	S310F	929C>T
ERBB2	9.5	Splice site 1899-1G>A	1899-1G>A
ERBB4	6.5	P854S	2560C>T
FANCG	16.1	A468V	1403C>T
FUBP1	5.2	Splice site 636+1G>A	636+1G>A
INPP4B	1.6	V125I	373G>A
KDM5A	1.7	A1052T	3154G>A
KDM5A	8.1	P325S	973C>T
KDM6A	10.5	R583fs*10	1748delG
KDM6A	13.7	Splice site 974+1G>A	974+1G>A
MAP2K4	1.4	R281*	841C>T
MAP3K13	31.1	W319*	957G>A
MCL1	1.2	D65N	193G>A
MEF2B	1.7	G105E	314G>A
MEN1	10.7	P545L	1634C>T
MLH1	77.9	T117M	350C>T
MLL2	11.7	Q1361*	4081C>T
MLL2	7.6	Splice site 13,839+1G>A	13,839+1G>A
MSH2	13.5	Splice site 1510+1G>A	1510+1G>A
MSH3	13.6	K383fs*32	1148delA
NF1	5.7	W267*	800G>A
NF1	2.1	T2017I	6050C>T
NOTCH1	3.6	A2441V	7322C>T
NOTCH1	3.4	G1166D	3497G>A
NOTCH3	13.9	W1495*	4485G>A
NTRK2	2.2	S257F	770C>T
PBRM1	29.3	Splice site 3616+1G>A	3616+1G>A
PIK3CA	26.6	A1035T	3103G>A
PMS2	7.5	Splice site 537+1G>A	537+1G>A
PTEN	7.6	G20E	59G>A
PTEN	12.94	Splice site 1026+1G>A	1026+1G>A
PTEN	7.8	G129E	386G>A
RB1	73.51	Splice site 1215+1G>A	1215+1G>A
RET	3.9	G911D	2732G>A
RNF43	29.3	P756L	2267C>T
SMARCB1	38.4	Splice site 500+1G>A	500+1G>A
SMARCB1	15.0	A327T	979G>A
SPEN	28.0	Q1346*	4036C>T
STAG2	38.6	Splice site 385+1G>A	385+1G>A
TP53	2.2	A129T	385G>A
TP53	82.7	R273H	818G>A
TP53	2.3	S183L	548C>T
TP53	0.87	D207N	619G>A
TP53	3.2	G262D	785G>A
TP53	1.0	Splice site 672+1G>A	672+1G>A
TP53	2.1	P295S	883C>T
TSC2	14.3	W257*	771G>A
TSC2	9.9	Splice site 3398-1G>A	3398-1G>A

TABLE 1 (Continued)

Gene	VAF (%)	Alteration	Coding sequence effect
VHL	26.0	Splice site 340+1G>A	340+1G>A

Abbreviation: VAF, variant allele frequency.

influence $CD8^+$ T-cell recruitment, occasionally resulting in immunotherapy resistance. In our case, the absence of enhanced $CD8^+$ T cells in the CGP-analyzed specimen (Figure S2A), coupled with a mere 10% prevalence of PD-L1 positivity among the cancer cells (Figure S2B), suggested that PD-L1 expression alone may not be a reliable biomarker for predicting the efficacy of pembrolizumab in GBM. Conversely, the 10% tumor proportion score (TPS) of PD-L1 may still be sufficient to expect some efficacy from pembrolizumab, as seen in other cancers like non–small-cell lung cancer. Although the 10% TPS of PD-L1 observed in this case aligns with indications for pembrolizumab use in non–small-cell lung cancer, the unique microenvironment of GBM may present different challenges for its efficacy. The detected tumor-infiltrating lymphocytes (TILs) within the CGP-analyzed tumors suggested a potential anti-tumor immune response; however, without the primary tumor specimen, a direct comparison of TIL levels remains a limitation. Nevertheless, the results in Figure S2 show the presence of TILs and moderate PD-L1 expression, which could indicate a mixed, but potentially responsive, tumor microenvironment. A more nuanced understanding of these findings may help to guide future strategies in integrating immunotherapy with other modalities for GBM treatment.

Notably, tumors characterized by substantial clonal neoantigen loads and low neoantigen diversity are more responsive to anti-PD-L1 therapies.[9] Conversely, heterogeneous tumors with substantial subclonal neoantigen quantities often exhibit a subdued response to ICIs, possibly progressing despite substantial neoantigen count.[10] Our case seems to correspond with the former, suggesting a more intricate interplay between tumor biology and the immunotherapeutic response. From immune-related biomarkers, the combination therapy with pembrolizumab and TMZ might be anticipated to be a promising therapy, as supported by previous case reports.[11,12]

In summary, irrespective of the origin of TMB-high status, GBM patients with considerable TMB (e.g., exceeding 100 mutations/Mb) and dMMR profiles may demonstrate susceptibility to ICIs, even in cases with a history of TMZ therapy. This highlights the critical need for additional prospective studies to ascertain the effectiveness and potential of ICIs in GBM treatment, contributing to the dynamic field of personalized oncology for this formidable disease.

AUTHOR CONTRIBUTIONS

Akihiro Nishiyama: Conceptualization; writing – review and editing. **Shigeki Sato:** Writing – review and editing. **Hiroyuki Sakaguchi:** Writing – review and editing. **Hiroshi Kotani:** Writing – review and editing. **Kaname Yamashita:** Writing – review and editing. **Koushiro Ohtsubo:** Writing – review and editing. **Tomoko Sekiya:** Writing – review and editing. **Atsushi Watanabe:** Writing – review and editing. **Atsushi Tajima:** Writing – review and editing. **Chie Shimaguchi:** Writing – review and editing. **Keishi Mizuguchi:** Writing – review and editing. **Hiroko Ikeda:** Writing – review and editing. **Masashi Kinoshita:** Writing – review and editing. **Mitsutoshi Nakada:** Writing – review and editing. **Shinji Takeuchi:** Writing – review and editing.

ACKNOWLEDGMENTS

We express our gratitude to the patients and their families for providing informed consent to use their personal data.

CONFLICT OF INTEREST STATEMENT

Mitsutoshi Nakada is an editorial board member for *Cancer Science* and has ties to Novocure GmbH. The remaining authors have no conflicts of interest.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: N/A.

Informed Consent: We have obtained the consent for publication in print and electronically from the patient and their family.

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Nishiyama A, Sato S, Sakaguchi H, et al. Pembrolizumab efficacy in a tumor mutation burden-high glioblastoma patient: A case study and implications for precision oncology. *Cancer Sci*. 2024;00:1-6. doi:10.1111/cas.16370