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► **To cite this version:**

Diego Prost, Franck Bielle, Keith L Ligon, Mehdi Touat. Mutational burden and immune recognition of gliomas. *Current Opinion in Oncology*, 2021, 33 (6), pp.626-634. 10.1097/CCO.0000000000000787 . hal-03474667

HAL Id: hal-03474667

<https://hal.sorbonne-universite.fr/hal-03474667>

Submitted on 10 Dec 2021

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2 **Mutational burden and immune recognition of gliomas**
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20 2800 words

21 1 Figure, 1 Table

22 90 references
23
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29 **Abstract**

30 ***Purpose of review***

31 Recent evidence suggests high tumor mutational burden (TMB-H) as a predictor of
32 response to immune checkpoint blockade (ICB) in cancer. However, results in TMB-H
33 gliomas have been inconsistent. In this article, we discuss the main pathways leading to
34 TMB-H in glioma and how these might affect immunotherapy response.

35 ***Recent findings***

36 Recent characterization of TMB-H gliomas showed that “post-treatment hypermutation”
37 related to mismatch repair (MMR) deficiency is the most common mechanism leading to
38 TMB-H in gliomas. Unexpectedly, preliminary evidence suggested no benefit with ICB
39 as compared to chemotherapy in this population. In contrast, ICB response was reported
40 in a subset of TMB-H gliomas associated with constitutional MMR or polymerase epsilon
41 (POLE) defects (e.g., constitutional biallelic MMRd deficiency). In other cancers, several
42 trials suggest increased ICB efficacy is critically associated with increased lymphocyte
43 infiltration at baseline which is missing in most gliomas. Further characterization of the
44 immune microenvironment of gliomas is needed to identify biomarkers to select the
45 patients who will benefit from ICB.

46 ***Summary***

47 Intrinsic molecular and immunological differences between gliomas and other cancers
48 might explain the lack of efficacy of ICB in TMB-H gliomas. Novel combinations and
49 biomarkers are awaited to increase immunotherapy response in these cancers.

50

51

52 **Key words**

53 Biomarkers; immune checkpoints; immunotherapy; chemotherapy; immune
54 microenvironment.

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59 **Introduction**

60 Gliomas are the most common primary tumors of the central nervous system (CNS) [1].
61 They can affect patients of any age. They are frequently aggressive and responsible for
62 high morbidity and mortality. The treatment of gliomas varies depending on accessibility
63 for surgical resection, tumor grade and molecular profile. It typically includes radiation
64 therapy and chemotherapy with alkylating agents such as temozolomide (TMZ) [2–4].
65 Despite these treatments, relapse is almost inevitable, especially in high-grade gliomas
66 (HGG). Recurrent HGGs are among the most challenging cancers to treat, commonly
67 harboring resistance to conventional, targeted therapies and immunotherapies [5].

68 The development of immune checkpoint blockade (ICB) has recently transformed
69 the care of various cancer types. Intensive efforts have focused on identifying predictive
70 biomarkers for clinical response to ICBs. Among several markers under investigation, a
71 number of studies showed a positive correlation between ICB response rates and the
72 presence of a high tumor mutational burden (TMB-H), defined as the number of coding
73 mutations per megabase (Mb) across the genome [6–11]. These data as well as
74 promising results from the Keynote-158 study led to the tumor-agnostic approval of the
75 anti-PD1 pembrolizumab for TMB-H tumors by the Food and Drug Administration (FDA)
76 in 2020 [12]. However, the correlation between TMB and ICB clinical benefit was mainly
77 driven by data from a limited number of cancers such as melanoma, lung carcinomas
78 and known mismatch repair deficient (MMR-d) cancers, and it remains unclear whether
79 TMB-H and MMR-d are universally predictive in rare cancers not represented in these
80 studies [13,14]. Gliomas are one of such cancers, as these tumors typically harbor a
81 strong immunosuppressive TME [15] and conflicting data has been reported regarding
82 their benefit from ICB, even in the presence of TMB[16–28].

83 In this review, we discuss recent data on hypermutated gliomas, their mechanism
84 of mutagenesis and the potential role of TMB-H as a prognostic and predictive biomarker
85 for response to chemotherapy and immunotherapy.

86

87 **TMB-H in gliomas: mechanisms and potential role in predicting prognosis and**
88 **response to conventional therapies.**

89 Cancer somatic mutations are caused by mutational processes of exogenous and
90 endogenous origin which happen during development of each tumor cell and its progeny
91 [29–32]. Each mutational process can involve components of DNA damage or
92 modification, abnormal DNA replication or repair and generates a “mutational signature”
93 (e.g., specific mutational pattern), which can include base substitutions, small insertions
94 and deletions (indels), chromosome rearrangements and copy number abnormalities.
95 Mutational signatures can be extracted from tumor sequencing data (e.g., exome or

96 genome sequencing) to infer the mutational processes responsible for mutations in
97 individual samples. Mutations are sometimes associated with the production of foreign
98 antigens (neoantigens) which are recognized by the immune system and can elicit T cell
99 immunoreactivity.

100 The frequency of mutations and underlying mechanisms causing them varies
101 greatly across cancers [13,33]. A small subset of cancers (<20% of cancers) show a
102 markedly elevated mutation burden which is referred to as TMB-H (or hypermutation,
103 often used for some cancers like gliomas). The mutation burden defining this varies
104 across assays used but is generally higher than 10 mutations per Mb of genome
105 sequenced. Exceptionally (<1% of cancers), an “ultra-hypermutated” (i.e. TMB higher
106 than 100 mutations per Mb) is observed [13]. TMB-H is prevalent in melanoma [34] and
107 lung cancer [35], where the increase in TMB is mainly related to environmental mutagens
108 exposure (tobacco smoke, UV light), and associated with ICB response [36]. In gliomas,
109 TMB-H is less common and observed in two distinct contexts associated with unique
110 biology: de novo (i.e., hypermutation present in the newly-diagnosed tumor) and post-
111 treatment (i.e. hypermutation only found at recurrence after treatment).

112

113 ***De novo hypermutation***

114 De novo hypermutation is found in less than 2% of all newly-diagnosed gliomas [14]. De
115 novo hypermutation in gliomas has been reported in tumors with inherited or somatic
116 defects of the DNA polymerases ϵ (POLE) and δ (POLD1) or the MMR system, which
117 lead to the loss of polymerase proofreading or DNA replication error repair, respectively
118 (Figure 1). Given its rarity and lack of dedicated prospective trials focusing on these
119 patients, the management of gliomas in patients with de novo TMB-H glioma is not well
120 codified [37].

121 DNA replication fidelity is primarily governed by the DNA polymerases POLE and
122 POLD1 catalytic and proofreading domains. Germline pathogenic mutations in the
123 exonuclease domains of polymerases POLE and POLD1 predispose to adenomatous
124 polyps, colorectal cancer (CRC), endometrial cancer, and more rarely to other
125 malignancies including glioblastoma [26,38], all of which are typically harboring
126 hypermutation or ultra-hypermutation [13]. Somatic POLE defects have also been
127 reported in glioblastoma [39]. Very little is known about the phenotype of POLE/POLD1-
128 deficient gliomas. Recent studies have suggested an association between POLE/POLD1
129 defects, increased inflammatory infiltrates, and longer survival in gliomas, but these data
130 need further confirmation in larger datasets [40].

131 The MMR system - consisting mainly of MSH2, MSH6, MLH1 and PMS2 proteins
132 - is responsible for recognizing base-base mismatches and indels occurring during DNA

133 replication and recruiting proteins which excise the newly-synthesized strand before DNA
134 is resynthesized by DNA polymerases [33]. Germline - and less commonly somatic -
135 MMR defects have both been reported in de novo hypermutated gliomas. Constitutional
136 (Biallelic) mismatch repair deficiency (CMMR-d) is a rare autosomal recessive disorder
137 caused by germline biallelic MMR mutations, most commonly affecting PMS2, and
138 characterized by early-onset cancers. Gliomas are one of the tumors commonly seen in
139 CMMR-d patients [26,41–45]. They develop at younger age (<10 years). The histology
140 is most commonly glioblastomas which are wild-type for other common defining driver
141 events such as *H3F3A*, *IDH1/2*, or infant-type receptor tyrosine kinase (RTK)
142 aberrations. The prognosis of CMMR-d patients is poor, especially once patients develop
143 brain tumors. In a subset of patients with CMMR-d glioma, secondary hits in the
144 polymerase *POLE/POLD1* are acquired, leading to a rapid burst in the mutational burden
145 (ultra-hypermutation) (Figure 1) [13,46].

146 Lynch syndrome is an autosomal dominant disorder caused by germline
147 heterozygous inactivating mutations of one of the MMR genes. Patients with Lynch
148 syndrome can develop cancers after a second hit occurring in the remaining wild-type
149 MMR allele leading to MMR loss of function, which typically occurs after the first decade
150 of life. Patients with Lynch syndrome most commonly develop colorectal, urinary, or
151 gynecological cancers but can also suffer from high-grade glioma [47,48]. Most gliomas
152 arising in patients with Lynch syndrome are *IDH1/2*-wild-type glioblastomas and seem to
153 have a poor prognosis, although *IDH1/2*-mutant astrocytomas with MMR-d have also
154 been reported [47,49].

155

156 ***Post-treatment hypermutation***

157 Post-treatment hypermutation is the most common cause of TMB-H in gliomas, ranging
158 from 5-60% of gliomas depending on tumor subclass, genetics, and treatment history
159 [50,51]. Post-treatment hypermutation is predominantly seen in gliomas which are known
160 to be the most responsive to chemotherapy [14,50,52–56]. TMZ is an alkylating agent
161 widely used to treat gliomas. Its mechanism of action is based on the production of an
162 intermediate metabolite reaching high concentration in the brain and producing methyl
163 groups in tumor cells DNA, particularly on N7 and O6 guanines residues [57]. The
164 enzyme O6-methylguanine DNA methyltransferase (MGMT) removes the O6 guanine
165 methyl groups (O6-meG) which are the most cytotoxic lesions [58]. The enzyme is a
166 “suicide” protein and each MGMT protein can only repair one O6-meG residue on DNA,
167 which means the levels of MGMT protein in cells is critical to DNA repair. MGMT
168 promoter methylation, which silences its expression, leads to an MGMT-deficient state

169 (MGMT-d), and is associated with increased sensitivity to TMZ and improved prognosis
170 in patients with glioma [59,60].

171 In the absence of O6-meG removal (e.g. in MGMT-d tumors), replication of O6-
172 meG-containing DNA results in the insertion of a thymine opposite the O6-meG, creating
173 a O6-meG:T mismatch that is recognized by the MMR machinery. Failed attempts to
174 repair O6-meG:T mismatch by MMR generate DNA strand breaks, ultimately leading to
175 cell cycle arrest and cell death. This means that TMZ cytotoxicity is critically dependent
176 on a functional MMR pathway. Consequently, MMR-d cells (e.g. cells lacking MMR
177 capacity due to mutations in genes encoding MMR proteins) display resistance to
178 alkylating agents such as TMZ [14].

179 MMR-d cells have increased burden of mutations of several types. Single base
180 substitutions are the most common and are the mutation type reported in “TMB” most
181 commonly calculated from sequencing. In addition, MMR deficiency also results in
182 mutations (e.g. indels) at tandemly repeated DNA motifs (microsatellites) which lead to
183 microsatellite instability (MSI), a well-known and widely used marker to diagnose MMR
184 deficiency in common MMR-d cancers such as colorectal cancer [61]. The degree to
185 which the MSI/indel repair and MMR are linked in TMB-H cancers is not well understood
186 and in the most common MMR-d cancers they co-occur in almost all cases.

187 In gliomas, TMZ-induced DNA damage and hypermutation is now known to be
188 characterized by the acquisition of MMR-d mutations or other alterations but shows a
189 lack of MSI or significantly increased indels. They also harbor a unique MMR-d
190 mutational signature (signature 11) specific to TMZ in a setting of cells which cannot
191 repair TMZ-related mismatches due to MMR deficiency. Interestingly, MMR-d gliomas
192 with TMB-H also lack significant inflammatory CD8⁺ infiltrates compared to the most
193 common MMR-d cancers [14,62–64], even when the latter are located in the CNS
194 [65,66].

195

196 ***Origins of MMR-d cells in post-treatment gliomas and clinical implications***

197 The highest rates of TMB-H have been reported in the most chemosensitive subtypes
198 where MGMT promoter methylation is more common (e.g., IDH1/2-mutant astrocytomas
199 and oligodendrogliomas). While recent studies help to define post-treatment TMB-H
200 gliomas as an MMR-d cancer, a question that remains unaddressed is whether pre-
201 existing MMR-d cells are selected for after treatment response or whether the initial MMR
202 mutations are induced by TMZ in cells and selected for during treatment, or a
203 combination of both processes. Although it is difficult to ascertain the origins of post-
204 treatment MMR deficiency in individual samples, indirect data suggests that in a subset
205 of samples TMZ induces mutations which cause MMR-d in cells. This is for instance

206 showed by the fact that the MMR-d inducing hotspot mutation most frequently found in
207 post-treatment hypermutated gliomas (~15%) is an MSH6 T1219I mutation with
208 dominant negative effect found exceptionally in patients with Lynch syndrome [67–71].
209 This variant results from a C > T transition similar to TMZ-associated lesions and is
210 inducible in in vitro models chronically exposed to TMZ [14].

211 Conflicting data has been reported regarding the prognosis of post-treatment
212 hypermutation. While the GLASS study found no prognostic significance [56,72], two
213 retrospective studies suggested that post-treatment hypermutated might have a worse
214 prognosis from recurrence when compared to non-hypermutated recurrences. However,
215 since secondary MMR defects occur in tumors most sensitive, and potentially with the
216 greatest beneficial responses to TMZ, the deleterious effect of secondary MMR
217 deficiency (poor prognosis after relapse) is unlikely to outweigh its positive effects. These
218 results therefore do not argue for the use of TMZ vs nitrosourea-based protocols (e.g.
219 PCV) in lower grade gliomas, especially given that randomized trial data demonstrated
220 survival benefit with TMZ in both IDH1/2-wild-type and -mutant tumors [73,74].

221 Regarding treatment response, while data regarding radiation therapy or
222 chemoradiation is currently insufficient [75], consistent evidence shows that MMR-d or
223 TMB-H in gliomas are both predictive biomarkers for resistance to single-agent TMZ.
224 Interestingly, experimental data from models and indirect evidence from clinical samples
225 suggest that at least a subset of MMR-d tumors might retain sensitivity to nitrosoureas
226 such as CCNU [14,57,76,77]. This observation might explain at least in part the
227 superiority of the TMZ/CCNU combination with radiation compared to standard
228 chemoradiation with TMZ recently reported in a randomized trial of newly-diagnosed
229 MGMT-d glioblastomas [78]. Further research is needed to address whether the
230 TMZ/CCNU combination or PCV might reduce the risk of post-treatment hypermutation
231 and to characterize the unique resistance mechanisms associated with this combination.
232 Another area of ongoing investigation is whether PARP inhibition might restore TMZ
233 sensitivity in MMR-d glioma cells and therefore prevent the development of post-
234 treatment hypermutation [79].

235

236 **Treatment of hypermutated gliomas with ICB**

237 The concept that TMB-H tumors are capable of presenting immunogenic neoantigens is
238 well established [6]. Neoantigens are recognized and processed by dendritic cells which
239 recruit lymphocytes against tumor neoantigens. In this context, ICB treatment enables
240 lymphocyte proliferation (anti-CTLA-4) and prevents lymphocyte inactivation (anti-PD-
241 1/PDL-1). Melanoma [80], NSCLC [81] and MMR-d tumors with MSI [82] are the classic
242 examples of TMB-H benefiting from ICB, but clinical data in hypermutated glioma as well

243 as other cancer types has been so far inconsistent. Research for biomarkers predicting
244 ICB response in the context of hypermutation is an area of intensive investigation
245 [11,83,84]. A recent pan-cancer study was able to categorize TMB-H tumors in two
246 groups with distinct pattern of ICB response [85]. The first group, enriched with ICB
247 responders, consisted of tumors in which increased TMB was associated with a great
248 number of CD8⁺ lymphocytes infiltrates. In contrast, the second group showed no
249 correlation between increased TMB and CD8⁺ infiltration. Of note, the latter group
250 represents the majority of cancers including TMB-H gliomas [16]. These important results
251 clearly indicate TMB is not a universal predictive marker and suggests that additional
252 biomarkers are required to appropriately select the patients most likely to benefit from
253 immunotherapy.

254

255 ***Current data in de novo and post-treatment hypermutated gliomas***

256 ICBs as a treatment has not shown improvement compared to standard of care in newly-
257 diagnosed and recurrent glioblastomas, although the majority of patients included in
258 these trials were TMB-low gliomas [86]. Unfortunately, similar results have been
259 observed even in TMB-H gliomas now. Indeed, while reports suggested that a subset of
260 hypermutated gliomas might benefit from ICB [17], recent retrospective analyses of
261 hypermutated or MMR-d gliomas - mostly post-treatment - treated with anti-PD1
262 suggested that the use of ICB does not translate into clinical benefit (Table 1) [14,24].
263 Nevertheless, given their retrospective nature, further confirmation of these results in
264 prospective studies is warranted (NCT03718767, NCT02658279, NCT04145115).

265 In contrast, in de novo TMB-H gliomas ICB has shown encouraging results
266 reported in series studies [17,20] and in several case reports [17,19,21,22,26–28]. These
267 findings were confirmed in a recent non-peer reviewed pre-print [20]. In this study, the
268 authors analyzed the responses to ICBs of pediatric CMMR-d patients, including patients
269 with glioma. Interestingly, in a subset of tumors with additional POLE/POLD1 defects,
270 significant recruitment of inflammatory CD8⁺ cells and ICB benefit was observed. Even
271 though CMMR-d gliomas response to ICBs was lower than in other tumor types
272 developed in the same patients, it remained in appearance superior to the one of TMB-
273 low gliomas [20].

274

275 ***Potential explanations for the low response rates in gliomas***

276 A number of unique characteristics of hypermutated gliomas with other MMR-deficient
277 tumors could at least in part explain the lack of response to ICB. First, the lack of clonal
278 MSI and predominantly subclonal mutational burden of gliomas with post-treatment
279 hypermutation could be associated with the absence of effective immune responses

280 against tumor neoantigens [10,14,83,87]. Interestingly, patients with CMMR-d are more
281 likely to harbor POLE/POLD1 defects which lead to ultra-hypermutation and
282 accumulation of indels which are much more immunogenic neoantigens [88] (Table 1).
283 This might explain the presence of CD8⁺ cells expressing PDL-1 and increased rate of
284 benefit from ICB in this setting. Furthermore, the absence of significant T lymphocyte
285 infiltrates and ICB response even in some gliomas with de novo MMR-d gliomas (Lynch
286 syndrome) suggest that beyond the nature of tumor neoantigens, specificities in the
287 immunosuppressive microenvironment of gliomas - especially in the population of
288 immunosuppressive microglial and macrophage cells which are the dominant immune
289 TME cell types in glial tumors [15] - contribute significantly to gliomas ICB resistance. A
290 proposed mechanism of resistance in non-responders derived from the study of
291 preclinical models is the expansion of Treg (FOXP3⁺) and macrophages in the tumor
292 microenvironment [89]. In this study, increased CD8⁺ infiltrates and INF- γ signaling was
293 observed in the responders, suggesting that additional biomarkers might enable further
294 subgrouping hypermutated gliomas and identifying ICB responders. The benefit of
295 neoadjuvant PD1 inhibitors may support this as surgery is known to increase the levels
296 of macrophages within the tumors.

297

298 **Conclusions**

299 Recent studies have improved our understanding of the mechanisms responsible for
300 TMB-H in gliomas and its potential role as prognostic and predictive biomarker. Efforts
301 are ongoing to determine the optimal strategy for use of radiation therapy and
302 chemotherapy in the context of TMB-H. Areas of investigation include the development
303 of non-invasive biomarkers to monitor hypermutation and the investigation of novel
304 therapeutic strategies that will prevent MMR-d acquisition in the most chemosensitive
305 such as oligodendrogliomas (e.g. by using CCNU or PARP inhibition in combination with
306 TMZ) to determine whether preventing TMB-H development might improve patients
307 outcome. As regard immunotherapy strategies, response and clinical benefit is driven by
308 a sum of complex factors which cannot be explained only with the number of mutations
309 in tumor exomes. Neoantigen quality seems determinant to responses as well as the
310 presence of effectors immune cells in the TME. Approaches aimed at increasing both
311 tumor infiltration by cytotoxic lymphocytes are therefore likely both necessary in order to
312 improve the response to immunotherapy in gliomas. Among several current strategies
313 under investigation, IL-12 gene therapy combined with ICB showed safety and biological
314 efficacy (production of IFN- γ) in HGG patients including one patient with post-treatment
315 hypermutation [22,90].

316

317 **Key points**

318 TMB-H in gliomas is observed in two distinct contexts associated with unique biology: de
319 novo and post-treatment.

320 De novo TMB-H is observed in tumors with inherited or somatic defects of the DNA
321 polymerases POLE/POLD1 or the MMR system.

322 TMZ together with MMR-d is responsible for TMB-H and resistance in post-treatment
323 gliomas.

324 TMB-H MMR-d gliomas harbor unique characteristics (e.g., low lymphocyte infiltration)
325 compared to other cancers where MMR-d is common.

326 ICBs response in TMB-H is uncommon except in rare contexts such as CMMR-d.

327

328 **Acknowledgements**

329 None

330

331 **Financial support and sponsorship**

332 None.

333

334 **Conflict of interest**

335 FB reports a next-of-kin employed by Bristol Myers Squibb and travel fees funded by
336 Bristol Myers Squibb. KLL reports consulting fees and institutional research support from
337 Bristol Myers Squibb and is a founder and consultant for Travera Inc. MT reports
338 consulting or advisory role from Agios Pharmaceutical, Integragen, and Taiho Oncology,
339 outside the submitted work; research funding from Sanofi, outside de submitted work.

340

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642 **Figures and tables legends**

643 **Figure 1. Characteristics associated with de novo (top) and post-treatment**
644 **(bottom) TMB-H in gliomas.** Frequency and distribution across ages represent
645 adults/adolescents/infants. De novo TMB-H tumors are rare and related to young
646 patients. In these tumors, the driver MMR/POLE mutations can be inherited or somatic.
647 TMB-H is found in the newly-diagnosed tumor. The combination of increased TMB,
648 increased indels burden (often observed in POLE-deficient tumors), and increased tumor
649 infiltration by T cells make these tumors more likely to benefit from ICBs, although the
650 relative contribution of each individual factor is unknown. Post-treatment TMB-H tumors
651 are strongly related to the use of TMZ in chemotherapy sensitive tumors (eg MGMT-d).
652 TMB-H is only found in the recurrent (post-chemotherapy) tumor. Increased tumor
653 infiltration and ICB response in this context are both rare.

654

655

656 **Table 1. Case reports and series of TMB-H gliomas and CNS tumors treated with**
657 **ICB and other immunotherapy approaches.**

658 § Defined by prolonged disease control or radiological response as assessed by authors.
659 A subset of patients in the Morgenstern et al. study [20] showed tumor control after initial
660 flare.

661 † Post-nivolumab progression data from patient reported in the Bouffet et al. 2016 study
662 [17].

663 †† POLE deficiency assessed based on mutational signature analysis. PFS of 9.9 months
664 and OS of 21.6 months reported for the overall dataset.

665 * PFS and OS not available for POLE-deficient vs POLE-proficient cases. PFS of 9.9
666 months and OS of 21.6 months reported for the overall dataset.

667 Abbreviations: IDH1/2-mut, IDH1/2-mutant; MMR-d, MMR-deficient; POLE-d, POLE-
668 deficient; TMZ, temozolomide; RT, radiation therapy; PFS, progression free survival; OS,
669 overall survival; na, not available

Table 1. Case reports and series of TMB-H gliomas and CNS tumors treated with ICB and other in

§ Defined by prolonged disease control or radiological response as assessed by authors. A subset of

† Post-nivolumab progression data from patient reported in the Bouffet et al. 2016 study [17].

†† POLE deficiency assessed based on mutational signature analysis.

* PFS and OS not available for POLE-deficient vs POLE-proficient cases. PFS of 9.9 months and OS of

Abbreviations: IDH1/2-mut, IDH1/2-mutant; MMR-d, MMR-deficient; POLE-d, POLE-deficient; TMZ,

Study	IDH1/2-mut	MMR-d	Stage	Prior TMZ
<i>POLE-deficient</i>				
[17] Bouffet et al. 2016 (n=2)	0 (0%)	2 (100%)	Recurrence	1 (50%)
[26] Johanns et al. 2017 (n=1)	0 (0%)	NA	Recurrence	1 (100%)
[28] Larouche et al. 2018 (n=1) †	0 (0%)	1 (100%)	Recurrence	1 (100%)
[27] Anghileri et al. 2021 (n=1) ††	0 (0%)	1 (100%)	Recurrence	1 (100%)
[19] Sathornsumetee et al. 2021 (n=1)	0 (0%)	NA	Recurrence	1 (100%)
[20] Morgenstern et al. 2021 (n=19)	na	19 (100%)	na	na
<i>POLE-proficient</i>				
[14] Touat et al. 2020 (n=11)	3 (27.2%)	11 (100%)	Recurrence	11 (100%)
[22] McCord et al. 2021 (n=1)	1 (100%)	1 (100%)	Recurrence	1 (100%)
[20] Morgenstern et al. 2021 (n=8)	na	8 (100%)	na	na
<i>POLE status na</i>				
[24] Lombardi et al. 2020 (n=13)	4 (30.7%)	13 (100%)	na	13 (100%)
[21] Rittberg et al. 2021 (n=1)	1 (100%)	1 (100%)	Primary	0 (0%)
[27] Alharbi et al. 2018 (n=1)	na	1 (100%)	Recurrence	1 (100%)
[20] Morgenstern et al. 2021 (n=4)	na	4 (100%)	na	na

immunotherapy approaches.

of patients in the Tabori et al. study [20] showed tumor control after init

of 21.6 months reported for the overall dataset.

Z, temozolomide; RT, radiation therapy; PFS, progression free survival; O

Prior RT	Clinical benefit §	PFS months	OS months
2 (100%)	2 (100%)	9; 11	NA
1 (100%)	1 (100%)	2	NA
1 (100%)	1 (100%)	30 months, ongoing	30 months, ongoing
1 (100%)	1 (100%)	13	80,4
1 (100%)	1 (100%)	15	20
na	13 (68.4%)	na *	na *
11 (100%)	0 (0%)	1,38	8,07
1 (100%)	0 (0%)	2	na
na	1 (12.5%)	na *	na *
13 (100%)	0 (0%)	2,2	5,4
0 (0%)	1 (100%)	20	NA
1 (100%)	1 (100%)	10	na
na	3 (75%)	na *	na *

ial flare.

OS, overall survival; na, not available

Notes

Both patients had combined MMR-d and POLE-d (CMMR-d); one of patients is included in Larouche et al. [86]

Patient with germline POLE deficiency

Response to combined nivolumab and ipilimumab after progression on nivolumab

De novo TMB-H patient with high lymphocyte infiltration and high burden of clonal variants in both primary and recurrent tumor samples

Clinical benefit with combined bevacizumab and pembrolizumab

Higher TMB, indel burden, T-cell infiltration and PDL1 expression in tumors with combined MMR-d and POLE-d as compared to MMR-d only tumors

De novo (5) and post-treatment (6) TMB-H samples

Loss of MMR-d clones after local IL-12 and anti-PD-1 combination therapy

Significantly less response seen compared to POLE-deficient cases

Neither TMB nor CD8+ T-cell infiltration associated with pembrolizumab activity

CMMR-d patient

CMMR-d patient

Tumor sequencing data not available. 2 Lynch and 2 CMMR-d patients

De novo Hypermutated

Gliomas associated with constitutional mismatch repair defects



Frequency and distribution

bMMRD <1%
Lynch 1-2%

Children and young adults



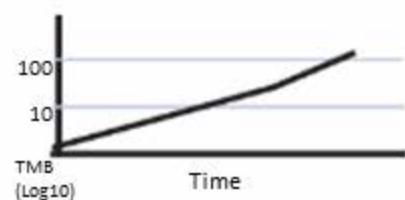
Pattern of DNA replication repair deficiency and mutation gain



Bi-allelic germline hit in MMR gene

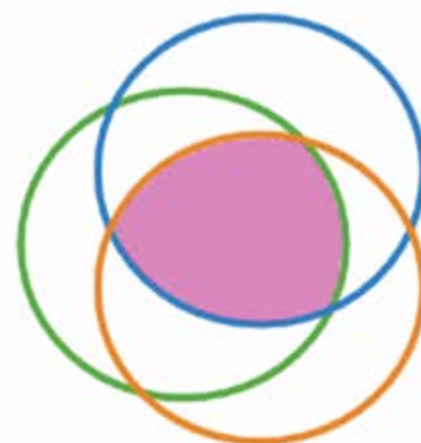


Germline MMR mutation + 2nd hit acquired during life



Response to ICI and putative predictive factors overlap

■ Hypermutation
■ PDL-1 expression
■ CD8+ infiltration
■ ICB's response



Hypermutation associated with an "inflamed" tumor microenvironment > frequent response to ICB

Gliomas associated with constitutional polymerase defects



<1%

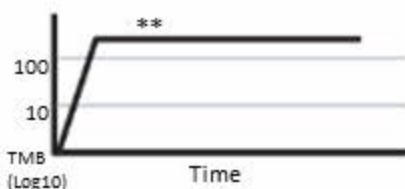
Children and young adults



Early POLE mutation



Late POLE mutation



Post-treatment Hypermutated

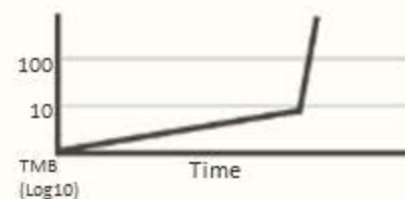


20%

All age



TMZ exposure associated with induction or selection of MMR-deficient clones



Hypermutation associated with a "cold" tumor microenvironment > uncommon response to ICB