Multi-institution analysis of tumor mutational burden and outcomes in pediatric CNS tumor patients

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Abstract

Background: Pediatric CNS tumors are the leading cause of pediatric cancer mortality. Research addressing genomic biomarkers and clinical outcomes is needed to inform therapeutic decision making. **Methods**: We conducted a retrospective analysis of pediatric patients (age <21) diagnosed with a primary CNS tumor at four upstate New York hospitals from 2008 to 2021. Clinical and histopathologic data were identified from each patient, including genomic analysis of somatic mutations and tumor mutation burden (TMB) where available. These variables were each compared with overall survival using cox-regression analyses. Multivariable analysis was conducted to identify patient characteristics that may independently predict survival. **Results**: We identified 119 patients. Common tumor types included low-grade glioma (N=51), high-grade glioma (N=29), and medulloblastoma (N=11). Common driver-mutations included *TP53* inactivation (N=16), *BRAF-KIAA1549* fusion (N=16), *FGFR1* amplification (N=12), *BRAF V600E* mutation (N=12), *NF1* loss (N=12), and *H3F3A K28M* mutation (N=6). Median TMB was 1 mutation/megabase (mut/Mb, range=0-132). Overall survival was 79.9%. Variables associated with poorer survival on univariable analysis were higher TMB (p=0.002, HR 4.97), high grade tumors (p=0.009, HR 84.3), and high-grade glioma histology (p=0.021, HR 3.14). Multivariable analyses further identified TMB (p=0.011, HR 4.46) and high-grade histology (p=0.015, HR 5.28) as independently predictive of worse survival. Tumor progression was more common in high TMB (N=15, 44%) than in low TMB tumors (N=19, 35%). **Conclusions**: High TMB is correlated with higher rates of progression and death as compared to low TMB tumors. These findings may help identify patients who may benefit from alternative treatments, such as immunotherapies.

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Key Words : Tumor mutational burden, driver mutation, survival, pediatric, CNS tumors

Abbreviation	Full Term
TMB	Tumor mutational burden
DM	Driver mutation
HGG	High grade glioma
LGG	Low grade glioma
ATRT	Atypical teratoid rhabdoid tumor
DIPG	Diffuse intrinsic pontine glioma
COSMIC	Catalogue of somatic mutations In cancer
ICI	Immune checkpoint inhibitor
EMR	Electronic medical record
OS	Overall survival
TTD	Time to death
TTP	Time to progression

Abstract:

Background: Pediatric CNS tumors are the leading cause of pediatric cancer mortality. Research addressing genomic biomarkers and clinical outcomes is needed to inform therapeutic decision making.

Methods : We conducted a retrospective analysis of pediatric patients (age <21) diagnosed with a primary

CNS tumor at four upstate New York hospitals from 2008 to 2021. Clinical and histopathologic data were identified from each patient, including genomic analysis of somatic mutations and tumor mutation burden (TMB) where available. These variables were each compared with overall survival using cox-regression analyses. Multivariable analysis was conducted to identify patient characteristics that may independently predict survival.

Results : We identified 119 patients. Common tumor types included low-grade glioma (N=51), high-grade glioma (N=29), and medulloblastoma (N=11). Common driver-mutations included *TP53*inactivation (N=16), *BRAF-KIAA1549* fusion (N=16), *FGFR1*amplification (N=12), *BRAF V600E* mutation (N=12), *NF1* loss (N=12), and *H3F3A K28M* mutation (N=6). Median TMB was 1 mutation/megabase (mut/Mb, range=0-132). Overall survival was 79.9%. Variables associated with poorer survival on univariable analysis were higher TMB (p=0.002, HR 4.97), high grade tumors (p=0.009, HR 84.3), and high-grade glioma histology (p=0.021, HR 3.14). Multivariable analyses further identified TMB (p=0.011, HR 4.46) and high-grade histology (p=0.015, HR 5.28) as independently predictive of worse survival. Tumor progression was more common in high TMB (N=15, 44%) than in low TMB tumors (N=19, 35%).

Conclusions : High TMB is correlated with higher rates of progression and death as compared to low TMB tumors. These findings may help identify patients who may benefit from alternative treatments, such as immunotherapies.

Introduction:

Pediatric central nervous system (CNS) tumors are the leading cause of pediatric cancer mortality.[1] Historically, pediatric CNS tumors were characterized by tumor location and histologic features. With advances in next-generation sequencing, these data may better inform tumor diagnosis and clinical decision making.[1,2]

Tumor mutational burden (TMB) is the total number of mutations per megabase (mut/Mb) in a tumor's genetic coding region.[3]Driver mutations (DM) are specific mutations that are thought to enable oncogenicity.[4] TMB and DMs have been studied across numerous cancer types as a means of risk stratification.[5] High TMB has been associated with poor outcomes in adult diffuse glioma patients.[6]Multiple types of high TMB tumors have demonstrated improved responses to immunotherapy.[7] More research is needed, especially in pediatric populations, to identify ways in which TMB and DMs may be used as prognostic and therapeutic determinants.[8]

Patel et al. were among the first investigators to analyze TMB and DMs in a large cohort of pediatric brain tumors patients. They provided evidence that a high TMB subsets exists in pediatric brain tumor patients and demonstrated a clear relationship between TMB and certain types of DMs. For example, low TMB tumors had a higher incidence of*BRAF* alterations, whereas high TMB tumors had a higher incidence of *TP53* mutations.[9]

The aim of this study is to identify specific patient and genomic variables that may be associated with outcomes in pediatric brain tumors. Specifically, we aim to determine the prognostic relevance of TMB and identify potential populations that may benefit from the addition of immunotherapy agents, including immune checkpoint inhibitors (ICI).

Methods:

We conducted a multi-institutional retrospective analysis of all pediatric patients <21 years with a primary diagnosis of a CNS tumor, who had available histopathology data from 2008 to 2021 at 4 upstate New York hospitals. Institutional Review Board approval was obtained from each respective institution; only de-identified data were shared across institutions. Tumors were classified based on molecular genetics and clinical features. DMs were determined based on Catalogue Of Somatic Mutations In Cancer (COSMIC) database Tier 1 genes (genes that are definitively implicated in cancer development).[10] Using COSMIC, the following mutations were identified in our subset of patients as DMs: *TP53*inactivation, *BRAF-KIAA1549* fusion, *FGFR1* amplification,*BRAF V600E* mutation, *NF1* loss and *H3F3A K28M*mutation. Patient demographics, clinical history, tumor histopathology and genomics, treatments, and outcomes were obtained from the

electronic medical record (EMR). Specific genomic alterations and TMB were identified using FoundationOne sequencing.

The prespecified primary analysis of this study was to compare TMB with survival outcomes. High TMB ([?]3 mut/Mb) and low TMB (<3 mut/Mb) were stratified based on patient TMB distribution in our study and also based on prior literature.[11] Time to progression (TTP) was defined as the days from date of diagnosis to first clinical documentation of progression. Time to death (TTD) was defined as the days from date of diagnosis to date of death. A cutoff date of July 1, 2021, was used for survival calculations. Secondarily, we sought to further characterize the genomics of pediatric patients with CNS tumors.

Frequencies, means, medians and confidence intervals were used to describe the patients, treatments, and genomics. Univariable cox-regression analyses were conducted to assess the relationship between patient/tumor characteristics and survival. Variables with $p<0.05$ on univariable analysis were included in a multi-variable cox-regression model. All analyses were performed via SPSS Statistics, Version 28.0.1.1 (IBM Corp., Armonk, NY).

Results:

One hundred and nineteen patients (60 male, 59 female) with a median age of 9 years (range 2 months-20 years) were diagnosed with a primary CNS tumor at 4 upstate New York hospitals between 2008 and 2021 and had FoundationOne testing (Table 1). There were 25 deaths observed (21%), median age at death was 13 years old (range 1.4 - 22 years). All death events were in high-grade tumor patients. Overall survival was 79.9%.

Tumor types included LGG (N=51, 43%), HGG (N=29, 24%), medulloblastoma (N=11, 9%), ependymoma (N=7, 6%), ganglioglioma (N=4, 3%), ATRT (N=4, 3%), embryonal tumor (N=3, 3%), choroid plexus (N=2, 3%), DIPG (N=1, 1%), and other CNS tumor types (N=7, 6%). Seventy patients had DMs, including *TP53* inactivation (n=16, 13%), *BRAF-KIAA1549*fusion (N=16, 13%), *FGFR1* amplification (N=12, 10%), *BRAF V600E* mutation (N=12, 10%), *NF1* loss (N=12, 10%), and*H3F3A K28M* mutation (N=6, 5%).

TMB data was available for most patients (N=89, 75%); median TMB was 1 mut/Mb (range=0-132). Low TMB was defined as <3 mut/Mb (N=55, 62% of tumors with TMB available); high TMB was defined as [?]3 mut/Mb (N=34, 38% of tumors with TMB available) (Table 1). Two tumors (2%), HGG and medulloblastoma, had DNA methylation data. Patient outcomes were categorized as progression (N=47, 40%), stable disease or cure (N=45, 38%), recurrence (N=29, 24%), or death (N=25, 21%) (Table 1).

High TMB tumors were most commonly HGG (N=11, 32% of patients with high TMB) and medulloblastomas (N=7, 21%). Low TMB tumors were most commonly LGG (N=30, 55% of patients with low TMB), however also prevalent in HGGs (N=11, 20%) (Table 1). High TMB tumors were more commonly identified in high-grade (N=26, 77%) than low-grade (N=8, 24%) histologies. High TMB tumors were more likely to have a *TP53*inactivation (N=10, 29%) as compared to low TMB tumors (N=3, 6%).*NF1* loss was similar amongst high TMB tumors (N=4, 12%) and low TMB tumors (N=5, 9%). All other DMs were more likely to be in low TMB tumors (Figure 1).

Patients with high TMB tumors were more likely to have a death event (N=14, 41%) as compared to patients with low TMB (N=5, 9%) (Figure 2). For patients with a death event and TMB data, the mean TTD was 25.8 months (range 3.27 months-15.5 years); patients with high TMB tumors had a shorter TTD (mean=20.7 months) as compared to those with low TMB (mean=3.3 years). Tumor progression was more common in high TMB (N=15, 44%) than in low TMB tumors (N=19, 35%) (Table 1). Mean TTP was 28.3 months (range 22 days-15.0 years). TTP was shorter in high TMB tumors (mean=17.6 months) as compared to low TMB tumors (mean=30.3 months).

The majority of patients were treated with surgery (N=99, 83.2%). Patients were also treated with chemotherapy (N=77, 64.7%), radiation (N=56, 47.1%), and/or targeted therapy (N=32, 26.9%). Few patients were treated with immunotherapy (N=3, 2.5%), types included were pembrolizumab, dendritic cell-based immunotherapy, and nivolumab; all were used as second-line therapy following progression. High

TMB tumors were more frequently treated with systemic therapy (N=29, 85%) compared to low TMB tumors (N=38, 69%) (Table 1). Most patients who were treated with systemic therapy were treated initially at the time of diagnosis (N=75, 87%) rather than after tumor progression (N=11, 13%).

Variables significantly associated with reduced OS on univariable analysis were high TMB (p=0.002, HR=4.97, 95% CI: 1.79-13.83), high grade tumor (p=0.009, HR=84.3, 95% CI: 3.05-2333), and HGG (p=0.021, HR=3.14, 95% CI: 1.19-8.26). Including these factors in a multivariable model, high TMB (p=0.011, HR=4.46, 95% CI: 1.40-14.15) and HGG (p=0.015, HR=5.28, 95% CI: 1.38-20.27) remained significantly associated with reduced OS (Table 2).

There were no statistically significant differences in OS with respect to age, gender, treatment modality (chemotherapy, surgery, radiation, targeted therapy, or immunotherapy), the presence of an oncogenic DM, or non-high-grade glioma histology (Table 2).

Discussion:

Previous studies have demonstrated a clear correlation between TMB and DM in pediatric brain tumors.[9] This multi-institutional analysis assessed whether higher TMB tumors correlate with outcomes in children with a broad range of brain tumors. We summarized genomic characteristics of pediatric CNS tumors and outcome data in 119 pediatric patients across 4 academic medical institutions between 2008-2021. We report a clear link between higher TMB and worse outcome. Higher TMB was a significant and independent predictor of reduced OS. Death events and tumor progression were more common in high TMB tumors as compared to low TMB tumors; high TMB tumors also had shorter TTD and TTP. These represent novel findings in understanding the genomics as they relate to outcomes in pediatric CNS tumors.

TMB and outcomes have previously been studied in many adult, and some pediatric, tumors. TMB has not always correlated to OS; there are wide ranges of solid tumor types and data has been inconsistent.[5] TMB has been associated with shorter OS in adult patients with gliomas, BRCA1 or BRCA2 mutated high-grade serous ovarian cancer, and lung cancer. Worse outcomes in high TMB tumors are likely attributed to mutations that inhibit cell cycle checkpoints and DNA repair mechanisms, leading to high proliferative activity.[6,9,12,13] These mutations contribute to more severe and rapid metastatic disease in high TMB tumors.[6] Our data further demonstrates poor prognostication of TMB.[6,14,15] Poorer outcomes may also be indicative of inadequate treatment options for this high-risk pediatric CNS tumor patient subset.

Similar to data presented by Patel et al., high TMB tumors in this analysis were more likely to have a *TP53* inactivation and low TMB tumors were more likely to have a *BRAF* mutation.[9] Patel et al., postulated this was because *TP53* inactivation causes an inability to repair DNA mutations and *BRAF* has a minimal effect on DNA repair.[9] *TP53* inactivation is likely associated with high TMB tumors given its facilitation of a multi-step malignant progression.[16] The current data further demonstrates that high TMB tumors often do not have driver mutations that can be therapeutically targeted; identifying a need for alternative therapies.

Although most high TMB tumors in our dataset were treated with systemic therapy, only a small fraction of patients were treated with targeted or immunotherapy. Targeted and immunotherapy may not have had a significant association with survival because such therapies were most commonly used second-line, post-tumor recurrence or progression; this is true for all three patients who received immunotherapy in our study. Literature supports that first-line immunotherapy may have better outcomes than when immunotherapy is used as second-line; the hypothesis being that upfront chemotherapy may destroy the host immune system, decreasing the efficacy of subsequent immunotherapy.[17] A lack of statistical significance may also be due to low sample size. The use of ICIs may be indicated for such patients, given our findings that high TMB tumors are more likely to be associated with loss of tumor suppressor genes and less likely to be associated with oncogene mutations.

Furthermore, TMB is a biomarker for ICI enrollment; TMB has been found to predict ICI response in 20-60% of patients.[3,7,18-21] High TMB (>3 mut/Mb) has also been demonstrated to predict improved progression-free survival in patients on ICI.[11] Although TMB has been associated with worse survival, tumors with high TMB

tumors are often extremely responsive to ICI therapy. Additionally, high TMB tumors associated with poor OS were found to have improved OS with ICI when compared to low TMB tumors. These responses have been demonstrated in non-small cell lung cancer, malignant melanoma, cervical cancer, breast cancer, gastric, esophageal and head and neck cancers.[18,19,22-24] In pediatrics, however, responses have been inconsistent, possibly because of the relatively low TMB of pediatric cancers.[25,26]

There are over 2,000 clinical trials investigating ICI in patients of all ages and with all tumor types (accessed June 2022). Of the 141 trials investigating ICI in pediatric patients, five of which have or are currently investigating ICI in pediatric CNS tumor patients.[27-31] Only one trial, specifically recruits pediatric patients with high TMB CNS and solid tumors to analyze the effect of ICI.[28] Pembrolizumab has been approved for high TMB solid tumors, including pediatric CNS tumors with PD-L1 expression.[32] In a post-hoc analysis of the HERBY trial, hypermutated pediatric HGGs treated with bevacizumab (anti-VEGF) had significantly more CD8+ tumor-infiltrating lymphocytes and improved OS as compared to non-hypermutated types.[33] This analysis supports that bevacizumab may be a treatment option for pediatric CNS tumor patients with high TMB and poor outcomes in need of more effective treatment regimens. It also emphasizes the importance of understanding tumor microenvironment as a means of further stratifying patients, which may be a future direction for studies.[34]

Our multi-institutional analysis shows a clear correlation between higher TMB and poor outcomes for children with brain tumors**.**Although variables were standardized, discrepancies may exist in data collection amongst institutions. Additionally, DNA methylation profiling has increasingly been used for pediatric CNS tumor classification.[2,35,36] Only two patients in our dataset had DNA methylation profiling and only 75% of patients had TMB data. In addition, the brain tumor tissue obtained at these institutions was not routinely sent to FoundationOne for TMB testing for all children so it is possible our sample is not representative of all children with brain tumors. We recommend an increase in TMB and DNA methylation testing, as both may be applicable toward stratifying treatments and also used to improve research and treatment developments in the future.[2] Lastly, although our analyses were pre-determined prior to data collection, results should be interpreted in the context of the retrospective nature of this study.

We identify pediatric CNS high TMB tumor patients as a unique high-risk patient subset that has poor outcomes: higher progression rates and poorer overall survival. Our data may help in identifying such patients who may require alternative treatments, such as ICI.

Conflict of Interest Statement: All authors have no conflicts of interest to disclose.

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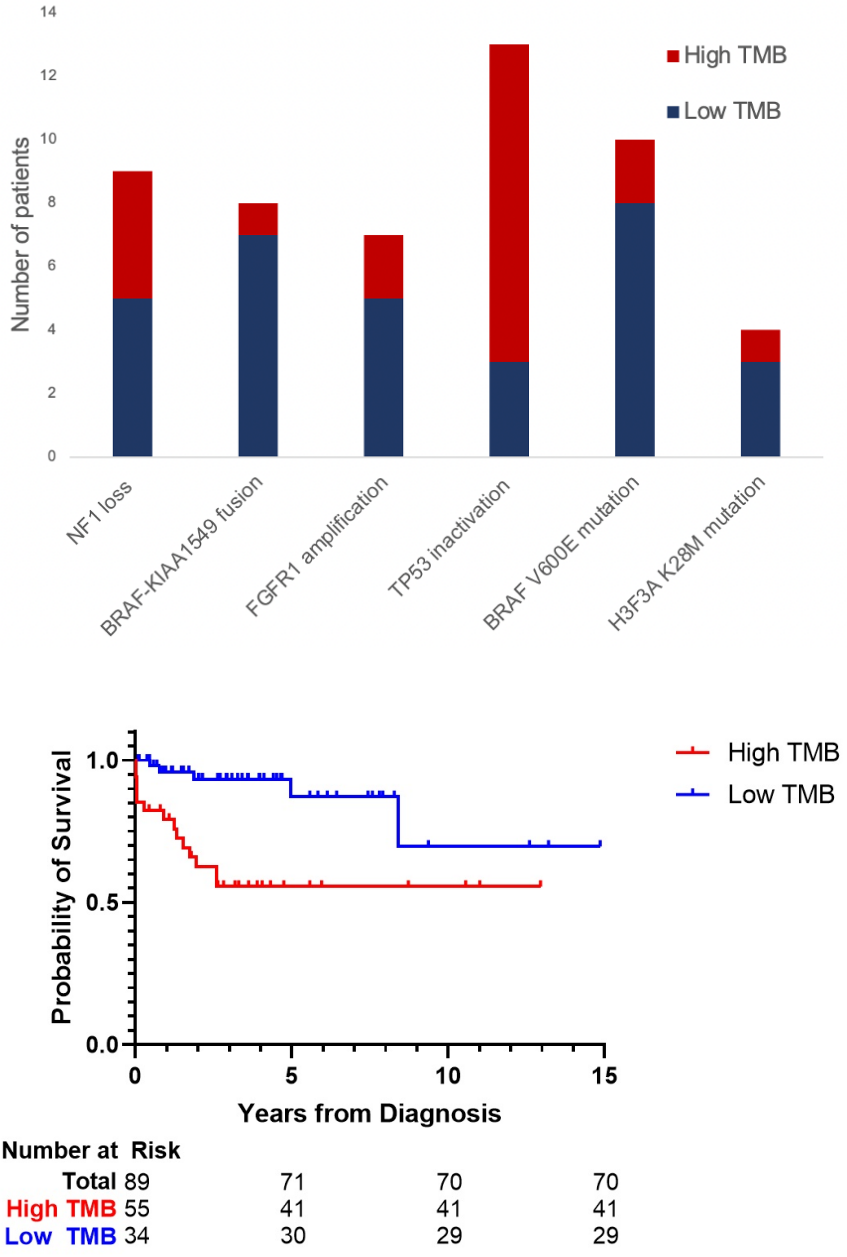
Figure Legends:

Figure 1. Driver mutations in pediatric CNS tumor patients characterized by proportion of high vs. low tumor mutational burden (TMB) tumors.

Figure 2. Overall survival of pediatric CNS tumor patients reported as time from diagnosis to last follow-up or death event with the last date of data collection being July 1 2021 (p=0.002) .



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