Molecular features of gliomas with high tumor mutational burden

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Background

While the overall tumor mutational burden (TMB) is low in gliomas, some tumors are observed with a very high TMB. High TMB in gliomas can be caused by underlying mutations in MMR mismatch repair genes and the defects in the POLE proofreading exonuclease domain, similar to other cancer types. Another driver of TMB in glioma, specifically recurrent glioma, are GC>TA alterations caused by the FDA-approved alkylating agent temozolomide, when coupled with gene mutations rendering the mismatch repair system futile, a large number of therapy-induced mutations accumulate.

Microsatellite instability high/deficient mismatch repair (MSI-H/dMMR) and elevated TMB are both favorable predictors of response to immune checkpoint inhibition in other cancers. In gliomas, however, large studies to support the link between MSI and TMB with immune checkpoint inhibitor response is lacking, with the exception of a few case reports.

We aim to study a large cohort of glioma tumors tested with comprehensive molecular profiling and to investigate the molecular context of TMB-H in glioma in order to further delineate the molecular features underlying TMB-H. A deeper molecular understanding will help further tailored treatment for TMB-H glioma patients.

Methods

- Gliomas were tested with molecular profiling at Caris Life Sciences (Phoenix, N=3129 tumors).
- NGS was performed on genomic DNA isolated from FFPE tumor samples using the NextSeq (592-genes).
- MGMT promoter methylation was tested by pyrosequencing.
- PD-L1 testing was performed using the SP142 anti-PD-L1 clone (Ventana, Tucson, AZ).
- MSI was tested by NGS, FA and IHC.
- The GC:AT transition rate was calculated as the prevalence of G:A and C:T changes seen in each tumor and > 80% was regarded as high .
- TMB was estimated from 592 genes (1.4 megabases [MB] sequenced per tumor) by counting all non-synonymous missense mutations found per tumor that had not been previously described as germline alterations. TMB values were compared using Wilcoxon Rank Sum.

Results

Table 1: Patient characteristics: A total of 3129 tumors were included.

	Female N (%)	Male N (%)	Average Age
GBM	911 (39)	1404 (61)	57.5
Astrocytoma-high grade	129 (40)	194 (60)	45.8
Astrocytoma-low grade/pilocytic	84 (43)	111 (57)	42.7
Oligodendroglioma-high grade	52 (54)	45 (46)	48.3
Oligodendroglioma-low grade	54 (47)	62 (53)	43.7
Unclear	38 (46)	45 (54)	40.2
Total	1268 (41)	1861 (59)	54.1

Table 2: TMB distribution in glioma subgroups: Q1-Q4 (quartiles) were defined by TMB distribution in the complete cohort; shown are prevalence of tumor subgroups in each quartile.

Cancer types	Q1 (0-5)	Q2 (6)	Q3 (7, 8)	Q4 (9-372)	Total
GBM	34.7%	26.5%	18.4%	20.3%	2315
Astrocytoma-high grade	34.4%	24.8%	19.2%	21.7%	323
Astrocytoma-low grade/pilocytic	49.7%	24.1%	19.0%	7.2%	195
Oligodendroglioma-high grade	33.0%	21.6%	14.4%	30.9%	97
Oligodendroglioma-low grade	41.4%	28.4%	14.7%	15.5%	116
unclear	49.4%	22.9%	14.5%	13.3%	83

Figure 1: TMB distribution in various glioma subgroups: median TMB=6 for all groups shown

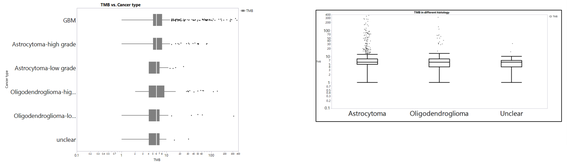


Figure 2: Molecular alterations and pathways in TMB-High gliomas. Q1-4: tumors in TMB quartiles in the tumors investigated. Shown are alterations significantly different in Q1-3 vs. Q4 after adjusting for multiple comparison. (adjusted p<0.05). **Only pathogenic/presumed pathogenic mutations are counted towards mutation rates.**

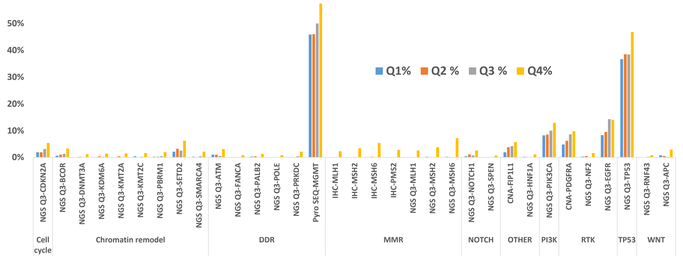


Figure 3: TMB in different molecular categories in Top Quartile tumors

Level	N	Median	Mean	Std Error	Lower 95%	Upper 95%
POLE	5	264	236.8	12.00	213.23	260.37
GC>TA-High/MSI-H	8	113.5	127	9.488	108.37	145.63
GC>TA-High/MSS	65	49	61.646	3.329	55.11	68.18
GC>TA-Low/MSI-H	26	23	31	5.263	20.66	41.34
GC>TA-Low/MSS	509	9	11.342	1.189	9.01	13.68

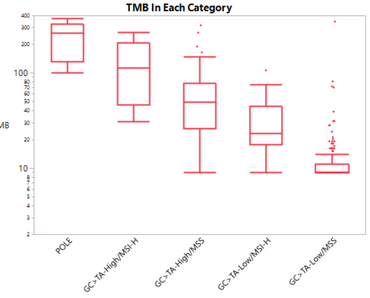
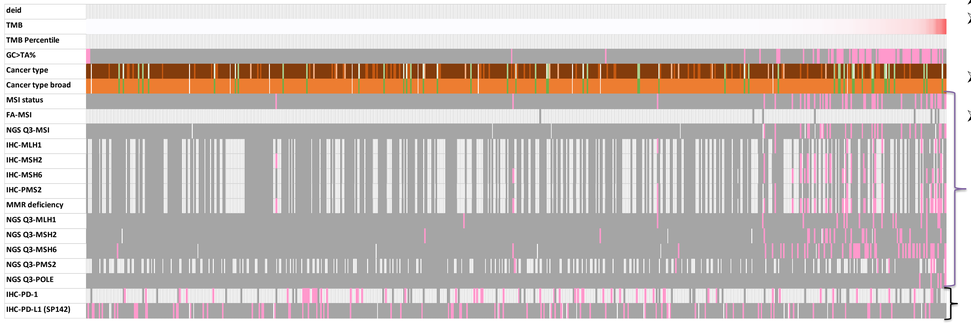


Figure 4: Distribution of immune checkpoint therapy-associated markers in the top TMB quartile. Tumors are displayed in ascending order of TMB.



- MSI-H/dMMR is rare
- POLE mutation is rare and only associated with very high TMB; mutually exclusive of high GC>TA transition rate
- PDL1 expression is not associated with MSI status, TMB or GC>TA transition rate
- GC>TA transition rate is highly correlated with MSI Status.

Increase with TMB

No association with increased TMB

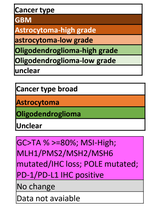
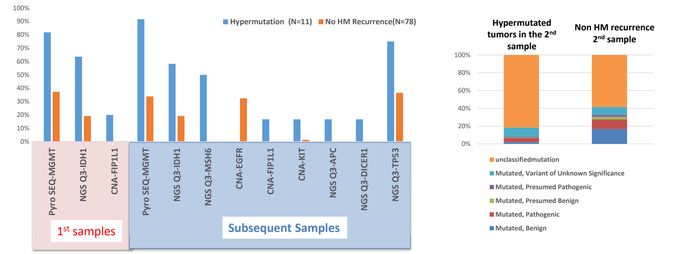


Figure 5: Paired sample analysis

- 110 patients with multiple tumors profiled with 592-NGS were identified; all with data collection intervals >150 days
  - 11 gained additional TMB of 10 or higher
  - 78 gained additional TMB 9 or lower or had no change
  - The first tumors of these patients were compared
  - The 2nd tumors were compared

Cancer type	Non-hypermutated recurrence	hypermutation prone	Total
Astrocytoma-high grade	3	1	4
astrocytoma-low grade	3	1	4
GBM	67	8	75
Oligodendroglioma-low grade	3	1	4
unclear	2		2
Total	78	11	89



Conclusions

- TMB-H is associated with POLE mutation, high rate of GC>TA transition and microsatellite instability.
- GC>TA high, an indicator of alkylator-induced phenotype, is associated with MSI-H; and the concurrent GC>TA high and MSI-H further increase TMB.
- MSI-H or TMB-H is not associated with increased PD-L1 expression.
- Paired sample analysis confirms that temozolomide sensitivity markers including MGMT-Me and IDH mutations are more prevalent in tumors acquiring hypermutation phenotype.
- Our results support the notion that unlike other cancer types, TMB-H in glioma may not associate with increased response to Immuno-Oncology therapy
- Further understanding of molecular and immune profile of the TMB-H may facilitate more individualized treatment planning
- A clinical trial of treating TMB-High glioma patients differentially according to the underlying molecular drivers is warranted.

References

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