Results of comprehensive molecular profiling of gliomas and the potential therapeutic implications.

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Abstract

Background: Gliomas are the most common type of primary brain tumors with underlying molecular heterogeneity contributing to differential treatment response. Our retrospective study was designed to interrogate biomarkers from a large cohort of glioma patients to identify alterations with therapeutic implications.
Methods: 871 glioma tumor samples (79% WHO grade IV glioblastoma, GBM) were analyzed with a multi-platform approach including sequencing, IHC, FISH/CISH and methylation assay to investigate actionable biomarker aberrations. Retrospective data analysis was performed on the complete cohort and molecular subgroups of patients.
Results: In the 871 patient samples, mutations in 27 genes were seen. Both common TP53 (39%), IDH1 (22%), PTEN (13%) and previously unreported mutations in gliomas were observed, including JAK3, SMO and ABL1. Co-mutation of 2 or more genes occurred in 37% of cases. TP53 mutation was suggestive of genetic instability and was frequently associated with other concurrent mutations (p=0.0006). IDH1 mutations were associated with MGMT promoter methylation, low expression of TS, RRM1 and TOP2A (p from <0.0001 to 0.0036), suggesting different responses to temozolomide, fluoropyrimidine, gemcitabine and etoposide. IDH1 mutation was also associated with TP53 mutation; whereas wild type IDH1 was associated with PTEN mutation (p=0.0309) and showed some association with EGFR mutations (p=0.0543). Distinct biomarker profiles by IHC, FISH and sequencing were also observed when comparing GBM to grade II/III gliomas, suggesting different biology from GBM and thus different treatment implications. 20 GBM patients were identified with pre and post treatment analyses performed (comparative analysis ongoing).
Conclusions: Gliomas exhibit a high degree of molecular heterogeneity as revealed by multi-platform profiling. IDH1 mutation identifies molecular subgroups of patients with different responses to therapeutic agents; while TP53 mutation suggests increased genetic instability. These results highlight the benefits of profiling in consideration of treatment options for glioma patients.

Background

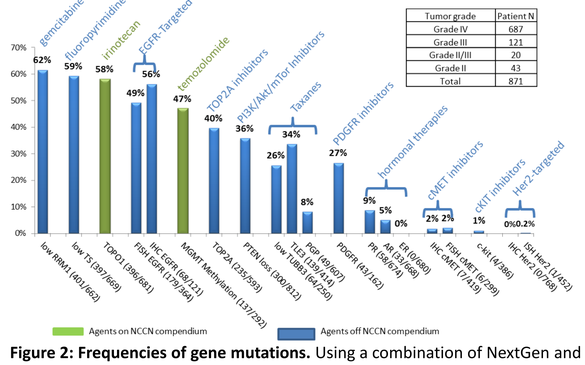
Gliomas are the most common type of primary brain tumors. They are classified by the World Health Organization (WHO) on the basis of histopathological criteria into four prognostic grades: I to IV. Based on the observation that histologically similar brain cancers can be driven by distinct genetic events and therefore manifest different clinical behavior and prognosis, recent efforts have been put into classifying gliomas according to molecular aberrations with the hope of better directing therapy. In our study, multi-platform biomarker analysis was performed on 871 glioma patients who had undergone molecular profiling to provide information to help inform therapeutic selection. Biomarker data as well as therapeutic agents associated are considered for the full cohort as well as subgroups defined by patient's tumor grade or their biomarker characteristics including IDH1 mutation and MGMT promoter methylation.

Methods

All 871 glioma cases (687 with GBM and 184 grade II/III) referred to Caris Life Sciences between 2009 thru 2013 from 50 states and 59 countries were evaluated; diagnoses were collected from referring physicians and classified at intake based on pathology and clinical history. Specific testing was performed per physician request and included a combination of sequencing (Sanger NGS), protein expression (immunohistochemistry), gene amplification (CISH or FISH), promoter methylation (pyrosequencing) and/or RNA fragment analysis. Biomarker associations were calculated by two-tailed Fisher Exact tests.

Results

Figure 1: Frequencies of biomarker results tested by IHC, FISH/CISH, promoter methylation in the complete cohort of 871 glioma patients. The associated therapies are shown in green (NCCN endorsed agents) and blue (agents off NCCN compendium).



Tumor grade	Patient N
Grade IV	687
Grade III	121
Grade II/III	20
Grade II	43
Total	871

Agents on NCCN compendium
Agents off NCCN compendium

Figure 2: Frequencies of gene mutations. Using a combination of NextGen and Sanger sequencing, 27 genes out of 45 tested harbored mutations.

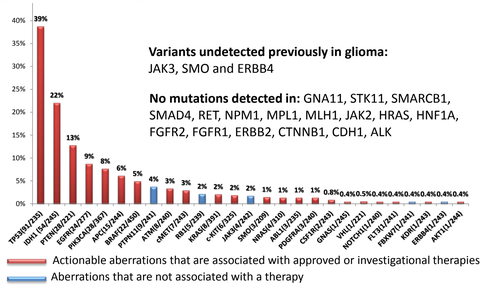


Figure 3: Differential biomarker expressions in GBM and grade II/III gliomas. A. Differential expression levels of 23 IHC's as well as EGFR ISH. Asterisks indicate the markers that are significantly associated with tumor grade. (GBM, N=687; Grade II/III gliomas, N=184); B: Differential mutation rates of 27 genes as well as MGMT promoter methylation. Asterisks indicate the three markers that are significantly associated with tumor grade. (GBM, N=189; Grade II/III tumors, N=71)

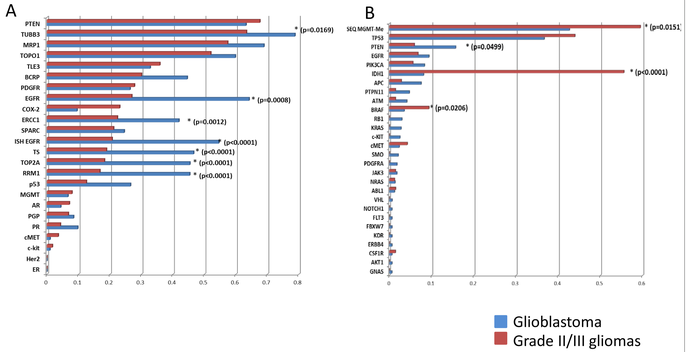
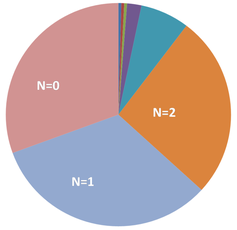


Figure 4: Frequencies co-occurring mutations.(N: number of simultaneous mutations found in one case). The 3 cases with the highest co-mutations are listed with the specific mutations found. The table in red shows that TP53-mutated tumors have a significantly higher chance to harbor additional mutations (75% vs. 52%), suggesting that TP53 mutation is indicative of genetic instability.



Genes mutated	Number of Cases	Frequency
N=7	1	0.4%
N=6	1	0.4%
N=5	1	0.4%
N=4	5	2.1%
N=3	17	7.0%
N=2	64	26.4%
N=1	79	32.6%
N=0	74	30.6%
Total	242	

Case: 7 genes mutated	
ABL1	E409K
CSF1R	S939I
GNAS	Q213H
KRAS	V8A
PDGFRA	Y582H
PTPN11	A72V
TP53	R110C
Case : 6 genes mutated	
APC	M1431T
EGFR	G627R
ERBB4	R306C
	P170S
KDR	P230L
SMO	R547C
TP53	P250L
	R273C
Case : 5 genes mutated	
cKIT	E583K
PIK3CA	D434N
PTEN	R11X
PTPN11	A72T
TP53	P177L

Presence of TP53 mutation is associated with additional mutations

	TP53 mutated	TP53 wild type	p value
Cases with additional mutations found	68 (75%)	75 (52%)	0.0006
Cases with no other mutations found	23 (25%)	70 (48%)	

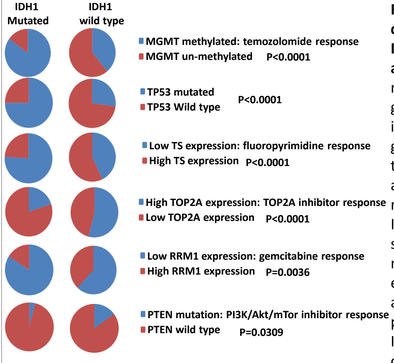


Figure 5: Significant biomarker differences in IDH1 mutated vs. IDH1 wild type cases and the associated therapies. IDH1 mutation occurs early in gliomagenesis and is an important prognostic marker in glioma. Our data suggest that temozolomide, fluoropyrimidine and gemcitabine are potentially more beneficial to patients with IDH1 mutations based on significantly high MGMT methylation, low TS and RRM1 expression; TOP2A inhibitors and PI3K/Akt/mTor inhibitors are potentially more beneficial to IDH1 wild type patients based on higher expression of TOP2A and a high mutation rate of PTEN.

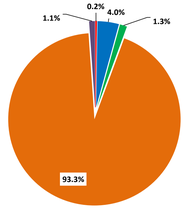


Figure 6: Summary of drug recommendations on glioma tumor profiling reports. (N=818) 99.8% of cases yielded drug recommendations using multiplatform tumor profiling.

Conclusions

1. Distinct biomarker profiles were observed in WHO grade IV and grade II/III gliomas. The differences included biomarkers measured by IHC, FISH, pyro-sequencing (promoter methylation) and NextGen sequencing, associated with potentially different treatment options including chemotherapies as well as targeted therapies.
2. A key mutation in isocitrate-dehydrogenase 1 (IDH1) identifies a patient group with different protein expression and gene mutation characteristics, and therefore potentially different treatment options. TP53 mutation is an indicator of genetic instability for additional mutations to occur in the genome.
3. Multiplatform tumor profiling is able to identify treatment options for glioma patients. The treatment recommendations include those that are part of standard of care as well as those that are not typically used for glioma treatment.

References

1. Brennan, C.W., 2013, Cell, *155*(2), 462-477.
2. Turcan, S., 2012, Nature, *483*(7390), 479-483