Tumor profiling on 1245 gliomas and paired tumor study on 19 high grade gliomas

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Abstract #2058

Background: Gliomas are molecularly heterogeneous with genetic alterations driving the growth of recurrences different from the initial tumor. Previous reports showed molecular changes during progression of lower grade gliomas to GBM, driving tumor growth and treatment resistance; however changes during progression of high-grade gliomas have not been systematically reported.

Methods: 1245 glioma tumors (934 GBM) were tested with multiple platforms including sequencing (SEQ), immunohistochemistry (IHC), fluorescent/chromogenic in-situ hybridization (FISH/CISH), fragment analysis (FA) and promoter methylation (Me) assay. Metachronous paired tumors from 19 patients (pts) were assessed for biomarker changes over time.

Results: EGFRvIII was seen exclusively in GBM (16% of GBM) while amplification was more common in GBM than grade II-III tumors (56% vs. 20%, p < 0.001). MGMT Me was seen in 47% of all, and was more common in grade II-III (64% vs. 42%, p < 0.001). PD-L1 expression on tumor cells was seen in 27% and was more common in tumors without MGMT Me (36% vs. 18%, p = 0.01). PD-1 expression on tumor-infiltrating lymphocytes was seen in 48% and was higher in GBM than grade II-III(54% vs. 30%, p = 0.005). 38 of 48 sequenced genes had mutations, including BRCA1 (8%) and BRCA2 (6%). 1p19q co-deletion was seen in 26% of grade II-III and 2.9% of GBM. Paired tumors from 19 pts (18 GBM and 1 grade III in both samples) taken at an average of 469 days apart (91-1400) showed that 17 pairs (89%) had one or more biomarker changes over time. 3 of 13 (23%) pairs lost MGMT Me, potentially indicating acquired resistance to temozolomide. EGFR aberrations including amplification (N = 1), mutations on the extracellular (EGFRvIII, N = 1) and intracellular domains (T790M, N = 1; Exon 20 insertion N = 1) were acquired in 3 pairs. One pt, presenting with a PTEN mutation, acquired three additional mutations: cKIT (E583K), PTPN11 (A72T) and PIK3CA (D434N).

Conclusions: Multiplatform tumor profiling on a large cohort of gliomas confirms tumor heterogeneity. Changes in MGMT Me and EGFR of potential therapeutic importance are frequently observed in high grade gliomas at the time of recurrence, suggesting the need for a re-biopsy for tumor profiling to direct the next line of therapy.

Results

Table 1: Patient characteristics

	Patient N	Average Age (range)	Gender
Glioma	1245	54.4 (21-91)	Female 41%; Male 59%
Grade IV	934	57.1 (21-91)	Female 40%; Male 60%
Grade III	155	47.3 (21-81)	Female 40%; Male 60%
Grade II	99	42.7 (21-76)	Female 44%; Male 56%
Glioma, Not Otherwise Specificied	57	49.7 (23-84)	Female 47%; Male 53%

Table 2: Biomarker aberration frequency in glioma and associated therapies

Testing platform - biomarker	Positive N	Total N	Biomarker Frequency	Associated therapies	Therapy status
Pyro SEQ-MGMT	294	625	47.00%	Temozolomide	Standard-of-care
SEQ-IDH1	129	559	23.10%	EGFR-Targeted therapies	Clinical trials
FA-EGFRvIII	29	262	11.10%		
ISH-EGFR	180	358	50.30%		
IHC-EGFR	154	213	72.30%		
SEQ-EGFR	36	577	6.20%		
IHC-TOPO1	539	1015	53.00%	Irinotecan	Standard-of-care
FISH 1p19q	9	97	9.30%	PCV combination therapy	Standard-of-care
IHC-PD-1	94	194	48.50%	Nivolumab, pembrolizumab	Clinical trials
IHC-PD-L1	53	193	27.50%		
SEQ-BRCA1	11	143	7.70%	Olaparib| cisplatin, carboplatin	Clinical trials| stand-of-care
SEQ-BRCA2	8	143	5.60%		
SEQ-ATM	22	547	4.00%		
Low IHC-ERCC1	179	479	62.60%		
IHC-TOP2A	385	929	41.44%	Doxorubicin	Clinical trials
Low IHC-TS	333	976	65.90%	Pemetrexed, fluorouracil	Clinical trials
Low IHC-TUBB3	427	545	21.70%	Docetaxel, paclitaxel, cabazitaxel	Clinical trials
IHC-TLE3	250	699	35.80%		
Low IHC-PTEN	926	1118	17.20%	Everolimus, temsirolimus	Clinical trials
SEQ-PTEN	59	517	11.40%		
SEQ-PIK3CA	51	674	7.60%		
SEQ-AKT1	2	556	0.40%		
IHC-cMET	11	751	1.50%	INC280, crizotinib	Clinical trials
SEQ-cMET	17	557	3.10%		
SEQ-BRAF	25	760	3.30%	Vemurafenib, dabrafenib	Clinical trials
SEQ-KRAS	13	704	1.80%	MEK inhibitors	Clinical trials
SEQ-NRAS	4	623	0.60%		
SEQ-GNAQ	1	353	0.30%		
SEQ-HRAS	1	428	0.20%		
SEQ-VHL	2	483	0.40%	Bevacizumab	Standard-of-care
SEQ-RET	5	543	0.90%	Vandetinib, cabozantinib	Clinical trials
SEQ-SMO	2	460	0.40%	SMO inhibitors	Clinical trials
SEQ-CKIT	10	640	1.60%	Multikinase inhibitors	Clinical trials
SEQ-ABL1	7	527	1.30%		
SEQ-PDGFRA	5	548	0.90%		
SEQ-RB1	10	549	1.80%	CDK inhibitors	Clinical trials

0-5%	5.1-10%	10.1-20%	20.1-50%	50.1-75%

Results

Figure 1: Differential biomarker features tested by promoter methylation, fragment analysis, in-situ hybridization and IHC (A) and by sequencing (B) in glioblastoma and grade II-III gliomas. Asterisks indicate the markers that are statistically significantly different in GBM and grade II-III gliomas. P-values in bold indicate comparisons that remain statistically significant after correction for multiple comparisons.

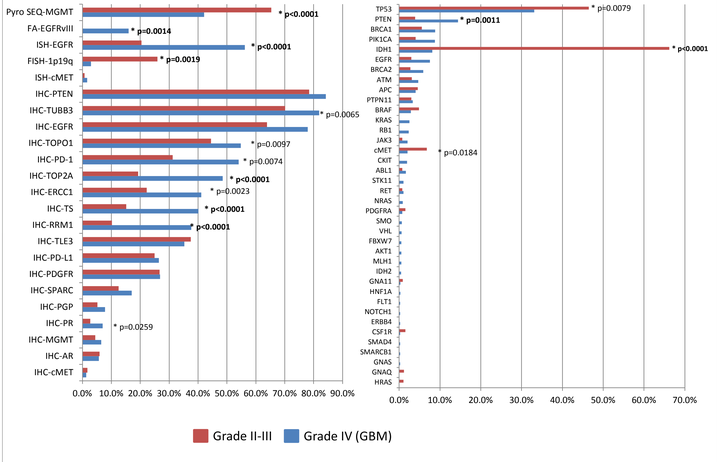
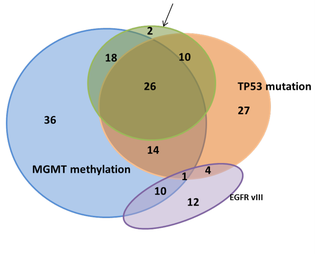


Table 3: Differential biomarker characteristics in IDH1-mutated and IDH1-wild type patient cohorts. (Asterisks indicate comparisons that remain statistically significant after correction for multiple comparisons.)

	All gliomas			
	IDH1 MT N/Total (%)	IDH1 WT N/Total (%)	RR [95% CI]	p value
MGMT Methylation	97/117 (83%)	150 /403 (37%)	5.36 (2.42-8.40)	<0.0001*
TP53 mutation	88/126 (70%)	113/425 (27%)	4.03 (2.87-5.66)	<0.0001*
EGFR vIII	0/62 (0%)	28/186 (15%)	0	<0.0001*
PTEN mutation	2/122 (1.6%)	57/401 (14%)	0.13 (0.03-0.52)	<0.0001*
BRAF mutation	0/127 (0%)	16/436 (3.7%)	0	0.0292
EGFR mutation	1/126 (0.8%)	34/428 (7.9%)	0.12 (0.02-0.8)	0.0015*
1p19q co-deletion	7/22 (32%)	2/68 (2.9%)	4.2 (2.36-7.46)	0.0006*

Figure 2: Venn diagram made from 234 glioma cases with IDH1, TP53, MGMT methylation and EGFRvIII evaluated. 160 cases showed at least one aberration.



Results

Figure 3: Comparison of biomarker profiles on paired tumor samples (N=19). Biomarkers that changed and did not change over time are shown.

Patient	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	increase %	decrease %	Total change %
MGMT-methylation																				8%	23%	31%
EGFR vIII																				17%	0%	17%
EGFR ISH																				7%	7%	14%
1p19q FISH																				0%	0%	0%
EGFR mutation																				29%	0%	29%
PTPN11 mutation																				17%	0%	17%
IDH2 mutation																				14%	0%	14%
c-KIT mutation																				14%	0%	14%
PIK3CA mutation																				14%	0%	14%
AR IHC																				0%	17%	17%
PR IHC																				0%	22%	22%
MGMT IHC																				0%	7%	7%
TS IHC																				40%	20%	60%
TOP2A IHC																				30%	30%	60%
TOPO1 IHC																				20%	27%	47%
ERCC1 IHC																				8%	38%	46%
PTEN IHC																				25%	19%	44%
SPARC IHC																				6%	35%	41%
TLE3 IHC																				20%	20%	40%
RRM1 IHC																				7%	14%	21%
PGP IHC																				8%	0%	8%
EGFR IHC																				0%	0%	0%

Biomarker decrease over time: loss of protein overexpression (IHC); gene amplification (ISH); or gene mutation (mutation)

Biomarker increase over time: acquisition of protein overexpression (IHC); gene amplification (ISH); or gene mutation (mutation)

no change over time

0%
1-10%
11-20%
21-30%
31-40%
41-50%
51-60%

Details of tumor mutational status changes: Patient a: Acquisition of EGFR D770_N771insN; Patient b: Acquisition of IDH2 P167L; Patient d: Acquisition of EGFRT790M; Patient e: Acquisition of cKIT (E583K), PTPN11 (A72T) and PIK3CA (D434N); with a concurrent increase of MGMT methylation level (7%→54%)

Conclusions

1. Well-recognized biomarkers in glioma, including MGMT promoter methylation, IDH1 mutation and 1p19q co-deletion are systematically studied in a large cohort of clinical glioma tumors. In addition, promising novel therapeutic targets, including EGFRvIII, PD1/PDL1 and BRCA1/2 are investigated and the aberration frequency is reported.
2. While standard chemotherapy options are limited for patients with gliomas, our data is of importance for both clinical consideration and for clinical trial design.
3. Distinct biomarker profiles observed in grade II-III glioma tumors and GBM, as well in IDH1-WT and IDH1-MT tumors may underlie the distinct clinical behavior of these groups and provide biological evidence to treat these cancers differently.
4. Frequent biomarker changes over time, especially those that carry important therapeutic implications, suggest the need for a re-biopsy for tumor profiling to direct the next line of therapy.

References

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