Biomarker Analysis of Glioblastoma and Potential Implications for Therapy

Joanne Xiu, PhD, Rebecca Feldman-Moreno, PhD, David Arguello, MD, Zoran Gatalica, MD, Anatole Ghazalpour PhD, Ryan P. Bender, PhD, Michael Castro, MD, Sandeep K. Reddy, MD, Gargi D. Basu, PhD, and Les Paul, MD, MS

Caris Life Sciences, Phoenix, AZ; Caris Life Sciences, Irving, TX

Abstract

Background: Glioblastoma multiforme (GBM), the most aggressive CNS cancer, has limited effective therapeutic options, with underlying molecular heterogeneity contributing to the differences in treatment response. Our study was designed to interrogate biomarkers from a large cohort of GBM patients to seek therapeutic implications.

Methods: Data were analyzed from 664 high grade astrocytoma patients (vast majority GBM) who received tumor profiling at Caris Life Sciences from 2009 to 13. Test methodologies included IHC, FISH, CISH, Sanger SEQ, MGMT promoter methylation (MGMT-Me) and NextGen SEQ (Illumina TruSeq).

Results: The combination of IHC, FISH, Sequencing and promoter methylation identified biomarker changes in GBM and the proportion of patients that can potentially benefit from biomarker-associated chemotherapies. MGMT-Me, low MGMT expression by IHC, and IDH mutation were observed in 47%, 71% and 31% respectively, suggesting the potential for temozolomide response; TLE3 overexpression, negative TUBB3 and Pgp were seen in 41%, 31% and 91%, associated with potential benefit from taxanes. Topo1 positivity, negative TS and RRM1 were seen in 49%, 38% and 19% of patients, indicating potential benefit from irinotecan, fluoropyrimidines and gemcitabine, respectively. EGFR FISH amplification was seen in 41% of patients, and is correlated with a high EGFR, a loss of p53, a lower MGMT, as well as a trend of high BCRP expressions. Mutations on the MAPK, KIT and mTor pathways are more frequently seen in EGFR non-amplified patients. Furthermore, gene mutations and MGMT methylation are frequent events and often co-occur. Correlation studies show that RRM1, TS and Top2A expressions are highly correlated; and that TS expression is more frequently observed in IDH1 wild type and MGMT unmethylated patient cohorts. These correlation studies reveal approved therapies that when used in combination, have the potential to benefit select GBM patient cohorts based on biomarker expression patterns, including fluoropyrimidines combined with gemcitabine as well as with temozolomide.

Conclusions: By profiling tumor biomarkers from a large cohort of GBM patients using validated assays in a single reference laboratory, we demonstrate the vast molecular heterogeneity of GBM and highlight the importance of individualized therapy based on a patient's unique tumor profile. Incorporating a comprehensive biomarker analysis into clinical management of this aggressive cancer allows for an informed selection of more effective therapies.

Background

Glioblastoma is the most lethal cancer of the CNS. Standard of care includes surgery followed by radiation and adjuvant temozolomide, improving patient survival to 14.6 months. Almost all GBM patients experience recurrence, for which the standard treatment is lacking, therefore novel treatment options are in great need. Distinct genetic events underlie the tumorigenesis and progression of symptomatically similar forms of GBM. EGFR amplification drives the most prevalent form, primary GBM, which occurs mostly in older patients, progresses rapidly and associates with a poor prognosis. Secondary GBMs are often initiated by TP53 mutations; they affect younger patients, progress slowly and associates with a better survival (Ohgaki 2007). Emerging genomic and proteomic data has revealed the molecular complexity underlying the subtypes of GBM demonstrating the need for treatment plans tailored to the predictive biomarker profile of each case (Verhaak 2009). Currently, MGMT methylation and IDH mutation status are important predictors for temozolomide. The purpose of our study is to interrogate the targetable biomarkers from a large cohort of GBM, and to further seek potential therapeutic options manifested by the presence of tumor targets.

Methods

Data was analyzed from 664 GBM patients who received tumor profiling at Caris Life Sciences from 2009 to 2013. IHC, FISH, CISH, Sanger SEQ, MGMT promoter methylation and NextGen SEQ (Illumina TruSeq) were performed on formalin-fixed, paraffin-embedded tumor samples in a CLIA certified lab and interpreted by board-certified pathologists and molecular geneticists. Correlation studies were performed by two-tailed Fisher Exact tests.

Results and Discussion

Four platforms (IHC, FISH, Methylation and Sequencing) reveal biomarker aberrations in GBM:

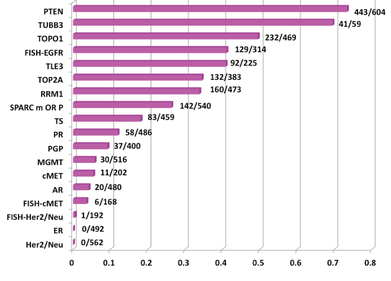

Figure 1: positivity rates of select IHC and FISH tests. IHC reveals TOPO1 over-expression in 49%, TLE3 in 41%, TOP2A in 34%, SPARC in 26% of cases, respectively, indicating potential benefit in these cohorts for irinotecan, taxanes, Top2A inhibitors, as well as nab-paclitaxel. TUBB3, RRM1, TS, MGMT are overexpressed in 69%, 34%, 18% and 5.8%, respectively, indicating potential resistance to taxanes, gemcitabine and fluoropyrimidines. While EGFR gene amplification is common (41%), cMET and Her2 amplifications (3.6%, 0.5%) are much rarer events, reflecting the reason for a great interest in EGFR targeted therapies.

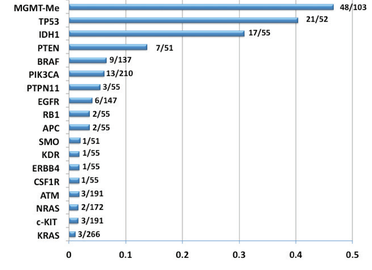

Fig 2: Methylation rate and mutation rates. MGMT methylation detected by pyrosequencing is present in 47% of cases, and IDH1 mutation in 31%; these patients are reported with better prognosis and response to temozolomide. IDH1 mutation in malignant astrocytoma also indicates a more aggressive surgery strategy for patients to achievelong-term survival (Cahill 2012). NextGen also identified novel mutations which may be targeted with therapy, including PTEN, BRAF, PIK3CA, EGFR, KDR (VEGFR2), SMO, CSF1R, NRAS, cKIT and KRAS, etc.

Biomarker features of EGFR amplified patients:

	EGFR Amplified		EGFR NOT Amplified		
	positive or mutated	Negative or Wild Type	positive or mutated	Negative or Wild Type	p value
IHC -EGFR	25 (93%)	2 (7.4%)	18 (46%)	21 (54%)	0.0013
IHC -p53	0	18 (100%)	9 (29%)	22 (71%)	0.0177
IHC -MGMT	2 (1.6%)	127 (98%)	13 (7.1%)	171 (93%)	0.0299
IHC -BCRP	24 (55%)	20 (45%)	17 (39%)	27 (62%)	0.0941
IHC -Ki67	13 (93%)	1 (7.1%)	11 (69%)	5 (31%)	n/s
BRAF SEQ	1 (4.2%)	23 (96%)	5(12%)	37 (88%)	n/s
c-KIT SEQ	0	2 (100%)	2 (33%)	4 (67%)	n/s
EGFR SEQ	0	8 (100%)	1 (9.1%)	10 (91%)	n/s
PIK3CA SEQ	0	5 (100%)	4 (31%)	9 (69%)	n/s
KRAS SEQ	0	4 (100%)	0	9 (100%)	n/s
Any mutation	1 (2.3 %)	42 (98%)	12 (15%)	69 (85%)	0.0331
cMYC FISH	0	12 (100%)	3 (10%)	27 (90%)	n/s
PIK3CA FISH	0	2 (100%)	2 (12.5%)	14 (88%)	n/s

Table 1: EGFR amplification by FISH is found to be mutually exclusive of p53 overexpression (p=0.017), and is associated with a higher EGFR protein expression (p=0.0013), a lower MGMT expression (p=0.0299), and a trend for a higher BCRP (p=0.0941). Mutations on BRAF, cKIT, EGFR or PIK3CA genes are more frequently seen in the EGFR non- amplified patient cohort (p=0.0331).Ki67 low expression, cMYC and PIK3CA FISH show a marginal trend of being more prevalent in EGFR not-amplified patients.

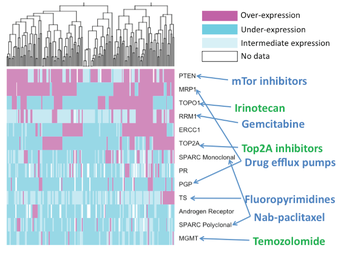

Figure 3: Select IHC test results in the EGFR amplified cohort with indicated chemotherapy. The heatmap of IHC expression highlights the heterogeneity of the protein expression profile. Irinotecan, Top2A inhibitors and temozolomide correlate with expression levels of Topo1, Top2A and MGMT respectively, are recommended by NCCN (shown in green). Novel therapies outside of NCCN are shown in blue.

Mutations co-occur frequently in GBM:

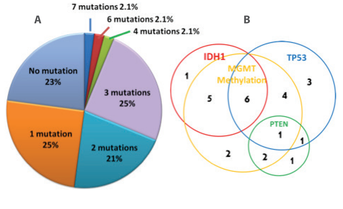

Figure 4: Frequent co-occurrence of mutations highlights the genetic instability in GBM. (A): The 44-gene NextGen panel as well as MGMT-Me is available in 48 cases. 77 % show at least one mutation, while over 30% show at least 3 genetic events. P53 mutation co-occurs significantly with MGMT-Me and IDH1 mutation. PTEN mutation is mutually exclusive of IDH1, but overlaps with MGMT methylation and P53 mutation. **(B):** P53, IDH1 mutations and MGMT-Me all carry favorable prognosis . Co-existence of IDH1 mutation and MGMT-Me show additional advantageous survival when treated with temozolomide compared with those that carry a single alteration. The significant molecular heterogeneity depicted here highlights the importance of a comprehensive molecular profiling for GBM.

Correlated biomarkers in GBM may reveal novel combination therapies:

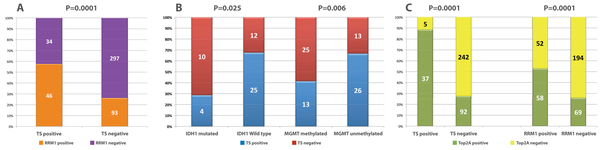

Figure 5: (A): In TS positive cohort, RRM1 is low in 52%, while in TS negative cohort , 75% shows low RRM1 (p=0.0001). The correlation of TS and RRM1 indicates the potential benefit of a combination therapy of fluoropyrimidine and gemcitabine. **(B):** In IDH1 mutated and MGMT-Me patients, TS is positive in 29% and 34% of cases, respectively, while in IDH1 WT and MGMT unmethylated patients, TS is positive in a significantly larger proportion of patients, 68% and 67%, respectively (p=0.025 and 0.006). These correlations indicate the potential benefit of treating with a combination of temozolomide and fluoropyrimidine. **(C):** Top2A is significantly associated with TS and RRM1 expressions(p=0.0001 and 0.0001), indicating that Top2A inhibitors may not be effective when combined with fluoropyrimidines or gemcitabine.

Conclusions and Study Highlights

- Immunohistochemistry of 664 GBM patient tumors reveal the heterogeneous protein expression profile. Proportions of responders to standard and novel therapies are identified; examples include 49% for irinotecan, 34% for Top2A inhibitors and 26% for nab-paclitaxel.
- EGFR amplification is seen in 41% of cases and associate with loss of p53 protein expression, representing two distinct cohort of primary and secondary GBM. EGFR amplified patients have higher EGFR, lower MGMT, and higher BCRP protein expressions.
- More than 50% of cases have more than 2 mutations per case, suggesting an increased genetic instability of GBM. MGMT methylation, IDH1 and TP53 mutation co-occur frequently, indicating a good prognosis and favorable response to temozolomide.
- High correlations of TS with RRM1, MGMT-Me and IDH1 mutation may reveal the opportunity for consideration of potentially favorable combination therapies for GBM including fluoropyrimidines + gemcitabine and fluoropyrimidines + temozolomide. These agents can cross the blood-brain barrier and have shown promising utility in GBM. The use of a combination of approved drugs based in part by tumor expression patterns holds promise in GBM and suggests for future study.

Key Citations

- Verhaak 2009, Cancer Cell 17, 98–110.
- The Cancer Genome Atlas Research Network 2008, Nature 455, 1061-8.
- Ohgaki 2007, Am J Pathol, 170(5):1445-53.
- Cahill 2012, J Clin Oncol 30, 2012 (suppl; abstr 2019).