Results of molecular profiling for recurrent malignant gliomas reveal significant changes in biomarkers compared to mostly treatment naïve tumors that could impact treatment decision

Lyndon Kim, MD[1,2], Joanne Xiu[3], Kevin Judy, MD[1], James Evans, MD[1], Christopher Farrell, MD[1] David Andrews, MD[1], Department of Neurological Surgery[1], Department of Medical Oncology[2] Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA, USA, Caris Life Sciences[3], Phoenix, AZ, USA

Background

There have been few studies done in systemic malignancies to look for differences in molecular profiling in newly diagnosis versus recurrence.

Molecular genetic profiling of ovarian cancer comparing first and recurrent paired tissues revealed significantly high discordance rate (up to 40 %) in some biomarkers which could impact the treatment strategy in recurrent setting.

The difference between treatment naïve and recurrent malignant gliomas profiling is not known.

Methods

- 10 patients (pt) were identified retrospectively with tumor profiling tests on multiple specimens.
- Molecular profiling was performed by Caris Life Sciences, Inc.
- Tests included immunohistochemistry (IHC), next generation sequencing, and fluorescence in situ hybridization.
- A total of 10 paired pts's results were obtained from March 2011 to May 2016.
- Male:Female 8:2 Age: 22-69 years old Median Age: 42 years old.
- 1pt had Anaplastic Oligodendroglioma (AO), 2 pt had Anaplastic Astrocytomas (AA), and 7 pts had Glioblastomas (GBM).
- 7/10 patients had tests performed at the time of 1st surgery and recurrence. Of 7 pts who had 1st test at the time of 1st surgery, 2 pt had second test at the 1st recurrence, 3 pts at the 2nd recurrence,2 pt at the 3rd recurrence and 2 pts at the 4th recurrence.
- 3 pts had the first test performed at the time of recurrence: 4 months (GBM pt) 8 months (GBM) and 8 years (AO pt) from the 1st diagnosis/surgery and the second test was performed after 2nd, 3rd and 3rd surgery 34 months, 6months and 13 months later, respectively.
- Average time between two tests/time of recurrences was 32.6 mo for AA/AO and 26.1 mo for GBM respectively.

Results

Decreased expression of PD-L1 2/8 (25%), ERCC1 2/7 (29%), PTEN3/9 (33%), RRM1 2/5 (40%), TLE3 4/6 (67%) and EGFR 2/6 (33%) were seen over, while no increase of expression were seen.

1 showed loss of EGFRvIII variant over time out of 5 pairs tested. In contrast, cMET expression and amplification, as well as TUBB3 expression were seen to increase over time in 1/5 (20%), 1/4 (25%) 2/10 (20%) samples, while no decrease was seen.

Expression of PD-1, TOPO2A, TOPO1, TS were seen to both increase and decrease over time.

In the 9 pairs with sequencing data available, acquisition of EGFR(V292L), FLT3(D324N), NOTCH1(G736R) and ATM (H231R) were seen in one tumor each.

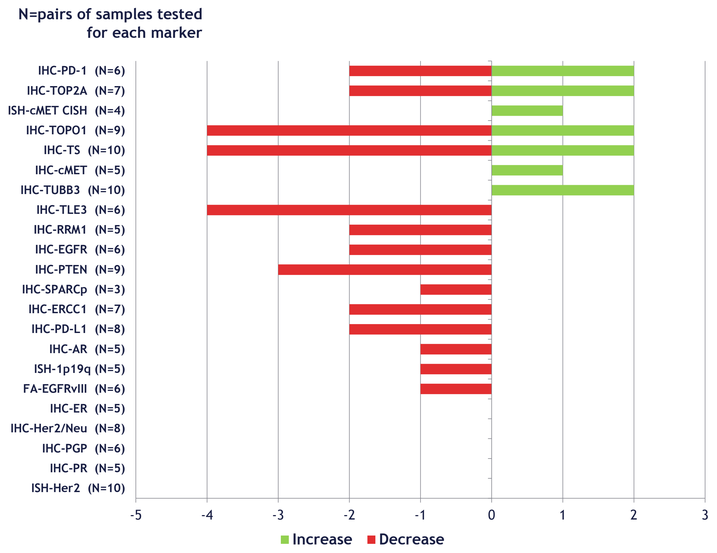
In one case, Tp53 R175H mutation was seen in the first sample and an R158H mutation was seen in addition to R175H was seen in a sample collected 3.3 years later; in the same case, PDGFRA (R841_I843del) was seen in the first sample and D842V is seen in a sample collected later. In the 8 pairs with MGMT methylation tests done, two samples showed decreased MGMT methylation.

Results

Patient #	1	2	3	4	5	6	7	8	9	10	Associated treatments	predicted change in patient response
Diagnosis	GBM	GBM	GBM	GBM	GBM	GBM	GBM	AO	AA	AA		
Time Interval (m)	46	11	23	34	34	5	15	13	55	17		
sex	m	f	m	m	m	m	m	m	f	m		
age	55	68	18	35	63	50	43	43	62	27		
Primary vs. Recurrence	primary | 1st rec	primary | 2nd rec	primary | 1st rec	1st rec | 2nd rec	primary | 4th rec	1st rec | 2nd rec	primary | 2nd rec	3rd recur | 4th recur	1st rec | 2nd rec	primary | 2nd rec		
Treatment	TMZ, Carboplatin, BCNU, Bevacizumab	TMZ, Carboplatin, Irinotecan	TMZ, Bevacizumab, Carboplatin, BCNU	TMZ, BCNU, Bevacizumab	TMZ, bevacizumab, Irinotecan	TMZ, BCNU, Carboplatin, Bevacizumab, Irinotecan	TMZ, carboplatin	TMZ, PCV, Everolimus, Pembrolizumab, Carboplatin	TMZ	TMZ, Carboplatin, BCNU, Bev		
TTP	51.4 m	>26.8m	39m	38 m	34m	16m	15m	129	69m	>17m		
Pyro SEQ-MGMT	Equivocal | Un-Met	Un-meth	Meth	un-meth	un-meth	un-meth	un-meth	Meth | Un-Me	Meth	un-meth	Temozolomide	more sensitive
FA-EGFRvIII		Present | N									EGFRvIII-targeted therapy	less sensitive
IHC-AR				Pos | N								
IHC-cMET	N | Pos										cMET-targeted therapy	more sensitive
ISH-cMET	N | Amp.											
IHC-EGFR	Pos | N	Pos					Pos	Pos | N	Pos		EGFR-targeted therapy	less sensitive
IHC-ER												
IHC-ERCC1	Pos | N				Pos | N				Pos		Plastinum agents	more sensitive
IHC-Her2/Neu												
IHC-PD-1	N | Pos			Pos | N			N | Pos	Pos | N			Immune-checkpoint inhibitors	mix
IHC-PD-L1	pos			Pos | N			Pos | N					
IHC-PGP												
IHC-PR												
IHC-PTEN	Pos	Pos | N	Pos | N	Pos	Pos	Pos | N	Pos	Pos		Pos	mTor inhibitors	More sensitive
IHC-RRM1			Pos | N				Pos | N				Gemcitabine	More sensitive
IHC-TOP2A	N | Pos	Pos | N	Pos				N | Pos	Pos | N			Top2A inhibitors	mix
IHC-TOPO1		Pos | N	Pos | N	N | Pos	Pos | N	N | Pos			Pos | N		irinotecan	mix
IHC-TS	Pos	Pos | N	Pos | N		N | Pos	N | Pos	Pos	Pos | N	Pos | N		fluoropyrimidine	mix
IHC-SPARCp	Pos | N										nab-paclitaxel, paclitaxel, docetaxel	less sensitive
IHC-TLE3	Pos | N			Pos | N			Pos | N		Pos | N			
IHC-TUBB3	Pos	N | Pos	Pos		Pos	Pos	Pos	Pos	N | Pos	Pos		
ISH-1p19q								Pos | N			PCV	less sensitive
SEQ-RB1					N | E746fs						Cell cycle inhibitors	less sensitive
ISH-Her2												
SEQ-APC					S1144R						Acquisition of variant of unknown significance are seen	
SEQ-ATM					N | H231R							
SEQ-c-KIT							H697Y					
SEQ-EGFR		WT | V292L										
SEQ-FLT3										WT | D324N		
SEQ-IDH1	R132H							R132S				
SEQ-JAK3												
SEQ-NOTCH1										WT | G736R		
SEQ-PDGFRA			R841_I843del | D842V									
SEQ-PTEN						N184fs						
SEQ-STK11							F354L					
SEQ-TP53	R248W		R175H | R175H, R158H		S215G	c.994-1G>C	V173M	N235S				

Figure 1: molecular test details of the 10 patients whose paired tumor samples were tested: Grey: two samples generated the same negative or wild type results; **Blue:** two samples generated the same positive results or the same mutation was seen in both samples; **Green:** a molecular alteration absent in the first sample was seen in the second tumor sample; **Red:** a molecular alteration seen in the first sample was lost in the second sample
TTP: time to tumor progression, calculated from diagnosis to the most recent tumor progression (TP)

Figure 2 – Summary of IHC and ISH marker changes seen in the paired samples. Y-axis: biomarkers tested followed by number of paired results available; X-axis: number of cases with biomarker changes observed. **Green:** protein overexpression or gene amplification absent in the first sample was seen in the second tumor sample; **Red:** protein overexpression or gene amplification seen in the first sample was lost in the second sample



Discussion

- MGMT Methylation seems to confer improved time to tumor progression and overall survival compared to pts with Unmethylated MGMT.
- IDH1 mutations are seen in the GBM and anaplastic gliomas with the longest TTP, confirming its predictive value for a better survival.
- Changes of TS and expression may have potential to associate with patient's outcome: decrease of protein expression vs. increase may suggest better patient outcome.
- Irinotecan treatment based on positive TOPO1 expression is often adopted and generated favorable outcome: e.g, patient 3 was treated with Irinotecan x 7 cycles at the third recurrence after patient has failed bevacizumab and achieved stable disease.
- Acquisition of variants of unknown significance by sequencing is seen as the tumor progresses, the therapeutic implication remains unknown.
- Further data analysis is ongoing.

Conclusions

- Although cohort is small, we show dynamic changes in recurrent malignant gliomas with high discordance rate of 29% compared to the first test.
- There was greater loss of targetable-biomarkers than gains over time (p=0.015).
- Frequent biomarker changes are seen when serial tumor samples are compared for biomarker aberrations; suggesting the need for a fresh specimen to guide next line of therapy.

References:

1) Treatment-Related Protein Biomarker Expression Differs between Primary and Recurrent Ovarian Carcinomas in Molecular Cancer Therapeutics 11(2):492-502 · December 2011

2) Mutational Analysis Reveals the Origin and Therapy-Driven Evolution of Recurrent Glioma. Science. 2014 Jan 10;343(6167):189-93