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A molecular approach to Glioblastoma Multiforme

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ABSTRACT

Glioma is a tumor of the central nervous system that occurs in the glial cells, Which it surrounds and protects the nerve cells. Glioblastoma Multiforme (GBM) is the most common and malignant sub-type of gliomas that arises from star-shaped cells called "astrocytes", which constitute the supportive tissue of the brain. GBM are known to be heterogeneous in outcome with majority having a poor prognosis, thus there is an urgent need for novel therapeutic approaches. The detailed understanding of GBM is established by the combination of histopathology and genomic information of the tumor that aids in the best choice of Personalized Medicine. In this article, seven GBM patients are discussed who underwent tissue diagnosis as well as tumor molecular profiling; the significance of the genes and associated mutations/variations picked up in each individual.

Key words: Brain, Genes, Genetic testing, Glioblastoma multiforme, Glioma, Mutations, Personalized medicine

Introduction

Glioma encompasses all tumors that are thought to be of glial cell origin. Glioblastoma multiforme (GBM) is usually highly proliferative because the cells reproduce quite fast and is assisted by a large network of blood vessels (neovascularization). They are generally found in the cerebral hemispheres of the brain, but can also be found in other parts of the brain and spinal cord.

The average incidence rate of GBM in the USA is 3.19 per every 100,000 of the population, and the median age of diagnosis is 64 years with incidence higher in men. Many genetic and environmental factors have been studied in GBM, but the majority are sporadic, and no risk factor accounting for a large proportion of GBMs has been identified.[1]

Types of gliomas include astrocytic tumors (World Health Organization classification) astrocytoma Grades I, II (astrocytoma), III (anaplastic astrocytoma), and IV (glioblastoma or GBM), oligodendrogliomas, ependymomas, and mixed gliomas.

GBMs are biologically aggressive tumors that present unique treatment challenges due to localization of tumors in the brain; inherent resistance to conventional therapy; limited capacity of the brain to repair itself; migration of malignant cells into adjacent brain tissue; variably disrupted tumor blood supply which inhibits effective drug delivery; tumor capillary leakage, resulting in an accumulation of fluid around the tumor; (peritumoral edema) and intracranial hypertension; a limited response to therapy and the resultant neurotoxicity of treatments directed at gliomas.[2]

The standard treatment for newly diagnosed glioblastoma (Grade IV) is biopsy or surgical resection, depending on the location of the tumor, followed by the treatment with radiotherapy (RT) and chemotherapy (CT) (temozolomide). Because surgery, radiation and CT are unlikely to result in a prolonged remission of GBM tumors owing to their nature, researchers are investigating the use of novel treatments when the first line of therapy has failed.

Various prognostic and predictive biomarkers which are currently used in GBM include O6-methylguanine-methyltransferase (MGMT) promoter methylation status and isocitrate dehydrogenase (IDH) 1 and 2 mutation status. Leibetseder *et al* reported a high frequency of IDH1 mutations and MGMT promoter methylation among young adult patients with primary GBM with favorable outcome. MGMT promoter methylation is also a predictive marker for response to adjuvant CT and RT.

All the cases discussed here were referred to Dr. Amit Verma who runs a specialised clinic for Personalized Cancer Medicine at Max Cancer Center, New Delhi. Here, patients with GBM underwent molecular profiling, where their tumor(s) was tested for either of the two somatic mutation gene panels: (i) 48 (6 patients); (ii) 315 with 28 translocations (1 patient) genes that are shown to be important drivers in the process

of oncogenesis. Certain changes in these genes are validated targets of therapy in various types of tumors and/or in clinical trials. Next-generation sequencing (NGS) is the molecular technique used here. See the supplement sheet for the panel of genes (Appendix 1). All the cases were discussed in the molecular tumor board meeting where medical opinion was taken from a team that consisted of Medical Oncologist, Radiation Oncologist, Surgical Oncologist, Pathologist, Radiologist, Neurosurgeon, and a Molecular Oncologist.

Molecular Subtypes of Glioblastoma Multiforme

Glioblastoma has been classified into subtypes based on their gene expression by The Cancer Genome Atlas (TCGA). Table 1 gives a clear understanding of the subtypes and the various genes mutations and copy number variations.

Mesenchymal

The mesenchymal subgroup contains the most frequent number of mutations in the neurofibromin (NF1) tumor suppressor gene. Patients in the mesenchymal group had significant increase in survival after aggressive treatment, unlike those in the proneural, and to an extent, in the neural subgroups.

Classical

Classical GBM tumors are characterized by abnormally high levels of epidermal growth factor receptor (EGFR). The EGFR abnormalities occur at a much lower rate in the three other GBM subtypes. However, *TP53*, the most frequently mutated gene in GBM, is not mutated in any of the classical GBM tumors. Clinically, the classical group survived the longest in response to aggressive treatment.

Neural

The neural group was characterized by the expression of several gene types that are also typical of the brain's normal, noncancerous nerve cells, or neurons. Patients in the neural group had some improvement in survival after aggressive treatment but not as much as the classical and mesenchymal groups.

Proneural

Proneural tumors are also characterized by having the most frequent mutations-in the *IDH1* gene. Platelet-derived growth factor receptor alpha is found to be mutated and expressed in abnormally high amounts in this subtype. Unlike the other types, whose patients were similar in age on average, the proneural subgroup was significantly younger. They also tended to survive longer and have the best prognosis among all subgroups. However, patients in the proneural group who received aggressive treatment with TMZ did not survive significantly longer than proneural patients who did not receive aggressive treatment.

Clinical Case Scenarios

Case 1

A 52-year-old female who is a known case of right frontal GBM was diagnosed 4 years ago. Surgery was performed with subtotal resection of the brain which was followed by temozolomide (TMZ) and RT. She was also on TMZ as maintenance therapy. On following up with the patient, she was found to progress on TMZ and received 4 cycles of PCV which was later discontinued because of serious adverse events. Thereafter, she was again put back on TMZ for 1 year but showed progression. Hence, TMZ was discontinued and was started on bevacizumab (Avastin). Since then, she was on Avastin with a stable disease and a good physical performance for more than 1½ years. Unfortunately, she died after 4 years due to aspiration pneumonia.

Clinical questions

1. With such a dismal prognosis of GBM, why did the patient have a good survival of approximately 4 years?
2. Why was there a progression on TMZ? Is there any additional benefit to continue TMZ beyond progression?

Molecular oncology approach

1. Somatic mutation 48 gene panel testing was carried out.

Result interpretation

The genomic alterations picked up are explained in detail in Table 2.

The identified variation p.Val2l6Met in *TP53* gene has been previously reported in primary glioblastoma[4] and also in esophageal cancer, hematopoietic and lymphoid cancers, upper aerodigestive tract cancer, breast cancer, and ovarian cancer. Details on the gene and the mutations can be obtained from

Table 1: Classification of primary glioblastoma (TCGA)

Transcriptional subtype	Mesenchymal	Classical	Neural	Proneural
IDH1/2 mutation	Wild type	Wild type	Wild type	Mutant
Other gene mutations	*NF1*	EGFR	-	TP53
Methylation status	-	-	-	G-CIMP
Copy number variation	*NF1* loss	*PTEN* loss *EGFR* amp	-	*PDGFRA* amp *MET* amp
Signaling pathway affected	*NF1* signaling, YKL-40, VEGF, CD44, IRS1	EGFR signaling, Notch signaling	-	PDGFB signaling

EGFR: Epidermal growth factor receptor, TCGA: The cancer genome atlas, NF1: Neurofibromin 1, PTEN: Phosphatase and tensin homolog, VEGF: Vascular endothelial growth factor

Table 2: Summary of gene mutations identified

Case number	Histology	Gene(s) involved	Genomic loci	cDNA alteration	Protein change	Likely molecular subtype[++]	Actionable mutation	Targeted therapy available
1	GBM	*TP53*	Chr17:7578203C>T	c.646G>A	p.Val21Met	Proneural[a]	None	None
2	GBM	*PIK3CA*	Chr3:I78952088A>C	c.3143A>G	p.His1048Arg	Classical[b]	Yes	*PIK3CA* - Everolimus, temsirolimus, buprasilib
3	GBM	*EGFR, PTEN*	*EGFR* amplification PTEN – Deletion (6 loci)*		EGFR over expression Loss of PTEN expression	Classical[b]	Yes	*EGFR* – Afatinib, cetuximab, erlotinib, gefitinib, lapatinib, panitumumab *PTEN* -Everolimus, temsirolimus,
4	GBM	*VHL, PTEN, RB1*	*VHL*-chr3: 10183817C>T chr3: 10183836C>T *PTEN*-Chr10: 89717681G>A *RB1*-chr13: 49027241C>T chr13: 49037870A>G chr13: 49037937C>T	*VHL* – c.286C>T c.305C>T *PTEN*-c.706G>A *RB1*-c.1808C>T c.2110A>G c.2177C>T	*VHL* – p.Gln96* p.Pro102Leu *PTEN* - p.Asp236Asn *RB1* - p.Ala603Val p.Met704Val p.Thr726Ile	Classical[b]	Yes	*VHL* - Bevacizumab, sorafenib, *PTEN* -Everolimus, temsirolimus *RB1* - None
5	Anaplastic Astrocytoma Grade III	*EGFR, TP53*	*EGFR* –Chr7: 55249003_55249011dupCAGCGTGGA TP53-Chr17: 7577538C>T	*EGFR* – c.2301_2309dupCAGCGTGGA *TP53* - c.743G>A	*EGFR* –p.Ser768_Asp770dup *TP53* - p.Arg248Gln	Classical[b] or Proneural[d]	Yes	*EGFR* – Afatinib, cetuximab, erlotinib, gefitinib, lapatinib, panitumumab *TP53* - None
6	Gliosarcoma	No mutation identified	-	-	-	Neural[c] or Classical[b]	No	-
7	GBM	EGFR, PTEN, CDKN2A/B, BCORL1,SETD2, TERT	EGFR - amplification, D46N, EGFRvII, EGFRvIII PTEN - Tyr68Hist CDKN2A/B loss BCORL1- Gln1076fs*6 SETD2 splice site 5278-2A>T TERT promoter -146C>T			Classical	yes	EGFR: Afatanib, Cetuximab,Erlotinib, Gefitinib, Lapatinib, Panitumumab PTEN - Everolimus, Temsirolimus

[a, b, c, d]Refer Table 1 for more details. Case 3: PTEN – Deletion (6 loci)*, chr10: 89685304_89685432; chr10: 89711891_89712012; chr10: 89717515_8917638; chr10: 89717638:89717772; chr10: 89720709_89720843 and chr10: 89720843_89720967, Case 5 - Other VUS genes mutations*, *ATRX* - H475D, Q883R; *CREBBP* - P1279L; *GLI* – P1668R; *MYCL1* - A117T; *BRCA2* - A1996T; *FGFR2* - A16T, *HGF* - D552del, *TGFBR2* - T1551I, EGFR: Epidermal growth factor receptor, GBM: Glioblastoma multiforme, PTEN: Phosphatase and tensin homolog

Table 3: Gene Information

Genes	Mutations/Alterations	Relevant Information
EGFR	Amplification - *EGFR*vII, Amplification - *EGFR*vIII p.Ser768_Asp770dup p.Asp46Asn	*EGFR* gene is located on chromosome 7p12 region and the protein encoded by this gene is a member of the tyrosine kinase superfamily. The binding of growth factors ligands results in the activation of multiple downstream pathways controlling proliferation and survival (Siegelin and Borczuk, 2013)
PTEN	Deletion of 6 loci p.Asp236Asn Tyr68His	*PTEN* gene is located on chromosome 10q23 and codes for the PTEN protein which directly antagonizes PI3K signaling. It undergoes genomic loss, mutation, or epigenetic inactivation in 40-50% of gliomas, resulting in high levels of PI3K activity and downstream signaling (Koul, 2008)
VHL	P.Gln96* p.Pro102Leu	A tumor supressor gene that plays an important role in mammalian oxygen sensing pathway through the polyubiquitinylation of hypoxia-inducible factor. Tumors linked to *VHL* inactivation are often highly vascular and can overproduce angiogenic factors such as VEGF
RB1	p.Ala603Val p.Met704Val p.Thr726Ile	Retinoblastoma transcriptional corepressor 1is a tumor suprressor gene and is located on chromosome 13q14.1-q 14.2.pRB interacts with other proteins to influence cell survival, the self-destruction of cells (apoptosis) and differentiation
TP53	p.Val21Met p.Arg248Gln	TP53, otherwise called tumor protein 53or p53 is located in the nucleus of cells throughout the body where it attaches directly to the DNA. When DNA is damaged, p53 activates other genes to fix the damage. Gene location - Chromosome 10
PIK3CA	p.His1048Arg	The *PIK3CA* gene provided instructions for making the p110 α protein, which is a subunit of an enzyme PI3K. It is a catalytic subunit which helps in phosphorylation. Studies suggest that PI3K signaling may be involved in regulation of several hormones and play a role in the maturation of fat cells (adipocytes)
SETD2	Splice site 5278-2A>T	*SETD2* locayed on chromosome 3 is a tumor supressor gene. It is a histone methyltransferase that is specific for lysine-36 of histone H3, and methylation of this residue is associated with active chromatin and has been found to been associated with hyperphosphorylated RNA polymerase II
TERT	TERT Promoter 146C>T	Telomerase reverse transcriptase is a catalytic subunit of enzyme telomerase, which is responsible for the lengthening of the DNA strands. It is normally repressed in somatic cells, resulting in progressive shortening of telomeres
CDKN2A/B	Loss	Cyclin dependant kinase inhibitor 2A gene situated on chromosome 9q21.3 provide instructions for several studies, p16 (INK4a) and p14 (ARF) which are the most studied. These proteins help in cell cycle and p16 binds to CDK4 and 6 while p14 protects a different protein called TP53, hence prevent tumor formation
BCORL1	Gln1076fs*6	BCL6 corepressor –like 1 encodes for a transcriptional repressor that exhibits homology to BCOR, but unlike BCOR does not interact with BCL-6, rather functions as a transcriptional repressor with Class II histone deacetylases, and potentially BRCA1

EGFR: Epidermal growth factor receptor, TCGA: The cancer genome atlas, PTEN: Phosphatase and tensin homolog, PI3K: Phosphatidylinositol-3-kinase, VEGF: Vascular endothelial growth factor, SETD2: SET domain containing 2

Tables 2 and 3. The identified variation is located in the DNA binding region of *TP53* and is known to cause loss of transactivation potential of the protein.[5] Functional TP53 is required for TMZ mediated apoptosis, thus rendering the patient insensitive to the cytotoxic effects of TMZ.

Based on the Recursive Partitioning Analysis (RPA) criteria (based on the clinical parameters), the patient lies in Group 3 with a median survival of 70 weeks. However, the patient survived for over 48 months (>220 weeks) since diagnosis, suggesting a favourable tumor biology. This is supported by the fact that EGFR expression (IHC was carried out) and amplification (NGS) were absent, which are indicators of good prognosis. Further, based on the presence of aberrant TP53 expression (IHC was carried out) and somatic gene mutation (NGS), the patient can be classified into "Proneural Molecular Subtype" [Table 1]. The median overall survival of proneural subtype is reported to be 36-48 months,[6] thus explaining the patient's natural course (favourable).

Further, a study by Le Mercier *et al*.[7] showed no additional benefit of adding TMZ to RT versus RT alone in proneural subtype in comparison to the classical subtype of glioblastoma [Figure 1]. Thus, this calls into question the continuation of TMZ beyond progression. To conclude, molecular profiling may help to analyze and predict the prognosis and possible response to conventional therapy and management.

Therapeutic implications

i. Proneural molecular subtype with expected median overall survival 36-48 months as observed in the case
ii. No additional benefit of continuing TMZ beyond progression.

Case 2

A 68-year-old male who is a known case of left temporal GBM underwent sub-total resection and was on TMZ and RT. He was put on maintenance TMZ.

Clinical questions

1. What is the patient-specific tumor biology? Can we predict the treatment response?

2. What if the disease progressed while on treatment? What else can be done?

Molecular oncology approach

1. Somatic mutation 48 gene panel testing was carried out.

Result interpretation

The genomic alterations picked up are reported in Table 2.

The identified variation in *PIK3CA* gene (p.His10484Arg) is within the hotspot region and highly conserved kinase domain and has been previously reported in endometrial and breast cancers.

Based on the clinical parameters using RPA criteria, it was found that the patient lies in the poor prognosis group with median survival of 40 weeks. Further, mutation in the *PIK3CA* gene, which known to be genetically altered in primary glioblastoma with poor prognosis [Figure 2] and distinguishes from secondary glioblastoma with good prognosis. Thus, both the clinical and molecular assessment suggest poor prognosis.

This variation may lead to constitutional activation of the phosphatidylinositol-3-kinase *(PI3K)/AKT* survival pathway thus resulting in growth factor-independent proliferation and protection from cell death. Activation of the *PI3K* pathway is known to play a role in radioresistance in glioma.[8]

Therapeutic implications

i. Based on the mutation identified, we can subtype the GBM into "classical molecular subtype" with a median survival of 40 weeks (poor prognosis). The patient may benefit

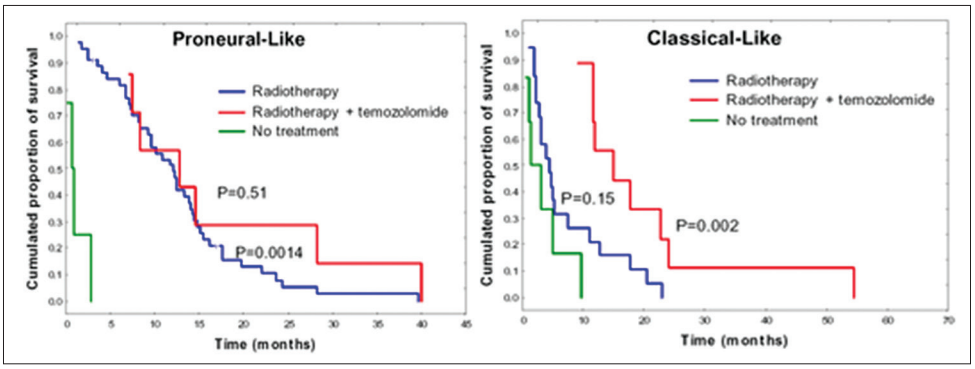

Figure 1: Predictive prognosis of Proneural subtype vs Classical subtype (Mercier et al)

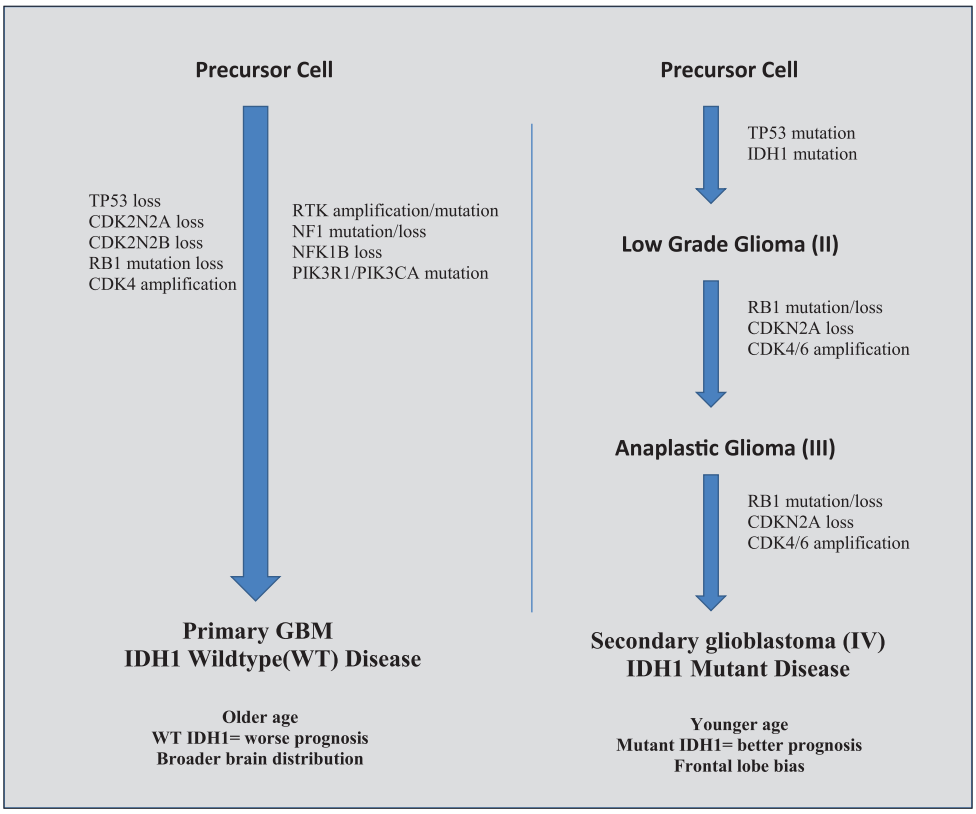

Figure 2: Stages in formation of Primary GBM vs Secondary GBM

from aggressive TMZ based treatment
ii. Beyond progression on Avastin, Everolimus may be the next choice.

Case 3

A 59-year-old male diagnosed with GBM of the left frontoparietal region, underwent surgery for the same and was undergoing RT along with CT (TMZ). He is a non-smoker and a non-tobacco user.

Clinical questions

i. What is the patient-specific tumor biology? Can we predict the treatment response?

Molecular oncology approach

1. Somatic mutation 48 gene panel testing was carried out.

Result interpretation

The genomic alterations picked up are reported in Table 2.

Epidermal growth factor receptor amplification

EGFR amplification was observed in this patient. It is frequently observed in glioblastoma and is been seen in about 36-40% of the tumors and is associated with resistance to CT and radiation therapy.[9] Prognostic significance of EGFR is not yet clear. Diverse observations are reported where EGFR amplification serves as poor prognostic factor. In contrast, it is a marker of prolonged survival in older glioblastoma patients.[10] EGFR amplification and overexpression are associated with resistance to CT and radiation therapy.[11] Mechanistically, connexion 43 is involved *EGFR*-mediated TMZ resistance.[12]

Deletion of both copies of phosphatase and tensin homolog gene

Both the copies of the phosphatase and tensin homolog *(PTEN)* gene on chromosome 10 have been deleted in this patient.

The deletion of *PTEN* genes has already been reported in prostate cancer, breast cancer, glioblastoma cell lines and as well as in primary glioblastomas. Di Nicolantonio *et al*.[13] demonstrated that patients with *PIK3CA* activating mutations or *PTEN* loss of expression showed clinical benefits from everolimus monotherapy.

Therapeutic implications

i. This patient falls under the "Classical Molecular Subtype" of GBM, hence making him a poor prognostic candidate
ii. Due to *EGFR* gene amplification, this patient would be benefited from Afatinib, Cetuximab, Erlotinib, Gefitinib, and radiation
iii. The loss of *PTEN* genes favors the use of mTOR inhibitors such as temsirolimus and everolimus.

Case 4

A 45-year-old female who presented with right frontal GBM, underwent surgery for the same and was undergoing RT along with CT (TMZ).

Clinical questions

1. What is the patient-specific tumor biology? Is it possible to know the potential therapy options by doing molecular testing of the tumor?

Molecular oncology approach

1. Somatic mutation 48 gene panel testing was carried out.

Result interpretation

The genomic alterations picked up are reported in Table 2.

p.Gln96* and p.Pro102Leu variations in the *VHL* gene were identified. p.Gln96* leads to the change of glutamine to a stop codon which truncates the protein, hence affecting the protein production and function; and p.Pro102Leu leads to a change in amino acid from proline to leucine, the effect still being unknown.

Mutations in *VHL* or loss of expression are most prominently found to be associated with glial tumors, hemangioblastomas, and renal cell carcinoma.[14] The identified mutations in the *VHL* gene have been previously reported mainly in sporadic renal cell cancer and hemangioblastomas.

The identified missense mutation at p.Asp236Asn in the *PTEN* gene has not been previously reported, making it a novel mutation. Hence, it is not known whether it would result in loss of function. The effect of the identified mutation in *PTEN* gene on targeted therapy cannot be conclusively ascertained since it is a novel missense mutation.

p.Ala603Val & p.Thr726Ile identified in *RB1* gene have also not been reported before, hence making them novel mutations. However, p.Met704Val variant identified in the *RB1* gene has been reported previously in OVCAR-3 cell lines.[15] Although mutations in *RB1* are well known in cancer, no specific therapeutic relevance has been found for *RB1* mutations in gliomas. Hence, the significance of these variants with regard to therapy or prognosis cannot be ascertained.

Therapeutic implications

i. This patient falls under the "Classical Molecular Subtype" of GBM, hence making him a poor prognostic candidate
ii. The mutation observed in *PTEN* genes favors the use of mTOR inhibitors such as temsirolimus and everolimus, and the patient maybe benefited from bevacizumab due to variations in the *VHL* gene.

Case 5

Clinical presentation

A 7-year-old young male with anaplastic astrocytoma, Grade III.

Clinical questions

1. What is the patient specific tumor biology? Is it possible to know the possible therapy options by doing a molecular testing of the tumor?

Molecular oncology approach

1. Somatic mutation 48 gene panel testing was carried out.

Results and interpretation

The genomic alterations picked up are reported in Table 2.

The identified mutation (p.Ser768_Asp770dup) in the EGFR gene represents duplication of 9 bases. EGFR variations are associated significantly associated with reduced survival in anaplastic astrocytoma.[16] Primary GBM tumors with simultaneous alterations in TP53 are associated with worse prognosis.

The observed TP53 mutation (p.Arg248Gln) has been reported in many tissues of the glioma.[21] It lies in the L3 domain of p53 that is involved in binding with 53BP2 protein which consists of evolutionary conserved regions that are frequently mutated in cancer The variation was reported to be dominant negative variation with a gain of function activity in human lung cancer NCI-H1299 cells and enhanced *in vitro*-invasiveness.[22]

Variations in EGFR are common in Grade IV glioblastoma than in anaplastic astrocytoma, Grade III. It is speculated that anaplastic astrocytoma with variations in EGFR gene represents undersampled GBM. It is recommended that anaplastic astrocytoma with variation in EGFR be treated like GBM, even though the histopathology criteria for GBM are not met.[9]

Therapeutic implications

i. Anaplastic astrocytoma patient with EGFR variation should be treated as a GBM patient, hence, subcategorizing this patient into "Classical or Proneural Molecular Subtype" based on the molecular information
ii. Since, this patient harbors both EGFR and TP53 variations, prognosis is worse but benefit from CT such as afatinib, cetuximab, erlotinib, gefitinib, and dacomitinib due to EGFR mutations is known.

Case 6

A 60-year-old male with progressive gliosarcoma Grade IV, Ki-67 is 30%. He underwent surgery which was followed by CT and radiation therapy assisted with avastin which was given for six cycles. However, it was noted that the patient was showing progression on this treatment. He has no family history for cancers, a non-smoker who follows a vegetarian diet. It was found that he strongly expresses GFAP with interspersed perivesicular GFAP negative sarcomatous foci.

Clinical questions

1. What is the patient-specific tumor biology? Can we predict the treatment response?
2. What if the disease progressed while on treatment? What else can be done?

Molecular oncology approach

1. Somatic mutation 48 gene panel testing was carried out.

Result and interpretation

The molecular analysis of 48 somatic genes identified no mutation.

Therapeutic implications

i. Since, this patient has *IDH*1/2 wildtype, *TP53* wildtype with no mutation in the *EGFR* gene, he belongs to "Classical or Neural Molecular subtype" of GBM
ii. No mutation picked up by this panel, hence one cannot comment on the choice of targeted treatment for this patient.

Case 7

A 54-year-old female with high-grade GBM status postsurgery followed by CT and RT with adjuvant TMZ. MGMT methylation status is positive.

Clinical questions

1. Will the analyses of the tumor help in deciding the course of treatment?

Molecular oncology approach

1. Somatic mutation 315 genes panel testing was carried out.

Result interpretation

The genomic alterations picked up are reported in Table 2.

EGFR amplification was found to be strongly correlating with *EGFR* protein expression in GBM. Mutation of the *EGFR* gene known as *EGFR*vIII is reported in about 4-46% of GBM cases and results from a gene rearrangement that deletes exons 2-7.[16] This alteration causes an in-frame deletion of 801 base pair encoding part of the extracellular binding protein ligand which results in activation of *EGFR* as well as tumorigenesis. *EGFR*vII is reported in ~15% of GBM patients and results from a gene arrangement that deletes exons 14 and 15, and consequently removes a part of the extracellular domain, which is been identified in glioblastoma rarely when compared to *EGFR* vIII, but is said to be oncogenic. The *EGFR D46N* mutation observed, however, has not been characterized and its effect on function remains unclear but has been reported in the context of cancer, which may indicate a biological reference.

PTEN alterations that disrupt the N-terminal PIP2 binding motif, the phosphatise domain (amino acids 14-185), C2 domain (amino acids 190-350), and/or C-terminal region observed, here, are predicted to cause loss of function. *PTEN* alterations are been reported in 31% of GBM in TCGA database including homozygous deletion in 8% of samples.[17] Decreased *PTEN* expression is associated with high-grade GBM

tumors. However, loss of *PTEN* is corelated with significantly worse prognosis in GBM.

In GBM, TCGA dataset, homozygous deletion of *CDKN2A/B* has been found in 54% of the GBM cases (cBioPortal, Apr 2015).

Somatic mutation of *BCORL1* gene has been observed in acute myelogenous leukemia patients,[18] suggesting a role of tumor suppressor in this disease.

Somatic inactivating alterations of SET domain containing 2 are documented at a low frequency in a number of solid tumors, most common in renal carcinoma[19] and 6-12% in acute lymphoblastic leukemia.

TERT promoter mutations have been reported in 51-59% of gliomas,[20] most frequently in GBM (54-84%) with poor prognosis. TERT mutation occurs with EGFR amplification on GBM and is associated with poor prognosis.

Therapeutic implications

i. This patient falls under the "Classical Molecular Subtype" of GBM
ii. Based on the molecular information from the patient's tumor, it is therefore concluded that this patient can benefit from afatinib, cetuximab, erlotinib, gefitinib, lapatinib, panitumumab due to an *EGFR* alteration and everolimus, temsirolimus due to alteration in the *PTEN* gene.

Discussion

In this upcoming era of molecular oncology, there is a paradigm shift from conventional histopathology to molecular subtyping. Prognosis and prediction of treatment response based on the clinical criteria and available single gene testing is not that precise. Beyond bevacizumab, options of targeted therapy in GBMs are limited. Hence, understanding the molecular basis may help in prognosticating and opening up more treatment options including use of other targeted therapies in an adjuvant set up.

Current clinical practice is limited with single gene testing like MGMT methylation, IDH mutations, 1p/19q co-deletion. With advent of advanced testing methods, like NGS, comprehensive large-scale gene information is readily available, which may give a better insight of the tumor biology. Few molecular signatures such as IDH1/2 mutation may aid to distinguish between true progression versus pseudoprogression. Further, these signatures may serve as follow-up marker especially in conjunct with newer technologies like liquid biopsy for early recurrence and treatment response evaluation.

The guidelines as of now do not recommend the use of multi-gene panel testing for treatment decision-making. Further, different panels of genes are offered and this varies from laboratory to laboratory. The choice of ordering the multi-gene panel is at the discretion of the treating physician. Currently, there is no standardized testing protocol and panel of genes to be analysed. Therefore, the implications of these alteration(s) in treatment needs more understanding and further validation.

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Appendix

List of 315 genes along with 28 translocations

ABL1	BRAF	CHEK1	FANCC	GATA3	JAK2	MITF	PCDC1LG2	RBM10	STAT4
ABL2	BRCA1	CHEK2	FANCD2	GATA4	JAK3	MLH1	PDGFRA	RET	STK11
ACVR1B	BRCA2	CIC	FANCE	GATA6	JUN	MPL	PDGFRB	RICTOR	SUFU
AKT1	BRD4	CREBBP	FANCF	GID4	KATBA	MRE11A	PDK1	RNF43	SYK
AKT2	BRIP1	CRKL	FANCG	GLI1	KDM5A	MSH2	PIK3C2B	ROS1	TAF1
AKT3	BTG1	CRLF2	FANCL	GNA11	KDM5C	MSH6	PIK3CA	RPTOR	TBX3
ALK	BTK	CSF1R	FAS	GNA13	KDM6A	MTOR	PIK3CB	RUNX1	TERC
AMER1	C11orf30	CTCF	FAT1	GNAQ	KDR	MUTTYH	PIK2CG	RUNX171	TERT (PROMOTER)
APC	CARD11	CTNNA1	FBXW7	GNAS	KEAP1	MTYDC	PIK3R1	SDHA	TET2
AR	CBFB	CTNNB1	FGF10	GPR124	KEL	MYC	PIK3R2	SDHB	TGFBR2
ARAF	CBL	CIUL3	FGF14	GRIN2A	KIT	MYCL	PLCG2	SDHC	TNFAIP3
ARFRP1	CCND1	CYLD	FGF19	GRM3	KLHL6	MYCN	PMS2	SDHD	TNFRSF14
ARID1A	CCDN2	DAXX	FGF23	GSK3B	KMT2A	MYD88	POLD1	SETD2	TOP1
ARID1B	CCND3	DDR2	FGF3	H3F3A	KMT2C	NF1	POLE	SF3B1	TOP2A
ARID2	CCNE1	DICER1	FGF4	HGF	KMT2D	NF2	PPP2R1A	SLIT2	TP53
ASXL1	CD27	DNMT3A	FGF6	HNF1A	KRAS	NFE2L2	PRDM1	SMAD2	TSC1
ATM	CD79A	DOT1L	FGFR1	HRAS	LMO1	NFKB1A	PREX2	SMAD3	TSC2
ATR	CD79B	EGFR	FGFR2	HAD3B1	LRP1B	NXX2-1	PPKAR1A	SMAD4	TSHR
ATRX	CDC73	EP300	FGFR3	HSP90AA1	LYN	NOTCH1	PRXCI	SMARCA4	U2AF1
AURKA	CDH1	EPHA3	FGFR4	IDH1	LZTR1	NOTCH2	PRKDC	SMARC91	VEGFA
AURKB	CDK12	EPHA5	FH	IDH2	MAGI2	NOTCH3	PRDSS8	SMO	VHL
AXIN 1	CDK4	EPHA7	FLCN	IGF1R	MAP2K1	NPM1	PTCH1	SNCAIP	WISP3
AXL	CDK6	EPHB1	FLT1	IGF2	MAP2K2	NRAS	PTEN	SOCS1	WT1
BAP1	CDK8	ERBB2	FLT3	IKBKE	MAP2K4	NAD1	PTPN11	SOX10	XPO1
BARD1	CDKN1A	ERBB3	FLLT4	IKIF1	MAP3K1	NTRK1	OKI	SOX2	ZBTB2
BCL2	CDKN1B	ERBB4	FOXL2	ILTR	MCL1	NTRK2	RAC1	SOX9	ZNF217
BCL2L1	CDKN2A	ERG	FOXP1	INNBA	MDM2	NTRK3	RAD50	SPEN	ZNF703
BCL2L2	CDKN2B	ERRF11	FRS2	INPP4B	MDM4	PAK3	RAD51	SPOP	
BCL	CKDN2C	ESR1	FUBP1	IRF2	MED12	PALB2	RAF1	SPTA1	
BCOR	CEBPA	EZHZ	GABRA6	IRF4	MEF2B	PARK2	RANBP2	SRC	
BCORL1	CHD2	FAM46C	GATA1	IRS2	MEN1	PAX5	RARA	STAG2	
BLM	CHD4	FANCA	GATA2	JAK1	MET	PBRM1	RB1	STAT3	

28 Gene rearrangements

ALK	FGFR3
BCL2	KIT
BCR	MSH2
BRAF	MYB
BRCA1	MYC
BRCA2	NOTCH2
BRD4	NTRK1
EGFR	NTRK2
ETV1	PDGFRA
ETV4	RAF1
ETV5	RARA
ETV6	RET
FGFR1	ROS1
FGFR2	TMPRSS2

List of 48 gene panel

ABL1	CSF1R	FGFR3	JAK2	NOTCH1	RET
AKT1	CTNNB1	FLT3	JAK3	NPM1	SMAD4
ALK	EGFR	GNA11	KDR	NRAS	SMARCB1
APC	ERBB2	GNAQ	KIT	PDGFRA	SMO
ATM	ERBB4	GNAS	KRAS	PIK3CA	SRC
BRAF	FBXW7	HNF1A	MET	PTEN	STK11
CDH1	FGFR1	HRAS	MLH1	PTPN11	TP53
CDKN2A	FGFR2	IDH1	MPL	RB1	VHL