INTRODUCTION

Glioblastoma

Brain tumors account for about 85–90% of all primary central nervous system (CNS) tumors. Worldwide, approximately 343,175 new cases of brain and other CNS tumors were diagnosed in the year 2012 (http://www.cbtrus.org/). Glioblastoma or glioblastoma multiforme (GBM) is the most lethal and clinically challenging of brain tumors. Most patients die of their ailment in less than a year (Stupp et al., 2005). Some of the reasons for high fatality are the complex nature and diffuse character of the tumor itself and the high rate of disease recurrence. As the name infers, it is multiforme microscopically showing regions of pseudopalisading and hemorrhage, multiforme genetically with various genetic alterations leading to its aggressive nature.

The standard of care for treatment of GBM includes surgical resection followed by radiation and chemotherapy. The addition of a chemotherapeutic agent, Temozolamide in recent years changed the median survival of for GBM patients to 14.6 months from 12.1 months with surgery and radiotherapy (Stupp et al., 2005). Also, currently there is no
standard of care available for recurrent disease and most of the patients die. Hence there is an urgent need to develop molecular targeted therapy for this devastating disease.

Some of the molecular alterations responsible for GBM progression and therapeutic resistance includes genetic and epigenetic alterations, activation of stem cell pathways, and changes in the tumor microenvironment and cellular metabolism. However, the functional consequences of many of these alterations are largely unknown in GBM tumorigenesis (Frattini et al., 2013; Schonberg et al., 2013).

**Omic**

With the sequencing of the human genome, the study of biological systems underwent a major genomic revolution. The major technological breakthroughs in high-throughput assay development, technological advancements in instrumentation and bioinformatics data analysis have reshaped how we view the cancer genome (Vucic et al., 2012). “Omics” refers to the study of cancer as a whole entity focusing on the various micro- and macro-molecules. It includes (but not limited to) DNA mutations, copy number changes, epigenetic changes like DNA methylation, transcriptome analysis and whole-genome DNA/RNA sequencing. The omics-based recent approaches including genomics, transcriptomics, epigenomics, proteomics and metabolomics have unveiled the molecular mechanisms behind various cancers and assisted in identification of next-generation molecular markers for early diagnosis, prognosis, predictive of response to treatments and predisposition to gliomas (Chin, 2013; Cho, 2010) (Fig. 1). The publically available multi-omics databases collected by International Cancer Genome Consortium

![Figure 1: Omics in glioblastoma.](image-url)
(ICGC) and The Cancer Genome Atlas (http://cancergenome.nih.gov/) a network group using a sample cohort of several hundred clinical specimens of GBM further elaborated the molecular processes fundamental to GBM pathogenesis (Hudson et al., 2010; Verhaak et al., 2010).

Genomics/Transcriptomics
Early work on gene expression analysis of gliomas employed DNA microarrays and attempted to correlate mRNA signatures with the grades of gliomas and their clinical behavior to aid in overall prognosis and treatment response of patients (Kim et al., 2002; Mischel et al., 2004). Transcriptomics is the study of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell using high-throughput methods, such as microarray analysis, allowing the identification of genes that are differentially expressed in distinct cell populations. Recent multi-omics (genomics, transcriptomics and proteomics) data integration studies have utilized patient derived samples and cell lines to reveal heterogeneity among the primary GBM, suggesting additional molecular subclasses: neural, proneural, classical and mesenchymal (Verhaak et al., 2010). These subtypes were defined on the basis of distinct gene signatures and also characterized by different molecular alterations and activated pathways (Brennan et al., 2013; Verhaak et al., 2010). The proneural subtype was mostly characterized by abnormalities in platelet-derived growth factor receptor-α (PDGFRA) or in isocitrate dehydrogenase 1 (IDH1); whereas mutation of the epidermal growth factor receptor (EGFR) was found in the classical subgroup, and mutations in neurofibromin 1 (NF1) were common in mesenchymal tumors. The neural subtype seemed to be similar to the classical subtype but with a higher frequency of TP53 mutations (Brennan et al., 2013). Cytogenetic and molecular studies have also identified a number of recurrent chromosomal abnormalities and genetic alterations in malignant gliomas, as well as novel candidates, particularly in GBMs. The identification of molecular subtypes has revealed a set of core signaling pathways commonly activated in GBM (Table 1) (Furnari et al., 2007) and could be used in molecular targeted therapies (Table 2).

Epigenomics
Epigenetic changes involve enzymatic modifications of DNA and associated histone proteins to regulate gene expression. In recent years these changes have been recognized as important causes of phenotypic changes in human cancers (Esteller, 2007). The epigenetic changes are dynamic in nature and play an important role in gene expression and DNA structure. Epigenetic alterations, especially those related to changes in histone acetylation, are a recent focus for therapeutic
drug targeting in clinical trials. Genomic-array (microarray) techniques studying DNA methylation have identified frequent promoter-associated hypermethylation of specific loci accompanying tumor suppression in GBM (Sturm et al., 2014), such as (CDKN2A), RB1, PTEN, TP53 (Amatya et al., 2005; Baeza et al., 2003; Costello et al., 1996; Nakamura et al., 2001) and other previously unrecognized regulatory genes EMP3, PDGFB (Alaminos et al., 2005; Bruna et al., 2007). Most significantly, O-6-methylguanine-DNA methyltransferase (MGMT) promoter hypermethylation was identified occurring in ~45% of adult patients with GBM (Brennan et al., 2013; Esteller et al., 1999). MGMT hypermethylation leads to gene silencing, and reduced gene expression levels which compromises its ability to repair damaged DNA by alkylating agents like Temozolomide (Felsberg et al., 2011). Thus, gene methylation could be used as a biomarker.

<table>
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<tr>
<th>GBM Biomarkers</th>
<th>Role in GBM prognosis</th>
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<tr>
<td>EGFR amplification</td>
<td>EGFR amplification is the most common event in primary GBM, with EGFRvIII being the most prominent mutated receptor tyrosine kinase receptor occurring in ~50% of GBM cases that overexpress EGFR (Verhaak et al., 2010). A potential predictive biomarker for molecular therapies.</td>
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<td>EGFRvIII mutation</td>
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<td>PDGFRA</td>
<td>PDGFRA is mainly mutated and expressed in abnormally high amounts in proneural tumors (Verhaak et al., 2010) and associated with poor prognosis in IDH1 mutant GBM (Brennan et al., 2013).</td>
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<td>TP53 mutation</td>
<td>TP53 gene although mutated, has no predictive or prognostic role. Can distinguish tumor grade (Brennan et al., 2013).</td>
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<td>1p/19q Co-deletion</td>
<td>1p/19q co-deletion is the most common genetic alteration in oligodendroglioma tumors and is associated with favorable response to chemotherapy, radiation and survival (Alaminos et al., 2005).</td>
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<td>MGMT promoter methylation</td>
<td>Promoter methylation of MGMT gene, inactivates DNA repair function (Esteller et al., 1999). It is the first predictive epigenetic biomarker with a putative diagnostic role in detecting pseudoprogression. MGMT methylation helps in molecular stratification of patients for Temozolomide therapy (Malstrom et al., 2012).</td>
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<td>VEGF</td>
<td>VEGF is considered to be the driving factor of tumor angiogenesis and has been identified in 64.1% GBMs. It is a strong predictor of survival, in patients with gliomas (Reynes et al., 2011)</td>
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<td>PTEN</td>
<td>A gene level biomarker with poor survival outcomes for GBM (Baeza et al., 2003). PTEN is deleted in 50–70% of primary and 54–63% of secondary GBM. Also mutated in 14–47% primary GBM. Mutation is linked to resistance to targeted EGFR inhibitors in GBM (Deberardinis et al., 2008).</td>
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<td>IDH1/2 mutation</td>
<td>IDH1 mutation is now recognized as an important driver in the etiology of low-grade and secondary brain tumors (48). Has prognostic value in WHO grade III and IV GBM. Accumulation of oncometabolite 2-hydroxyglutarate (2HG) considered as metabolomic imaging biomarker for mutant IDH1 gliomas (Chen et al., 2014).</td>
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to predict sensitivity to chemo-radiotherapy (Malmström et al., 2012; Wick et al., 2012). Glioma CIMP (G-CIMP) is a powerful determinant of tumor aggressiveness (Brennan et al., 2013; Riemenschneider et al., 2010). These epigenomic and other omics analyses have revealed several mutations, altered proteins, miRNA expressions and pathways associated with GBM pathogenesis and prognosis.

Table 2: Molecular targeted therapies for glioblastoma

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<tr>
<th>Pathways targeted</th>
<th>Agents</th>
<th>Molecular targets</th>
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<tr>
<td><strong>Epidermal Growth Factor Pathway</strong></td>
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<td>EGFR is amplified and frequently mutated in ~50% of GBMs and is overexpressed in many malignant gliomas. Therefore could be used as a therapeutic targeted agent in GBM patients.</td>
<td>Erlotinib (Roche)</td>
<td>Kinase inhibitors of EGFR</td>
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<td></td>
<td>Gefitinib (AstraZeneca)</td>
<td></td>
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<tr>
<td><strong>VEGF Pathway</strong></td>
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<td>Targeting vascular endothelial growth factor (VEGF) pathways to induce anti-angiogenic effects in the treatment of malignant gliomas has been in focus for past few years.</td>
<td>Bevacizumab (Avastin; Genentech)</td>
<td>Recombinant human neutralizing monoclonal antibody to VEGF</td>
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<td></td>
<td>Vatalanib (Novartis)</td>
<td>Kinase inhibitor of VEGFR/PDGFR</td>
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<td></td>
<td>Cediranib (AstraZeneca)</td>
<td>pan-VEGF inhibitor</td>
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<td><strong>Transforming Growth Factor β (TGF-β) Pathway</strong></td>
<td>Trabedersen (Antisense Pharma)</td>
<td>Anti-sense TGF-β2 mRNA</td>
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<td>TGF-β is a multifunctional cytokine, which regulates glioma cell motility, invasion, and immune surveillance. Several small molecule inhibitors of TGF-β receptors have shown antitumor efficacy in preclinical models of gliomas.</td>
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<td><strong>PI3K–AKT–mTOR Pathways</strong></td>
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<td>PI3K pathways regulate several malignant phenotypes including antiapoptosis, cell growth, proliferation, and invasion. Activated PI3K phosphorylates several downstream effectors, including AKT. mTOR is a major player connecting multiple pathways downstream from AKT.</td>
<td>Rapamycin (Sirolimus)</td>
<td>inhibitors of m-TOR</td>
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<td></td>
<td>Tensirolimus (Sirolimus)</td>
<td></td>
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<td></td>
<td>Everolimus (Novartis)</td>
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<td><strong>PKC Pathways</strong></td>
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<tr>
<td>Protein kinase C (PKC) is a serine/threonine kinase that regulates cell proliferation, invasion, and angiogenesis.</td>
<td>enzastaurin (Eli-Lilly)</td>
<td>PKC-β inhibitor with activity against glycogen synthase kinase 3β</td>
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Note: Several of the above agents are being evaluated in clinical trials as monotherapies or in combination with other treatment modalities such as chemotherapy or radiation in patients with malignant gliomas.
Proteomics

Proteomic profiling represents the large-scale examination of protein expression, post-translational modification, and understanding how different proteins interact with each other. Using various bioinformatics techniques, the information can be unified into protein networks. Currently, histopathology represents the gold standard for the typing and grading of gliomas and depends largely on certain architectural similarities of tumor cells with normal glial cells (Riemenschneider et al., 2010; Tohma et al., 1998). We feel that the underlying disease pathology would result into differential proteomic profiling of diseased tissue and the surrounding disease-free normal tissue. Recent technological advances in proteomics has allowed analysis of glioma patient biopsies, proximal fluids, cerebrospinal fluid (CSF) and cyst fluid, plasma, glioma cell lines. This has allowed a comprehensive proteomic profiling of glioma biology to aid the traditional histopathology in improving our understanding of glioma processes and to better evaluation of drug responses to treatment (Somasundaram et al., 2009). The techniques involve evaluation of protein arrays, including antibody and aptamer arrays. This allows simultaneous detection of multiple proteins/phosphoproteins. These high throughput techniques can be used for efficient biomarker validation, treatment monitoring and can be translated into clinical applications in an affordable manner. Various plasma/serum biomarkers have been identified earlier for GBM including YKL-40, GFAP and matrix metalloproteinase-9 (Jayaram et al., 2014). Reynes et al. (2011) reported inflammatory markers (C-reactive protein, IL-6 and TNF-) and angiogenesis markers such as VEGF and soluble VEGF receptor 1 to be significantly elevated in the plasma of GBM patients. Jung et al. (2007) identified GFAP as a discriminatory serum biomarker for GBM. Similarly, serum osteopontin (OPN), validated using IHC and ELISA in GBM patients, was shown to correlate with poor prognosis (Sreekanthreddy et al., 2010). In an extended effort, the TCGA group also generated protein expression data from 214 GBM patient samples using a high throughput antibody-based reverse phase protein arrays (RPPAs) (Brennan et al., 2013) revealing several mutations, altered genes, proteins and their pathways underlying GBM pathophysiology (Dong et al., 2010).

Some of the challenges in using protein profiling more commonly in characterizing and quantifying accepted protein biomarkers includes high costs, lengthy production times and most importantly lack of high specificity antibodies. Moreover, the proteomic approach has the potential to identify novel diagnostic, prognostic, and therapeutic biomarkers for human gliomas. The application of proteomics in neuro-oncology is still in its developing stage. Please refer recent reviews by Whittle et
al. (2007) and Niclou et al. (2010) for more on the current status of glioma proteomics and its clinical applications.

Metabolomics
Nearly a century ago, Otto Warburg made a seminal observation that even in the presence of adequate oxygen cancer cells metabolize glucose by aerobic glycolysis, termed as Warburg effect (Warburg et al., 1924; 1927). Moreover, very recently disease-related altered cellular metabolism has come into forefront of cancer research. Now, there is increasing evidence that the underlying genetic alterations contributing to glioma pathogenesis is also responsible for altered cellular metabolism (Parsons et al., 2008). Metabolomics refers to the global quantitative assessment of endogenous enzyme kinetics, cellular biochemical reactions, and synthesis of cellular metabolites within a biologic system, (Boros et al., 2005; Griffin and Shockcor, 2004). Although considerable progress has been made in understanding GBM biology through genetic analysis, little is known about the underlying metabolic alterations in glioma. In recent years, several biochemical and biophysical techniques such as Mass Spectrometry (MS), liquid- and gel-chromatography, Magnetic Resonance Spectroscopy (MRS), Nuclear Magnetic Resonance (NMR), have helped in profiling global metabolomic signatures in cancers including glioma (Dunn et al., 2005; Serkova and Niemann, 2006). Several key differences in metabolite profiles have been identified in GBM cancer cells when compared to normal controls, providing a novel insight into GBM tumorigenesis (Spratlin et al., 2009). As metabolomics reflect underlying altered genotype-phenotype, it can be used as a predictive biomarker for measure of efficacy and as a pharmacodynamic marker, for both traditional chemotherapy and hormonal agents. Using the 1H-NMR spectra and neural networks, human glioma cell cultures can be separated into drug-resistant and drug-sensitive groups before treatment with nitrosourea treatment (El-Deredy et al., 1997).

Frequent genetic alterations in glioma such as MYC amplification, PTEN deletion or protein loss and EFRG amplification are associated with multiple downstream metabolic targets (Deberardinis et al., 2008). IDH1 and IDH2 metabolic genes are mutated in ~12% of primary gliomas, 86% of grade II and III gliomas and secondary glioblastoma through a gain-of-function mutation that alters the enzymatic activity of the protein product, which results in the production of 2-hydroxyglutarate (Dang et al., 2009). The detection of 2-HG metabolic product has been proposed to be a potential tool for in vivo distinction of secondary from primary glioblastomas (Esmaeili et al., 2013). More recently Chen et al. (2014) showed that IDH1-mutant glioma growth is facilitated by overexpression of glutamate dehydrogenase 2
gene (GLUD2) and it could be targeted for growth inhibitory effects. Hence, metabolomics applications in a clinical perspective may have a favorable impact on glioma grade, metabolic state and treatment stratification of glioma patients.

Other Omics: microRNAs
In recent years, microRNAs (miRNAs) have emerged in the forefront of cancer molecular biology. MicroRNAs are key post-transcriptional regulators that inhibit gene expression by promoting mRNA decay or suppressing translation (Iorio and Croce, 2012). Experimental and clinical evidence supports that miRNAs play pivotal role in cancer gene regulation, proliferation, apoptosis and metastasis (Cho, 2011; Iorio and Croce, 2012). The functional role of miRNAs was first discovered in human gliomas (Li et al., 2013). Several miRNA expressions are found to be dysregulated in GBM. TCGA group identified alterations in 149 miRNAs (Dong et al., 2010) and an expression signature comprising 10 miRNAs with prognostic prediction (Srinivasan et al., 2011). miR-128, miR-342 and miR-21 are known to play both oncogenic and tumor suppressive roles and are being explored as possible markers for GBM (Dong et al., 2010; Srinivasan et al., 2011). More recently several lines of evidence have implicated over-expression of miR21 with chemo and radioresistance of GBM cells. Its expression levels have been associated with glioma grade and as a candidate independent marker for overall survival (Chao et al., 2013; Wu et al., 2013). Thus, integrative omics analysis has revealed the importance and scope of translational repression in microRNA-mediated GBM pathogenesis. Please refer to additional reviews (Karsy et al., 2012; Nikaki et al., 2012; Sana et al., 2011) for more detailed coverage on miRNA expression and function in GBM.

Omnics data integration methods
The post-human genome project era has generated enormous heterogeneous and large data sets. As vast gene profiling datasets and technologies are being developed, they have created an unprecedented need to develop technologies to process the data in a meaningful way. The efforts have yielded meaningful results in cancer biomarker discovery, protein interactions and genotype to phenotype correlations (Park et al., 2005). However, current omics technologies cannot model interactions between multiple molecules by analyzing individual genes, proteins or metabolites. This is often not very effective due to the complex and heterogeneous nature of human cancers. Cancer is a complex biological system and requires a better understanding of the disease's complexity at systems-level (Faratian et al., 2009; Hu et al., 2013). Pathway and network based methods have taken more important role
in analysis of high-throughput data, that can provide a global and systematical way to explore the relationships between biomarkers and their interacting partners (Wang et al., 2015). Integration of data from multiple omic studies can not only help unravel the underlying molecular mechanism of carcinogenesis but also identify the signature of signaling pathway/networks characteristic for specific cancer types that can be used for diagnosis, prognosis and designing tumor targeted therapy.

Most recently, attempts at integration of multiple high-throughput omics data have concentrated on comparing data acquired using various experimental conditions/platforms to explore functional and regulatory associations between genes and proteins (Faith et al., 2007; McDermott et al., 2009). This has culminated into combining functional characterization and quantitative interactions extracted from various biomolecules such as DNA, mRNA, proteins and metabolites (Chen et al., 2011; Coban and Barton, 2012; Mitchell et al., 2013) (Fig. 1). Some analysis utilizes pathways in the form of connected routes through a graph-based representation of the metabolic network (Blum and Kohlbacher, 2008). Other approaches focus on the functional module of protein interaction network and analyze experimental data in the context of pathways using multiple source omics data (Wang et al., 2012; Blazier and Papin, 2012; Federici et al., 2013). Although currently there are tools available to process large datasets generated by one platform, it is expected that soon tools combining data across multiple platforms will be available to researchers. This will help in integrating research results into a framework of whole biological systems to support translation of research into clinical applications.

Omics advantages in GBM therapy
So far clinical translation of an effective GBM therapy has been hindered by multiple factors, including diffuse infiltration at the time of diagnosis, significant cellular heterogeneity (both intratumoral and intertumoral), difficulty in crossing the blood-brain barrier by effective drugs, and the role of tumor progenitor cells in reestablishment of resistant disease following chemo and radiotherapy. Current standard treatment of GBM consists of attempted gross total surgical resection followed by concurrent temozolomide and radiation therapy (RT) (Clarke et al., 2010). Although, RT provides good local control, it is not very beneficial in controlling the disease recurrence. In case of GBM, majority of patients die from recurrent disease, as currently there is no effective therapy for recurrent GBM. Therefore, the addition of systemic chemotherapy to RT can help in controlling recurrence and offering an additional radiosensitization benefits in GBM, benefiting both definitive and palliative strategies for disease management (Clarke et
So far non-omics studies have identified few GBM targets at the protein level, but fail to see an overall role of molecules in signaling pathways, protein-protein interactions, and role in metabolic processes. Unfortunately so far only one drug has been identified (Temozolomide) which can radiosensitize GBM patients. Thus, non-omics techniques will compliment whole genomic/epigenomics/metabolomics approach of omics technologies. Without publically available databases, the surge of preclinical and clinical information seen in the GBM field over last few years, would have not been possible. As omics studies expand our understanding of the molecular pathways driving GBM tumorigenesis, more druggable targets will be identified to treat GBM patients. Also, understanding of ionizing radiation at the level of molecular biology will lead to development and production of targeted radiosensitizers. Temozolomide is currently the only radiosensitizing agent used for GBM with class I evidence of benefit (Mrugala and Chamberlain, 2008). It is a novel oral bioavailable second-generation alkylating agent. At physiologic pH it undergoes hydrolysis to its active form methyl traizeno-imidazole carboxamid (MTIC). The mechanism of action of MTIC, is to transfer a methyl group to the middle guanine in a GGG sequence to convert it to O6-methylguanine. Temozolomide exerts its antineoplastic activity by interfering with repair of damaged DNA after radiation treatment (Mrugala and Chamberlain, 2008). In a recent randomized trial, concomitant and adjuvant Temozolomide chemotherapy with radiation, significantly improved progression free survival from 12.1 months to 14.6 months, for GBM patients (Clarke et al., 2010; Stupp et al., 2005). The consequent analysis of these patients by Hegi et al. (2005) reported that patients with methylated MGMT gene promoter were benefited from this treatment compared to patients with unmethylated MGMT promoter. The MGMT promoter methylation silences the gene function required to reverse the O6-guanine methylation and therefore cannot counteract the action of Temozolomide. Thus, omics has been helpful in predicting tumor response to Temozolomide and to guide clinical decision making. The other most common types of chemotherapies for GBM under investigation include targeted molecular therapies, antiangiogenic therapies, immunotherapies, gene therapies, radiation-enhancement therapies and drugs to overcome resistance (Table 2).

**Challenges and Prospective**

Oncogenic transformation is a complex, multistep process that differs widely between and even within cancer types. Advances in the large scale omics technologies have led to identification of promising GBM disease biomarkers. The major challenge is how to
bring omics research into accurate and reliable clinical use. In case of GBM, omics technologies have their limitations due to late diagnosis of disease, intrinsic molecular complexity and genetic heterogeneity of GBMs. To find consistencies that can be therapeutically targeted on the basis of molecular analysis, poses a major problem. However, we are optimistic that the wealth of information generated by omics techniques has paved a roadmap for designing new therapeutic agents for GBM. The advances with respect to gene expression profiling, signaling pathway characterization, glioma stem cell identification, regulatory RNA studies, metabolomic changes and immunomodulation approaches, have resulted in several ongoing clinical studies evaluating new therapeutic agents for GBM (Table 2). It is evident that omics based cancer research is going to play a pivotal role in diagnosis, treatment and monitoring of GBM patients.

CONFLICT OF INTEREST
The authors claim no conflict of interest.

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