# The Promise of Molecular Profiling for Identification and Treatment of Glioblastoma


## Abstract

Glioblastomas are the most aggressive tumours of the central nervous system, with an average life expectancy of 15 months following diagnosis. Despite several advances in treatment and standard of care, patient survival remains poor, mainly due to the inherent heterogeneity of tumours and the lack of personalized therapies. Molecular profiling has shown potential in predicting patient outcomes and providing insights into clinically relevant biomarkers for the development of targeted therapies. The clinical integration of biomarkers such as MGMT, IDH1/2 and EGFR has enabled more accurate stratification on the basis of tumour aggressiveness and patient survival. This review will cover innovations in the molecular profiling of GBM tumours and new insights in overall and progression-free survival (OS and PFS respectively) of patients. As well, sensitivity and response to therapies such as temozolomide will be assessed in relation to molecular profiles that have been identified. More specifically, the review outlines the current identified molecular biomarkers as well as their prognostic value, in order to determine whether they should be used when diagnosing and treating patients.



## Key Words

Glioblastoma; Molecular Profiling; Mutation; Biomarker


## Introduction

Glioblastoma (GBM) is the most common malignant tumour of the central nervous system in adults, accounting for 46.6% of all primary brain tumours (Ostrom et al. 2016). In GBM, glial cells, cells that support and insulate neurons, undergo a series of changes to become tumorigenic. Ninety percent arise *de novo* (primary glioblastoma), developing quickly and without any lower grade precursor. Ten percent arise as secondary GBMs which develop more slowly from lower-grade tumours and have a much more favourable prognosis (Urbańska et al. 2014). Although the molecular pathways of the two are different, they have the same structure and physical characteristics. Both forms of GBM most often occur in the cerebral hemispheres, with few occurring in the cerebellum, brainstem and spinal cord (Hanif, 2017).

GBM is the most aggressive tumour of the central nervous system, classified as a grade IV glioma by the World Health Organization (WHO) (Diamandis and Aldape, 2018). Due to its highly invasive nature, the median survival rate is only 15 months, with only 5.5% of patients surviving 5 years after diagnosis (Ostrom et al. 2016). GBM more commonly occurs in older adults (aged 75 to 84) with lower chances of survival with increasing age. Incidence is 1.57 times higher in males and 1.93 times higher in Caucasians than in African Americans (Ostrom et al. 2016). Risk factors may include previous exposure to radiation, certain immune disorders and occupational exposure to vinyl chloride or synthetic rubber, although evidence remains inconclusive (Davis, 2016). The current standard of care for primary GBM

involves maximum surgical resection, followed by radiotherapy and temozolomide, a medication effective in containing GBM tumour growth (Dresemann, 2010). However, no standard of care has been established for secondary or recurring GBM (Hottinger et al. 2016). Although advances in surgical approaches and chemotherapy have resulted in small improvements in the past decade, GBM remains fatal and incurable.

Traditionally, GBM has been classified histopathologically, a classification system based on physical characteristics such as areas of necrosis (cell death), microvascular proliferation (duplication of cells lining the interior of blood vessels) and cellular morphology (potential cell of origin) (Urbańska et al. 2014). However, there are limitations to how accurate and effective these diagnostic methods can be as there is no evidence that supports correlations between these characteristics to responses to various therapies (Abedalthagafi et al. 2018). Recently, molecular profiling has become an important area of research, exploring specific biomarkers or changes in the genetic profile of tumours, being used as another method of classifying GBM (Diamandis and Aldape, 2017). Using a biopsy sample retrieved by surgical resection, testing determines the molecular makeup of cancer cells and determines the mutations that have taken place (Kummar et al. 2015). Various molecular characteristics such as DNA copy number, gene expression and DNA methylation drive the transformation of healthy cells into malignant cancer cells. Understanding the mechanisms of its aggressive nature has the potential of leading to better management, more targeted therapies and improved patient outcomes of GBM. Because of this, the use of molecular profiling has become recognized more recently as a powerful diagnostic tool.

## Materials and Methods

Databases entitled PubMed, Neuro-Oncology, Google Scholar and ScienceDirect were searched using the following search terms: “Glioblastoma”, “Biomarkers”, “Overall Survival” and the individual names of biomarkers studied. Studies were filtered based on the types of tissue used. Studies using cell lines were discarded as they do not fully represent a tumour’s genomic profile. As well, more studies using fresh frozen tissue were chosen, if available, over those using paraffin-embedded blocks to ensure the quality of the samples. All pediatric studies, defined as using patients less than 18 years old, were also discarded as their genomic profiles are completely different from that of adult Glioblastomas. Additionally, studies with samples size of less than 10 were discarded as their genomic profiles are not representative of diverse populations.

## Results

### *O6-methylguanine–DNA methyltransferase (MGMT)*

The MGMT gene encodes for a critical repair protein that removes harmful substances, such as alkylating agents, that may be attached to DNA during DNA replication (Rivera et al. 2009). While this protein protects normal cells, it is also able to protect tumour cells from alkylating agents such as temozolomide, making the tumour resilient to therapy. Since the protein is responsible for ensuring proper replication of DNA, tumour cells benefit from the

protein as they can divide without being damaged by temozolomide (Hegi, 2005). Thus, the silencing of MGMT through a process called methylation results in a loss of cell repair activity, which allows easier and more effective control of the tumour (Hegi, 2005). In GBM, MGMT methylation is identified as an important independent prognostic factor, associated with a more favourable outcome. MGMT methylation leads to improved OS, with an average increase of 7-9 months in survival between patients with methylated versus unmethylated MGMT (Kim, 2012). In a study conducted where the genomes of long term survivors (longer than 30 months) and short term survivors (less than 30 months) were compared, MGMT methylation was detected in a significant portion of long term survivors because of the patients' improved responses to temozolomide (Smrdel, 2016). There were varying results in regards to PFS, with the majority of data concluding that MGMT methylation does lead to an increase in PFS (Rivera et al. 2009)(Kim, 2012). However, one study which only used data from patients older than 70, concluded that it results in no significant difference in PFS (Cao, 2009). This discrepancy is attributable to the narrow chosen age range, yet may depict that MGMT methylation does not make a difference in PFS for much older patients specifically, while increasing PFS overall.

*Isocitrate dehydrogenase (IDH)*

IDH1/2 are genes that encode for enzymes and proteins which serve various functions such as aiding in cell metabolism and DNA repair (Molenaar, 2018). Through a series of reactions, IDH1/2 produce nicotinamide adenine dinucleotide phosphate (NADPH), which is responsible for protecting the cell from harmful molecules called reactive oxygen species (ROD) (Brandes, 2009). When IDH1/2 are mutated, they can no longer catalyze the reactions necessary to produce NADPH, resulting in increased ROD levels and subsequently resulting in apoptosis (Cohen, 2014). Mutated IDH1/2 have also been shown to produce new enzymes that consume NADPH, further depleting its supply (Bleeker, 2010). As NADPH is no longer able to repair DNA, cells become more sensitive to various therapies. Overall, IDH1/2 mutations have shown to be independent and favourable prognostic factors. IDH1 mutations are primarily observed in younger patients, leading to a significantly better prognosis than older patients with unmutated IDH1 (Bleeker, 2010). As well, IDH1/2 mutations were primarily observed in secondary GBM, being detected in less than 6% of primary GBM (Yao, 2012). IDH1/2 mutations resulted in an improved OS, being shown as a favourable prognostic factor in patients under the age of 50, with OS significantly decreasing with an increase in age (Christians, 2019). While data regarding PFS varied, stronger evidence showed that PFS increased with an IDH1/2 mutation, almost doubling (Songtao, 2011). A study with inconclusive results regarding PFS has insufficient amounts of data due to a lack of proper follow up (Dunn, 2009). Interestingly, IDH1/2 mutations were heavily correlated with MGMT methylation as patients identified with having both these mutations showed the best response to temozolomide and consequently, had the best OS and PFS (Molenaar, 2014). The best OS was observed in patients with: both an IDH1 mutation and MGMT methylation, only an IDH1 mutation, only MGMT methylation, and without an IDH1 mutation or MGMT methylation (Songtao, 2011).

*Tumour protein 53 (TP53)*

TP53 is a gene that codes for protein 53, which regulates the cell cycle and plays a crucial role in cell apoptosis. In this way, the protein maintains cellular stability and is triggered by DNA damage, resulting in the arrest of further tumour growth by preventing the replication of damaged DNA (Jesionek-Kupnicka et al. 2014). If the TP53 gene is mutated, the protein is inactive and cannot block the proliferation of damaged cells. The TP53 mutation occurs in about 30% of cases of primary GBM and is found in 65%–90% of cases with secondary GBM (Uhm, 2009). TP53 mutations are not a strong prognostic factor, showing no significant difference in the OS or PFS of mutated and unmutated patients (Shiraishi et al. 2002). One study found that TP53 mutations were a poor prognostic factor evaluated patients who were not treated with temozolomide as it was not the standard of care at the time, explaining the inconsistency in results (Newcomb et al. 1998). While the role of TP53 in chemo- and radiosensitivity remains controversial, it was noted that silencing of mutant TP53, led to a five-time increase in sensitivity to temozolomide as mutated TP53 directly increases MGMT protein activity (Wang et al. 2013). Interestingly, there is a correlation shown between the importance of TP53 as a biomarker and the patient's age. A study which stratified tumour samples according to age found that patients with a median age of 40 with normal TP53 expression had much higher OS than any other group, whose data showed no significance (Soussi, 2000). However, this, as well as the value of silencing mutated TP53 require further exploration and evidence.

*Phosphatase and tensin homolog (PTEN)*

PTEN is a tumour suppressor gene that creates enzymes responsible for preventing the rapid growth of tumour cells. Through various chemical pathways, PTEN enzymes cause apoptosis and prevent DNA loss in healthy cells (Koul, 2008). Most importantly, they inhibit the activation of Phosphoinositide 3-kinase (PI3K), which is involved in cell growth and survival (Endersby, 2008). Thus, when PTEN is mutated, tumour aggressiveness and growth are increased. PTEN is most commonly one of the first genes to be mutated in human cancers, with mutations being identified in 34% of GBM (Smith, 2001). However, the prognostic value of PTEN in regards to OS and PFS remains controversial. Many studies conclude that it is not a statistically significant factor in OS as patients with unmutated and mutated PTEN have an average survival of 289 days compared to 198 days, respectively (Bäcklund, 2003). Carico C. et al attributes the lack of prognostic value to the use of temozolomide, as tumours with a PTEN mutation are inefficient at repairing cell damage caused by temozolomide (Carico et al. 2012). Thus, the damage caused by temozolomide balances the tumour suppressing effects of PTEN, resulting in no difference being detected between patients with PTEN mutated and unmutated. There is also evidence that PTEN enzyme underexpression leads to reduced OS and PFS. Multiple studies with this conclusion have statistically insignificant sample sizes or use cell cultures, which are unrepresentative of GBM tumour growth. However, this discrepancy is also attributable to the factors that can lead to PTEN underexpression, being that other chemical pathways can result in the loss of PTEN enzyme

activity besides PTEN mutation (Sano, 1999). Therefore, PTEN mutation is not statistically significant in determining OS and PFSand thus is not a prognostic factor (Bäcklund, 2003).

*Epidermal growth factor receptor (EGFR):*
EGFR gene encodes for a type of protein that spans the cell membrane in order to receive signals from the surrounding environment. One end of the protein remains inside the cell, while the other binds to proteins outside the cell, triggering cell growth and division (An, 2018). EGFR is a powerful tool for tumour cells as any alterations to it can promote tumour growth, survival and division. Overexpression of the proteins can increase drug resistance and the aggressiveness of the tumour (Saadeh, 2017). Specifically, EGFRvIII (a type of EGFR mutation), is resistant to preexisting inhibitors in the cell, increasing the tumour cell's ability to proliferate easily (Hatanpaa, 2019). Overall, EGFR alterations are evident in approximately 60% of GBM (An, 2018). EGFR amplification (an increase in proteins), is associated with decreased OS, with the mean survival for EGFR amplified and unamplified being 315.73 days and 657.91 days, respectively (Tripathy, 2014). EGFR amplification coupled with a mutation of EGFRvIII shows the worst OS, attributable to the increased invasiveness of the tumour (Tripathy, 2014). Interestingly, age plays an important factor in the prognostic value of EGFR. When stratified, EGFR amplification showed an unfavourable prognostic outcome for younger patients (less than 60 years old) while showing a better outcome for older patients (greater than 60 years old) (Smith, 2001). While there's inconclusive evidence as to why this discrepancy occurs, EGFR amplification is shown to have an even worse prognostic value when coupled with TP53 unmutated genes (Simmons, 2001). Furthermore, EGFR amplification is correlated with shorter PFS as high expression levels lead to greater resistance against various therapies (Tini et al. 2015).

*Telomerase reverse transcriptase (TERT)*
TERT gene encodes for an enzyme called telomerase, which maintains structures called telomeres. Telomeres line the ends of chromosomes, preventing them from breaking down while shortening after every cell division (Yuan et al. 2016). Eventually, telomeres become so short that they induce apoptosis, which maintains the proper lifespan of various cells. Telomerase adds segments of DNA to chromosomes to counteract the effects of the shortening telomeres, thus, resulting in continued cell division (Heidenreich et al. 2014). While mainly inactive in healthy cells, the mutated TERT gene results in the overproduction of telomerase and therefore, allows a tumour cell to divide indefinitely (Liu et al. 2018). TERT mutations occur in approximately 83% of primary GBM and 10% of secondary GBM, with the mutation increasing with age (Simon et al. 2014). There is a significant decrease in OS with TERT mutated vs. unmutated being 13.8 months compared to 18.4 months, respectively (Chamberlain and Sanson, 2015). Its prognostic value is even more unfavourable when paired with an IDH mutation with median OS decreasing from 37.6 months to 13.8 months. This is attributable to the fact that the favourable impact of an IDH mutation is balanced with the unfavourable impact of the TERT mutation, making the OS the same as standard GBM. Furthermore, TERT mutation is correlated with reduced PFS, with less of an impact than OS (Simon et al. 2014). Overall, a TERT mutation is an independent and unfavourable prognostic factor.

## Discussion

*Histopathological Analysis Vs. Molecular Profiling*

Currently, histopathological diagnosis affects therapeutic decisions and prognostic estimation the most out of any other factor (Nutt et al. 2003). Molecular profiling has proven to be an effective approach over traditional histopathological diagnosis and treatment of GBM, especially when attempting to predict important factors such as OS and PFS. Histopathological diagnosis offers many advantages; it is cost-effective, less time consuming and is generally a more established practice worldwide (Coons et al. 1997). However, guidelines for this analysis, such as evidence of necrosis (cell death) and microvascular proliferation (the formation of blood vessels), remain vague and susceptible to various interpretations by clinicians (Bent, 2010). Molecular profiling has allowed the identification and classification of GBM on a more accurate level and has increased understanding of various clinical behaviours.

MGMT methylation is an independent and favourable prognostic factor, increasing response to temozolomide in patients. IDH1/2 in conjunction with MGMT methylation provides an increased chance of survival in patients. EGFR amplification has been shown to be a poor prognostic factor, especially in younger patients. While data for other identified biomarkers such as TP53, PTEN and TERT remains inconclusive, there is a multitude of biomarkers that have not yet been identified or explored. Therefore, molecular profiling provides advantages such as more specific and reproducible classifications of GBM as well as less variability in the interpretation of identified characteristics. However, there are still several barriers to the implementation of complete molecular profiling in the GBM standard of care. Mainly, a larger amount of tissue is required to complete a molecular profile, which is difficult to acquire from GBM patients during surgical resection, as GBM has undefined edges, blending in with surrounding healthy tissue (Shirahata et al. 2007). A growing field called radiogenomics presents the possibility of eradicating this concern as it allows non-invasive genomic profiling based on imaging such as MRI, rather than tissue extracted by surgical resection (Jamshidi et al. 2014). Cost is also another key factor as routine genome sequencing costs around $500-$800 per case and methylation profiling costs $500 per case (Diamandis, 2017). In contrast, histopathological analysis costs a total of $220-$300, proving far more cost-effective (Diamandis, 2017). Furthermore, it takes almost three times as long for profiling of tissue to be completed when compared to histopathological analysis. However, despite these challenges, molecular profiling provides far more accuracy and specificity than histopathological analysis, something much deserved by GBM patients who face such a dismal prognosis.

*Therapeutic Applications*

Several therapies targeting these biomarkers have entered clinical trial phases, showing their applicability. A novel idea is the introduction of tumour suppressing genes such as TP53 and PTEN into GBM cells through a process called non replicating adenovirus serotype 5 (Hong et al. 2001). This technique, in combination with gene therapy which includes the

introduction of genes that promote apoptosis, shows a decrease in tumour progression and cell growth (Natsume and Yoshida, 2008). The main downside to this approach is the high risk of failed gene transfer into the cells. As well, the use of EGFRvIII vaccine has shown to trigger the body's own immune system in more aggressively attacking the tumour, almost doubling OS in patients administered the vaccine (Sampson et al. 2009). Voxtalisib is another drug being tested, which inhibits PI3K activation when PTEN has been mutated and is unable to do so (Zhao et al. 2017). As discussed previously, PI3K is responsible for promoting cell growth, thus Voxtalisib results in tumour suppression. Lastly, AMG232 is a drug recently developed in an attempt of reactivating a mutated PTEN gene, which contributes to controlling the transformation of normal cells into cancerous ones (Her et al. 2018). Although still limited, developing targeted therapies indicate an exciting new era in the treatment of GBM as they bridge the gap between molecular profiling and biological behaviour.

**Conclusion**

Despite several advancements, GBM remains fatal and available treatment options are far from satisfactory. This is mainly due to the fact that GBMs are diverse, composed of various complex genotypes. Identifying the mechanisms that cause tumour development, expansion and aggressiveness can help subclassify GBM more accurately and develop new therapies that are tailored to each patient. Already, identified biomarkers show the potential to predict OS and PFS of patients, as well as resistance to various drugs. With decreasing costs and increased accessibility to advanced genomic technology, the implementation of regular molecular profiling for GBM is now a more realistic goal. Further research must be done to investigate the role of such biomarkers in relation to one another and as part of greater molecular pathways, rather than single mutations. As well, more studies and clinical trials must be conducted on larger and more diverse groups of patients to develop well-established conclusions about the prognostic value of such biomarkers. Although there are several years of work to follow, molecular profiling and its potential for leading to the discovery of new therapeutics show promising results in lengthening and bettering the lives of GBM patients.

**Abbreviations**

GBM: Glioblastoma
WHO: World Health Organization
OS: Overall survival
PFS: Progression-free survival
MGMT: The O6-methylguanine–DNA methyltransferase
IDH: Isocitrate dehydrogenase
TP53: Tumour protein 53
PTEN: Phosphatase and tensin homolog
EGFR: Epidermal growth factor receptor
TERT: Telomerase reverse transcriptase
NADPH: Nicotinamide adenine dinucleotide phosphate
ROD: Reactive oxygen species

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**References**


Ostrom, Q. T.; Gittleman, H.; Xu, J.; Kromer, C.; Wolinsky, Y.; Kruchko, C.; Barnholtz-Sloan, J. S. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2009–2013. *Neuro-Oncology* [online] **2016**, *18*, 1–75. https://pubmed.ncbi.nlm.nih.gov/28475809/.

Urbańska, K.; Sokołowska, J.; Szmidt, M.; Sysa, P. Glioblastoma Multiforme – an Overview. *Contemp Oncol (Pozn)* [online] **2014**, 307–312. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4248049/.

Hanif, F.; Muzaffar K.; Perveen, K.; Malhi, S.; Simjee, S. Glioblastoma Multiforme: A Review Of Its Pathogenesis And Treatment through Clinical Presentation and Treatment. *Asian Pac J Cancer Prev* [online] **2017,** *18* (1), 3–9. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5563115/.

Diamandis, P.; Aldape, K. World Health Organization 2016 Classification of Central Nervous System Tumors. *Neurologic Clinics* [online] **2018**, *36* (3), 439–447. https://pubmed.ncbi.nlm.nih.gov/29200109/.

Kummar, S.; Williams, P. M.; Lih, C.-J.; Polley, E. C.; Chen, A. P.; Rubinstein, L. V.; Zhao, Y.; Simon, R. M.; Conley, B. A.; Doroshow, J. H. Application of Molecular Profiling in Clinical Trials for Advanced Metastatic Cancers. *JNCI Journal of the National Cancer Institute* [online] **2015**, *107* (4). https://pubmed.ncbi.nlm.nih.gov/30072064/.

Davis, M. Glioblastoma: Overview of Disease and Treatment. *Clinical Journal of Oncology Nursing* **2016**, *20* (5). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5123811/.

Dresemann, G. Temozolomide in Malignant Glioma. *OncoTargets and Therapy* [online] **2010**, 139. https://pubmed.ncbi.nlm.nih.gov/20856849/.

Hottinger, A. F.; Abdullah, K. G.; Stupp, R. Current Standards of Care in Glioblastoma Therapy. Glioblastoma **2016**, 73–80.

Abedalthagafi, M.; Barakeh, D.; Foshay, K. M. Immunogenetics of Glioblastoma: the Future of Personalized Patient Management. *npj Precision Oncology* [online] **2018**, *2* (1). https://www.nature.com/articles/s41698-018-0070-1.

Diamandis, P.; Aldape, K. D. Insights From Molecular Profiling of Adult Glioma. *Journal of Clinical Oncology* [online] **2017**, *35* (21), 2386–2393. https://pubmed.ncbi.nlm.nih.gov/28640696/.

Rivera, A. L.; Pelloski, C. E.; Gilbert, M. R.; Colman, H.; Cruz, C. D. L.; Sulman, E. P.; Bekele, B. N.; Aldape, K. D. MGMT Promoter Methylation Is Predictive of Response to Radiotherapy and Prognostic in the Absence of Adjuvant Alkylating Chemotherapy for Glioblastoma. *Neuro-Oncology* [online] **2009**, *12* (2), 116–121. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2940581/.

Hegi M.; Diserens A.; Gorlia T.; Hamou M.; Tribolet N.; Weller M. MGMT Gene Silencing and Benefit from Temozolomide in Glioblastoma. *The New England Journal of Medicine* [online] **2005**, 352, 997-1003. https://www.nejm.org/doi/full/10.1056/nejmoa043331.

Kim, Y. S.; Kim, S. H.; Cho, J.; Kim, J. W.; Chang, J. H.; Kim, D. S.; Lee, K. S.; Suh, C.-O. MGMT Gene Promoter Methylation as a Potent Prognostic Factor in Glioblastoma Treated With Temozolomide-Based Chemoradiotherapy: A Single-Institution Study. *International Journal of Radiation Oncology*Biology*Physics* [online] **2012**, *84* (3), 661–667. https://pubmed.ncbi.nlm.nih.gov/22414280/.

Smrdel, U.; Popovic, M.; Zwitter, M.; Bostjancic, E.; Zupan, A.; Kovac, V.; Glavac, D.; Bokal, D.; Jerebic, J. Long-Term Survival in Glioblastoma: Methyl Guanine Methyl Transferase (MGMT) Promoter Methylation as Independent Favourable Prognostic Factor. *Radiology and Oncology* [online] **2016**, *50* (4), 394–401. https://pubmed.ncbi.nlm.nih.gov/27904447/.

Cao, V. T.; Jung, T.-Y.; Jung, S.; Jin, S.-G.; Moon, K.-S.; Kim, I.-Y.; Kang, S.-S.; Park, C.-S.; Lee, K.-H.; Chae, H.-J. The Correlation And Prognostic Significance Of Mgmt Promoter Methylation And Mgmt Protein In Glioblastomas. *Neurosurgery* [online] **2009**, *65* (5), 866–875. https://pubmed.ncbi.nlm.nih.gov/19834398/.

Sanson M, Marie Y, Paris S, Idbaih A, Laffaire J, Ducray F et al. Isocitrate Dehydrogenase 1 Codon 132 Mutation Is an Important Prognostic Biomarker in Gliomas. *Journal of Clinical Oncology* [online] **2009**, 27, 4150-4154. https://pubmed.ncbi.nlm.nih.gov/19636000/.

Cohen, A.; Holmen S.; Colman H. IDH1 And IDH2 Mutations in Gliomas. *Curr Neurol Neurosci Rep* [online] **2014**, *13*, 345. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4109985/.

Bleeker, F. E.; Atai, N. A.; Lamba, S.; Jonker, A.; Rijkeboer, D.; Bosch, K. S.; Tigchelaar, W.; Troost, D.; Vandertop, W. P.; Bardelli, A.; Noorden, C. J. F. V. The Prognostic IDH1 R132 Mutation Is Associated with Reduced NADP -Dependent IDH Activity in Glioblastoma. *Acta Neuropathologica* [online] **2010**, *119* (4), 487–494. https://pubmed.ncbi.nlm.nih.gov/20127344/.

Yao, Y.; Chan, A. K.-Y.; Qin, Z. Y.; Chen, L. C.; Zhang, X.; Pang, J. C.-S.; Li, H. M.; Wang, Y.; Mao, Y.; Ng, H.-K.; Zhou, L. F. Mutation Analysis of IDH1 in Paired Gliomas Revealed IDH1 Mutation Was Not Associated with Malignant Progression but Predicted Longer Survival. *PLoS ONE* [online] **2013**, *8* (6). https://pubmed.ncbi.nlm.nih.gov/23840696/.

Christians, A.; Adel-Horowski, A.; Banan, R.; Lehmann, U.; Bartels, S.; Behling, F.; Barrantes-Freer, A.; Stadelmann, C.; Rohde, V.; Stockhammer, F.; Hartmann, C. The Prognostic Role of IDH Mutations in Homogeneously Treated Patients with Anaplastic Astrocytomas and Glioblastomas. *Acta Neuropathologica Communications* [online] **2019**, *7* (1). https://actaneurocomms.biomedcentral.com/articles/10.1186/s40478-019-0817-0.

Dunn, J.; Baborie, A.; Alam, F.; Joyce, K.; Moxham, M.; Sibson, R.; Crooks, D.; Husband, D.; Shenoy, A.; Brodbelt, A.; Wong, H.; Liloglou, T.; Haylock, B.; Walker, C. Extent of MGMT Promoter Methylation Correlates with Outcome in Glioblastomas given Temozolomide and Radiotherapy. *British Journal of Cancer* [online] **2009**, *101* (1), 124–131. https://pubmed.ncbi.nlm.nih.gov/19536096/.

Molenaar, R. J.; Verbaan, D.; Lamba, S.; Zanon, C.; Jeuken, J. W.; Boots-Sprenger, S. H.; Wesseling, P.; Hulsebos, T. J.; Troost, D.; Tilborg, A. A. V.; Leenstra, S.; Vandertop, W. P.; Bardelli, A.; Noorden, C. J. V.; Bleeker, F. E. The Combination of IDH1 Mutations and MGMT Methylation Status Predicts Survival in Glioblastoma Better than Either IDH1 or MGMT Alone. *Neuro-Oncology* [online] **2014**, *16* (9), 1263–1273. https://pubmed.ncbi.nlm.nih.gov/24510240/.

Songtao, Q.; Lei, Y.; Si, G.; Yanqing, D.; Huixia, H.; Xuelin, Z.; Lanxiao, W.; Fei, Y. IDH Mutations Predict Longer Survival and Response to Temozolomide in Secondary Glioblastoma. *Cancer Science* [online] **2011**, *103* (2), 269–273. https://pubmed.ncbi.nlm.nih.gov/22034964/.

Jesionek-Kupnicka, D.; Szybka, M.; Malachowska, B.; Fendler, W.; Potemski, P.; Piaskowski, S.; Jaskolski, D.; Papierz, W.; Skowronski, W.; Och, W.; Kordek, R.; Zawlik, I. TP53 Promoter Methylation in Primary Glioblastoma: Relationship with TP53 MRNA and Protein Expression and Mutation Status. *DNA Cell Biol.* [online] **2014**, 217–226. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3967380/.

Uhm, J. Comprehensive Genomic Characterization Defines Human Glioblastoma Genes and Core Pathways. *Yearbook of Neurology and Neurosurgery* [online] **2009**, *2009*, 117–118. https://www.nature.com/articles/nature07385.

Shiraishi, S.; Tada, K.; Nakamura, H.; Makino, K.; Kochi, M.; Saya, H.; Kuratsu, J.-I.; Ushio, Y. Influence of p53 Mutations on Prognosis of Patients with Glioblastoma. *Cancer* [online] **2002**, *95* (2), 249–257. https://pubmed.ncbi.nlm.nih.gov/12124823/.

Newcomb, E. W.; Cohen, H.; Lee, S. R.; Bhalla, S. K.; Bloom, J.; Hayes, R. L.; Miller, D. C. Survival of Patients with Glioblastoma Multiforme Is Not Influenced by Altered Expression of P16, P53, EGFR, MDM2 or Bcl-2 Genes. *Brain Pathology* [online] **1998**, *8* (4), 655–667. https://pubmed.ncbi.nlm.nih.gov/9804374/.

Wang, X.; Chen, J.-X.; Liu, J.-P.; You, C.; Liu, Y.-H.; Mao, Q. Gain of Function of Mutant TP53 in Glioblastoma: Prognosis and Response to Temozolomide. *Annals of Surgical Oncology* [online] **2013**, *21* (4), 1337–1344. https://pubmed.ncbi.nlm.nih.gov/24248532/.

Soussi, T. Analysis of p53 Gene Alterations in Cancer: A Critical View. *25 Years of p53 Research* [online] **2000**, 255–292.

Koul, D. PTEN Signaling Pathways in Glioblastoma. *Cancer Biology & Therapy* [online] **2008**, *7* (9), 1321–1325. https://pubmed.ncbi.nlm.nih.gov/18836294/.

Endersby, R.; Baker, S. J. PTEN Signaling in Brain: Neuropathology and Tumorigenesis. *Oncogene* [online] **2008**, *27* (41), 5416–5430. https://pubmed.ncbi.nlm.nih.gov/18794877/.

Smith, J. S.; Tachibana, I.; Passe, S. M.; Huntley, B. K.; Borell, T. J.; Iturria, N.; Ofallon, J. R.; Schaefer, P. L.; Scheithauer, B. W.; James, C. D.; Buckner, J. C.; Jenkins, R. B. PTEN Mutation, EGFR Amplification, and Outcome in Patients With Anaplastic Astrocytoma and Glioblastoma Multiforme. *JNCI Journal of the National Cancer Institute* [online] **2001**, *93* (16), 1246–1256. https://academic.oup.com/jnci/article/93/16/1246/2519475.

Bäcklund, M.; Nilsson, B.; Goike, H.; Schmidt, E.; Liu, L.; Ichimura, K.; Collins, P. Short Postoperative Survival for Glioblastoma Patients with a Dysfunctional Rb1 Pathway in Combination with No Wild-type PTEN. *Clin Cancer Res* [online] **2003**, *9* (11), 4151-4158. https://pubmed.ncbi.nlm.nih.gov/14519639/.

Carico, C.; Nuño, M.; Mukherjee, D.; Elramsisy, A.; Dantis, J.; Hu, J.; Rudnick, J.; Yu, J. S.; Black, K. L.; Bannykh, S. I.; Patil, C. G. Loss of PTEN Is Not Associated with Poor Survival in Newly Diagnosed Glioblastoma Patients of the Temozolomide Era. *PLoS ONE* [online] **2012**, *7* (3). https://pubmed.ncbi.nlm.nih.gov/22479427/.

Sano, T.; Lin, H.; Chen, X.; Langford, L.; Koul, D.; Bondy, M.; Hess, K.; Myers, J.; Hong, Y.-K.; Yung, A.; Steck, P. Differential Expression of MMAC/PTEN in Glioblastoma Multiforme. *Cancer Research* [online] **1999**, *59* (8). https://pubmed.ncbi.nlm.nih.gov/10213484/.

An, Z.; Aksoy, O.; Zheng, T.; Fan, Q.-W.; Weiss, W. A. Epidermal Growth Factor Receptor and EGFRvIII in Glioblastoma: Signaling Pathways and Targeted Therapies. *Oncogene* [online] **2018**, *37* (12), 1561–1575. https://pubmed.ncbi.nlm.nih.gov/29321659/.

Saadeh, F. S.; Mahfouz, R.; Assi, H. I. EGFR as a Clinical Marker in Glioblastomas and Other Gliomas. *The International Journal of Biological Markers* [online] **2017**, *33* (1), 22–32. https://pubmed.ncbi.nlm.nih.gov/28885661/.

Hatanpaa, K. J.; Burma, S.; Zhao, D.; Habib, A. A. Epidermal Growth Factor Receptor in Glioma: Signal Transduction, Neuropathology, Imaging, and Radioresistance. *Neoplasia* [online] **2010**, *12* (9), 675–684. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2933688/.

Tripathy, K. Prognostic Significance of Epidermal Growth Factor Receptor in Patients of Glioblastoma Multiforme. *Journal Of Clinical And Diagnostic Research* [online] **2017**. https://pubmed.ncbi.nlm.nih.gov/28969132/.

Simmons, M. L.; Lamborn, K. R.; Takahashi, M.; Chen, P.; Israel, M. A.; Berger, M. S.; Godefrey, T. Analysis of Complex Relationships between Age, p53, Epidermal Growth Factor Receptor, and Survival in Glioblastoma Patients. *Cancer Research* [online] **2001**, 1222–1228. https://pubmed.ncbi.nlm.nih.gov/11221842/.

Tini, P.; Cerase, A.; Cevenini, G.; Carbone, S. F.; Miracco, C.; Pirtolli, L. Epidermal Growth Factor Receptor Expression May Correlate with Survival Through Clinical and Radiological Features of Aggressiveness in Glioblastoma Treated with Radiochemotherapy. *Anticancer Research* [online] **2015**, 4117–4124. https://pubmed.ncbi.nlm.nih.gov/26124364/.

Yuan, Y.; Qi, C.; Maling, G.; Xiang, W.; Yanhui, L.; Ruofei, L.; Yunhe, M.; Jiewen, L.; Qing, M. TERT Mutation in Glioma: Frequency, Prognosis and Risk. *Journal of Clinical Neuroscience* [online] **2016**, *26*, 57–62. https://pubmed.ncbi.nlm.nih.gov/26765760/.

Heidenreich, B.; Rachakonda, P. S.; Hemminki, K.; Kumar, R. TERT Promoter Mutations in Cancer Development. *Current Opinion in Genetics & Development* [online] **2014**, *24*, 30–37. https://www.sciencedirect.com/science/article/pii/S0959437X13001548.

Liu, J.; Zhang, X.; Yan, X.; Sun, M.; Fan, Y.; Huang, Y. Significance of TERT and ATRX Mutations in Glioma. *Oncology Letters* [online] **2018**. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6313213/.

Chamberlain, M. C.; Sanson, M. Combined Analysis of TERT, EGFR, and IDH Status Defines Distinct Prognostic Glioblastoma Classes. *Neurology* [online] **2015**, *84* (19), 2007–2007. https://pubmed.ncbi.nlm.nih.gov/25964481/.

Simon, M.; Hosen, I.; Gousias, K.; Rachakonda, S.; Heidenreich, B.; Gessi, M.; Schramm, J.; Hemminki, K.; Waha, A.; Kumar, R. TERT Promoter Mutations: a Novel Independent Prognostic Factor in Primary Glioblastomas. *Neuro-Oncology* [online] **2014**, *17* (1), 45–52. https://pubmed.ncbi.nlm.nih.gov/25140036/.

Nutt, C. L.; Betensky, R.; Tamayo, P.; Cairncross, G.; Ladd, C. Gene Expression-Based Classification of Malignant Gliomas Correlates Better With Survival Than Histological Classification. *Cancer Res* [online] **2003**, 1602–1607. https://pubmed.ncbi.nlm.nih.gov/12670911/.

Coons, S. W.; Johnson, P. C.; Scheithauer, B. W.; Yates, A. J.; Pearl, D. K. Improving Diagnostic Accuracy and Interobserver Concordance in the Classification and Grading of Primary Gliomas. *Cancer* [online] **1997**, *79* (7), 1381–1393. https://pubmed.ncbi.nlm.nih.gov/9083161/.

Bent, M. J. V. D. Interobserver Variation of the Histopathological Diagnosis in Clinical Trials on Glioma: a Clinician's Perspective. *Acta Neuropathologica* [online] **2010**, *120* (3), 297–304. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2910894/.

Shirahata, M.; Iwao-Koizumi, K.; Saito, S.; Ueno, N.; Oda, M.; Hashimoto, N.; Takahashi, J. A.; Kato, K. Gene Expression-Based Molecular Diagnostic System for Malignant Gliomas Is Superior to Histological Diagnosis. *Clinical Cancer Research* [online] **2007**, *13* (24), 7341–7356. https://pubmed.ncbi.nlm.nih.gov/18094416/.

Jamshidi, N.; Diehn, M.; Bredel, M.; Kuo, M. D. Illuminating Radiogenomic Characteristics of Glioblastoma Multiforme through Integration of MR Imaging, Messenger RNA Expression, and DNA Copy Number Variation. *Radiology* [online] **2014**, *270* (1), 1–2. https://pubs.rsna.org/doi/full/10.1148/radiol.13130078.

Hong, Y.; Joe, Y.; Lee, K.; Son, B.; Jeun, S.; Chung, D.; Cho, K.; Park, C.; Kim, M.; Kim, H.; Yung, W.; Kang, J. Potentials and Limitations of Adenovirus-p53 Gene Therapy for Brain Tumors. *J Korean Med Sci.* [online] **2001**, 315–322. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3054646/.

Natsume, A.; Yoshida, J. Gene Therapy for High-Grade Glioma: Current Approaches and Future Directions. *Cell Adh Migr.* [online] **2008**, *2* (3), 186–191. https://pubmed.ncbi.nlm.nih.gov/19262115/.

Sampson, J. H.; Archer, G. E.; Mitchell, D. A.; Heimberger, A. B.; Herndon, J. E.; Lally-Goss, D.; Mcgehee-Norman, S.; Paolino, A.; Reardon, D. A.; Friedman, A. H.; Friedman, H. S.; Bigner, D. D. An Epidermal Growth Factor Receptor Variant III-Targeted Vaccine Is Safe and Immunogenic in Patients with Glioblastoma Multiforme. *Molecular Cancer Therapeutics* [online] **2009**, *8* (10), 2773–2779. https://pubmed.ncbi.nlm.nih.gov/19825799/.

Zhao, H.-F.; Wang, J.; Shao, W.; Wu, C.-P.; Chen, Z.-P.; To, S.-S. T.; Li, W.-P. Recent Advances in the Use of PI3K Inhibitors for Glioblastoma Multiforme: Current Preclinical and Clinical Development. *Molecular Cancer* [online] **2017**, *16* (1). https://pubmed.ncbi.nlm.nih.gov/28592260/.

Her, N.-G.; Oh, J.-W.; Oh, Y. J.; Han, S.; Cho, H. J.; Lee, Y.; Ryu, G. H.; Nam, D.-H. Potent Effect of the MDM2 Inhibitor AMG232 on Suppression of Glioblastoma Stem Cells. *Cell Death & Disease* [online] **2018**, *9* (8). https://www.nature.com/articles/s41419-018-0825-1.

## Appendix

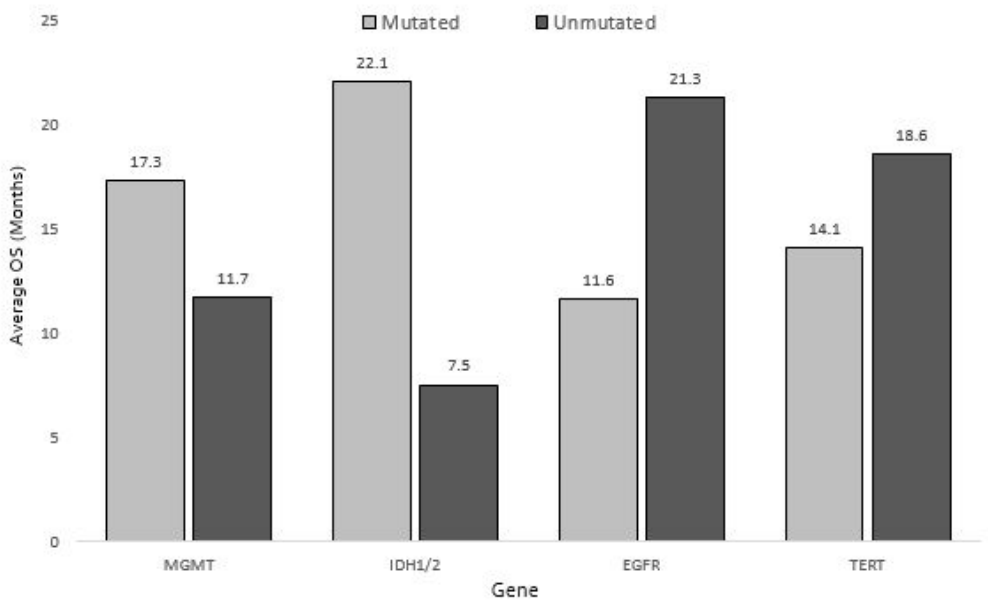


***Figure 1***: Average overall survival of patients based on data collected from studies reviewed, showing the prognostic value of each gene and whether a mutation of each gene is favourable or unfavourable. TP53 and PTEN were excluded due to inconclusive data.

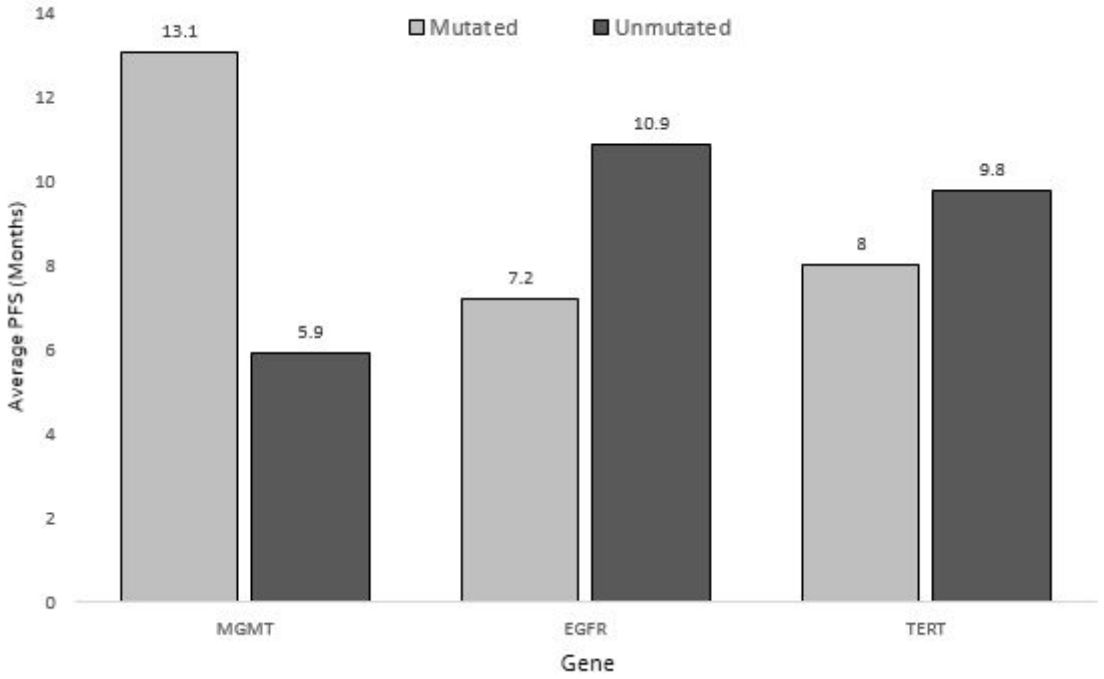


***Figure 2:*** Average progression-free survival of patients based on data collected from the studies reviewed, showing whether each mutation determines a favourable or unfavourable prognosis. IDH1/2, TP53 and PTEN were excluded due to inconclusive data.