Title name: Mechanisms of drug resistance in Glioblastoma Multiforme

Running Title: Therapeutic resistance of Temozolomide in Glioblastoma

Author Information:

Dr. TimothyAllen, MD,Ph.D.

Affiliation: Global Allied Pharmaceutical, Center for Excellence in Research & Development. 160 Vista Oak Dr.Longwood, FL32779, USA

Phone: 1-321-945-4283

Email: timothy.allen@gapsos.com

Corresponding Authors:

Dr. Giridhar M.N.V, MD, MBA.

Lead Medical Officer,

Affiliation: Global Allied Pharmaceutical

Phone: 1-321-445-1969

Email: giridhar.maddirevula@gapsos.com

Abstract:

Glioblastoma multiforme (GBM) is a group of low-grade as well as high-grade brain tumors that originate from the glia;neuroglial cells and are the most common and crucial brain tumor, specially in adults. The main causes are deemed to be associated with: Age, race, pre- existence of low grade brain tumor, which over the time may turn into a higher/more progressive grade, and genetic pre- dispositions(Neurofibromatosis, Tuberous sclerosis,Von Hippel-Lindau disease, Li-Fraumeni syndrome, and Turcot syndrome).Current standard of care includes surgical resection, which is followed by radiation therapy and chemotherapy with agent temozolomide (TMZ). These malignant glioblastomic tumor cells have the ability of intrinsic or acquired

resistance to surgical, radiation and chemotherapy. Despite the advancement in the therapeutic area, all tumors recur and have a median survival rate of around 12- 14 months only. [21,23] *Temozolomide (TMZ) plays an important role as a chemotherapeutic agent for the treatment of GBM, but the satisfaction rate is not up to the mark. This review article focuses on the role of resistance of Temozolomide in the GBM.*

Keyword: DNA repair, glioblastoma, temozolomide, O6-methylguanine-DNA methyltransferase, apoptosis, autophagy, resistance, Anticancer Drug, Cancer Therapy, Cell Death, DNA Methylation

Introduction:

Tumor originating from the glial cells or supportive tissue of the brain is known as "glioma". Tumors of astrocytes are known as "Glioblastomas", because of their star shape.These tumors are well connected to the various blood vessels, therefore they grow very quickly and are highly malignant. Location of Glioblastomas is mainly in the cerebral hemispheres, but can also present anywhere in the brain or spinal cord. Glioblastomas (GBM) are highly malignant heterogenous type of cells that metastasize by ample blood supply, but along with the presence of dead cells at the center of the tumor. There are two main types of glioblastomas: "Primary" and "Secondary"

tumor. Primary tumors are prompt in the action and very common, while secondary usually have longer and slower growth history. [1] Among all types of Astrocytoma, grade IV is classified as glioblastoma, developed from gial cells. [2] Glioma accounts for approximately 80% of primary tumor, out of which 50% glioma are gliobastomas. They are common in adults of age range 45-65. [3] Gliomas are primarily found in men than women in adults, although 9 % of the children experience GBMs. Approximately 18000 new cases are diagnosed with GBM every year in the US only. In comparison, around 13000 deaths are reported every year in US alone. [4]

The median overall survival times of the patients are 8.1 months in 2000-03 to 9.7 months in 2005-08 after the implementation of temozolomide. [5]

Without any treatment and diagnosis, patient's overall survival is limited to 3 months. Age and KPS scale (karnofsky performance status (refer appendix:02) of the patient have an impact on the prognosis of the patients once diagnosed. [6] The WHO has classified a grading system for the tumor of astrocytes: Grade I gliomas are less aggressive, whereas grade IV is most malignant and aggressive, which is GBM.

Staging of Gliobastoma

The WHO has developed a grading system of Gliomas, based on hypercellularity, presence of necrosis, mitosis rate and vascular proliferation.

Grade	Description	EGFR response to Glioma(%)
Grade I- (juvenile pilocytic astrocytoma)	Slow growing tumor, Non-cancerous, Benign,	15-20 %
Grade II (tumor astrocytoma)	Absence of necrosis, Hypercellular, absence of vascular proliferation,	
Grade III (anaplastic astrocytoma)	Rapid mitosis, high rate of hypercellualrity, tumor recurrence	30-35 %
Grade IV (glioblastoma)	High rate of hypercellualrity and mitosis, presence of necrosis and vascular proliferation	40-50 %

Table 1: Stages of GBM[5]

Characteristics of GBM

Clinical symptoms experienced by patients include, slow progressive neurological deficit along with headache, seizures and increased intracranial pressure. Its etiology is not well understood, but few causes are noted: Genetic factors (Neurofibromatosis, Tuberous sclerosis,Von Hippel-Lindau disease, Li-Fraumeni syndrome, and Turcot syndrome), head injury, electromagnetic field exposure, etc. Genetic abnormalities of different chromosomes may lead to the development of tumor. Symptoms of GBM are related to the site of the tumor more than its pathological properties. [8] The main characteristic of GBM depends upon the abnormal level of EGFR (epidermal growth factor receptor). EGFR plays very significant role in the growth of cells. It is a type of protein which is found on the surface of cells and binds with the growth factor and helps in the signaling. [9] Genomic profiling of GBM categorizes it into four subtypes, based on the genetic alteration and different molecular profiles: Classical; mesenchymal, proneural and neural respectively. [10] Gene expression of EGFR, NF1 and PDGFRA/IDH1 describes classical; mesenchymal, proneural and neural respectively. [11](Figure 1)

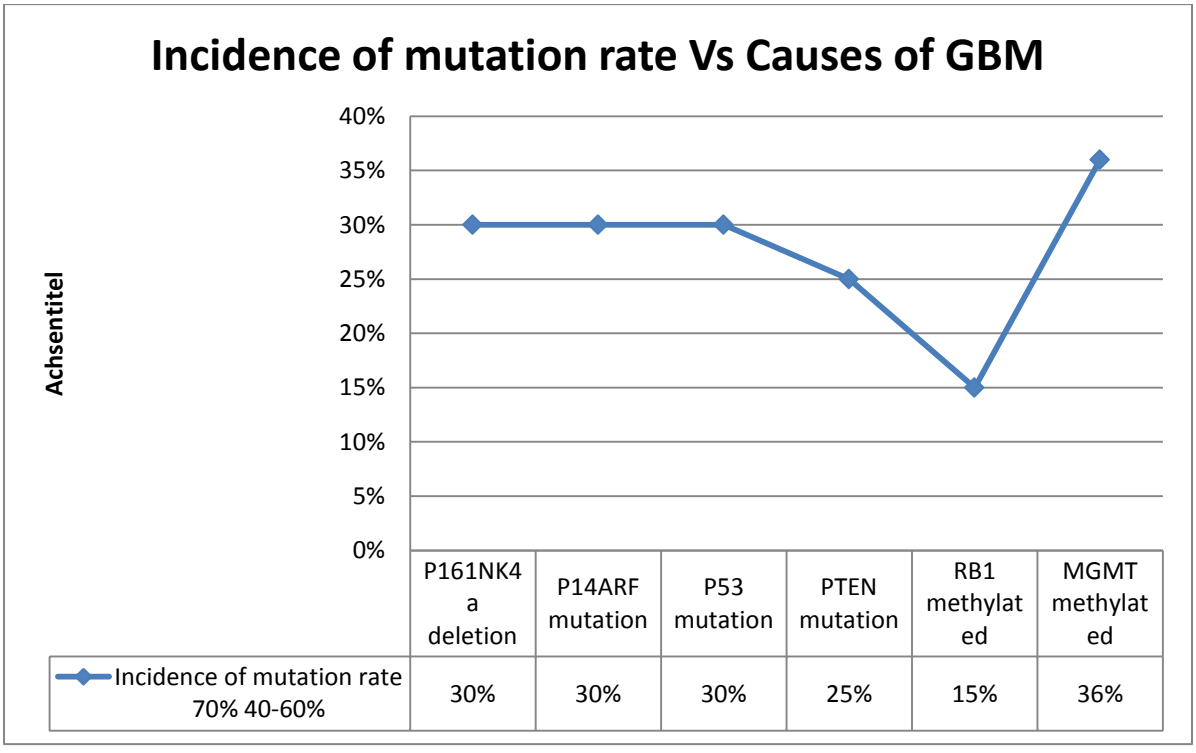

Figure 1: Statisical representation of incidence Vs common causes of GBM [12]

Pathogenesis of Glioblastoma:

Malignant GBM are standardized as primary and secondary. Primary GBM is reported to be around 60% in people with 50 years of age and above, whereas 40% of secondary GBM develops in younger patients. Primary or *de novo* type of glioblastoma are very aggressive and accounts for the majority of the cases. The GBM's overall progression rate ranges from 1- 10 years. Primary tumors show their effects suddenly and without histopathologic evidence of a preexisting lesion. Secondary GBM is the result of pathological evaluation of tumors such as low grade extracts and anaplastic atrocities over the 4-5 years of time. Mainly development of GBM occurs, when the induration of anaplastic matter has been seen along with presence of gray matter, nearby the hemorrhagic necrosis where neoplastic cells are generally located. These areas are denominated as a progression region for anaplastic astryocytoma. The predominant anaplastic form of cells contains elongated nucleus and also have gray matter in the tumor, which diffuses easily through fibrous tracts to the different part of the body. [13]

Treatment Option of Glioblastoma

1. Primary treatment:

Although MRI investigates the proper appearance of GBM, but histological diagnosis is mandatory. GTR (gross tumor resection) is a diagnostic technique, in which contrast enhancing tumor is considered an important prognostic factor for the patients with glioblastoma. The primary goal of surgical treatment is to remove all visible tumors and least remnants should be present. These days, new technique like intraoperative MRI, helps in the complete resection. The microscopic nature of the tumor cells is the main problem of the procedure to achieve successful result of GTR. The microscopic invisible tumor cells sometimes migrate from the tumor boundaries. In this case, surgical GTR is not efficient. [14]

Maximum possibility of safe resection can be achieved along with the temozolomide and radiation therapy, which is significant in the treatment. GTR has a median rate of survival of 6 months, whereas, combined therapy of radiation and resection therapy showed 12.1 months. The best result has come through the combination therapy with resection, radiation and chemotherapy with tolozolomide in which, the median survival rate increased till 14.6 months. [15]

2. Radiation and chemotherapy:

Standard care of treatment with radiation therapy is EBRT (external beam radiation therapy), in which, radiation is directed into the body from outside at the cancerous tissues. Characteristic of

EBRT is focal and fractionated. Radiation therapy is usually used alone or in combination with chemotherapy or surgery. Stereotactic radiation therapy and brachytherapy, also used for the treatment of the brain cancer. In this therapy, patients are exposed to 60 Gy IMRT or 3 Dimensional conformal radiation therapy. However, the clinical difference cannot be measured. IMRT are preferred due to ease in planning. [16]

Treatment protocol: Management's opinion differs for grade II lesions, especially for grade II astrocytoma. Dosage differs from institute to institute, although a standard protocol has been defined. (Please refer appendix 1)

Resistance to drugs in glioblastoma cells:

Malignant glioblastoma is highly chemo-resistant. So, it has become a great challenge for the oncologist today. Due to the inherent resistance to radio and chemotherapy, median survival is less than 12 months. Even in advancement in treatment option like, surgery, chemotherapy and radiotherapy, a palliation of symptoms has been seen in the case of GBM, which leads to the death of the patients. [17,18] In the last two decades, chemotherapy has become the considerable treatment option of glioblastoma. Some alkalating substances such as carmustine, lomustine, and nimustine have been used since long time. [19, 20] However, the use of temozolomide is more common in the market these days and is considered as the main drug for the treatment of GBM in chemotherapy. [21. 22] TMZ along with surgical resection and radiotherapy shows improved survival and progression free metabolic function in patients with GBM. [21].On the other hand, it provides 12-14 months of survival rate.[21,23] After all types of treatment option in GBM patients, 90% of the chance remains for recurrence of the tumor. [24]

Drug- Resistance mechanism:

The main reason behind the resistance in GBM includes DNA damage repair, hypoxic areas of tumor cells, cancer stem cells and miRNA. Many of the therapeutic aspects focus on this area of specification for the treatment aspects. [25]

TMZ (Temozolomide), key role in chemotherapy for GBM patients

TMZ is 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-as-tetrazine-8-carboxamide and acts as an alkylating agent, which is indicated for the treatment of GBM along with the radiotherapy and acts as a maintenance agent. It does not affect directly, but it goes under rapid non-enzymatic

conversion to the 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC) at a particular pH and thus, results in alkylation of the DNA. [26]

Methylation of DNA is the main principal mechanism for the cytotoxicity of TMZ to the malignant tumor cells of the brain. Conversion of the TMZ to the MTIC is initiated by the effect of water on the C4 position of the TMZ. Due to this action, CO_2 is released and converted into the MTIC. [24, 25] MTIC further degrades to the methyldiazonium cation, which undergoes the changes by transferring methyl group from the DNA and as a degradation product AIC (5-aminoimidazole-4-carboxamide) is made, which is degraded by kidney. [27,28]

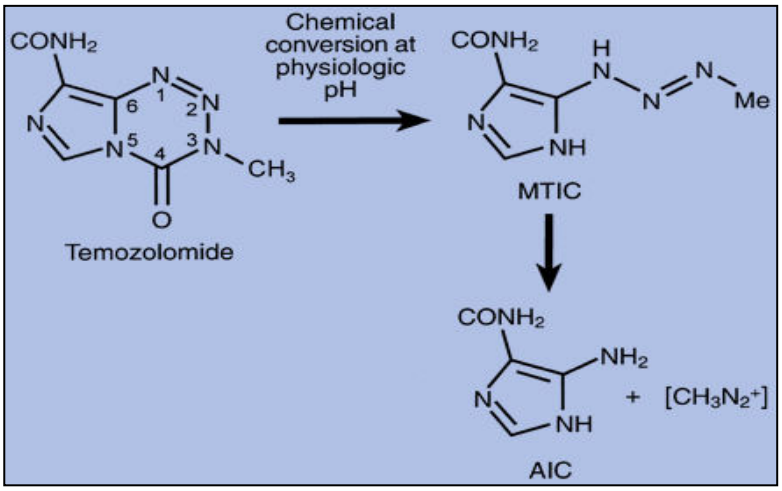

Figure 1: Mechanism of action of TMZ

The most common methylation occurs in the N7-guanine position followed by N3-adenine and O6 guanine. [28, 29]

Mechanism of action in TMZ:

Most explained resistance mechanism to TMZ is regulated by DNA repair protein, also known as MGMT (Methyl Guanine Methyl Transferase) or AGT (O6-alkylguanine DNA alkyltransferase). It acts on the methyl groups from O6-MeG lesions which is the end result of temozolomide treatment. [30]

O6-alkylguanine DNA alkyltransferase (AGT): There are two basic mechanisms of resistance, involved for alkylating agents and temozolomide: AGT enzyme and the MMR (mismatch repair) pathway. AGT plays a pivotal role in the resistance mechanism to temozolomide by the removal of the alkyl groups from the O^6 position of guanine, which induces the reverse effect of temozolomide. Alkylating agents DTIC ((3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, or dacarbazine), BCNU (bis-chloroethylnitrosourea) or carmustine and temozolomide (TMZ) can be correlated with AGT level in terms of sensitivity caused by the tumor cell lines. Beside this, transfer of retrovirus mediated human AGT gene to the cells which are active in the AGT activities, induce a high level of resistance on temozolomide and other chloroethylating and methylating agents. [30]

Poly(ADP)-ribose polymerase(PARP): Base excision repair pathway is the another possible mechanism of resistance to the TMZ. Human tumor cells when treated with temozolomide, increases the activity of PARP, which is functionally involved in the neucleotide excision repair and inhibition of PARP reports the enhancement of the cytotoxicity of the methylating agents. PARP inhibitors, along with the cell lines were deficit in excision repair or MMR pathway, which was indicated for the role of repair mechanism of N7-methylguanine and O3-methyladenine, which adducts in the resistance for TMZ and other alkylating agents for the antitumor activity. [30]

Probable target agents for the GBM

Due to the highly resistant nature of GBM cells to the cytotoxic chemotherapy and radiation therapy, treatment of GBM became a major concern to overcome. Aberrant signalling pathways play a potential role in targeting tumor cells,as shown in figure 2.[31]

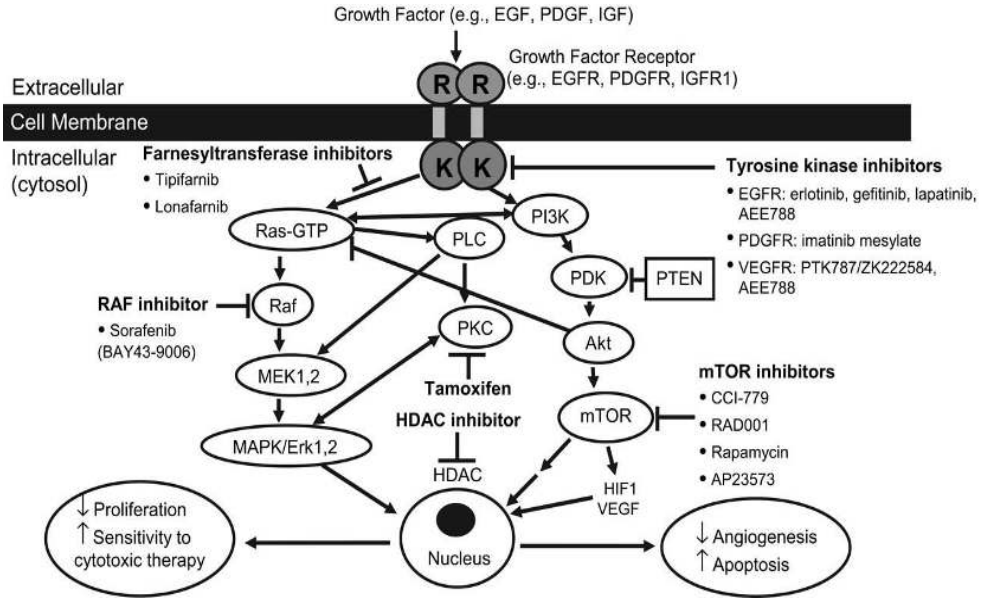

Figure 2: Signalling Pathway

These therapeutics involve three major steps [32]

- Activation of apoptosis.
- Inhibition of growth factor and receptor.
- Blockage of angiogenesis.

David A. Reardon and his associates in 2005, conducted a phase II study for the investigation of the combination therapy of imatinib mesylate and hydroxyurea, which is a tyrosine kinase inhibitor and ribonucleotide reductase inhibitor respectively. For the treatment of patients having recurrent GBM, 500 mg of a combination of imatinib mesylate plus hydroxyurea was administrated twice in a day and imatinib mesylate was given alone to the patients on EIAEDs (enzyme-inducing antiepileptic drugs). The assessment time was every 28 days and the primary end point was 6 months. From the results, it was observed that 27 % of patients who had progression free rate at 6 months, 9 % of patients achieved radiographic response, whereas 42 % stayed at a stable stage of disease. Most common adverse events were neutropenia, thrombocytopenia and edema. Altogether, imatinib mesylate plus hydroxyurea was considered well tolerated in the recurrent GBM. [33]

Future aspects on therapeutic targets:

The main mechanism of cell deaths involved in the TMZ is still little known. Despite of enhancement in therapy such as of O^6-methylating agents TMZ, the median survival level is low. Hence, there is a need to enhance therapy aspects of the GBM patients. Target of achieving a success rate in the medical industry focuses on the knowledge related to mechanism of cell death, which is caused due to the action of glioblastoma cells. Recently discovered AMPK (5' AMP-activated protein kinase) pathway may play a crucial role, [34-36]

Conclusion:

GBM is the most lethal and aggressive form of the brain tumor. It is highly resistant to the chemo, radio and surgical therapeutic technique. The most common, chemo drug Temozolomide is considered as best suited therapeutic option, but even it has also less median survival rate. In patients with GBM, resistance depends on the effect of MGMT (O(6) -methyl-guanine DNA methyltransferase) and defective MMR pathway. The clinical benefit of patients depends on the methylation process of MGMT promoters. Enhancement of different molecular mechanism and research may play role in overcoming the resistance in GBM.

Abbreviations:

GBM: Glioblastoma Multiforme
MGMT: O (6) -methyl-guanine DNA methyltransferase
AGT: O6-alkylguanine DNA alkyltransferase
MMR :mismatch repair pathway
EGFR: Endothelial growth factor receptor
EBRT: external beam radiation therapy
TMZ: Temozolomide
GBM: glioblastoma multiforme
AMPK : 5' AMP-activated protein kinase
EIAED: enzyme-inducing antiepileptic drugs
PARP: Poly(ADP)-ribose polymerase

DTIC : 3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, or dacarbazine

BCNU : bis-chloroethylnitrosourea

MTIC: 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide

References:

1. American Brain Tumor Associations, 'Glioblastoma' Available at: http://www.abta.org/brain-tumor-information/types-of-tumors/glioblastoma.html

2. National Brain Tumor Society, Tumor Types. Available at: http://www.braintumor.org/brain-tumor-information/understanding-brain-tumors/tumor-types/#glioblastoma-multiforme

3. Glioblastoma and Malignant Astrocytoma. Publication: About Brain Tumors: A Primer for Patients and Caregivers. Available at: http://www.abta.org/secure/glioblastoma-brochure.pdf

4. Schwartzbaum JA, Fisher JL, Aldape KD et al.Epidemiology and molecular pathology of glioma. Nature Clinical Practice Neurology. 2006;2:494-503

5. Brown GL, Eckley M and Wargo K.A. A Review of Glioblastoma Multiforme. US Pharm. 2010;35(5)(Oncology suppl):3-10

6. Mirimanoff RO, Gorlia T, Mason W, et al. Radiotherapy and temozolomide for newly diagnosed glioblastoma: recursive partitioning analysis of the EORTC 26981/22981-NCIC CE3 phase III randomized trial. Journal of Clinical Oncology. 2006;24(16):2563–2569

7. Jeffrey N Bruce, Glioblastoma Multiforme Available at: http://emedicine.medscape.com/article/283252-overview updated on : May 2, 2014

8. Verhaak R.G, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. 2010;17(1):98-110

9. Stommel J.M, Kimmelman A.C, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science. 2007;318:287–290

10. Wilson TA, Karajannis MA, and Harter DH. Glioblastoma multiforme: State of the art and future therapeutics, SurgNeurol Int. 2014; 5: 64.

11. Verhaak RGW, Hoadley KA, Purdom E, et al. An integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR and NF1. Cancer Cell. Jan 19, 2010; 17(1): 98.

12. Porter A. A Dead End: A Review of Glioblastoma Multiforme, Eukaryon, Vol. 8, March 2012, Lake Forest College

13. Tutt B. Glioblastoma Cure Remains Elusive Despite Treatment Advances. OncoLog, March 2011;56(3).

14. Cohen KJ. High-Grade Gliomas (2010). Available at: http://www.childhoodbraintumor.org/medical-information/brain-tumor-types-and-imaging/item/80-high-grade-gliomas

15. Stupp R, Mason WP, van den Bent MJ et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352:987–96.

16. Aguilar LK, Arvizu M, Aguilar-Cordova E et al. The spectrum of vaccine therapies for patients with glioblastoma multiforme. Curr Treat Options Oncol. 2012;13:437–50.

17. Ziegler DS, Wright RD, Kesari S, et al. Resistance of human glioblastoma multiforme cells to growth factor inhibitors is overcome by blockade of inhibitor of apoptosis proteins. J Clin Invest. 2008;118(9):3109–3122.

18. Quezada C, Garrido W, Oyarzún C, et al. 5′-ectonucleotidase mediates multiple-drug resistance in glioblastoma multiforme cells. March 2013;228(3):602–608.

19. Weller M, Muller B, Koch R, et al. Neuro-Oncology Working Group 01 trial of nimustine plus teniposide versus nimustine plus cytarabine chemotherapy in addition to involved-field radiotherapy in the first-line treatment of malignant glioma. J ClinOncol 2003; 21:3276-3284.

20. Walker MD, Alexander E Jr, Hunt WE, et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. J Neurosurg 1978;49:333-343.

21. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-996.

22. Yung WK, Albright RE, Olson J, Fredericks R, Fink K, Prados MD, Brada M, Spence A, Hohl RJ, Shapiro W, Glantz M, Greenberg H, Selker RG, Vick NA, Rampling R, Friedman H, Phillips P, Bruner J, Yue N, Osoba D, Zaknoen S, Levin VA: A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. Br J Cancer 2000, 83:588-593

23. Stupp R, Hegi M.E, Mason W.P.et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-Year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009;10:459–466

24. Wen, P.Y, Kesari, S. Malignant gliomas in adults. N. Engl. J. Med. 2008;359:492–507.

25. Haar CP, Hebbar P, Wallace GC et al. Drug resistance in glioblastoma: a mini review. Neurochem Res. 2012 Jun;37(6):1192-200

26. FDA package Insert for TEMODAR, updated on 05/05/2014. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/021029s026lbl.pdf

27. Spassova M. K., Golovinsky E. V. Pharmacobiochemistry of arylalkyltriazenes and their application in cancer chemotherapy. Pharmacol. Ther., 1985;27: 333-352.

28. Denny B. J., Wheelhouse R. T., Stevens M. F. G., et al. NMR and molecular modeling investigation of the mechanism of activation of the antitumor drug temozolomide and its interaction with DNA. Biochemistry, 1994; 33: 9045-9051.

29. Zhang J, Stevens, M.F.; Bradshaw, T.D. Temozolomide: Mechanisms of action, repair and resistance. Curr. Mol. Pharmacol. 2012; 5: 102–114.

30. Park C.K.; Kim J.E.; Kim J.Y et al. The Changes in MGMT Promoter Methylation Status in Initial and Recurrents Glioblastomas. Transl. Oncol. 2012; 5: 393–397.

31. Reardon DA, Tisch PR, and Wen PY. Therapeutic Advances in the Treatment of Glioblastoma: Rationale and Potential Role of Targeted Agents. The Oncologist February 2006;11(2):152-164.

32. Sarafian V, Koev I and Staykov D. Mechanisms Of Cell Resistance In Glioblastoma Multiforme. Journal of IMAB - Annual Proceeding (Scientific Papers) 2009, book 1.

33. Reardon DA, Egorin MJ, Quinn JA. Phase II Study of ImatinibMesylate Plus Hydroxyurea in Adults With Recurrent Glioblastoma Multiforme. JCO December 20, 2005; 23(36):9359-9368.

34. Roos W. P, Batista L. F, Naumann S. C, et al. Oncogene 2007;26:186–197.

35. Garcia-Gil M, Pesi R, Perna S, et al. Neuroscience 2003; 117: 811–820.

36. Luo Z., Saha A. K., Xiang X., et al. Trends Pharmacol. Sci. 2005; 26: 69–76.

37. Jeffrey N Bruce et. Al, Brain Cancer Treatment Protocols; medscape; http://emedicine.medscape.com/article/2005182-overview , last updated on Sep 12, 2013.

38. Hollen PJ, Gralla RJ, et al. Measurement of quality of life in patients with lung cancer in multicenter trials of new therapies. Cancer. 1994; 73: 2087-2098.

Appendix 1: Treatment option and dosage in comparsion to grade of GBM tumor [37]

Grade	Characteristics OF Tumor	Treatment option	Comments	Dosage for radiation therapy
Grade I	*pilocyticastrocytomas*	Surgery and radiation therapy*	**In case primary treatment is not possible entirely*	NA
Grade II	*low-grade infiltrative astrocytomas, oligodendroglioma, mixed gliomas*	Surgery, radiation therapy and adjuvant chemotherapy	1) Low risk or patient with ≤ 40y- survillance 2) high risk - treated with fractionated external-beam radiation therapy or adjuvant chemotherapy 3) chemotherapy is used for low-grade oligodendrogliomas, particularly with 1p19q	1) low-grade astrocytomas- 45-54 Gy, delivered in 1.8 to 2.0 Gy fractions 2) temozolomide 150-200 mg/m^2/day PO on days 1-5 of 28-d cycle for 6-8 cycle 3) for recurrence or

			deletion, which is a marker for tumor susceptibility to chemotherapy	progressive or untreated tumor-temozolomide 75 mg/m^2 PO daily on days 1-21 or 150-200 mg/m^2 PO on days 1-5 of 28-d cycle until disease progression or for maximum of 24 cycles
Grade III	*anaplastic astrocytoma or oligoastrocytoma*	surgical resection followed by external-beam radiation therapy and adjuvant chemotherapy	*NA*	1) Radiation therapy-60 Gy in 30-35 fractions 2) adjuvant chemotherapy with temozolomide 75 mg/m^2/day PO on days 1-42, usually 1-1.5h before radiation and 3) post radition therapy - continue temozolomide at higher doses of 150-200 mg/m^2/day PO on days 1-5 every 28d
Grade IV	*glioblastoma*	surgical resection followed by external-beam radiation therapy and adjuvant chemotherapy	*NA*	1) radiation therapy (60 Gy in 30-35 fractions) adjuvant chemotherapy temozolomide 75 mg/m^2/day PO on days 1-42, usually 1-1.5h before radiation 3) post radiation therapy: continue temozolomide at higher doses of 150-200 mg/m^2/day PO on days 1-5 every 28d

Appendix: 02: KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS

RATING (%) CRITERIA

Status of the patients	Definition rating % Criteria

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

The Karnofsky Performance index classified the patients according to the functional impairment. This is used for the comparison of the effectiveness of various therapies to know the prognosis of the patients. Higher score results for the normal condition whereas, lower for serious. [38]