Congress of Neurological Surgeons Systematic Review and Evidence-based Guidelines Update on the Role of Neuropathology in the Management of Progressive Glioblastoma in Adults

Abigail Goodman (✉ abigail.goodman@atriumhealth.org)

Atrium Health <https://orcid.org/0000-0003-2596-5514>

José E. Velázquez Vega

Children's Hospital of Atlanta

Chad Glenn

The University of Oklahoma

Jeffrey J. Olson

Emory University Hospital

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Abstract

Target population

These recommendations apply to adult patients with progressive or recurrent glioblastoma (GBM).

Question

For adult patients with progressive glioblastoma does testing for *Isocitrate Dehydrogenase (IDH) 1* or *2* mutations provide new additional management or prognostic information beyond that derived from the tumor at initial presentation?

Recommendation

Level III: Repeat IDH mutation testing is not necessary if the tumor is histologically similar to the primary tumor and the patient's clinical course is as expected.

Question For adult patients with progressive glioblastoma does repeat testing for *MGMT* promoter methylation provide new or additional management or prognostic information beyond that derived from the tumor at initial presentation and what methods of detection are optimal?

Recommendation

Level III: Repeat *MGMT* promoter methylation is not recommended.

Question For adult patients with progressive glioblastoma does *EGFR* amplification or mutation testing provide management or prognostic information beyond that provided by histologic analysis and if performed on previous tissue samples, does it need to be repeated?

Recommendation

Level III: In cases that are difficult to classify as glioblastoma on histologic features *EGFR* amplification testing may help in classification. If a previous *EGFR* amplification was detected, repeat testing is not necessary. Repeat *EGFR* amplification or mutational testing may be recommended in patients in which target therapy is being considered.

Question

For adult patients with progressive glioblastoma does whole genome or large panel sequencing provide management or prognostic information beyond that derived from histologic analysis?

Recommendation

Level III: Primary or repeat whole genome or large panel sequencing may be considered in patients who are eligible or interested in molecularly guided therapy or clinical trials.

Question

For adult patients with progressive glioblastoma should immune checkpoint biomarker testing be performed to provide management and prognostic information beyond that obtained from histologic analysis?

Recommendation

Level III: The current evidence does not support making PD-L1 or mismatch repair (MMR) enzyme activity a component of standard testing.

Question

For adult patients with progressive glioblastoma are there meaningful biomarkers for bevacizumab responsiveness and does their assessment provide additional information for tumor management and prognosis beyond that learned by standard histologic analysis?

Recommendation

Level III: No established Bevacizumab biomarkers are currently available based upon the inclusion criteria of this guideline.

Introduction

Rationale

Glioblastoma is the most common primary brain tumor in adults, it is also one the most malignant and fatal brain cancers with a median survival time of only 15 months.[1; 2] Because of the dismal prognosis, intensive standard therapy including surgical resection, radiotherapy, and chemotherapy is employed early in the course of the disease.[3] Despite this, nearly all glioblastomas will eventually recur and no effective standard treatment strategy against recurrent glioblastoma has been established.[4]

While highly variable among institution and clinical setting approximately 25-40% of patients with recurrent glioblastoma will undergo repeat surgery.[5–7] Unfortunately the radiation and adjuvant temozolomide therapy that is part of the current standard treatment for primary glioblastoma may produce tissue injury and necrosis that can be difficult to distinguish from recurrence radiologically.[8–10] These same therapy related effects that can cause radiographic uncertainty can also cause challenges for classification and grading of progressive glioblastoma histologically as well. Knowledge of the patient's clinical history and treatment status, neurosurgical impression, and the neuroradiologic findings are crucial.[8; 11] Additionally, it frequently means the patient has progressed after primary standard therapy and there may be increased interest in determining if the patient would be eligible for or may benefit from alternative treatment options.

We evaluated the current literature addressing the diagnosis of progressive GBM, including histologic alterations present in response to therapy. Ancillary studies including immunohistochemistry and molecular diagnostic techniques in this setting will also be evaluated. With the success of immunotherapy and targeted treatment options in other tumor types there is a greater interest in more comprehensive molecular-genetic evaluation so literature pertaining to these subjects will be reviewed. Since the publication of the previous guideline the impact of select molecular features on the prognosis and progression of primary malignant brain tumors have been better recognized, some of which have been integrated into the revised 4th edition of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS).[12]

Objectives

While the previously published guidelines[8] thoroughly delineated the histologic and immunohistochemical features of progressive glioblastoma limited studies were available at that time to assess what additional molecular diagnostics should be considered. Since that publication numerous studies looking at the role of ancillary and molecular studies for the diagnosis, prognosis, and treatment of glioblastoma have been published. This review addresses these advancements and reviews the recent literature to evaluate what ancillary testing is most appropriate in progressive glioblastoma to help guide treatment and prognosis and when ancillary testing is most appropriate.

Methods

Writing Group and Question Establishment

The evidence-based clinical practice guideline taskforce members and the Joint Tumor Section of the American Association of Neurological Surgeons (AANS) and the Congress of Neurological Surgeons (CNS) have prioritized an update of the guidelines for management of progressive glioblastoma. A series of writers were identified and screened for conflict of interest. This group in turn agreed on a set of questions addressing the role of neuropathology in the diagnosis of progressive GBM and conducted a systematic review of the literature relevant to the histopathologic diagnosis of progressive GBM in addition to immunohistochemical and molecular testing that can be used for either for diagnostic, prognostic, or treatment markers.

Literature Search

A search of PubMed, EMBASE, and Cochrane Library searches of the National Library of Medicine database of scientific literature published between July 1, 2012 and March 31, 2019. A broad search strategy using the following search terms was employed: "Progressive glioblastoma" OR "recurrent glioblastoma" OR "relapsing glioblastoma" OR "treated glioblastoma" OR "recurrent glioma" OR "relapsing glioma" OR "treated glioma" AND diagnosis OR pathology OR cytopathology OR "frozen section" OR radionecrosis OR "radiation necrosis" OR pseudoprogression OR gliosis OR immunohistochemistry OR proliferation OR genetics OR genomics OR prognosis OR accuracy OR "predictive value" OR sensitivity OR

grading OR histology OR molecular OR genetic OR IDH OR biomarker OR ki-67 OR morphology.” We limited our searches to human studies published in the English language. Key words were searched in multiple combinations. Links to “related articles” from highly relevant studies were utilized to broaden the search. Articles were also identified from the reference lists from articles uncovered in initial searches.

Study Selection and Eligibility Criteria

Original articles providing information to establish histopathologic diagnostic criteria for progressive glioblastomas and addressing immunohistochemical and molecular testing, and biomarkers in infiltrating gliomas and progressive glioblastomas were selected for review. A greater focus was put on studies looking at progressive and recurrent IDH-wildtype glioblastomas.

The citations were screened for the following inclusion and exclusion criteria:

Inclusion Criteria

Fully published peer-reviewed primary studies, that were published in English between July 1, 2012 and March 31, 2019 that focused on adult patients (>18 years of age).

Exclusion Criteria:

- Published in abstract form only
- In vitro studies only
- Animal studies only
- Studies focused on non-infiltrative gliomas or other CNS tumors

Those abstracts that met with the selection criteria mentioned above were retrieved in full text form. The adherence to the selection criteria were confirmed. The information was then used for construction of the evidence tables the text below.

Data Collection Process

The search resulted in 923 articles, which were reviewed yielding 283 potentially eligible articles. Links to “related articles” from highly relevant studies were utilized to broaden the search. Articles were also identified from the reference lists from references uncovered in initial searches. We also analyzed the references from prior evidence-based reports on glioblastomas and progressive glioblastomas.[8; 13] (See figure 1.)

Assessment for Risk of Bias

Inherent in research related to pathologic studies in patients with progressive glioblastoma is that they represent patients who were able to undergo a second surgery, likely those who were in a better state of health or younger patients at the time of 2nd surgery. Additionally, these frequently represent patients with a relatively definable lesion, patients who present with more infiltrative disease are often less optimal

patients for repeat surgery. The relatively recent identification of IDH mutations and *MGMT* promoter methylation status and their marked prognostic implications and the reclassification of infiltrating gliomas based on IDH and other molecular features has made it difficult to use historical samples and publications in which molecular testing was not performed. Thus there is a relatively limited time frame of data in which publications assessing progressive glioblastomas with molecular features incorporated into the results and makes using older studies that did not differentiate between these entities difficult to apply to the current classification system. Additionally, pathologic studies are very frequently retrospective, these biases are noted by the authors and the evidence level stratification does attempt to highlight these drawbacks to the reader.

Classification of Evidence and Recommendation Levels

The concept of linking evidence to recommendations has been further formalized by the American Medical Association (AMA) and many specialty societies, including the American Association of Neurological Surgeons (AANS), the Congress of Neurological Surgeons (CNS), and the American Academy of Neurology (AAN). This formalization involves the designation of specific relationships between the strength of evidence and the strength of recommendations to avoid ambiguity. We utilized the “Classification of Evidence on Diagnosis” to evaluate the literature and a summary of this classification can be viewed at <https://www.cns.org/guidelines/guideline-development-methodology>. Much of the pathology literature addressed below are well designed and studied large cohorts giving meaningful outcome data. However, due to the retrospective nature and lack of prospective validation will qualify them as Class III. Generally, Level I recommendations are based on Class I evidence, Level II recommendations are based on Class II evidence and Level III recommendations are based on Class III evidence.

Results

Summary and Commentary on Previously Published Neuropathology Guideline

As noted above this review is an update to the previously published guidelines for progressive glioblastoma by Brat et al.[8] It is useful to briefly review the questions and results from that paper.

The first topic addressed the diagnostic considerations in reporting progressive glioblastoma. A level III recommendation that the pathologist consider the patient’s previous diagnosis and treatment, as well as the current clinical and neuroimaging features that led to a second biopsy or resection. In the setting of prior radiation and chemotherapy, it was recommended the pathologist adhere to strict histologic criteria for microvascular proliferation and necrosis in order to establish a diagnosis of a glioblastoma.

For patients undergoing biopsy or neurosurgical resection at the time of radiologic or clinical progression, reporting the presence and extent of progressive neoplasm as well as the presence and extent of necrosis

within the pathologic material examined was recommended.[8] This recommendation continues to be supported in this update and with recent publications adding further credence to these as described below.

There is often a combination of radiation necrosis and progressive glioma in biopsy and resection specimens and it can be difficult to histologically assess disease status. Additional manuscripts published since the last version of this guideline refine the understanding of this issue. This was particularly emphasized by the study conducted by Holdhoff, et al., in which only “marginal agreement” by Fleiss’ kappa statistics was observed when 48 pathologists (92% of whom were neuropathologists) reviewed 13 cases of suspected recurrence of glioblastoma. [14] Much of this may be due to differential understanding of the terminology used in this study. The terminology of “active tumor”, “inactive tumor”, and “treatment effect” are not well established in the literature[14] yet this is commonly used terminology.

Multiple studies have aimed to determine what features give the greatest prognostic information on second resection specimen, methodology and results have varied. Azoulay et al., Dalle Ore et al., Hu et al., and Woodworth et al., show that the majority of re-resection specimens demonstrate a mixture of tumor and therapy related effects (ranging from 49-85% of specimens), with only a minority demonstrating absence of active residual tumor (ranging from 5-29% of patients).[9; 10; 15; 16] Azoulay et al. and Woodworth et al. demonstrated that patients in whom the re-resection specimen demonstrated only therapy related changes without active tumor had an increased survival.[15; 16] Hu et al. demonstrated a significant association between percent tumor and overall survival,[10] however Bagley et al. and Dalle Ore et al. were not able to reproduce these findings[5; 9] (See Table I). Another suggested marker of prognosis present in re-resection specimens has been the proliferative index as assessed by Ki-67 / MIB-1 immunohistochemistry. Okita et al. were able to demonstrate that MIB-1 indices significantly correlated with overall survival in a multivariate analysis ($p=0.004$)[17] (See Table II). Thus, it is recommended that the percentage of viable tumor and the percentage of radiation necrosis is documented. If available the proliferative index as assessed by MIB-1 immunohistochemistry may also be informative, but it is not felt there is enough evidence at this time to include this as part of the recommendation (See Table II).

The second question in the prior version of this guideline addressed what ancillary studies are most useful in differentiating progression from treatment effect. Immunohistochemistry, including Ki-67, IDH, p53, and WT1, and genetic studies, specifically *EGFR* amplification or gain of chromosome 7, were selectively recommended for distinguishing neoplastic cells from atypical reactive cells in progressive glioblastoma.[8] This update does not alter this recommendation but numerous additional studies that have been published since then and have better elucidated the impact some of these features have on the behavior of infiltrating gliomas. Indeed, this was reflected in the World Health Organization 2016 Classification of Tumors of the Central Nervous System[12] which incorporates selected immunohistochemical and genetic features in the classification system. Thus, while the prior review focused on using these markers to determine the presence and in some cases quantity of tumor present, several of these studies are now indicated for classification and prognostic uses as well.

The integration of ancillary studies is expected to expand with the publication of the next World Health Organization Classification of Tumors of the Central Nervous System with some of the expected updates already published in the form of cIMPACT-NOW update recommendations.[18] For the current review of progressive glioblastoma, criteria of the World Health Organization classification with the addition of the published cIMPACT-NOW update 3 recommendations will be used, since they represent a recent and updated international standard for classifying and grading.[12; 18] An additional cIMPACT-NOW update 5 was published after the designated time interval of the search for this guideline and the information in this document are not used to formulate the updated recommendations.[19]

It is of value to briefly expand on the evolution of the immunohistochemical and molecular markers alluded to in the second question of the prior guideline as it provides background on the new questions asked in this update as noted below. The 2016 WHO classification divides the diffuse gliomas into IDH-wildtype astrocytomas, *IDH*-mutant astrocytomas, IDH-mutant and 1p/19q codeleted oligodendrogliomas, and H3K27M-mutant diffuse midline glioma. A 3-tiered grading system is used in grading diffuse astrocytic gliomas, and a 2-tiered grading system of oligodendrogliomas.[12]

IDH-wildtype diffuse astrocytomas tend to arise in an older patient population with large majority of them presenting as *de novo* glioblastomas.[20] As such IDH-wildtype diffuse astrocytoma, WHO grade II and anaplastic astrocytoma (WHO grade III) are recognized as provisional entities in the 2016 WHO and multiple studies have concluded that a substantial subset of these demonstrate an aggressive clinical course most akin to IDH-wildtype glioblastoma, WHO grade IV. Recently the cIMPACT-NOW update 3 addressed this by recommending that *IDH*-wildtype infiltrating astrocytomas that would histologically be classified as WHO grade II or III which carry and *EGFR* amplification, combined whole chromosome 7 gain and whole chromosome 10 loss, or a *TERT* promoter mutation be given an integrated diagnosis of “diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV.” It is critical to note that other IDH-wildtype glial tumors that may enter the differential have been reported to harbor *TERT* promoter mutations and thus histologic examination of the specimen remains critical.[18]

IDH mutant diffuse astrocytomas tend to arise in younger patient population and tend to present as either a diffuse astrocytoma WHO grade II or anaplastic astrocytoma WHO grade III. They are associated with *TP53* mutations and *ATRX* alterations and are relatively slowly progressive.[12; 20; 21]

Diffuse midline glioma (H3K27-mutant) and H3 G34 mutant diffuse gliomas do not have *IDH*-mutations and occur predominantly in childhood and adolescents and are associated with an aggressive clinical course. Diffuse midline gliomas (H3K27M-mutant) are by definition WHO grade IV. While the 2016 World Health Organization Classification of Tumors of the Central Nervous System did not provide a separate classification or grade for H3 G34-mutant diffuse glioma, this mutation in a diffuse glioma indicates a high-grade biology with only modestly longer survivals than other *IDH*-wildtype glioblastomas. In most cases the assessment of prognostic genetic markers will have been performed on the initial specimen, however, if they were not performed previously they should be performed at the time of progression. Specific alterations that have current diagnostic, prognostic, or therapeutic implications in diffuse

astrocytic gliomas include *IDH1* and *IDH2* mutations, *EGFR* amplification, whole chromosome 7 gain with concurrent whole chromosome 10 loss, *TERT* promoter mutation, *CDKN2A* homozygous deletion, 1p/19q co-deletion, and *MGMT* promoter methylation status.[8; 12; 18]

Question: For adult patients with progressive glioblastoma does testing for Isocitrate Dehydrogenase (IDH) 1 or 2 mutations provide new additional management or prognostic information beyond that derived from the tumor at initial presentation?

Study selection and Characteristics:

Four studies focused on IDH mutation status in primary and progressive glioblastoma were uncovered in the initial screen.[22; 23] Only two of these papers focused on the use of IDH mutations in recurrent glioblastoma. Two additional papers were identified from relevant review articles that addressed maintenance of IDH mutations during glioma progression[24; 25] (Table IV).

Results of individual studies, discussion of study limitations and risk of bias

Mutations in *IDH* are frequent in lower grade astrocytomas as well as glioblastomas that progress from these lower grade precursor lesions. *IDH* mutations most commonly occur in *IDH1* as a substitution of histidine at R132, which accounts for approximately 90% of all *IDH* mutations.[12; 23] The *IDH1* R132H mutation can be detected by either immunohistochemical or molecular methods. Approximately 10% of *IDH* mutations are due to alternate mutations in *IDH1* or on *IDH2*, including *IDH1* R132C, *IDH1* R132G, *IDH1* R132S and *IDH1* R132L and *IDH2* R172K,[26] which can currently only be detected by molecular testing. *IDH* mutations tend to occur early in glioma development and they are retained upon recurrence, even after therapy.[24; 25; 27] *IDH* mutations contribute to glioma development through overproduction of the oncometabolite 2-hydroxyglutarate leading to induction of the HIF-1 pathway and genome wide histone and DNA methylation alterations.[12; 28-30] Testing for *IDH1/2* mutations may be useful in the context of progressive glioblastoma when attempting to determine if residual disease is present in a resection specimen and to assess the approximate percentage of tumor.[8; 22] It is also feasible to use *IDH* mutation testing to aid in frozen section diagnosis, however, the turnaround time is approximately 1 hour and may not be practical for all laboratories.[22] It should be noted that infiltrating gliomas, and glioblastomas are heterogeneous tumors and rare instances of alterations in IDH status from that seen in the original lesion have been noted, usually a loss of the IDH mutation[31–33](See Table III).

Synthesis

Due to the early occurrence and conserved nature of IDH mutations repeat testing is not necessary if the tumor is histologically similar to the primary tumor and the patient's clinical course is as expected.

Question: For adult patients with progressive glioblastoma does repeat testing for *MGMT* promoter methylation provide new or additional management or prognostic information beyond that derived from the tumor at initial presentation and what methods of detection are optimal?

Study selection and Characteristics

Thirteen papers were uncovered in the screening process that discussed alterations in *MGMT* promoter methylation status in progressive/recurrent glioblastoma. Eleven were selected for inclusion as 1 was a review article and 1 paper only had four paired tumors and did not specifically note if *MGMT* promoter methylation status was retained in those four cases.

Results of individual studies, discussion of study limitations and risk of bias

Temozolomide, a chemotherapeutic agent that is part of the standard therapy for glioblastoma, and other alkylating agents cause DNA crosslinking through alkylation of the O⁶ position guanine. These alkyl adducts are removed by O⁶-methylguanine-DNA methyltransferase (MGMT). Methylation of the *MGMT* promoter is one of the major mechanisms for *MGMT* regulation and leads to transcriptional silencing. Thus, glioblastomas with *MGMT* promoter methylation and thus lower expression of MGMT would be expected to have better response to alkylating agents.[13; 34] *MGMT* promoter methylation has become a clinically relevant prognostic and predictive marker in patients with glioblastoma treated with temozolomide or other alkylating agents and is associated with a statistically significant improvement in progression free survival and overall survival in patients receiving standard therapy.[34; 35]

MGMT promoter methylation status is fairly consistent from original tumor presentation to recurrence, with no status change in approximately 66–82% of glioblastomas.[17; 36] No significant intra-tumor heterogeneity of *MGMT* promoter methylation status has been found.[36] Brandes et al. demonstrated that overall survival correlated with *MGMT* promoter methylation status determined at the primary surgery but not at recurrence and Okita et al. found no correlation between *MGMT* promoter methylation status at recurrence and survival time or progression free survival.[17; 36] A few earlier studies did show correlation with *MGMT* promoter methylation at recurrence and chemoresistance and survival,[37] however, the majority of the data suggests that repeat testing is not needed.

Multiple methods of *MGMT* promoter methylation are available, including pyrosequencing, quantitative real-time methylation specific PCR, and methylation specific PCR. Recent studies have compared the multiple methods and tried to determine optimal stratification. The best performance was seen using a 3 tiered system of unmethylated, low level methylation, and high level methylation using either pyrosequencing or methylation specific PCR, with pyrosequencing being the preferred method.[34; 38-41]

An immunohistochemical stain for MGMT protein expression is also available, however MGMT protein expression can be upregulated by glucocorticoids, chemotherapy, and radiotherapy and thus may not reflect true MGMT status. It is also prone to high inter-observer variability and shows inconsistent correlation with clinical outcomes[38; 39; 42-44](See Table IV).

Synthesis

Repeat *MGMT* promoter methylation testing does not need to be repeated upon recurrence. Either pyrosequencing or methylation specific PCR can be used to assess *MGMT* promoter methylation, with pyrosequencing being the preferred method. Immunohistochemical testing for MGMT protein expression is not recommended for clinical use.

Question: For adult patients with progressive glioblastoma does EGFR amplification or mutation testing provide management or prognostic information beyond that provided by histologic analysis and if performed on previous tissue samples, does it need to be repeated?

Study selection and Characteristics

Twenty articles were uncovered in the screening process of which 9 articles were selected for inclusion in this review and 1 additional article was identified from the references of the included articles. Reasons for exclusion from the included publications included animal or in-vitro studies, phase 1 clinical trials that were ended early or in which therapeutic outcomes were not discussed, review articles or single case studies, or did not address pathology (studies with only imaging data).

Results of individual studies, discussion of study limitations and risk of bias

EGFR is the most commonly amplified and overexpressed proto-oncogene in glioblastoma, with amplification present in approximately 40-50% of glioblastomas.[45–47] Amplification is primarily seen in de novo glioblastomas[45] and appears to be mutually exclusive of *IDH* mutations.[30; 48] *EGFR* is located on the short arm of chromosome 7 and encodes a cell-surface receptor tyrosine kinase. EGFR activation initiates signal transduction through several major pathways including RAS-MAPK and PI3K-AKT signal transduction cascades leading to increased DNA transcription, anti-apoptosis, angiogenesis and cellular proliferation.[49–51] EGFR overexpression was found to contribute to gliomagenesis and poor survival in patients with glioblastoma.[52]

Mutations of *EGFR* are also seen in IDH-wildtype glioblastoma and appear to occur exclusively in the setting of *EGFR* amplified glioblastomas. *EGFRvIII* is the most common mutation in glioblastoma and results in the creation of a tumor-specific antigen that is detectable in 23–33% of IDH-wildtype glioblastomas,[53; 54] and in approximately 50% of *EGFR* amplified glioblastomas.[46; 55; 56] *EGFRvIII* is the result of a deletion of *EGFR* exons 2–7, which generates a constitutively active tyrosine kinase with a truncated extracellular domain.[46] The truncated extracellular domain creates a new unique targetable peptide sequence.[53; 57] *EGFRvIV* mutation is less common, seen in approximately 20% of *EGFR* amplified glioblastomas. EGFRvIV results due to deletion of the carboxyl terminal domain and also exhibits constitutive activation.[48]

Due to high frequency of *EGFR* alterations and the success in targeting EGFR in other tumors EGFR is an attractive target, however, EGFR inhibitors and an EGFRvIII vaccine have so far had disappointing results in glioblastomas.[58–60] It is reasonable to think that clinical trials targeting this pathway will continue and information regarding *EGFR* amplification and mutation may be desired. Felsberg et al., van den Bent

et al., and Cioca et al. demonstrated that *EGFR* amplification was retained in recurrent glioblastomas after standard treatment and does not need to be retested. *EGFRvIII* more commonly shows loss or reduced expression at recurrence and retesting should be considered if targeted therapy is being contemplated.[46; 52; 61] *EGFR* amplification can be detected by FISH, CGH, or PCR-based assays and *EGFR* mutations can be detected by PCR or IHC[8; 47; 61; 62] (See Table V).

Synthesis

Testing for *EGFR* amplification should be performed in cases of IDH-wildtype tumors that are difficult to grade as it may help classify the tumor as a glioblastoma. If a previous *EGFR* amplification was detected, retesting is not necessary. Repeat *EGFR* testing may be indicated for patients in which targeted therapy is being considered, particularly therapies targeting specific *EGFR* mutations.

Question: For adult patients with progressive glioblastoma does whole genome or large panel sequencing provide management or prognostic information beyond that derived from histologic analysis?

Study selection and Characteristics

16 studies were uncovered by the search criteria and ten studies were included in this review. The remaining studies were excluded because they were review articles (4) or were focused on lower grade infiltrating gliomas (2).

Results of individual studies, discussion of study limitations and risk of bias

Currently the only Federal Drug Administration (FDA) approved therapeutic agents for the treatment of progressive glioblastoma are bevacizumab, and in selected patients carmustine-wafers, thus patients are often encouraged to go on clinical trials.[63–65] Glioblastoma is molecularly heterogeneous and appears to be highly mutable with progressive glioblastoma displaying inherent or acquired resistance to treatment.[63; 64; 66]

Whole genome and large panel sequencing have expanded our understanding of the alterations that occur in de novo glioblastoma and progressive glioblastoma. Most studies demonstrate a gain of genetic alterations in recurrent glioblastomas[67] with approximately 17% of recurrent glioblastomas showing hypermutation[68]. Hypermutated tumors with alterations in the retinoblastoma (RB) and mammalian target of rapamycin pathways were noted after temozolomide therapy by Johnson et al.[24] while Wood et al. noted a subset of recurrent glioblastomas demonstrated an increased expression of *CHI3L1*, *TIMP1*, and *CD44*, whose expression has been associated with a more aggressive course[69]. Interestingly in one study 43% of recurrent gliomas showed a loss of 50% or more of the mutations present in the initial tumor, some of which were driver mutations, including *TP53*, *ATRX*, *SMARCA4*, and *BRAF*[24].

The increase in genetic understanding has also sparked hope that more effective novel or targeted therapies may be available for particular subsets of patients. To this end multiple studies looking at possible targets, alterations in recurrence, and clinical trials involving targeted therapies have been

performed or are underway. Most studies were performed using next generation sequencing to assess genomic alterations either using data from whole transcriptome sequencing, whole exome sequencing, or targeted panels. Targeted panels have the advantage of being the most cost effective with the analysis of the data being the most straight forward and having relatively high specificity however the genes included on the targeted panels can vary and requires enrichment of the target regions. Whole exome sequencing requires enrichment of the exons and analysis of the data to ensure optimal processing and sequencing reaction. Whole genome sequencing while giving a comprehensive view of all alterations is the most expensive and requires the most data analysis and interpretation and many of the identified alterations may not be therapeutically targetable or relevant to diagnosis. Additional methods of genomic profiling that can be utilized include chromosomal microarray analysis and DNA methylation. It has become clear that progressive glioblastoma demonstrates an evolution of molecular alterations relative to primary glioblastoma.[24; 67; 69-71] Byron et al. demonstrated that genome wide molecular testing to guide therapy was feasible, and had promising results in 2 patients, in this study of a very limited size. [65] The ability of chosen therapies to cross the blood-brain barrier was taken into account in this study and is an important consideration when designing treatment recommendations. Most targeted therapies, particularly when used as single agent therapy, seem to have limited activity in the setting of progressive glioblastoma.[64] However, given the limited response to therapy in the recurrent setting repeat molecular testing would be of value in patients who are eligible for clinical trials based on a targeted therapy[65; 67; 68](See Table VI).

Synthesis

Primary or repeat whole genome or large panel sequencing should be considered in patients in whose management may be impacted including those who are eligible or interested in targeted therapy based on a particular oncogenic pathway anomaly or pathway member or for assessment of eligibility in clinical trials based on a particular molecular characteristic.

Question: For adult patients with progressive glioblastoma should immune checkpoint biomarker testing be performed to provide management and prognostic information beyond that obtained from histologic analysis?

Study selection and Characteristics

Twenty-seven articles were uncovered during the screening process, however the majority of these represented review articles and in vitro or animal models. Other studies were excluded due to a lack of pathology data (radiology studies), phase 1 studies that did not include pathology or outcomes, and studies focused on pediatric patients. Two additional studies were identified from references of review articles identified in the screening process. Ultimately eight studies were included in the review.

Results of individual studies, discussion of study limitations and risk of bias:

Immune checkpoint inhibitors have shown marked success in the treatment of a variety of solid cancer types by blocking immune checkpoint signaling and allowing a T-cell response against the tumor.[72] Glioblastoma is known to cause host immunosuppression through a variety of mechanisms[63] and glioblastomas show frequent genetic and epigenetic alterations which potentially may produce numerous neoantigens,[72] thus immune checkpoint inhibitors sound like a promising treatment modality for progressive glioblastoma.

Variable PD-L1 staining patterns are seen in glioblastoma ranging from clear membranous staining to diffuse cytoplasmic staining[72; 73] and shows heterogeneity within the tumor.[72] The clinical significance of these differential staining patterns is yet to be elucidated but likely contributes to the wide range of PD-L1 expression in glioblastomas being reported, from 10–88%.[73–76] A large proportion of tumors have been reported to demonstrate the diffuse pattern of staining, up to 88% of primary glioblastomas and 72% of recurrent glioblastomas .[73] While the clear membranous staining pattern is only seen in a subset of primary and progressive glioblastomas, approximately 37% of primary glioblastomas and 11-16.7% of progressive glioblastomas[73; 77], with Berghoff et al. noting a significant decrease in progressive glioblastomas demonstrating clear membranous PD-L1 expression. Heyneckes et al. found a significant decrease in both the PD-L1 mRNA expression and number of PD-L1 positive cells in progressive glioblastomas and that this reduction was more pronounced in patients who received extended temozolomide therapy .[72] The recent CheckMate 143 clinical trial, the first large randomized clinical trial of nivolumab, a PD-1 inhibitor, failed to extend overall survival in the setting of progressive GBM.[78–80] PD-L1 expression was not included as a criteria for inclusion in this study, and in the phase II CheckMate 143 studies while 68% of patients had PD-L1 expression over 1% only 27% of patients had PD-L1 expression greater than 10%. Confounding these values is that the PD-L1 expression was measured on the primary resection, not the recurrent tumor.[78] A phase 1a study of atezolizumab in progressive glioblastoma also demonstrated dismal results with 100% of patients discontinuing therapy due to progressive disease. However, a more recent trial using pembrolizumab in patients with progressive glioblastoma as neoadjuvant therapy prior to re-resection and continuing as adjuvant therapy demonstrated improved overall survival (13.7 months) and progression free survival (3.3 months) when compared to patients receiving pembrolizumab as adjuvant therapy only after re-resection (7.5 months and 2.4 months respectively). Further studies will need to be done to further validate the use and efficacy of pembrolizumab in the neoadjuvant setting as this was a small study, it is also notable that PD-L1 expression was not a criteria and expression was not reported in either cohort.[81]

Another biomarker of interest is loss of mismatch repair (MMR) proteins and in 2017 the FDA approved pembrolizumab (Keytruda) for the treatment of unresectable or metastatic solid tumors harboring mismatch repair deficiency (dMMR) or microsatellite instability-high (MSI-H) regardless of site.[82] MMR enzymes, including MSH2, MSH6, MLH1, and PMS2, are involved in inducing programmed cell death in tumor cells damaged by alkylating agents, including temozolomide.[83] Multiple studies have shown that progressive glioblastoma has an increased prevalence of inactivating mutations of mismatch repair genes, particularly involving MSH6 and there is some evidence that MMR gene alterations are caused by or selected for by temozolomide therapy .[84-86] Indraccolo et al. found that the majority (78.5%) of

cases lacking MMR protein expression at recurrence had *MGMT* promoter methylation at diagnosis.[84] Loss of MMR enzyme expression is also associated with a hypermutant genotype, while this is a small subset of patients (~10%), it is hypothesized that the increased mutagenesis may make these tumors more immunogenic and thus more amenable to immunotherapy[74; 84] (See Table VII). However, the efficacy of pembrolizumab or other immune checkpoint inhibitors has yet to be investigated in MMR deficient progressive glioblastomas has yet to be fully investigated.

Synthesis

If immune checkpoint inhibitors are being considered PD-L1 expression or loss of MMR enzyme activity should be determined but due to the limited benefit demonstrated by immune checkpoint agents in glioblastoma (primary or progressive) standard testing is not currently necessary.

Question: For adult patients with glioblastoma are Bevacizumab biomarkers available and should they be performed in the setting of progressive glioblastoma?

Study selection and Characteristics

Numerous studies (greater than 75) looking at Bevacizumab were identified however most were clinical trial papers, without examination of pathology, studies looking at radiologic biomarkers, or review articles. Eight articles looking at tissue biomarkers were identified and included in this review.

Results of individual studies, discussion of study limitations and risk of bias

Glioblastoma demonstrates marked up-regulation of VEGF-A and displays rapid vascularization.[4; 87] Bevacizumab is a monoclonal antibody against vascular endothelial growth factor (VEGF) that has been approved for treatment of recurrent glioblastoma. However, bevacizumab has not been shown to improve OS and there is concern that glioblastomas treated with bevacizumab are more aggressive and show increased infiltration.[4; 88; 89] A subset of patients do show a favorable clinical response following bevacizumab and biomarkers are being investigated to identify these patients.[4] Choi et al. and Hovinga et al. both found that classical subtype glioblastomas did not respond as well to bevacizumab.[4; 47] Choi et al. and Erdem-Eraslan et al. identified possible biomarkers in predicting response to bevacizumab (COL4A2) and bevacizumab combined with lomustine (FM04/OSBPL3), however, both of these are retrospective studies on primary tumor specimens and further studies need to be performed to confirm these findings.[4; 90] YKL-40 mRNA expression and plasma levels have been found to be associated with a worse response to carmustine plus bevacizumab or bevacizumab therapy alone, respectively[91; 92] in retrospective studies but no prospective studies have been performed. At this time no established biomarkers are available to predict response to bevacizumab[88; 93; 94] (See table VIII).

Synthesis

Bevacizumab biomarker testing at this time remains experimental, markers which may be indicative of response include YKL-40, COL4A2, and FM04 expression although confirmatory studies are warranted.

Discussion

If prior ancillary and molecular testing including *IDH* mutation status, *MGMT* promoter methylation status, and other relevant testing including chromosomal alterations and histone mutations were not performed on the primary resection specimen, it is recommended they be performed at the time of progression for definite classification.

All of the recommendations presented within this review are based on class III data, usually due to the retrospective nature of the studies or small cohort size, however, the recommendations are based upon multiple class III studies whose results are coherent. Repeat testing of commonly assessed alterations, including *IDH* mutation, *MGMT* promoter methylation, and *EGFR* amplification, usually do not need to be repeated upon progression as they tend to remain stable, or in the case of *MGMT* promoter methylation alterations present at the time of progression are of unclear clinical and therapeutic significance. Less common studies including *EGFR* mutations, large panel sequencing, and immune checkpoint biomarker testing may be warranted in patients who are interested in targeted therapy, immunotherapy, or other select clinical trials where presence or absence of a particular molecular marker is included in the eligibility criteria. While a few studies have shown benefit from targeted therapy and immunotherapy there is currently not enough evidence to suggest that these studies be considered part of standard workup. This is particularly true in the case of immune checkpoint inhibitors, in which the majority of clinical trials have been disappointing and the relevance of PD-L1 or MMR expression in the efficacy of these agents is unclear as the clinical trials have not required PD-L1 expression or MMR loss as a criteria and no association between PD-L1 expression and response.[72; 73; 78; 80; 95-97] However, promising results have recently emerged using pembrolizumab as neoadjuvant therapy in progressive glioblastoma[81] and investigations of targeted therapies will undoubtedly continue to be pursued.

While many of the clinical trials for targeted therapy have been disappointing the recommendations in this updated guideline highlight the progress that has been made in identifying and interrogating key alterations in glioblastoma and the significant advancements that have been made in further understanding the heterogeneity within glioblastomas.

Conclusions And Key Issues For Future Investigations

Continuing work using whole genome, large-scale molecular studies, and methylation profiling continues to further elucidate the alterations that occur in glioblastomas over time and following therapy. Further understanding of the genetic landscape of infiltrating gliomas has already allowed us a greater understanding of the heterogeneity within these tumors and allowed us to begin integrating these molecular alterations into a classification system[30; 98-103] and targeted therapy. However, even within the current classification system there remains extensive molecular and behavioral diversity. An update of the WHO classification of Tumors the Central Nervous System is expected to be released later this year which is expected to further integrate molecular features into the classification system as has been evident in the c-IMPACT now publications.[18; 19; 98; 104; 105] This observation has been reinforced in

recent clinical trials where molecular characteristics were not part of the eligibility criteria and yet the overall results have been disappointing with only a minority of patients demonstrating a meaningful response. Identification of useful biomarkers that predict response or resistance for these targeted therapeutic agents will be critical for further progress.

One of the major hindrances within studies related to progressive glioblastoma is that patients who are candidates for re-resection are often younger and healthier patients with a definable mass. Tissue from these cases may not reflect alterations present in more aggressive diffuse glioblastomas and further work to identify what alterations are present in these tumors will need to be undertaken.

Abbreviations

BEV

Bevacizumab

CNS

central nervous system

EGFR

epidermal growth factor receptor

GBM

glioblastoma

IHC

immunohistochemistry

MGMT

O6-methylguanine-methyltransferase

MMR

mismatch repair

NGS

next-generation sequencing

OS

overall survival

PD-1

programmed cell death protein

PD-L1

programmed death-ligand 1

PFS

progression free survival

Pts

patients

RT

radiotherapy

TMZ

temozolomide

Declarations

Conflict of Interest (COI)

All Guideline Task Force members were required to disclose all potential COIs prior to beginning work on the guideline, using the COI disclosure form of the AANS/CNS Joint Guidelines Review Committee. The CNS Guidelines Committee and Guideline Task Force Chair reviewed the disclosures and either approved or disapproved the nomination and participation on the task force. The CNS Guidelines Committee and Guideline Task Force Chair may approve nominations of task force members with possible conflicts and restrict the writing, reviewing, and/or voting privileges of that person to topics that are unrelated to the possible COIs. The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this series of articles.

Data transparency

The author has ensured all data and materials as well as software applications or custom code supports their published claims and comply with field standards.

Author Contributions

The author listed on this publication agrees with the content included and gives explicit consent to the submission of this publication. The author obtained consent from the responsible authorities at the institute/organization where the work has been carried out, before the work was submitted.

The author whose name appear on this submission:

- 1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;
- 2) drafted the work or revised it critically for important intellectual content;
- 3) approved the version to be published; and
- 4) agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Compliance with Ethical Standards

Funding: These guidelines were funded exclusively by the Congress of Neurological Surgery and the Joint Section on Tumors of the Congress of Neurological Surgeons and the American Association of

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Ethical Approval

This article does not contain any studies with human participants performed by any of the authors.

Data Availability

The data generated during and/or analyzed during the current study are available via www.cns.org/guidelines.

Disclosures

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Disclaimer of Liability

This clinical systematic review and evidence-based guideline was developed by a multidisciplinary physician volunteer task force and serves as an educational tool designed to provide an accurate review of the subject matter covered. These guidelines are disseminated with the understanding that the recommendations by the authors and consultants who have collaborated in their development are not meant to replace the individualized care and treatment advice from a patient's physician(s). If medical advice or assistance is required, the services of a competent physician should be sought. The proposals contained in these guidelines may not be suitable for use in all circumstances. The choice to implement any particular recommendation contained in these guidelines must be made by a managing physician in light of the situation in each particular patient and on the basis of existing resources.

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Tables

Table I. Neuropathologic techniques/ Radionecrosis in progressive glioblastoma

Abbreviations: **BEV**, Bevacizumab; **ECOG PS**, Eastern Cooperative Oncology Group performance status; **GBM**, glioblastoma; **HR**, hazard ratios; **MGMT**, O6-methylguanine-methyltransferase; **MRI**, Magnetic resonance imaging; **MVD**, mean vascular density; **OS**, overall survival; **pMRI-FTB**, perfusion MRI-fractional tumor burden; **PTRE**, post treatment radiation effect; **pts**, patients; **RT**, radiation therapy; **TMZ**, temozolomide

Table II. MIB-1 quantification in progressive glioblastoma

Abbreviations: **GBM**, glioblastoma; **OS**, overall survival; **pts**, patients

Table III. Repeat IDH testing in progressive glioblastoma

Abbreviations: **CNA**, copy number abnormalities; **GBM**, glioblastoma; **HMGA1**, high mobility group A1; **IHC**, immunohistochemistry; **PI3K**, phosphoinositide 3-kinase; **pts**, patients; **RTK**, receptor tyrosine kinase

Table IV. Repeat *MGMT* promoter methylation testing in progressive glioblastoma

Abbreviations: **dBisSeq**, direct bisulfite sequencing; **dd-TMZ**, dose-dense temozolomide; **FFPE**, formalin fixed paraffin embedded; **GBM**, glioblastoma; **GKS**, gamma knife surgery; **IHC**, immunohistochemistry; **MGMT**, O6-methylguanine-methyltransferase; **mo.**, months; **mOS**, median overall survival; **mPFS**, median progression free survival; **MSP**, methylation-specific polymerase chain reaction; **msPCR**, methylation-specific PCR; **MST**, median survival time; **OS**, overall survival; **PCR**, polymerase chain reaction; **PFS**, progression free survival time; **PSQ**, pyrosequencing; **pts**, patients; **qMSP**, quantitative Methylation-Specific PCR; **RT**, radiation therapy; **sqMSP**, semi-quantitative methylation specific PCR; **TMZ**, temozolomide; **unmethyl**, unmethylated; **wt**, wild type

Table V. EGFR amplification and mutational testing in progressive glioblastoma

Abbreviations: **BEV**, bevacizumab; **CR**, complete response; **ECD**, extracellular domain **EGFR**, epidermal growth factor; **EGFRvIII**, epidermal growth factor receptor variant III; **EGFRvIV**, epidermal growth factor receptor variant IV; **FISH**, fluorescence in situ hybridization; **GBM**, glioblastoma; **IHC**, immunohistochemistry; **msPCR**, methylation specific PCR; **OS**, overall survival; **PCR**, polymerase chain reaction; **PFS**, progression free survival; **PR**, partial response; **p-SRC**, phosphorylated-*SRC*; **pts**, patients; **RR**, radiologic response; **RT**, radiation therapy; **SNV**, single nucleotide variant; **TMZ**, temozolomide; **wt**, wild type

Table VI. Whole genome or large panel sequencing in progressive glioblastoma

Author (year):	Description of study:	Data class:	Conclusions:
Hu, L.S., et al., 2012	<p><u>Study Description:</u></p> <p>Single center retrospective study of 25 pts with recurrent GBM in whom histologic tumor fraction upon recurrence is correlated to perfusion MRI and OS.</p> <p>Post treatment radiation effect was classified as paucicellularity, scattered rare / no atypical cell, preponderance of reactive cells including astrocytes (gemistocytes), microglia and macrophages and vascular hyaline fibrosis. Necrosis circumscribed in non-neoplastic tissue</p> <p>Tumor recurrence included cellular sheets and/or nests of atypical cells often w/ mitotic figures. Few atypical cells in a linear infiltrative configuration in parenchyma w/out prominent reactive changes.</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=25)</p> <p>Median age: 50</p>	III	<p><u>Results:</u></p> <p>OS was significantly associated with histologic tumor fraction and pMRI-FTB. Significantly shorter OS in pts with histologic tumor fraction above 49% and pMRI-FTB above 63%. 4 pts (16%) had only PTRE, while 2 demonstrated only tumor (8%), the remainder demonstrated a mix of tumor and PTRE (76%).</p> <p><u>Author's Conclusions:</u></p> <p>pMRI-FTB metric reliably estimates histologic tumor fraction and correlates with OS in the context of recurrent GBM.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as it is a retrospective study with a limited number of patients, additionally interrogating histologic factors predictive of OS was not the primary aim of the study.</p>

<p>Woodworth, G.F. et al., 2013</p>	<p><u>Study Description:</u></p> <p>Single center retrospective study of 59 pts with recurrent GBM in whom pathology was reviewed for treatment related effects at the time of re-resection.</p> <p>Treatment effect is defined as paucicellular coagulation necrosis, gliosis, pleomorphism in low density areas, and vascular changes including fibrinoid necrosis, telangiectasia, and hyalinization.</p> <p>Active tumor was defined as highly cellular tumor similar to GBM prior to treatment, however, necrosis or MVP was required. Mitosis were usually present but not required in a small specimen.</p> <p>Presence of only low grade tumor was not considered as "active tumor".</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=59)</p> <p>Median age at dx: 53</p>	<p>III</p>	<p><u>Results:</u></p> <p>71% of pts had at least focal high grade glioma, with only 29% of pts with no evidence of active high grade tumor. Pathologic pseudoprogression at re-operation and gross total resection were associated with survival.</p> <p><u>Author's Conclusions:</u></p> <p>Histopathologic assessment of pseudoprogression can be used to prognostically categorize pts and histologic assessment is superior to standard radiologic assessment.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because study is a retrospective study that does not interrogate what features of "active tumor" are prognostic nor does it interrogate if their proposed definition is ideal.</p>
<p>Azoulay, M, et al., 2017</p>	<p><u>Study Description:</u></p> <p>Single center retrospective study of 183 pts with recurrent GBM, 69 of whom underwent re-</p>	<p>III</p>	<p><u>Results:</u></p> <p>Pathology at re-resection:</p> <p>85% of pts had residual GBM, 15% of pts had only RT induced necrosis, and 1 pt. had developed a sarcoma. Absence of residual GBM on repeat</p>

	<p>resection. Tumor present on re-resection histology was correlated with survival</p> <p><u>Patient Population:</u></p> <p>GBM patients (n = 183)</p> <p>Re-resection (n=69)</p> <p>Median age: 58</p>		<p>pathology specimen (HR 0.23) were shown to affect survival.</p> <p><u>Author's Conclusions:</u></p> <p>Surgery allows for confirmation of histopathology when recurrence cannot be distinguished from radionecrosis based on imaging alone. Surgery at the time of progression leads to increased survival in this study and having more than one surgery following recurrence can significantly improve patient outcome.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as this is retrospective study in which histopathology was not the main focus, only +/- for residual tumor was interrogated in this study.</p>
Blumenthal, D.T., et al., 2018	<p><u>Study Description:</u></p> <p>Single center study of 15 pts treated w/ TMZ + RT followed by BEV. Re-resection after BEV samples were compared to the pts initial were evaluated for morphologic changes and mean vascular density (MVD).</p> <p><u>Patient Population:</u></p> <p>BEV treated patients (n = 15)</p> <p>Median age: 42 (20-73)</p> <p>BEV-naive (n=44)</p> <p>Median age: 68 (24-77)</p>	III	<p><u>Results:</u></p> <p>No morphologic differences were apparent between pre- and post-BEV-treated tumor samples. There was a statistically significant decrease in mean vascular density overall between pre- and post BEV treated specimens (pooled, $p < 0.001$). No significant difference was identified when looking at the individual cases ($p = 0.2$), however, 7 patients did demonstrate a significant decrease in MVD.</p> <p><u>Author's Conclusions:</u></p> <p>No overt changes in histopathology were observed after antiangiogenic therapy, but mean vascular density was present decreased status post BEV treatment. No predictive or prognostic clinical or imaging value correlated with "response" to BEV as assessed by MVD.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because study is retrospective review with a limited number of specimens. No significant impact of BEV treatment on histology.</p>
Holdhoff, M., et al., 2018	<p><u>Study Description:</u></p> <p>Study of variability between 48 pathologists (44 of which were neuropathologists) on</p>	III	<p><u>Results:</u></p> <p>Agreement in overall assessment of disease activity within this survey, determined using Fleiss' kappa statistics was 0.228 (95% CI 0.22–0.24). This is consistent only with "marginal agreement" between observers.</p>

	differentiating active tumor and inactive tumor/treatment effect in 13 cases of recurrent glioblastoma.		<p><u>Author's Conclusions:</u></p> <p>Formal criteria and terminology need to be developed to help improve consistency and reliability.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because study is a prospective study with determination of Kappa statistics, however, the cut-off points and delineations made by the authors limited the utility of the study and they either did not report or did not analyze all the data collected.</p>
Bagley S.J. et al., 2018	<p><u>Study Description:</u></p> <p>Single institution retrospective study of 37 pts with recurrent GBM who underwent re-resection.</p> <p>Tumor burden was assessed by extent of viable tumor, mitoses per 10 high-power fields, Ki-67 proliferative index.</p> <p>Treatment related effects were categorized as vascular hyalinization, rarefaction, hemosiderin, and geographic necrosis.</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (n = 37)</p> <p>Median age: 61</p>	III	<p><u>Results:</u></p> <p>Variables associated with OS in multivariate analysis included the Ki-67 proliferation index (p=0.003), time from initial diagnosis to repeat surgery (p=0.017), and ECOG PS (0.002). The extent of viable tumor showed a trend (p=0.06) toward decreased OS on univariate analysis but not multivariate.</p> <p><u>Author's Conclusions:</u></p> <p>In patients with glioblastoma undergoing repeat resection following TMZ + RT, high Ki-67 index in the recurrent specimen, short time to recurrence, and poor ECOG PS are independently associated with worse OS. Histopathologic quantification of viable tumor versus therapy-related changes were not associated with OS.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because study is retrospective review with a limited sample size, but did assess histologic findings on a scale (not +/-).</p>
Dalle Ore, C.L., et al., 2019	<p><u>Study Description:</u></p> <p>Single center retrospective study of 110 pts with recurrent GBM status post TMZ</p>	III	<p><u>Results:</u></p> <p>Treatment effects were noted in 54% of cases, with 49% of cases having tumor and treatment effect, and 5.4% of cases with only treatment effect. Sarcoma elements were present in 7% of pts. The</p>

+ RT, 60 pts also were treated with BEV in whom pathology was reviewed for treatment related effects at the time of re-resection.

Treatment related changes are characterized by coagulative necrosis, fibrinous vascular necrosis, and hyalinized vessels.

Patient Population:

Recurrent GBM pts (N=110)

Median age at 1st surgery: 54.9

presence of treatment effect was significantly associated with more recent RT therapy (8.9 mo. vs 13.8 mo., $p=0.003$). Patients treated with BEV prior to reoperation were less likely to have treatment effect (20% vs 65%, $P < .001$). The presence of treatment effect was not correlated with *MGMT* promoter methylation status or IDH-1 mutation. Treatment effect was associated with increased survival after re-operation ($p=0.014$) but not OS from primary resection ($p=0.172$).

Author's Conclusions:

Treatment related changes are present in the majority of patients and is associated with earlier re-resection, improved survival from re-resection and unchanged OS from primary resection.

Comments and Conclusions:

Classified as Class III because study is a retrospective study and % tumor/treatment effect was not reported for the majority of pt's nor considered in the statistics, however there is a large number of pts.

Author (year):	Description of study:	Data class:	Conclusions:
Okita, Y., et al., 2012	<p><u>Study Description:</u></p> <p>Retrospective study of 32 pts with recurrent GBM in which prognostic factors were assessed.</p> <p>Therapy related changes were defined as pseudopalisading necrosis, coagulation necrosis, gemistocytic cells, and giant cells.</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=32)</p> <p>Median age: 57 (19-71)</p>	III	<p><u>Results:</u></p> <p>MIB-1 indices in recurrent tumors significantly correlated with OS in multivariate analysis ($p=0.004$). Median survival time was not significantly associated with <i>MGMT</i> promoter methylation ($p=0.8$). 6/18 (33%) pts showed a change in methylation status upon recurrence.</p> <p><u>Author's Conclusions:</u></p> <p>Only MIB-1 index in recurrent GBM is a significant prognostic factor. <i>MGMT</i> promoter methylation status and degenerative changes in tumor cells in recurrent tumors had no correlation with survival time or PFS.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as it is a retrospective study with a limited sample size, histologic features were examined only by +/- of particular findings of treatment effect.</p>

Author (year):	Description of study:	Data class:	Conclusions:
Kanamori, M., et al., 2014	<p><u>Study Description:</u></p> <p>Single center prospective study of the use of intraoperative <i>IDH</i> mutation analysis in cases of suspected non-neoplastic lesion (18), low grade gliomas (5), low grade infiltrating gliomas (3), high grade gliomas (10), and radiation necrosis (3).</p> <p><u>Patient Population:</u></p> <p>Use for determining residual tumor in treatment effect (N=3)</p>	III	<p><u>Results:</u></p> <p><i>IDH</i> PCR was performed at the time of frozen section, could be performed and resulted in 60-65 min, and had excellent correspondence with IHC. Successfully used to determine tumor necrosis vs residual tumor in 3 cases, in which tumor was identified in all 3 cases, Detects an <i>IDH</i> mutation even in cases of low tumor burden</p> <p><u>Author's Conclusions:</u></p> <p>Per prior report (Capper et al., 2010) glioma cells are present in most cases of radiation necrosis, even when definitive tumor is not present on histology. Thus, while this technique is not needed for intraoperative purposes it can help estimate the proportion of tumor cells in these specimens.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III, while prospective, there is a limited number of pts, particularly in the arm of study of most interest for our review.</p>
Johnson, B., et al., 2014	<p><u>Study Description:</u></p> <p>Retrospective study of 23 <i>IDH</i> mutant grade II glioma, favoring astrocytoma, and pts with progressive disease in whom the primary and recurrent specimen were sequenced to assess mutational differences.</p>	III	<p><u>Results:</u></p> <p><i>IDH</i> mutation was retained in all 23 cases. 54% of mutations detected in the primary tumor were present upon recurrence. <i>IDH1</i>, <i>TP53</i>, and <i>ATRX</i> were the most commonly retained mutations. Of the 10 pts treated with TMZ 6 (60%) demonstrated a hypermutated phenotype upon progression and progressed to glioblastoma. 97% of TMZ associated mutations were C>T/G>A. Mutations involving the RB and AKT-mTOR pathways were identified only in pts who progressed to glioblastoma and none of the grade II-III recurrences. In a subset of patients these mutations seem to be linked to TMZ induced mutations.</p> <p><u>Authors' Conclusions:</u></p> <p>A significant proportion of mutations in primary gliomas are lost at recurrence. TMZ-treated pts showed TMZ-induced</p>

	<p>-</p> <p><u>Patient Population:</u></p> <p>Paired progressive <i>IDH</i> mutant glioma pts (N=23)</p> <p>TMZ treated pts (N=10)</p>		<p>mutagenesis and a proportion hypermutagenesis and appeared to follow an evolutionary path to high-grade glioma distinct from that in untreated patients.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as it is a retrospective study with a limited sample size and little reported demographic information. This study attempted to get predominantly astrocytomas but likely included some oligodendrogliomas.</p>
Liu, B., et al., 2015	<p><u>Study Description:</u></p> <p>Retrospective study of 25 recurrent high grade gliomas assessing expression of HMGA1 and IDH in matched primary resection and recurrent GBM specimens.</p> <p>-</p> <p>HMGA1 and IDH-1 R132H expression were assessed by IHC.</p> <p>-</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=25)</p> <p>Median age: 44.5 (30-62)</p>	III	<p><u>Results:</u></p> <p>HMGA1 overexpression was found significantly more in recurrent GBM (15/25) than in initial GBM (9/25; $P = 0.002$), no correlation was identified between HMGA1 expression and adjuvant therapy ($P = 0.516$). For IDH-1 R132 mutation, 3 cases (3/25, 12%) were found in both initial and recurrent GBM groups. No correlation was identified between HMGA1 expression and IDH-1 R132H mutation in initial GBM group ($P = 0.922$). Nine pts who had initial GBM overexpressing HMGA1 had a median PFST of 7.3 months (95% CI: 2.1–13.2 months), while pts with initial GBM with little or no HMGA1 expression had a median survival time of 11.1 months (95% CI: 4.6–26.8 months; log-rank test: $P = 0.044$)</p> <p>-</p> <p><u>Author's conclusions:</u></p> <p>No difference of IDH-1R132H mutations was found between initial and recurrent GBM patients. HMGA1 may be a promising target in the prognosis and treatment of GBMs, especially recurrent GBMs.</p> <p>-</p> <p><u>Comments and conclusions:</u></p> <p>Classified as class III as it is a retrospective study with a limited number of pts. Conclusions about the role and expression of HMGA1 need to be further investigated.</p>
Bai, H., et al., 2016	<p><u>Study Description:</u></p> <p>Retrospective study of 41 progressive <i>IDH1</i> mutant gliomas by</p>		<p><u>Results:</u></p> <p>Progressed tumors acquired an average of 21 mutations compared to the initial tumor and 27% of mutations and 30% of CNAs present in the initial tumor were no longer present in the progressed sample. <i>IDH1</i> mutation was not lost in any of the paired tumors. 27% of progressive gliomas acquired different coding mutations in the same glioma</p>

exome sequencing of paired specimens from initial diagnosis and their progressed counterpart.	driver genes, such as <i>TP53</i> , <i>ATRX</i> , <i>CIC</i> and <i>FUBP1</i> , in which mutations were observed in their lower-grade counterparts. During glioma progression, the receptor tyrosine kinase (RTK)-RAS–phosphoinositide 3-kinase (PI3K) pathway, the NOTCH pathway and FAT receptors were most frequently altered (in 22%, 12% and 10% of the patients, respectively). The most frequently amplified genomic region during progression was the MYC locus on chromosome 8q (22% of pts), and amplification was significantly associated with progression ($q = 1.7 \times 10^{-3}$, likelihood-ratio test)
-	-
<u>Patient Population:</u>	<u>Author's Conclusions:</u>
Progressive glioma pts (N=41)	During progression numerous convergent alterations occur, including activation of the MYC and RTK-RAS-PI3K signaling pathways, alterations in cell cycle regulators such as <i>CDKN2A-CDKN2B</i> , upregulation of FOXM1- and E2F2-mediated cell cycle transitions, and epigenetic silencing of key developmental transcription factors.
Average age at initial resection: 38.9 (21-58)	-
-	<u>Comments and Conclusions:</u>
	Classified as Class III as it is a retrospective study with a limited samples size. The cohort included oligodendrogliomas and not all tumors progressed to glioblastoma.

alterations; **GBM**, glioblastoma; **GEO**, Gene Expression Omnibus; **GGN**, German glioma network; **KPS**, Karnofsky Performance Status Scale; **mRNA**, messenger RNA; **OS**, overall survival; **PI3K**, Phosphoinositide 3-kinase; **pts**, patients; **RT**, radiation therapy; **TCGA**, The Cancer Genome Atlas; **TMZ**, temozolomide; **TTP**, time to progression

Table VII. Immune checkpoint biomarker testing in progressive glioblastoma

Abbreviations: **BEV**, bevacizumab; **GBM**, glioblastoma; **G-CIMP**, cytosine-phosphate-guanine (CpG) island methylator phenotype; **IHC**, immunohistochemistry; **MGMT**, O6-methylguanine-methyltransferase; **MMR**, mismatch repair; **mo.**, months; **mOS**, median overall survival; **mPFS**, median progression free survival; **mRNA**, messenger RNA; **MSI**, microsatellite instability; **NIVO1+IPI3**, nivolumab 1 mg/kg + ipilimumab 3 mg/kg every 3 weeks (Q3W) for 4 doses, then nivolumab 3 mg/kg Q2W; **NIVO3**, nivolumab 3 mg/kg every 2 weeks; **NIVO3+IPI1**, nivolumab 3 mg/kg + ipilimumab 1 mg/kg Q3W for 4 doses, then nivolumab 3 mg/kg Q2W; **OS**, overall survival; **PD-L1**, programmed death-ligand 1; **PFS**, progression free survival; **pts**, patients; **qRT-PCR**, Real-time quantitative PCR ; **RT**, radiation therapy; **TIL**, tumor infiltrating lymphocytes; **TMB**, tumor mutational burden; **TMZ**, temozolomide

Table VIII. Bevacizumab biomarker testing in progressive glioblastoma

Abbreviations: **GBM**, glioblastoma; **pts**, patients; **BEV**, bevacizumab; **FTM**, fotemustine; **RR**, radiologic

Author (year):	Description of study:	Data class:	Conclusions:
Okita, Y., et al., 2012	<p><u>Study Description:</u></p> <p>Retrospective study of 32 pts with recurrent GBM in which prognostic factors were assessed.</p> <p>Therapy related changes were defined as pseudopalisading necrosis, coagulation necrosis, gemistocytic cells, and giant cells.</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=32)</p> <p>Median age: 57 (19-71)</p>	III	<p><u>Results:</u></p> <p>MIB-1 indices in recurrent tumors significantly correlated with OS in multivariate analysis (p=0.004). MST was not significantly associated with <i>MGMT</i> promoter methylation (p=0.8). 6/18 (33%) pts showed a change in methylation status upon recurrence.</p> <p><u>Author's Conclusions:</u></p> <p>Only MIB-1 index in recurrent GBM is a significant prognostic factor. <i>MGMT</i> promoter methylation status and degenerative changes in tumor cells in recurrent tumors had no correlation with survival time or PFS.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as it is a retrospective study with a limited sample size, histologic features were examined only by +/- of particular findings of treatment effect.</p>
Yang, P., et al., 2015	<p><u>Study Description:</u></p> <p>Prospective study of 274 pts with GBM looking at the effect <i>IDH</i> mutation and <i>MGMT</i> promoter methylation on treatment.</p> <p><u>Patient Population:</u></p> <p>GBM pts (N=274)</p> <p>Median age: 48 (18-81)</p>	II	<p><u>Results:</u></p> <p><i>IDH</i> mutations was observed in 56 cases (21%) of cases and <i>MGMT</i> promoter methylation was observed in 95 cases (40%). The <i>IDH1</i> and <i>MGMT</i> promoter methylation status of the study populations were: 32 (14%) <i>IDH</i> mutant and methylated <i>MGMT</i> promoter, 54 (25%) wild type <i>IDH</i> and methylated <i>MGMT</i> promoter, 15 (7%) <i>IDH</i> mutant and unmethylated <i>MGMT</i> promoter pts, and 128 (56%) wild type <i>IDH</i> and unmethylated <i>MGMT</i> promoter pts.</p> <p>TMZ and Radiation Therapy, OS and PFS was most favorable for those with tumors harboring both <i>IDH</i> mutant and methylated <i>MGMT</i> promoter (median OS: 35.8 mo., median PFS: 27.5 mo.); either <i>IDH</i> mutant or methylated <i>MGMT</i> promoter exhibited intermediate OS and PFS (mOS: 36 and 17.1 mo.; mPFS: 12.2 mo. and 9.9 mo., respectively); poorest OS and PFS was observed in wild type <i>IDH</i>, <i>MGMT</i> promoter unmethylated (mOS: 15 mo., mPFS: 9.7 mo.).</p> <p><u>Author's Conclusions:</u></p>

IDH mutation and *MGMT* promoter methylation status are independently associated with favorable outcome in TMZ+RT treated GBM and have distinct biologic functions. TMZ shows the greatest efficacy in wild type *IDH* tumors.

Comments and Conclusions:

Classified as Class II as it is a prospective study with a relatively large sample size in which multivariate analysis was performed.

Kristensen, L.S., et al., 2016

Study Description:

III

Retrospective study of 151 primary GBM pts treated by the Stupp protocol in which different methods of *MGMT* methylation status was assessed. Survival analysis was also performed.

-

Patient Population:

Primary GBM pts (n=151)

Results:

34% (51) of pts were methylation positive by qMSP, of which the mean methylation level was 15.2% and median was 2.4%.

36% (47) of pts were methylated (>9%) by standard pyrosequencing, with mean and median methylation levels of 37.3% and 30% respectively.

There is a strong association between the methods and methylation levels ($p < 0.001$, $p = 0.002$).

Methylation by qMSP and pyrosequencing and IHC was strongly associated ($p = 0.009$ and $P < 0.001$ respectively), IHC was positive in 43.9% and 48.8% of qMSP and pyrosequencing unmethylated samples, and negative in the majority of methylated 78% and 84.4% respectively.

All 3 methods correlate well with OS qMSP ($p = 0.006$), pyrosequencing ($p = 0.002$), and IHC ($p = 0.009$).

MGMT promoter methylation was associated with better OS ($p = 0.006$; qMSP and $p = 0.002$; standard pyrosequencing), and the presence of the protein was associated with worse OS ($p = 0.009$).

Author's Conclusions:

Use of both *MGMT* promoter methylation and *MGMT* IHC but not allelic methylation data as prognostic markers in patients with TMZ-treated glioblastoma.

Comments and Conclusions:

Classified as Class III because it is a retrospective study with a relatively large

			number of specimens, however, factors other than methylation status were not evaluated.
Millward, C.P., et al., 2016	<p><u>Study Description:</u></p> <p>Retrospective study of 100 GBM pts to assess for a correlation with <i>MGMT</i> promoter methylation status and <i>IDH</i> mutation response to chemo radiotherapy.</p> <p>-</p> <p><u>Patient Population:</u></p> <p>Primary GBM pts (N=100)</p> <p><i>IDH</i> mutant (N=5)</p> <p>Median age at dx: 54 (18-68)</p> <p>53% had <i>MGMT</i> promoter methylation >9%</p>	III	<p><u>Results:</u></p> <p>Independent prognostic variables for OS and PFS were female sex (only for OS, $p=0.019$), <i>MGMT</i> promoter methylation ($p<0.0001$, $p=0.001$, respectively), and <i>IDH</i> mutation ($p=0.023$, $p=0.018$, respectively).</p> <p>Kaplan-Meier survival analysis showed that <i>MGMT</i> methylated/<i>IDH1</i> mutant gliomas were associated with a significantly longer OS 66.8 months and PFS 16.9 months when compared with <i>MGMT</i> methylated/<i>IDH1</i> wild type gliomas (OS 15.5 months and PFS 9.4 months) and <i>MGMT</i> unmethylated/<i>IDH1</i> wild type gliomas (OS 11.1 months and PFS 6.3 months) ($p = 0.000$).</p> <p><u>Author's Conclusions:</u></p> <p>Combination of <i>MGMT</i> promoter methylated/<i>IDH1</i> mutant glioma is associated with considerably longer OS and PFS in this series of chemoradiotherapy-treated glioblastoma tumors.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as it is a retrospective study with a limited sample size.</p>
Quillien, V., et al. 2016	<p><u>Study Description:</u></p> <p>Prospective multi-institution trail in which analysis of the <i>MGMT</i> promoter methylation status by pyrosequencing and methylation-specific PCR on frozen section tissue and formalin-fixed paraffin-embedded tissue from 139 GBM patients and the correlation of PFS and OS with specific cut-off thresholds.</p> <p><i>MGMT</i> promoter methylation was assessed by pyrosequencing and</p>	III	<p><u>Results:</u></p> <p><i>MGMT</i> promoter methylation as determined by PSQ and MS-PCR was concordant in 85% of cases. For PSQ a cut-off of 8% was determined to best correlate with OS, using this cut-off 51% of patients were classified as methylated. For msPCR a cut-off of 13% was determined to best correlate with OS, using this cut-off 37% of patients were classified as methylated. Both methods demonstrated statistically significant correlation with OS.</p> <p><u>Author's Conclusions:</u></p> <p>PSQ is the ideal method due to strong inter-laboratory reproducibility, increased sensitivity, and multiple studies demonstrating concordant threshold levels.</p> <p><u>Comments and Conclusions:</u></p>

quantitative
methylation specific
PCR (msPCR)

Classified as Class III, it is a multi-institutional study with a relatively large sample size, however statistical methods demonstrating superiority of one method was not performed. Note that cut-off values were determined based on FFPE and fresh frozen tissue.

Patient Population:

Primary GBM pts
(N=139)

Median age at surgery:
55.9 (23-71)

Brandes, A.A.
et al. 2017

Study Description:

III

Results:

Single center
retrospective study of
108 pts who underwent
TMZ + RT with
recurrent GBM in whom
methylation status was
tested in both the
primary tumor and the
recurrence.

MGMT promoter was methylated in 44.4% (79) of primary GBMs and unmethylated in 55.6% (99). *MGMT* promoter was methylated in 46.7% (64) of recurrent GBMs and unmethylated in 53.3% (73). In paired samples the findings were as follow:

MGMT promoter
methylation was
detected by microscale
thermophoresis

methylated > methylated = 35.2% (n=38, OS=35.2 m), unmethyl > unmethyl = 39.8% (n=43, OS= 20.1 m), methylated > unmethyl = 14.8% (n=16, OS=27.3 m), unmethyl > methylated 10.2% (n=11, OS=23.3 m). *MGMT* promoter methylation was stable in 75% of the cases, and there was significant concordance between the first and the second *MGMT* promoter methylation assessments (K = 0.500, p < .001). In multivariate analysis *MGMT* promoter methylation at first surgery (p<0.001) (but not at 2nd surgery) was significantly correlated with survival.

Patient Population:

Primary GBM pts
(N=178)

Recurrent GBM pts
(N=137)

Paired GBM pts
(N=108)

Mean age: 50.8

Author's Conclusions:

MGMT promoter methylation status remains stable during the clinical course in the majority of GBM pts., thus re-testing this biomarker at recurrent does not provide further information. OS from diagnosis is correlated with *MGMT* promoter methylation status at 1st surgery but not recurrence; a potential explanation for this may be that *MGMT* promoter methylation increased PFS but not post progression survival.

Comments and Conclusions:

Classified as Class III because study is retrospective review with a relatively large

			number of specimens in which hazard ratios were able to be determined.
Kim, B.S., et al., 2017	<p><u>Study Description:</u></p> <p>Single center retrospective study of 61 pts who underwent gamma knife surgery (GKS) for local recurrent GBM pts to determine in <i>MGMT</i> promoter methylation was a prognostic marker of gamma knife radiosurgery</p> <p><i>MGMT</i> promoter methylation was detected by methylation specific PCR</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=61)</p> <p>Median age at GKS: 58</p>	III	<p><u>Results:</u></p> <p>41 % (25) pts were methylated and 59% (36) were unmethylated. <i>MGMT</i> promoter methylation status was the strongest predictor of OS (p=0.03) on multivariate analysis, preplanning tumor volume was correlated with PFS (p=0.005) on multivariate analysis regardless of methylation status. The time to progression was longer after GKS was longer in pts with <i>MGMT</i> promoter methylation.</p> <p>-</p> <p><u>Author's Conclusions:</u></p> <p><i>MGMT</i> promoter methylation conferred a profound delay in tumor progression compared to unmethylated (8.9 to 4.6 mo., p=0.016) and survival benefit (OS 14 vs 9 mo., p= 0.03). <i>MGMT</i> promoter methylation was also an independent prognostic factor for progression and survival after GKS.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because it is a retrospective study with a small number of pts, they only measure methylation at initial resection, and did not have a control arm of untreated pts Does not address re-assessing methylation at recurrence.</p>
Hsu, C. et al., 2017	<p><u>Study Description:</u></p> <p>Retrospective study comparing the prognostic power of <i>MGMT</i> promoter methylation assays including MSP, qMSP, PSQ, and IHC in 121 primary GBM pts.</p> <p>Median age: 55 (40-65)</p> <p>IDH-wt: N=111 (91.7%)</p> <p>IDH-mutant: N=10 (8.3%)</p>	III	<p><u>Results:</u></p> <p>Unmethylated pts:</p> <p>IHC: 46.3% (of note histiocytes demonstrate strong staining and must be excluded); MSP PCR: 48.8% (59); qMSP: 50.4% (61), PSQ: 55.4% (67)</p> <p>Methylated pts:</p> <p>IHC: 53.7%; MSP PCR: 51.2% (62); qMSP: 49.6% (60), PSQ: 44.6% (54)</p> <p>Complete concordance was observed between methods in 73.6% of cases (89), of which 49.4% were methylated. Concordance among MSP, qMSP, and PSQ was observed in 81.8% (99) of cases. PSQ and MSP had the highest predictive power for PFS and OS, respectively and IHC had the lowest for both PFS and OS, but was not significantly different between methods. Pricing: IHC: \$35/test, MSP: \$100/test, qMSP: \$105/test, PSQ: \$200/test.</p>

			<p>-</p> <p><u>Author's Conclusions:</u></p> <p>MGMT status evaluated by IHC, MSP qMSP, and PSQ all showed significant correlation with PSF and OS and their predictive powers were not significantly different.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because it is a retrospective study but has a relatively large study size but looks just at methylated vs unmethylated with no additional granularity.</p>
Johannessen, L.E.et al., 2018	<p><u>Study Description:</u></p> <p>Single center retrospective study of 48 pts with primary GBM in whom commercially available <i>MGMT</i> promoter methylation assay (PSQ theascreen, PSQ 96, MSP, MS-HRM, and qMSP) were compared.</p> <p>-</p>	III	<p><u>Results:</u></p> <p>Significant differences in OS in methylated vs. unmethylated <i>MGMT</i> promotor GBM was observed for the two PSQ kits (p=0.011) and MSP (p=0.037), but not for MS-HRM and qMSP (p=0.482 and p=0.113, respectively). Median OS using MSP was 11.5 months for the group with unmethylated <i>MGMT</i> and 13.5 months for the group with methylated <i>MGMT</i>, whereas 2-year OS was 12% and 33.8%, respectively. For PSQ, median OS was 11.6 months in the group with unmethylated <i>MGMT</i> promoter and 19.5 months in the group with methylated <i>MGMT</i>, whereas 2-year OS was 7.4% and 41.90%, respectively.</p> <p>-</p> <p><u>Author's Conclusions:</u></p> <p>PSQ is the best technique for prognostication.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because it is a retrospective study with a small number of pts and only univariate analysis was performed.</p>
Napoleoni, L., et al., 2019	<p><u>Study Description:</u></p> <p>Retrospective study of <i>MGMT</i> expression in 36 pts with progressive GBM as assessed by IHC.</p> <p>MGMT was assessed by IHC</p> <p>-</p>	III	<p><u>Results:</u></p> <p>19.4% (7) pts were negative for <i>MGMT</i> expression by IHC, and 80.6% (29) were positive. 33.3 % (12) had expression in >50% of cells and 66.7% (24) in <50%, 19.4% (7) had expression in >70% and 80.6% (<70%). <i>MGMT</i> expression was shown to be significantly associated with disease control rate when expression was <70% on multivariate analysis, but with PFS only on univariate analysis, and not significantly associated with OS.</p>

	<p><u>Patient Population:</u></p> <p>Primary GBM pts (N=36)</p> <p>Median age at dx: 61 (24-75)</p>		<p><u>Author's Conclusions:</u></p> <p>MGMT IHC high expression (>70%) might be used as a "surrogate" negative predictor for response dd-TMZ treatments. However, validation is still a matter of debate.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as it is a retrospective study with a limited sample size and did not compare different methods of <i>MGMT</i> promoter methylation assessment.</p>
Radke, J. et al., 2019	<p><u>Study Description:</u></p> <p>Retrospective study of 111 IDH-wt GBM pts in whom <i>MGMT</i> promoter methylation status was correlated with outcome to determine a predictive cut-off of clinical decision making.</p> <p><i>MGMT</i> promoter methylation was assessed by pyrosequencing, sqMSP, and dBisequ</p> <p><u>Patient Population:</u></p> <p>Primary GBM pts (N=111)</p> <p>Median age at dx: 61.2 (18-85.4)</p>	III	<p><u>Results:</u></p> <p>49% (55) of pts were <i>MGMT</i> promoter unmethylated (<10%), and 51% (56) were methylated (≥10%). mPFS and OS in unmethylated GBM was 7.2 mo. and 13.4, for low level methylation (10-20%) PFS and OS was 10.04 and 17.9, respectively, and high level methylation (>20%) has PFS and OS of 19.83 and 29.93. PFS was significantly different in all 3 groups but OS was only significantly different between unmethylated and high level methylated. Methylation was also determined by sqMSP and dBisequ and results were discordant as compared to pyrosequencing in 53.1% and 54.5% of cases, however in cases >16% good consistency was seen between the 3 methods.</p> <p><u>Author's Conclusions:</u></p> <p><i>MGMT</i> promoter methylation between 10-20% represents a transition zone in terms of PFS and OS relative to unmethylated or highly methylated patients. Of patients with low methylation PSQ results could only be validated in 51.5% of cases by another method to be clearly methylated. Recommend a 3-tier system of unmethylated (0-9%), low level methylation (10-20%), and high level methylation (>20%).</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as it is a retrospective single center study with a large relatively homogenous pt population.</p>

response; **CR**, complete response; **PR**, partial response; **PFS**, progression free survival; **PFS-6**, 6 month progression free survival; **OS**, overall survival; **CCNU**, lomustine

Figures

Author (year):	Description of study:	Data class:	Conclusions:
Lv, S. et al., 2012	<p><u>Study Description:</u></p> <p>Retrospective review of 35 recurrent GBM pts in a phase II clinical trial of cetuximab (EGFR-targeted mab) to determine correlation between the <i>EGFR</i> gene amplification, <i>EGFR</i> variant III and <i>EGFR</i> variant IV mutations, expression of PTEN and <i>IDH1</i> in response to cetuximab.</p> <p><i>IDH</i>, <i>EGFRvIII</i> and <i>EGFRvIV</i> were detected by PCR, and PTEN by IHC</p> <p>-</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=35)</p> <p>Median age: 54 (33-73)</p>	III	<p><u>Results:</u></p> <p><i>EGFR</i> amplification was detected in 54% of GBMs, <i>EGFRvIII</i> expression 31.4%, and <i>EGFRvIV</i> expression in 20%. <i>EGFRvIII</i> and <i>EGFRvIV</i> were exclusively found in <i>EGFR</i> amplified GBMs. PTEN was positive in 21% of pts. Patients with an <i>EGFR</i> amplification lacking <i>EGFRvIII</i> had a significantly superior PFS and a better OS following treatment with cetuximab [median PFS 3.03 vs. 1.63 months (p=0.006); median OS 5.57 vs. 3.97 months (p=0.12)]. Patients with <i>EGFRvIII</i> positive GBM had a worse survival [median PFS 1.63 vs. 3.03 months (p=0.01); median OS 3.27 vs. 5.57 months (p=0.08)].</p> <p><u>Author's Conclusions:</u></p> <p>Pts with an <i>EGFR</i> amplification without <i>EGFRvIII/vIV</i> may show a higher sensitivity to cetuximab. Previous reports demonstrate PTEN expression (by IHC) in ~60% of primary gliomas and 17% of recurrent GBMs (comparable to the 21% found in this study).</p> <p><u>Comments and Conclusions:</u></p>

Classified as Class III as it is a retrospective study with a limited sample size.

D'Alessandris, Q.G., et al., 2013

Study Description:

Single center prospective study of 10 pts with recurrent GBM in whom BEV and erlotinib were administered based on VEGF and *EGFRvIII* expression.

EGFRvIII was assessed by rt-PCR on primary tumor, VEGF was assessed by IHC on primary tumor

Patient Population:

Recurrent GBM pts (N=10)

Median age= 53 (30-77)

VEGF overexpressed N=10

EGFRvIII (+) N=4

III

Results:

All the pts who received BEV + erlotinib achieved a radiological response (RR) (3 complete responses (CRs) and 1 partial response (PR) with RR and PFS-6 of 100% (4/4 pts). Of the 6 pts treated with BEV alone, three had a radiological response (two CRs and one PR) with RR and PFS-6 of 50% (3/6 cases), 2 had progressive disease, and 1 pt had stable dx w/ intra-tumoral hemorrhage. The RR was 70% of cases (7/10), with 5 CRs and 2 PRs. PFS-6 was 70% (7/10 cases). Median PFS and OS were 8.0 mo. (range 3.0 to 31.0 months) and 9.5 mo. (range 5.0 to 31.0 mo.). BEV + erlotinib median PFS was 10.5 mo. (range 7.0–31.0 mo.) and OS was 17.0 mo. (range 8.0–31.0 mo.). BEV only median PFS was 5.5 mo. (range 3.0–9.0 mo.) and OS was and 6.75 mo. (range 5.0–15.0 mo.).

Author's Conclusions:

Expression of *EGFRvIII* is a reliable biomarker for activation of EGFR-related tyrosine kinase, which is the target of erlotinib. Conversely, anti-EGFR immunostaining, though indicative of

			<p>EGFR overexpression, does not necessarily indicate activation of EGFR-related tyrosine kinase. RR and PFS-6 of 70% was achieved in the whole cohort, 100% in the group treated with bevacizumab and erlotinib, and 50% in the group treated with bevacizumab.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III, while it is a prospective study there is a very limited number of pts.</p>
Chi, A., et. al., 2013	<p><u>Study Description:</u></p> <p>Multicenter, open-label, nonrandomized study of Dacomitinib (irreversible, small-molecule EGFR tyrosine kinase inhibitor) in 30 pts with first-recurrent <i>EGFR</i>-amplified GBM who were anti-VEGF naïve.</p> <p>Serum extracellular vesicle–derived genes were assessed between 7 clinical responders and 7 rapid progressors</p> <p>-</p> <p><u>Patient populations:</u></p> <p>Recurrent GBM pts (n=30)</p> <p>Median age = 61</p>	III	<p><u>Results:</u></p> <p><i>EGFRvIII</i> status was determined by rtPCR in 20 (67%) all pts had GFR ECD hotspot mutations was determined by Sanger sequencing. Five (17%) pts achieved PFS6, and thus, this arm did not meet the primary efficacy end point of 30% PFS6. Presence of <i>EGFRvIII</i> was not associated with clinical benefit. There was no association between any ECD mutant cohort and clinical benefit. In addition, there was no association between clinical response and the presence of any <i>EGFR</i> mutation (<i>EGFRvIII</i> and/or ECD hotspot mutation; P = .2391. <i>MGMT</i> promoter methylation in the archival tumor specimen was not associated with clinical response.</p>

Dacomitinib reaches concentrations in contrast-enhancing tumor tissue well above the IC50 values for cells with sensitizing *EGFR* mutations. Presence of *EGFR* mutation did not predict a lack of benefit. Genes differentially expressed between clinical responders and rapid progressors. 32 genes that were significantly differentially expressed in serum extracellular vesicle-derived in pts who remained stable > 6 months (responders) compared with pts with disease progression < 3 months (rapid progressors).

LAMTOR2 (late endosomal/lysosomal adaptor, mitogen-activated protein kinase [MAPK], and mammalian target of rapamycin [mTOR] activator 2), which is an activator of MAPK and mTOR signaling, and CSF1, which encodes macrophage colony-stimulating factor (M-CSF), were elevated in rapid progressors.

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Author's Conclusions:

Only a small subset of patients with *EGFR*-amplified GBM derived a clinically meaningful benefit from Dacomitinib. We did not find *EGFR* amplification, *EGFRvIII* or ECD mutation status to be

			<p>associated with clinical response.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because study is a retrospective study of a small number of pts. <i>EGFR</i> status was determined on archival tissue and may not reflect the pts current <i>EGFR</i> status.</p>
Lassman, A.B., et al., 2015	<p><u>Study Description:</u></p> <p>Phase II study in 50 pts of dasatinib (multi-target tyrosine kinase inhibitor) as monotherapy for BEV-naïve recurrent GBM harboring overexpression/activity assessed by IHC of SRC, PDGFR, EPHA2, and/or c-KIT following TMZ+RT</p> <p>-</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (n=50)</p> <p>Median age = 51 (33-81), 54 (26-75)</p>	III	<p><u>Results:</u></p> <p>36% of pts had 1 molecular marker, 48% had 2, and 16% had 3. p-SRC was positive in 60% of pts, PDGFR in 52%, EPHA2 in 86%, and c-Kit in 82%. Response was not seen in any pts, 24% showed stable dx, and 72% progressed.</p> <p><u>Author's Conclusions:</u></p> <p>Dasatinib failed to demonstrate efficacy as monotherapy for recurrent GBM.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as there was a limited number of pts and full statistical analysis to qualify as class II was not performed.</p>
van den Bent, M., et al., 2015	<p><u>Study Description:</u></p> <p>Retrospective study of 55 cases of matched recurrent glioblastomas treated with radiation and TMZ in which <i>EGFR</i> expression/mutation was assessed.</p>	III	<p><u>Results:</u></p> <p><i>EGFR</i> amplification status remains stable in the majority (84%) of tumors. The relative expression of <i>EGFRvIII</i> was often lower in recurrent tumors (53% showed a >20%</p>

EGFR amplification and *EGFRvIII* mutation was detected by qRT-PCR

Patient Population:

Recurrent GBM

- *EGFR* amp (N=55)
- *EGFRvIII* (N=42)

Median age: 51.2

decrease). Within the tumors expressing *EGFRvIII* at initial diagnosis, approximately 47% lose their *EGFRvIII* expression at tumor recurrence.

Author's Conclusions:

The relative stability of *EGFR* amplification indicates that molecular data obtained in the primary tumor can be used to predict the *EGFR* status of the recurrent tumor, but care should be taken in extrapolating *EGFRvIII* expression from the primary tumor.

Comments and Conclusions:

Classified as Class III as it is a retrospective study with a limited sample size.

Cioca, A, et al
2016

Study Description:

Single center retrospective study of 24 patients with recurrent GBM in whom *EGFR* expression was assessed by immunohistochemistry in the original and recurrent sample to assess for alterations in *EGFR* expression.

EGFR assessed by IHC

Patient Population:

Recurrent GBM pts (N=24)

Median age at dx: 54.3 (26-78)

III

Results:

EGFR immunopositivity was present in 96% of cases with newly diagnosed GBM; strong reactivity in 15 (62.5%) cases, intermediate in 7 (29.1%), and weak positivity in 1 (4.1%). All the recurrent tumors expressed *EGFR*, with strong reactivity in 9 cases (37.5%), moderate positivity in 10 cases (41.6%) and weak positivity in 5 cases (20.8%). Ten recurrent tumors (42%) had a lower expression than their correspondent

pair, 13 tumors (54%) had similar expression, and only one case (2%) had increased expression at recurrence.

Author's Conclusions:

EGFR is overexpressed in glioblastoma and this overexpression is maintained but decreased at recurrence. Tumors with strong EGFR expression in the primary tumor was correlated with longer relapse free interval compared to cases with low EGFR expression (11.46 mo., $p=0.017$).

Comments and Conclusions:

Classified as Class III because study is a retrospective study of a small number of pts with a suboptimal method of EGFR amplification detection. Treatment varied with 32% not given standard therapy.

Sepulveda-Sanchez, J.M., et al., 2017

Study Description:

Phase II clinical trial of 49 recurrent GBM pts with *EGFR* amplified +/- *EGFRvIII* mutation treated with Dacomitinib (pan-HER tyrosine kinase inhibitor).

Patient Population:

Recurrent GBM pts (N=49)

Median age: 59 (39-81)

III

Results:

Median PFS of 2.7 months, with 4 pts progression free at 6 months and 3 pts were at 12 months. Median OS was 7.4 months. The best overall response included 1 complete response and 2 partial responses (4.1%). Stable disease was observed in 12 patients (24.5%) and progressive disease

was seen in 61.2% of pts.

Author's Conclusions:

Despite the rationale to target EGFR in (recurrent) glioblastoma, dacomitinib has limited efficacy as a single agent, even in *EGFR* amp +/- vIII mutation. The response to other EGFR inhibitors has also been disappointing.

Comments and Conclusions:

Classified as Class III as it is a prospective study with a limited sample size.

Felsberg, J., et al., 2017

Study Description:

Single center retrospective study of 106 IDH-wt gliomas to assess the prognostic significance of *EGFR* amplification and *EGFRvIII* mutation. Changes in *EGFR* amplification and *EGFRvIII* status from primary to recurrent glioblastomas were evaluated in 40 patients with *EGFR*-amplified tumors and 33 patients with *EGFR*-nonamplified tumors.

EGFR status was determined by IHC, PCR or both.

Patient Population:

EGFR amplified GBM pts (N=106)

Recurrent GBM pts (N=73)

- *EGFR* amplified (N=40)
- Non-*EGFR* amplified (N=33)

Median age at dx: 63 (29-86)

III

Results:

57% of *EGFR*-amplified glioblastomas were *EGFRvIII*-positive. *EGFRvIII* positivity was not associated with different progression-free or overall survival. *EGFRvIII* status was unchanged at recurrence in 88% of patients with *EGFR*-amplified primary tumors. Four patients lost and one patient gained *EGFRvIII* positivity at recurrence. None of 33 *EGFR*-nonamplified glioblastomas acquired *EGFR* amplification or *EGFRvIII* at recurrence. PCR showed slighted increased sensitivity for *EGFRvIII* over IHC.

			<p><u>Author's Conclusions:</u></p> <p><i>EGFRvIII</i> and EGFR SNVs are not prognostic in <i>EGFR</i>-amplified glioblastoma patients. EGFR amplification is retained in recurrent glioblastomas. Most <i>EGFRvIII</i>-positive glioblastomas maintain <i>EGFRvIII</i> positivity at recurrence. However, <i>EGFRvIII</i> expression may change in a subset of patients at recurrence, thus repeated biopsy with reassessment of <i>EGFRvIII</i> status is recommended for pts with recurrent glioblastoma to receive EGFRvIII-targeting agents.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class II because study is a retrospective study of a relatively large number of patients, however, data was not suitable for NPV, PPV, sensitivity or specificity.</p>
Hovinga, K.E. et al, 2019	<p><u>Study Description:</u></p> <p>Retrospective study of 80 GBM pts treated with BEV for recurrence in whom genomic subtype analysis was available.</p> <p><i>EGFR</i> amplification was determined by FISH, EGFRvIII by IHC, <i>MGMT</i> promoter methylation by msPCR performed on the recurrent specimen</p>	III	<p><u>Results:</u></p> <p>Genomic subtypes: 26% classical, 36% mesenchymal, 6% neural, and 31% proneural. The classical subtype has a higher risk of progression on multivariate analysis ($p<0.001$). <i>EGFR</i> was amplified in 43% of tumors and was more often present in the classical subtype ($p<0.001$). Amplified <i>EGFR</i> was associated w/ higher risk of</p>

progression on multivariate analysis ($p=0.01$). Multifocal change on BEV was seen in 92% of cases.

Author's Conclusions:

Classical subtype and *EGFR* gene amplification are associated with significantly shorter time to progression for patients with recurrent GBM when treated with BEV.

Comments and Conclusions:

Classified as Class III because it is a retrospective study with some factors not available on all pts and with different treatment regimens.

	<p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=80)</p> <p>Mean age at treatment: 60.4 (25.1-80.33)</p>		
Lassman, A.B., et al., 2019	<p><u>Study Description:</u></p> <p>Phase I multi-center study of depatuxizumab mafodotin + TMZ of 60 patients with <i>EGFR</i>-amplified recurrent GBM, BEV naïve pts</p> <p><i>EGFR</i> status was assessed by FISH, whole exome sequencing, RT-PCR and IHC on the primary resection</p> <p>-</p> <p><u>Patient Population:</u></p> <p>Primary GBM pts (n=60)</p> <p>Median age: 56 (20-79 years)</p> <p>-</p>	III	<p><u>Results:</u></p> <p>50% of GBMs harbor <i>EGFR</i> amplification. The median duration of response was 5.6 months (95% CI = 1.5, 9.7). Notable reduction in tumor size of at least 25% was observed in 22% of patients; median time to progression in this group was 3.7 months. The overall PFS was 2.1 months and the OS was 7.4 months.</p> <p><u>Author's Conclusions:</u></p> <p>Depatux-m + TMZ displayed an AE profile similar to what was described previously. Antitumor activity in this TMZ-refractory population was encouraging. No association with <i>EGFRvIII</i> was seen.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because of the relatively limited number of pts and lack of a comparative arm</p>

Author (year):	Description of study:	Data class:	Conclusions:
Johnson, B.E., et al., 2014	<p><u>Study Description:</u></p> <p>Single center retrospective study of 23 pts with grade II gliomas at initial diagnosis and their recurrences resected from the same patients. Genome sequence analysis was performed on paired samples to interrogate alterations.</p> <p><u>Patient Population:</u></p> <p>Recurrent glioma pts (N=23)</p>	III	<p><u>Results:</u></p> <p>54% of mutations were detected in the primary and recurrent tumor and included <i>IDH</i>, <i>TP53</i>, <i>ATRX</i>. 6 tumors underwent TMZ-induced hypermutation and underwent malignant progression to GBM.</p> <p><u>Author's Conclusions:</u></p> <p>Recurrent tumors are often seeded by cells derived from the initial tumor at a very early stage of their evolution. TMZ-induced mutagenesis lead to hypermutated tumors with mutations in the RB and AKT-mTOR pathways.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because it is a retrospective study with a limited number of pts. It should be noted that none of these pts had an initial diagnosis of GBM and not all pts progressed to GBM in this study.</p>
Meng, J., et al., 2014	<p><u>Study Description:</u></p> <p>Retrospective study of 276 GEO cohort and 436 TCGA cohort glioma pts in which a 31 gene signature was assessed as a biomarker for estimating OS in radiation-treated pts</p> <p>-</p> <p><u>Patient Population:</u></p> <p>glioma pts (N=276; 436)</p> <p>Median age at dx: 50.3; 56.3</p>	III	<p><u>Results:</u></p> <p>Radiosensitive pts had a superior OS compared with RR pts either in radiotherapy-treated subset or in the patient subset that did not receive RT. Nevertheless, in the multivariate Cox regression analysis to assess for independent predictors of the relation between the gene signature and clinicopathologic features, we found that the gene signature is the strongest predictor (p=0.0093) in the subgroup of patients with radiotherapy, whereas it does not remain significant (p=0.202) in the non RT group when taking age and KPS into account.</p> <p><u>Author's Conclusions:</u></p> <p>The radiosensitivity gene signature is mainly predictive in patients treated with radiation therapy. Radioresistant phenotype was enriched for genes of EMT, whereas radiosensitive phenotype correlated strongly with decrease of genes of EMT.</p> <p><u>Comments and Conclusions:</u></p>

Classified as Class III as it is a retrospective study, but does have a very large cohort, however, not all pts within the GEO cohort have GBM.

Bleeker, F.E. et al., 2014

Study Description:

II

Results:

Single center study of 109 patient samples and 16 high grade glioma cells lines studies with between 9 and 80 pts (159 pts in total) with primary or secondary GBM. 4 patients with recurrent GBM were also included with comparison to the primary specimen.

No additional mutations were observed in the four recurrent tumors compared to their primary. 67% of GBMs displayed at least one somatic mutation. Somatic mutations were found in *TP53* (61 mutations), *PTEN* (39), *IDH1* (20), *PIK3CA* (13), *EGFR* (7), *BRAF* (3), *EPHA3* (1), *NRAS* (1), *TGFRB2* (1), *FLT3* (1) and *RPS6KC1* (1). *PIK3CA* and *PTEN* mutations were mutually exclusive.

Author's Conclusions:

Examined 174 exons including the following genes: *IDH1*, *IDH2*, *NRAS*, *PTEN*, *TP53*, *AKT2*, *ATM*, *ATR*, *BRAF*, *BRD2*, *DDR1*, *DYRK2*, *EGFR*, *EPHA3*, *EPHA5*, *EPHA6*, *EPHB2*, *ERBB2*, *ERBB4*, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *FLT1*, *FLT3*, *FRAP1*, *IDH1*, *IDH2*, *KDR*, *KIT*, *MAP2K4*, *MET*, *NRAS*, *NTRK2*, *NTRK3*, *PAK4*, *PDGFRA*, *PDPK1*, *PIK3CA*, *PTEN*, *RPS6KC1*, *STK11*, *TGFRB2* and *TP53*.

Most of these mutations likely represent 'driver' mutations. *PIK3CA* and *PTEN* mutations are mutually exclusive. Strong clustering of mutations in genes belonging to the PI3K-AKT pathway, however, due to the development of resistance mechanisms, kinase inhibition studies targeting the PI3K-AKT pathway for relapsing GBM have mostly failed thus far.

Comments and Conclusions:

Classified as Class III because study is retrospective with a limited clinical correlation. PI3K-AKT pathway is frequently mutated but no target therapies have shown promise. Testing for treatment purposes is not necessary at this time.

Patient Population:

GBM pts (N = 109)

Recurrent GBM (N=4)

Mean age: 54 (15-81)

Li, R. et al., 2015

Study Description:

III

Results:

Single center retrospective study of whole transcriptome sequencing in primary and recurrent GBM. Paired tumors were analyzed for molecular subtype and biological progression

36% of primary GBM belonged to the classical subtype vs 22% in recurrent GBM. 15% of primary GBMs were of the proneural subtype vs 23% of recurrent GBMs. The neural subtype made up 8% of primary GBM and 9% of recurrent GBM. 41% of primary and 45% of recurrent GBM were identified as the mesenchymal subtype.

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	<p><u>Patient Population:</u></p> <p>Primary GBM pts (N=88)</p> <p>Recurrent GBM pts (N=22)</p> <p>Median age at dx: 48</p>		<p><u>Author's Conclusions:</u></p> <p>Gene set enrichment analysis revealed that chromatin fracture, repair, and remodeling genes were enriched in recurrent glioblastoma.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as it is a retrospective study with a very young population and conclusions are drawn that likely reflect re-operation bias more so than natural progression of the disease.</p>
Kolodziej, M.A., et al., 2016	<p><u>Study Description:</u></p> <p>Single center retrospective study of 44 GBMs were analyzed for NDRG2 and NDRG4 expression (by PCR and IHC) and correlated with progression free survival</p> <p>-</p> <p><u>Patient Population:</u></p> <p>Primary GBM pts (n=40)</p> <p>Recurrent GBM pts (N=4)</p> <p>Median age at dx: 57.4</p>	III	<p><u>Results:</u></p> <p>Low protein expression of NDRG2 with <i>MGMT</i> promoter methylation was associated with a poor PFS of 10 mo. vs 22 mo. with high NDRG2 protein expression and <i>MGMT</i> promoter methylation. NDRG2 and NDRG4 expression positively correlated with <i>MGMT</i> promoter methylation. Pts with low NDRG4 protein expression (≤ 5.0) and <i>MGMT</i> promoter methylation had PFS of 12 (range=6.9-17.1) months compared with 17 (range=7.3-26.7) months for those with high NDRG4 protein expression (>5.0) and <i>MGMT</i> promoter methylation. The low NDRG4 protein expression in combination with unmethylated <i>MGMT</i> promoter was associated with a mean PFS of 8 months (range: 1.6-14.4). vs. 5 (range=0.2-9.8) months for those with high protein NDRG4</p> <p><u>Author's Conclusions:</u></p> <p>Expression of NDRG2 and NDRG4 genes at the protein or mRNA level changes in response to radiochemotherapy and hypothesize that radio resistance of tumor cells overexpressing NDRG2 gene is associated with down-regulation of NDRG2 after neoadjuvant therapy. Low NDRG2 expression leads to longer PFS independent of methylation status. NDRG4 acts as a tumor suppressor gene in <i>MGMT</i> methylated cells and as an oncogene in unmethylated <i>MGMT</i> cells.</p> <p><u>Comments and Conclusions:</u></p> <p>-</p> <p>Classified as Class III because it is a retrospective study with a moderate number of specimens</p>
Wood, M.D., et al., 2016	<p><u>Study Description:</u></p>	III	<p><u>Results:</u></p>

Retrospective study of 20 pts with *IDH*-wildtype recurrent GBM in which proteomic and genetic features are examined in pre and post treatment specimens.

Patient Population:

Recurrent GBM pts (N=20)

Median age: 52.3 (31-73)

Immunopositivity for Ki-67 in > 20% of tumor cells was associated with shorter progression-free and OS. Recurrent tumors showed decreased staining for CD34 suggesting lower vessel density. A subset of tumors showed increased staining for markers associated with the mesenchymal gene expression pattern, including CD44, phosphorylated STAT3, and YKL40. Recurrent tumors with the greatest increase in mesenchymal marker expression had rapid clinical progression, but no difference in overall survival after second surgery. There was poor correspondence between IHC (protein) and mRNA.

Author's Conclusions:

GBM progression is associated with a shift toward a mesenchymal phenotype in a subset of tumors and this may portend a more aggressive behavior.

Comments and Conclusions:

Classified as Class III as it is a retrospective study with a limited sample size.

Narsia, N., et al., 2017

Study Description:

Retrospective study of GEO cohort data to identify genetic alterations present in infiltrating gliomas.

III

Results:

3 genes were unique to recurrent GBM, overexpression of *FTL* and *CTSL* and under-expression of *MT1JP*. Recurrent GBM showed select alterations from primary GBM with enrichment of gap junction and hypoxia regulation genes, and WNT-beta catenin pathway genes and relative downregulation of cytokine-cytokine receptor interaction, ERBB signaling, ERK1/2/MAPK signaling, PDGF, FGFR, syndecan, and VEGF pathway signaling.

Patient Population:

Primary GBM pts (N=58)

Recurrent GBM (N=19)

Author's Conclusions:

Distinct gene expression pattern exists between grade II astrocytoma and GBM. Gene set enrichment analysis revealed a distinct expression pattern of transcriptional regulators in primary GBM. Further investigation into molecular processes showed that the genes involved in cell proliferation and invasion were shared across all subtypes of astrocytoma.

Comments and Conclusions:

Classified as Class III as it is a retrospective study with a limited sample size, the proportion IDH mutant recurrent GBM is not noted

<p>Byron, S.A. et al., 2018</p>	<p><u>Study Description:</u></p> <p>Single center prospective study of 16 pts with recurrent GBM in whom genome wide tumor sequencing and molecular tumor board to determine feasibility of creating individual treatment plans within 35 calendar days.</p> <p>Whole genome sequencing was performed on recurrent specimen</p> <p><u>Patient Population:</u></p> <p>GBM pts (N=16)</p> <p>Median age at dx: 51 (29-66)</p>	<p>III</p>	<p><u>Results:</u></p> <p>Therapeutically informative alterations were identified in 16 patients. The most common genes altered included <i>EGFR</i>, <i>PTEN</i>, <i>CDKN2A</i>, <i>NF1</i>, <i>RB1</i>, and <i>TP53</i>. Median time from surgery to molecular results and treatment recommendation was 27 calendar days, and was completed within 35 days for 81% of pts. In 1 pt molecular profiling needed to be repeated and in 2 pts RNA did not meet quality control measures. 47% (7) of pts decided to pursue the tumor board treatment recommendations, with 2 performing exceptionally well. Hyper-mutation is reported in ~17% of GBMs post TMZ exposure and is associated w/ mutations in MMR genes.</p> <p><u>Author's Conclusions:</u></p> <p>Genome wide molecular test to guide treatment is feasibility and 2 patients showed prolonged TTP.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because while it is prospective only a small number of pts were included and endpoint was not amenable to class II statistics</p>
<p>Cimino, P.J. et al, 2018</p>	<p><u>Study Description:</u></p> <p>4 cohort (TCGA, GGN, ARTE trial, and paired recurrent) retrospective study of <i>IDH</i>-wildtype glioblastoma were analyzed for gain of whole chromosome 1, gain of whole chromosome 19, and <i>CDK4/MDM2</i> co-amplification.</p> <p>Results of the paired recurrent cohort was compared to the TCGA+GGN cohorts (who were assumed to be representative of the population).</p> <p><u>Patient Population:</u></p>	<p>III</p>	<p><u>Results:</u></p> <p>Pts in the recurrent cohort are biased to better functional status and survival tend to have more chromosomal alterations and specifically the following alterations: whole chromosome 1 gain, whole chromosome 19 gain, and/or mutations in <i>TP53</i>, with lack of <i>CDK4</i> co-amplification (W3 subtype).</p> <p><u>Author's Conclusions:</u></p> <p>It is important to include molecular profiling, including copy number, when enrolling patients for clinical trials in order to balance arms and extrapolate relevance to the general glioblastoma population.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because study compares relatively large cohorts, however it is retrospective.</p>

GBM pts (N=620)

Recurrent GBM (N=62)

Neilsen,
B.K., et
al., 2019

Study Description:

Retrospective study of 10 matched *IDH*-wildtype recurrent glioblastomas interrogated by genomic profiling, all pts were treated with standard chemo-radiation.

Patient Population:

Recurrent GBM (N=10)

Median age at initial surgery: 60 (46-73)

III

Results:

All matched tumor pairs demonstrated differences in GA between the primary and recurrence including one resected without any intervening therapy. Four genes that were commonly altered in both primary and recurrent GBM *CDKN2A* (86%) and *CDKN2B* (86%) deletions, *EGFR* activating mutation (52%) or amplification (81%), and *TERT* mutation (95%). PI3K pathway activating mutations were also commonly seen in our cohort (67%). *EGFR* alterations correlated with shorter pt. survival but statistics were not performed due to low pt. #

Author's Conclusions:

Genetic alterations in GBM changed over time and with treatment, although some mutations are common to both the primary and recurrence. The loss of *CDKN2A* inhibits both the p53 and Rb pathways, in cases that did not show *CDKN2A* deletion other mechanisms of p53 and Rb disruptions was present (*CDKN2A* mutation, *p53* + *Rb* mutations, and *MDM2* + *CDK4* amplifications). *TERT* promoter mutation was the most common mutation (20/21). Screening both the primary and recurrent GBM may provide information that guides therapy.

Comments and Conclusions:

Classified as Class III as it is a retrospective study with a limited sample size.

Author (year):	Description of study:	Data class:	Conclusions:
Berghoff, A.S. et al 2015	<p><u>Study Description:</u></p> <p>Single institution retrospective study of 117 newly diagnosed GBM and 18 matched local recurrences assessing for CD3, CD8, CD20, HLA-DR, PTEN, PD-1, and PD-L1 IHC expression and pyrosequencing for assessment of the <i>MGMT</i> promoter methylation status.</p> <p>PD-L1 expression was measured by non-commercial 5H1 antibody</p> <p><u>Patient Population:</u></p> <p>GBM patients (N=117)</p> <p>Recurrent GBM patients (N=18)</p> <p>Median age at primary dx: 60 years</p>	III	<p><u>Results:</u></p> <p>Diffuse/fibrillary PD-L1 was observed in 88.0% (103) newly diagnosed and 72.2% (13) of recurrent GBM. Strong membranous staining was seen in 37.6% (44) of primary and 16.7% (3) of recurrent GBMs. Sparse-to-moderate density TILs was found in 72.6% (85) of primary and 83.3% (15) of recurrent GBMs and were primarily located in perivascular areas and zones of tumor invasion into surrounding parenchyma. PD-1 positive TIL density correlated positively with CD3 ($P < .001$), CD8 ($P < .001$), and CD20 TIL density ($P < .001$), and PTEN expression ($P = .035$) but not <i>MGMT</i> promoter methylation status or PD-L1 expression. PD-L1 expression did not correlate with <i>MGMT</i> promoter methylation status or PTEN expression. There was decreased epithelioid tumor cells with membranous PD-L1 labeling at recurrence but no differences were identified in diffuse/fibrillary PD-L1 staining or PD-1 positive TILs. Proneural and G-CIMP glioblastoma subtypes had lower levels of <i>PD-L1</i> gene expression. The mesenchymal subtype GBMs had high <i>PD-L1</i> gene expression ($P = 5.966e-10$). No significant differences in TIL density between initial and recurrent GBM specimens were evident. Diffuse/fibrillary PD-L1 expression did not differ between newly diagnosed and recurrent glioblastoma specimens ($P = .411$), however, epithelioid tumor cells with anti-PD-L1 membrane labeling were more common in initial tumors (9/18, 50%) than in recurrent tumors (3/18, 16.7%; $P = .034$).</p> <p><u>Author's Conclusions:</u></p> <p>TILs and PD-L1 expression are detectable in the majority of glioblastoma samples and provides rationale for investigating immune checkpoint inhibitors in GBM. The results between primary and recurrent GBM needs to be further investigated. PD-L1 expression was enriched in the mesenchymal subtype.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because study is retrospective review with a limited number of specimens. Used a noncommercial anti-PD-L1 antibody (5H1).</p>
Hodges, T. et al., 2017	<p><u>Study Description:</u></p> <p>327 glioma samples were profiled for TMB, MMR, and immune checkpoint expression. IHC</p>	III	<p><u>Results:</u></p> <p>Only 15 (4.6%) of gliomas demonstrated a high TMB, 4 grade III gliomas, 8 GBM or gliosarcomas, and 3 high grade NOS. Of the GBMs with a high TMB 4 (57%) were primary and 3 (43%) were recurrent. 3.5% of GBMs (7 of 198) had high TMB and 10% of GBMs (20 of 198) had moderately elevated TMB. Including the</p>

and next generation sequencing were used to determine tumor infiltrating PD-1 positive lymphocyte expression, PD-L1 expression, MMR protein expression and mutations, and DNA polymerase epsilon (*POLE*) mutations.

MMR IHC performed in 30 GBMs

PD-1 staining performed in 94 GBMs.

PD-L1 staining performed 189 GBMs.

Patient Population:

GBM (N=198)

gliosarcoma cases, 3.8% of grade IV cases (8 of 208) had high TMB and 10% (21 of 208) had moderately elevated TMB. No association between TMB and age or sex was found.

MMR protein expression was intact in 80% of GBM cases. Of the cases with loss of MMR expression 6.7% (2 of 30) demonstrated a loss of MLH1, 10% a loss of MSH2 (3 of 30), 13.3% (4 of 30) a loss of MSH6, and 6.7% (2 of 30) a loss of PMS2. A high TMB was significantly associated with loss of MMR IHC expression.

Of the GBM patients, 43 of 94 patients (45.7%) were PD-1 positive and 19 of 189 patients (10.1%) were PD-L1 positive. No association was found between TMB and PD-1/PD-L1 expression.

5 of 182 (2.7%) IDH1 wild-type GBM pts had high TMB versus 2 of 16 (12.5%) IDH1 mutated GBM pts ($P = .1014$). PD-1 positive T cells and PD-L1 expression were predominantly detected in IDH1 wild-type tumors and were not associated with high TMB.

Author's Conclusions:

There is an association between high TMB and loss of MMR protein expression, we found no statistically significant association among TMB, influx of cytotoxic CD8+ T cells, and immune checkpoint expression. Among the high TMB-expressing GBMs, 57% were newly diagnosed and 43% were recurrent. The recurrent GBMs with high TMB may not respond to immune checkpoint blockade, as radiation and chemotherapy may induce de novo mutations that do not elicit an effective antitumor immune response.

Comments and Conclusions:

Classified as Class III due to the limited clinical information available and particularly pertaining to this guideline the lack of investigation into alterations in progressive GBM, however there is a large sample size.

Heynckes, S., et al., 2017

Study Description:

III

Single center retrospective study of 64 pts with primary GBM and 28 pts with recurrent GBM in which PD-L1 expression was interrogated at the protein and mRNA level.

Results:

Primary GBMs showed a highly variable expression of PD-L1 gene expression, which was decreased upon each recurrence, with a 54% decrease in median expression upon 1st recurrence ($p=0.0041$). PD-L1 IHC showed a similar reduction of 66.71% in recurrent tumors ($p=0.0046$). Recurrent GBM showed a strongly reduced expression of PD-L1 (de-novo GBM 20.8%, recurrent 7.6% PD-L1 positive cells) and less lymphocytic infiltration (de-novo GBM 17.5%, recurrent 5.3% CD8 positive cells). Age, original surgical procedure and IDH status was not correlated with PD-L1 expression. Extended TMZ therapy beyond the

STUPP protocol was associated with PD-L1 downregulation (p=0.02).

PD-L1 was measured by qRT-PCR and E1LRN ab by Cell Signaling

Author's Conclusions:

20.83% of primary tumors were positive for PD-L1, with variable staining patterns. PD-L1 expression was reduced at both the mRNA and protein level in recurrent tumors. Greater reduction in PD-L1 was seen in pts with extended TMZ therapy.

Comments and Conclusions:

Classified as Class III because study is a retrospective study with a limited number of specimens.

Patient Population:

Primary GBM pts (N=64)

Recurrent GBM pts (N=38)

2nd recurrence (N=18)

3rd recurrence (N=10)

Mean age at dx: 49

Rahman, M. et al., 2018

Study Description:

III

Results:

38 primary and 11 recurrent GBM cases, including 4 matched cases, were evaluated for CD3, CD8, FoxP3, CD68, CD163, PD-1, PD-L1, CTLA4 and CD70 by IHC. *IDH*, *p53*, *ATRX* and *MGMT* promoter methylation status were considered in the analysis.

IDH-mutant tumors showed a lower expression of CD163 and CD70 and a trend toward decreased PD-1, CTLA4, and Foxp3 (regulatory T-cells). And TCGA demonstrate these has lower RNA for *PDC1* (gene for PD-1), *CD274* (gene for PD-L1) *CD3*, *CD8*, *CD163*, and *CD70*. *MGMT* promoter methylated GBM cases were found to have significantly lower odds of expressing CD8 (p = 0.0489) and CD68 (p = 0.0192) than *MGMT* unmethylated tumors. There was no statistical difference between primary or recurrent tumors in the tested variables.

Authors Conclusions:

Immune marker expression between primary and recurrent GBM were not significantly different. *IDH*-1 mutated tumors which are known to have a better prognosis were less likely to express immune checkpoint receptors, monocyte markers and

Patient Population:

Primary GBM pts (N=38)

	<p>Recurrent GBM pts (N=11)</p> <p><i>IDH</i> mutant (N=9)</p> <p>Age of pts was not provided</p>		<p>regulatory T cells markers which are known to be immunosuppressive.</p> <p><u>Comments and conclusions:</u></p> <p>Classified as class III due to the limited number of patients, particularly in the recurrent GBM group and matched samples, the lack of clinical data (including age and prior treatment). The data between matched samples is also not clearly delineated.</p>
Lukas, R. et al, 2018	<p><u>Study Description:</u></p> <p>Phase 1a study of 16 pts with recurrent GBM previously treated with RT+TMZ and 50% also treated with Bevacizumab who received atezolizumab (anti-PD-L1) therapy.</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=16)</p> <p>Mean age at treatment: 52 (31-75)</p>	III	<p><u>Results:</u></p> <p>All pts had at least 1 adverse event. 16/16 (100%) of pts discontinued therapy due to progressive disease. 1 (6%) pt had a partial response, 3 (19%) had stable disease. 3 (19%) of pts had longer term survival of 16+ months. Most pts had low or no PD-L1 expression. 12/12 tumors were microsatellite stable. 1/12 patients had a hypermutated tumor, this pt showed prolonged survival (17.7 months) and decrease in the initial lesion but then demonstrated new lesions.</p> <p><u>Author's Conclusions:</u></p> <p>Atezolizumab is well tolerated in patients, however, there is a lack of clinical efficacy with anti-PD-1 antibody monotherapy in biomarker-unselected patients with recurrent GBM. Combined therapy or selecting for pts with MMR deficiency may be more effective.</p> <p><u>Comments and conclusions:</u></p> <p>Classified as class III due the limited number of pts</p>
Omuro, A., et al., 2018	<p><u>Study Description:</u></p> <p>Phase I clinical trial of 40 pts with recurrent GBM treated with Nivolumab monotherapy (n=10), Nivolumab1 + Ipilimumab3 (n=10), or Nivolumab3 + ipilimumab1 (N=20).</p>	III	<p><u>Results:</u></p> <p>68% of patients had a PD-L1 expression level >1% and 27% >10%. 3 patients achieved a partial response, 1 pt on NIVO3 (11%) and 2 pts on NIVO3+IPI1 (10%) and 8 had stable disease for ≥ 12 weeks, NIVO3, n = 2 (22%), NIVO1+IPI3, n = 2 (20%), NIVO3+IPI1, n = 4 (20%). Progressive disease was seen in 4 (44%) NIVO3 pts, 7 (70%) NIVO1+ IPI3, and 9 (45%) NIVO3+IPI1. 68% of pts had PD-L1 expression levels >1% and 27% had PD-L1 expression >10% by IHC, but no association was observed between PD-L1 expression and response.</p> <p><u>Author's Conclusions:</u></p>

	<p>PD-L1 was assessed by Dako IHC on the primary resection specimen</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=40)</p> <p>Age range: 27-73 (median 58)</p> <p>-</p>		<p>Nivolumab monotherapy was better tolerated than nivolumab + ipilimumab; the tolerability of the combination was influenced by ipilimumab dose. These safety and exploratory findings merit further investigation of immunotherapies in glioblastoma. In the Phase III CheckMate 143 of Nivo3 vs BEV the endpoint of superior OS was not met</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III as it is a prospective study with a limited sample size, primarily to assess safety.</p>
Indraccolo, S., et al., 2019	<p><u>Study Description:</u></p> <p>Single center retrospective study of 57 pts having matched (diagnosis/relapse) GBM samples in which expression of MMR proteins was evaluated by IHC, followed by whole exome sequencing.</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=57)</p> <p>Mean age at treatment: 53.6</p>	III	<p><u>Results:</u></p> <p>3.6% (2) of primary tumors had a partial loss of MMR protein pair. In recurrent GBMs 25.9% (14) demonstrated loss of MMR IHC staining, with loss of MSH6 being the most common (12 tumors). No cases had loss of MSI. Tumors that had a complete loss of MSH6 staining by IHC showed a “hypermutable” genotype with a 135 fold increased in mutational load vs the matched primary, while tumors with only partial loss of MSH6 or no loss did not show this marked change in mutational load. The majority of cases lacking MMR protein expression at relapse (78.5%) had methylated <i>MGMT</i> promoter at diagnosis. No <i>IDH</i> mutant tumors had loss of MMR expression (N=3). Telomere shortening was seen in MMR deficient tumors but not MMR intact tumors.</p> <p><u>Author’s Conclusions:</u></p> <p>Complete loss of MSH6 correlated with increased TMB, which has been shown to correlate with response to immune checkpoint inhibitors in other tumor types. MMR deficient tumors have increased telomere shortening which might underscore genomic instability. Immune-checkpoint inhibitors have to this point not shown marked improvement over BEV, and further efforts to identify patients who would be good responders should include MMR expression, TMB, and MHC class I expression.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because it is a retrospective study with a limited number of pts.</p>
Cloughesy, T. et al., 2019	<p><u>Study Description:</u></p> <p>Multi-institutional clinical trial of 35 recurrent surgically</p>	III	<p><u>Results:</u></p> <p>Pts in the neoadjuvant arm demonstrated a statistically significant increase in OS with a hazard ratio of 0.39 compared to the adjuvant only group. Pts</p>

resectable GBM pts. 19 of whom were treated with adjuvant post-surgery pembrolizumab and 16 who were treated with neoadjuvant and adjuvant post-surgery pembrolizumab.

Patient population:

Recurrent GBM pts (N=35)

Mean age at enrollment: 57.4

IDH wild type N=25 (78%)

IDH mutant N=5 (16%)

IDH status unknown N=2 (6%)

in the adjuvant-only group had a mOS of 228 days (7.5 mo.), whereas those in the neoadjuvant arm had a mOS of 417 days (13.7 mo.). mPFS was 72.5 days (2.4 mo.) in the adjuvant-only group and 99.5 d (3.3 mo.) in the neoadjuvant group (P = 0.03). In pts that received surgery and had histologic evidence of tumor (n = 15 patients per group), the mOS of the neoadjuvant treatment cohort was 400 days (13.2 mo.) from registration date, while that of the adjuvant treatment cohort was 192 d (6.3 mo.) (P = 0.03).

The density of tumor-infiltrating CD8+ T cells was not different between groups but demonstrated significant variability in the neoadjuvant cohort.

Author's Conclusions:

PD-1 monoclonal antibody blockade was associated with statistically significant improvements in OS and PFS when administered in the neoadjuvant setting to pts with recurrent GBM. Neoadjuvant PD-1 monoclonal antibody blockade induces functional activation of tumor-infiltrating lymphocytes, producing an interferon response within the tumor microenvironment.

Comments and Conclusions:

Classified as Class III as it is a prospective study but has a limited number of participants and control arm is not standard therapy. PD-L1 expression was not assessed.

Author (year):	Description of study:	Data class:	Conclusions:
D'Alessandris, Q.G., et al., 2013	<p><u>Study Description:</u></p> <p>Single center prospective study of 10 pts with recurrent GBM in whom BEV and erlotinib were administered based on VEGF and <i>EGFRvIII</i> expression.</p> <p><i>EGFRvIII</i> was assessed by rt-PCR on primary tumor, VEGF was assessed by IHC on primary tumor</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=10)</p> <p>Median age= 53 (30-77)</p> <p>VEGF overexpressed N=10</p> <p><i>EGFRvIII</i> (+) N=4</p>	III	<p><u>Results:</u></p> <p>All of the pts who received BEV + erlotinib achieved a RR (3 CRs and 1 PR) with RR and PFS-6 of 100% (4/4 pts). Of the 6 pts treated with BEV alone, three had a RR (two CRs and one PR) with RR and PFS-6 of 50% (3/6 cases), 2 had progressive disease, and 1 pt had stable dx w/ intra-tumoral hemorrhage. The RR was 70% of cases (7/10), with 5 CRs and 2 PRs. PFS-6 was 70% (7/10 cases). Median PFS and OS were 8.0 mo. (range 3.0 to 31.0 months) and 9.5 mo. (range 5.0 to 31.0 mo.). <u>BEV + erlotinib</u> median PFS was 10.5 mo. (range 7.0–31.0 mo.) and OS was 17.0 mo. (range 8.0–31.0 mo.). <u>BEV only</u> median PFS was 5.5 mo. (range 3.0–9.0 mo.) and OS was and 6.75 mo. (range 5.0–15.0 mo.).</p> <p><u>Author's Conclusions:</u></p> <p>Expression of <i>EGFRvIII</i> is a reliable biomarker for activation of EGFR-related tyrosine kinase, which is the target of erlotinib. Conversely, anti-EGFR immunostaining, though indicative of EGFR overexpression, does not necessarily indicate activation of EGFR-related tyrosine</p>

			<p>kinase. RR and PFS-6 of 70% was achieved in the whole cohort, 100% in the group treated with bevacizumab and erlotinib, and 50% in the group treated with bevacizumab.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III, while it is a prospective study there is a very limited number of pts.</p>
Takano, S, et al., 2014	<p><u>Study Description:</u></p> <p>Retrospective study of 19 pts to examine the expression of VEGF in brain tumors and if it correlates with survival.</p> <p>-</p>	III	<p><u>Results:</u></p> <p>Strong expression of VEGF is seen in the blood vessels in the tumor and in the edge of GBM. In addition, strong expression is seen in the cytoplasm of tumor cells and around the areas of necrosis. Expression is weaker in the anaplastic astrocytoma and in the low-grade astrocytoma than in the GBM. VEGF is not expressed in the normal brain. VEGF concentration in the tissue of GBMs is significantly higher than that in the tissues of other types of tumors, and in the tissue of the normal brain. (*P<0.01). A VEGF concentration of more than 1,000 pg/mg was a prognostic factor. Median OS of the patients with VEGF concentration $\geq 1,000$ pg/mg (number [n] =20) was significantly shorter than in those with <1,000 pg/mg</p>

			<p>(n=17) at 11.8 months and 24.8 months, respectively (P=0.0025).</p> <p>-</p> <p><u>Authors Conclusions:</u></p> <p>VEGF is localized in tumor cells and tumor endothelial cells in glioma, especially in GBM, and its concentration predicts malignant glioma survival.</p> <p>-</p> <p><u>Comments and conclusions:</u></p> <p>Classified as class II as it is a retrospective study with a few patients and the patient number and demographics are not clear.</p>
Erdem-Eraslan, L., et al., 2016	<p><u>Study Description:</u></p> <p>Retrospective study of 114 specimens from the BELOB trial. All pts were treated with temozolomide and radiotherapy and at 1st recurrence were then treated with BEV, CCNU or BEV+CCNU. The cohorts were interrogated to determine possible biomarkers of response to the different treatment arms.</p> <p>All studies were performed on the primary tumor specimen</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=113)</p> <p>Median age at dx: 58 (37-77)</p>	III	<p><u>Results:</u></p> <p>Increased FM04/OSBPL3 expression was significantly correlated with treatment response and OS in the BEV+CCNU arm, but not the BEV or CCNU monotherapy arms. Trend toward increased survival in pts with the "classical" subtype of GBM treated with BEV+CCNU, but it was not significant.</p> <p><u>Author's Conclusions:</u></p> <p>Classical GBMs showed a significant benefit in PFS and a trend toward benefit in OS from</p>

			<p>BEV+CCNU treatment; other subtypes did not show such benefit. Expression of <i>FMO4</i> and <i>OSBPL3</i>, genes were associated with treatment response and increased OS in response to BEV+CCNU.</p> <p><u>Comments and Conclusions:</u></p> <p>Classified as Class III because study is a retrospective study of a relatively large number of ps.</p>
Choi, S.W. et al., 2018	<p><u>Study Description:</u></p> <p>Single center retrospective study of 9 pts treated with BEV at recurrence in whom genomic traits were analyzed to determine differences between the 2 groups. 5 pts were “BEV responders” and 4 were “non-responders”.</p> <p>All studies were done on the primary tumor specimen</p> <p><u>Patient Population:</u></p> <p>Recurrent GBM pts (N=9)</p> <p>Median age at dx: 58 (42-72)</p>	III	<p><u>Results:</u></p> <p>No somatic variants were identified between the 2 groups using whole exome sequencing. 3 of 4 (75%) of the non-responder tumors were classified as the classical subtype, whereas only 1 of 5 (20%) responder tumors were classical subtype. <i>PTGS2</i>, <i>COL4A2</i>, type 1 interferon pathway, immune response, and angiogenesis were significantly upregulated in the responder group, while the non-responder group showed upregulation of angiogenesis and extracellular matrix disassembly. In an independent GBM cohort <i>COL4A2</i> mRNA expression was significantly correlated with poor OS in pts who received BEV but was not prognostic when applied to pts not treated with BEV.</p>

Author's Conclusions:

Classic subtype tumors may not respond as well to BEV. Angiogenesis related genes may be composed of distinct subgroups with distinct functional roles. *COL4A2* demonstrates prognostic value in pts treated with BEV. *COL4A2* encode for alpha-2 chain of type IV collagen which has been previously shown to be involved in vascular stability.

Comments and Conclusions:

Classified as Class III because study is a retrospective study of a small number of pts and analysis was performed on the primary tumor specimen

Prelaj, A., et al., 2018

Study Description:

17 recurrent GBM (12, 70.6%) and anaplastic glioma (5, 29.4%) pts, underwent first-line therapy with Stupp regimen. BEV was administered as third-line therapy after second-line therapy with FTM (13 pts) or as second-line therapy in combination with FTM (4 pts). The assessment of *MGMT* promoter methylation and IDH1 mutation was conducted.

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Patient Population:

Recurrent GBM pts (N=12)

Anaplastic astrocytoma (N=5)

Mean age at recurrence: 50 (26-66)

-

III

Results:

MGMT promoter was methylated in 9 patients (52.9%) and unmethylated in 3 patients (17.5%). The assessment of the IDH1 mutation status was conducted in 8 patients (47%). IDH1 was mutated in 5 patients and wild-type in 3 patients. Subgroup analysis to identify correlations between responder/non-responder patients and clinical characteristics or tumor biomarkers such as sex, histology, tumor side, *MGMT* promoter methylation, IDH1 mutation and OS. A significant

			<p>correlation between the response to BEV and OS ($p < 0.001$) and between the response to BEV and <i>MGMT</i> promoter methylation ($p < 0.05$).</p> <p>-</p> <p><u>Author's conclusions:</u></p> <p>This study shows the efficacy and the safety of BEV alone or in association with FTM in the treatment of MGs.</p> <p>-</p> <p><u>Comments and conclusions:</u></p> <p>Classified as Class III due to the retrospective nature with a small number of pts. More in-depth analysis of the different tumors might have been informative.</p>
Hovinga, K.E. et al., 2019	<p><u>Study Description:</u></p> <p>Retrospective study of 80 GBM pts treated with BEV for recurrence in whom genomic subtype analysis was available.</p> <p><i>EGFR</i> amplification was determined by FISH, <i>EGFRvIII</i> by IHC, <i>MGMT</i> promoter methylation by msPCR performed on the recurrent specimen</p>	III	<p><u>Results:</u></p> <p>Genomic subtypes: 26% classical, 36% mesenchymal, 6% neural, and 31% proneural. The classical subtype has a higher risk of progression on multivariate analysis ($p < 0.001$). <i>EGFR</i> was amplified in 43% of tumors and was more often present in the classical subtype ($p < 0.001$). Amplified <i>EGFR</i> was associated w/ higher risk of progression on multivariate analysis ($p = 0.01$). Multifocal change on BEV was seen in 92% of cases.</p>

Author's Conclusions:

Classical subtype and *EGFR* gene amplification are associated with significantly shorter time to progression for patients with recurrent GBM when treated with BEV.

Comments and Conclusions:

Classified as Class III because it is a retrospective study with some factors not available on all patients and with different treatment regimens.

Patient Population:

Recurrent GBM pts (N=80)

Mean age at treatment: 60.4 (25-80)

Cardona A.F.
et al., 2019

Study Description:

Retrospective study of 59 recurrent GBM pts treated with carmustine plus BEV as second line therapy. Response was evaluated in relation to their molecular expression profile, including *CD133* mRNA, *MGMT* promoter methylation status, *PDGFR* amplification, *YKL40* mRNA expression, *IDH* mutation, *p53*, and *EGFRvIII*.

Patient Population:

Recurrent GBM pts (n=59)

IDH mutant pts (n=18)

Mean age: 43 (23-70)

III

Results:

Progression-free survival of patients with gliomas derived from low-grade tumors was 14.2 months (95% CI 11.3–17.1) and for those with primary GBMs it was 8.2 months (95% CI 6.2–10.1; $p = 0.0001$). Almost all patients with *YKL40* overexpression exhibited higher levels of *CD133* mRNA (85%; $p = 0.10$) and an absence of *MGMT* methylation (85.7%; $p = 0.027$). Higher *CD133* mRNA expression was correlated ($p=0.009$) with improved PFS, while *YKL40* mRNA expression correlates with a worse PFS ($p=0.01$).

Author's Conclusions:

High *YKL40* mRNA expression was related to a worse prognosis and diminished response to BCNU/bevacizumab therapy. *CD133*, and *YKL40* mRNA expression have prognostic role in the response to carmustine/BEV therapy.

Comments and Conclusions:

This is classified as class III as it is retrospective and not all case may truly represent recurrent GBMs, some may

represent anaplastic oligodendrogliomas.

Indraccolo, S., et al., 2019

Study Description:

Single center retrospective study of 57 patients having matched (diagnosis/relapse) GBM samples in which expression of MMR proteins was evaluated by IHC, followed by whole exome sequencing. IDH was assessed by sanger sequencing.

Patient Population:

Recurrent GBM pts (N=57)

Mean age at treatment: 53.6

III

Results:

3.6% (2) of primary tumors had a partial loss of MMR protein pair. In recurrent GBMs 25.9% (14) demonstrated loss of MMR IHC staining, with loss of MSH6 being the most common (12 tumors). No cases had loss of MSI. Tumors that had a complete loss of MSH6 staining by IHC showed a "hypermutable" genotype with a 135 fold increased in mutational load vs the matched primary, while tumors with only partial loss of MSH6 or no loss did not show this marked change in mutational load. The majority of cases lacking MMR protein expression at relapse (78.5%) had methylated *MGMT* promoter at diagnosis. No *IDH* mutant tumors had loss of MMR expression (N=3). Telomere shortening was seen in MMR deficient tumors but not MMR intact tumors.

Author's Conclusions:

Complete loss of MSH6 correlated with increased TMB, which has been shown to correlate with response to immune checkpoint inhibitors in other tumor types. MMR deficient tumors have increased

telomere shortening which might underscore genomic instability. Immune-checkpoint inhibitors have to this point not shown marked improvement over BEV, and further efforts to identify patients who would be good responders should include MMR expression, TMB, and MHC class I expression.

Comments and Conclusions:

Classified as Class III because it is a retrospective study with a limited number of patients.

Figure 1

PRISMA Diagram: